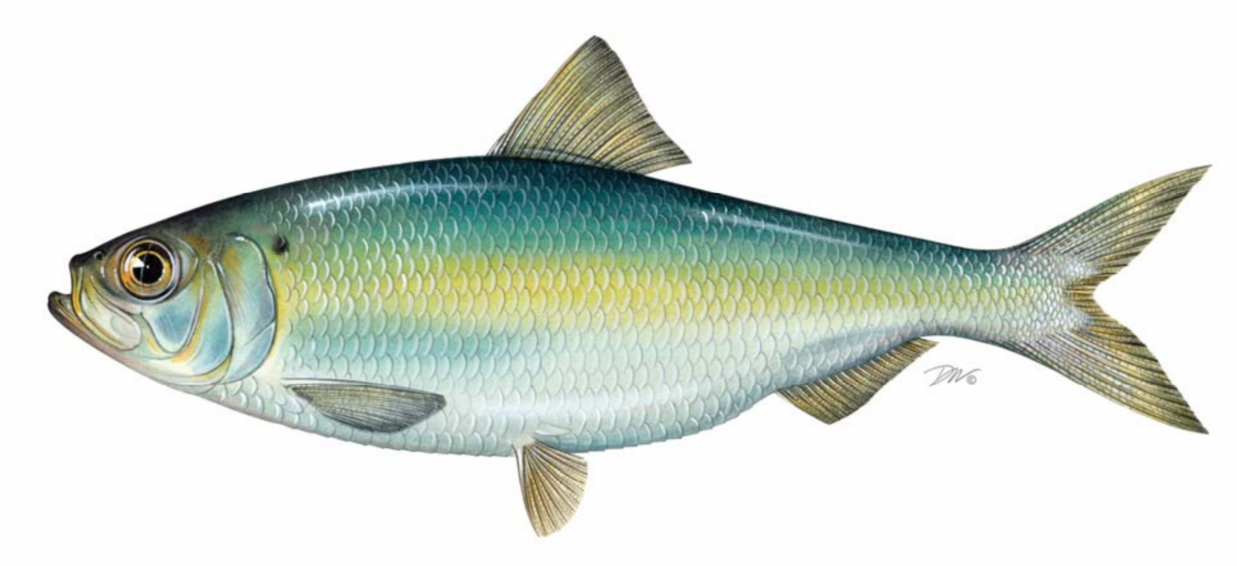
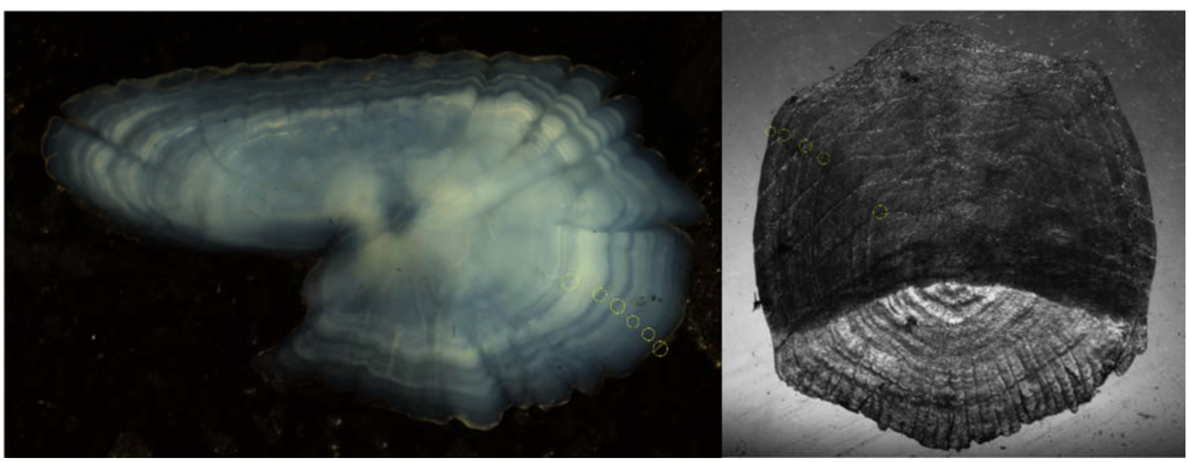
Atlantic States Marine Fisheries Commission

2013 River Herring Ageing Workshop Report





August 2014

Vision: Sustainably Managing Atlantic Coastal Fisheries

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Acknowledgements

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Statement of Problem

River herring species, alewife (*Alosa pseudoharengus*) and blueback herring (*Alosa aestivalis*), underwent an ASMFC benchmark stock assessment in 2012. Age data available for the assessment from ME, NH, MA, RI, NY, DE Bay & River, MD, VA, NC, and SC were derived from scales. All states contributing age data to the assessment cited Cating's (1953) publication on ageing American shad scales as a reference for river herring ageing. Cating's method relies on transverse groove frequencies on scales to assist with identifying the location of freshwater zones and the first three annuli for age determination and some states followed these guidelines for production ageing. Marcy (1969) applied Cating's method to river herring species and was also a reference for state river herring ageing. In addition to age estimates from scales, repeat spawner marks were recorded and were available for the stock assessment. Age estimates and spawner marks were used for maximum age and length-at-age analyses, Chapman-Robson mortality estimators, and statistical catch-at-age models. No information on ageing precision or bias was available for the assessment and ageing data was assumed to be accurate in age-structured analyses.

The stock assessment peer review panel expressed several concerns with age data used in the assessment (ASMFC 2012). There have been few collaborative efforts by states to develop a standardized, validated ageing methodology. Some age readers involved in the ageing workshop emphasized that river herring ageing had only recently become a priority within their agencies and official protocols had not been implemented. In the absence of a standardized methodology, there is the potential for bias and low precision of age determinations made between labs and readers within labs. Bias and precision is likely to change due to personnel changes over time.

Recent work has investigated the validity of applying Cating's method to river herring. Duffy et al. (2011) found that transverse groove frequencies are more closely related to scale size than age and appear to vary over time and between latitudinal locations. These findings suggest that Cating's method is not appropriate as a standardized ageing methodology for alosine populations along the Atlantic coast that experience different growth rates and life histories. There were no alternative age estimates (i.e., otolith age estimates) for comparison in the assessment, though several labs have since started ageing otoliths or have expressed interest in ageing otoliths in the future. Repeat spawning marks were used in place of age estimates to avoid the concerns with age data, but other concerns arose that could impact the reliability of any analyses with spawning mark data, particularly skip spawning.

The stock assessment included several recommendations focused on ageing:

1. Continue to assess current ageing techniques for river herring, using known-age fish, scales, otoliths, and spawning marks.

2. Conduct biannual ageing workshops to maintain consistency and accuracy in ageing fish sampled in state programs.

Workshop Objectives and Goals

The objectives of the exchange and workshop were to (1) share techniques and lessons learned from river herring ageing, (2) investigate age determinations made between labs and structures, and (3) make recommendations to improve river herring ageing practices.

Agencies were contacted about the availalbity of know-age fish for validating ageing methodologies, a concern noted during the stock assessment. No known-age fish were available, so the goals of the workshop were to provide initial information on ageing precision and bias between labs and structures and to improve standardization of ageing practices between states.

Agency Ageing Information

Maine Department of Marine Resources
Alewife and blueback herring scales are collected from spawning adults at all ME DMR managed fishways during the spring spawning run. Some otoliths were collected in 2012, but this is not a regular practice and otoliths are not aged during production ageing. Scale samples have also been collected by harvesters and submitted to ME DMR since 2008. Scales are cleaned with soap and water and blotted dry with paper towels. 3-4 scales are mounted between glass slides and viewed with microfiche readers. Depending on staff availability, 1-2 staff members read each sample. If multiple readers cannot come to a consensus age, the sample is excluded from production ageing or a third staff member provides the final age. Regenerated scales are excluded from production ageing.

New Hampshire Fish and Game
Alewife and blueback herring scales are collected from spawning adults in NH river systems during the spring spawning run. Scales are brushed clean with water and dried with paper towels. 8 scales are mounted on glass slides and viewed with microprojectors. All samples are read by at least two readers. Readers review any conflicting age determinations together and come to a consensus age. If ages cannot be determined from a mounted scale sample, they are remounted and reread. If age still cannot be determined, the sample is discarded from production ageing. Digital imaging software has been used in addition to microprojectors since 2012.

Massachusetts Division of Marine Fisheries
Alewife and blueback herring scales and otoliths are collected from spawning adults in MA river systems during the spring spawning run. Scales are cleaned in ultrasonic cleaners with 5% pancreatin solution and otoliths are rinsed with water and allowed to air dry overnight. Scales are mounted between glass slides and viewed with digital imaging software and a camera on a macro mirror stand. Otoliths are submersed in mineral oil and viewed with a stereomicroscope. Unreadable samples are excluded from production ageing. See Appendix A for more details on the MA DMF river herring ageing protocol. MA DMF has also produced a video (https://www.youtube.com/watch?v=wkGeh-q_Jgo) detailing extraction of river herring otoliths.

Rhode Island Division of Fish and Wildlife
Alewife and blueback herring scales are collected from spawning adults in RI river systems during the spring spawning run. Scales are cleaned and mounted between glass slides and viewed with microfilm readers. Regenerated scales and samples with poor quality scales are excluded from production ageing.

Connecticut Department of Energy and Environmental Protection
Alewife and blueback herring scales are collected from spawning adults during the spring spawning run. Scales are rinsed with water and wiped clean. 6-10 scales are mounted between glass slides and viewed with a microfiche reader. Unreadable samples (i.e., regenerated scales, scales with heavily eroded edges) are excluded from production ageing. Any scale samples of poor quality that two readers cannot reach a consensus age determination for are excluded from production ageing.

New York State Department of Environmental Conservation
Alewife and blueback herring scales and otoliths are collected from spawning adults in NY river systems during the spring spawning season. Currently, scales are the only structures aged during production ageing. The MA DMF protocol has been adopted as a model for the NY DEC protocol. Consistent river herring ageing began in 2012. Some sporadic ageing was done prior to 2012 using scale impressions on acetate. Archived scale samples are currently being remounted according to the new ageing protocol.

New Jersey Division of Fish and Wildlife
Alewife and blueback herring otoliths are collected during the NJ DFW Ocean Trawl Survey in January and April. This survey collects smaller fish than sampled in other state programs. Scale collection during this survey is not feasible, as few scales are retained on fish collected. At the time of the workshop, NJ DFW staff had just started reviewing river herring ageing materials provided by the MA DMF and practicing ageing otoliths, but had not begun production ageing. Digital imaging software is used to store images of each otolith sample collected.

Maryland Department of Natural Resources
Alewife and blueback herring scales are collected from spawning adults in MD river systems during the spring spawning season. Scales are cleaned with soap and water and rubbed dry with paper towels. 3-4 scales are mounted between glass microscope slides and viewed with microfiche readers.

North Carolina Division of Marine Fisheries
Alewife and blueback herring scales and otoliths are collected from spawning adults in NC river systems during the spring spawning season. Otoliths and scales are collected during fishery-dependent monitoring (pound net survey) and scales are collected during fishery-independent monitoring (gill net survey).

South Carolina Department of Natural Resources
Blueback herring scales have been collected from spawning adults since 2009. Currently, no production ageing is done by the SC DNR and all samples are sent to MD DNR to be aged.

Hard Part Exchange and Workshop Sample Evaluation

Prior to the ageing workshop, the participating labs conducted an exchange of age structures from both species. Samples were provided by ME DMR, MA DMF, CT DEEP, NY DEC, NJ DFW, MD DNR, NC DMF, SC DNR, and FL FWC. All samples, except for those contributed from NJ DFW, were paired samples (i.e., otoliths and scales) from spawning adults collected during spring spawning runs. Paired samples were collected from alewife ranging from 228-382 mm TL and from blueback herring ranging from 196-314 mm TL (tables 1 and 2). There were 69 paired alewife samples and 79 paired blueback herring samples (the otoliths for one blueback herring were missing, so there were 80 blueback herring scale samples). NJ DFW samples were collected during January and April and only included otoliths (table 3). NJ DFW samples were collected from alewife ranging from 82-314 mm TL and blueback herring ranging from 67-294 mm TL. There were 43 alewife otolith samples and 31 blueback herring otolith samples from NJ DFW. See Appendix B for guidelines to exchange participants on providing samples for the exchange. Exchange participants were asked to read each structure following their labs protocol, if applicable (Appendix C).

The group focused on inter-lab and inter-structure comparisons for this workshop. Intra-lab comparisons will provide important information on ageing error as well, but can be done within labs on a regular basis. NJ DFW samples were primarily from fish smaller than fish aged by the other labs and only included otoliths, so comparisons were of paired samples from the other states. Some labs/readers did not read both structures and/or the majority of samples for a particular structure (<85%) and comparisons of those labs/readers were not examined. If multiple readers within a lab participated in the exchange and did not provide a consensus age for the lab, the lab was not included in inter-lab comparisons. Symmetry around the diagonal 1:1 line (exact agreement) for each comparison was tested with a Bowker's test of symmetry (Evans and Hoenig 1998). Significant p-values (<0.05) indicate systematic bias between two sets of age determinations. It is important to keep in mind that, without a validated ageing method, a significant Bowker's p-value indicates the two readers read samples systematically different from each other. Both age determinations may still be biased from the true age and this cannot be determined without a validated ageing method (i.e., known-age fish). Mean CVs and percent agreement were calculated for each comparison to provide measures of precision. Summaries of bias and precision are available in tables 4-9. Age frequency and age bias plots for each comparison are in figures 1-150.

Systematic bias was commonly detected for inter-lab comparisons and paired sample comparisons. CVs were generally greater than 5%, which Campana (2001) suggests as a generic ageing precision reference point. Examination of the age frequency and age bias plots indicate that MD DNR scale ages were consistently older than the other labs scale ages (figures 21-30, 71-80). NH F&G scale ages were generally younger than MD DNR scale ages, but older than the other labs scale ages (figures 37-38, 43-44, 47-50, 87-88, 93-94, 97-100). Of the labs that provided a consensus age (or had only one reader), comparisons of NY DEC and MA DMF age determinations consistently had the lowest CVs (<10%) and highest percentage agreement (>56%). Systematic bias was detected for two of four comparisons of MA DMF and NY DEC age determinations (tables 4-7).

The paired sample comparisons resulted in typical patterns commonly observed in paired sample comparisons of other species. Otoliths were generally aged younger than scales for younger fish and otoliths were generally aged older than scales for older fish. As fish grow, annuli are compacted towards the edge of the scale and can become more difficult to identify. However, comparisons of age determinations by some states that had experience reading both structures (e.g., MA DMF) resulted in relatively high precision (tables 8 and 9). Systematic bias was detected for the MA DMF blueback herring structure comparison, but not the alewife structure comparison.

The exchange was intended to facilitate discussion at the workshop and provide baseline information on river herring ageing error. There are several caveats that likely impacted the results of the comparisons. Experience reading river herring age structures varied considerably between labs and readers and not all readers had read both age structures prior to the exchange. The exchange also included samples from fish along the entire coast which have noticeable differences due to different growth rates, distance traveled and time spent in freshwater systems, and other environmental variables. Reading samples from unfamiliar river systems and regions likely reduced precision and increased bias compared to reading samples within a familiar river system. Once readers and labs develop more experience and have additional materials to assist with ageing (i.e., reference collections), new comparisons can be assessed against the baseline information in this report.

During the workshop the group examined several paired samples from both species with digital imaging software. A mix of samples with excellent and poor agreement during the exchange were selected to identify issues with age determinations. Consensus age determinations, statistics from the exchange, and other notes on the samples evaluated are available in Appendix D.

Several issues of reading scales were attributed to interpretation of the freshwater zone, including identification of the first annulus. Two different interpretations of the actual freshwater zone were made for one scale, though the group agreed that both interpretations ultimately resulted in the same age determination and were, therefore, not of concern for age estimates (e.g., alewife 4 in Appendix D). The first annulus is often very indistinct and during the exchange and workshop some participants counted the first distinct annulus as age-1, while others counted the first distinct annulus as age-2 and counted the age-1 annulus as an indistinct ring that was difficult to distinguish from the freshwater zone. This disagreement was apparent in the exchange results for several samples that were assigned one of two subsequent ages by the majority of the participants. The group discussed the need to carefully examine the area adjacent to the freshwater zone for an indistinct annulus. The assumed second annulus is usually the most defined annulus and is often mistaken for the first annulus (e.g., alewife 2 in Appendix D). Issues relating to the quality of scale samples were regeneration, erosion (e.g., alewife 5 in Appendix D), and poor preparation (e.g., dirty, not enough scales, scales of different sizes). Implementing official protocols with improved preparation methods should result in better quality scale samples for production ageing.

Common issues identified for reading otoliths included identification of the first annulus and differentiating between true annuli and check marks, or false annuli (e.g., blueback 2 in Appendix D). The group agreed that all true annuli should be continuous around the core of the otolith and should not merge with other annuli. Experience and reference collections should assist readers with differentiating between true annuli and check marks. Participants also discussed crystallization, fragility, and storage in residual mineral oil from reading as having adverse impacts on the quality of the otoliths in the exchange. Crystalized otoliths should be excluded from production ageing and care should be exercised when extracting, preparing, and storing otoliths.

Workshop Recommendations

Following review of the exchange results and evaluation of samples during the workshop, the group emphasized the need for standardized ageing protocols between labs. The labs agreed to adopt the MA DMF protocol to serve as a model for collecting, preparing, and ageing river herring (see appendix A for MA DMF protocol). MA DMF has previously implemented their protocol in production ageing and provided this information to two other labs (NY DEC and NJ DFW) for developing their protocols. Comparison of NY DEC and MA DMF age determinations from the exchange suggest that standardized protocols reduce ageing error between labs. Several additional recommendations were made during the workshop and are included below.

- When possible, paired ageing structures (otoliths and scales) should be collected for comparisons. If otoliths are collected, both should be collected for assistance during age determination. Readers can compare both otoliths, if necessary, to differentiate between true and false annuli. Scales should always be collected because of the presence of spawning marks.
- Otoliths should be cleaned with water immediately after being extracted, air dried overnight, and stored dry.
- Transverse grooves may not correlate well with annuli in certain regions or river systems and should not be used for age determination.
- Microscope magnification should be standardized for reading otoliths. Magnification should only be changed to interpret the edge of the otolith.
- When using digital imaging for scales, standardize magnification used to read scales. The same magnification should be used to avoid interpreting marks on scales at different levels of detail.
- The rostrum of the otolith should be avoided during age determination, as it grows faster than other parts of the otolith causing false annuli to appear more like true annuli.
- Reference collections of ageing structures should be developed by river system. Reference collections should be read by all new readers as well as experienced readers that have not read samples for an extended period of time (i.e., only preform production ageing following spring spawning runs). Precision and bias benchmarks should be established and met by all readers before production ageing.
- Age samples should be read for one river at a time during production ageing. Ageing should also be done for one species of river herring at a time.

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Tables

Table 1. Sample size of paired alewife otoliths and scales in the exchange.

Total Length (mm)	ME	MA	CT	NY	MD	NC	Length Bin Total
220-229	0	1	0	1	1	1	**4**
230-239	0	1	1	1	1	2	**6**
240-249	0	1	2	1	1	1	**6**
250-259	1	1	2	1	1	2	**8**
260-269	1	1	2	1	1	2	**8**
270-279	3	1	2	1	1	2	**10**
280-289	2	1	2	1	1	1	**8**
290-299	1	1	1	1	1	1	**6**
300-309	2	1	0	1	1	2	**7**
310-319	0	1	0	0	0	3	**4**
320-329	0	1	0	0	0	0	**1**
380-389	0	0	0	0	0	1	**1**
State Total	**10**	**11**	**12**	**9**	**9**	**18**	**69**

Table 2. Sample size of paired blueback herring otoliths and scales in the exchange.

Total Length (mm)	ME	MA	NY	MD	NC	SC	FL	Length Bin Total
190-199	2	1	0	0	0	0	0	**3**
200-209	0	1	1	0	0	0	0	**2**
210-219	1	1	1	0	0	0	0	**3**
220-229	0	1	1	0	1	0	1	**4**
230-239	3	1	1	1	2	2	1	**11**
240-249	4	1	1	1	1	2	1	**11**
250-259	3	1	1	1	2	2	1	**11**
260-269	1	1	1	1	1	2	1	**8**
270-279	0	1	1	1	4	2	1	**10**
280-289	0	1	1	1	0	2	1	**6**
290-299	0	0	1	1	2	2	0	**6**
300-309	0	0	0	0	1	2	0	**3**
310-319	0	0	0	0	0	1	0	**1**
State Total	**14**	**10**	**10**	**7**	**14**	**17**	**7**	**79**

Table 3. Sample size of river herring otoliths from NJ DFW in the exchange.

Total Length (mm)	Alewife	Blueback Herring
60-69	0	1
70-79	0	2
80-89	3	2
90-99	3	2
100-109	3	2
110-119	3	2
120-129	3	2
130-139	3	2
140-149	3	2
150-159	3	2
160-169	3	2
170-179	3	3
180-189	3	3
190-199	3	2
200-209	3	1
210-219	3	0
230-239	0	1
260-269	1	0
Species Total	**43**	**31**

Table 4. Sample size (n), Bowker's p-value, mean CV, and exact percent agreement for inter-lab age comparisons of alewife otoliths. Significant Bowker's p-values (<0.05) are indicated with an asterisk.

Laboratories	n	Bowker's p-value	CV (%)	Exact Agreement (%)
MD and NY	64	0.366	22	25
MD and CT	64	0.063	26	31
MD and MA	64	0.011*	21	27
MD and NEFSC	64	<0.001*	24	28
NY and CT	66	0.496	22	36
NY and MA	67	0.109	8	63
NY and NEFSC	67	<0.001*	14	46
CT and MA	66	0.137	22	38
CT and NEFSC	66	0.024*	23	36
MA and NEFSC	68	<0.001*	11	50

Table 5. Sample size (n), Bowker's p-value, mean CV, and exact percent agreement for inter-lab age comparisons of alewife scales. Significant Bowker's p-values (<0.05) are indicated with an asterisk.

Labs	n	Bowker's p-value	CV (%)	Exact Agreement (%)
MD and NY	68	<0.001*	14	29
MD and CT	68	<0.001*	29	4
MD and MA	69	<0.001*	19	12
MD and NEFSC	68	0.775	21	13
MD and NH	68	0.001*	10	34
NY and CT	68	<0.001*	17	31
NY and MA	68	<0.001*	8	56
NY and NEFSC	68	0.003*	14	31
NY and NH	67	<0.001*	10	49
CT and MA	68	<0.001*	13	46
CT and NEFSC	68	0.006*	17	32
CT and NH	67	<0.001*	24	18
MA and NEFSC	68	0.775	10	50
MA and NH	68	<0.001*	15	35
NEFSC and NH	67	<0.001*	16	24

Table 6. Sample size (n), Bowker's p-value, mean CV, and exact percent agreement for inter-lab age comparisons of blueback herring otoliths. Significant Bowker's p-values (<0.05) are indicated with an asterisk.

Labs	n	Bowker's p-value	CV (%)	Exact Agreement (%)
MD and NY	78	0.011*	17	33
MD and CT	73	<0.001*	24	25
MD and MA	78	<0.001*	17	35
MD and NEFSC	76	<0.001*	32	24
NY and CT	73	0.038*	16	48
NY and MA	78	0.008*	6	68
NY and NEFSC	76	<0.001*	23	33
CT and MA	73	0.369	15	47
CT and NEFSC	72	<0.001*	20	38
MA and NEFSC	76	<0.001*	20	36

Table 7. Sample size (n), Bowker's p-value, mean CV, and exact percent agreement for inter-lab age comparisons of blueback herring scales. Significant Bowker's p-values (<0.05) are indicated with an asterisk.

Labs	n	Bowker's p-value	CV (%)	Exact Agreement (%)
MD and NY	70	0.028*	14	33
MD and CT	77	<0.001*	23	17
MD and MA	80	<0.001*	16	24
MD and NEFSC	80	0.004*	13	31
MD and NH	78	0.021*	9	46
NY and CT	69	0.006*	14	35
NY and MA	70	0.344	7	67
NY and NEFSC	70	0.086	10	50
NY and NH	70	<0.001*	12	49
CT and MA	77	0.004*	15	36
CT and NEFSC	77	0.001*	18	30
CT and NH	75	<0.001*	22	21
MA and NEFSC	80	0.004*	11	52
MA and NH	78	<0.001*	13	36
NH and NEFSC	78	<0.001*	10	49

Table 8. Sample size (n), Bowker's p-value, mean CV, and exact percent agreement for paired alewife otolith and scale comparisons. Significant Bowker's p-values (<0.05) are indicated with an asterisk.

Reader	n	Bowker's p-value	CV (%)	Exact Agreement (%)
NC R1	66	0.1	19	21
NC R2	66	0.682	14	30
MD	65	<0.001*	25	22
NJ R1	68	0.103	21	35
NJ R2	68	<0.001*	29	19
NJ R3	68	0.003*	22	21
NY Consensus	67	0.001*	14	37
CT	66	0.696	26	26
MA Consensus	68	0.039*	9	57
NEFSC	67	<0.001*	19	27
ME R1	68	<0.001*	17	22
ME R2	68	0.002*	18	25
ME R4	69	0.001*	19	28

Table 9. Sample size (n), Bowker's p-value, mean CV, and exact percent agreement for paired blueback herring otolith and scale comparisons. Significant Bowker's p-values (<0.05) are indicated with an asterisk.

Reader	n	Bowker's p-value	CV (%)	Exact Agreement (%)
NC R1	74	0.069	15	36
NC R2	79	0.593	12	48
MD	79	0.339	15	30
NJ R1	78	0.375	18	41
NJ R2	79	<0.001*	35	4
NJ R3	78	0.031*	19	23
NY Consensus	69	0.317	11	54
CT	70	0.255	25	23
MA Consensus	78	0.123	6	69
NEFSC	76	<0.001*	27	32
ME R1	66	<0.001*	14	39
ME R4	66	0.046*	15	35

Figures

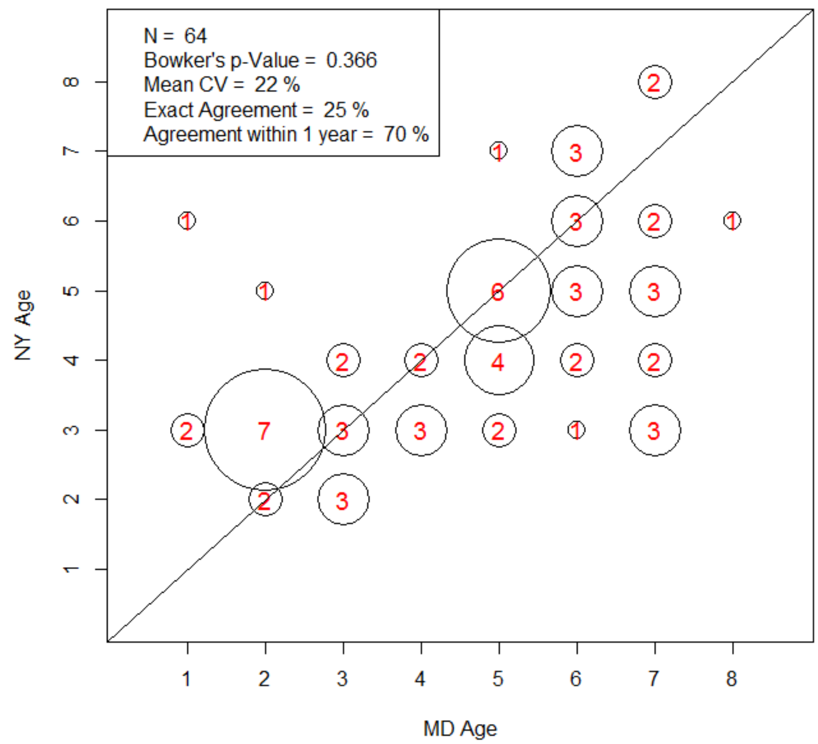


Figure 1. Age frequency plot for MD and NY alewife otolith age determinations.

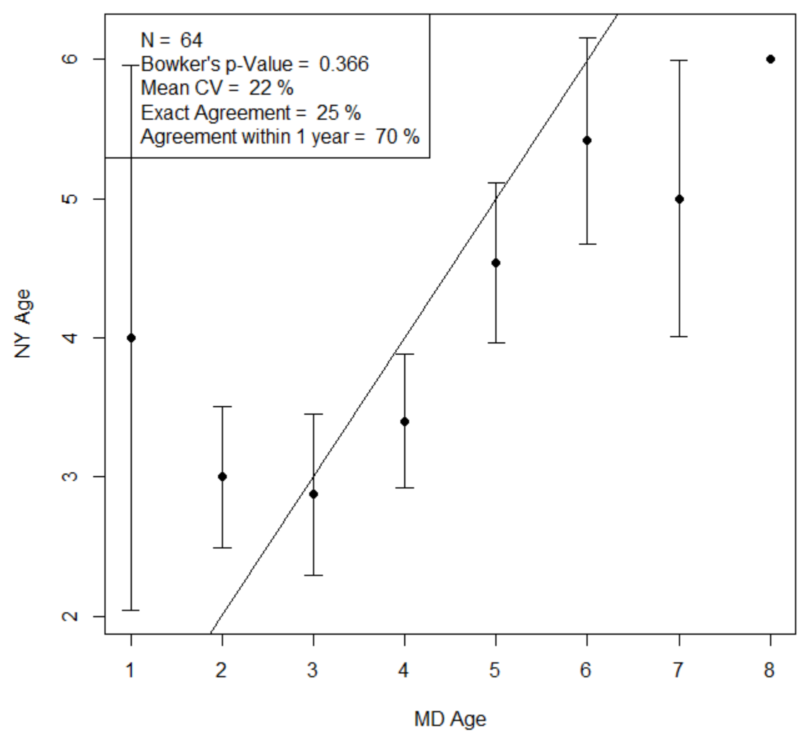


Figure 2. Age bias plot for MD and NY alewife otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

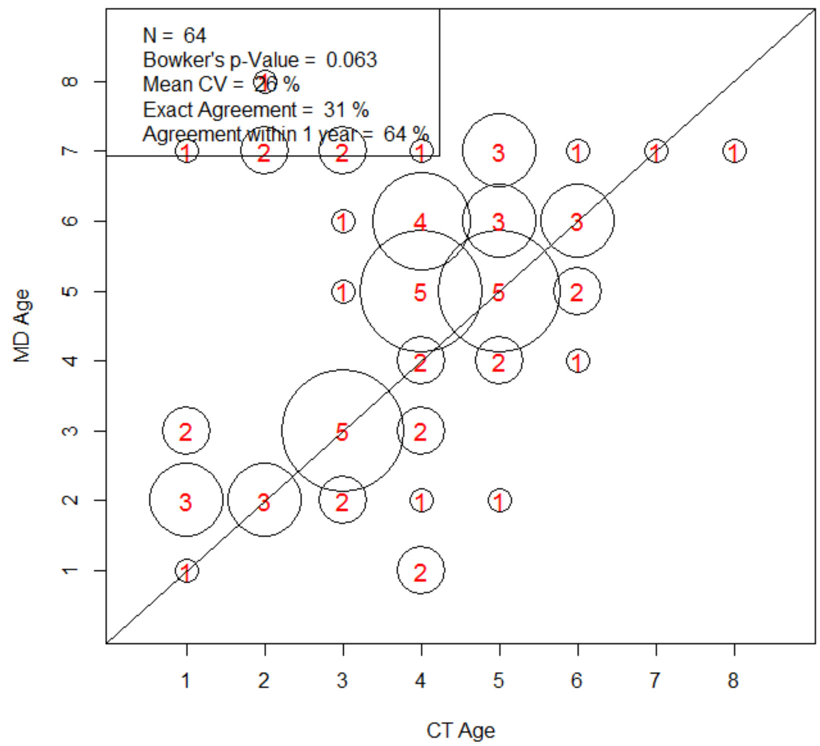


Figure 3. Age frequency plot for MD and CT alewife otolith age determinations.

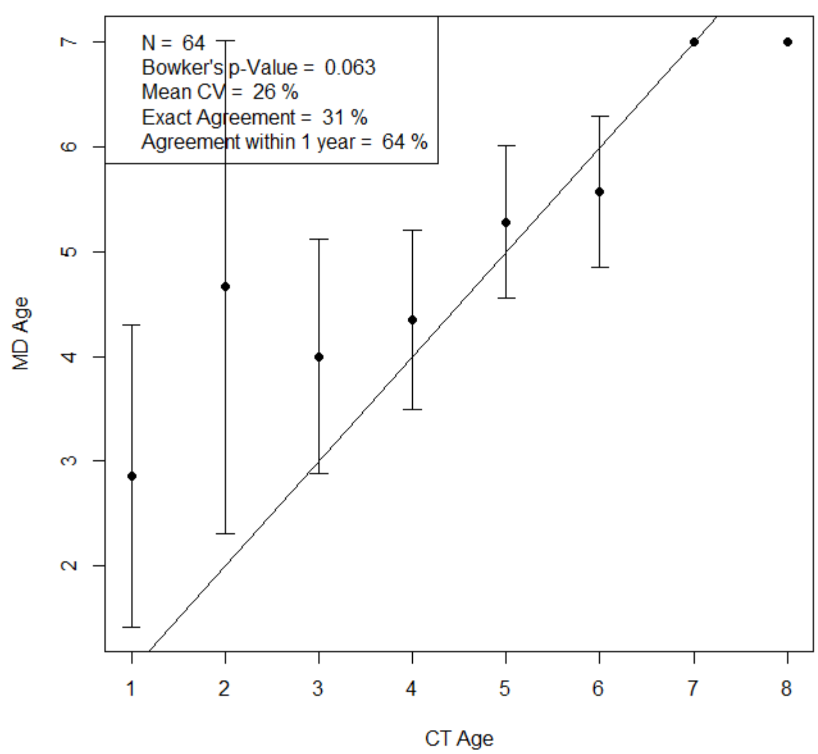


Figure 4. Age bias plot for MD and CT alewife otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

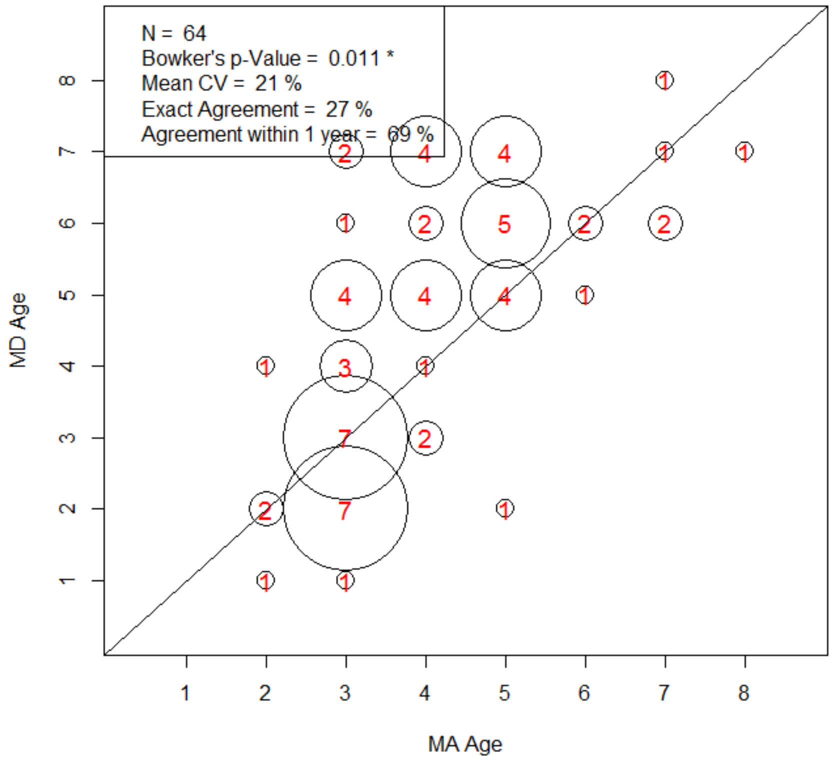


Figure 5. Age frequency plot for MD and MA alewife otolith age determinations.

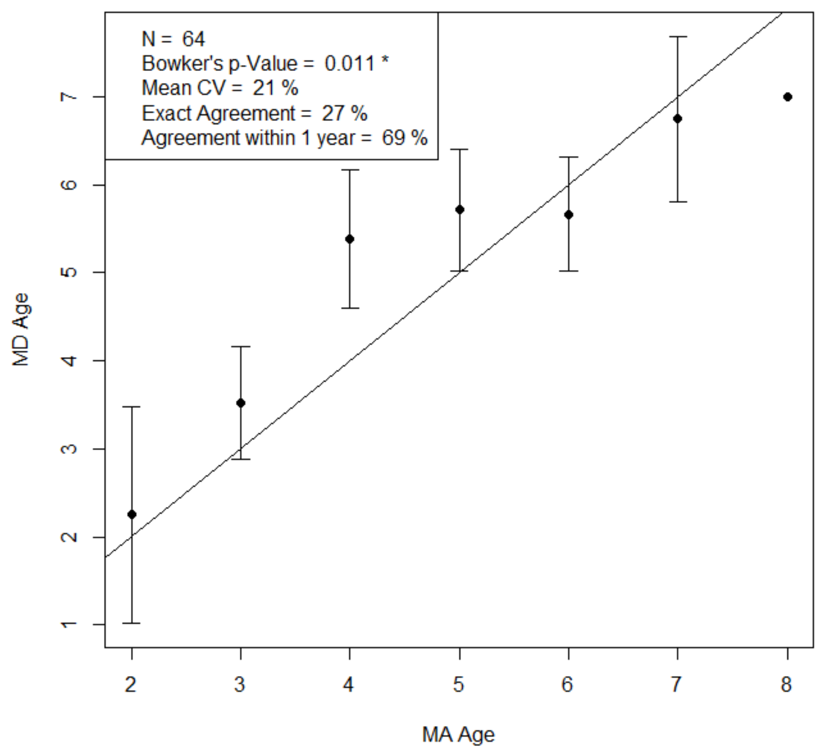


Figure 6. Age bias plot for MD and MA alewife otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

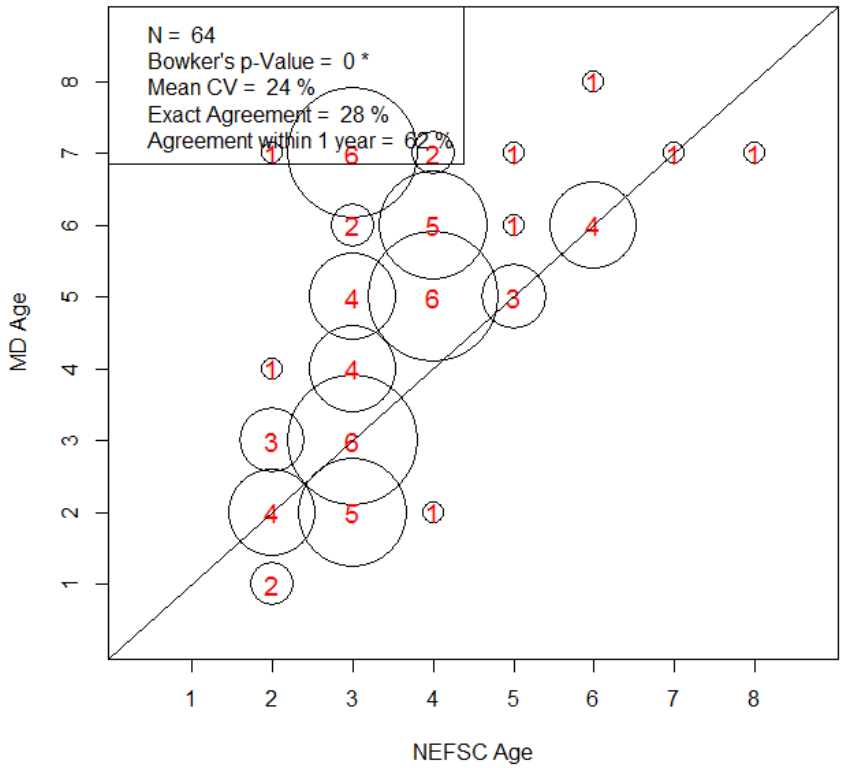


Figure 7. Age frequency plot for MD and NEFSC alewife otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

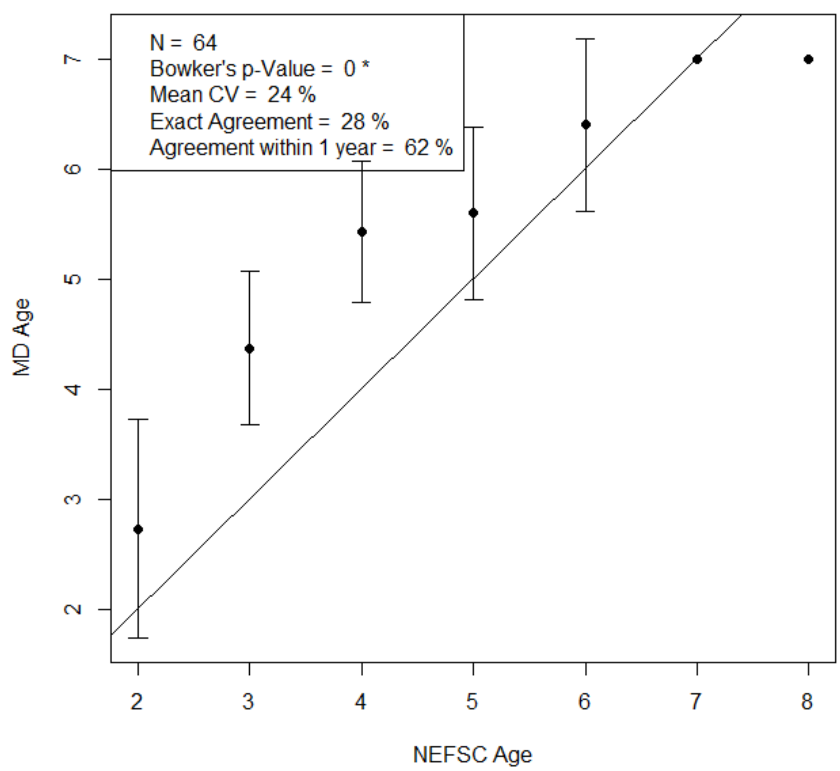


Figure 8. Age bias plot for MD and NEFSC alewife otolith age determinations.

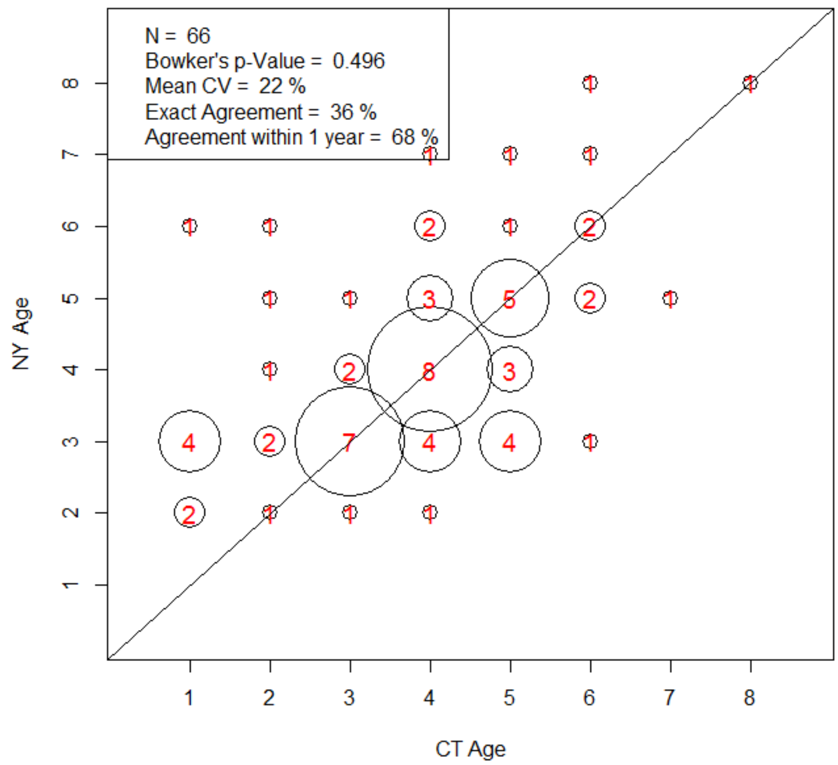


Figure 9. Age frequency plot for NY and CT alewife otolith age determinations.

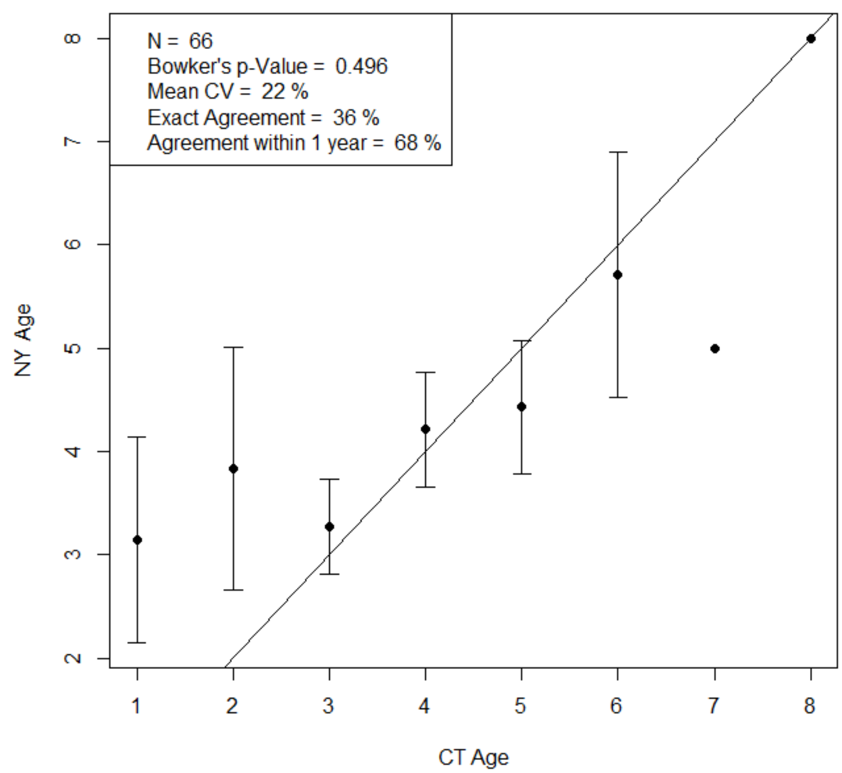


Figure 10. Age bias plot for NY and CT alewife otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

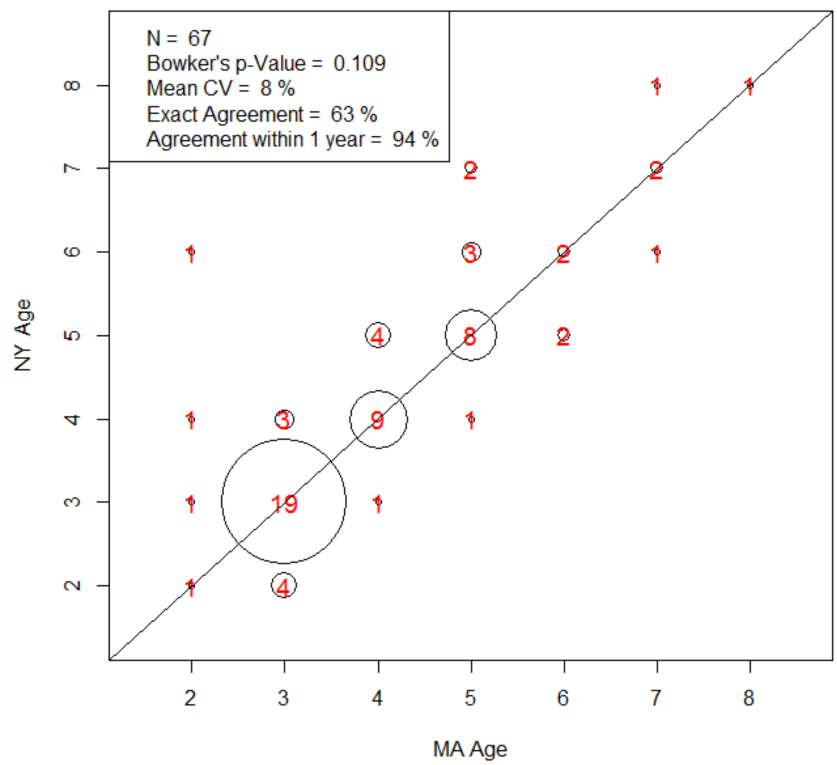


Figure 11. Age frequency plot for NY and MA alewife otolith age determinations.

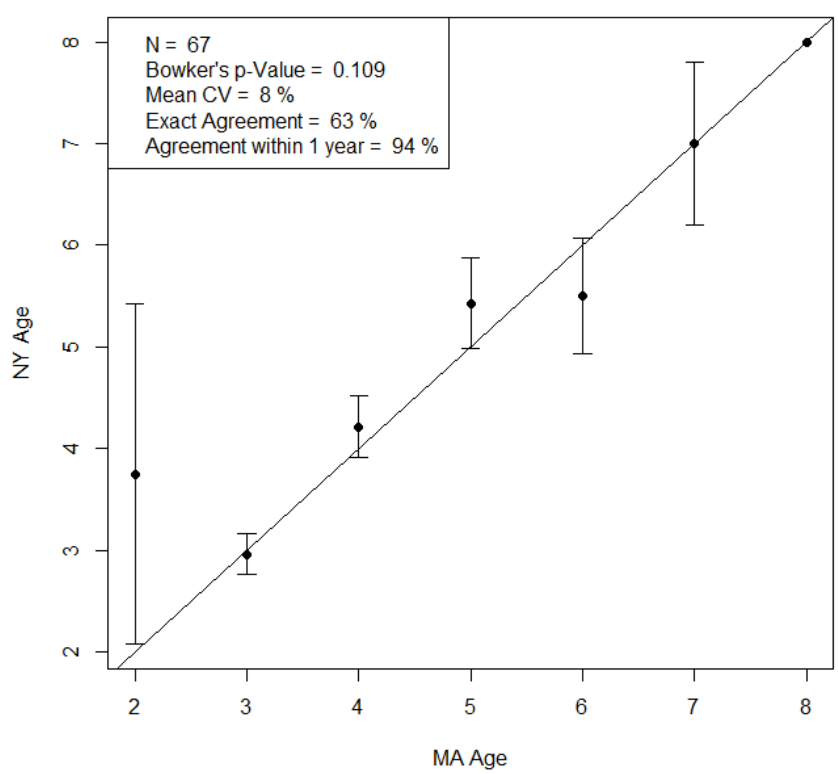


Figure 12. Age bias plot for NY and MA alewife otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

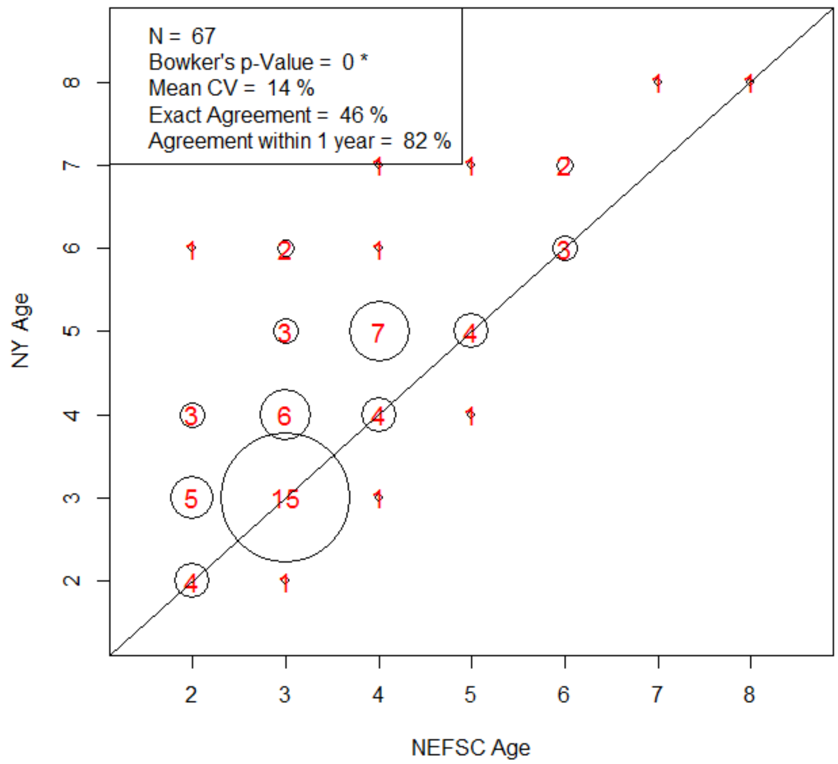


Figure 13. Age frequency plot for NY and NEFSC alewife otolith age determinations.

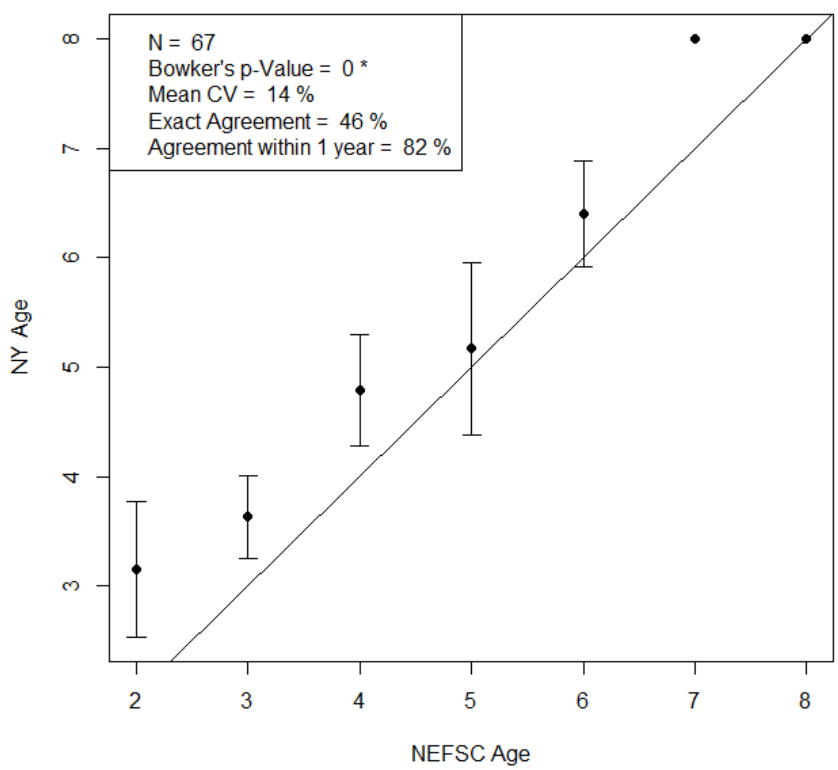


Figure 14. Age bias plot for NY and NEFSC alewife otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

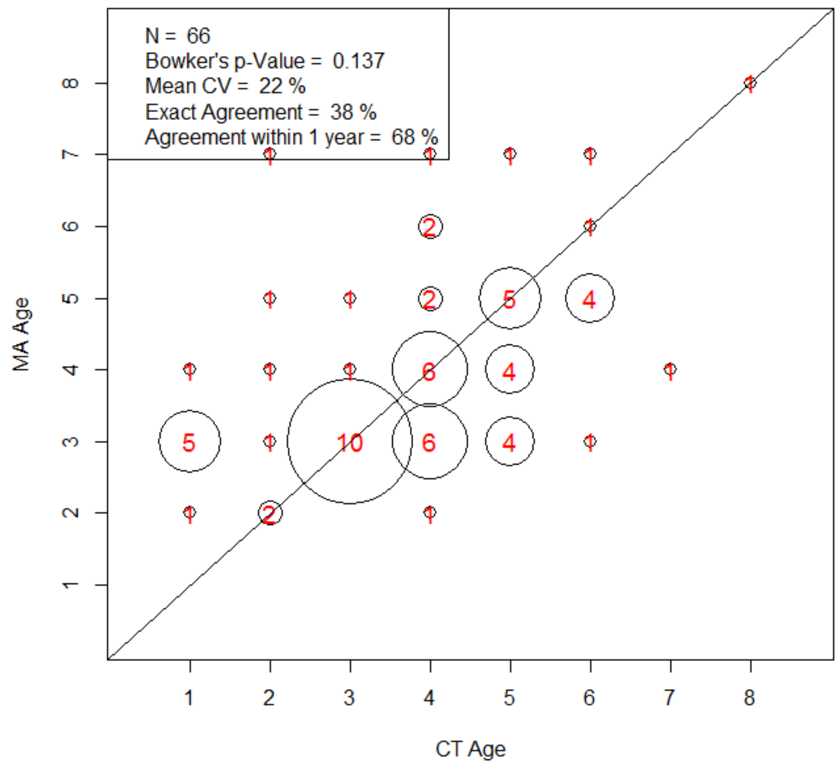


Figure 15. Age frequency plot for CT and MA alewife otolith age determinations.

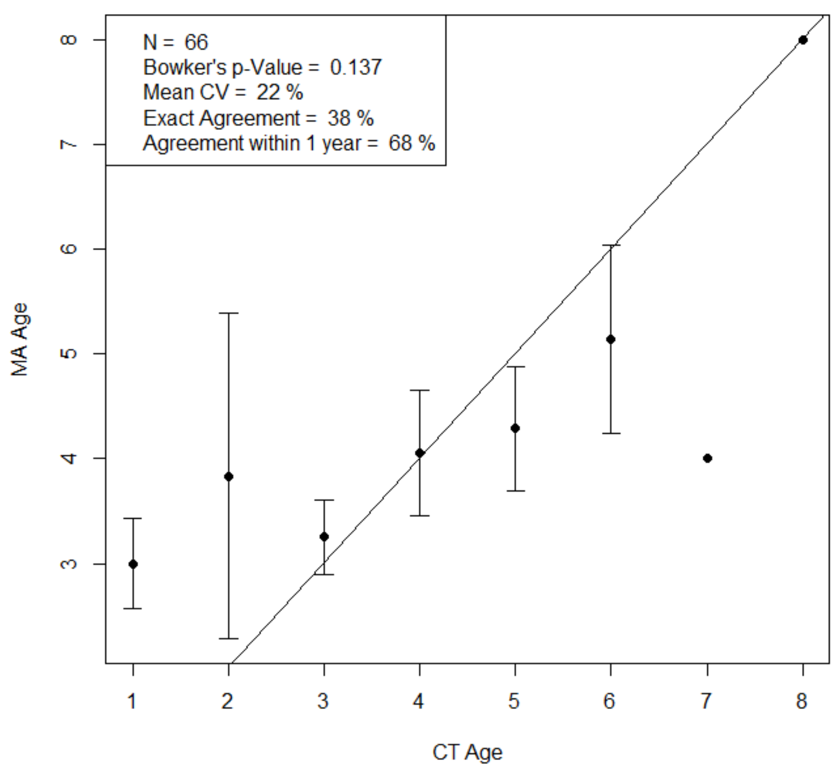


Figure 16. Age bias plot for CT and MA alewife otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

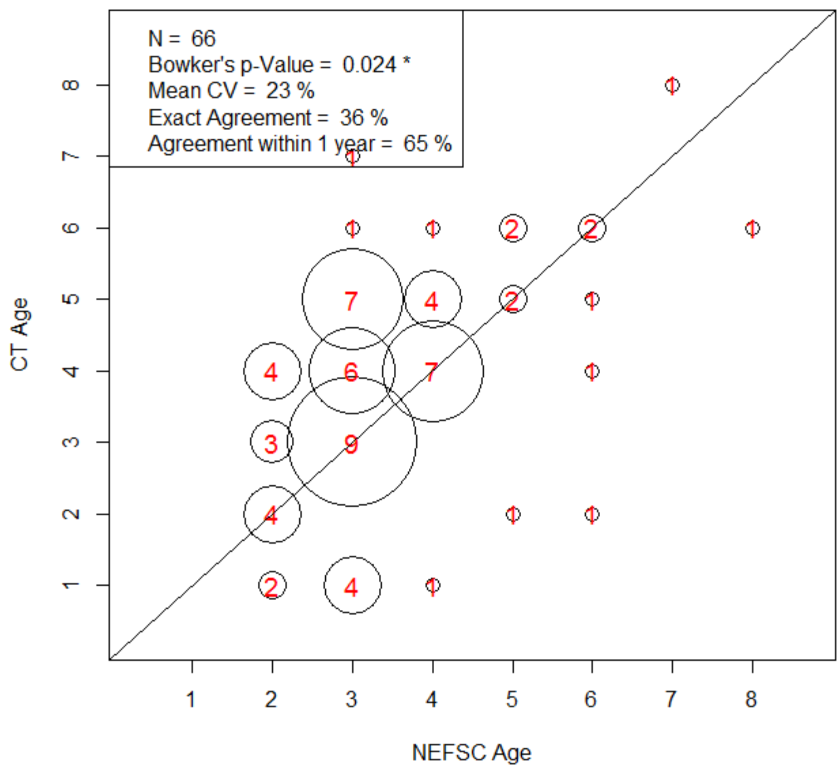


Figure 17. Age frequency plot for CT and NEFSC alewife otolith age determinations.

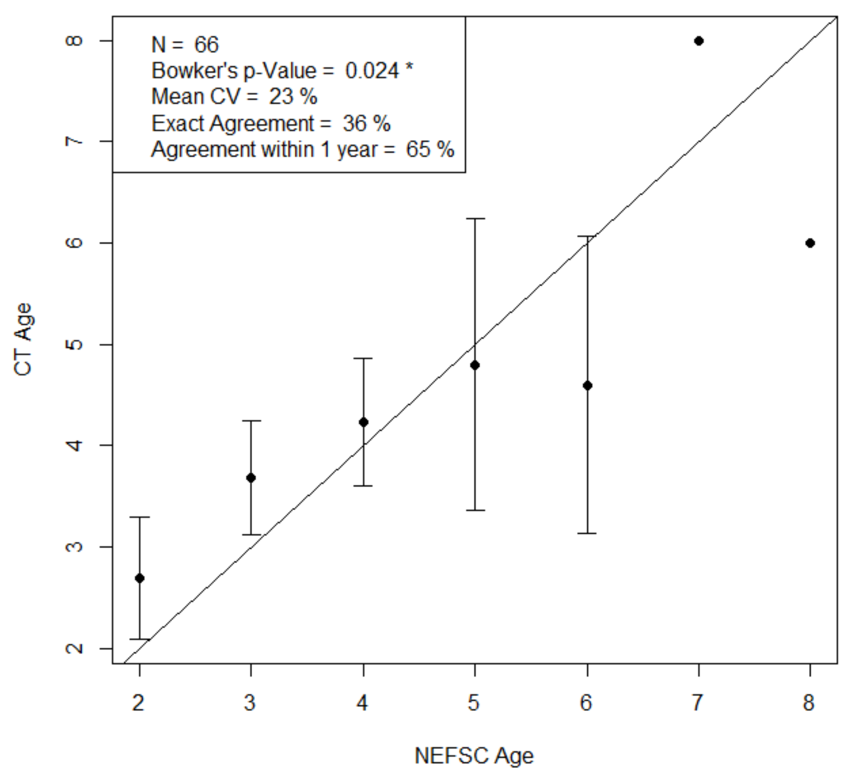


Figure 18. Age bias plot for CT and NEFSC alewife otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

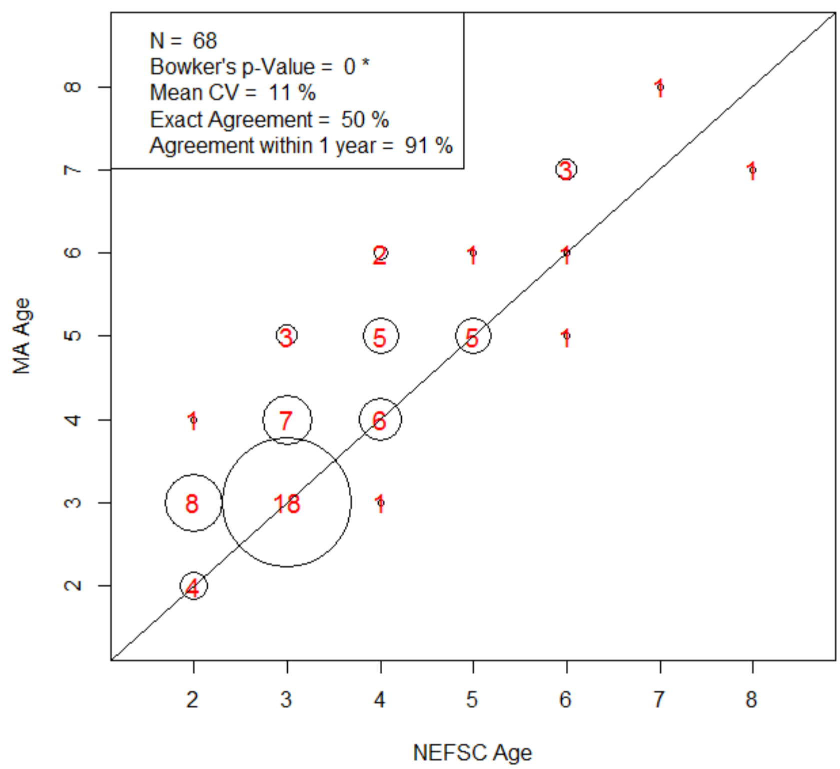


Figure 19. Age frequency plot for MA and NEFSC alewife otolith age determinations.

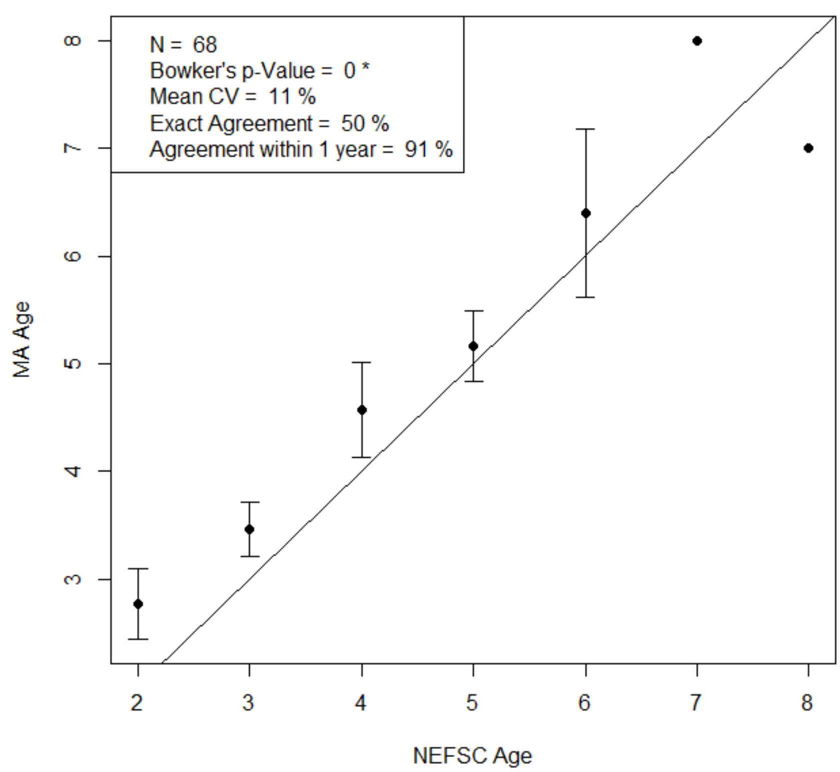


Figure 20. Age bias plot for MA and NEFSC alewife otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

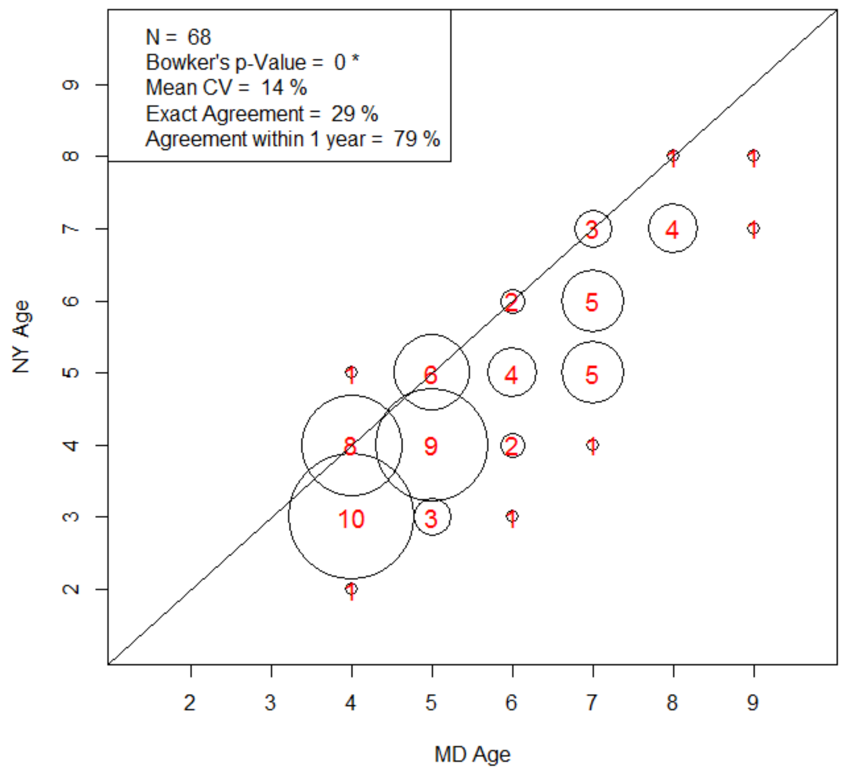


Figure 21. Age frequency plot for MD and NY alewife scale age determinations.

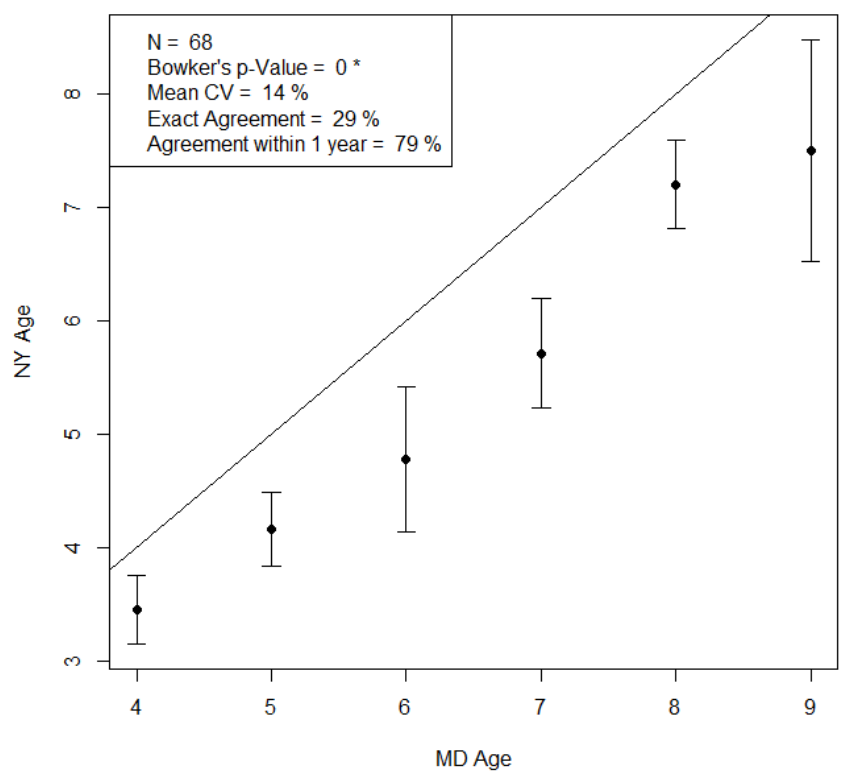


Figure 22. Age bias plot for MD and NY alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

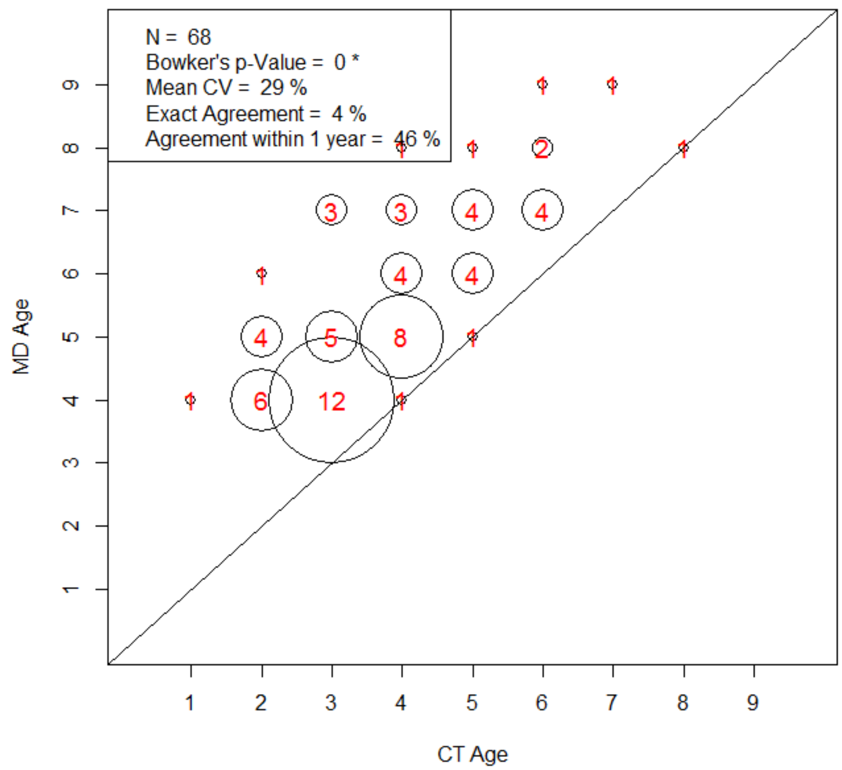


Figure 23. Age frequency plot for MD and CT alewife scale age determinations.

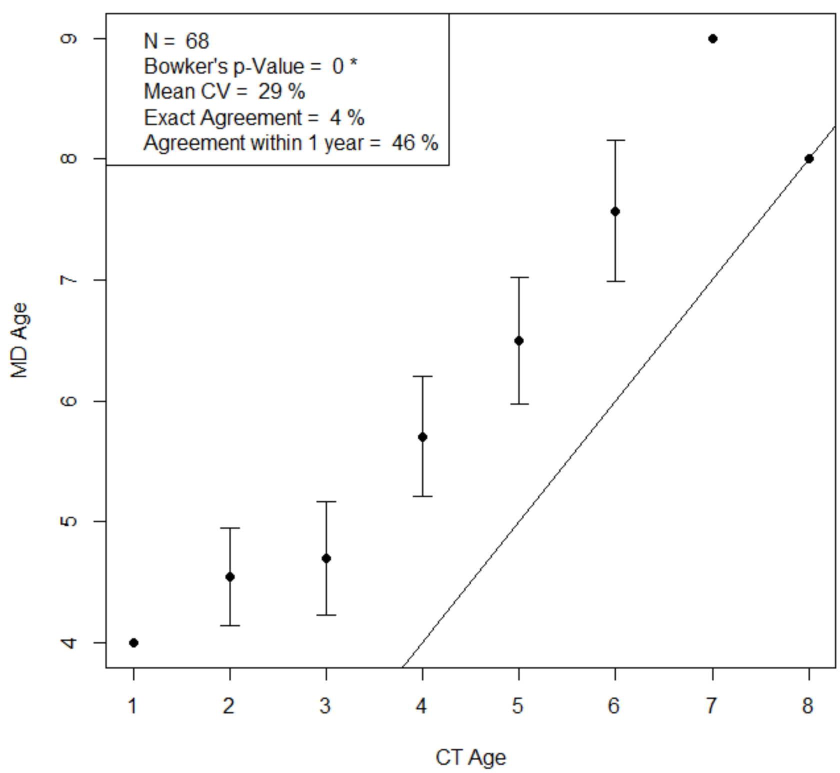


Figure 24. Age bias plot for MD and CT alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

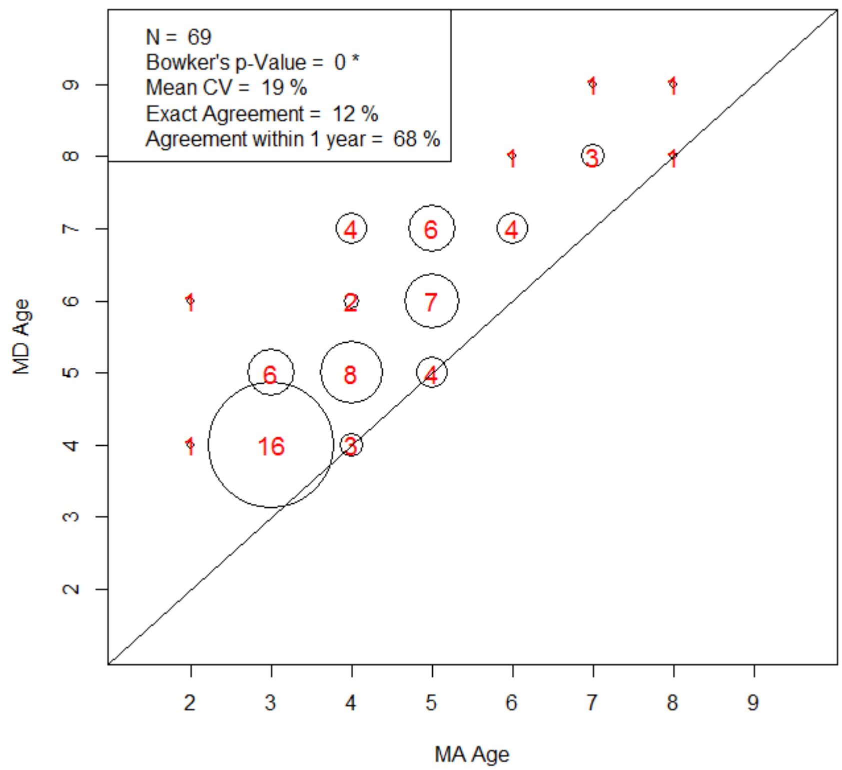


Figure 25. Age frequency plot for MD and MA alewife scale age determinations.

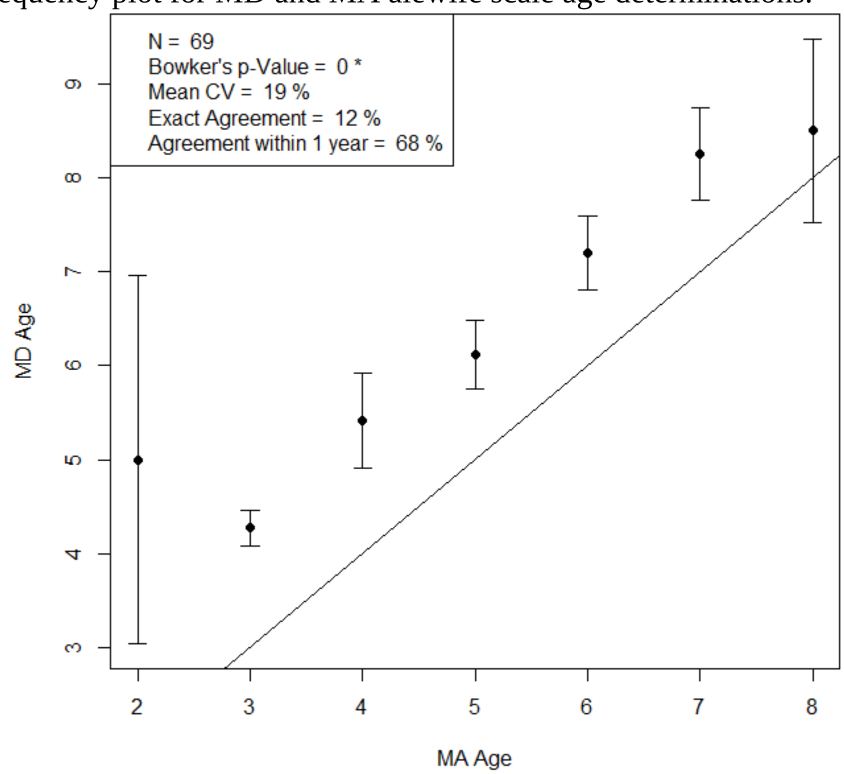


Figure 26. Age bias plot for MD and MA alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

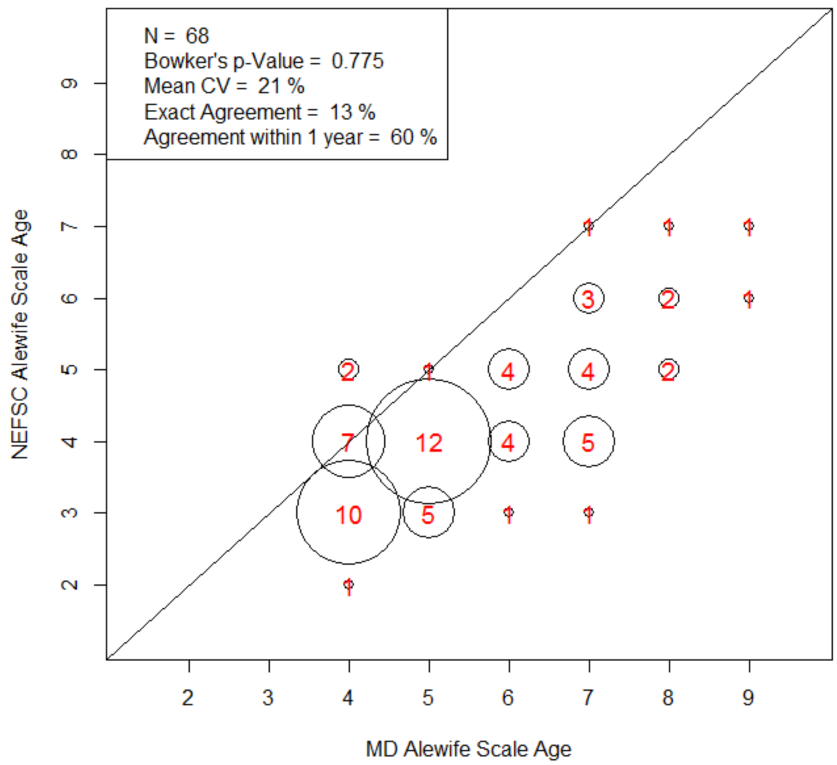


Figure 27. Age frequency plot for MD and NEFSC alewife scale age determinations.

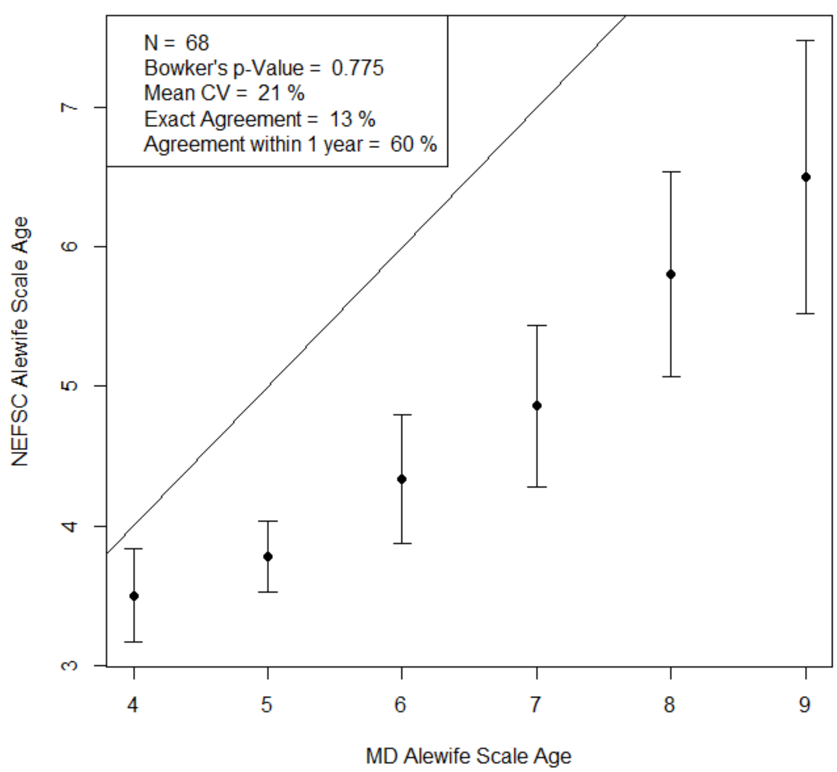


Figure 28. Age bias plot for MD and NEFSC alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

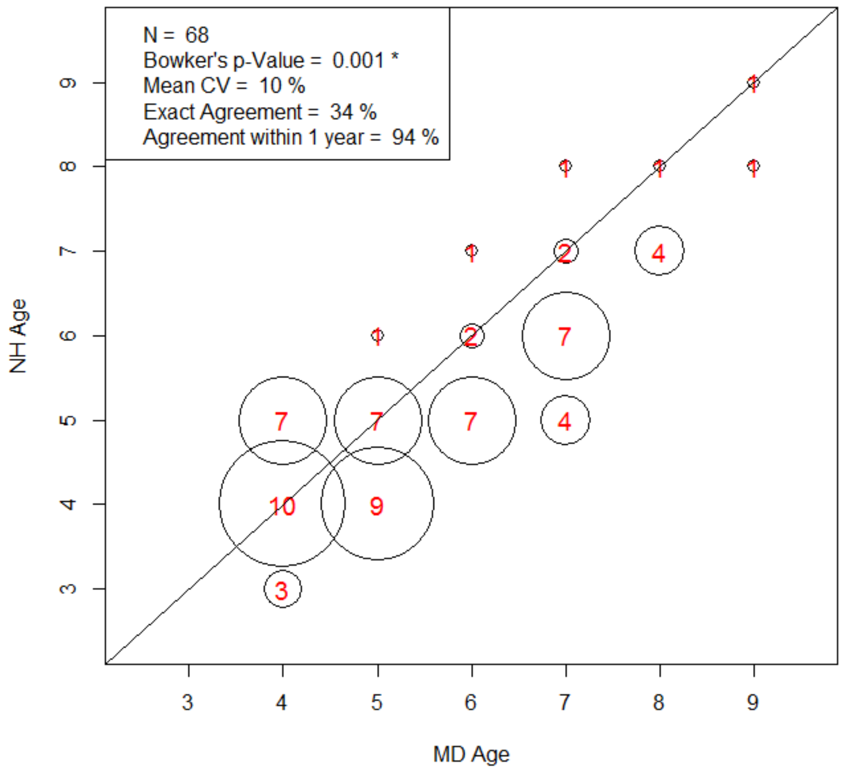


Figure 29. Age frequency plot for MD and NH alewife scale age determinations.

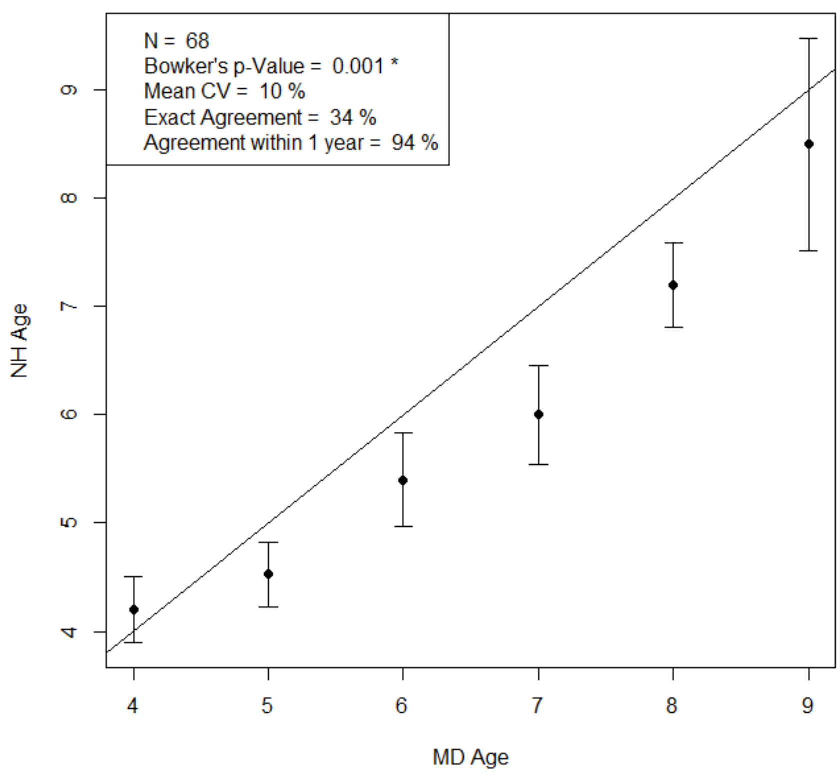


Figure 30. Age bias plot for MD and NH alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

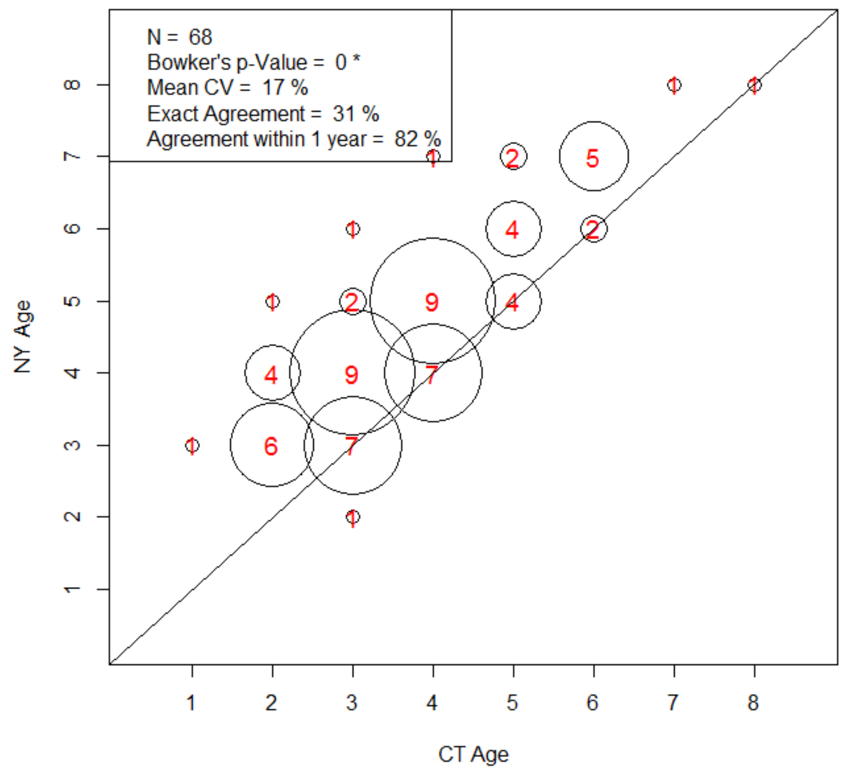


Figure 31. Age frequency plot for NY and CT alewife scale age determinations.

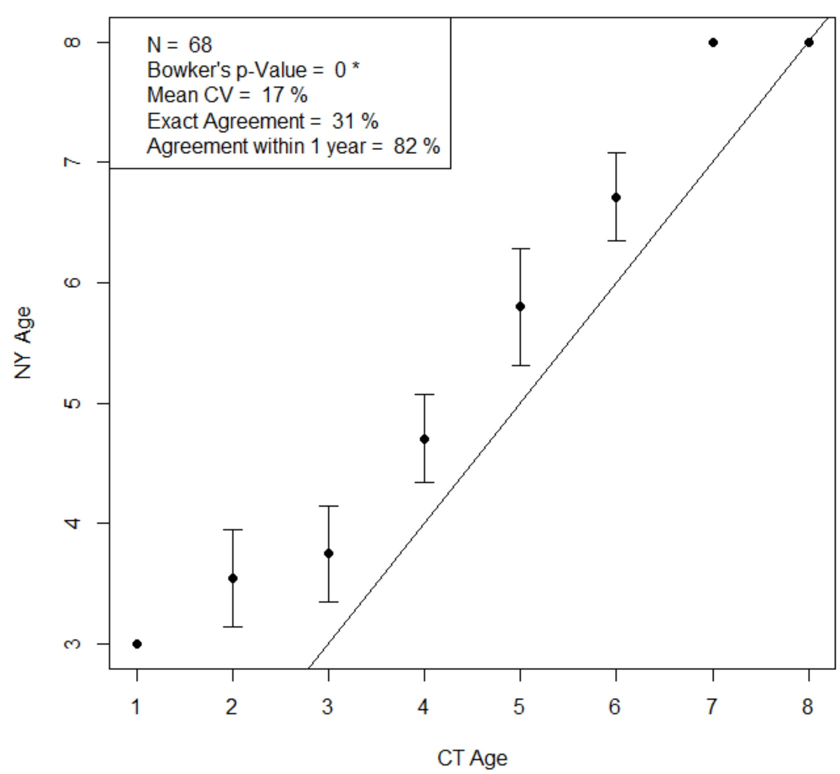


Figure 32. Age bias plot for NY and CT alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

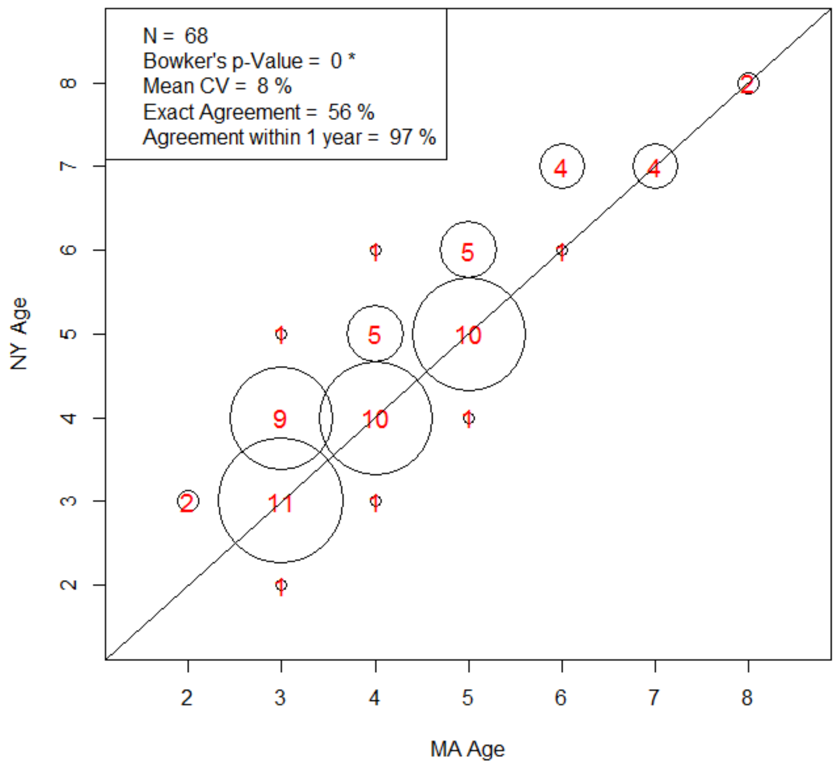


Figure 33. Age frequency plot for NY and MA alewife scale age determinations.

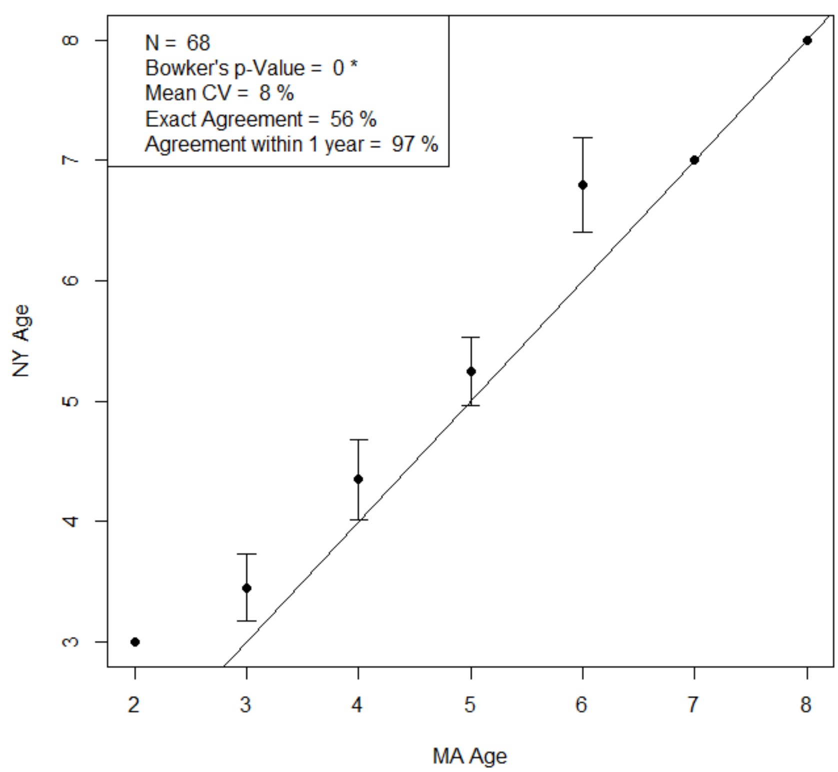


Figure 34. Age bias plot for NY and MA alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

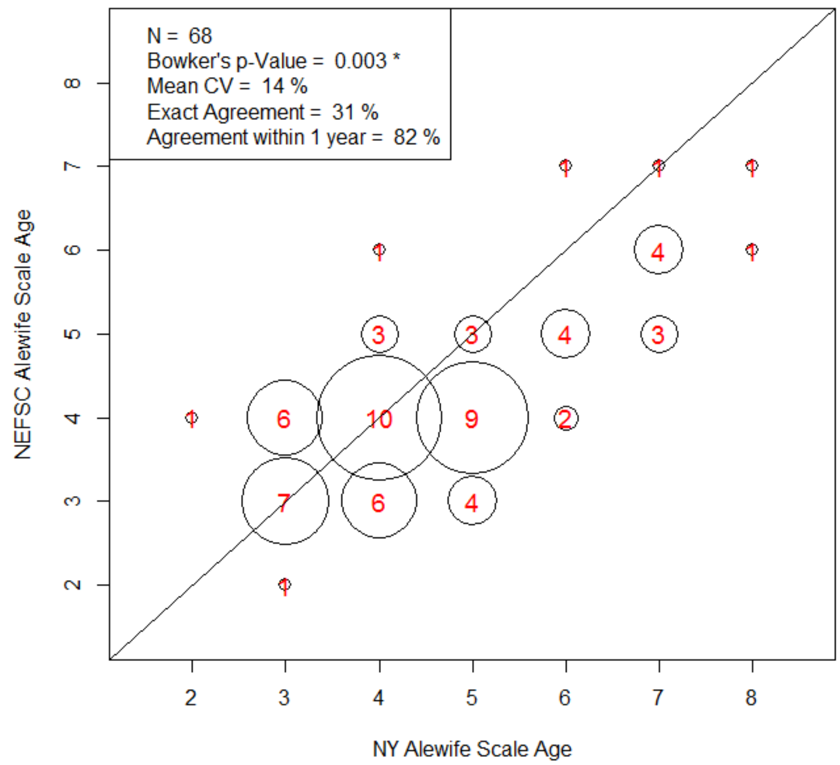


Figure 35. Age frequency plot for NY and NEFSC alewife scale age determinations.

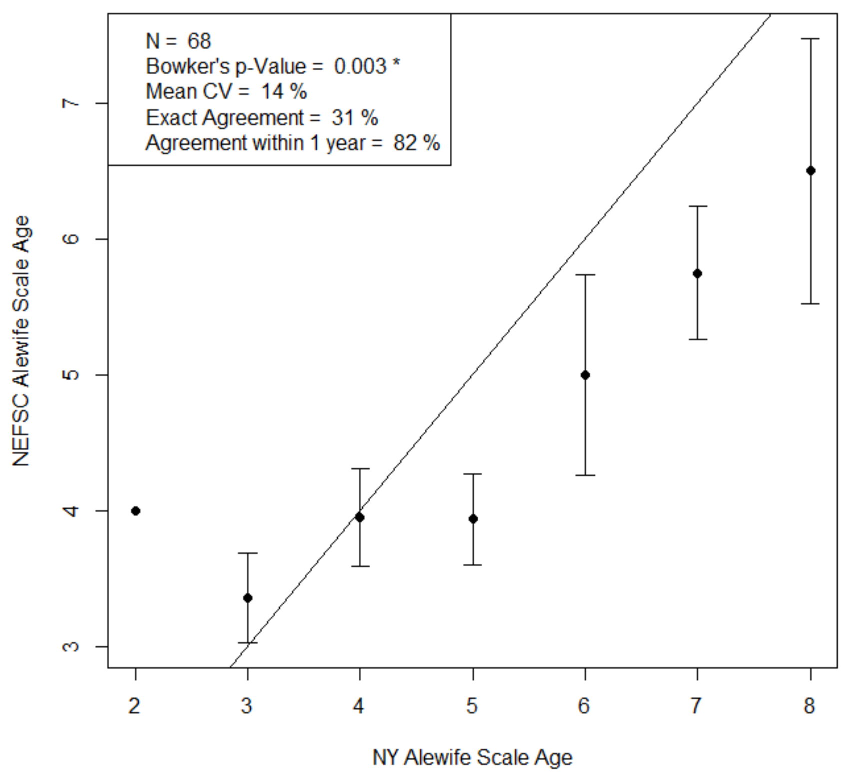


Figure 36. Age bias plot for NY and NEFSC alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

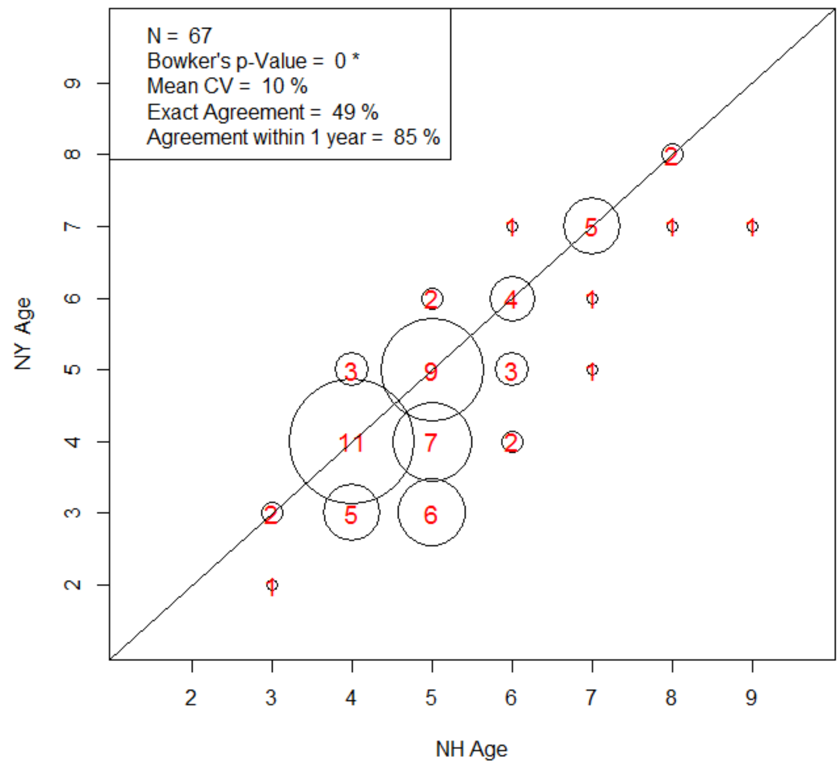


Figure 37. Age frequency plot for NY and NH alewife scale age determinations.

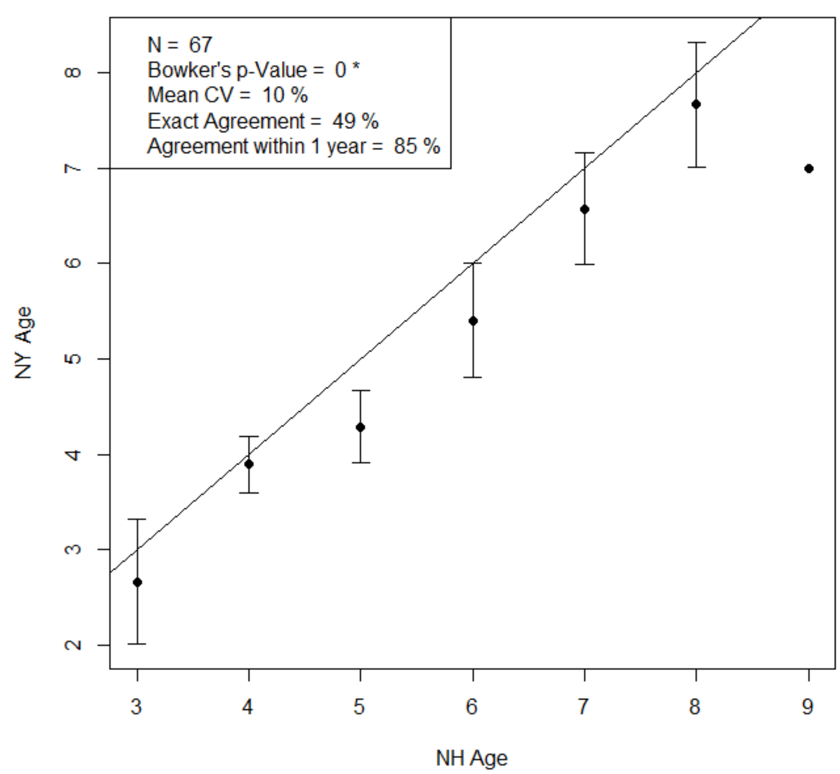


Figure 38. Age bias plot for NY and NH alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

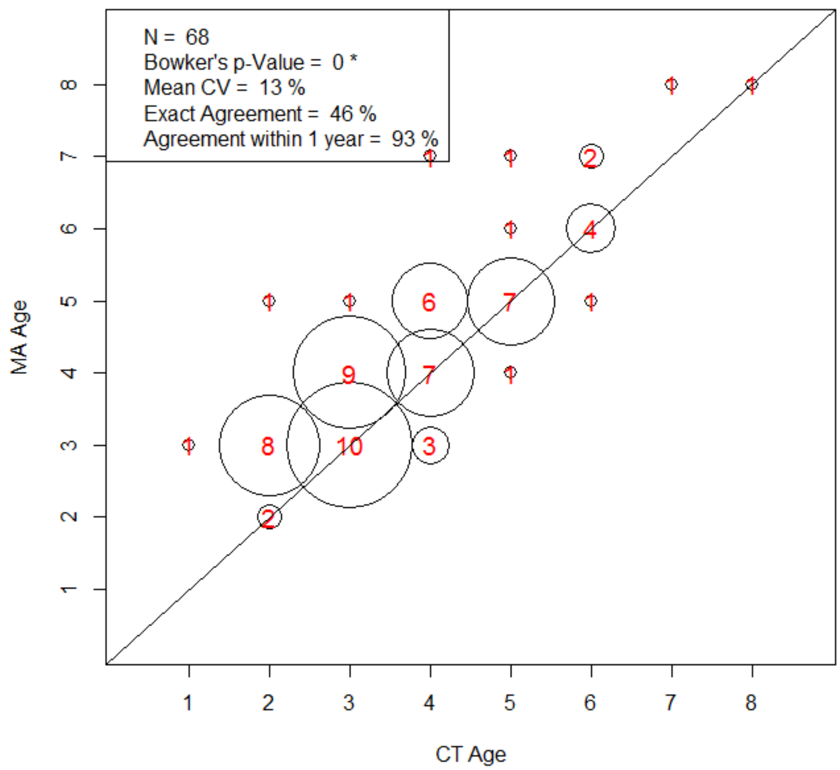


Figure 39. Age frequency plot for CT and MA alewife scale age determinations.

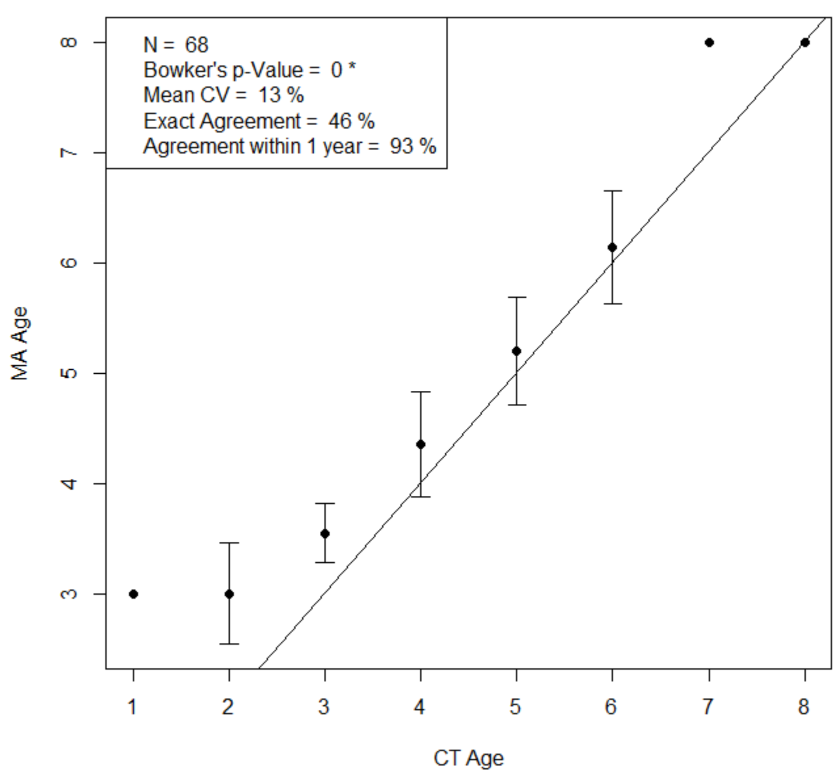


Figure 40. Age bias plot for CT and MA alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

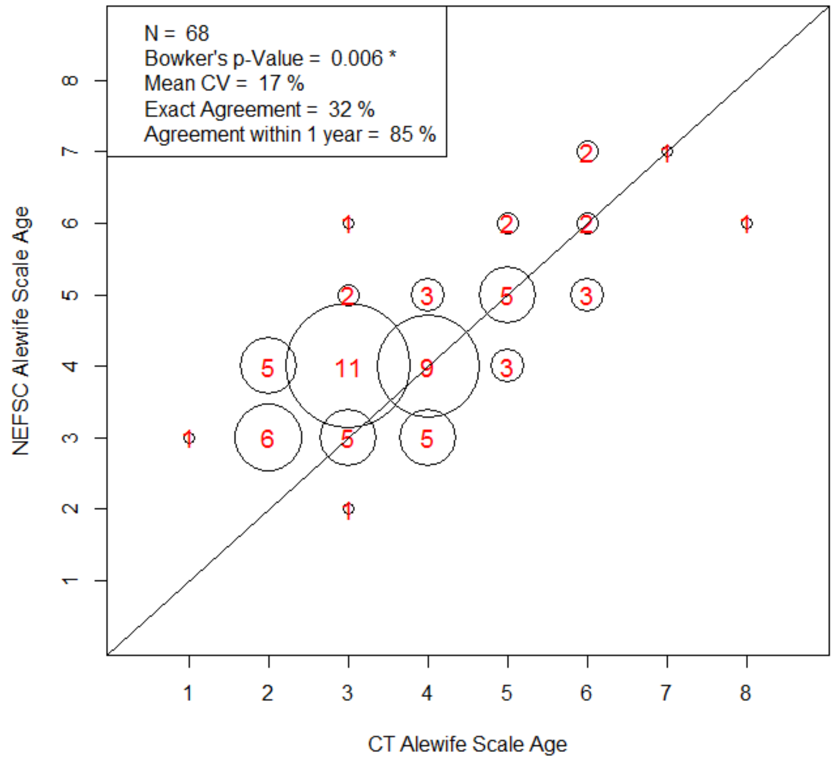


Figure 41. Age frequency plot for CT and NEFSC alewife scale age determinations.

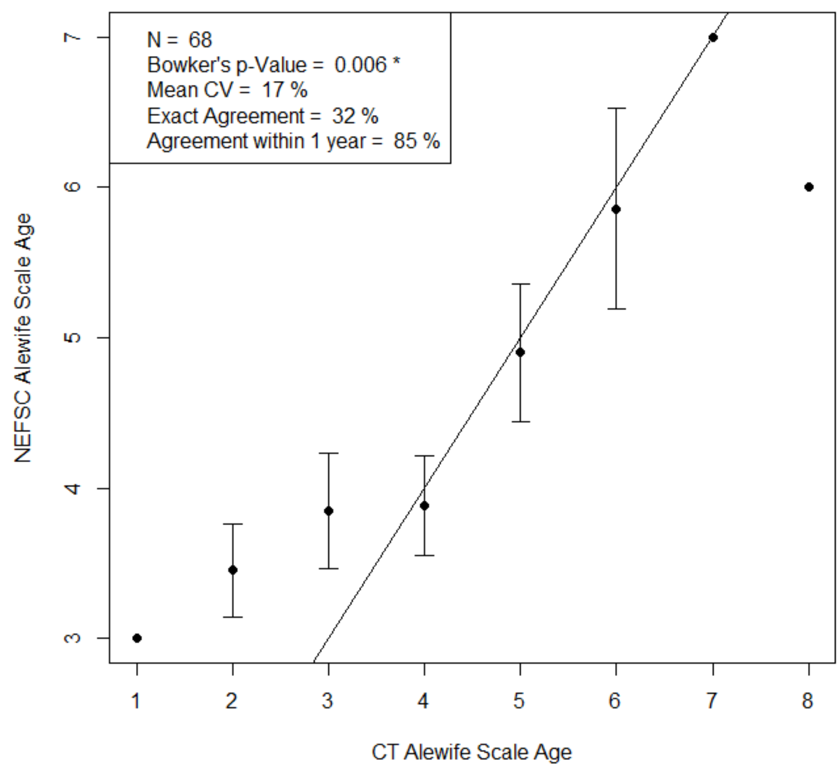


Figure 42. Age bias plot for CT and NEFSC alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

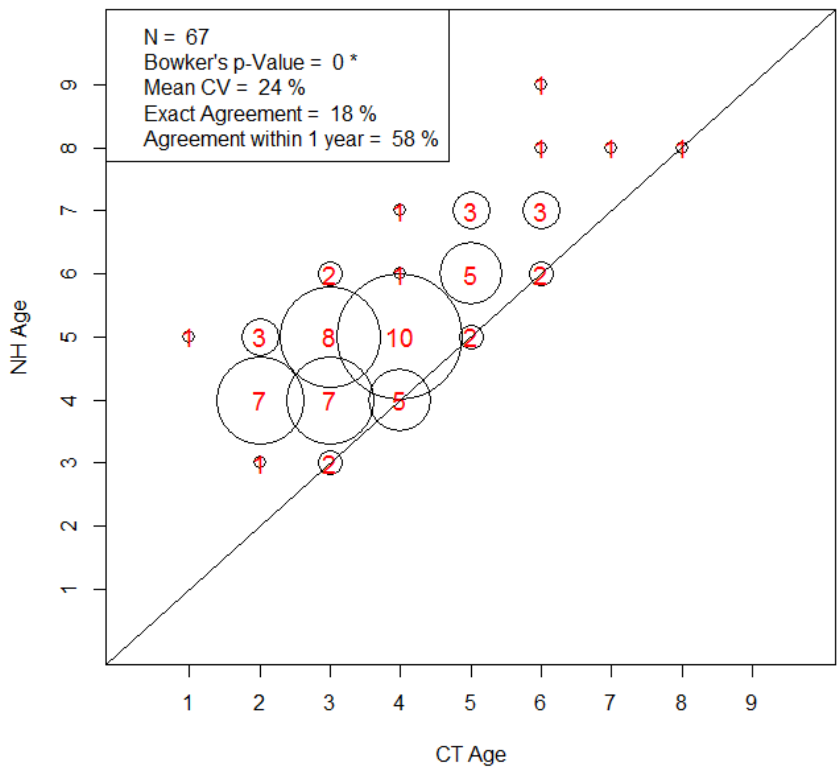


Figure 43. Age frequency plot for CT and NH alewife scale age determinations.

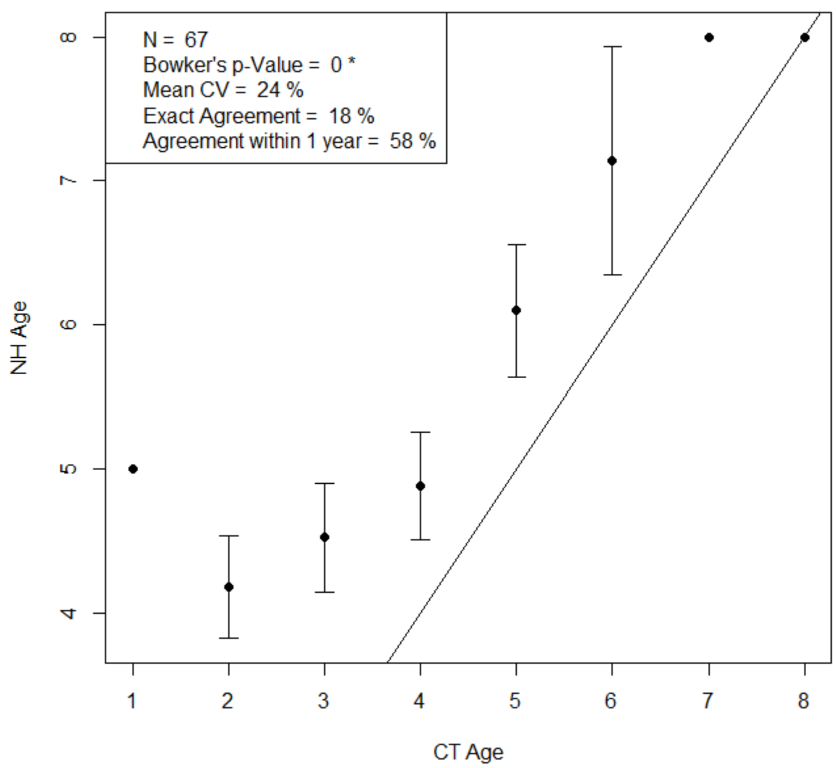


Figure 44. Age bias plot for CT and NH alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

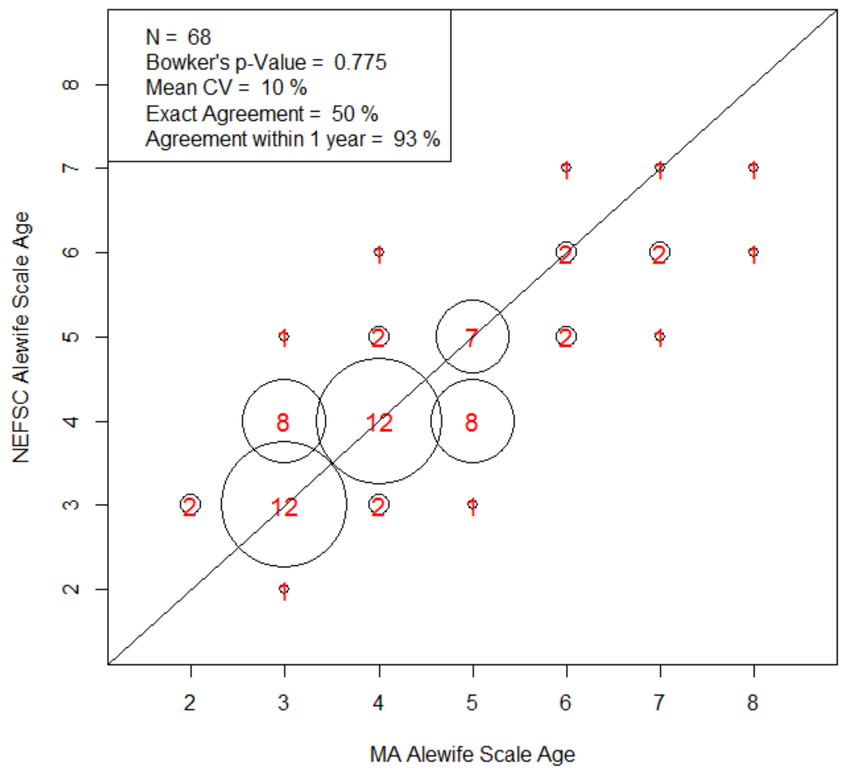


Figure 45. Age frequency plot for MA and NEFSC alewife scale age determinations.

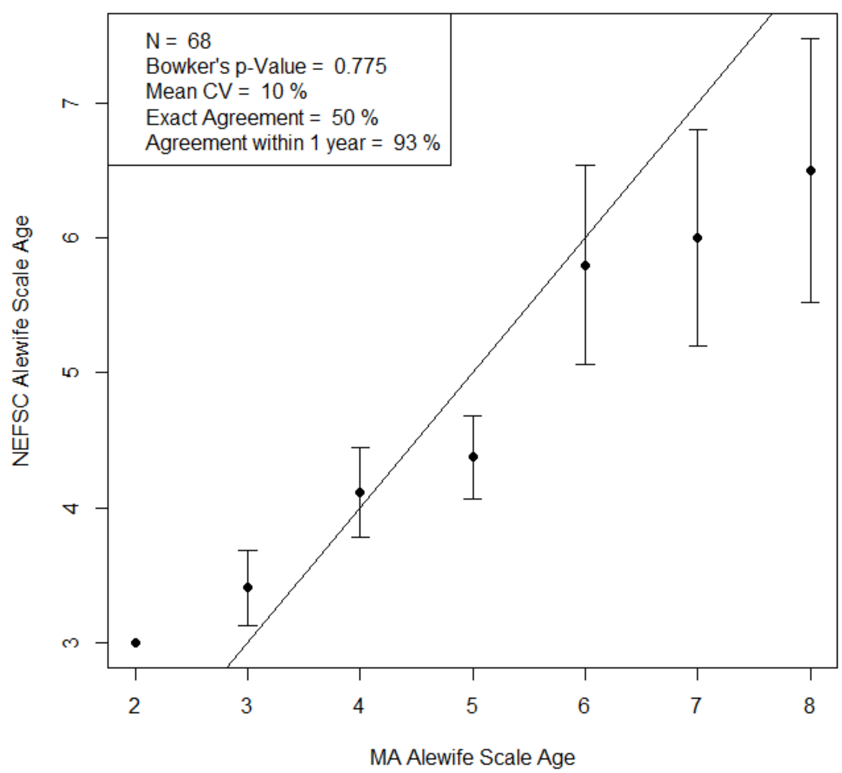


Figure 46. Age bias plot for MA and NEFSC alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

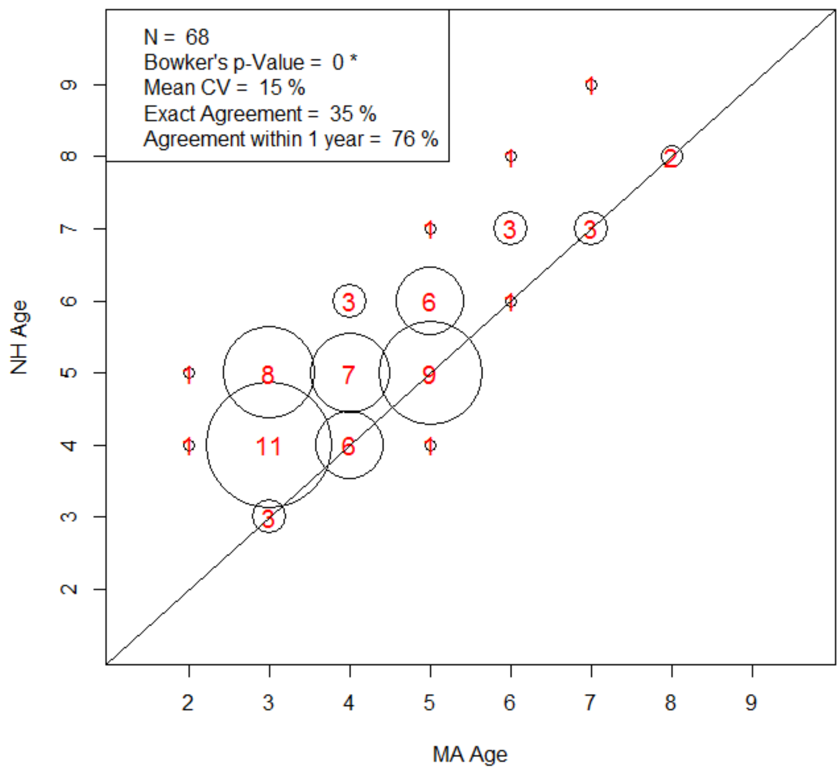


Figure 47. Age frequency plot for MA and NH alewife scale age determinations.

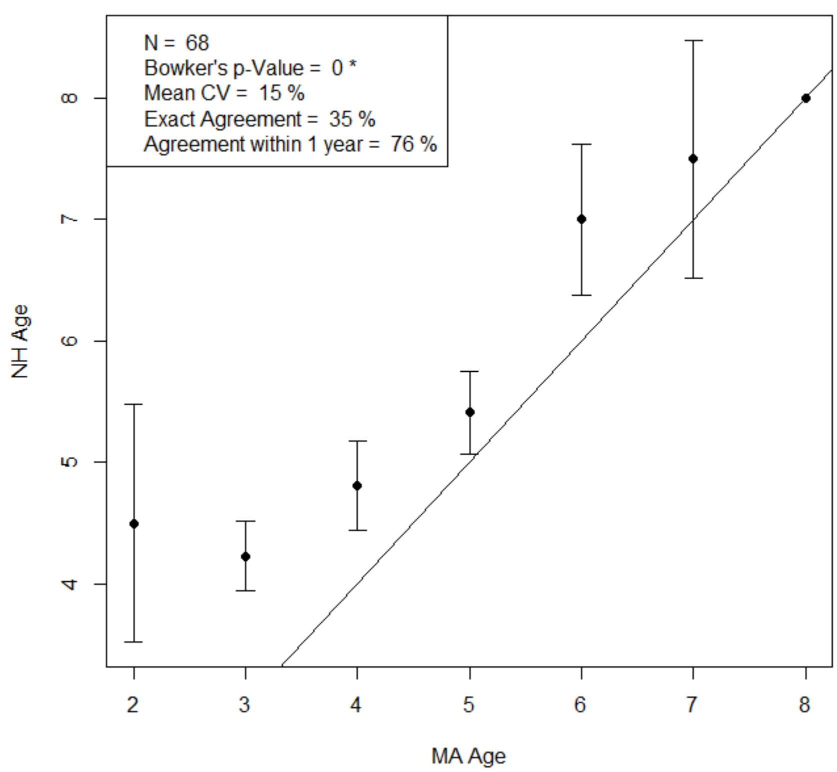


Figure 48. Age bias plot for MA and NH alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

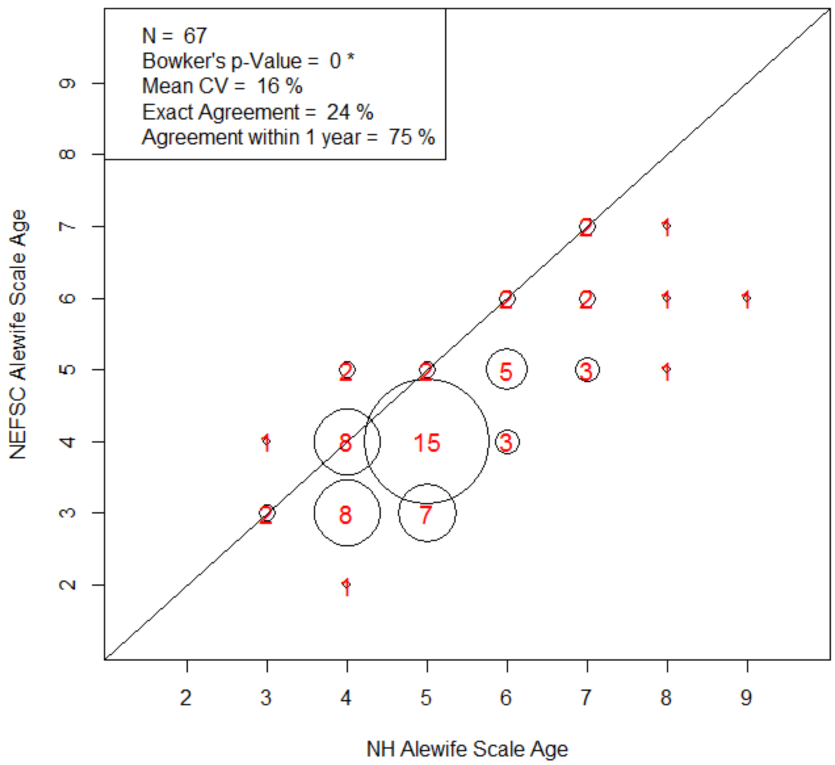


Figure 49. Age frequency plot for NEFSC and NH alewife scale age determinations.

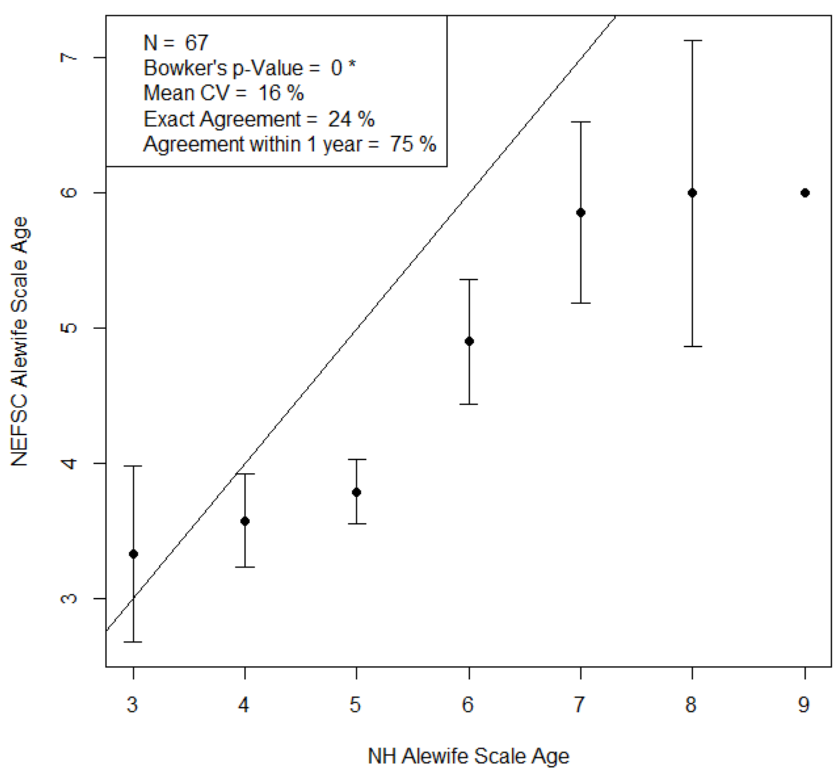


Figure 50. Age bias plot for NEFSC and NH alewife scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

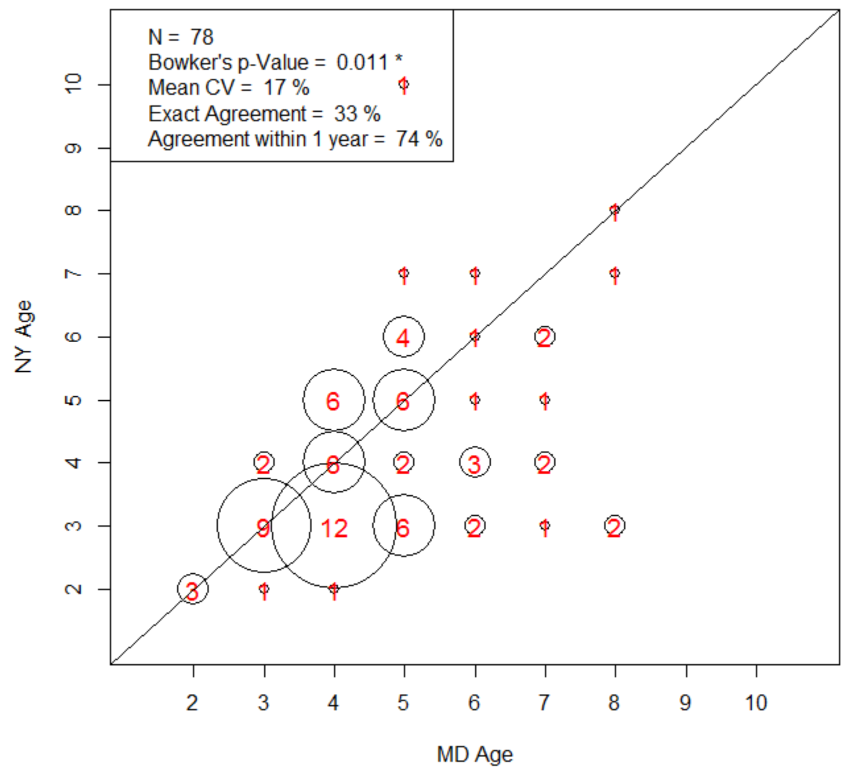


Figure 51. Age frequency plot for MD and NY blueback herring otolith age determinations.

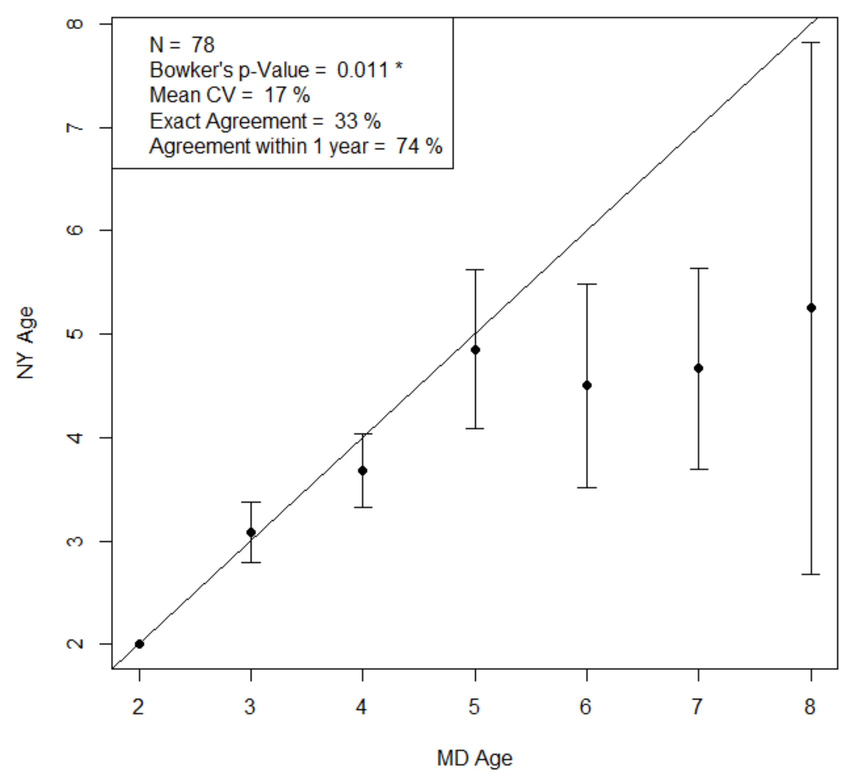


Figure 52. Age bias plot for MD and NY blueback herring otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

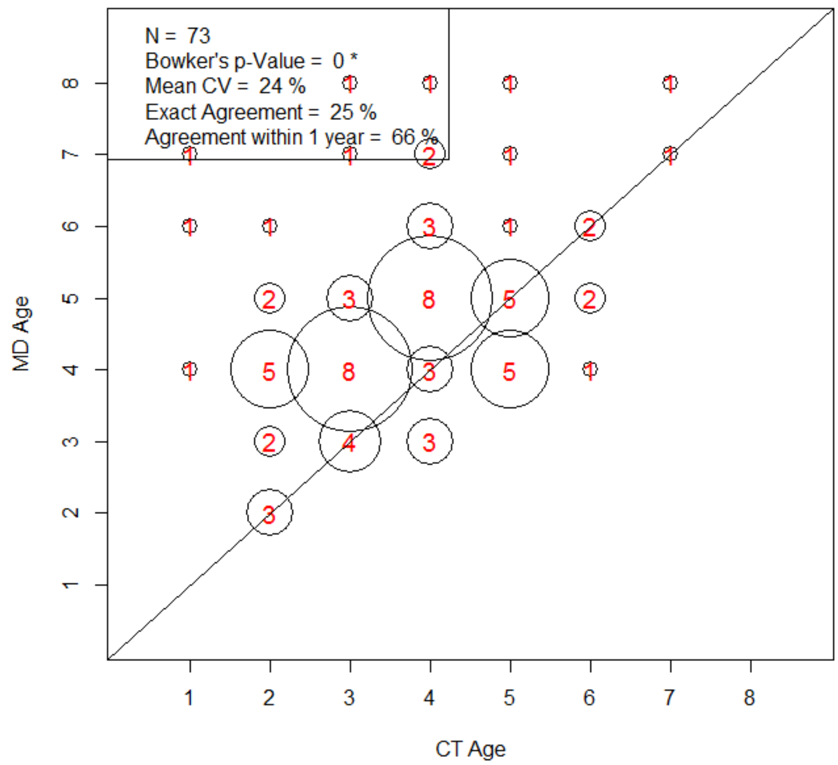


Figure 53. Age frequency plot for MD and CT blueback herring otolith age determinations.

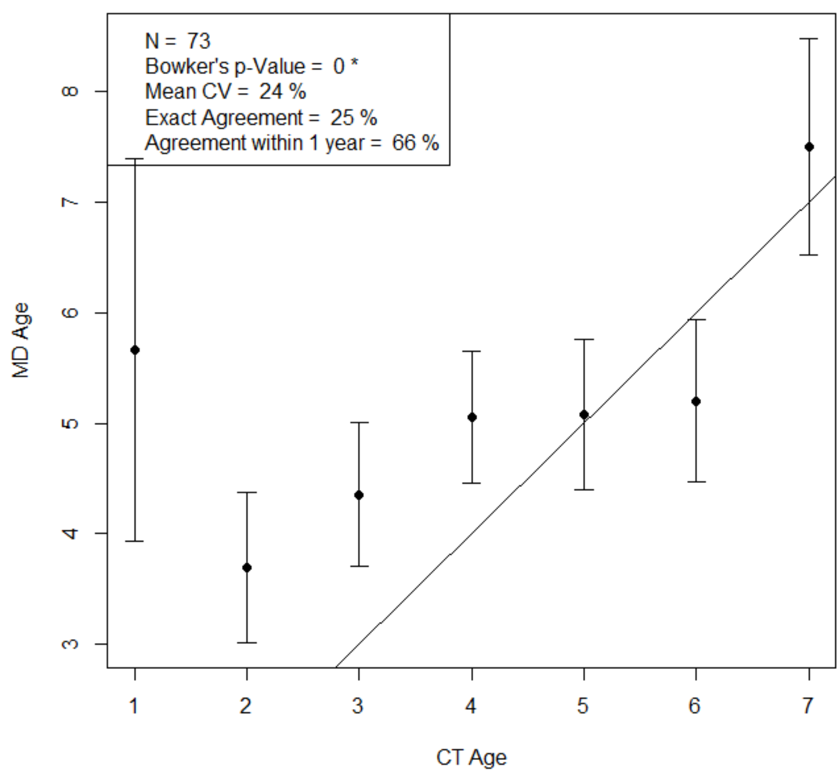


Figure 54. Age bias plot for MD and CT blueback herring otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

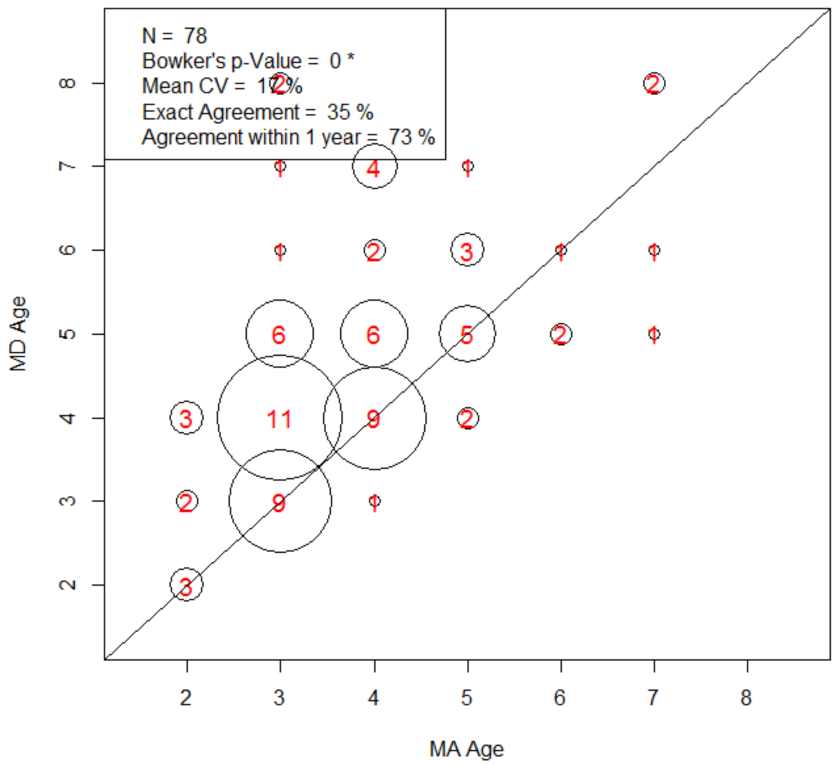


Figure 55. Age frequency plot for MD and MA blueback herring otolith age determinations.

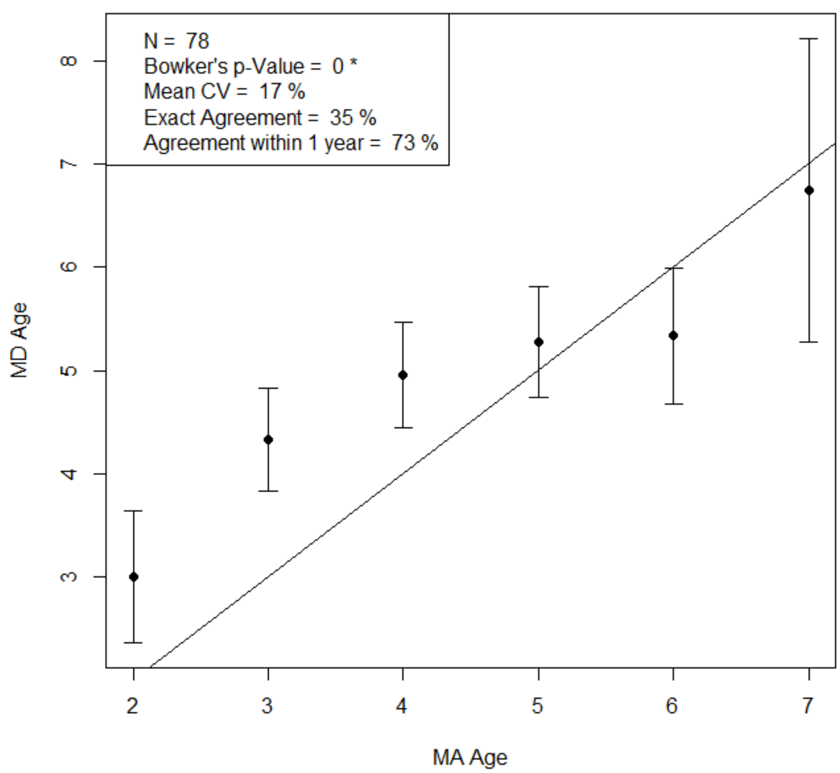


Figure 56. Age bias plot for MD and MA blueback herring otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

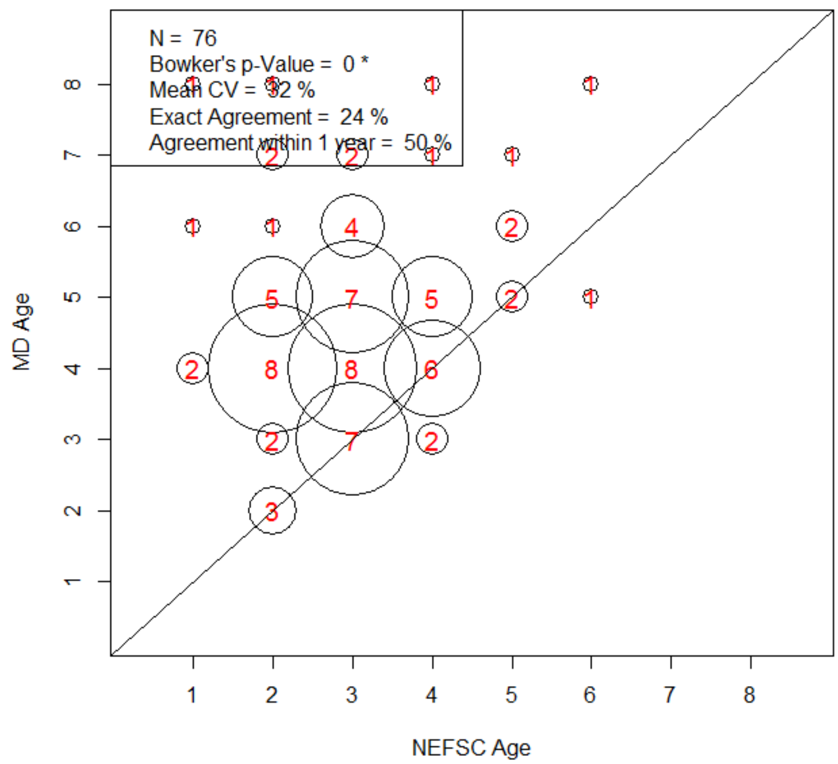


Figure 57. Age frequency plot for MD and NEFSC blueback herring otolith age determinations.

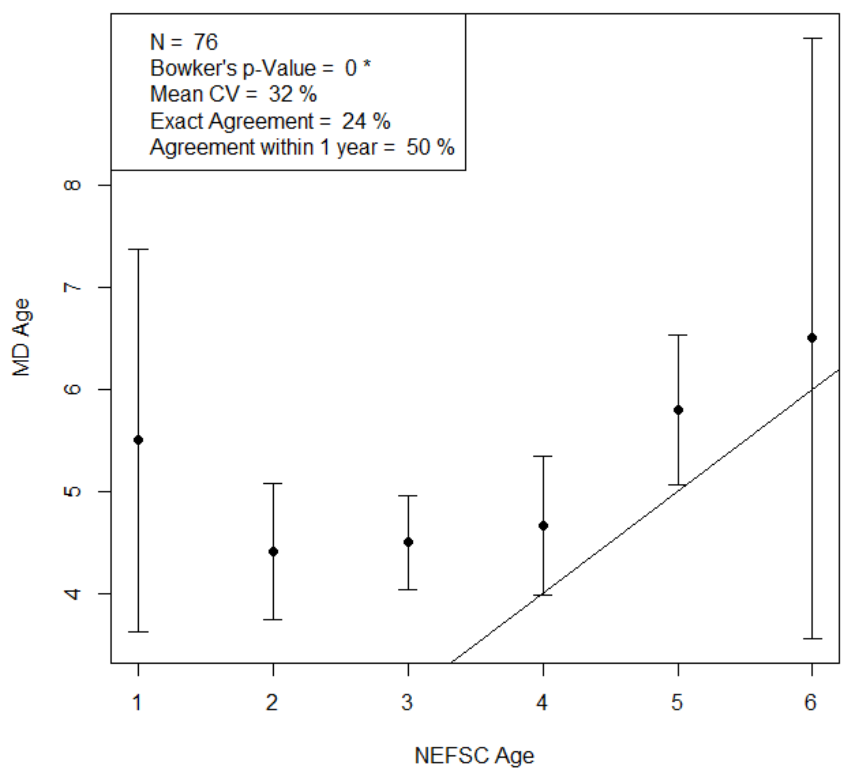


Figure 58. Age bias plot for MD and NEFSC blueback herring otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

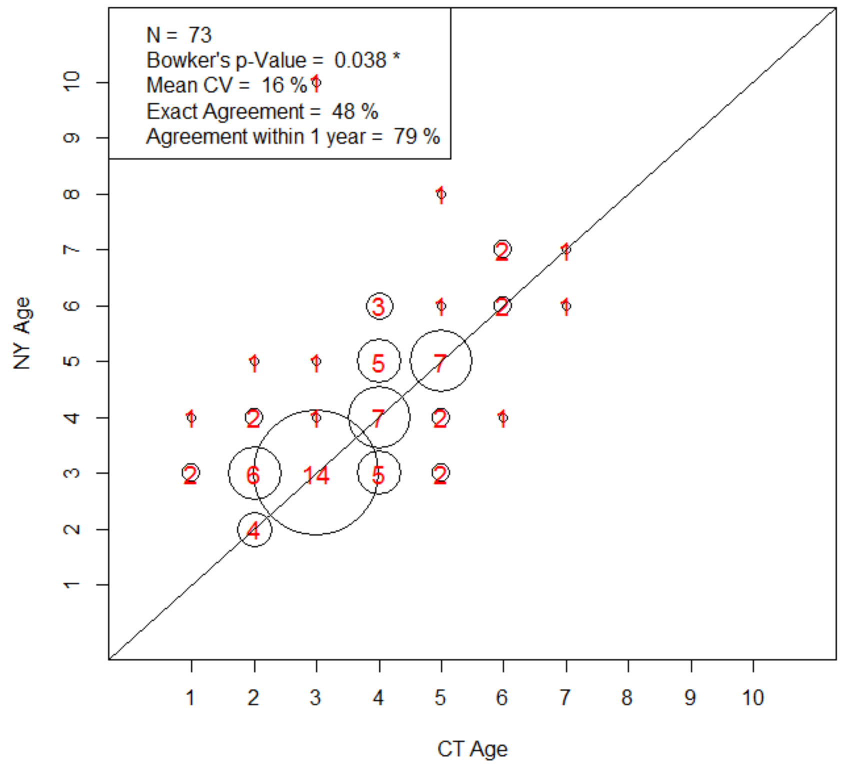


Figure 59. Age frequency plot for NY and CT blueback herring otolith age determinations.

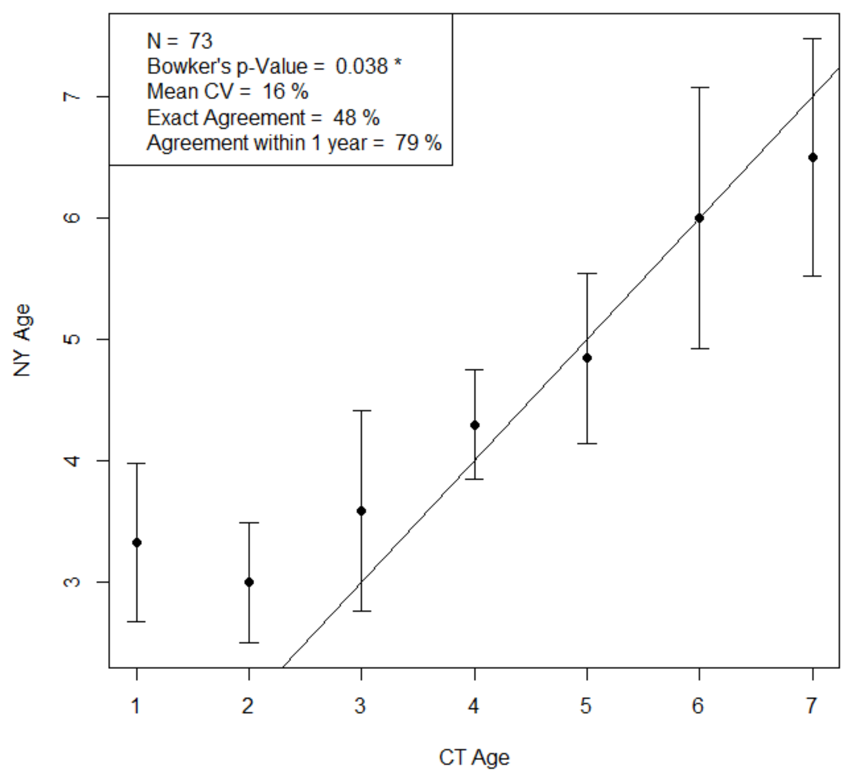


Figure 60. Age bias plot for NY and CT blueback herring otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

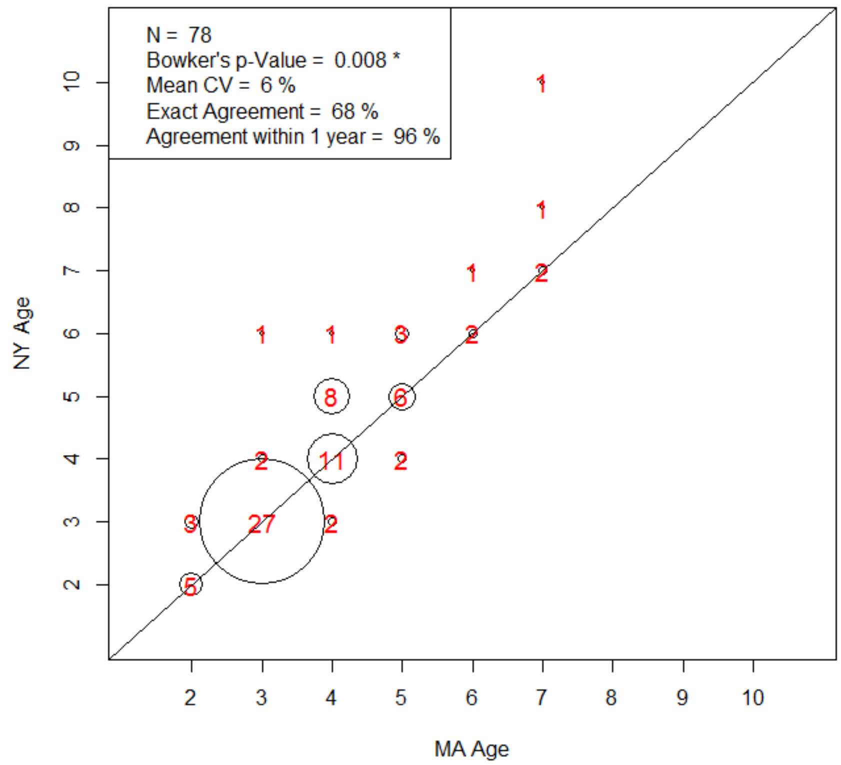


Figure 61. Age frequency plot for NY and MA blueback herring otolith age determinations.

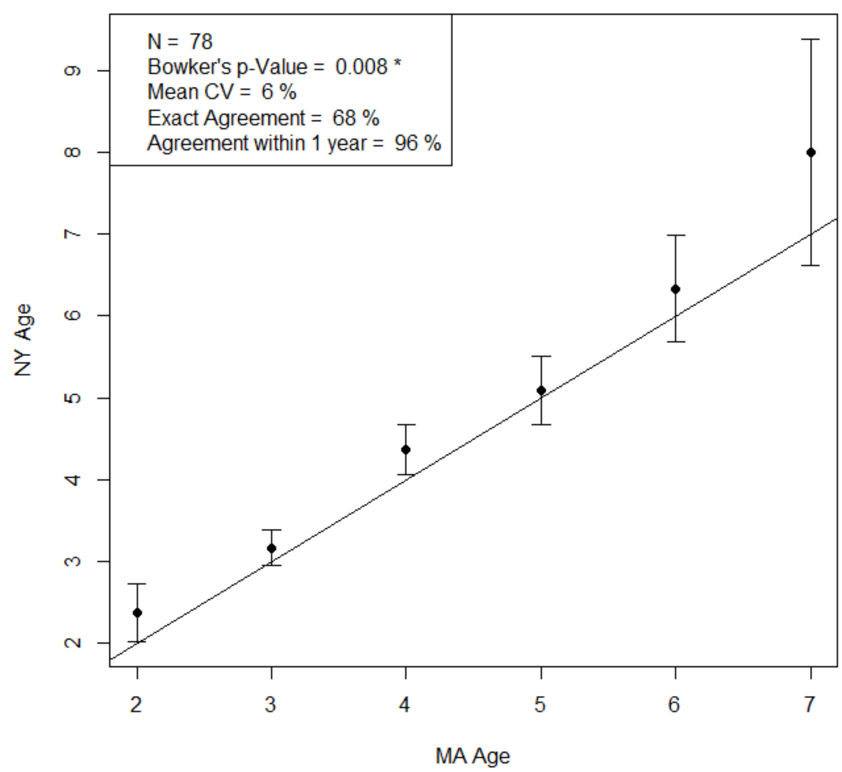


Figure 62. Age bias plot for NY and MA blueback herring otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

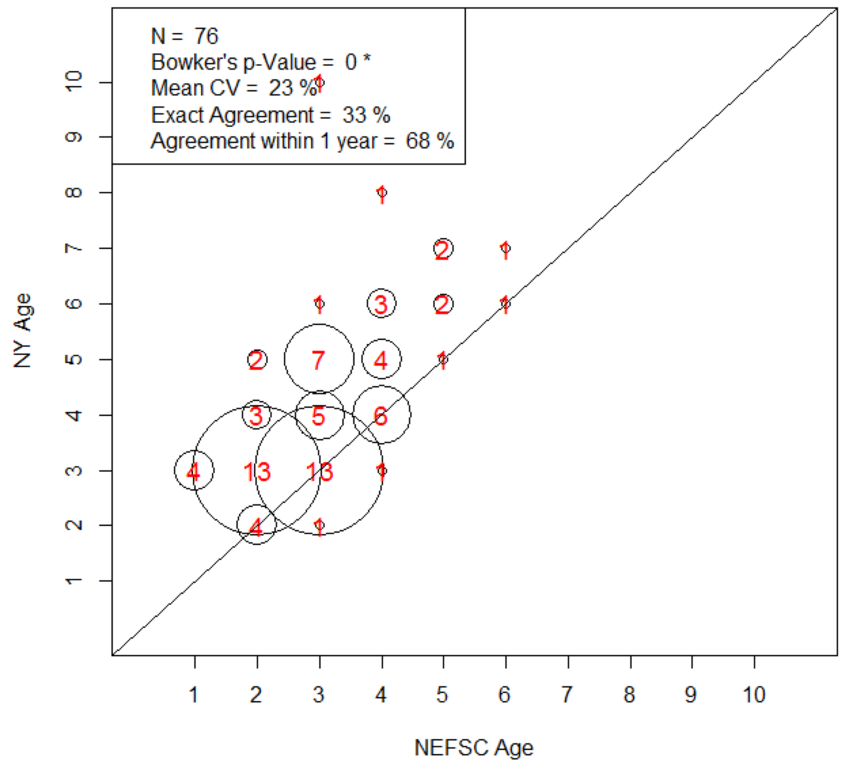


Figure 63. Age frequency plot for NY and NEFSC blueback herring otolith age determinations.

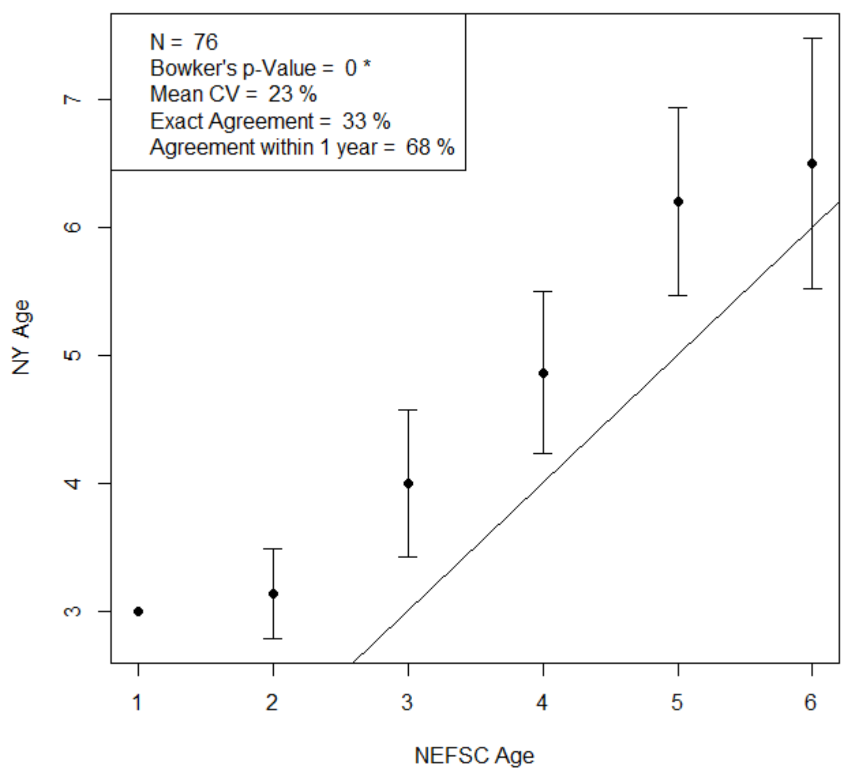


Figure 64. Age bias plot for NY and NEFSC blueback herring otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

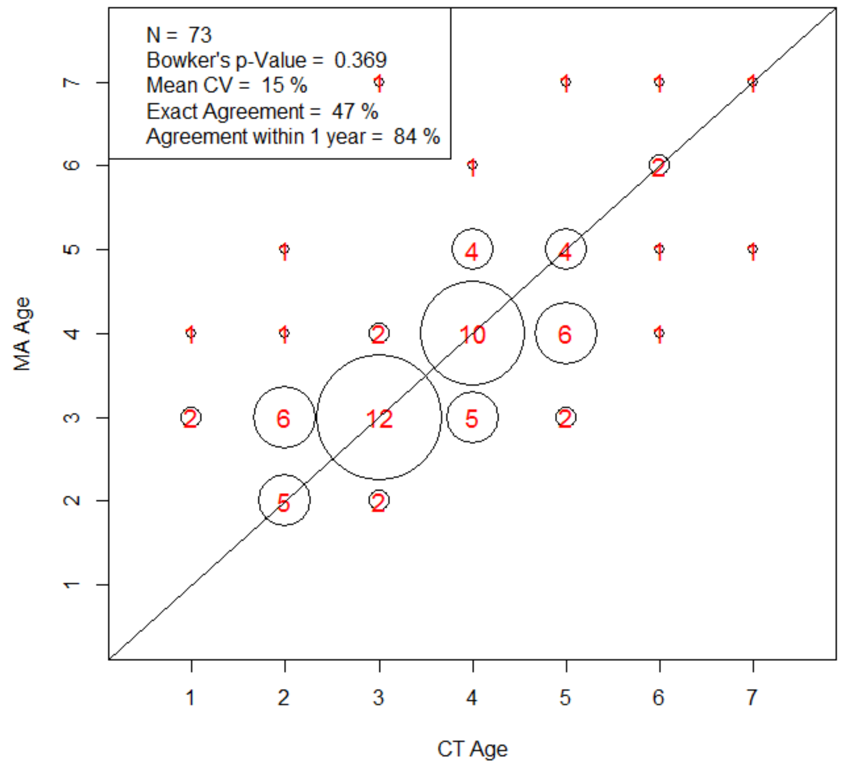


Figure 65. Age frequency plot for CT and MA blueback herring otolith age determinations.

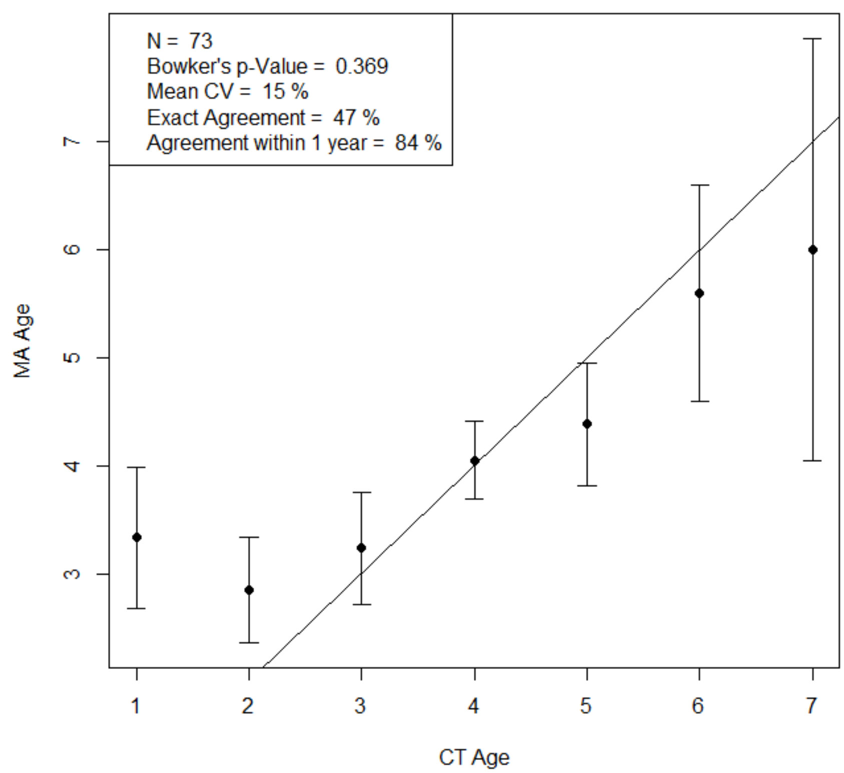


Figure 66. Age bias plot for CT and MA blueback herring otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

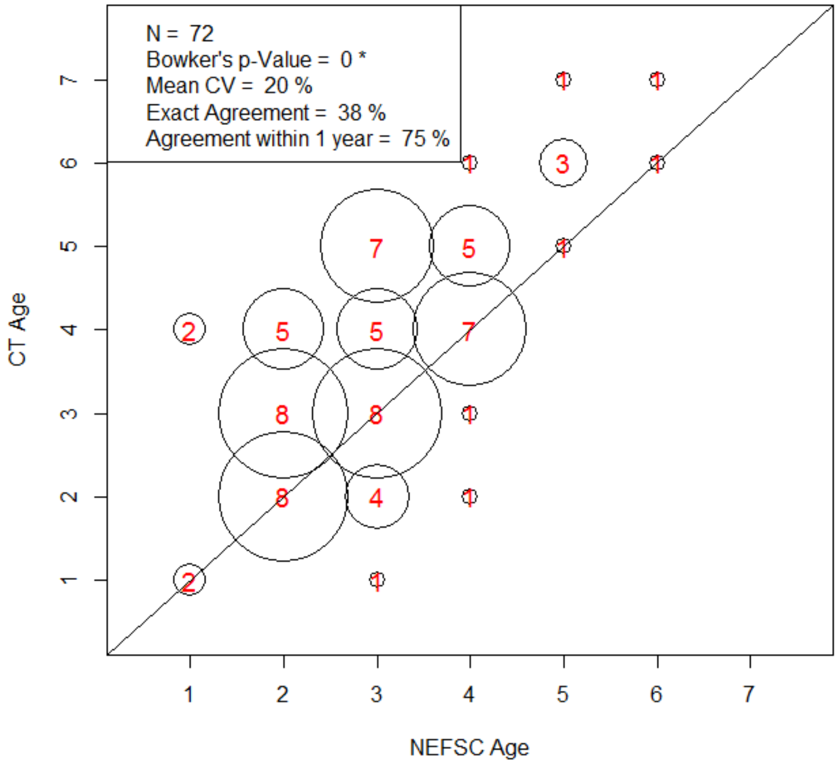


Figure 67. Age frequency plot for CT and NEFSC blueback herring otolith age determinations.

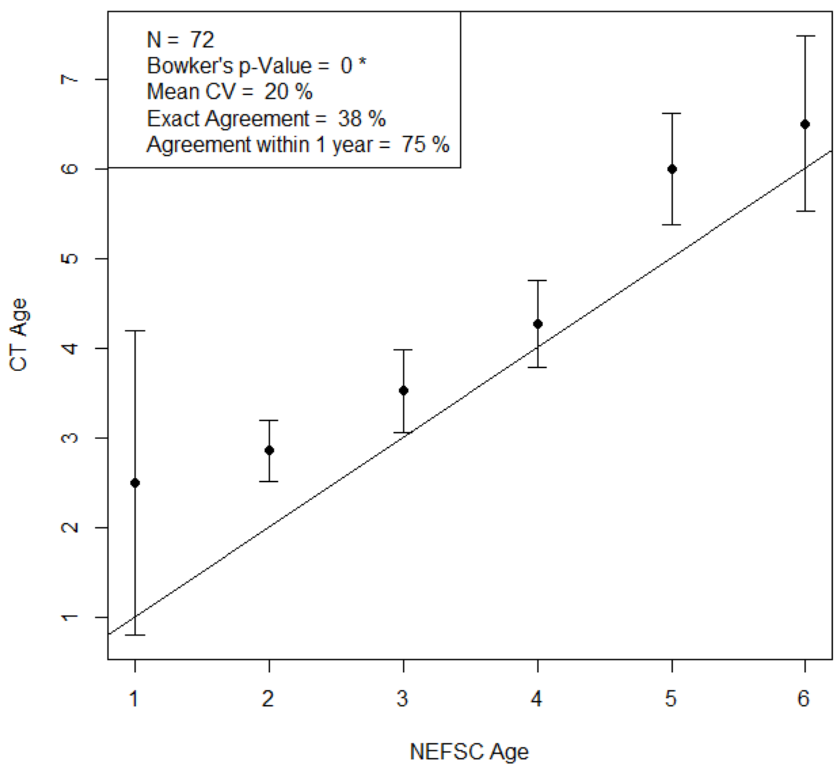


Figure 68. Age bias plot for CT and NEFSC blueback herring otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

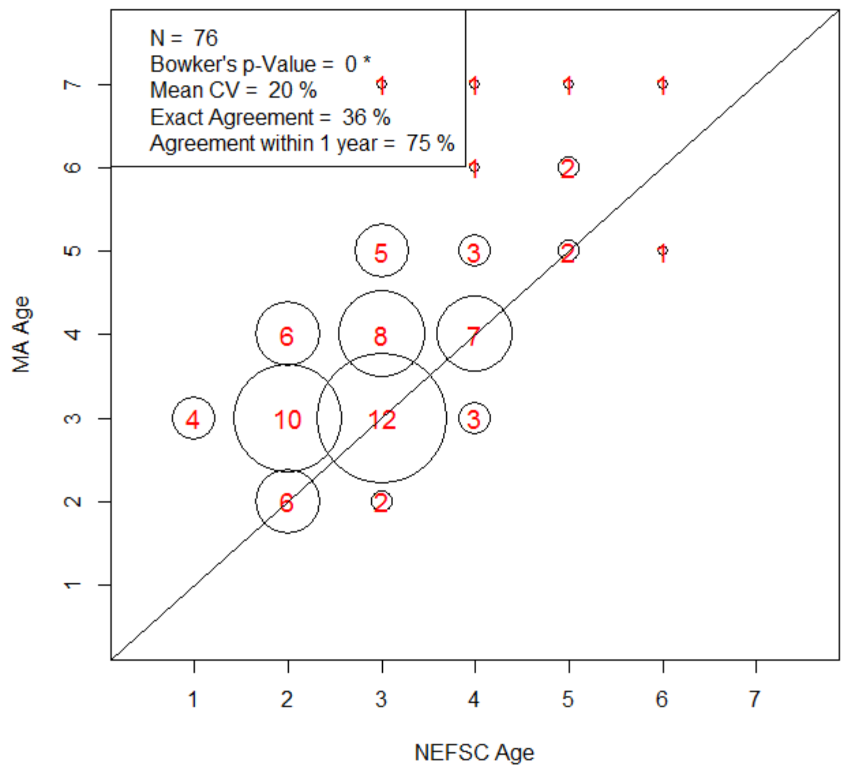


Figure 69. Age frequency plot for MA and NEFSC blueback herring otolith age determinations.

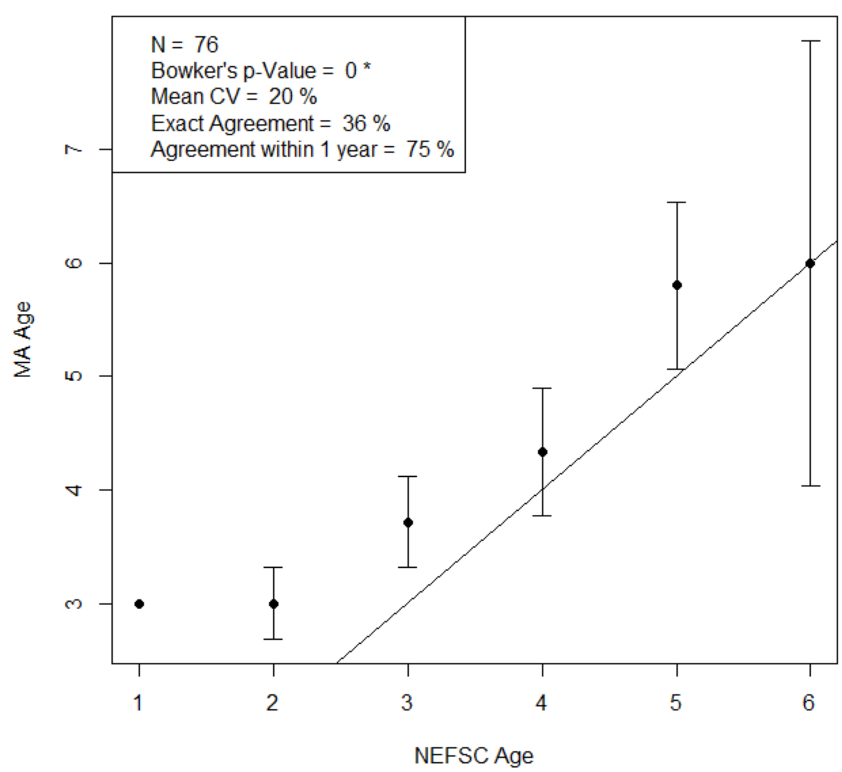


Figure 70. Age bias plot for MA and NEFSC blueback herring otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

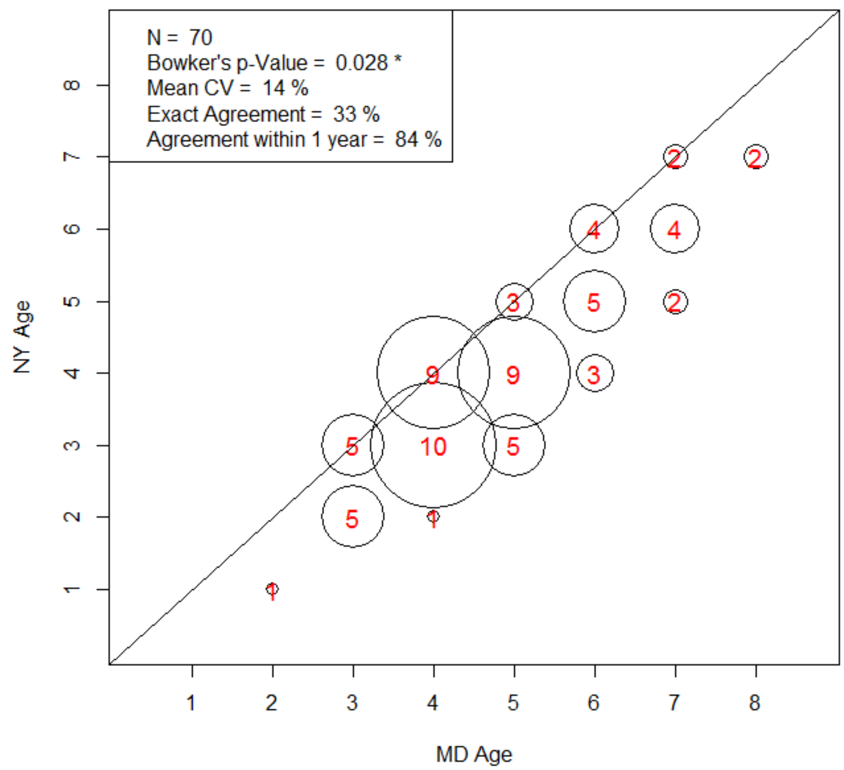


Figure 71. Age frequency plot for MD and NY blueback herring scale age determinations.

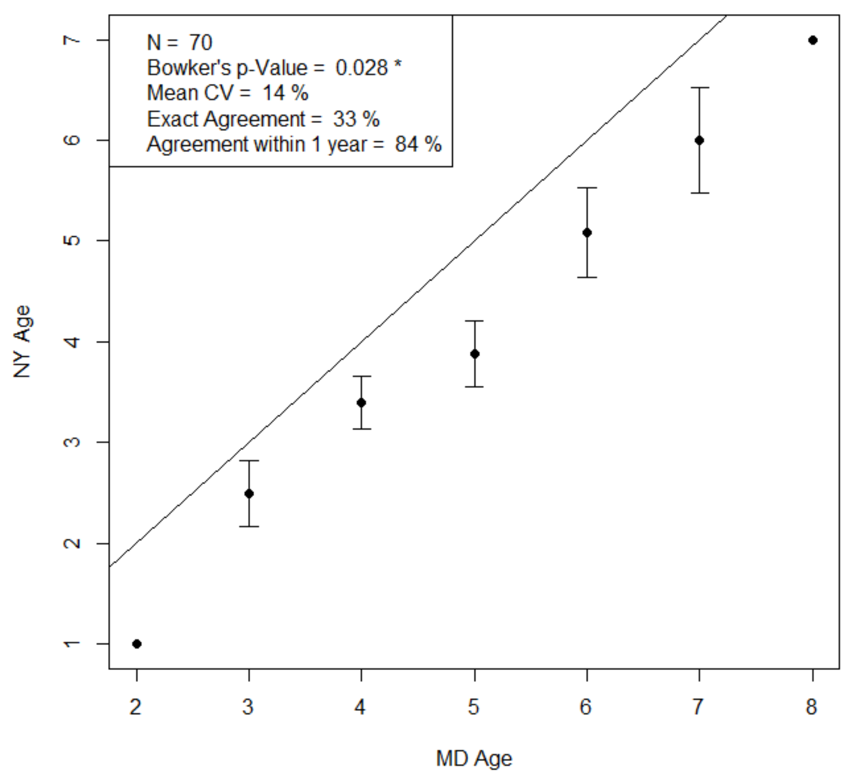


Figure 72. Age bias plot for MD and NY blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

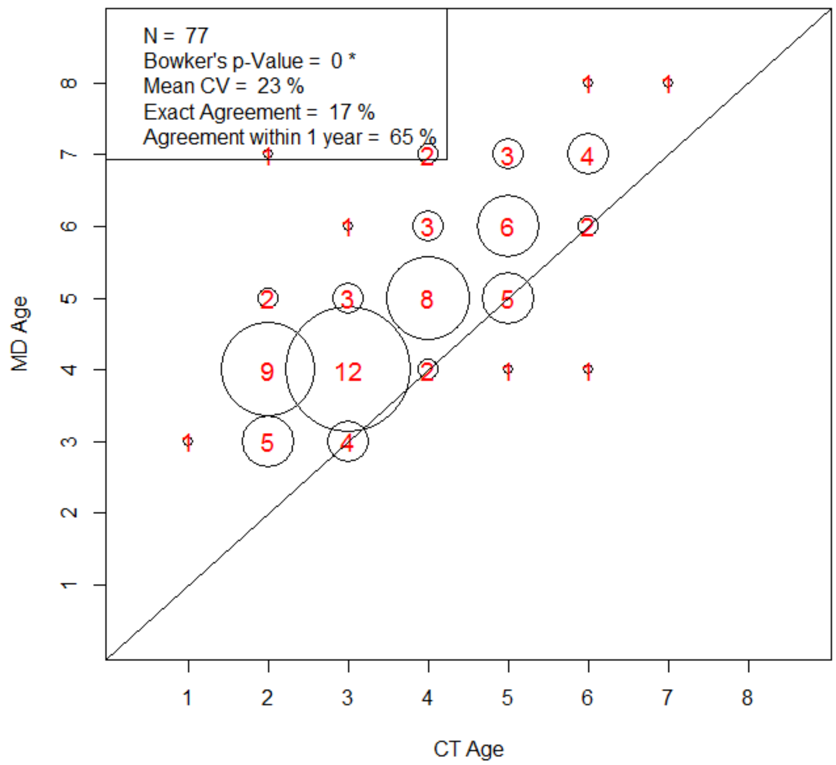


Figure 73. Age frequency plot for MD and CT blueback herring scale age determinations.

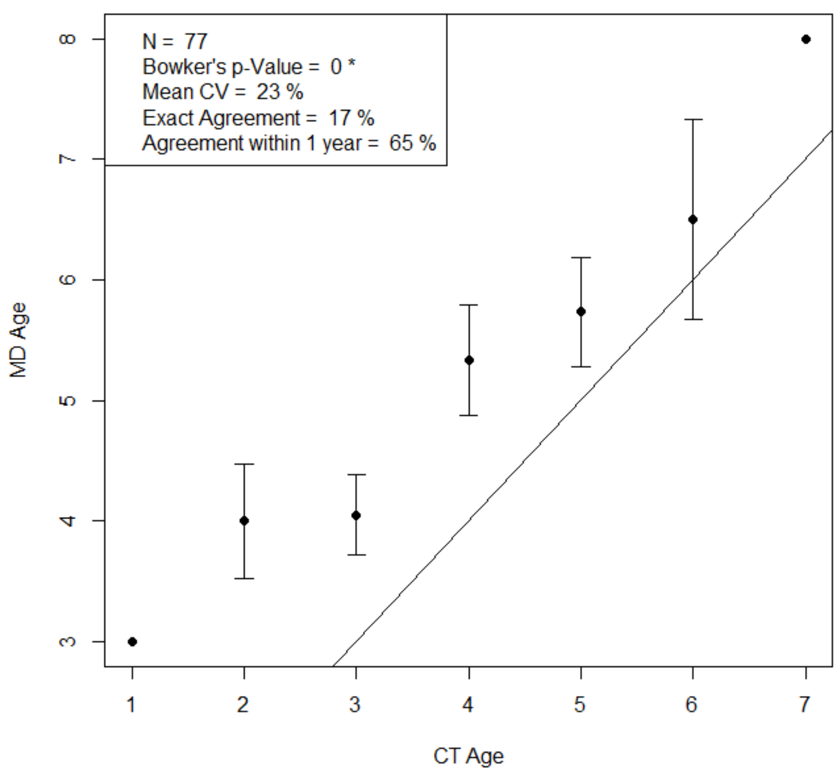


Figure 74. Age bias plot for MD and CT blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

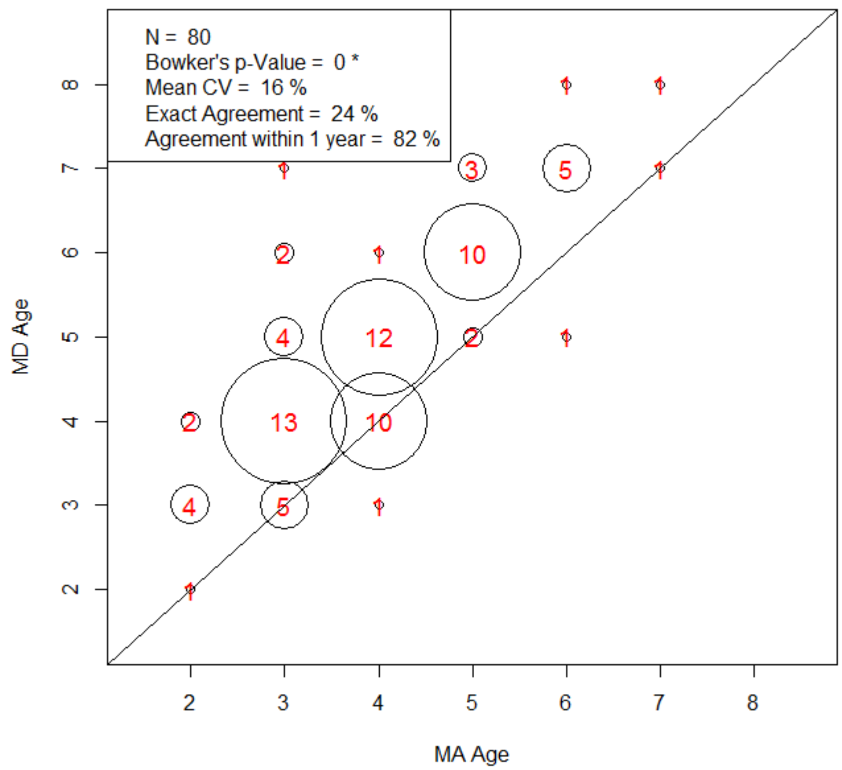


Figure 75. Age frequency plot for MD and MA blueback herring scale age determinations.

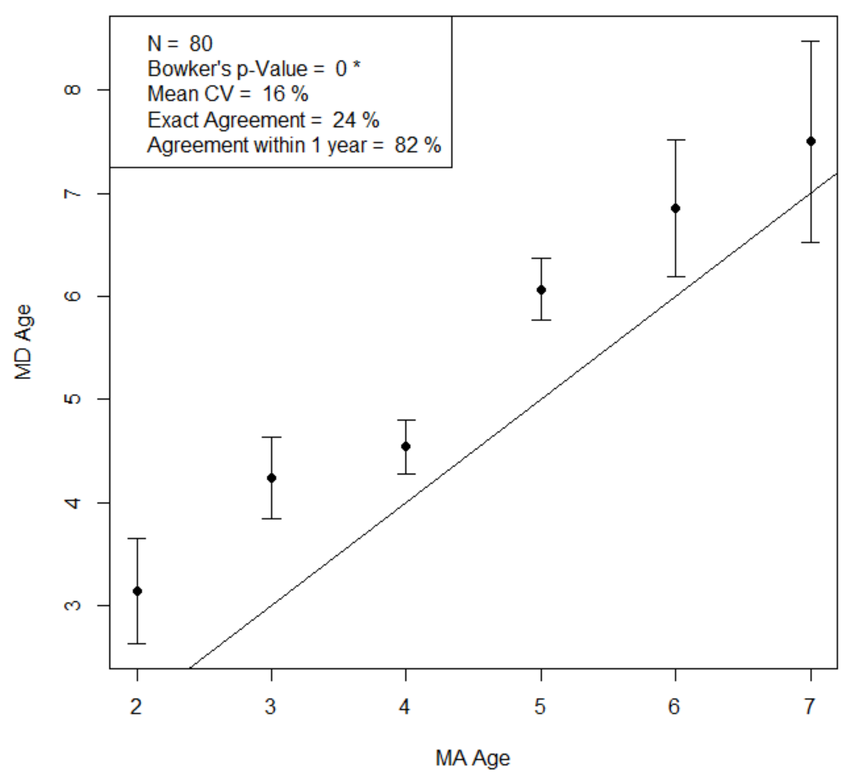


Figure 76. Age bias plot for MD and MA blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

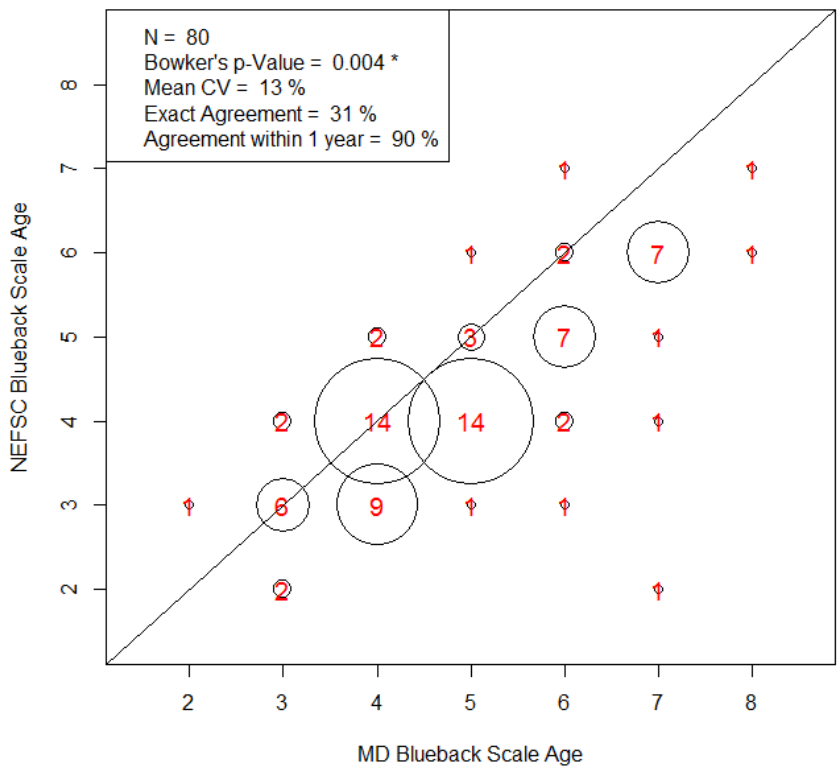


Figure 77. Age frequency plot for MD and NEFSC blueback herring scale age determinations.

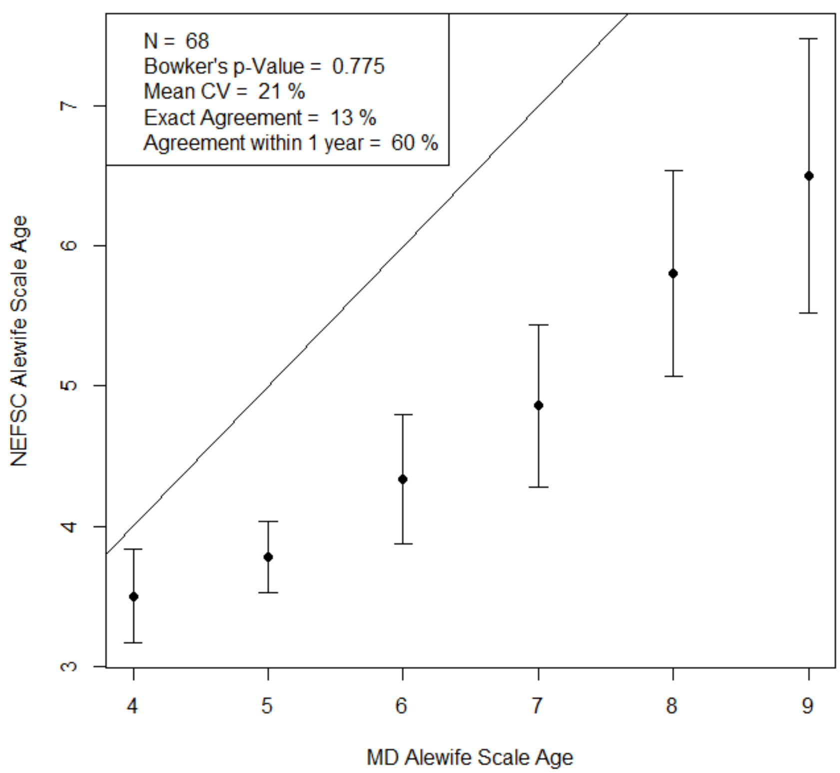


Figure 78. Age bias plot for MD and NEFSC blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

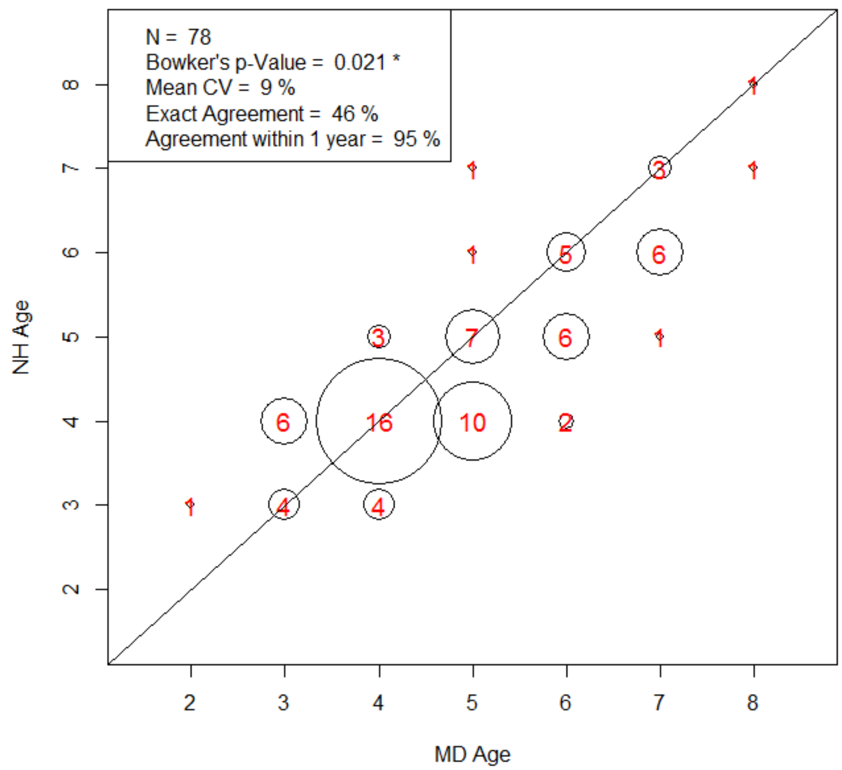


Figure 79. Age frequency plot for MD and NH blueback herring scale age determinations.

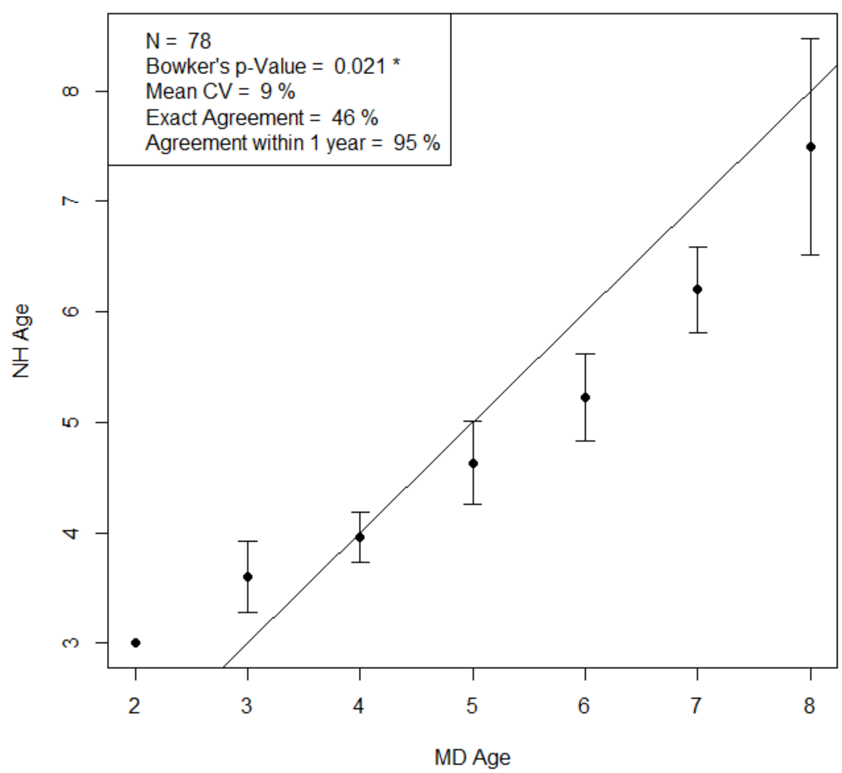


Figure 80. Age bias plot for MD and NH blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

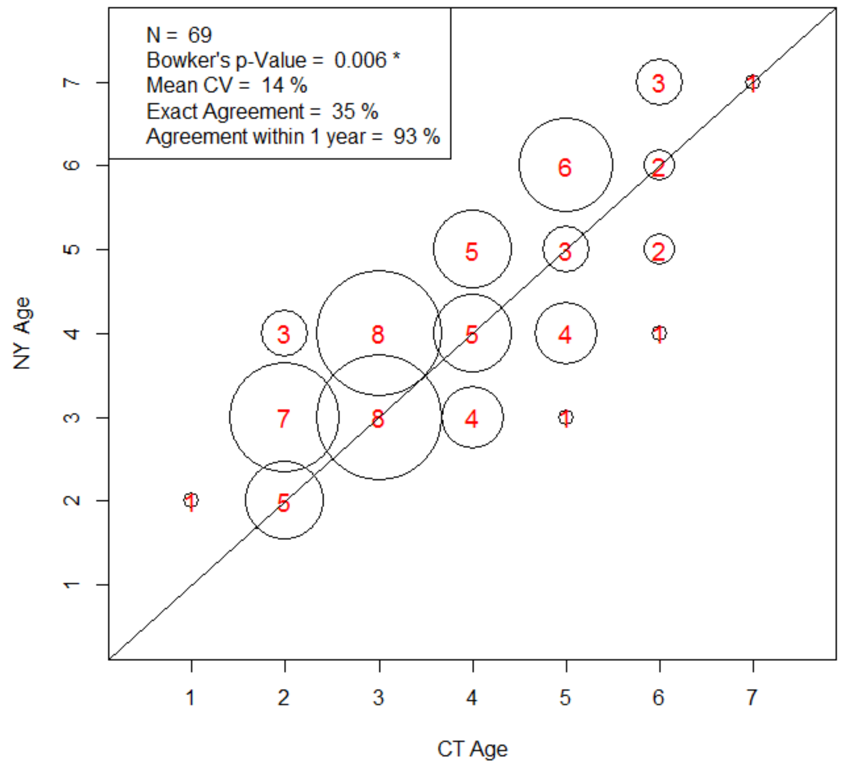


Figure 81. Age frequency plot for NY and CT blueback herring scale age determinations.

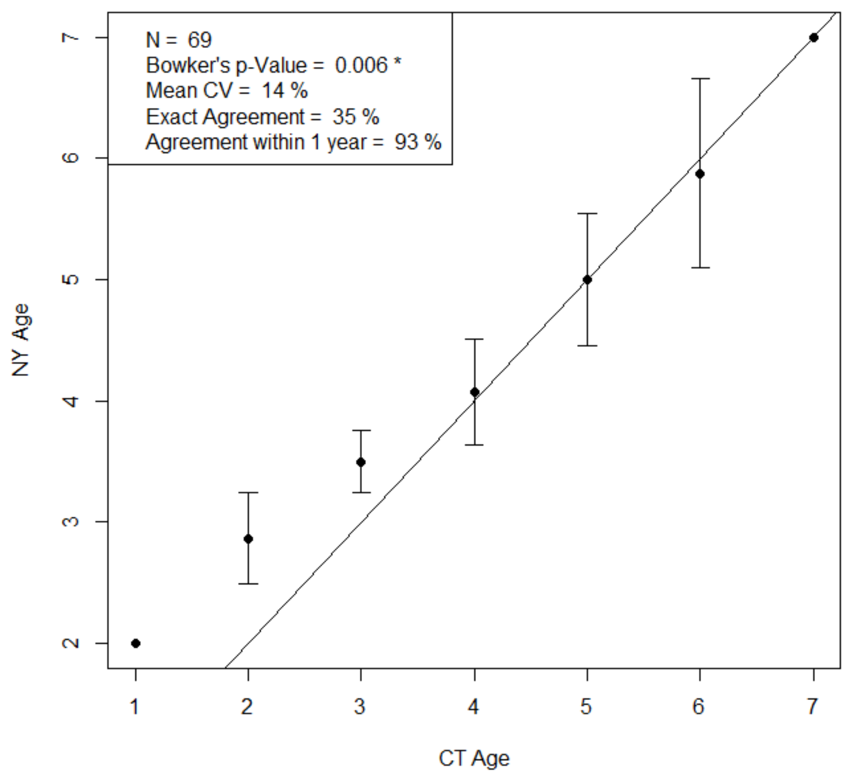


Figure 82. Age bias plot for NY and CT blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

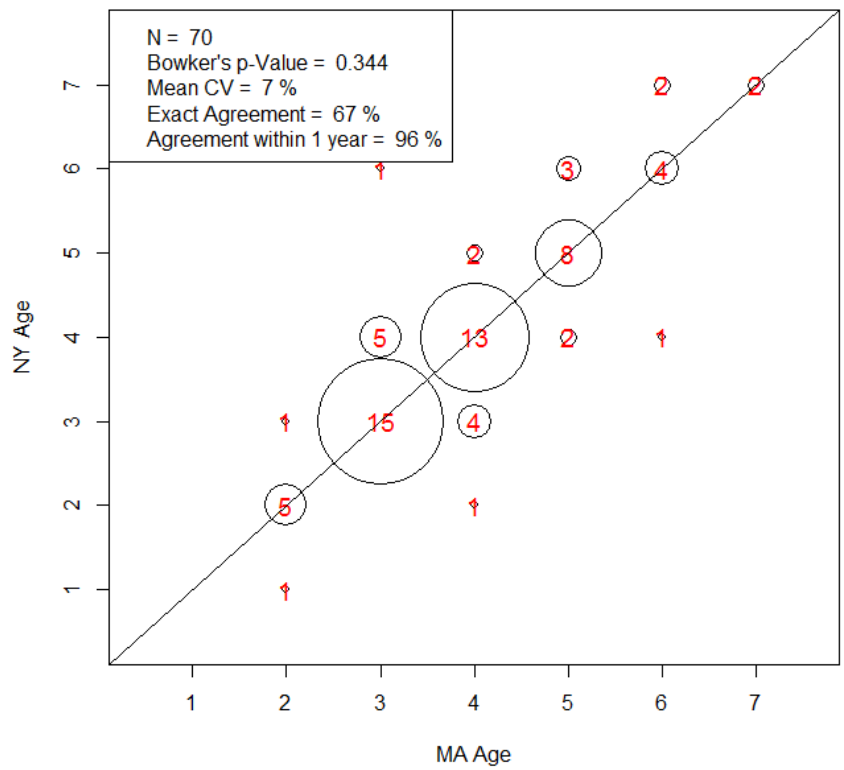


Figure 83. Age frequency plot for NY and MA blueback herring scale age determinations.

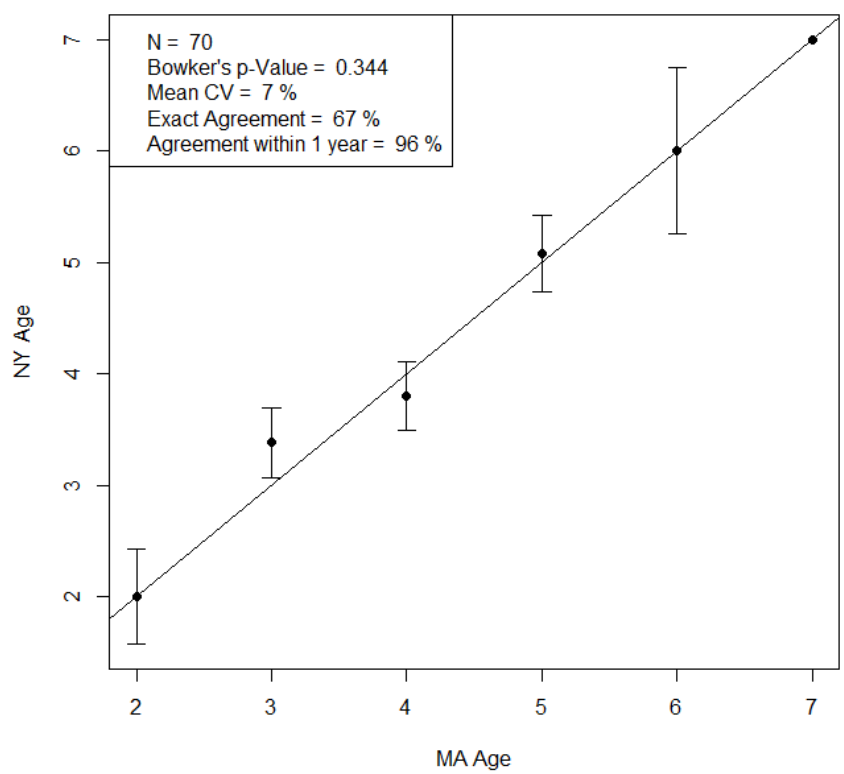


Figure 84. Age bias plot for NY and MA blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

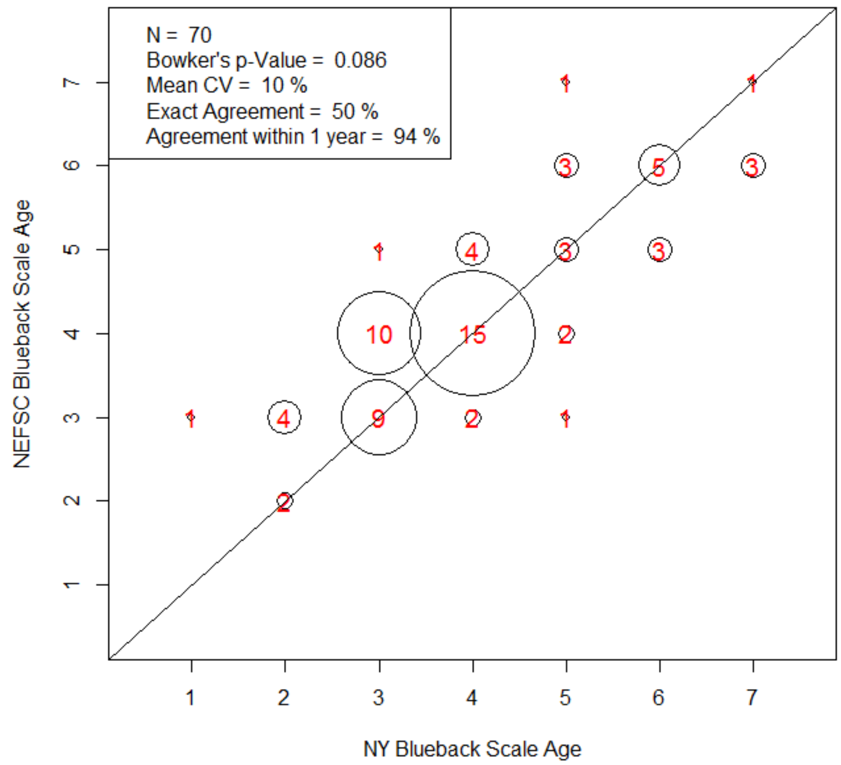


Figure 85. Age frequency plot for NY and NEFSC blueback herring scale age determinations.

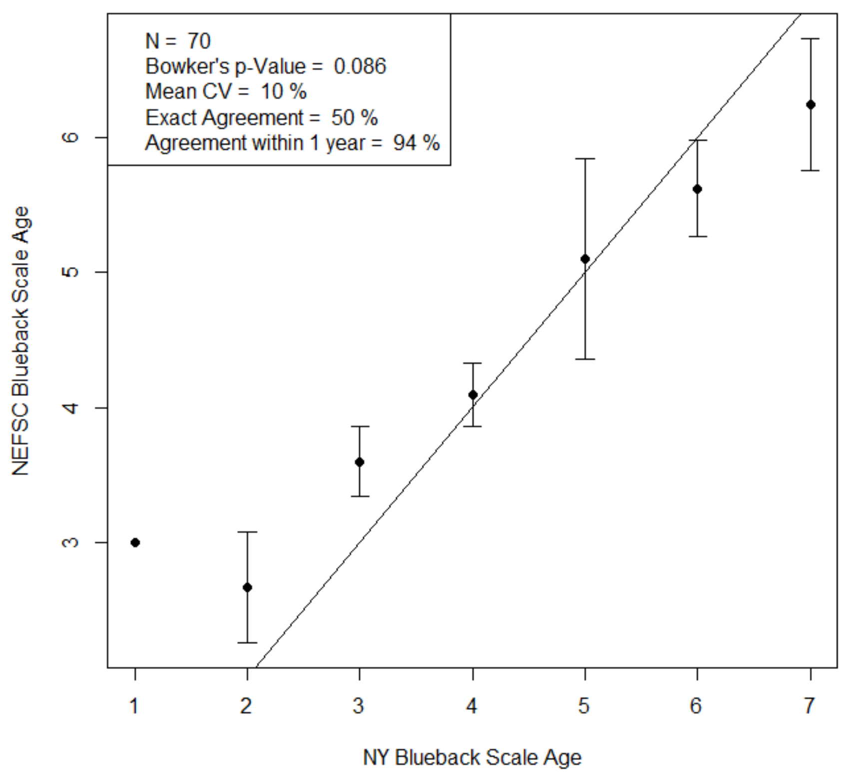


Figure 86. Age bias plot for NY and NEFSC blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

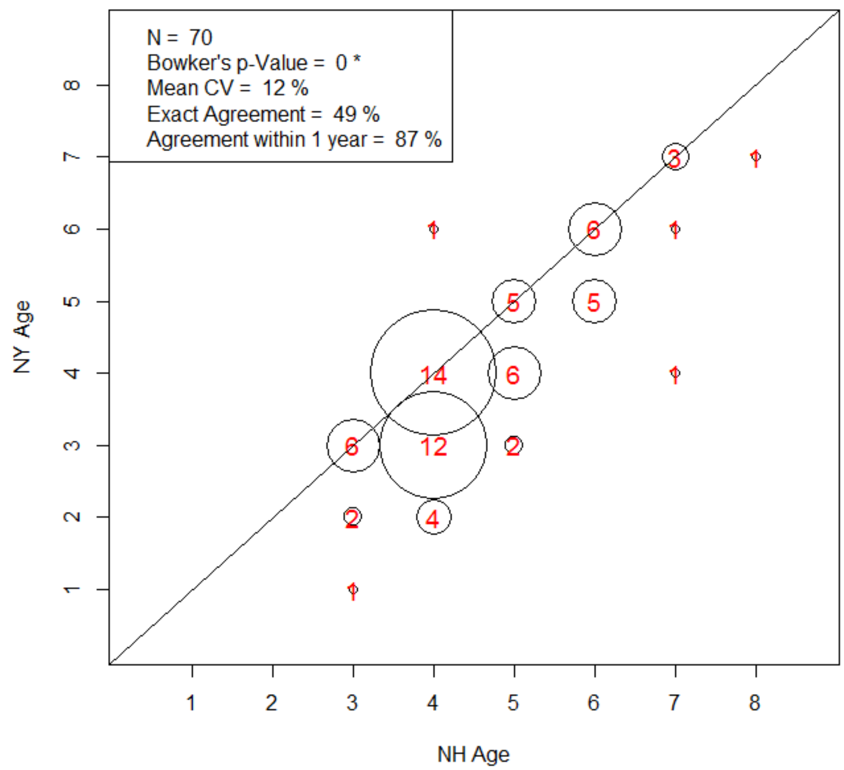


Figure 87. Age frequency plot for NY and NH blueback herring scale age determinations.

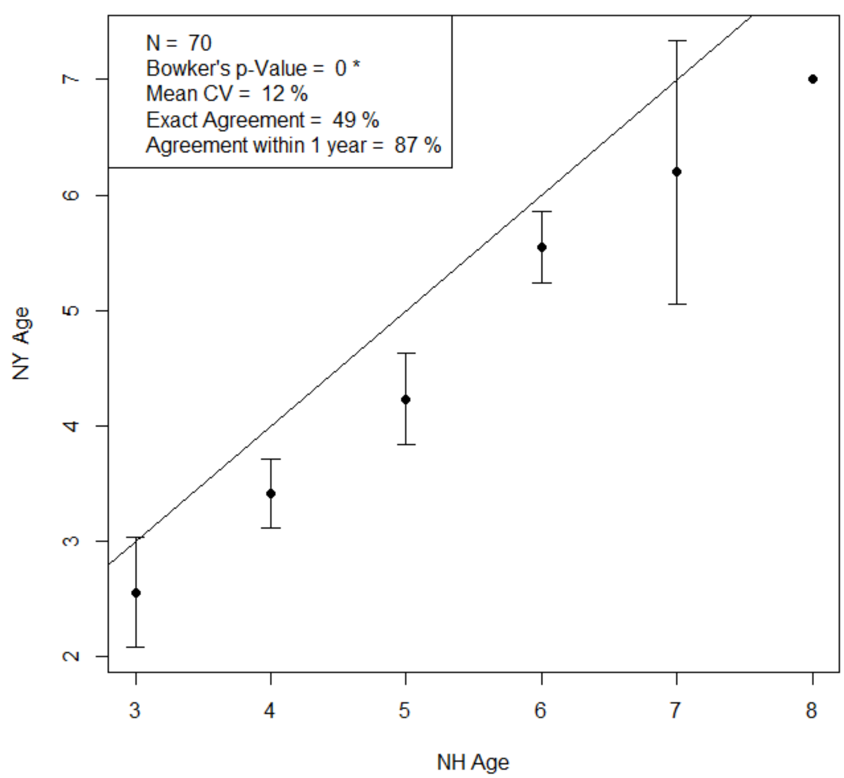


Figure 88. Age bias plot for NY and NH blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

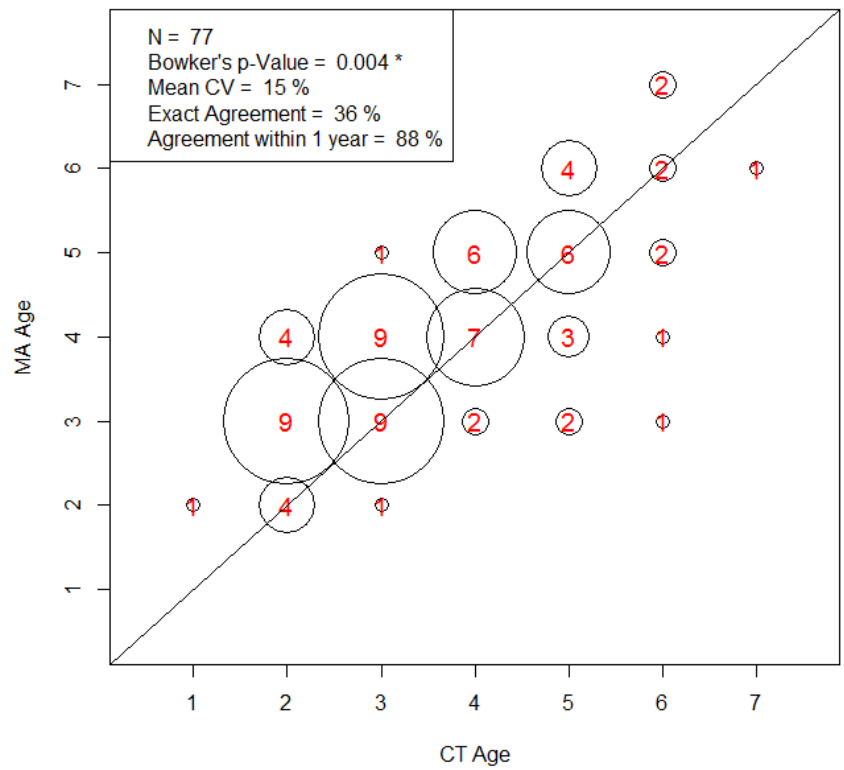


Figure 89. Age frequency plot for CT and MA blueback herring scale age determinations.

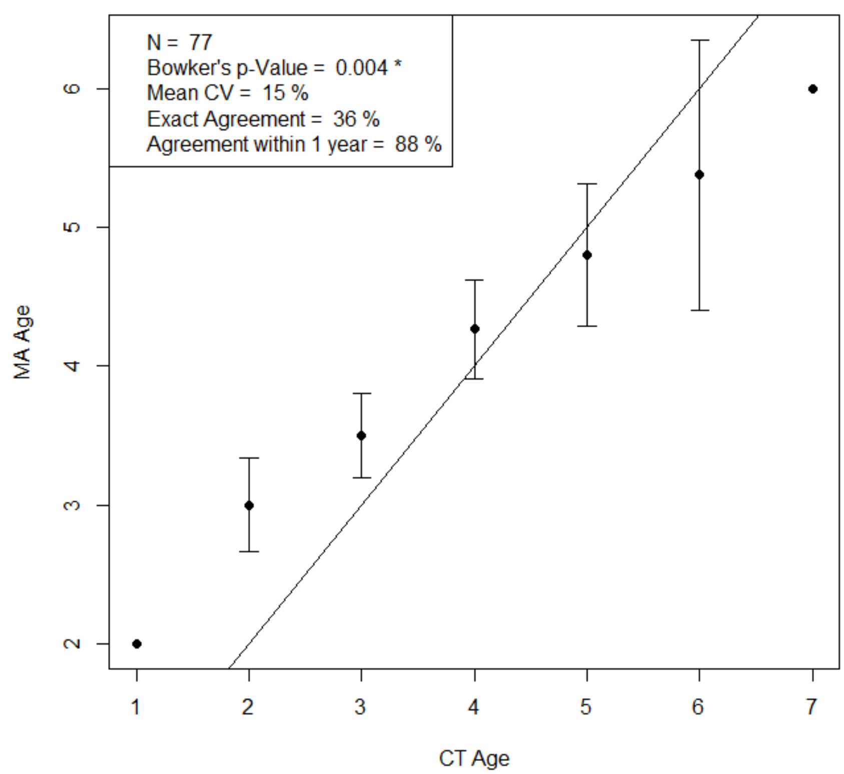


Figure 90. Age bias plot for CT and MA blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

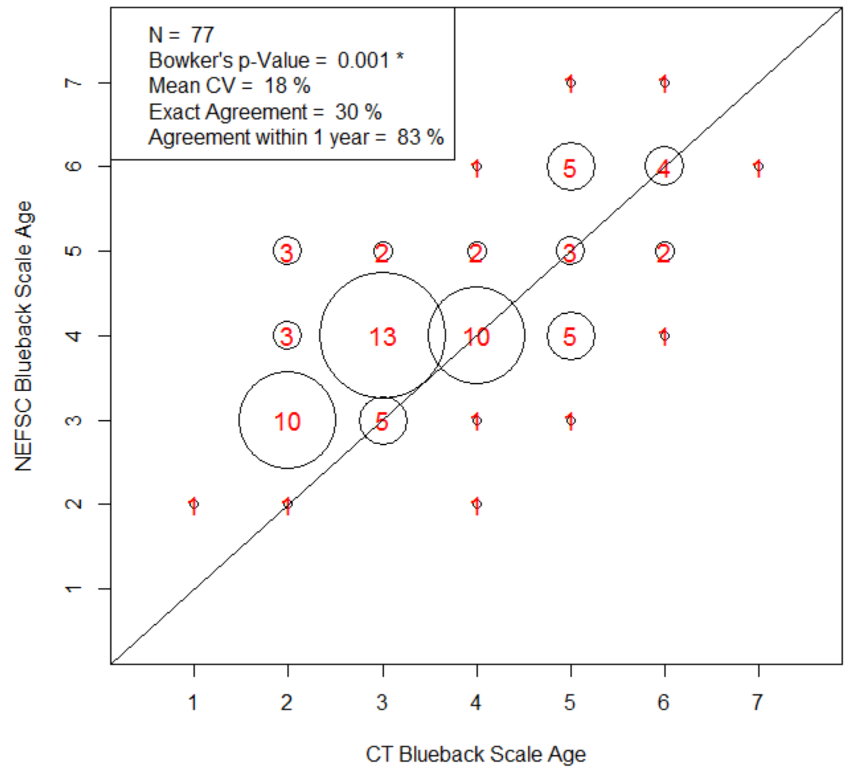


Figure 91. Age frequency plot for CT and NEFSC blueback herring scale age determinations.

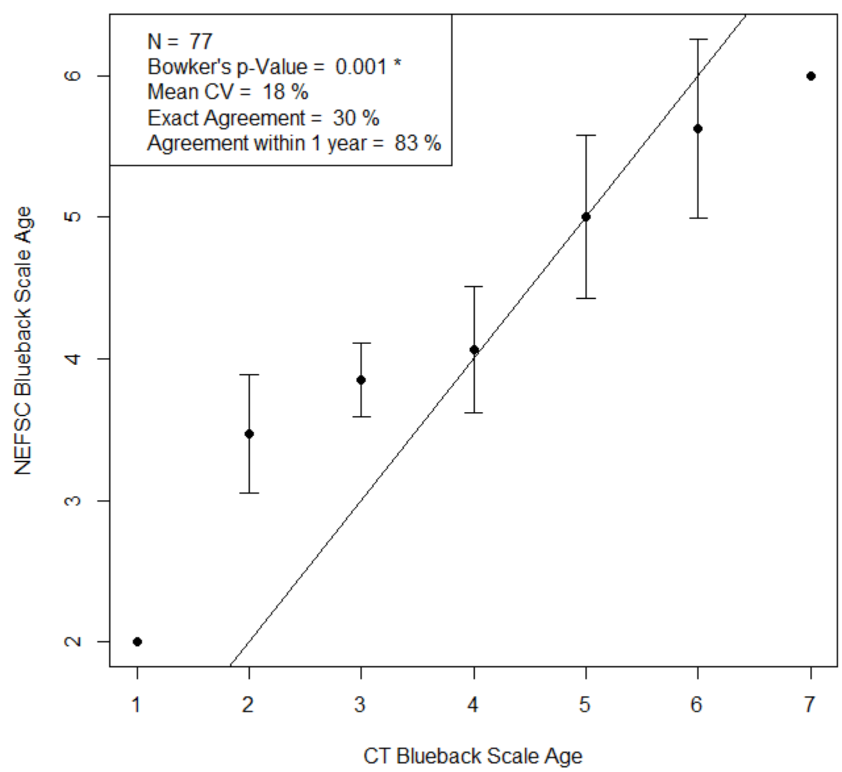


Figure 92. Age bias plot for CT and NEFSC blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

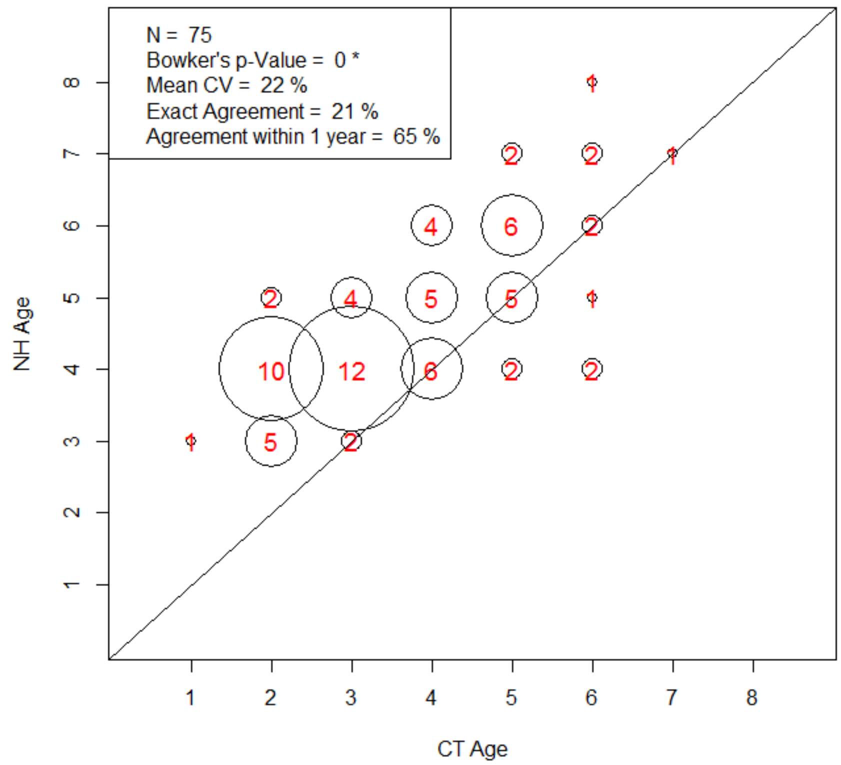


Figure 93. Age frequency plot for CT and NH blueback herring scale age determinations.

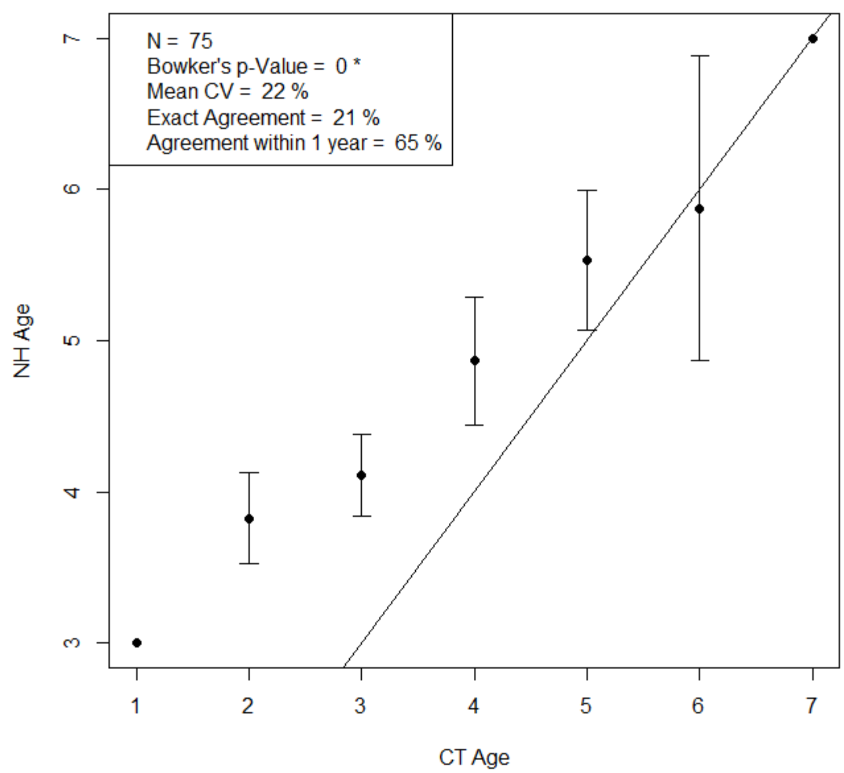


Figure 94. Age bias plot for CT and NH blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

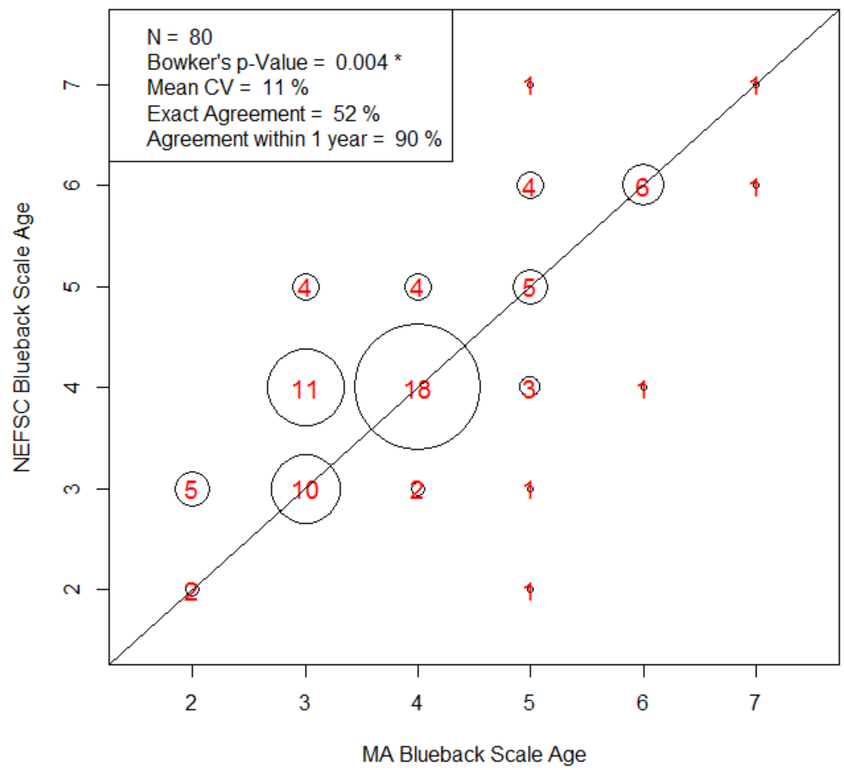


Figure 95. Age frequency plot for MA and NEFSC blueback herring scale age determinations.

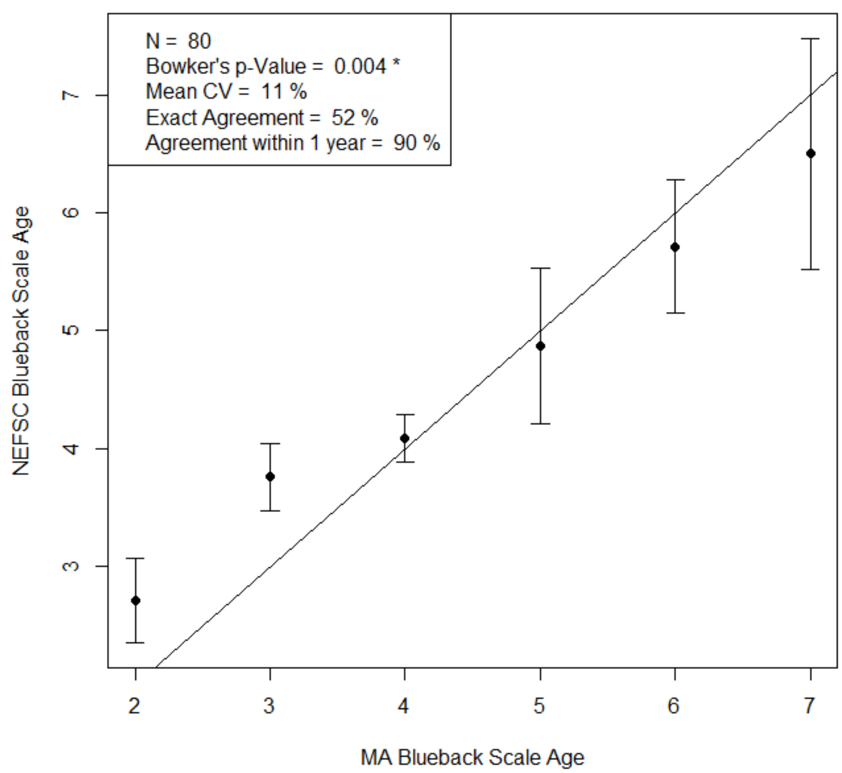


Figure 96. Age bias plot for MA and NEFSC blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

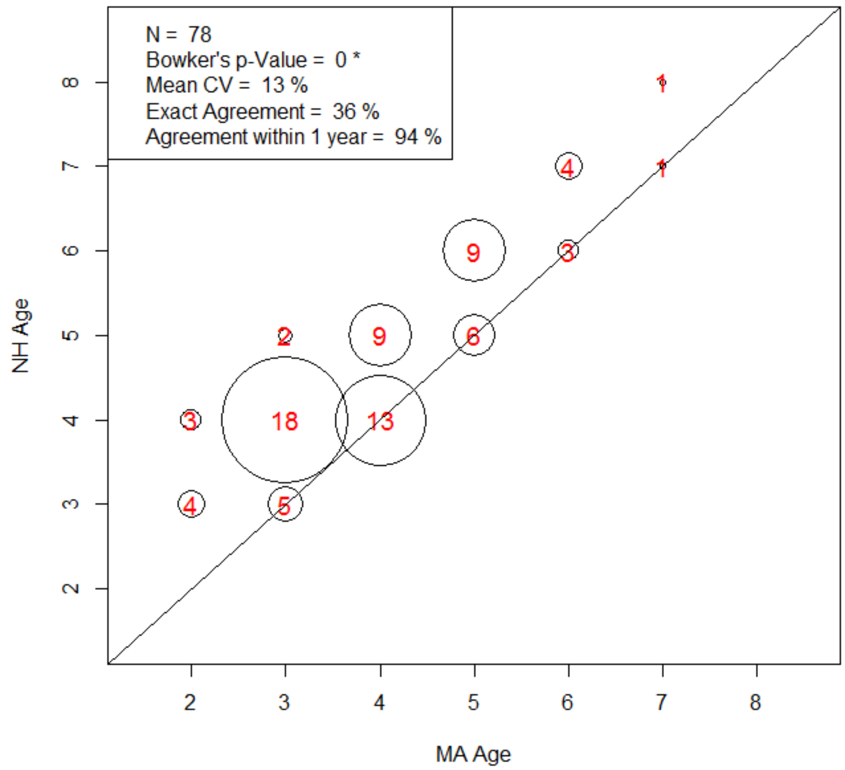


Figure 97. Age frequency plot for MA and NH blueback herring scale age determinations.

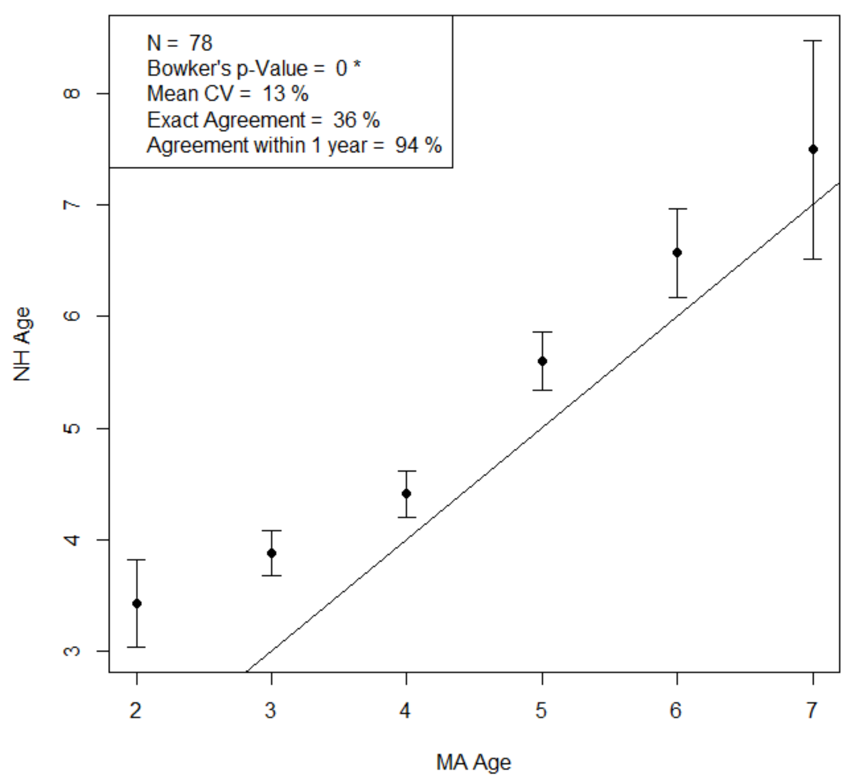


Figure 98. Age bias plot for MA and NH blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

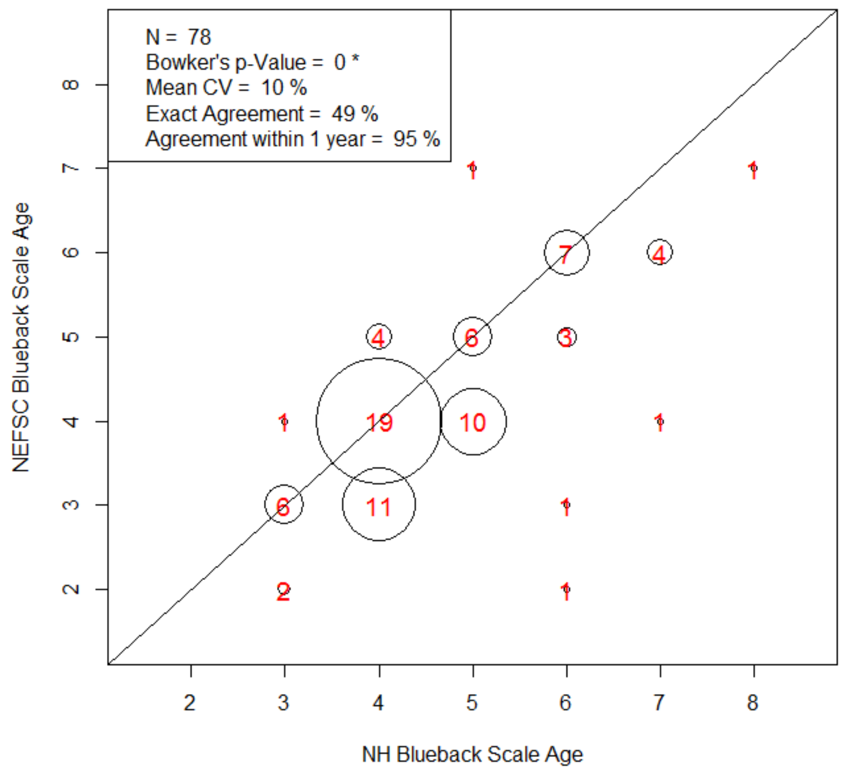


Figure 99. Age frequency plot for NEFSC and NH blueback herring scale age determinations.

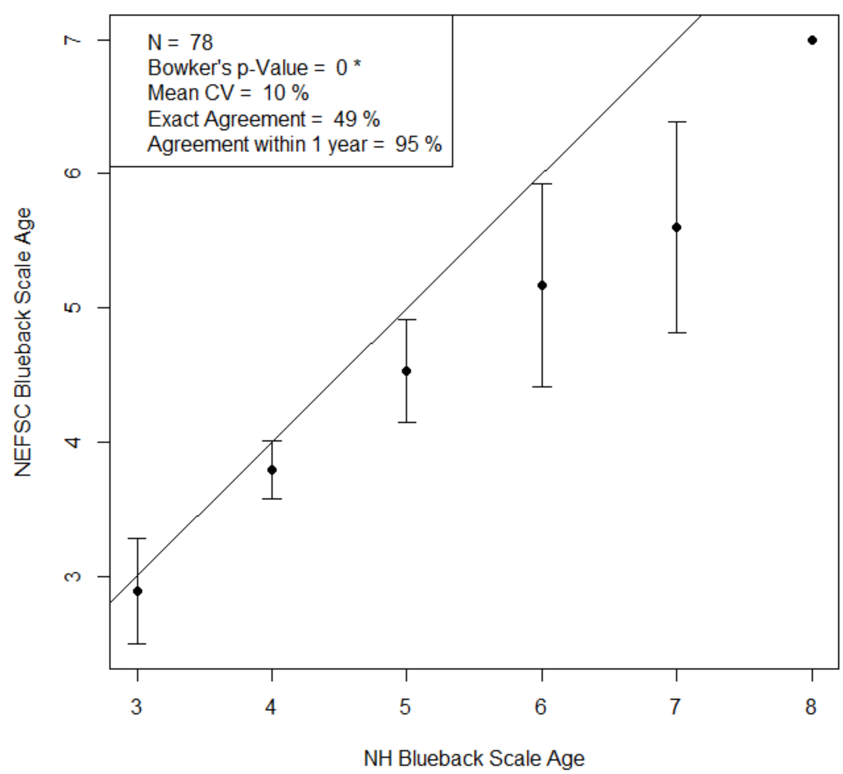


Figure 100. Age bias plot for NEFSC and NH blueback herring scale age determinations. Error bars in the age bias plots are 95% confidence intervals.

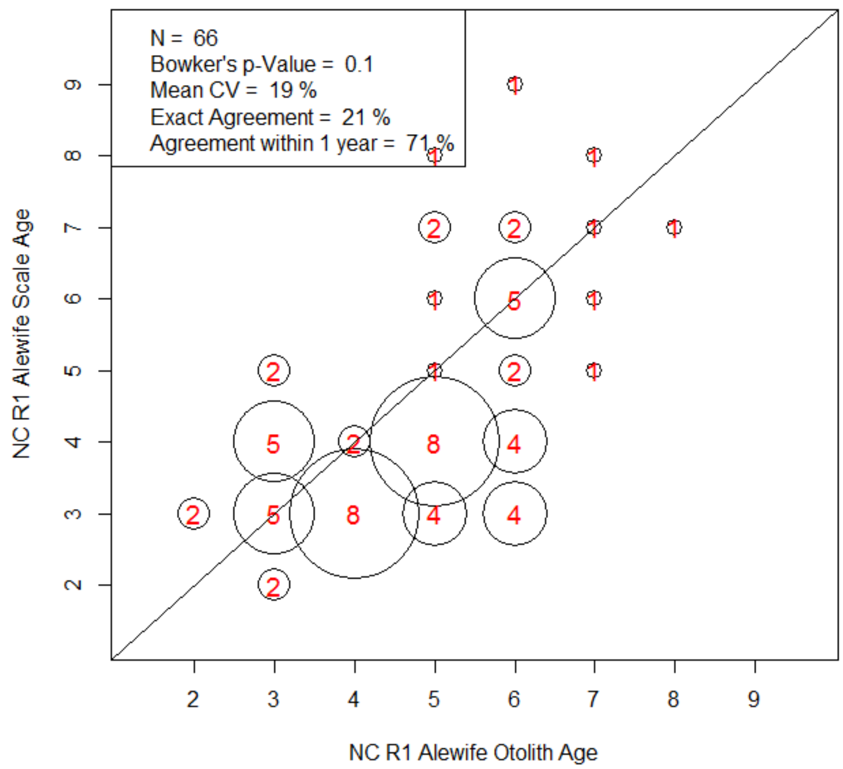


Figure 101. Age frequency plot for NC reader 1 paired alewife scale and otolith age determinations.

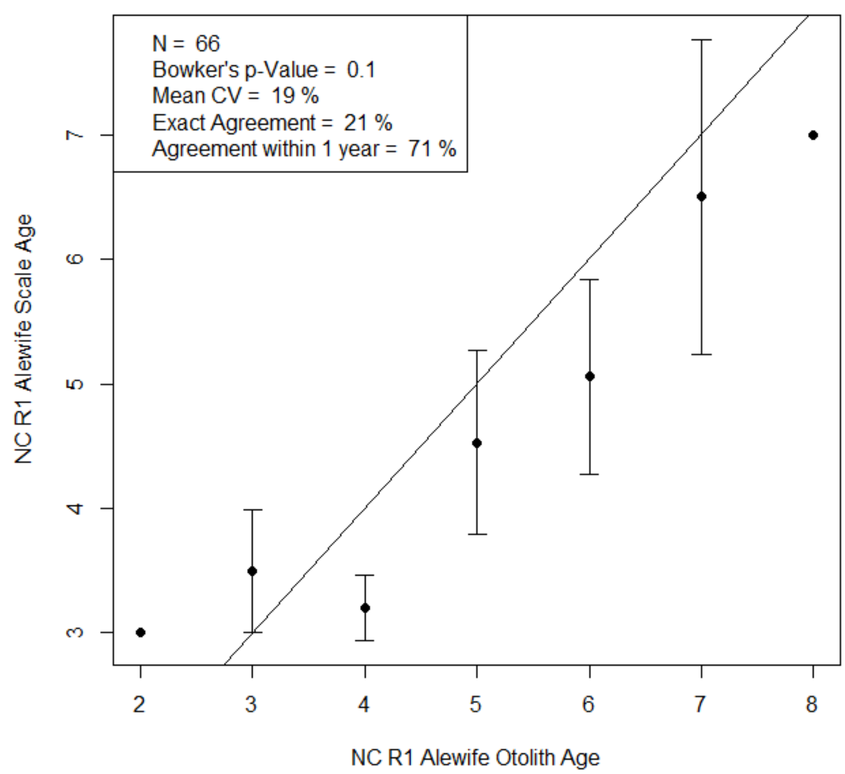


Figure 102. Age bias plot for NC reader 1 paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

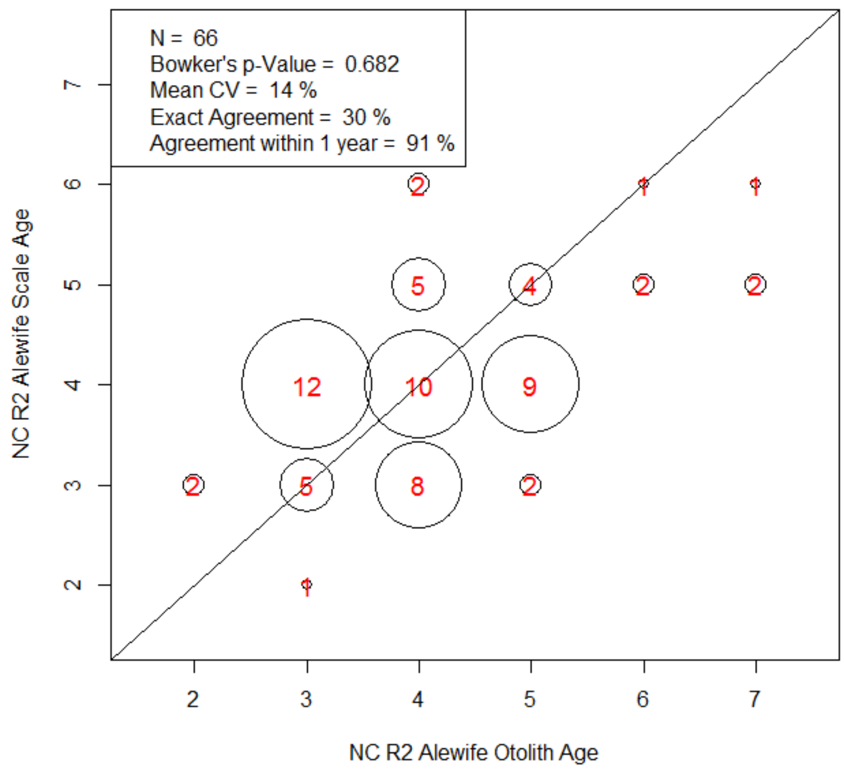


Figure 103. Age frequency plot for NC reader 2 paired alewife scale and otolith age determinations.

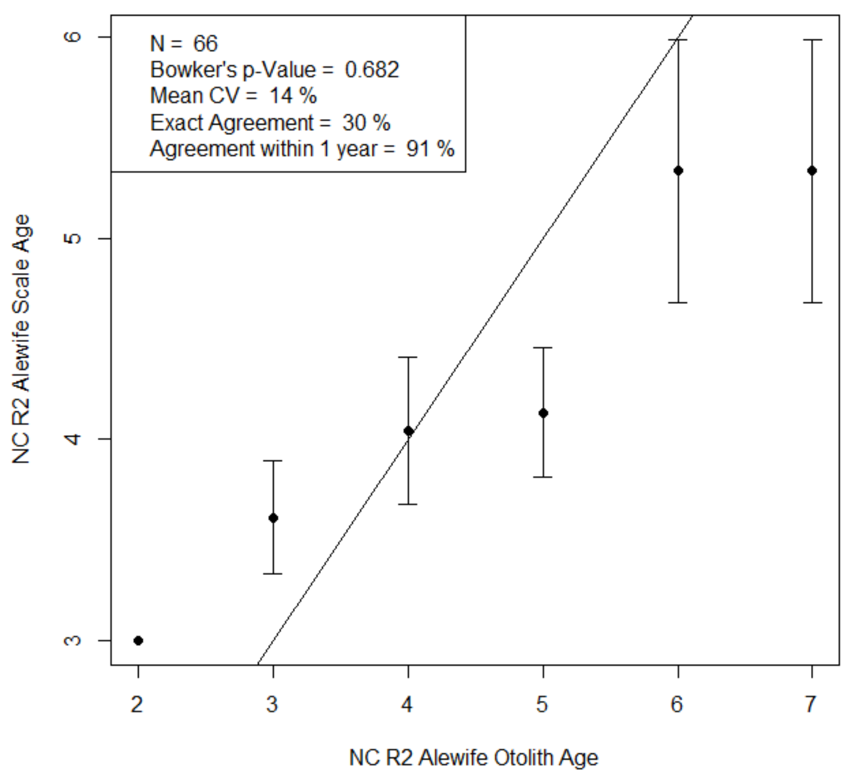


Figure 104. Age bias plot for NC reader 2 paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

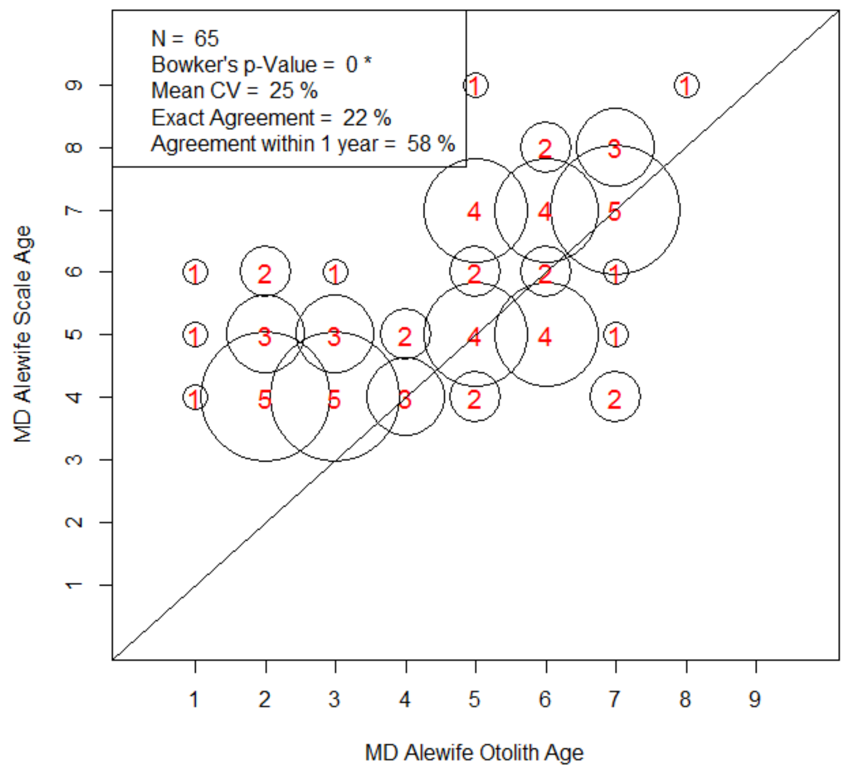


Figure 105. Age frequency plot for MD reader paired alewife scale and otolith age determinations.

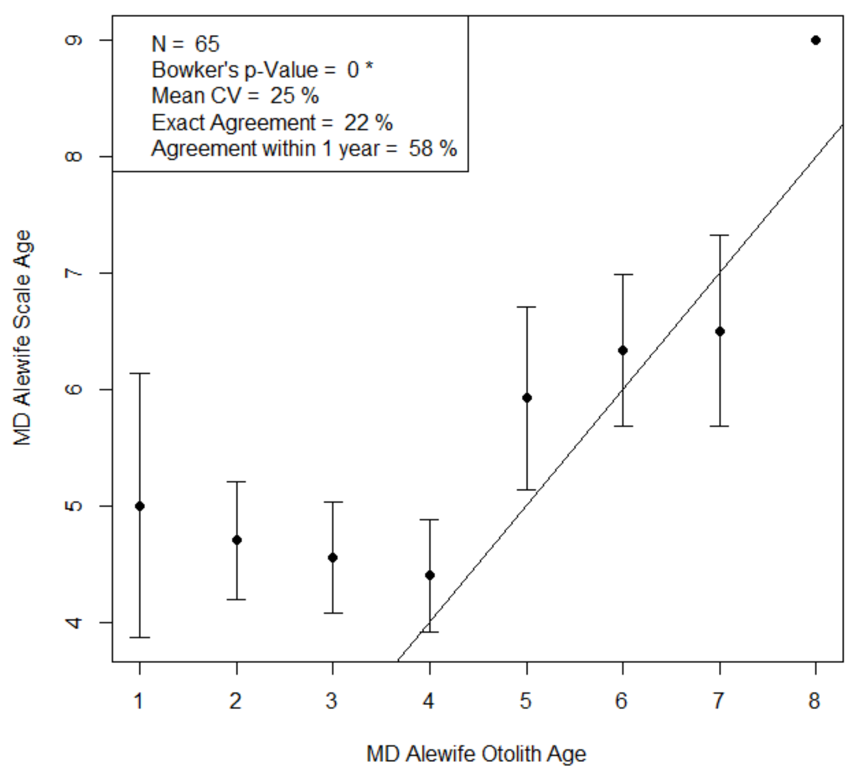


Figure 106. Age bias plot for MD reader paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

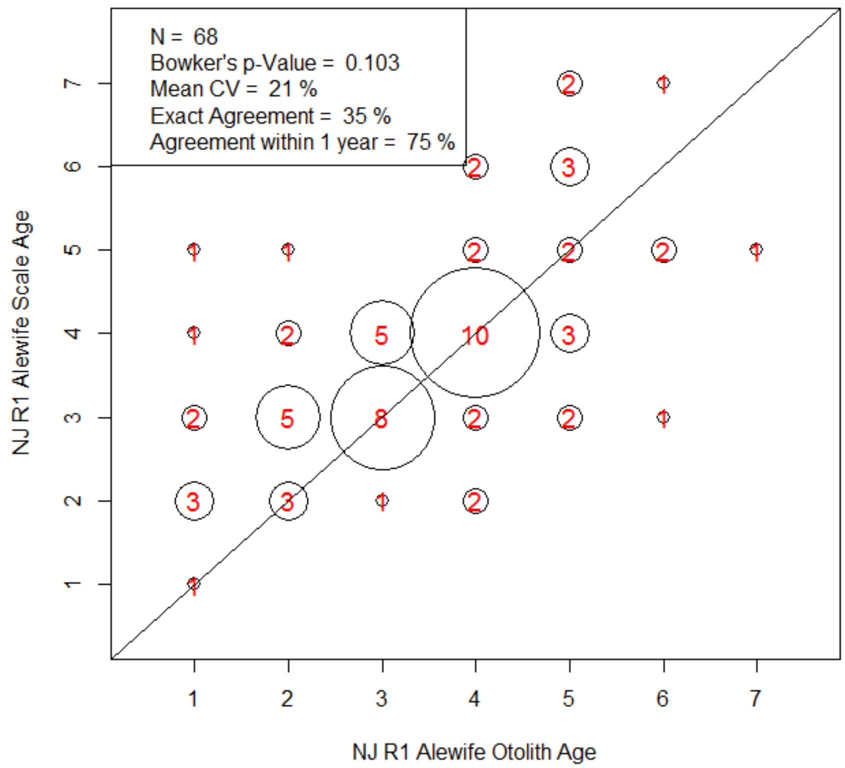


Figure 107. Age frequency plot for NJ reader 1 paired alewife scale and otolith age determinations.

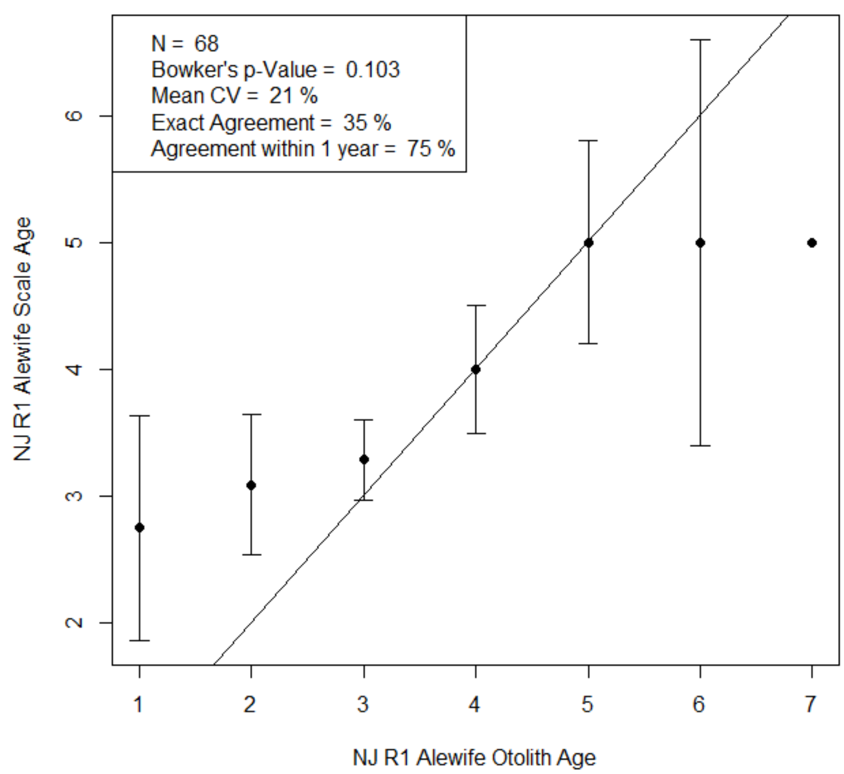


Figure 108. Age bias plot for NJ reader 1 paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

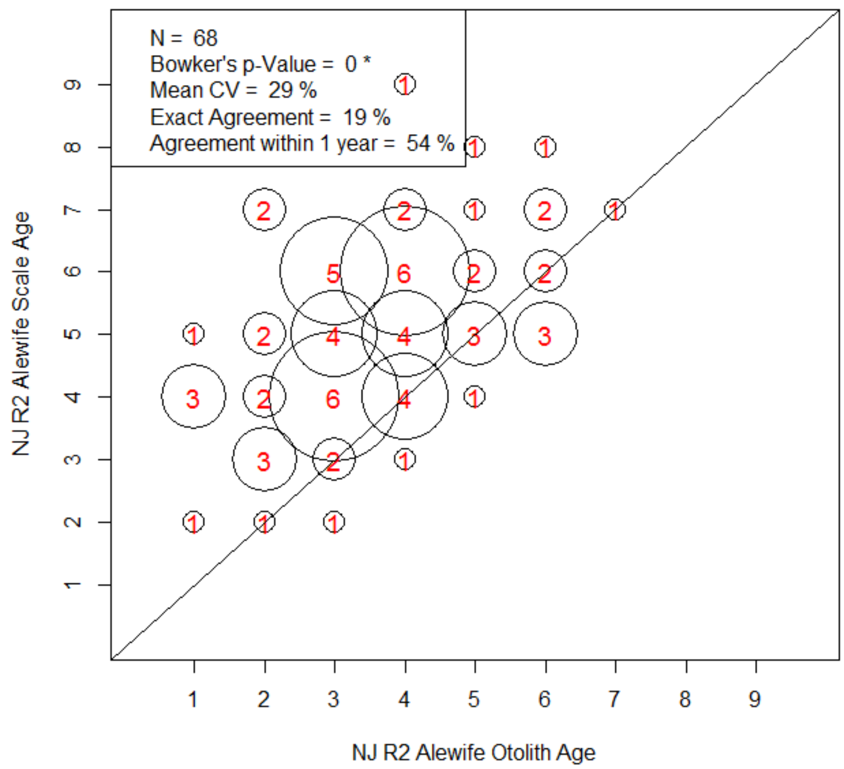


Figure 109. Age frequency plot for NJ reader 2 paired alewife scale and otolith age determinations.

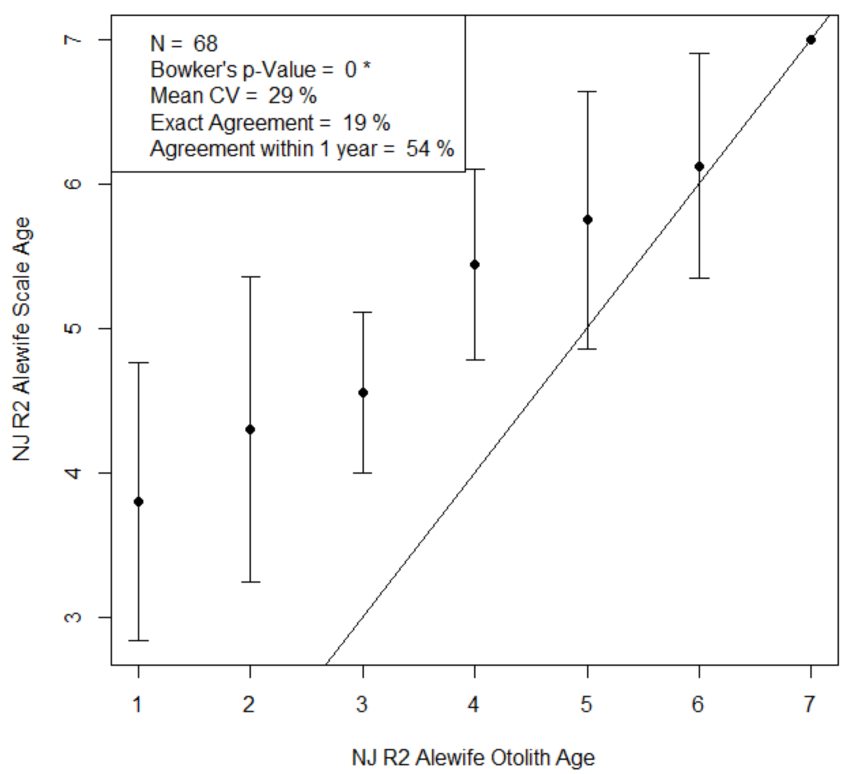


Figure 110. Age bias plot for NJ reader 2 paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

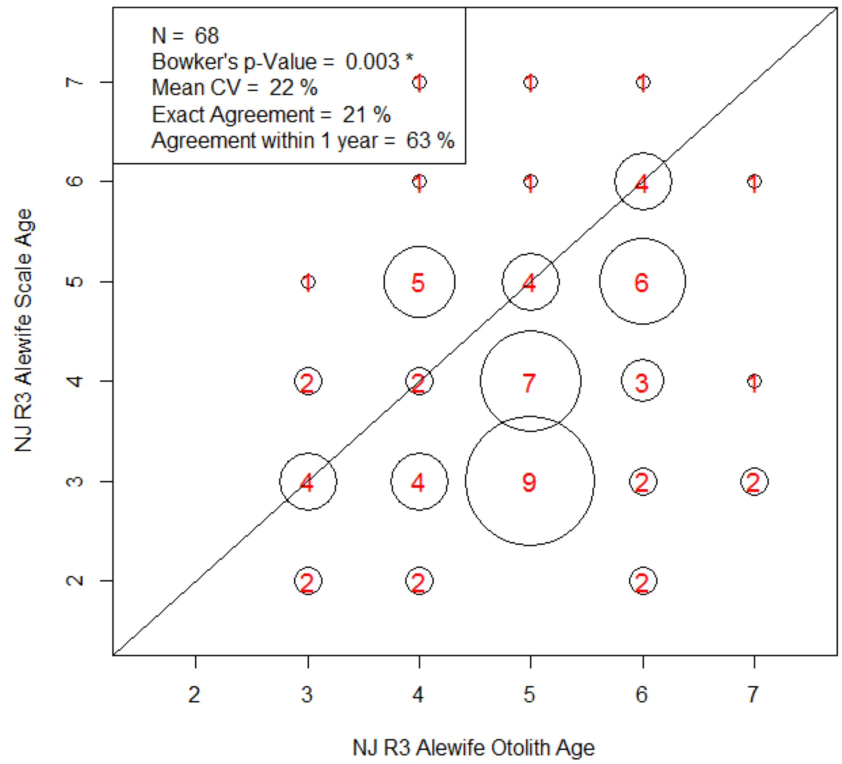


Figure 111. Age frequency plot for NJ reader 3 paired alewife scale and otolith age determinations.

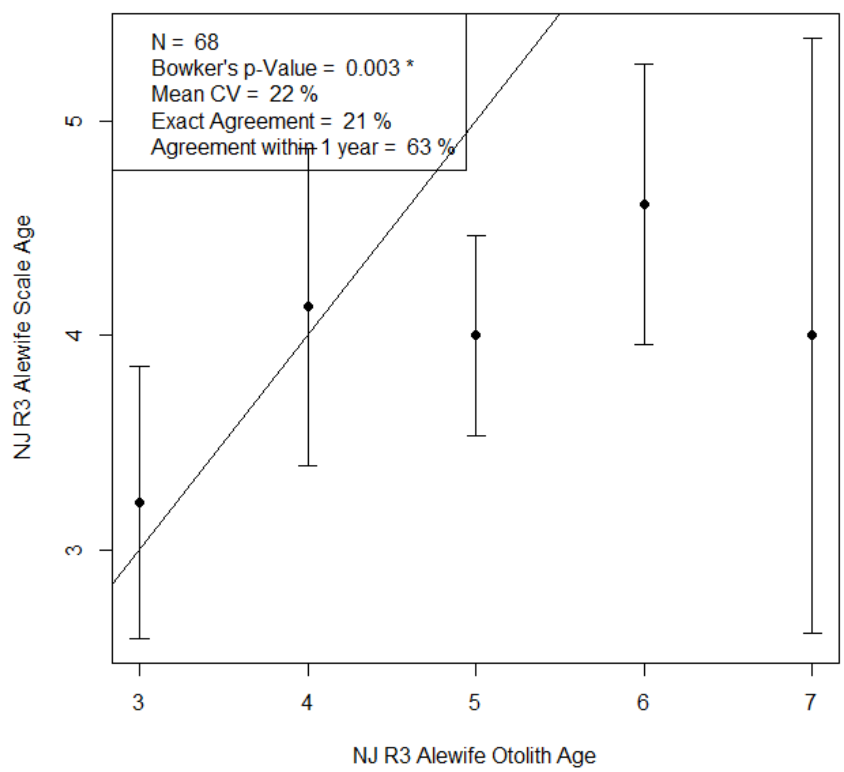


Figure 112. Age bias plot for NJ reader 3 paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

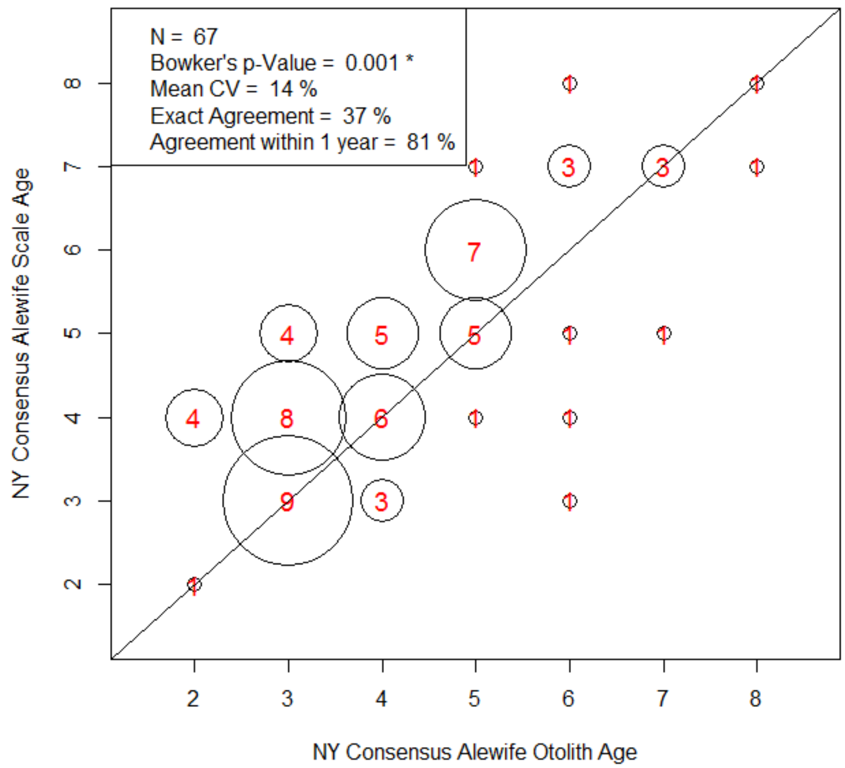


Figure 113. Age frequency plot for NY consensus paired alewife scale and otolith age determinations.

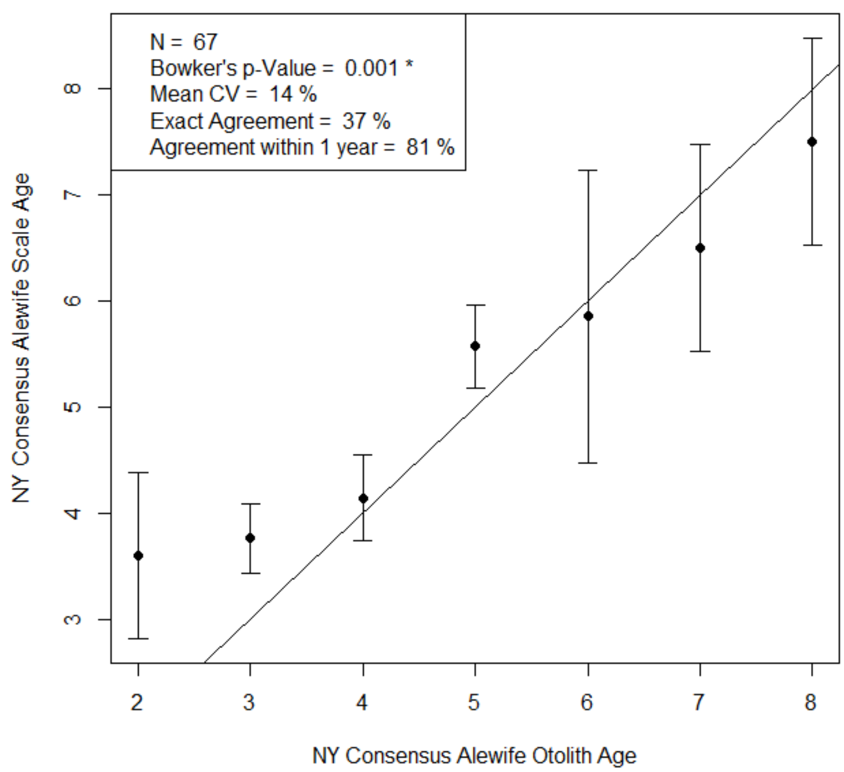


Figure 114. Age bias plot for NY consensus paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

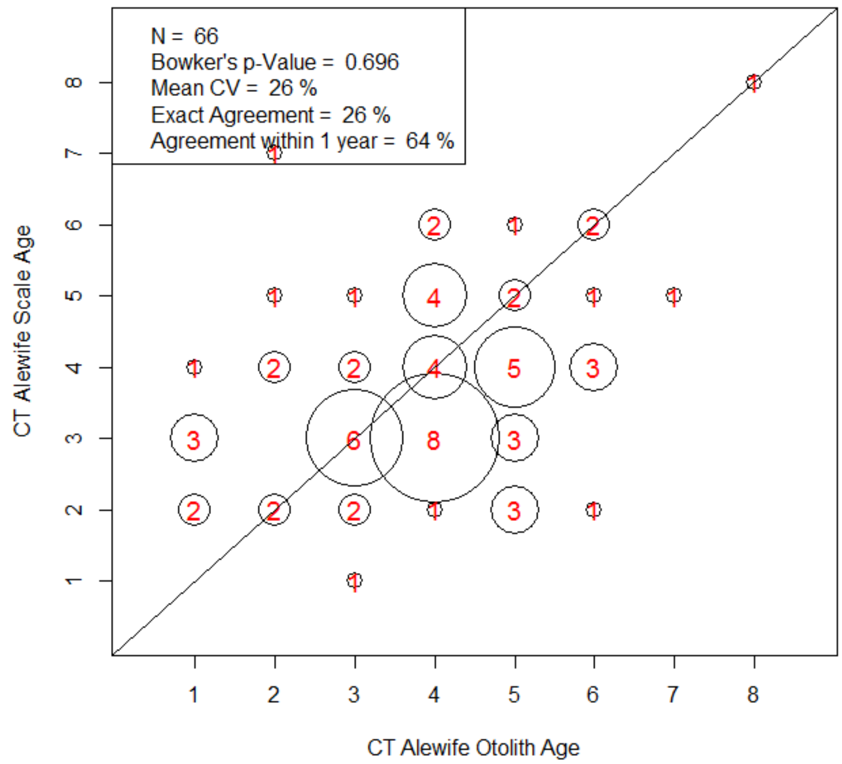


Figure 115. Age frequency plot for CT reader paired alewife scale and otolith age determinations.

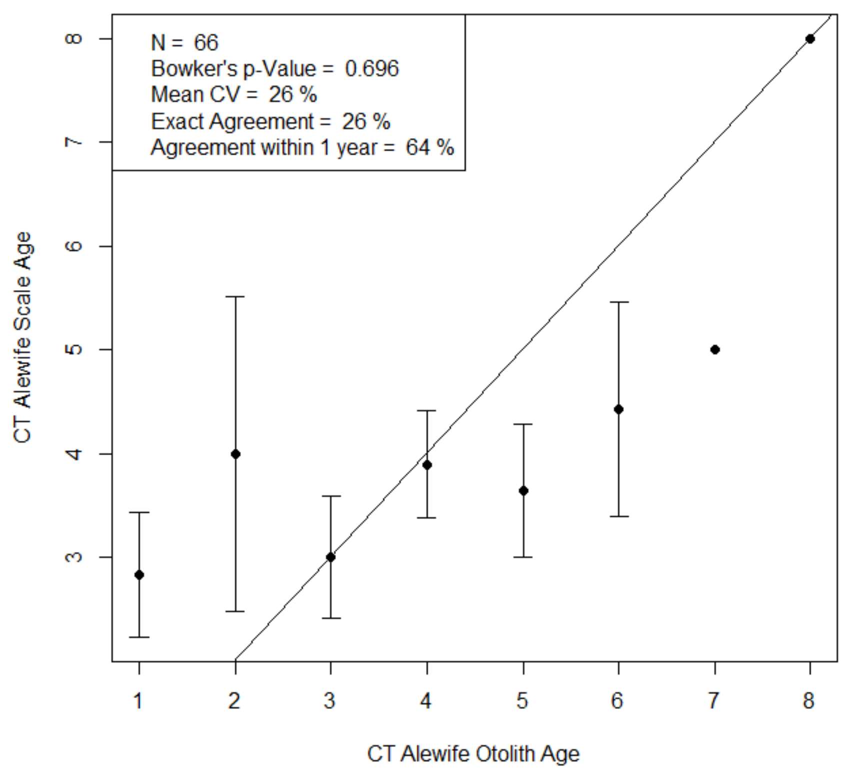


Figure 116. Age bias plot for CT reader paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

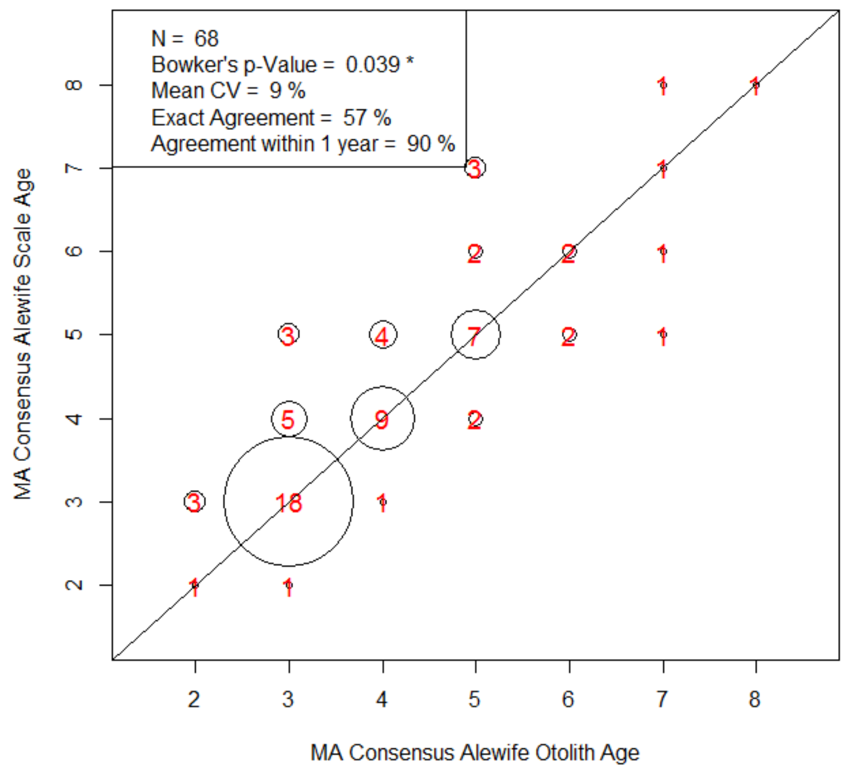


Figure 117. Age frequency plot for MA consensus paired alewife scale and otolith age determinations.

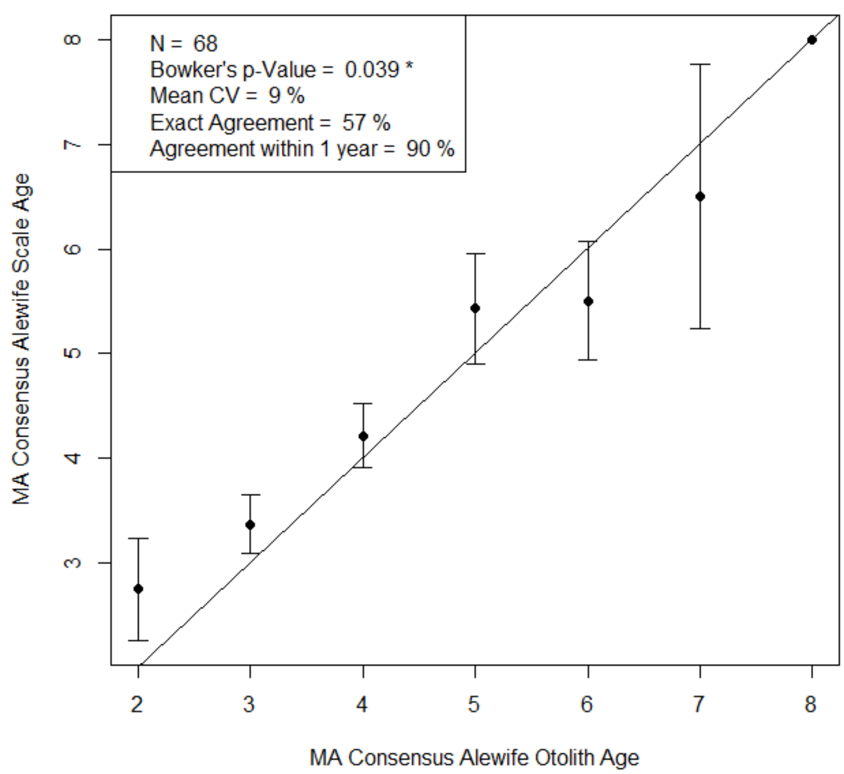


Figure 118. Age bias plot for MA consensus paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

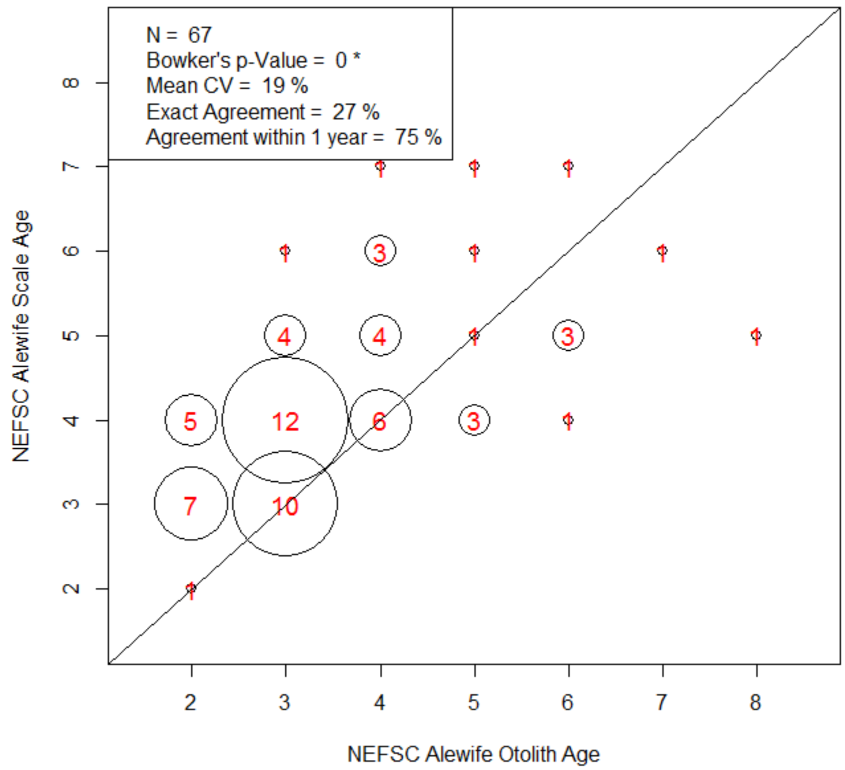


Figure 119. Age frequency plot for NEFSC reader paired alewife scale and otolith age determinations.

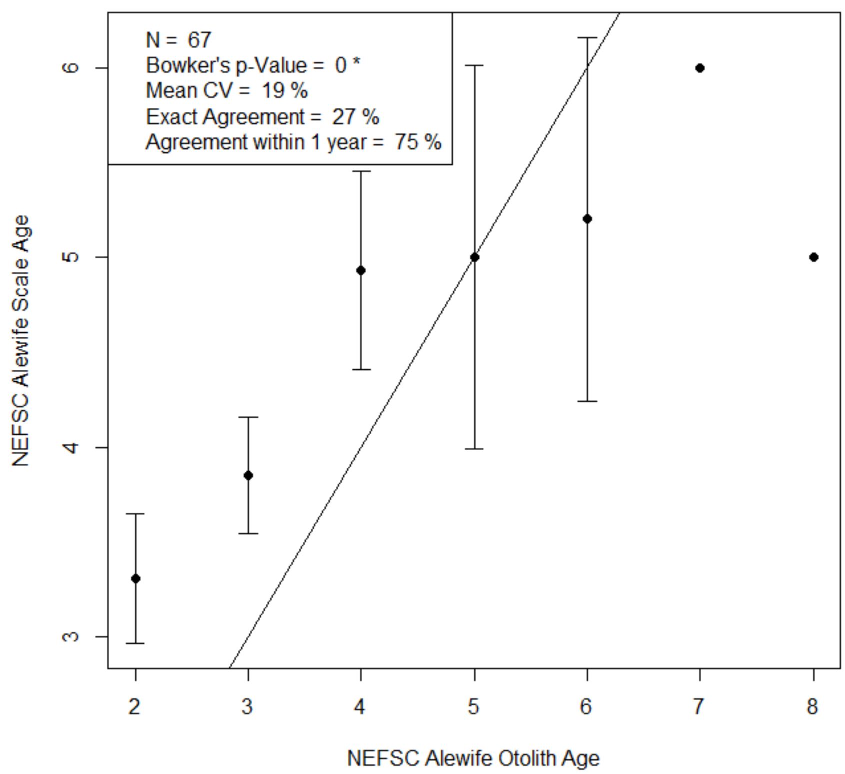


Figure 120. Age bias plot for NEFSC reader paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

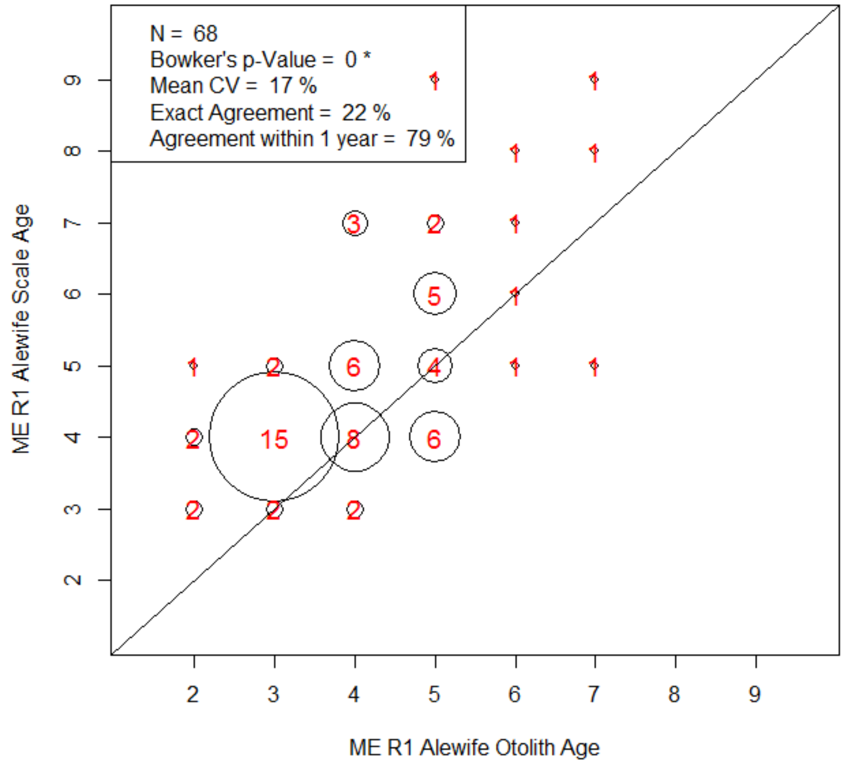


Figure 121. Age frequency plot for ME reader 1 paired alewife scale and otolith age determinations.

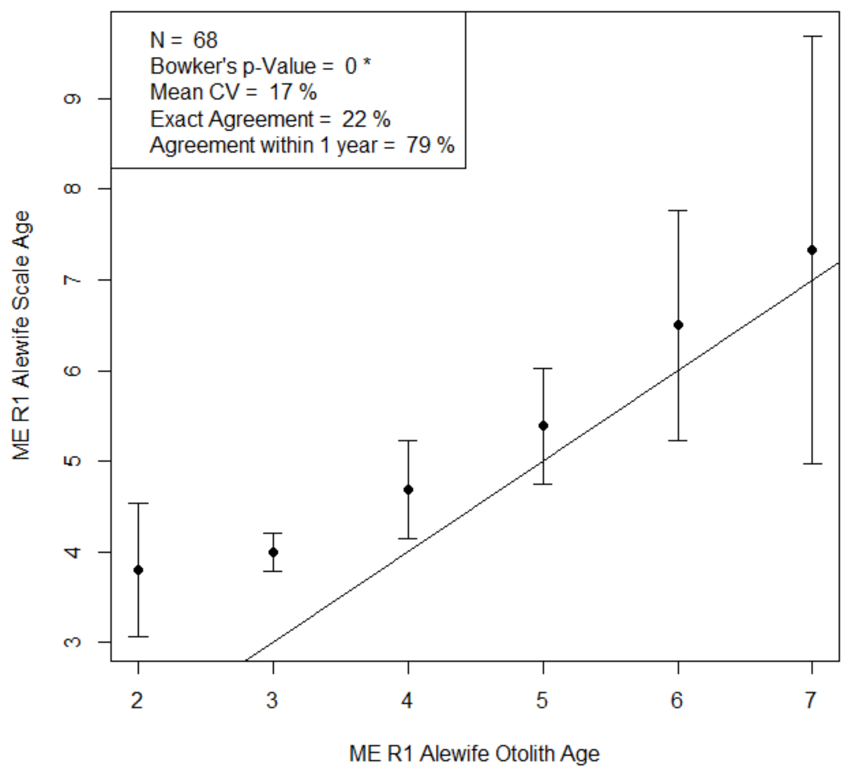


Figure 122. Age bias plot for ME reader 1 paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

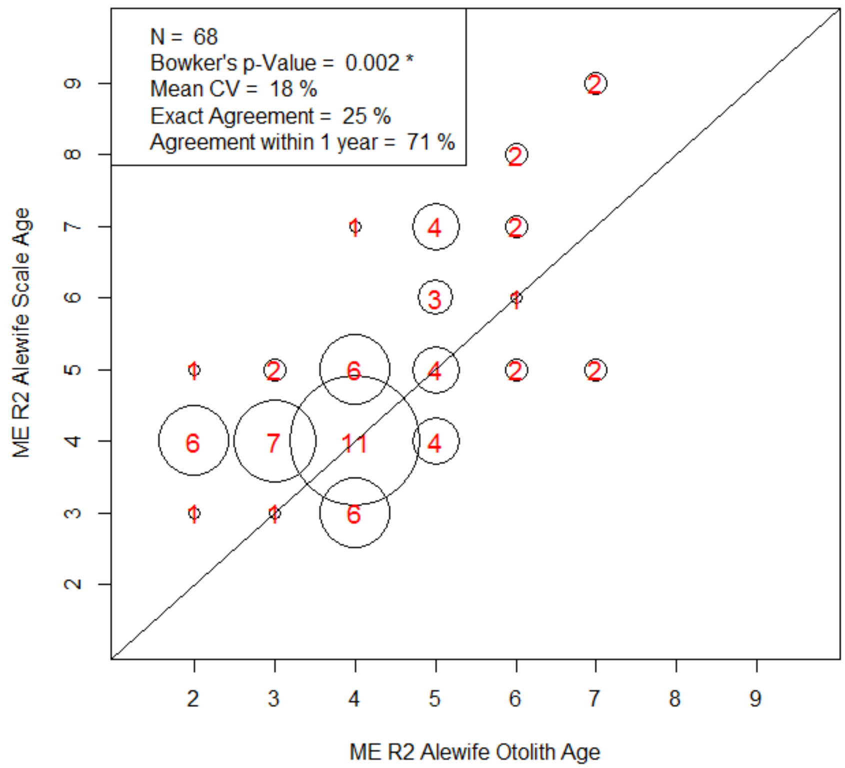


Figure 123. Age frequency plot for ME reader 2 paired alewife scale and otolith age determinations.

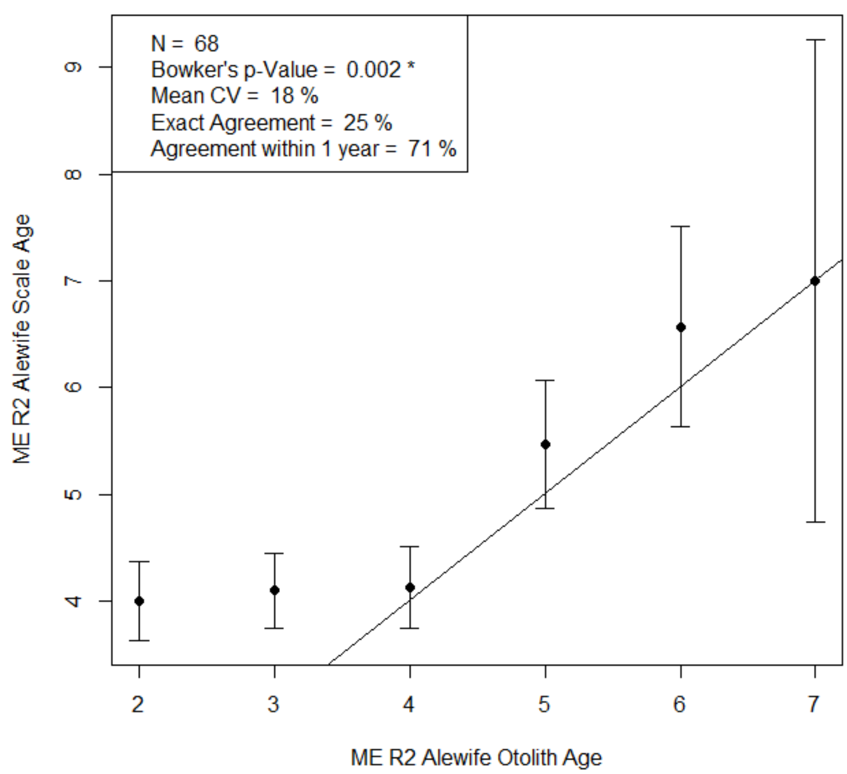


Figure 124. Age bias plot for ME reader 2 paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

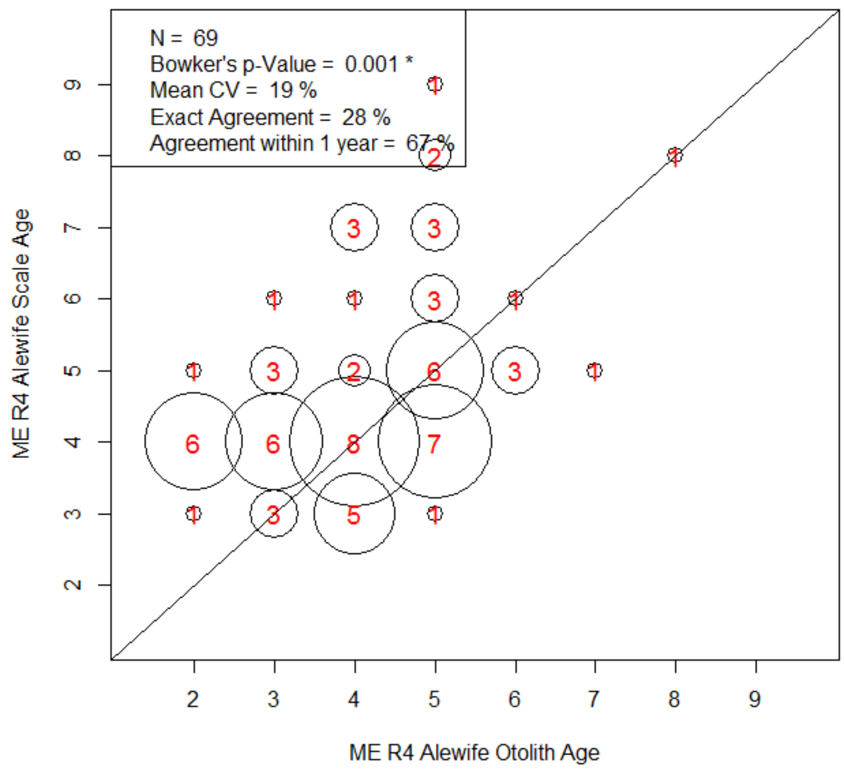


Figure 125. Age frequency plot for ME reader 4 paired alewife scale and otolith age determinations.

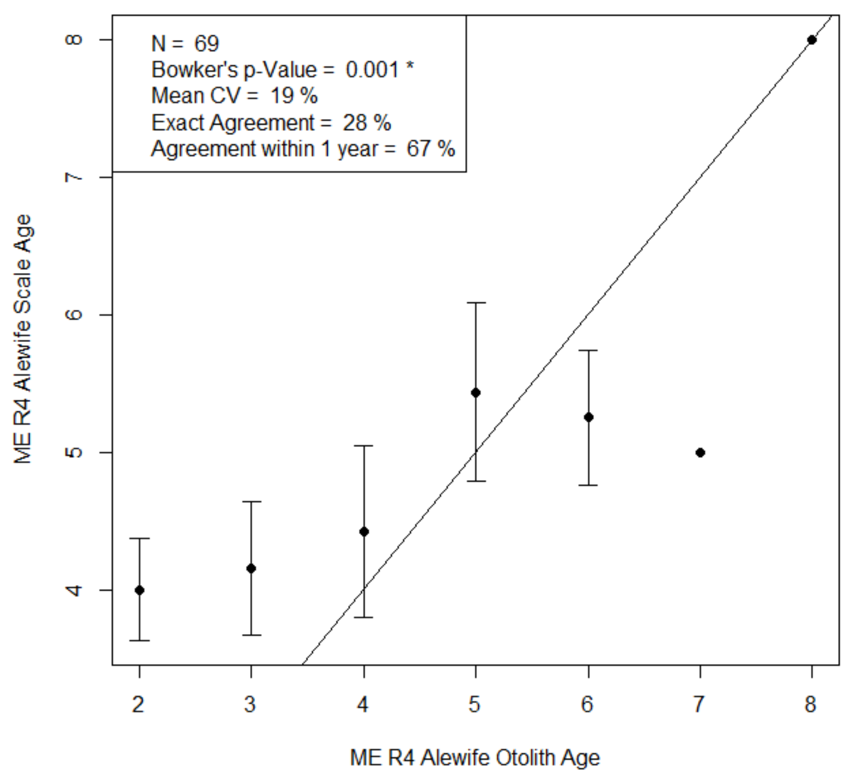


Figure 126. Age bias plot for ME reader 4 paired alewife scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

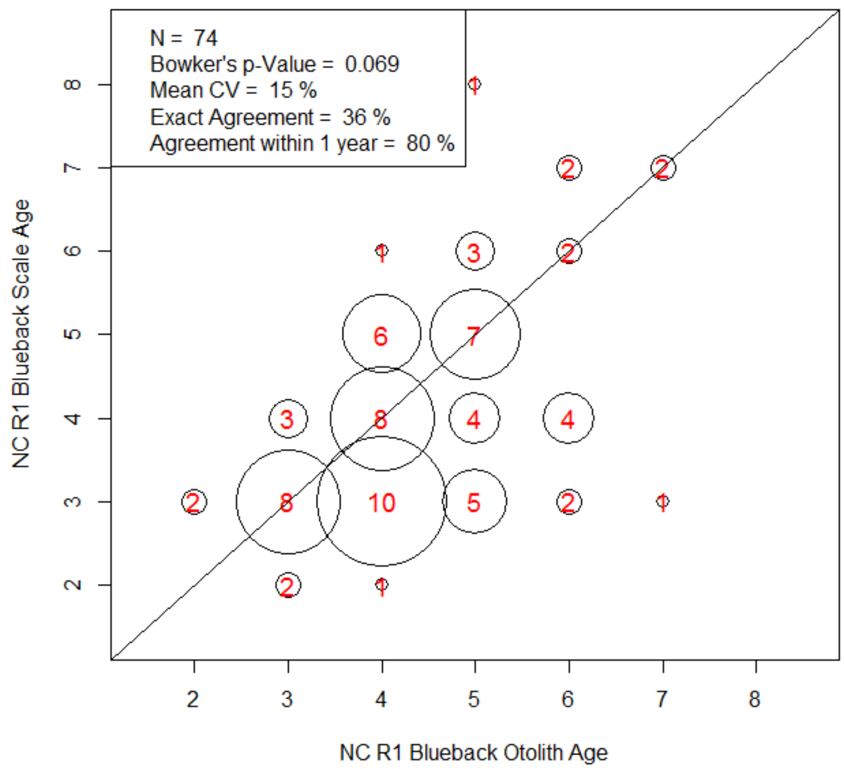


Figure 127. Age frequency plot for NC reader 1 paired blueback herring scale and otolith age determinations.

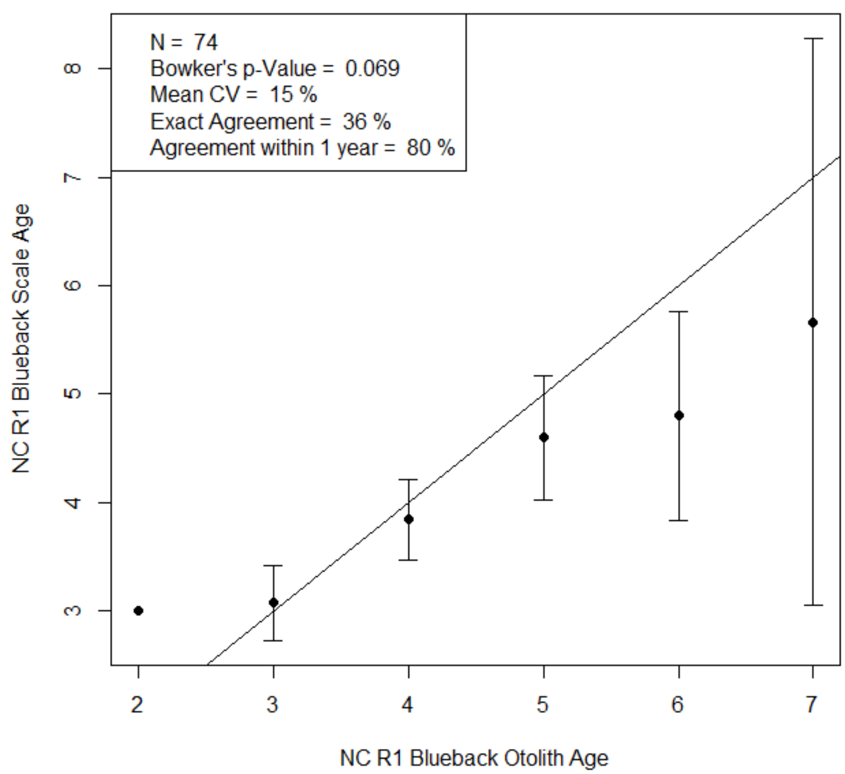


Figure 128. Age bias plot for NC reader 1 paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

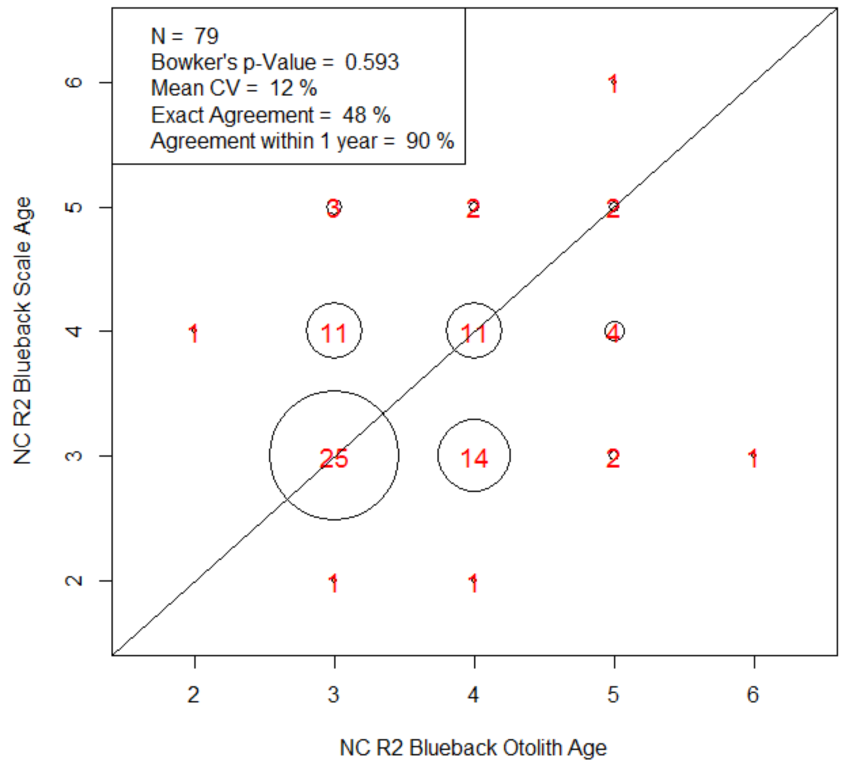


Figure 129. Age frequency plot for NC reader 2 paired blueback herring scale and otolith age determinations.

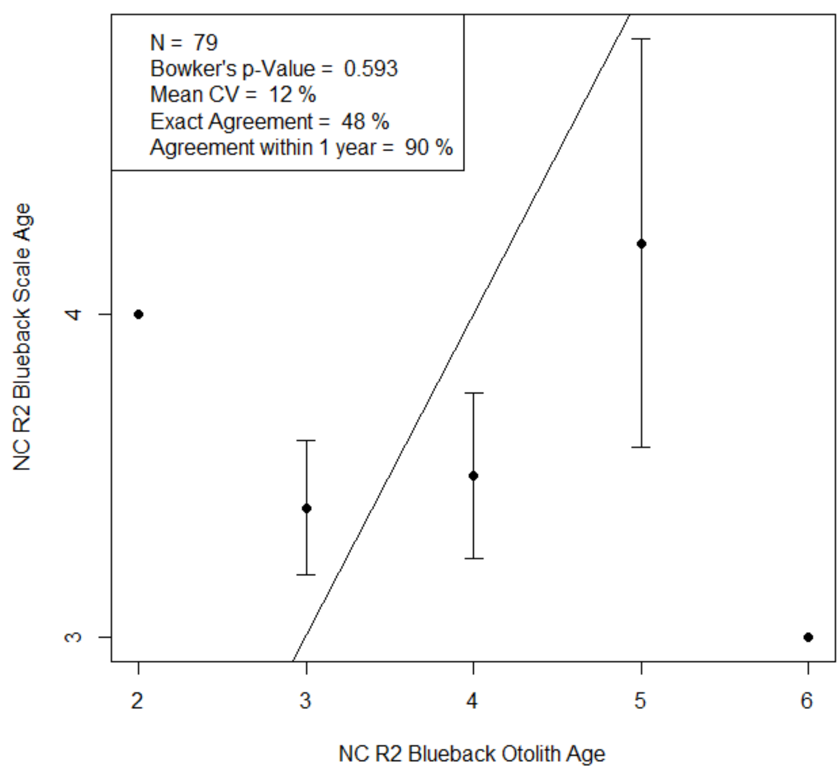


Figure 130. Age bias plot for NC reader 2 paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

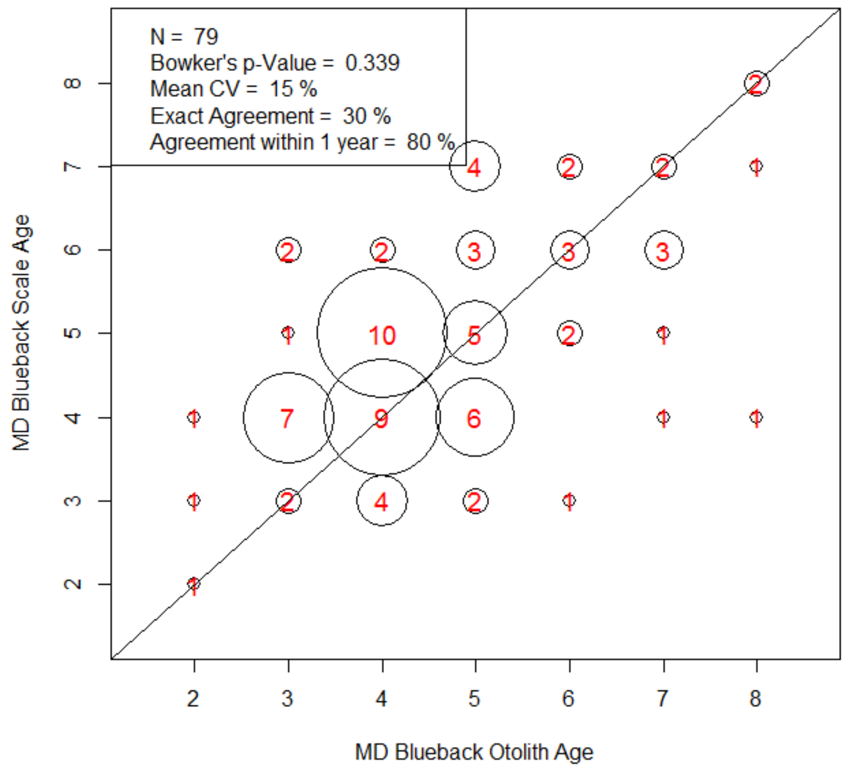


Figure 131. Age frequency plot for MD reader paired blueback herring scale and otolith age determinations.

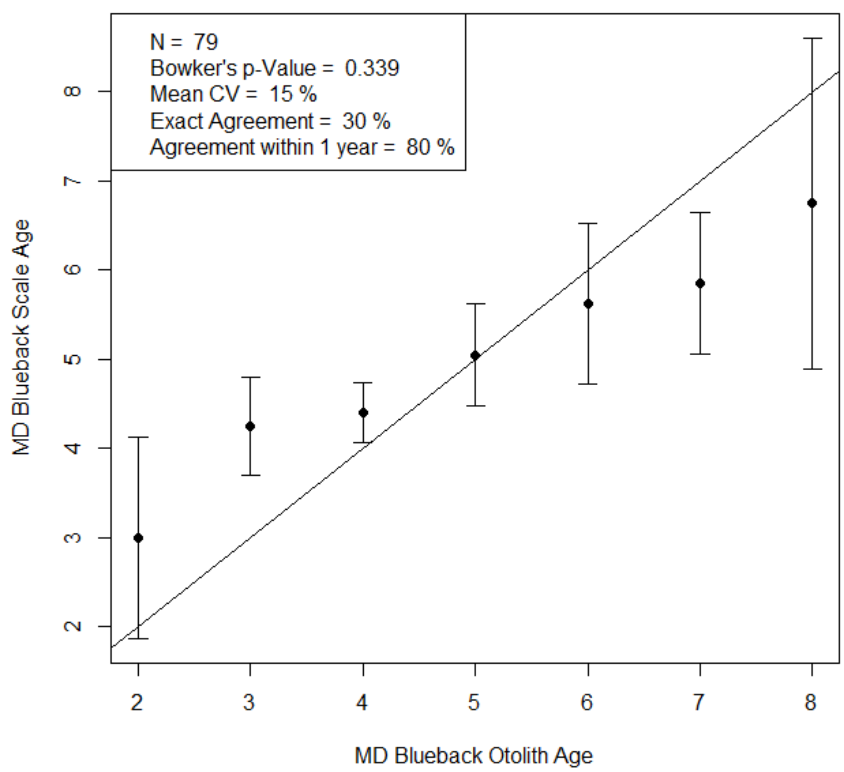


Figure 132. Age bias plot for MD reader paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

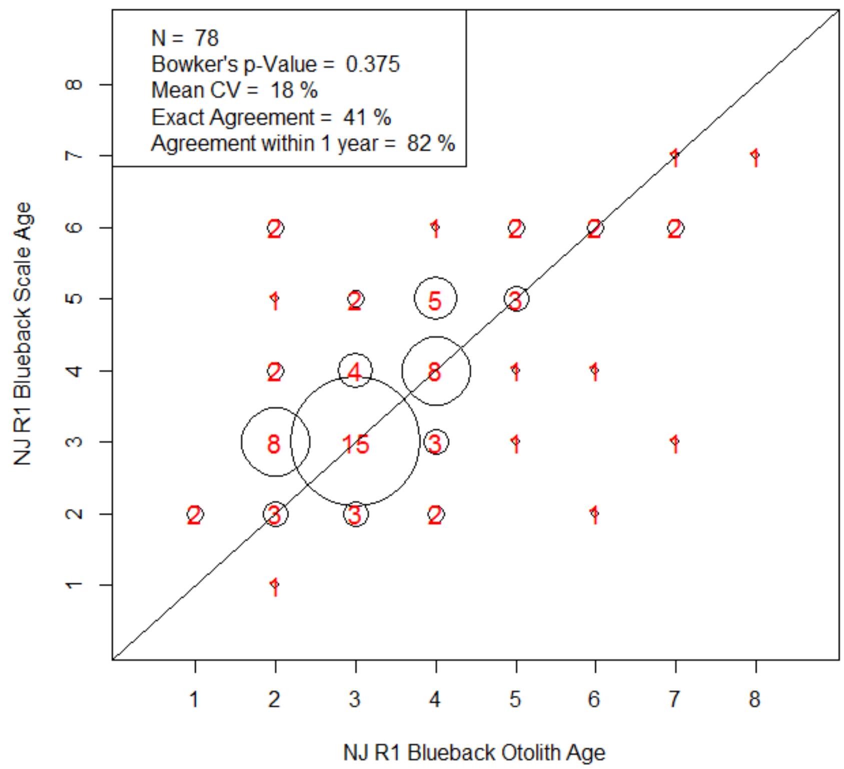


Figure 133. Age frequency plot for NJ reader 1 paired blueback herring scale and otolith age determinations.

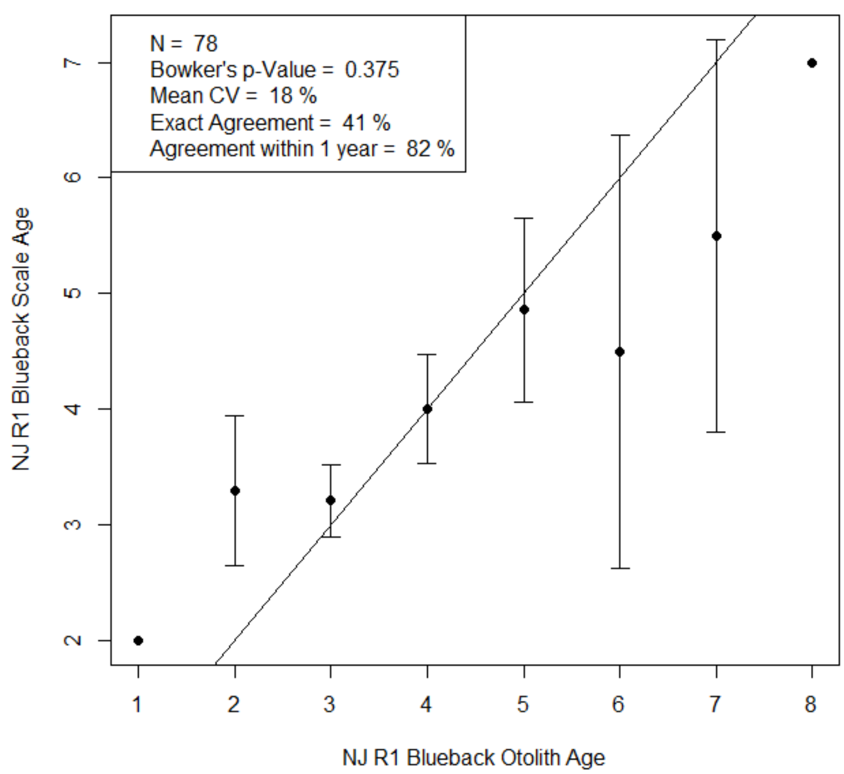


Figure 134. Age bias plot for NJ reader 1 paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

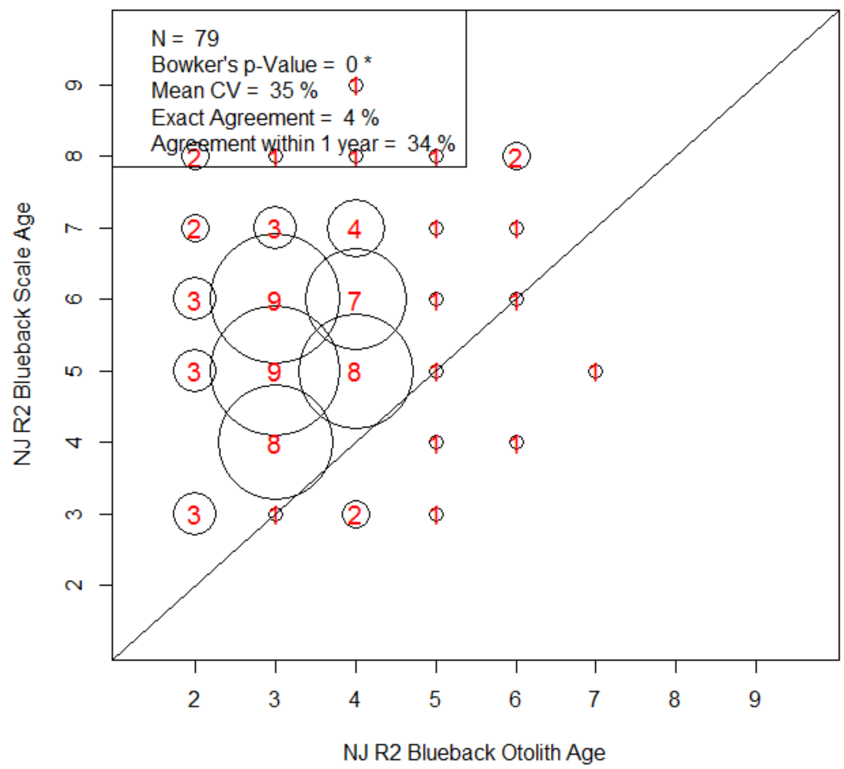


Figure 135. Age frequency plot for NJ reader 2 paired blueback herring scale and otolith age determinations.

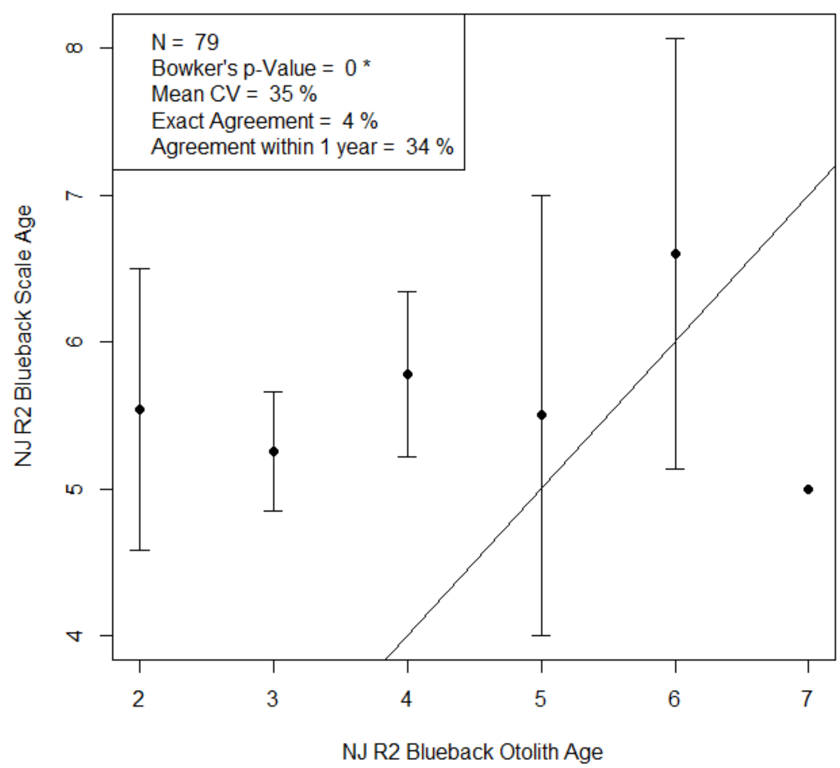


Figure 136. Age bias plot for NJ reader 2 paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

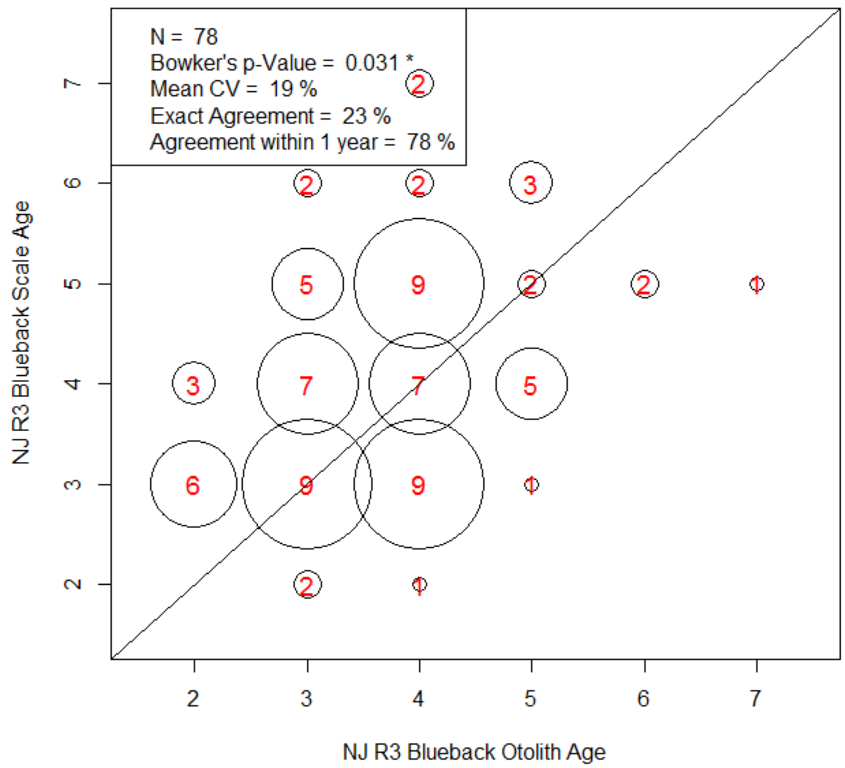


Figure 137. Age frequency plot for NJ reader 3 paired blueback herring scale and otolith age determinations.

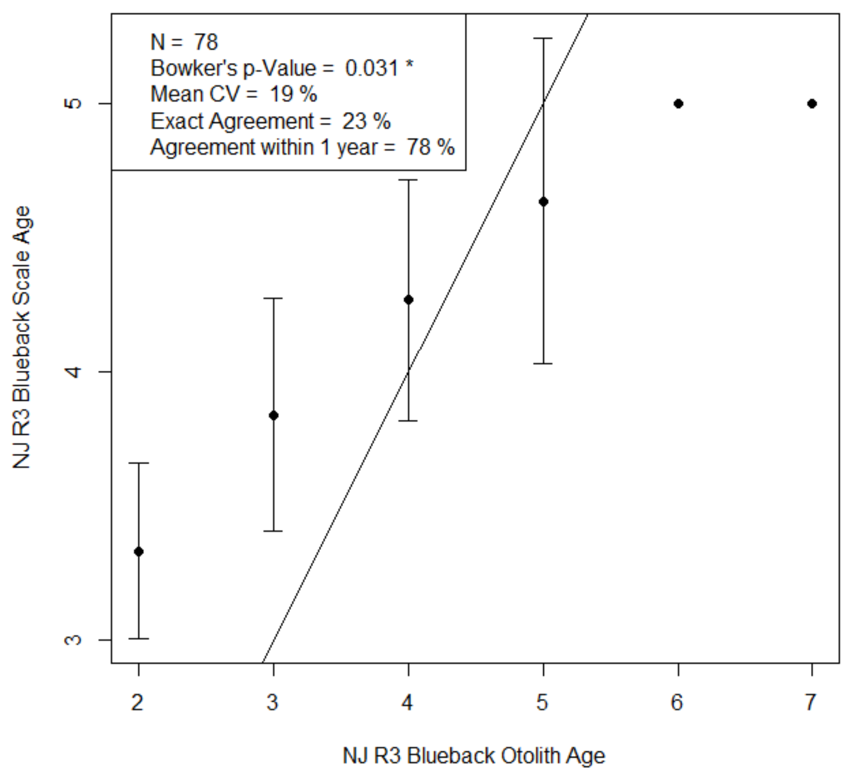


Figure 138. Age bias plot for NJ reader 3 paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

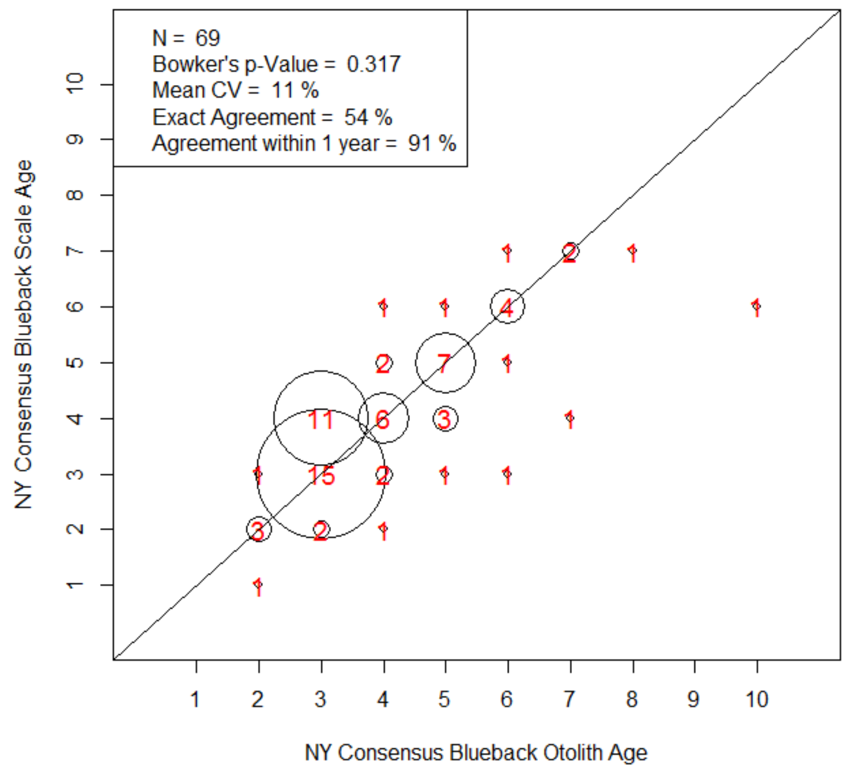


Figure 139. Age frequency plot for NY consensus paired blueback herring scale and otolith age determinations.

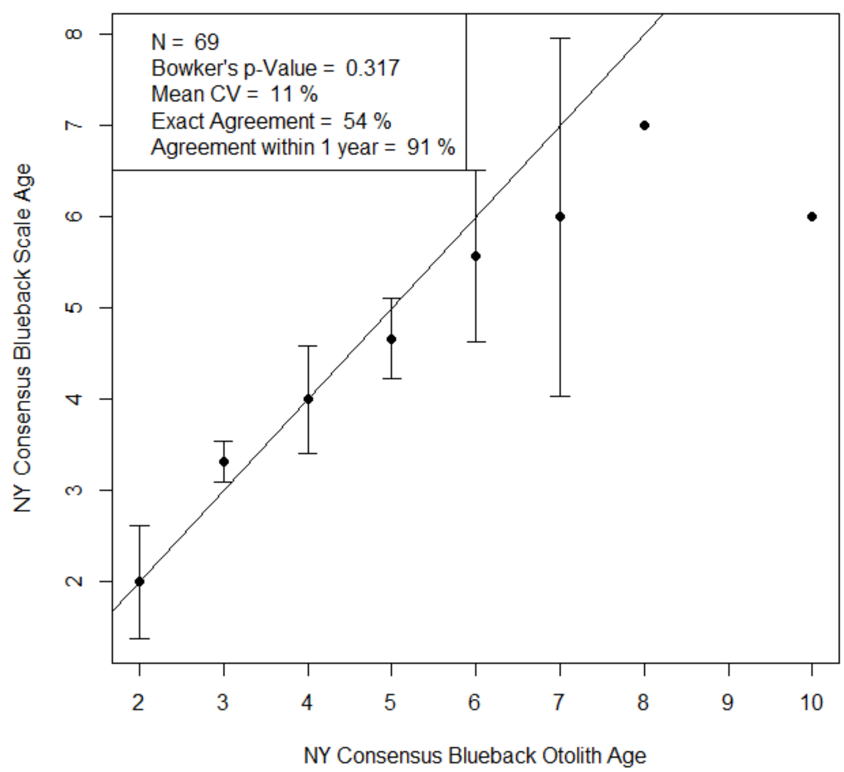


Figure 140. Age bias plot for NY consensus paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

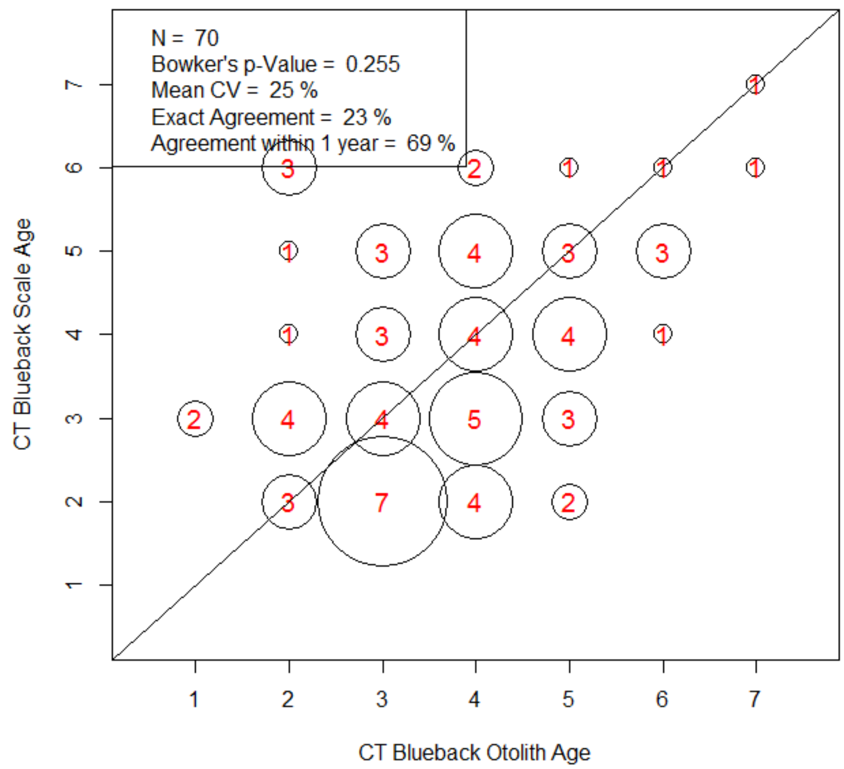


Figure 141. Age frequency plot for CT reader paired blueback herring scale and otolith age determinations.

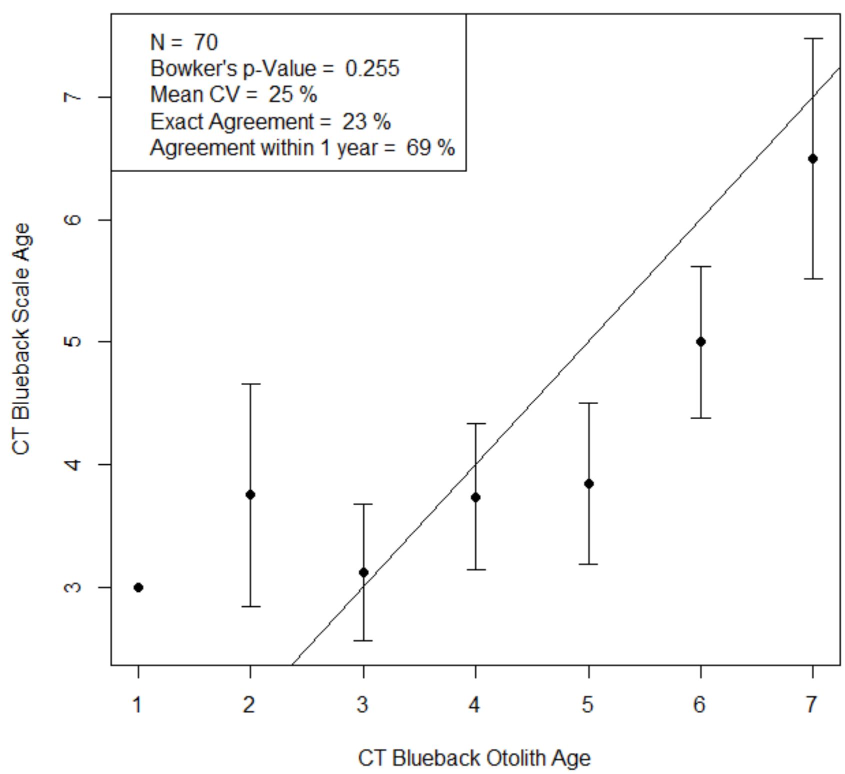


Figure 142. Age bias plot for CT reader paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

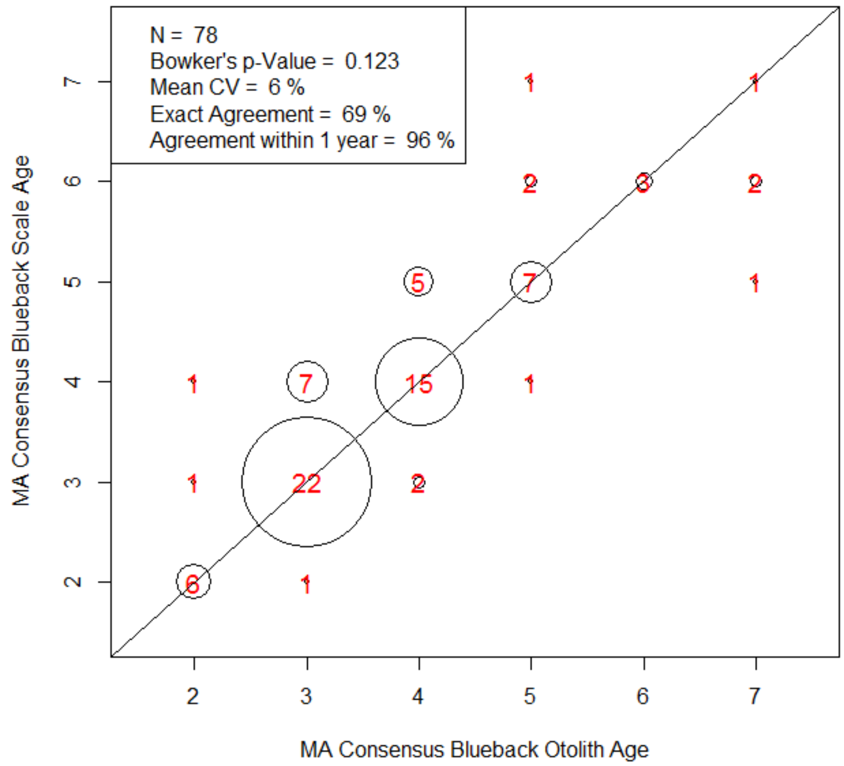


Figure 143. Age frequency plot for MA consensus paired blueback herring scale and otolith age determinations.

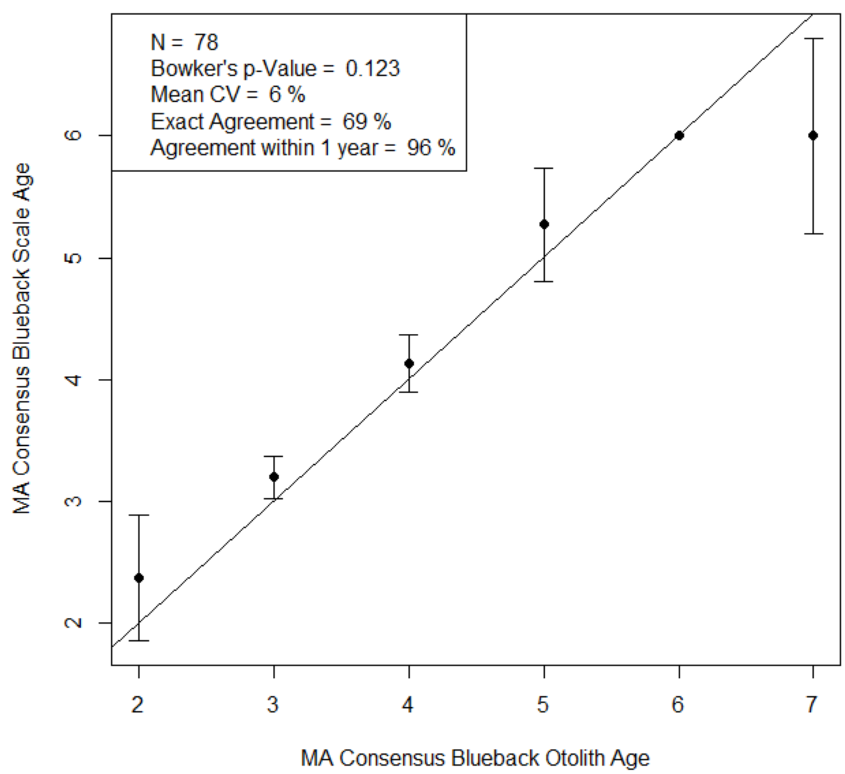


Figure 144. Age bias plot for MA consensus paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

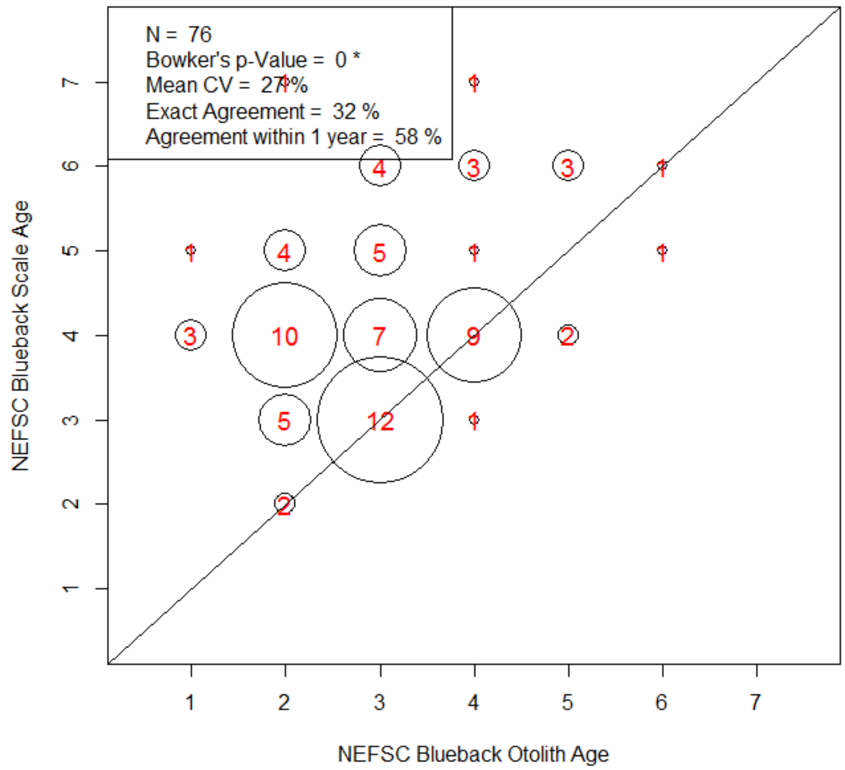


Figure 145. Age frequency plot for NEFSC reader paired blueback herring scale and otolith age determinations.

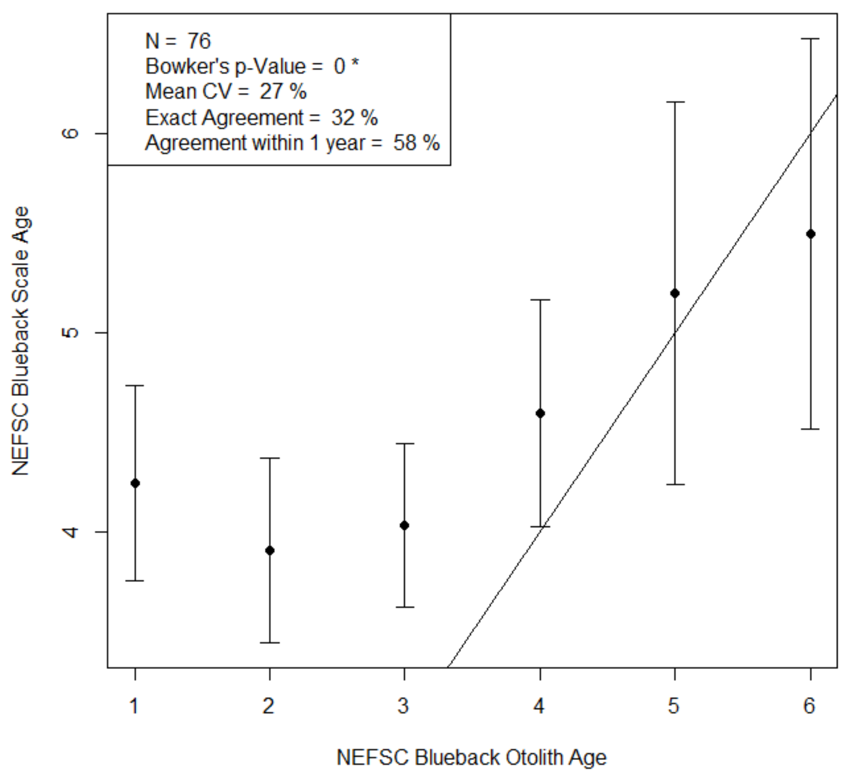


Figure 146. Age bias plot for NEFSC reader paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

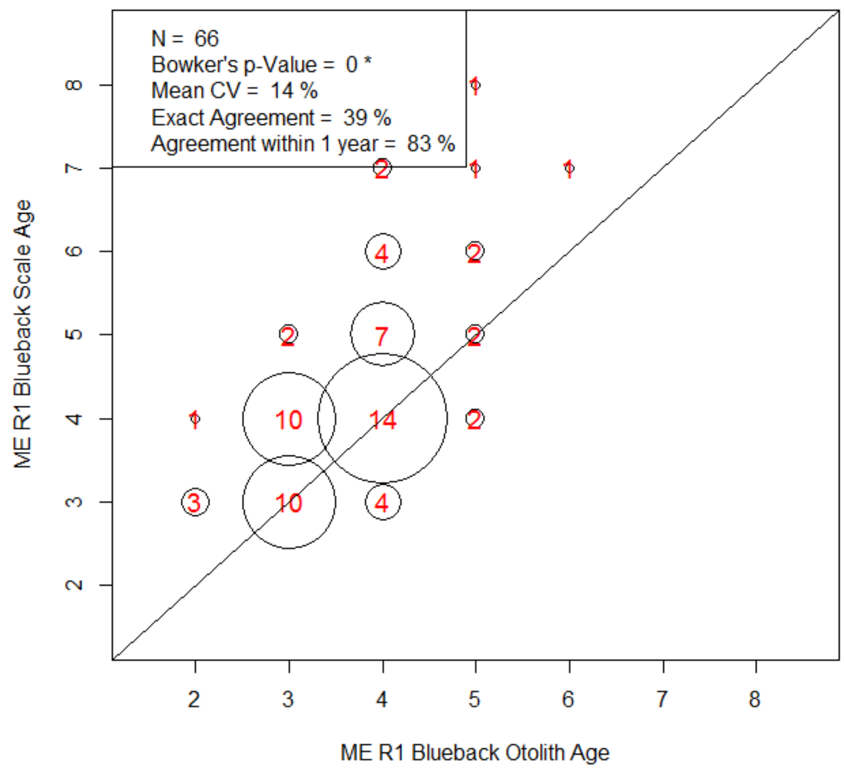


Figure 147. Age frequency plot for ME reader 1 paired blueback herring scale and otolith age determinations.

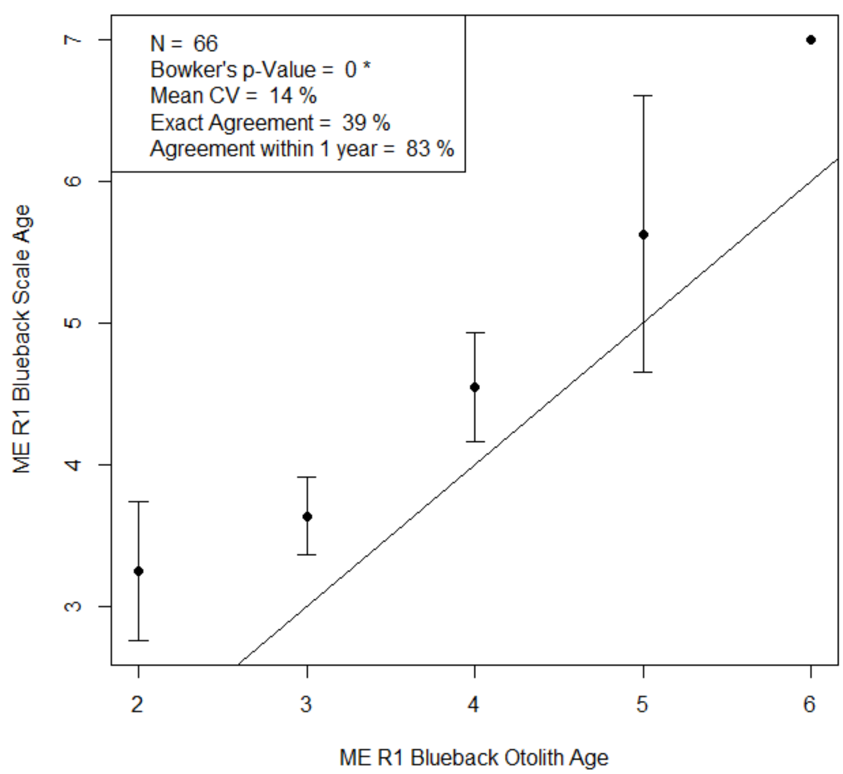


Figure 148. Age bias plot for ME reader 1 paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

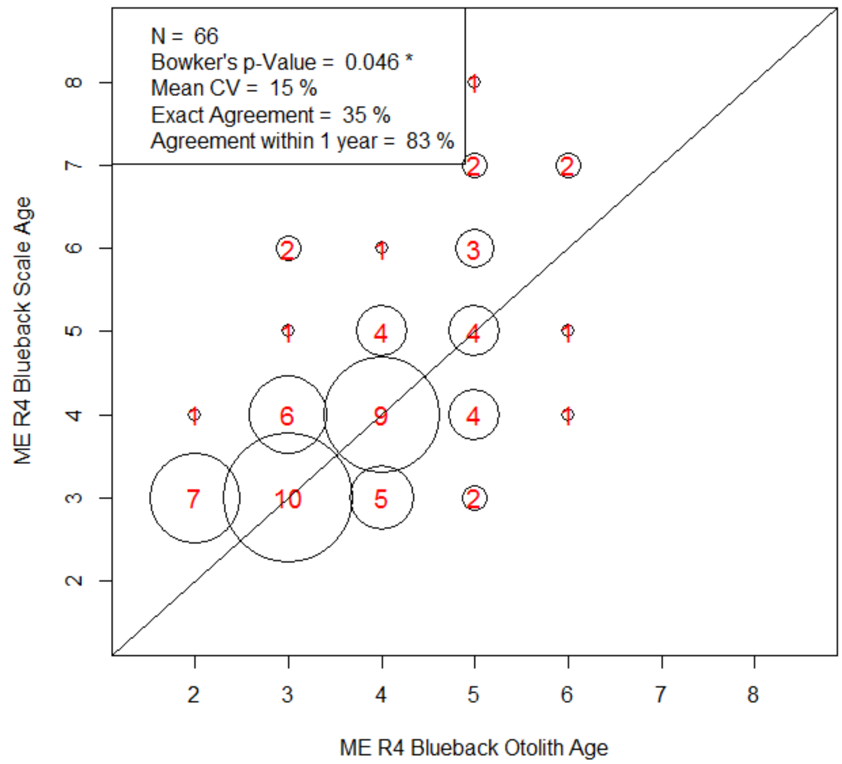


Figure 149. Age frequency plot for ME reader 4 paired blueback herring scale and otolith age determinations.

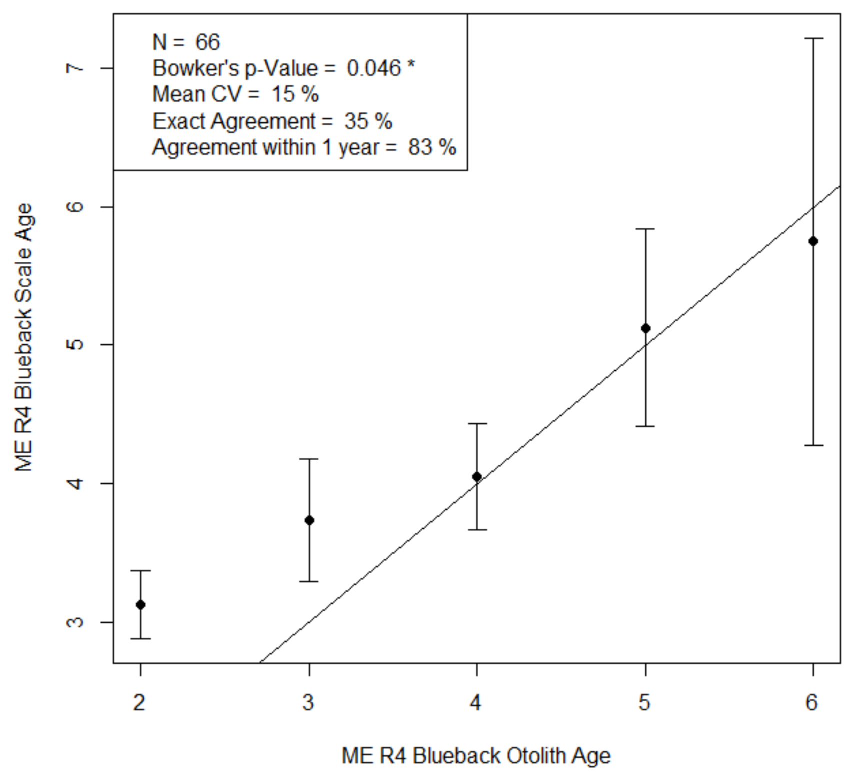


Figure 150. Age bias plot for ME reader 4 paired blueback herring scale and otolith age determinations. Error bars in the age bias plots are 95% confidence intervals.

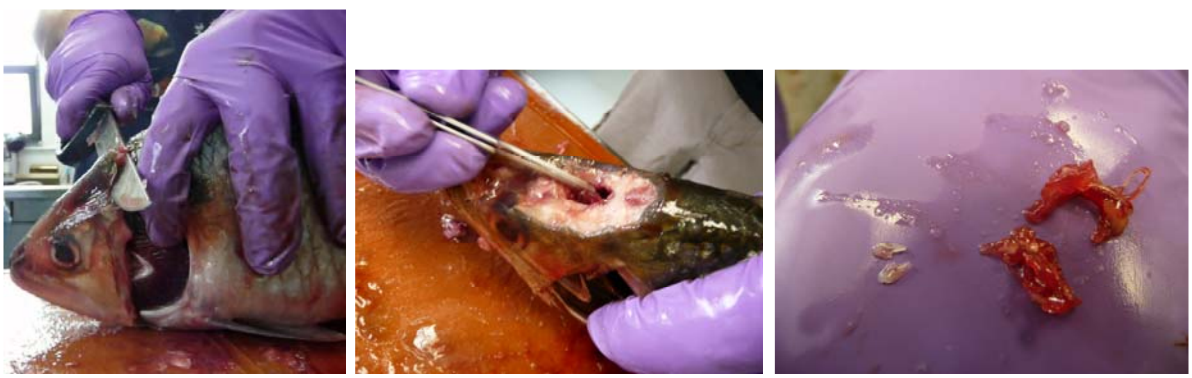
Appendix A: Massachusetts DMF River Herring Ageing Protocol

River Herring Ageing Protocol

Blueback (*Alosa aestivalis*), Alewife (*Alosa pseudoharengus*)
Massachusetts Division of Marine Fisheries Sclerochronology Lab

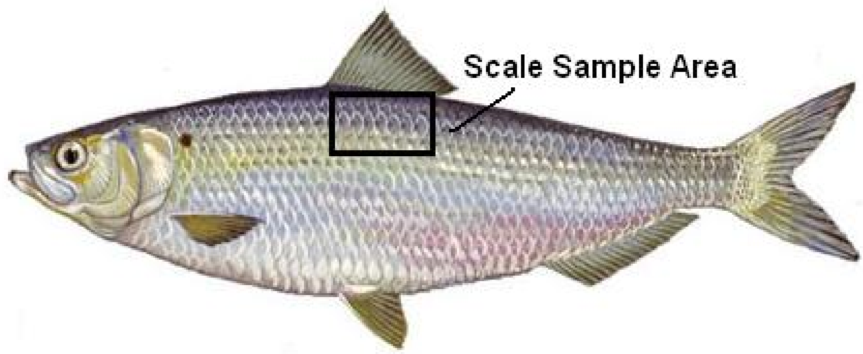
<u>Sample Collection</u>

- Each fish is given a unique sample id (river, year, and fish number).
- Weight, fork length, total length, sex, species, capture date and sample id number are recorded on envelopes and data sheet.
- Species is determined by the color of the peritoneum and the size of the eye.
- Otolith extraction:
    - Using a scalpel or knife, slice off the top part of the head exposing the brain cavity.
    - The slice should be shallow starting at the back of the skull slicing forward. See left image below.
    - Scoop out the brain matter using forceps.
    - Extract the otic membranes, one on each side. See center and right image below.
    - Otoliths will be found in the otic membrane. If they aren't, they may still be in the bony case where the membrane was removed from.
    - Otoliths should be rinsed with water and stored dry in labeled microcentrifuge tubes.



Images of the otolith extraction procedure for alosids using an American shad.

- Scale collection:
    - Collected ventrally of the dorsal fin as shown below.
    - Swipe collection area with scalpel or knife to remove slime, other fish's scales and any debris.
    - Collect 20-30 scales with clean knife or scalpel and place into corresponding envelope.



Scale Preparation and Ageing

- **Preparation**
  - Examine and select 6 readable scales per fish, avoid regenerated scales.
  - Prepare a 5% pancreatin solution and clean scales in a sonic cleaner (Whaley, 1991).
  - Drain pancreatin from scales (can be used multiple times). Put scales in dish of clean water. Wipe and dry them as needed.
  - Place dry scales between two glass slides labeled with the appropriate ID number.

- **Age Interpretation**
  - View with transmitted light on image processing software and a camera on a macro mirror stand.
  - Adjust the mirror and lighting so the annuli can be viewed crossing over the baseline.
  - Annuli appear as continuous strong bands that cross the transverse grooves and continue past the baseline.
  - The first dark band is usually the freshwater zone. (Fig. 1&2)
  - The first annulus is frequently weak and doesn't always follow the annulus criteria.
  - The edge is counted as the last annuli if captured in spring. (Fig. 1&2)
  - False annuli will not cross over the baseline and cannot be followed throughout the scale. (Fig. 3)
  - Typically the second annulus is the "strongest" looking. (Fig. 4&5)
  - On older fish annuli can become crowded together at the edge of the scale but will separate beneath the baseline. These should be counted as separate annuli. (Fig. 6)
  - Scales can be resorbed back over previous annuli during spawning, these annuli will still separate beneath the baseline. These should be counted as separate annuli. (Cating 1954). (Fig. 6)
  - Spawning marks are identified as annuli that appear fuzzy and jagged above the baseline or appear that they've been resorbed over another annulus above the baseline. (Fig. 6)

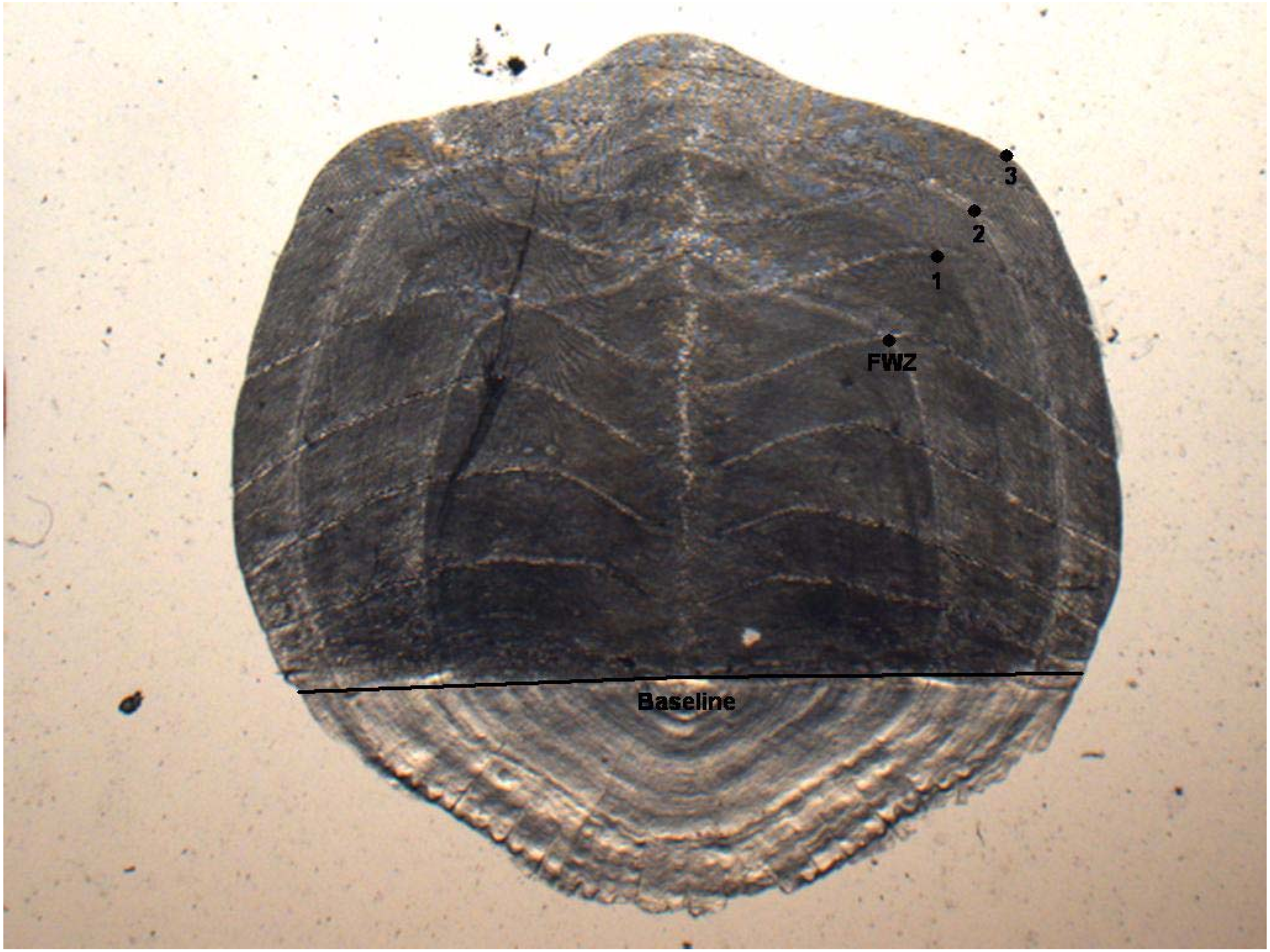


Figure 1. This 3 year old alewife has its baseline, fresh water zone (FWZ) and annuli all marked. Note the straight baseline and large FWZ typical of alewives.

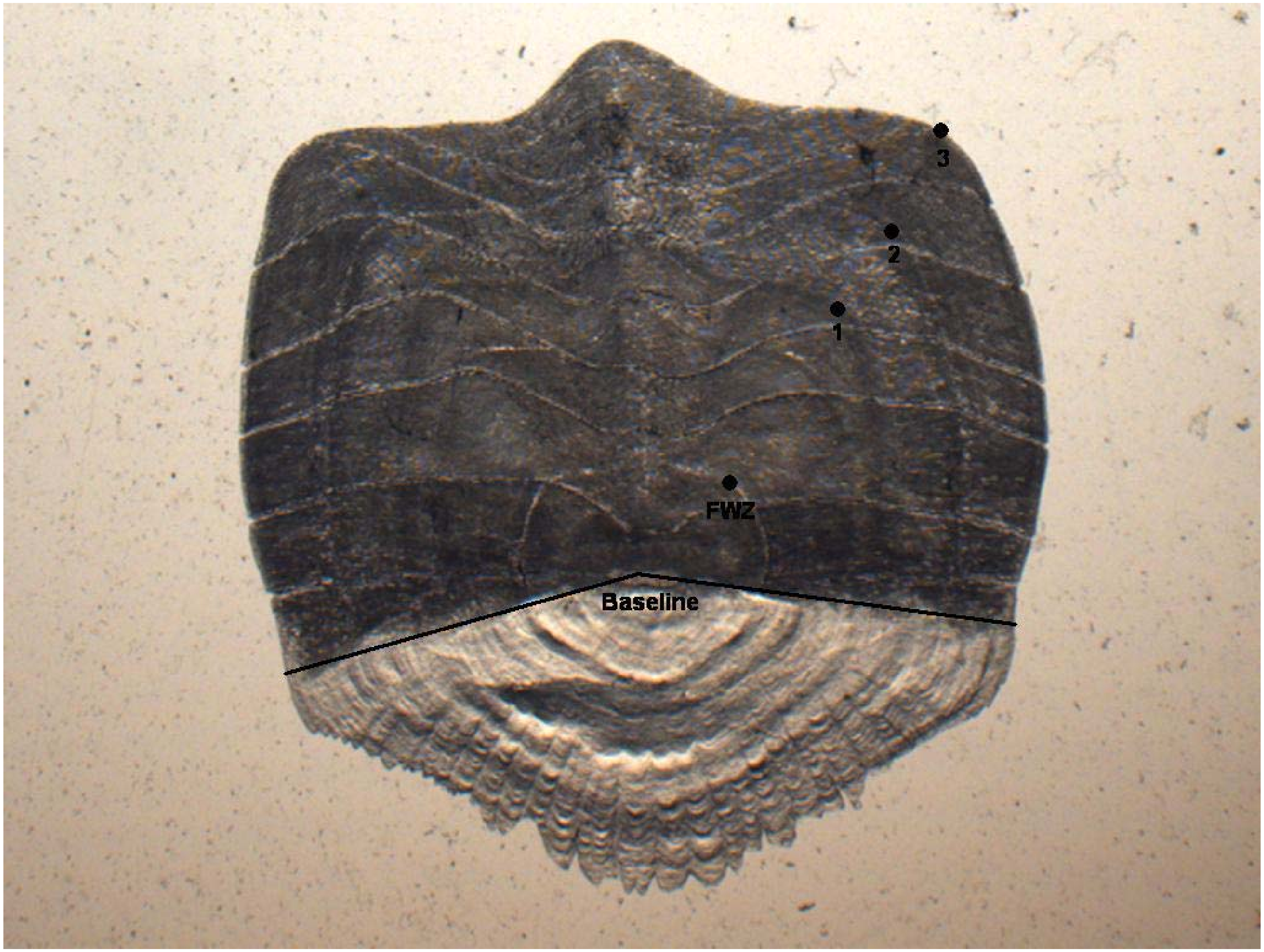


Figure 2. The baseline, fresh water zone (FWZ) and annuli are all marked on this blueback scale. Note the small FWZ and angled baseline typical of bluebacks.

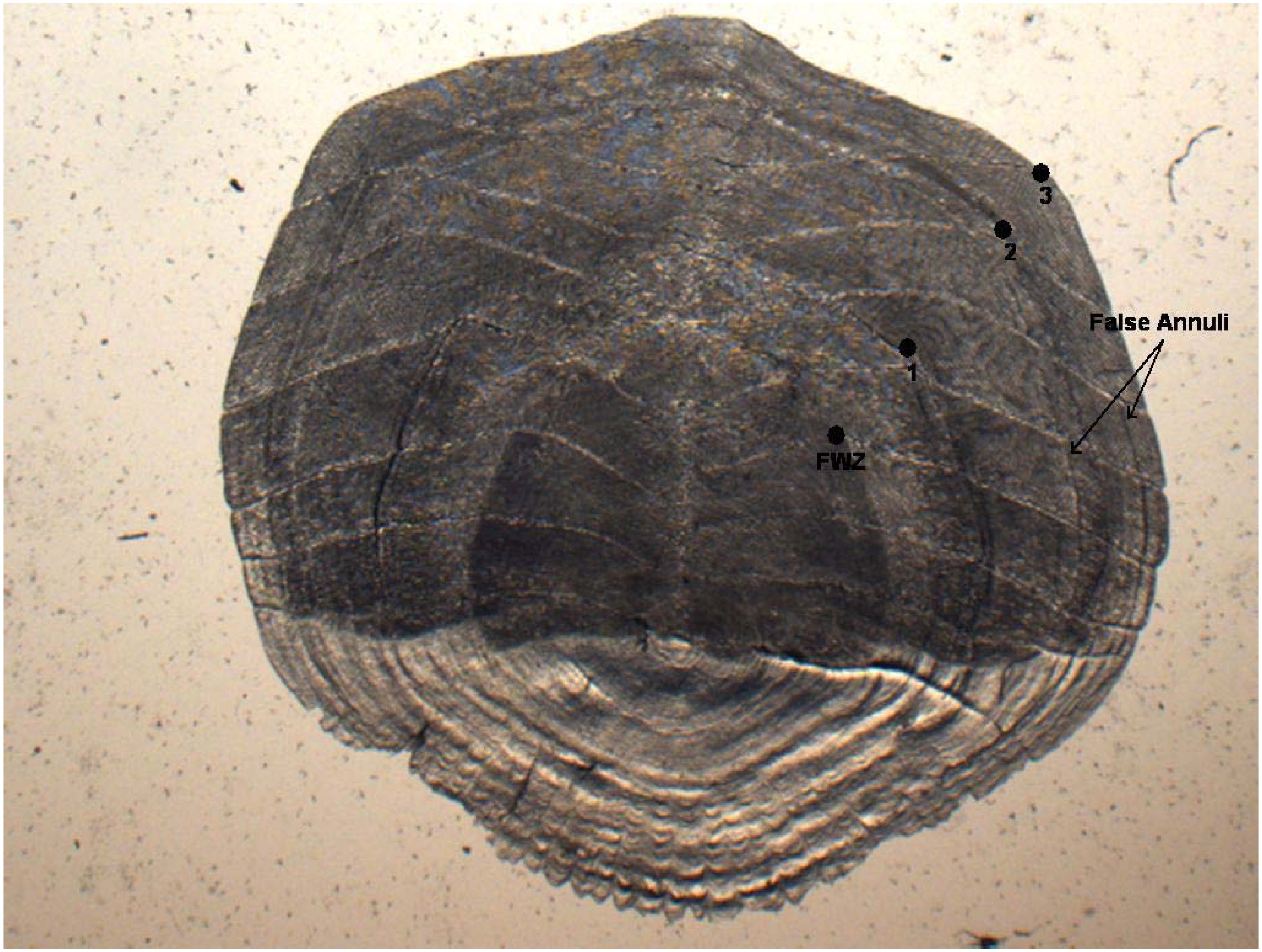


Figure 3. This three year old alewife has two false annuli, one on either side of annulus 2.

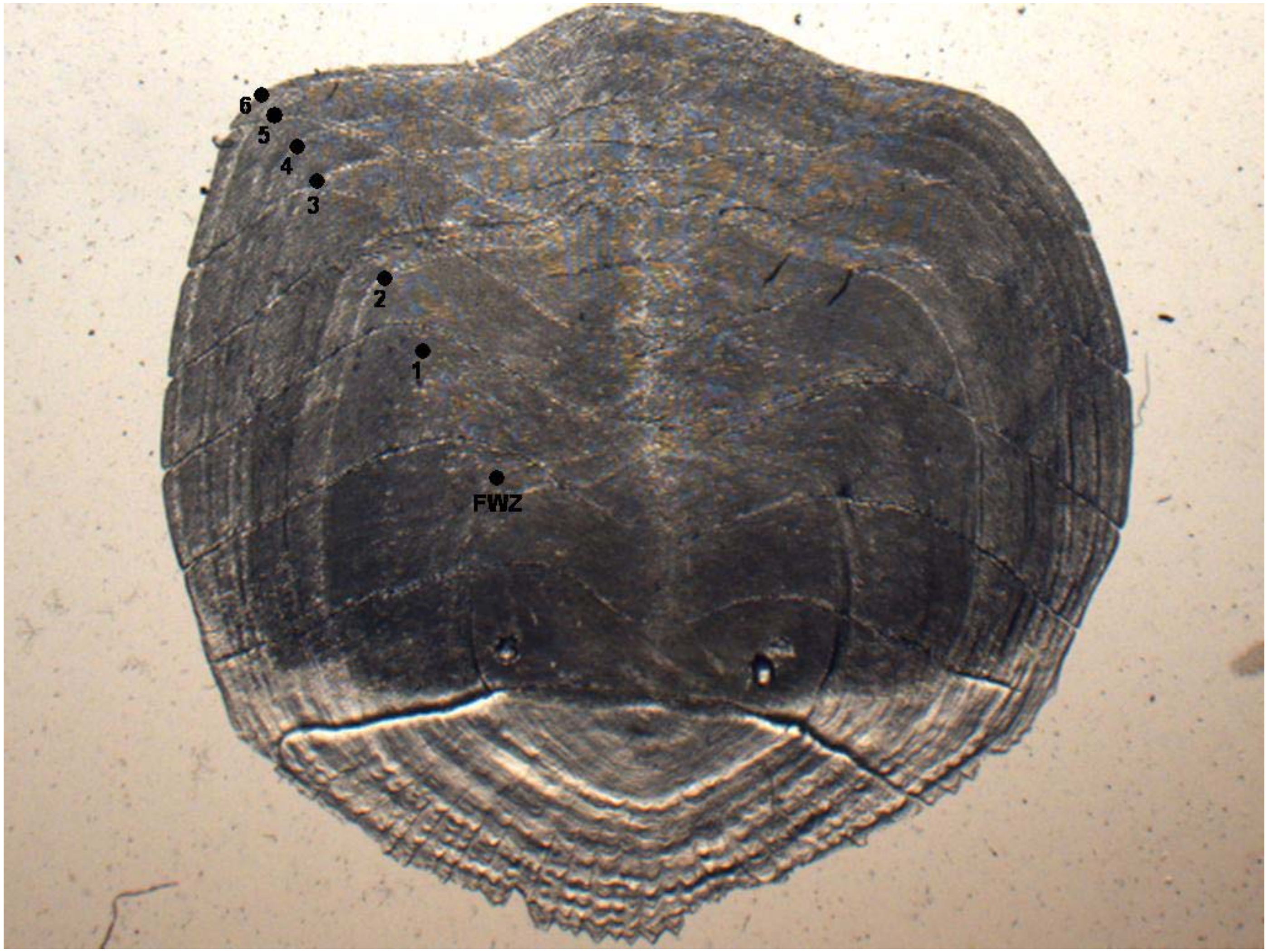


Figure 4. A six year old alewife. Note how weak the first annulus appears compared to the second.

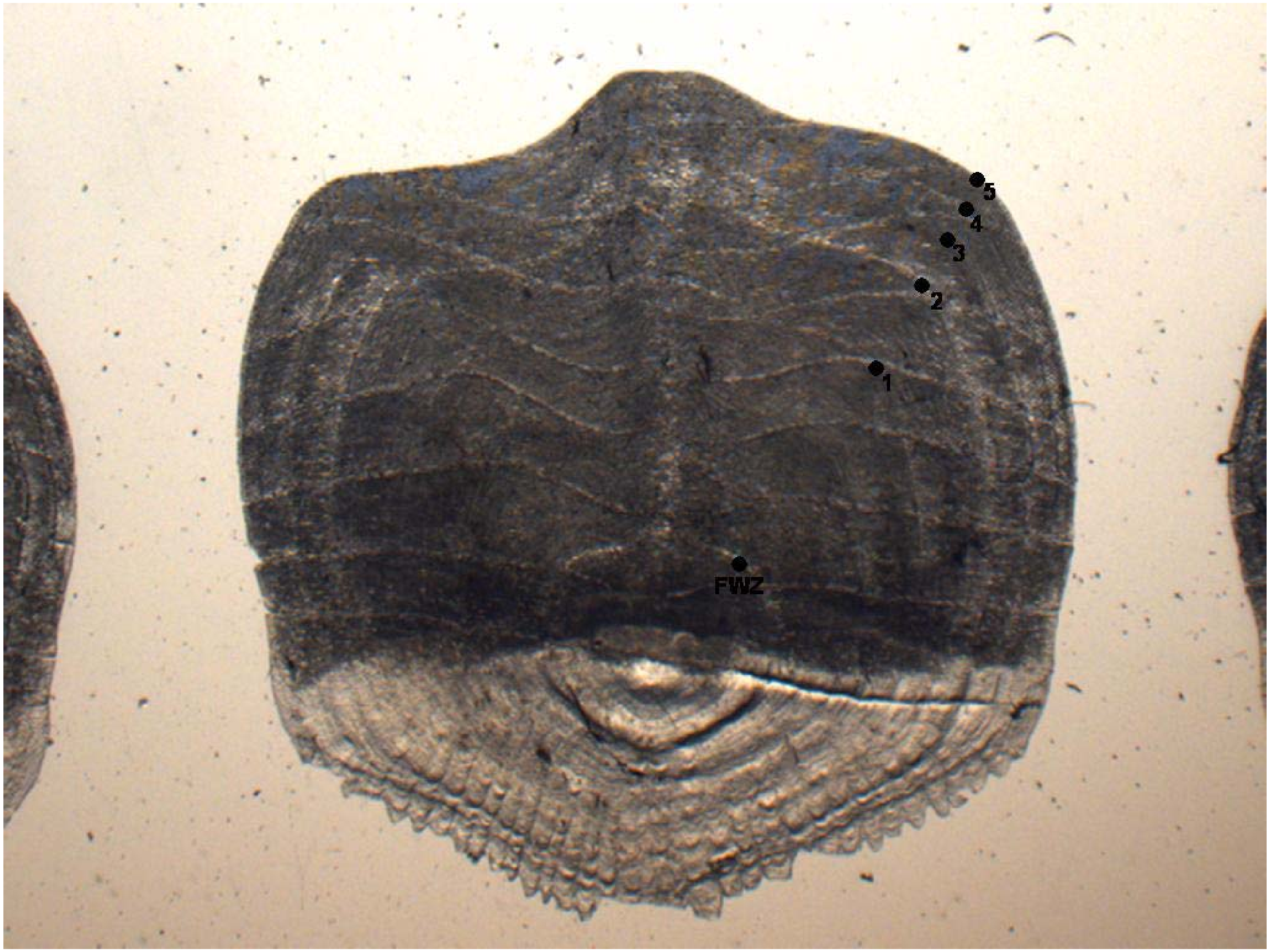


Figure 5. This five year old blueback has the typical strong second annulus.

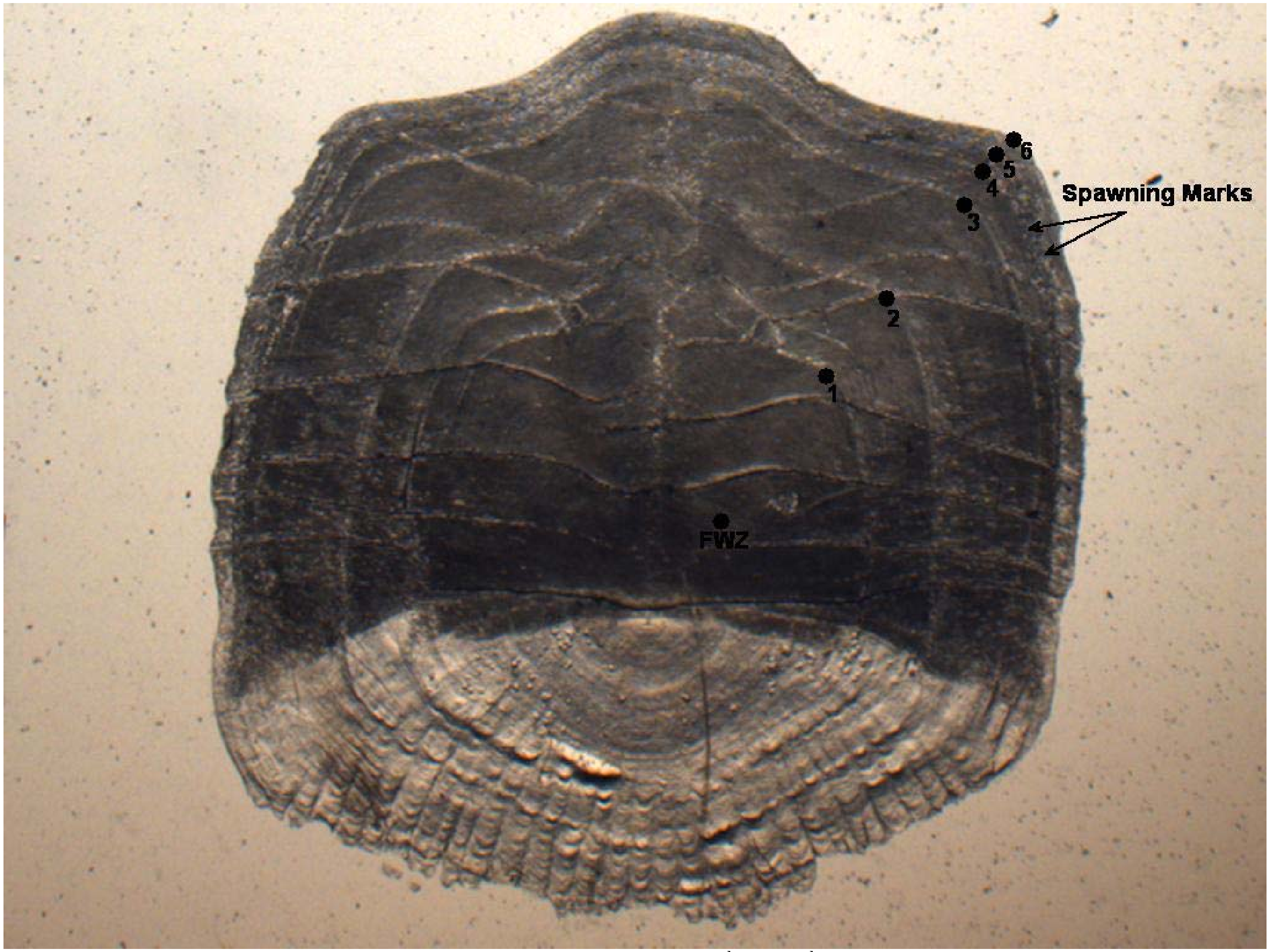


Figure 6. This six year old blueback has spawning marks at its 4th and 5th annuli.

Otolith Preparation and Ageing

- **Preparation**
  - Water is used to clean off any dried blood.
  - Dry gently with a paper towel then place into a labeled microcentrifuge tube for storage.

- **Age Interpretation**
  - Immerse otoliths in mineral oil, sulcus down, on a black background.
  - View under a stereomicroscope with reflected light.
  - Annuli are defined as continuous hyaline (dark) bands with no breaks. (Fig.7,8,9)
  - Annuli counted from the middle outward along the pararostrum or antirostrum.
  - False annuli typically are not continuous, appear outside of expected growth rates, lack a defined edge or connect with true annuli. (Fig 9 & 10)

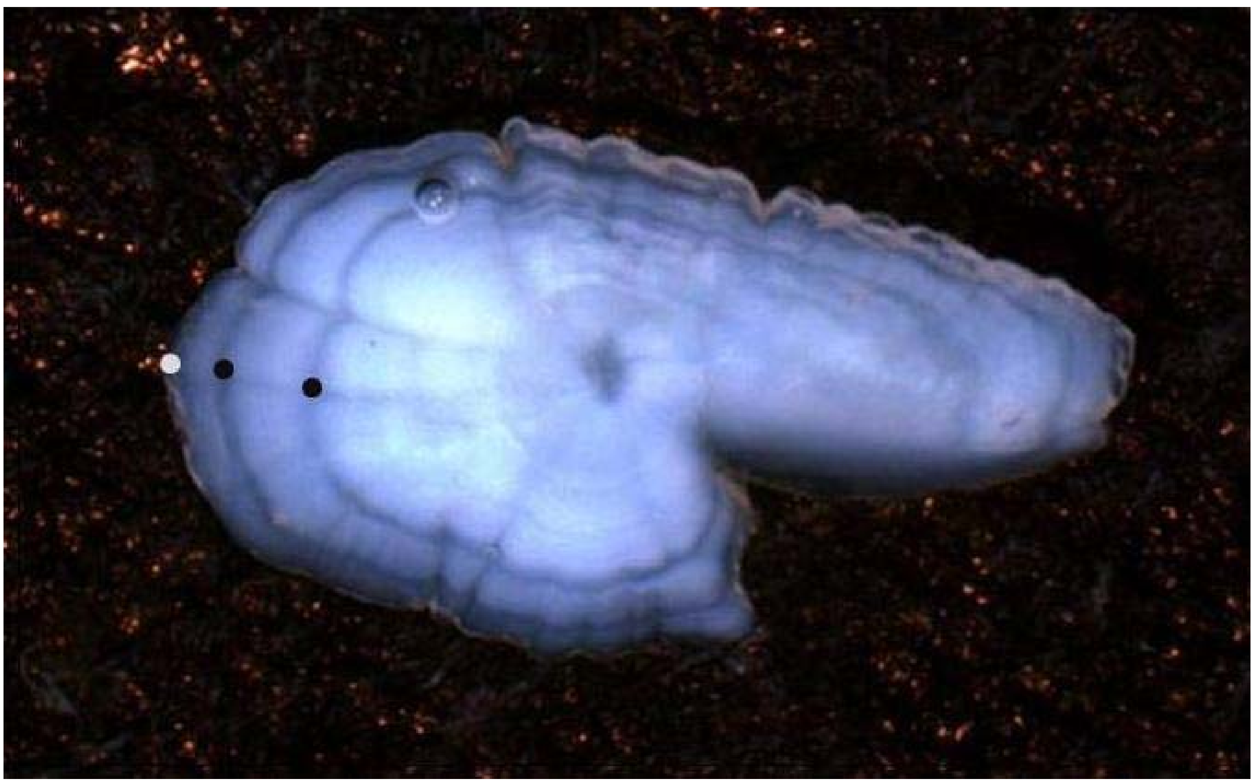


Figure 7. An otolith from a 3 year old blueback. Black dots mark the first and second annuli and the gray dot marks the edge. The edge is counted in this case because the fish was caught in the spring.

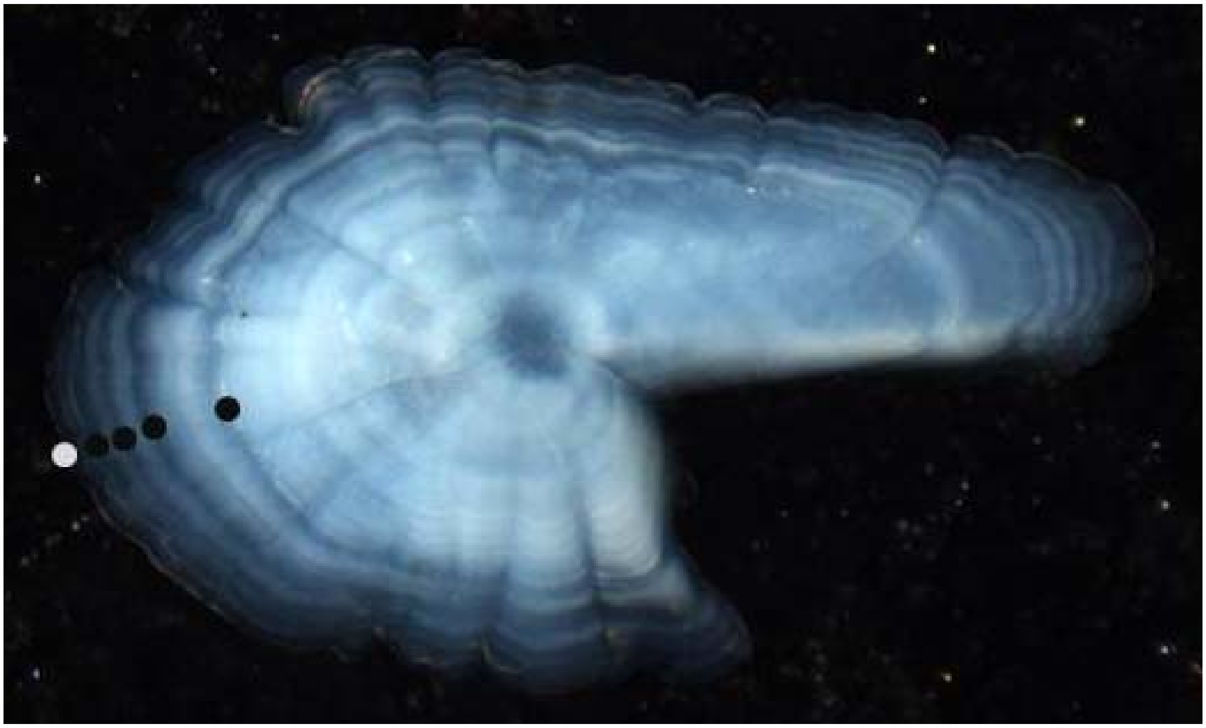


Figure 8. An otolith from a 5 year old alewife. Annuli one through four are marked with black dots and the edge is marked with a gray dot. This fish was caught in the spring so we count the edge.

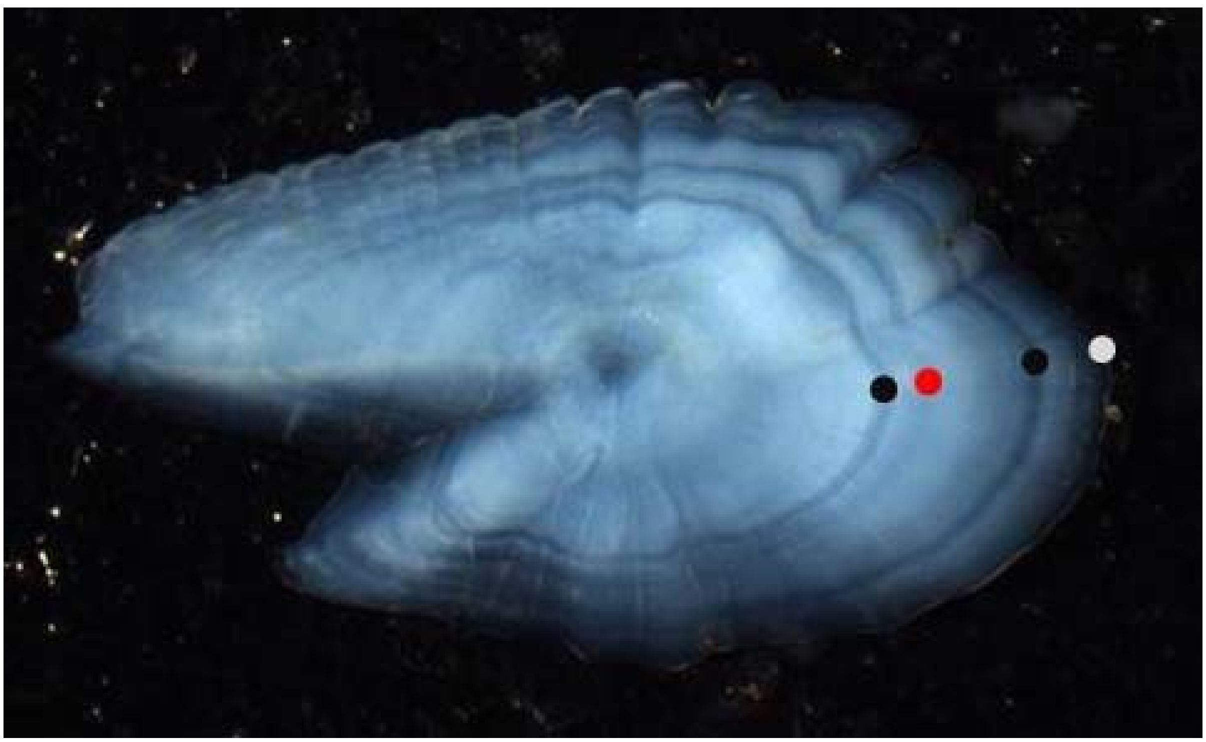


Figure 9. An otolith from a 3 year old blueback. The red dot marks a false annulus. Note how it is not continuously dark and the growth between the first annulus and the false isn't as much as expected for it to be the second annulus. The black dots mark annuli one and two and the gray dot marks the edge. We count the edge because this fish was caught in the spring.

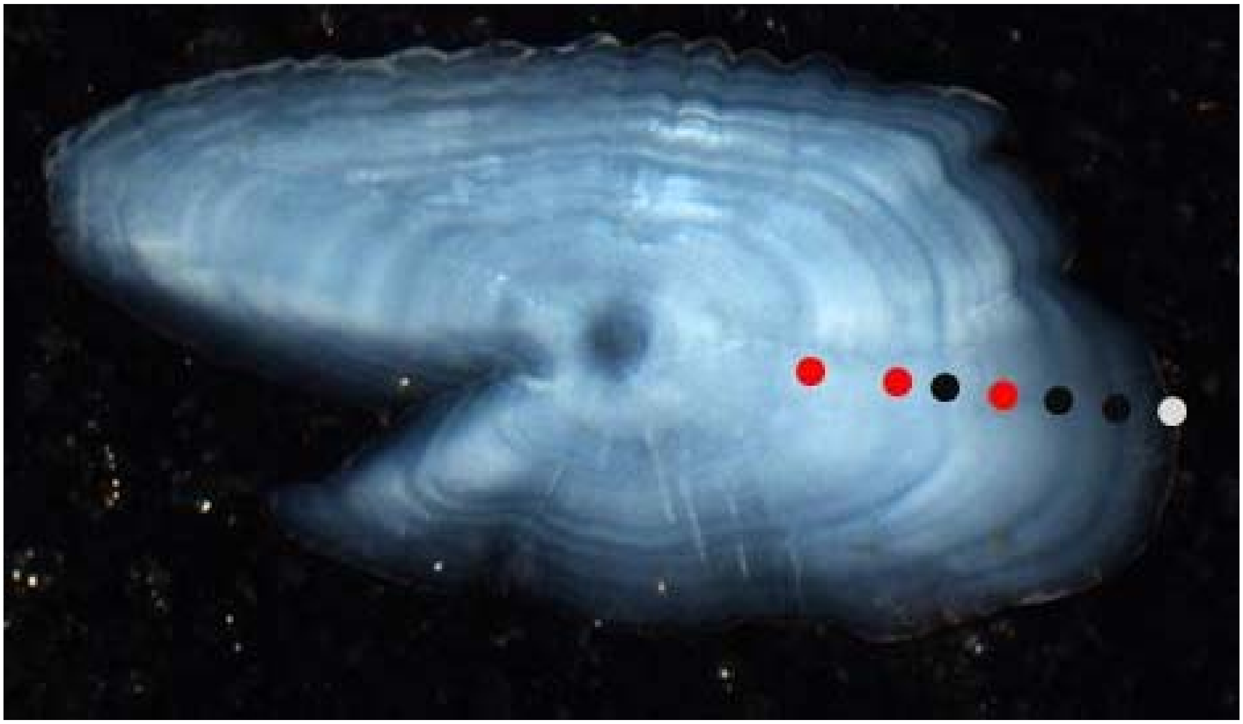


Figure 10. An otolith of a 4 year old blueback. The red dots mark false annuli, the black dots mark the first, second and third annulus and the gray dot marks the edge. We count the edge as an annulus because this fish was caught in the spring.

Literature Cited

Cating, J. P. 1953. Determining the age of Atlantic shad from their scales. U.S. Fishery Bulletin 54 85:187-199.

Whaley, R. A. 1991. An Improved Technique for Cleaning Fish Scales. North American Journal of Fisheries Management 11:234-236.

Appendix B: Paired Sample Instructions for River Herring Ageing Exchange

For paired samples collected this year:
Please collect one paired sample for each 10mm length bin that you encounter for blueback herring and alewives. Please use total length and floored length bins (0-9, 10-19, 20-29, etc.). If you have run into problem samples in past sampling and can take additional samples for quality assurance, please do so as you see necessary.

Please prepare your samples in the same method that you indicated on your questionnaire. If your lab does not prepare otoliths, please wash the otoliths with water to clean off any dried blood or other dirt. Let the otoliths air dry overnight and then store one otolith in a plastic centrifuge vial for shipping. If you do not have centrifuge vials, please let me know and I can send you some. For scales, please mount the scales on glass slides and ensure they will be secure for shipment. Please examine the prepared otolith (if your lab ages otoliths) and scales for each sample to make sure they are representative of your past samples (a sample you would typically have aged and not discarded). If the structures are not representative of past samples, select a different sample, if possible, until you have a representative sample. The goal is to have a random, representative sample of structures of varying qualities that would typically be aged for stock assessments.

For archived paired samples:
If you have multiple samples for any length bins, please randomly select a paired sample for that bin. If your lab does not prepare otoliths, please wash the otoliths with water to clean off any dried blood or other dirt. Let the otoliths air dry overnight and then store one otolith in a plastic centrifuge vial for shipping. If you do not have centrifuge vials, please let me know and I can send you some. For scales, please mount the scales on glass slides and ensure they will be secure for shipment. Please examine the prepared otolith (if your lab ages otoliths) and scales for each sample to make sure they are representative of your past samples (a sample you would typically have aged and not discarded). If the structures are not representative of past samples, select a different sample, if possible, until you have a representative sample. The goal is to have a random, representative sample of structures of varying qualities that would typically be aged for stock assessments.

For all paired samples:
Record the date (for assigning ages), sample number, species, state, and total length of the fish on a piece of paper with the glass slide or in the vial with otoliths. Please be sure to use the same sample number for the paired scales and otolith from the same fish. Please pack your samples in a box with packing material (crumpled newspaper, etc.) to keep secure in shipping and email me the weight of the package (estimate is fine). I will forward you a shipping label. Thanks so much for helping this exchange and workshop come together! Please let me know if there is anything I can do to help or if you have any questions.

Appendix C: River Herring Exchange Guidelines

The package contains two envelope boxes and three vial storage boxes. Alewife scale samples are in the Staples envelope box and blueback scale samples are in the blank envelope box. There are a few blueback scale samples (BB 125 – BB 132) that are not in envelopes, but are on laminated sheets loose in the envelope box. The otoliths that are part of the paired samples are in vial storage boxes #1 (alewife) and #2 (blueback). Vial storage box #3 contains the additional New Jersey otoliths for both species. There is only one otolith per sample. All samples should be in order by sample number.

Each sample should be aged with your labs typically procedure for ageing river herring. Please have each person (if multiple agers) record the number of annuli and the final age, based on the date collected and edge type, on the data sheet (attached to my email). If your lab usually comes to a consensus age, please record the consensus age information as well (located in a separate spreadsheet tab). If your lab has not aged otoliths, please refer to the PowerPoint developed by Scott Elzey and the Massachusetts DMF as a reference. Again, we will make note of the lack of experience ageing otoliths, but this will at least give us a place to start. Please record any notes pertaining to the readability/quality of the sample (e.g., cleanliness, false annuli, regenerated, unable to define freshwater zone, unclear outer edge, equipment limitations, etc.) and assign each sample a readability ranking of 1-4. 1 is for poor readability and 4 is for the best readability.

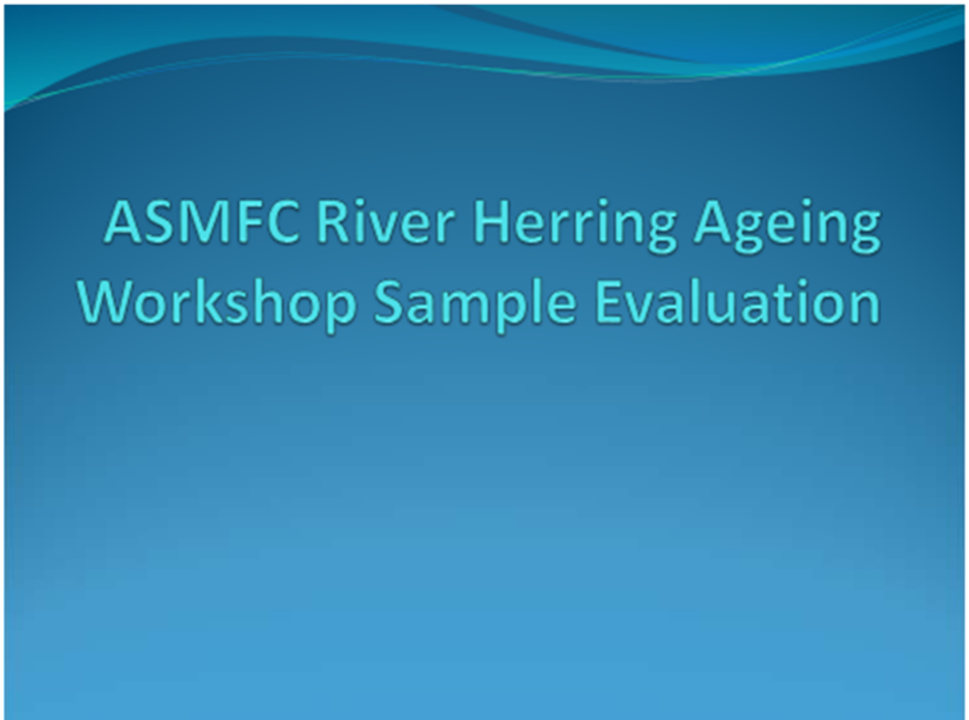
If there is no possibility of reading all samples in the collection before you are scheduled to ship to the next lab, focus on reading the paired samples (ALE 1 – ALE 67 & ALE 111 – ALE 181 for alewife, BB 1 – BB 79 & BB 111 – BB 190 for blueback) and read the additional otoliths from New Jersey (ALE 68 – ALE 110 for alewife, BB 80 – BB 110 for blueback) as time allows. The paired samples cover the length range that is typically collected and aged by the majority of the labs, but New Jersey collects smaller fish (and a few of the biggest fish in the collection) in their ocean trawl survey and ageing these fish during the exchange would provide beneficial information.

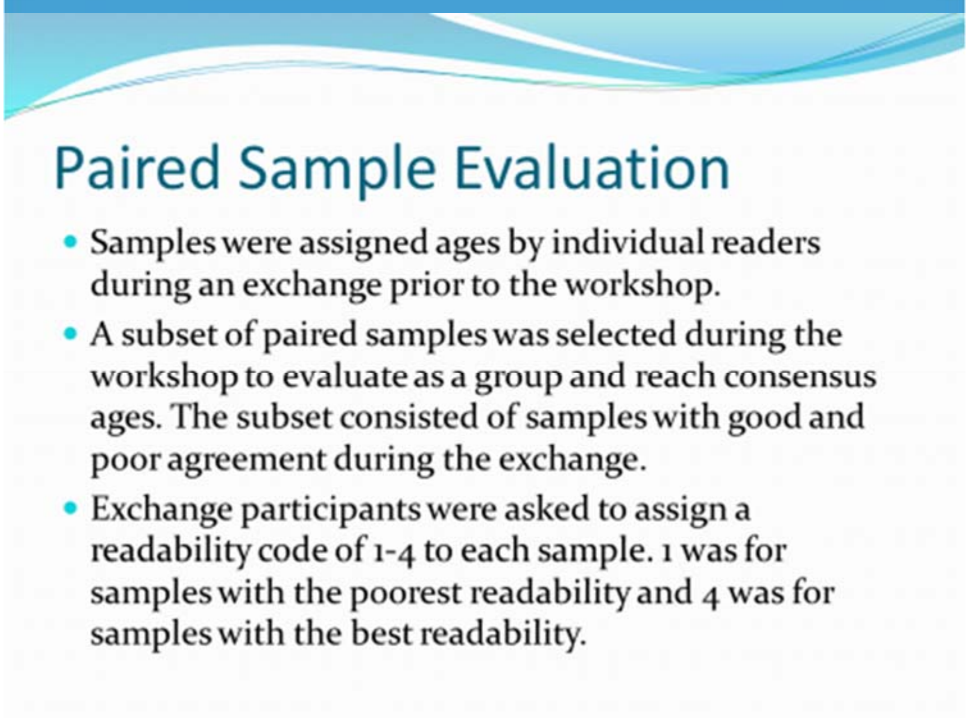
Both otoliths were damaged for a few samples, but I've included these in case an age determination can be made. Those that are broken are denoted on the data sheet. Please keep all samples in order for the next lab. The otoliths only contain a paper slip with the sample number in the vial and the scale samples are only numbered on the envelopes. Please be careful not to mix up the samples or lose the sample numbers so all samples can get back to their owners.

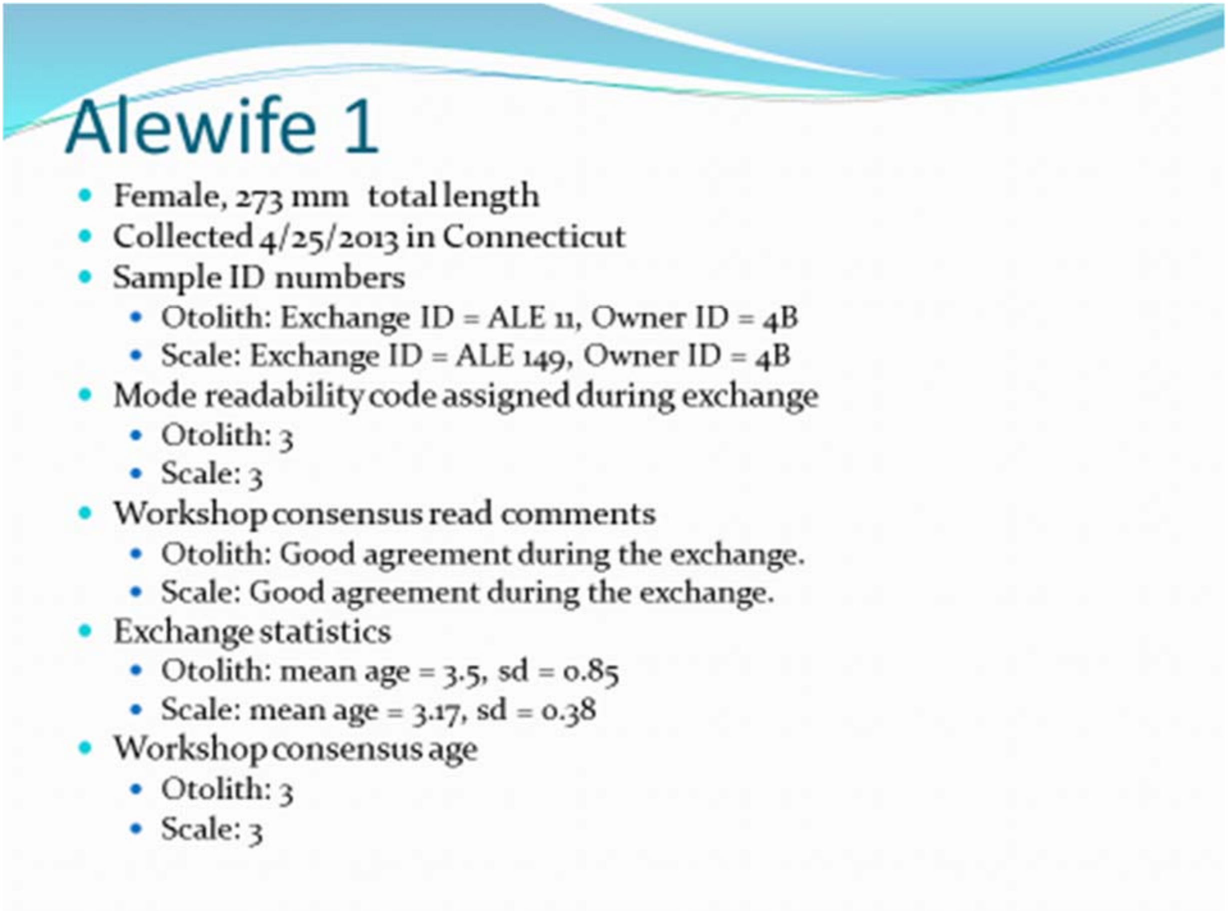
Please allow the otolith samples to air dry overnight before shipping. I will have a shipping label prepared and sent to you 3 days before you are supposed to ship to the next lab on the schedule.

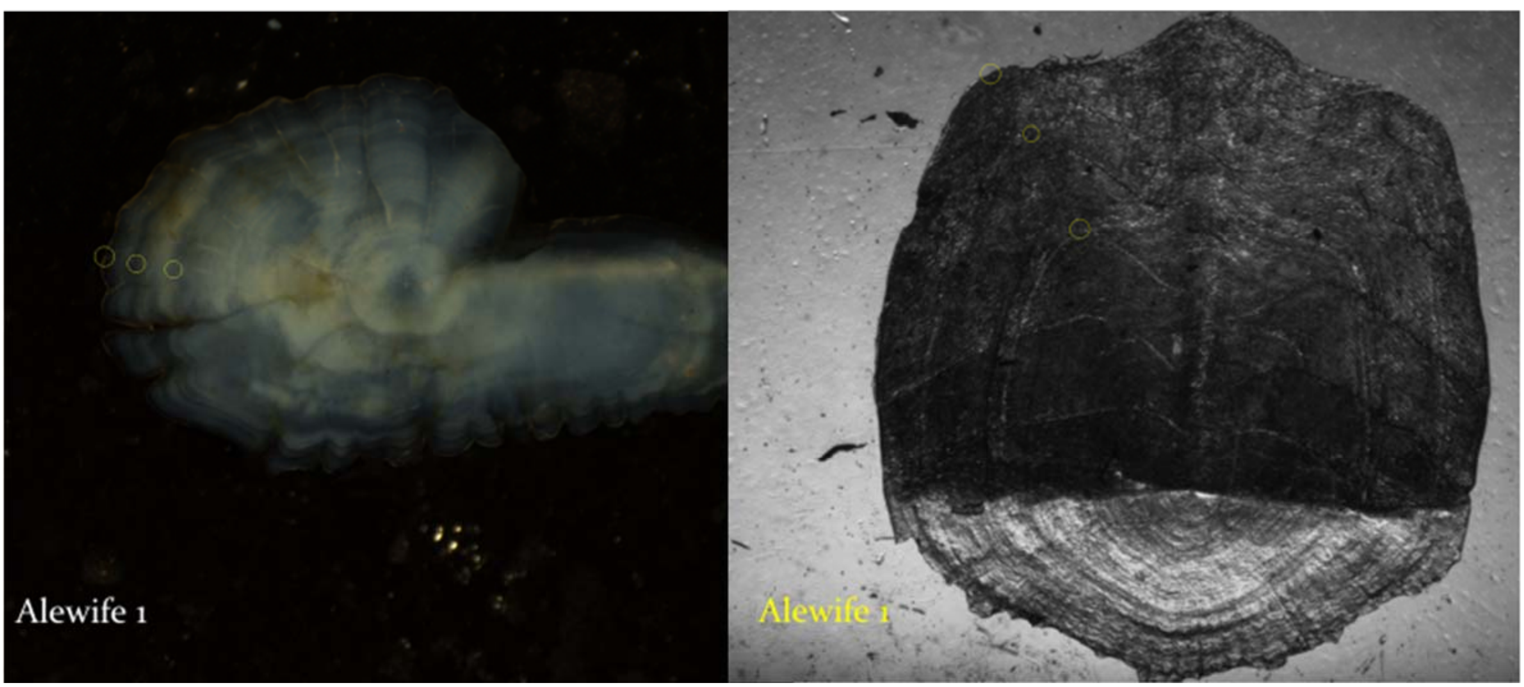
Once you have completed reading all samples, please email me the completed data sheet. Please let me know if you have any questions or if you run into any problems. Thanks for participating in the exchange!

Appendix D: River Herring Ageing Workshop Sample Evaluation



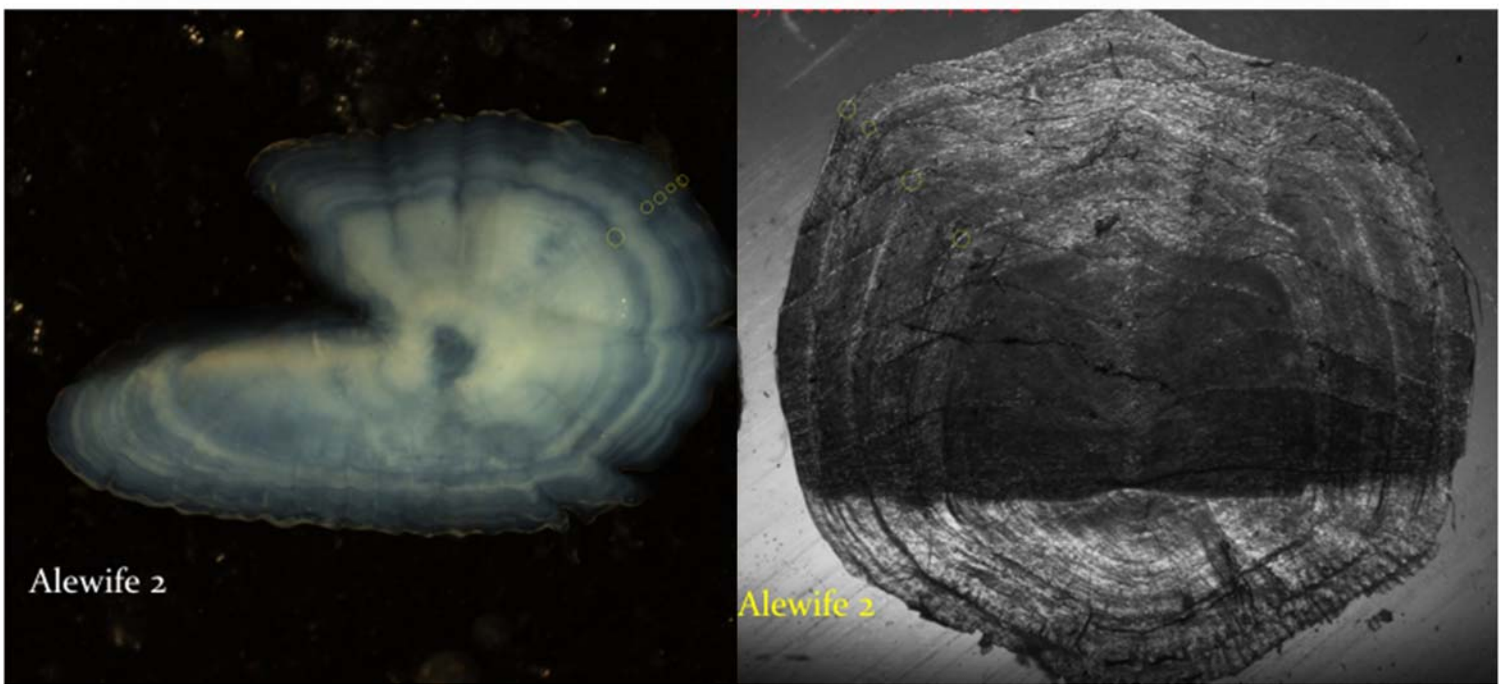



Alewife 1
Female, 273 mm total length
Collected 4/25/2013 in Connecticut
Sample ID numbers
Otolith: Exchange ID = ALE 11, Owner ID = 4B
Scale: Exchange ID = ALE 149, Owner ID = 4B
Mode readability code assigned during exchange
Otolith: 3
Scale: 3
Workshop consensus read comments
Otolith: Good agreement during the exchange.
Scale: Good agreement during the exchange.
Exchange statistics
Otolith: mean age = 3.5, sd = 0.85
Scale: mean age = 3.17, sd = 0.38
Workshop consensus age
Otolith: 3
Scale: 3


Alewife 1
Alewife 1

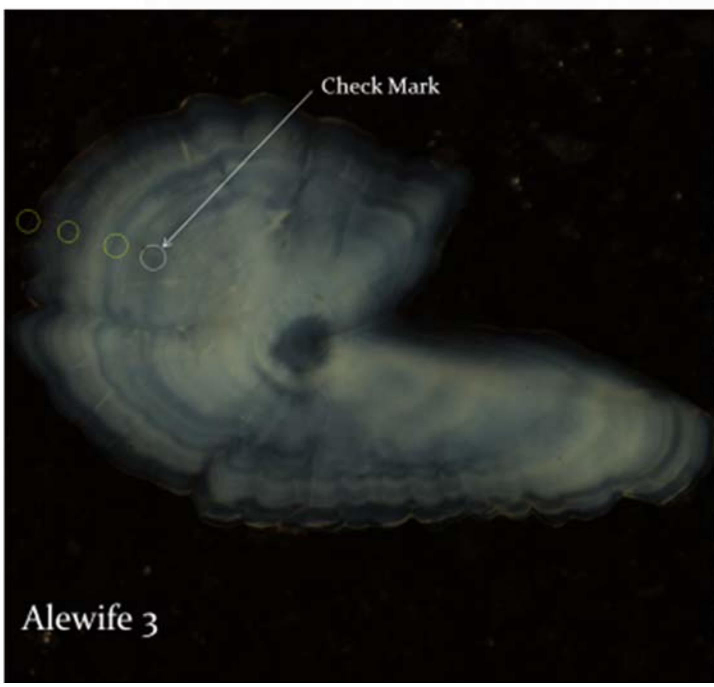
Alewife 2

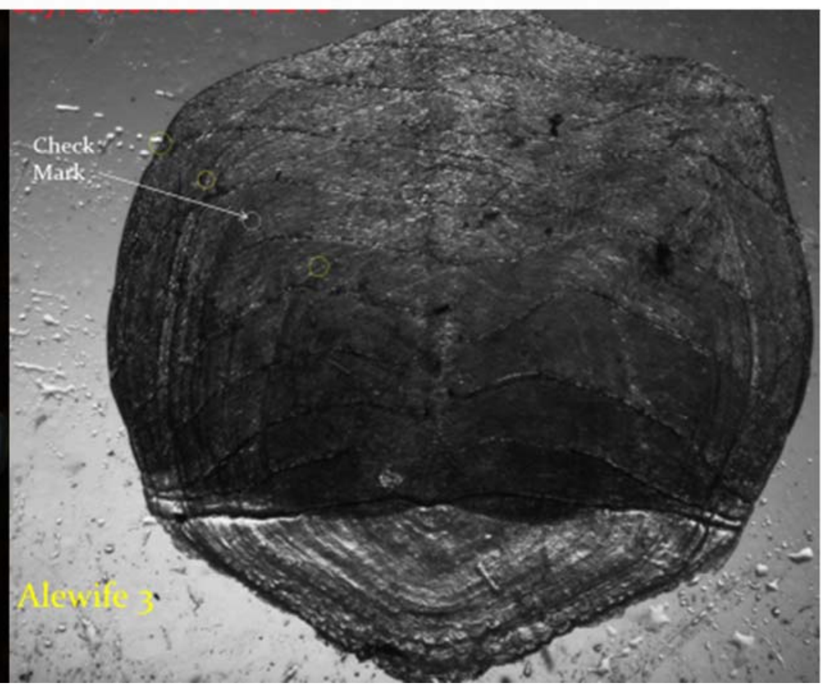
- Female, 313 mm total length
- Collected 3/26/2012 in Massachusetts
- Sample ID numbers
  - Otolith: Exchange ID = ALE 14 , Owner ID = NR-12-177
  - Scale: Exchange ID = ALE 135, Owner ID = NR-12-177
- Mode readability code assigned during exchange
  - Otolith: 3
  - Scale: 3
- Workshop consensus read comments
  - Scale: Some exchange participants counted the first annulus as an indistinct annulus within the area of the freshwater zone, others likely counted the first distinct annulus outside the area of the freshwater zone as the first annulus. Age determinations during the exchange were split evenly between 4 and 5. The consensus was to count an indistinct annulus within the area of the freshwater zone as the first annulus.
- Exchange statistics
  - Otolith: mean age = 4.93, sd = 0.96
  - Scale: mean age = 4.47 sd = 0.51
- Workshop consensus age
  - Otolith: 5
  - Scale: 4



Alewife 3

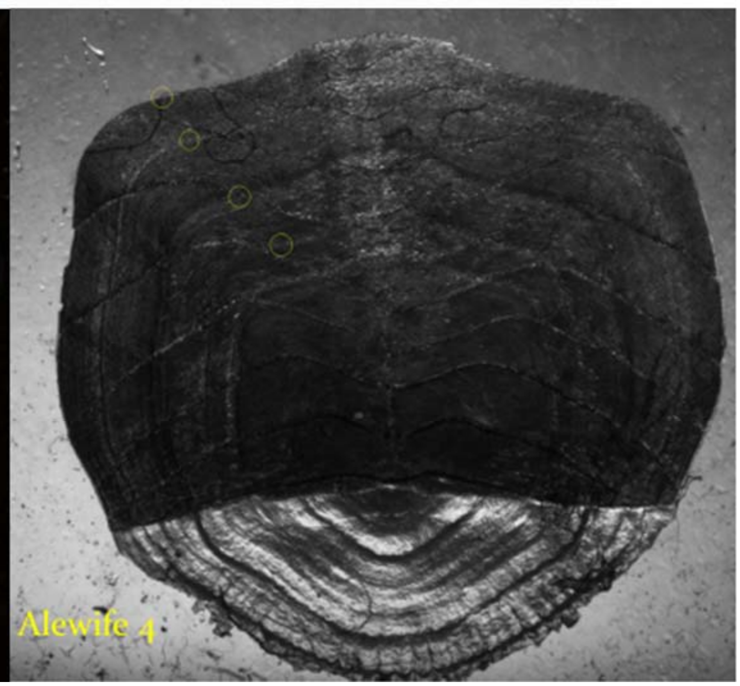
- Male, 248 mm total length
- Collected 4/19/2012 in Massachusetts
- Sample ID numbers
  - Otolith: Exchange ID = ALE 17, Owner ID = BD-12-56
  - Scale: Exchange ID = ALE 131, Owner ID = BD-12-56
- Mode readability code assigned during exchange
  - Otolith: 3
  - Scale: 3
- Workshop consensus read comments
  - Similar check marks in scale and otolith
  - Scale: Some exchange participants counted the first annulus as an indistinct annulus within the area of the freshwater zone, others likely counted the first distinct annulus outside the area of the freshwater zone as the first annulus. Age determinations during the exchange were split evenly between 3 and 4. The consensus was to count an indistinct annulus within the area of the freshwater zone as the first annulus. There was some debate on whether the second ring was a true annulus or a check mark. The consensus was that it was a check mark.
- Exchange statistics
  - Otolith: mean age = 3.73, sd = 0.80
  - Scale: mean age = 3.41, sd = 0.62
- Workshop consensus age
  - Otolith: 3
  - Scale: 3





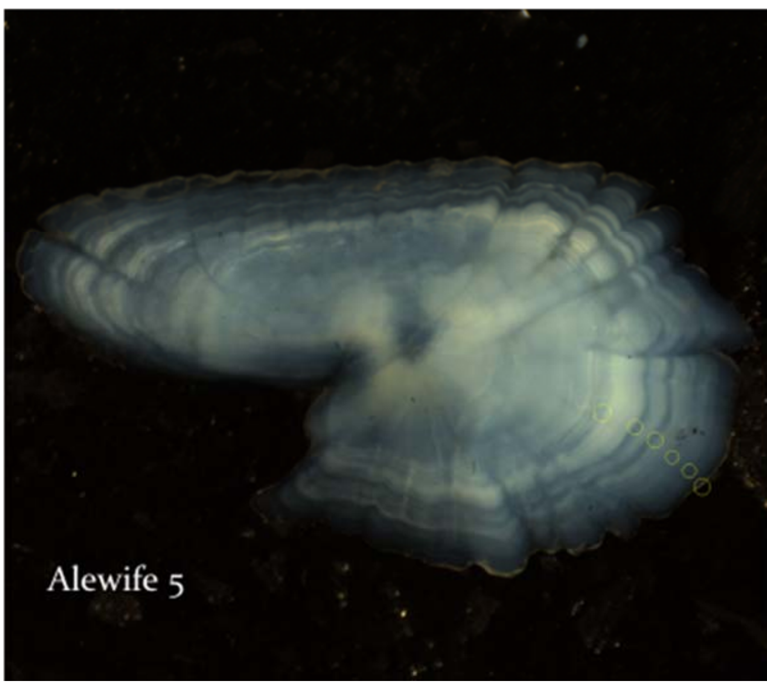
Alewife 4

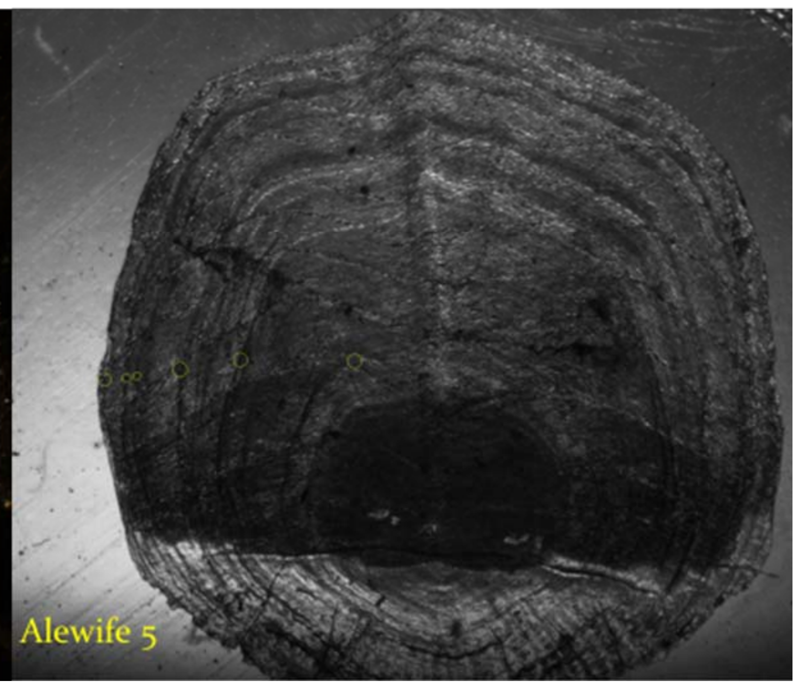
- Male, 241 mm total length
- Collected 3/4/2013 in North Carolina
- Sample ID numbers
  - Otolith: Exchange ID = Ale 26, Owner ID = (5) 1309752
  - Scale: Exchange ID = ALE 121, Owner ID = (5) 1309752
- Mode readability code assigned during exchange
  - Otolith: 2
  - Scale: 4
- Workshop consensus read comments
  - Otolith: Checky, not as clear as the scale.
  - Scale: The freshwater zone can be interpreted two ways, but both interpretations lead to an age of 4. The first annulus and freshwater zone are counted as one general area and the first distinct annulus is counted as the second annulus.
- Exchange statistics
  - Otolith: mean age = 4.13, sd = 0.99
  - Scale: mean age = 4.00, sd = 1.00
- Workshop consensus age
  - Otolith: 4
  - Scale: 4



Alewife 5

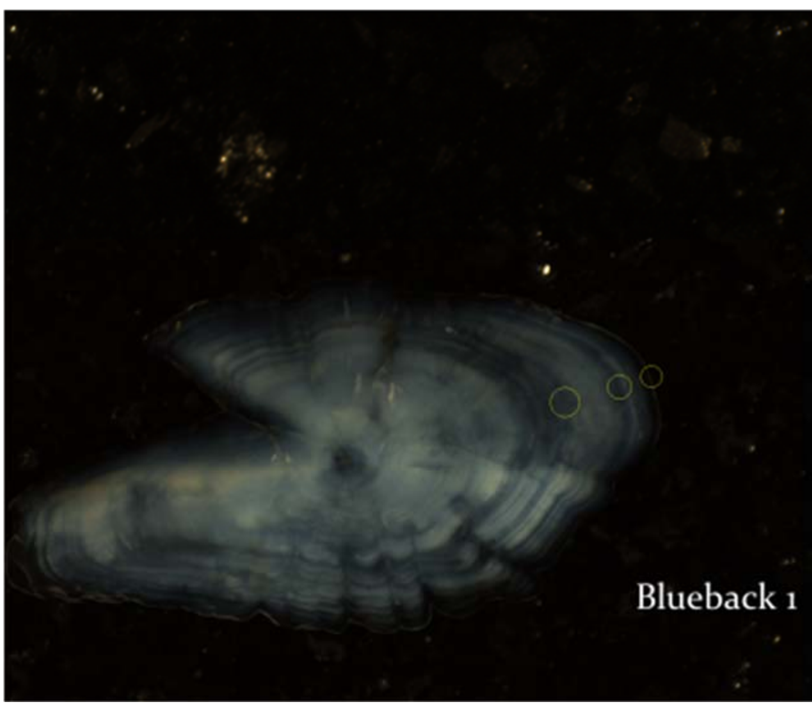
- Male, 292 mm total length
- Collected in New York during the spring run
- Sample ID numbers
  - Otolith: Exchange ID = ALE 47, Owner ID = 1324
  - Scale: Exchange ID = ALE 163, Owner ID = 24
- Mode readability code assigned during exchange
  - Otolith: 2
  - Scale: 3
- Workshop consensus read comments
  - Otolith: Trouble reaching a consensus age.
  - Scale: Possible erosion on edge. Trouble reaching a consensus age due to possible erosion. Additional scales needed.
- Exchange statistics
  - Otolith: mean age = 5.2, sd = 0.94
  - Scale: mean age = 6.33, sd = 1.14
- Workshop consensus age
  - Otolith: 6
  - Scale: 6

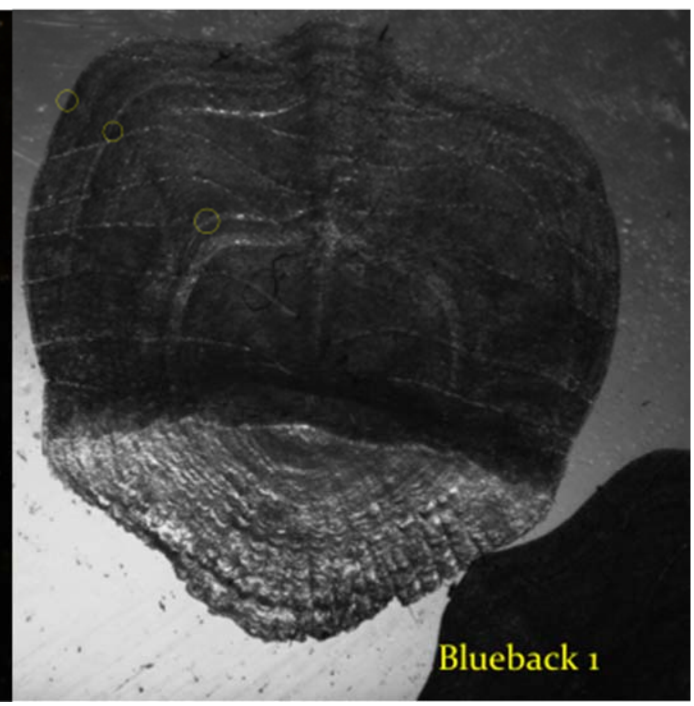




Blueback 1

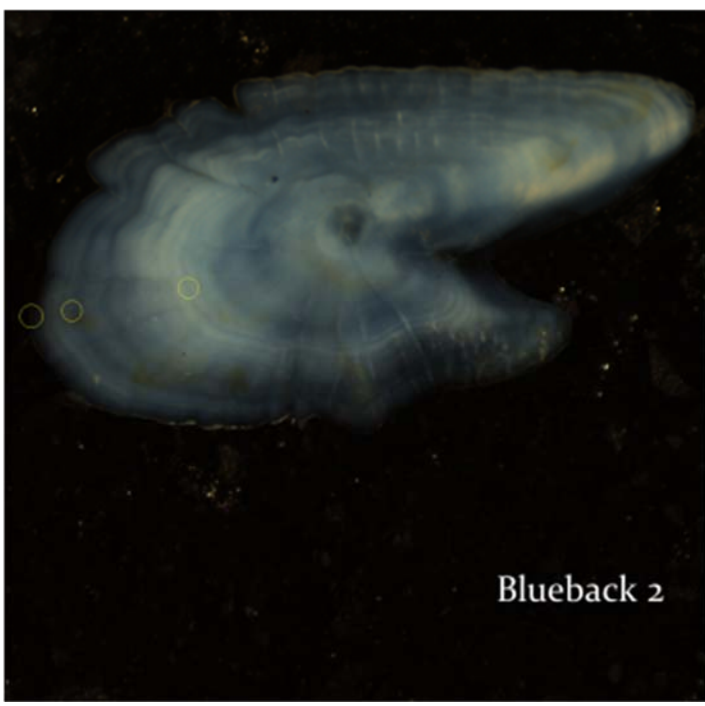
- Male, 231 mm total length
- Collected in New York during the spring run
- Sample ID numbers
  - Otolith: Exchange ID = BB2, Owner ID = 48
  - Scale: Exchange ID = BB184, Owner ID = 2444
- Mode readability code assigned during exchange
  - Otolith: 3
  - Scale: 3
- Workshop consensus read comments
  - Otolith: First year very unclear.
  - Scale: Good agreement during the exchange and workshop.
- Exchange statistics
  - Otolith: mean age = 4.73, sd = 1.03
  - Scale: mean age = 2.94, sd = 0.25
- Workshop consensus age
  - Otolith: 3
  - Scale: 3

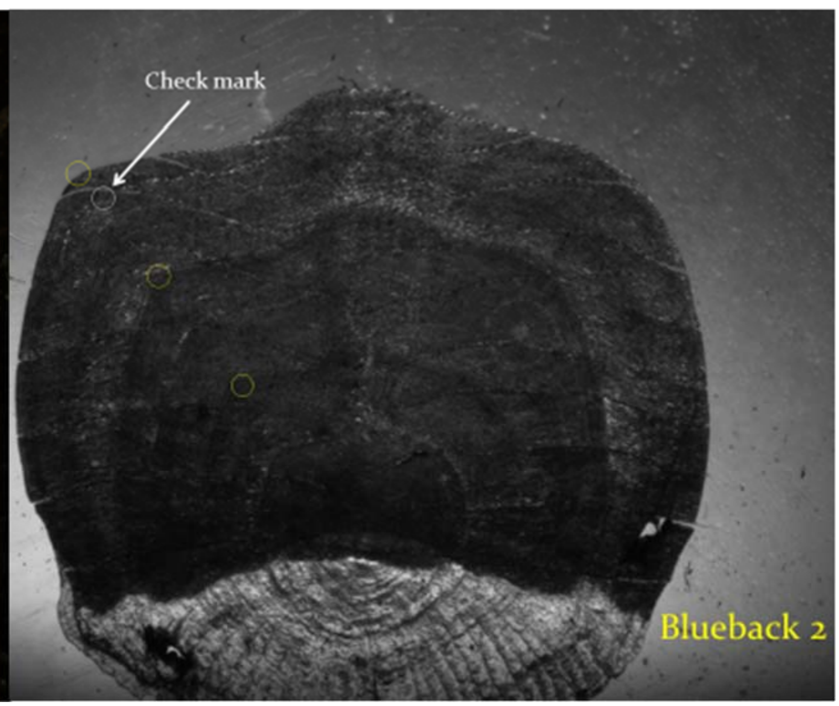




Blueback 2

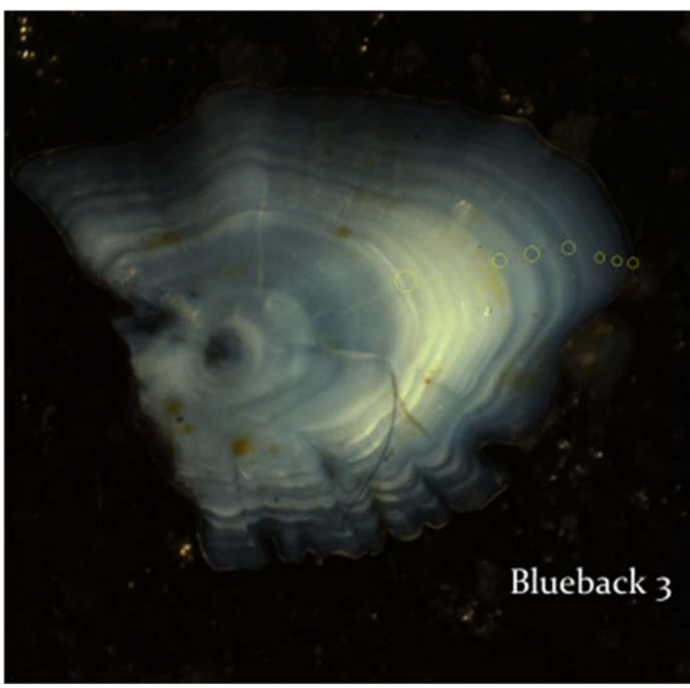
- Female, 254 mm total length
- Collected in New York during the spring run
- Sample ID numbers
  - Otolith: Exchange ID = BB 6, Owner ID = 1332
  - Scale: Exchange ID = BB 186, Owner ID = 32
- Mode readability code assigned during exchange
  - Otolith: 3
  - Scale: 3
- Workshop consensus read comments
  - Otolith: Check mark between 1st and 2nd annuli.
  - Scale: Check mark between 2nd and 3rd annuli.
- Exchange statistics
  - Otolith: mean age = 3.44, sd = 0.81
  - Scale: mean age = 3.63, sd = 0.81
- Workshop consensus age
  - Otolith: 3
  - Scale: 3

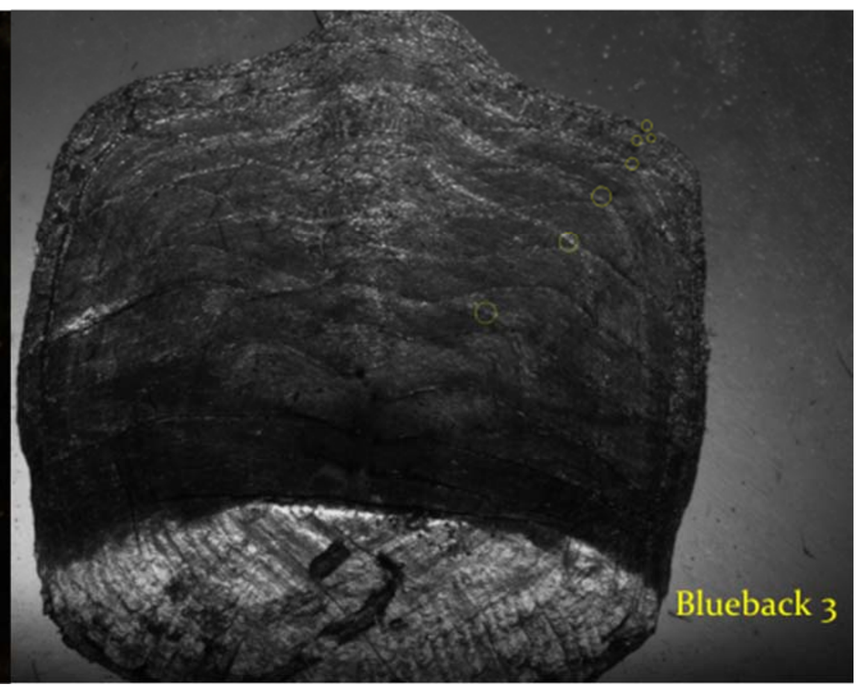




Blueback 3

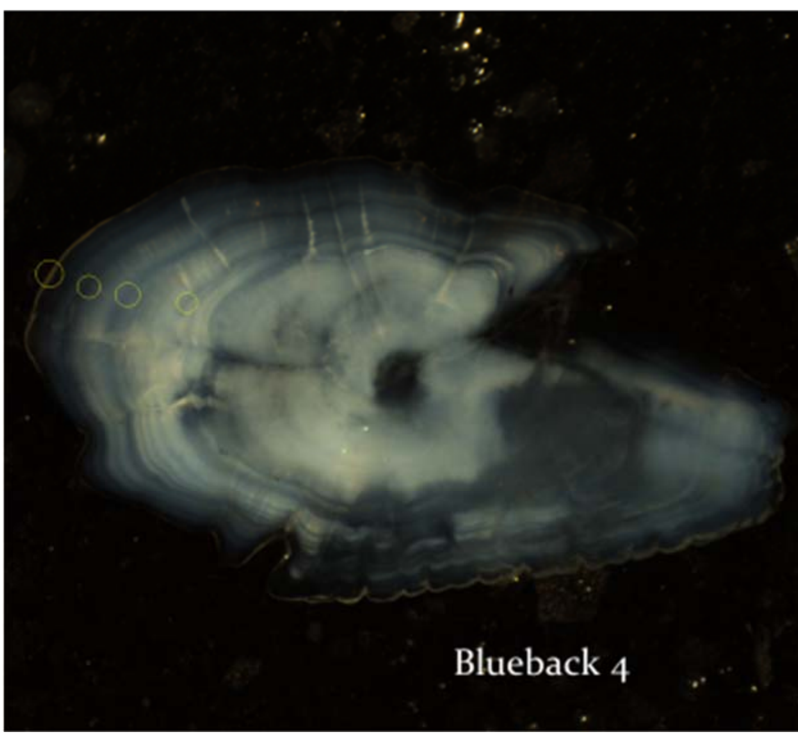
- Female, 309 mm total length
- Collected 4/25/2013 in North Carolina
- Sample ID numbers
  - Otolith: Exchange ID = BB 34, Owner ID = (7) 1306787
  - Scale: Exchange ID = BB 178, Owner ID = (7) 1306787
- Mode readability code assigned during exchange
  - Otolith: 3
  - Scale: 3
- Workshop consensus read comments
  - Otolith: Poor agreement during exchange. The otolith is broken.
  - Scale: Poor agreement during exchange.
- Exchange statistics
  - Otolith: mean age = 6.5, sd = 0.97
  - Scale: mean age = 6.88, sd = 0.99
- Workshop consensus age
  - Otolith: 7
  - Scale: 7

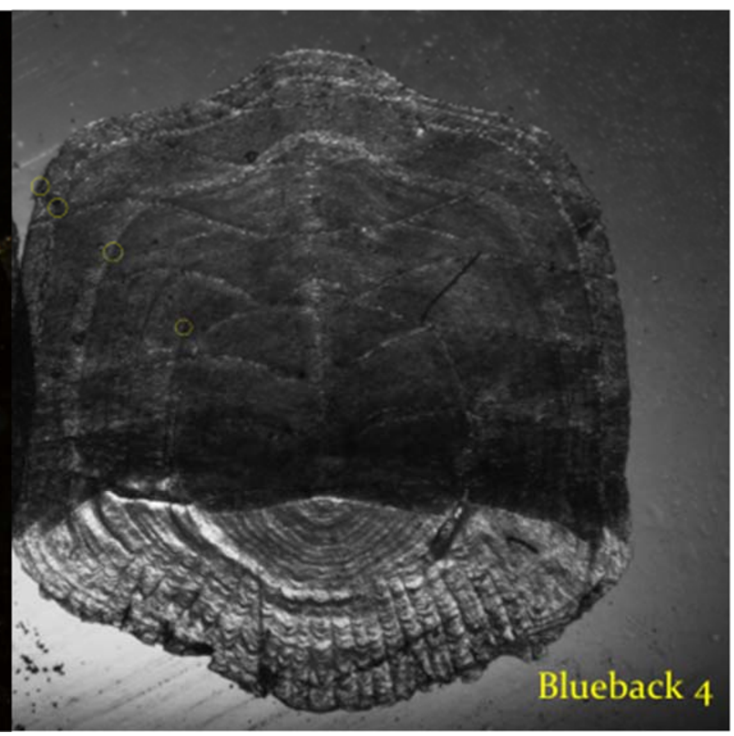




Blueback 4

- Female, 237 mm total length
- Collected 6/20/2013 in Maine
- Sample ID numbers
  - Otolith: Exchange ID = BB 77, Owner ID = 13
  - Scale: Exchange ID = BB 122, Owner ID = 13
- Mode readability code assigned during exchange
  - Otolith: 3
  - Scale: 3
- Workshop consensus read comments
  - Scale: There was confusion identifying spawning marks.
- Exchange statistics
  - Otolith: mean age = 4.17, sd = 0.58
  - Scale: mean age = 4.18, sd = 0.33
- Workshop consensus age
  - Otolith: 4
  - Scale: 4





Appendix E: River Herring Ageing Workshop Agenda

River Herring Ageing Workshop
Atlantic States Marine Fisheries Commission
December 16-17, 2013
CT DEEP Marine Fisheries Headquarters
333 Ferry Road
Old Lyme, CT 06371

Draft Agenda

<u>Monday, December 16 (1:00 pm – 5:00 pm)</u>

1. Welcome and Introductions
2. Workshop Goals and Objectives
3. Review Lab Ageing Methodologies (Sample Collection, Sample Preparation, Age Determination)
4. Review Results of Scale and Otolith Exchange

<u>Tuesday, December 17 (9:00 am – 12:30 pm)</u>

1. Examine Scale and Otolith Samples from Exchange Collection
2. Make Recommendations to Standardize Ageing Practices
3. Adjourn