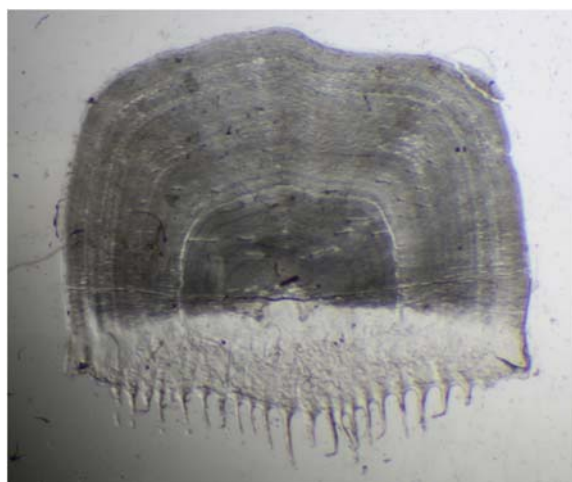
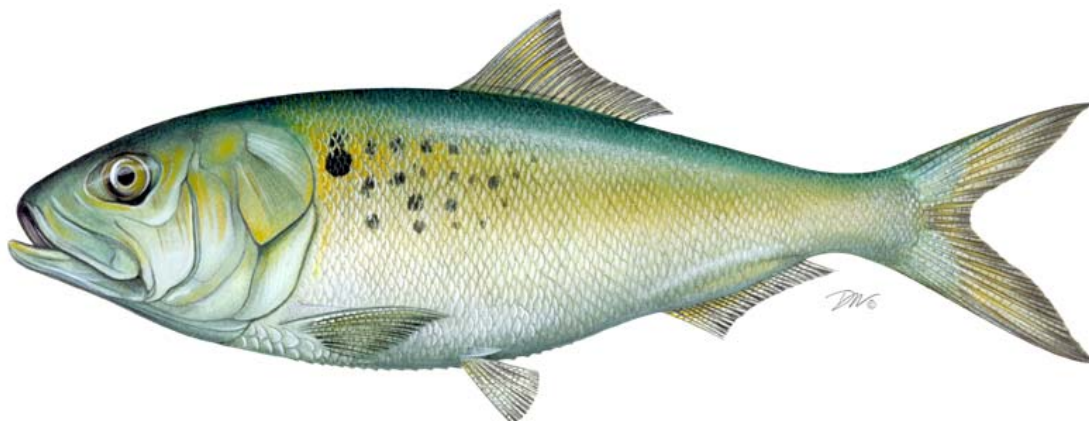


Atlantic States Marine Fisheries Commission

2015 Atlantic Menhaden Ageing Workshop Report



June 2015



Vision: Sustainably Managing Atlantic Coastal Fisheries

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Statement of Problem

The Atlantic Menhaden Advisory Committee and, subsequently, the Atlantic Menhaden Technical Committee have identified the need for increased biological sampling (i.e., length and age) of the menhaden bait fishery (ASMFC 2012). In response, Amendment 2 to the Instate Fishery Management Plan for Atlantic Menhaden has required jurisdictions from NC to ME to collect biological samples proportional to bait landings since 2013. Most jurisdictions have sent these samples to the NOAA-Beaufort Lab for processing and ageing.

A SouthEast Data, Assessment, and Review (SEDAR) benchmark stock assessment of Atlantic menhaden was completed in 2015. Age data available for the assessment were derived from scales. All age determinations were made by the NOAA-Beaufort Lab following a study conducted by June and Roithmayr (1960). The study validated annual ring deposition on scales of menhaden reared in ponds for fourteen months. Age data are available back to 1955 and have been provided by one reader, Ethel Hall, since the 1970s (SEDAR 2015). Ethel reread some scale samples in 2008 to develop estimates of intra-reader precision, which showed a general decline in precision with age. Ethel is scheduled to retire in fall 2015 and ageing responsibilities will transition to Jennifer Potts for the near future. The lack of other readers providing age data for the SEDAR precluded the need for inter-reader error assessments. Intra-reader precision estimates were used to incorporate ageing uncertainty in the assessment model, but model configurations with this uncertainty did not converge. The accepted model configuration assumes all ages are known without error.

Several research recommendations related to ageing Atlantic menhaden were made during the stock assessment and are identified below.

1. Continue current level of sampling from bait fisheries, particularly in the Mid-Atlantic and New England. Analyze sampling adequacy of the reduction fishery and effectively sample areas outside of that fishery (e.g., work with industry and states to collect age structure data and biological data outside the range of the fishery).
2. Ageing:
 - a. Conduct an ageing validation study (e.g., scale-otolith comparison), making sure to sample older age classes. Use archived scales to do radio isotope analysis.
 - b. Ageing precision: conduct an ageing workshop to assess precision and error among readers.**
3. Develop a menhaden specific coastwide fishery-independent index of adult abundance at age.

Additionally, the peer review panel noted that direct estimates of fishery-independent survey age composition could improve the assessment. Currently, only length samples are available from fishery-independent surveys.

Due to Ethel's scheduled retirement in 2015 and the requirements and recommendations for collecting biological samples from menhaden fisheries and fishery-independent surveys, there

was the need to exchange a reference set of scales among labs that have recently started to age their samples or plan to age their samples in the future and to convene an ageing workshop.

Objectives

The objectives of the reference set exchange were to (1) provide a general idea of inter-lab precision of age determinations for Atlantic menhaden scales, (2) identify any systematic bias between labs ageing Atlantic menhaden, and (3) identify ageing uncertainty issues to focus on during the workshop. The objectives of the workshop were to (1) utilize and share the experience, knowledge, and techniques of the NOAA-Beaufort Lab personnel, (2) assess precision and interchangeability of inter-lab age determinations, (3) evaluate reference set samples as a group to identify similarities and differences in age determination methods, and (4) make recommendations to improve and standardize ageing practices.

Agency Ageing Information

NOAA-Beaufort

Scale samples have been collected from fishery-dependent sampling of reduction fisheries since 1955. Samples are collected by port samplers and processed according to protocol (Appendix A) before being sent to the Lab for ageing. Scale samples collected in recent years from bait fisheries by state sampling programs have also been sent to the Lab for processing and ageing. The Lab ages an average of about 2,000 samples from reduction fisheries and about 1,500 samples from bait fisheries a year.

An Eberbach macro-projector is used to read scale samples. This is a rare piece of equipment that is uncommon in most other ageing labs. All scales mounted on a slide from each fish are viewed for age determination. The most symmetrical scale in the sample is typically selected for measurement. Historically, the scale selected for age determination and measurement was recorded, but this practice was discontinued in 1989. Illegible samples (e.g., all regenerated scales) are assigned a classification of 40 and not aged in production ageing. Occasionally, an illegible sample will be assigned an age based on supplemental information (i.e., fish length). Illegible samples due to regeneration are more commonly from older fish. Other than the illegible classification, no quality or confidence rating is provided for each sample. The Eberbach macro-projector is equipped with a sonic digitizer to record distances between each annulus and the focus and the edge of the scale and the focus (see ageing instructions, Appendix B). These distances are recorded from one scale in each legible sample.

North Carolina Division of Marine Fisheries (NC DMF)

Scale samples are currently collected from fishery-dependent sampling of bait fisheries. No scale samples are collected during fishery-independent sampling. Scales are processed and aged in-house with a microfiche reader.

Virginia Institute of Marine Science (VIMS)

Otolith samples have been collected on the fishery-independent ChesMMAP Survey since 2002 and the fishery-independent NEAMAP Trawl Survey since 2007 (approximately 2000 to date). Scales were collected alongside otoliths in 2012 on the NEAMAP Survey for future comparison of paired samples (approximately 200 to date). All age samples are currently unprocessed and have been archived for future ageing. The reference set was aged with a microfiche reader.

Old Dominion University (ODU)/Virginia Marine Resources Commission (VMRC)

Scale samples are currently collected by the VMRC from fishery-dependent sampling of bait fisheries and sent to ODU for ageing. All samples are currently unprocessed and have been archived for future ageing. The reference set was aged with a microfiche reader.

Potomac River Fisheries Commission (PRFC)

Raw samples of fish are currently collected from fishery-dependent sampling of bait fisheries. The raw specimens are processed by the NOAA's port agent at Reedville, VA.

Maryland Department of Natural Resources (MD DNR)

Scale samples are currently collected from fishery-dependent sampling of bait fisheries (pound nets). Sample size was reduced from 25 to 10 in 2013, as this sample size meets the requirements established in Amendment 2. Samples are processed and aged in-house with a microfiche reader. Ages are submitted in annual compliance reports. Age samples are not currently collected from fishery-independent sampling. A fishery-independent gill net survey conducted in the lower Choptank River has sampled menhaden lengths and could be expanded to collect scales.

Delaware Division of Fish and Wildlife (DE DFW)

Scale samples are currently collected from fishery-dependent sampling of bait fisheries (gill nets) and two fishery-independent trawl surveys. Some samples in 2013 were sent to the NOAA-Beaufort Lab for ageing. The reference set was aged with a microfiche reader.

New Jersey Division of Fish and Wildlife (NJ DFW)

Scale samples are currently collected from fishery-dependent sampling of bait fisheries (purse seines and pound nets); they are stored in coin envelopes. Age samples are not currently collected from fishery-independent sampling. Bait scale samples are mounted and read at the NOAA-Beaufort Lab, then archived there. The reference set was aged with a microfiche reader.

New York State Department of Environmental Conservation (NY DEC)

Scale samples are collected from fishery-dependent sampling of bait fisheries through Cornell Cooperative Extension. All scales collected in 2013 and 2014 are currently being processed to age. Scales collected in years prior to 2013 are stored in coin envelopes and have been archived. In the future, menhaden scales will also be collected from the fishery-independent Western Long Island Juvenile Striped Bass Beach Seine Survey. The reference set was aged with a microfiche reader.

Connecticut Department of Energy and Environmental Protection (CT DEEP)

Scale samples are currently collected on the fishery-independent Long Island Sound Trawl Survey and infrequently from fishery-dependent sources. In 2013, samples were sent to the NOAA-Beaufort Lab for paired ageing with the CT ages. CT DEEP participated in the exchange, but not the workshop. Samples are processed and aged in-house with a microfiche reader.

Rhode Island Division of Fish and Wildlife (RI DEM DFW)

Scale samples are currently collected from fishery-dependent sampling of bait fisheries. Scales are stored dry in coin envelopes, and shipped to the NOAA-Beaufort Lab where they are mounted, read, and archived. The annual sample size target is 100. Age samples are not currently collected from fishery-independent sampling. Duplicate scale samples have been archived in-house since 2014 for future ageing and comparison to NOAA-Beaufort age determinations. Some scale samples have been prepared with a heat press and the standard protocol for pressing scales of other species, but the quality was found to be poor. The reference set was aged with a microfiche reader.

Massachusetts Division of Marine Fisheries (MA DMF)

Scale samples are currently collected from fishery-dependent sampling of bait fisheries (cast nets). Samples are sent unprocessed to the NOAA-Beaufort Lab for processing and ageing. Due to the low number of samples collected annually, MA DMF does not have plans in the immediate future to age menhaden in-house. MA DMF participated in the exchange, but not the workshop.

Maine Department of Marine Resources (ME DMR)

Scale samples are currently collected from fishery-dependent sampling of bait fisheries. Age samples are not currently collected from fishery-independent sampling. Samples are sent unprocessed to the NOAA-Beaufort Lab for processing and ageing. ME DMR did not participate in the exchange or workshop.

Reference Set Exchange

Prior to the workshop, the labs conducted an exchange of 98 scale samples. Samples were provided by the NOAA-Beaufort Lab. Samples were collected from menhaden ranging from 131-300 mm fork length (FL; table 1, figure 1). The majority of samples (n=74) were collected from the reduction fishery in Reedville, VA (table 1, figure 1). There were also twelve samples collected from the bait fishery in MD, eleven samples collected from the bait fishery in NJ, and one sample collected from the bait fishery in RI/MA. Samples were collected during all months except January, February, and October, with the majority (n=43) collected in November (table 1, figure 2). Exchange participants were asked to age each sample following the provided instructions (Appendix B).

Consensus ages were used for labs with more than one reader (ODU, VIMS, MD DNR, and NJ DFW). NC DMF did not have consensus ages, thus the ages for reader 2 were used given that all samples were read by reader 2. However, a comparison of NC DMF readers showed high

agreement (exact agreement = 84%, agreement within 1 year = 100%, mean CV = 5%) and no systematic bias. DE DFW ages are annuli counts only and may be less than calendar ages for a few fish caught in spring. Systematic bias between age determinations from two labs was assessed with Bowker's test of symmetry (Bowker 1948) and Evans and Hoenig's test of symmetry (Evans and Hoenig 1998). Bowker's test compares disagreeing age determinations at each reference set age in a contingency table and is useful for detecting age-specific biases. Evans and Hoenig's test pools disagreeing age determinations over all reference set ages and is useful for detecting age-independent biases. Evans and Hoenig's test may be more appropriate when the number of disagreeing age determinations at each reference set age is small, as the chi square approximation breaks down (Evans and Hoenig 1998). Significant p-values (<0.05) indicate systematic bias between two sets of age determinations and violation of the assumption of interchangeability (i.e., one lab's age determinations are not reproducible by the other labs). It is important to keep in mind that systematic bias indicates labs' age determinations are systematically different from each other. Both age determinations may still be biased from the true age. Mean CVs and percent agreement were calculated for each comparison to provide measures of precision. Summaries of bias and precision are available in tables 2-4. Age frequency and age bias plots for each comparison are in figures 3-88.

Mean CVs were generally greater than 5%, which Campana (2001) suggests as a generic ageing precision reference point. However, no mean CVs exceeded 15%. Exact agreement was generally between 80% and 60%, and averaged 73%. Agreement within one year was generally above 95%, except in MD DNR and CT DEEP comparisons with other labs. Systematic bias was detected for MD DNR and CT DEEP comparisons to other labs with both Bowker's and Evans and Hoenig's symmetry tests. Examination of the age frequency and age bias plots indicate that MD DNR and CT DEEP ages were generally greater than other labs ages for fish approximately 2 and older. There was no systematic bias detected between MD DNR and CT DEEP age determinations. There was systematic bias detected for the VIMS and ODU comparison and the NY DEC and VIMS comparison with Bowker's test of symmetry, but not Evans and Hoenig's test of symmetry.

The exchange was intended to facilitate discussion at the workshop and provide baseline information on Atlantic menhaden ageing error between labs not previously providing age. Several labs are new to or have never aged Atlantic menhaden prior to the exchange and the results in this report will provide a benchmark to compare future reference set exchange results.

Workshop Sample Evaluation and Discussion

During the workshop the group examined samples with a dissecting microscope equipped with a polarizer and digital imaging software and, subsequently, the Eberbach macro-projector. The dissecting microscope and digital imaging software allowed the group to examine samples together, whereas the Eberbach is located in a confined space where only part of the group could examine samples together. The NOAA-Beaufort Lab readers noted there are differences when viewing samples with the different equipment and that the Eberbach is preferred for

Atlantic menhaden scales. Samples with poor agreement during the exchange were selected to address issues discussed at the workshop, including: (1) samples that were generally split between two ages, particularly those collected in the spring, (2) samples that were aged the same by CT DEEP and MD DNR, but differently than most other labs, (3) samples with relatively small distances to the first annulus, and (4) samples with ages across a wide range. The issues discussed at the workshop included: (1) differentiating between accessory rings (false annuli) and true annuli, (2) counting virtual rings in fish caught during spring, (3) determining if a first annulus was unrealistically close to the focus, (4) the bias detected between MD DNR and CT DEEP and other labs, and (5) quality/confidence issues. Consensus age determinations, statistics from the exchange, and other notes on the samples evaluated are available in Appendix C.

Samples with excellent agreement during the exchange where consensus aged quickly at the workshop with the dissecting microscope. Several samples aged the same by CT DEEP and MD DNR (sample # 45030 and 44780, Appendix C), but differently than most other labs were examined, but no pattern to the disagreeing age determinations during the exchange could be identified. Future exchanges will need to be completed to determine if the bias in the exchange is persistent. The group discussed the method for counting a virtual ring. If a fish is caught in the spring and has experienced significant growth since depositing the last annulus, a “virtual” annulus should be counted to assign the fish to the correct year class. It is assumed that an annulus would have been deposited in the coming weeks or months if the fish had not been captured. The NOAA-Beaufort Lab tends to get few spring fish, but the recommendations to collect more age samples from fishery-independent surveys may result in more samples collected in spring that need to be considered for virtual rings. Two samples collected on the same day in May were evaluated by the group (sample # 45022 and 45030, Appendix C). One was identified as a sample with a virtual ring due to a wide band of growth and another with no virtual ring due to very little growth after the most recent annulus. The relative margins of growth were also examined by looking at the measured distances between the last annulus and the scale edge. There are two rules of thumb used by the NOAA-Beaufort Lab to count a virtual ring, but there is also judgement outside these rules of thumb depending on the sample. The first rule of thumb is that the growth between two annuli is approximately one half the growth of the prior year. The second rule of thumb is that any fish caught after June 1 would not be assigned a virtual ring, no matter how much growth there is after the last annulus. Subjectivity of assigning virtual rings across labs may be addressed with development of a standard edge code classification. Participants noted they don’t have the capabilities to take measurements between annuli on their microfiche reader, so any potential edge codes may need to be qualitative. A preliminary marginal increment analysis completed following the workshop is in table 5. Further analysis would be useful for informing an edge code classification.

Accessory rings (false annuli) were identified in several samples examined by the group (sample # 45022 and 44780, Appendix C). NOAA-Beaufort Lab readers noted that rings must be continuous around the lateral and anterior fields of the scale to be counted a true annuli. The distance between rings (growth) can also be used to help differentiate accessory rings from true annuli. If the distance between the two rings varies significantly from one half the distance between the two previous rings, and the ring is not continuous, there is justification to identify

a ring as an accessory ring. Harry Rickabaugh mentioned that MD DNR readers have seen some samples with what appeared to be a first annulus unrealistically close to the focus, relative to other scale samples collected in Maryland. They have considered discounting this ring as an accessory ring. Several samples were evaluated to determine if a relatively small distance between the first ring and focus supported an accessory ring (sample # 42773 and 13969, Appendix C). The group discussed several potential reasons for a relatively small distance between a first annulus and the focus, including late spawned fish from the northern extent of the species range. Table 6 includes a preliminary analysis following the workshop of the distribution of the distance between the focus and first annulus in Eberbach units. The group agreed to maintain the rule of counting all first rings that are continuous around the focus, despite the distance to the focus.

Joe Smith pointed out issues with sample preparation that port samplers have experienced when processing samples sent from other jurisdictions. Scales are often stored in coin envelopes for long periods of time before being sent to the port samplers for processing. These samples are more difficult and time consuming to process and can be damaged (i.e., tears to the edges) during processing. Port samplers have also noticed that if scales from age-0 fish are not mounted immediately, they may cup up and result in difficulties mounting.

The group discussed the use of otoliths as alternative ageing structures. VIMS has typically collected otoliths from their fishery-independent surveys and ODU has done some preliminary work comparing otoliths and scales. For some samples evaluated at the workshop with poor quality/regeneration (sample #44816, Appendix C), it was noted that otoliths may be used as alternative structures to determine age. Additional work is needed to determine how otolith age determinations compare to scale age determinations. The NOAA-Beaufort Lab has archived several thousand paired scale and otolith samples collected during the mid-1990s that could be available for a comparison study. The scales have already been read for these samples. There were some concerns that the quality of the small, fragile otoliths may degrade with time similar to what has been observed in river herring otoliths, but there was no indication the archived otoliths had degraded.

Workshop Recommendations

Following review of the exchange results and evaluation of samples during the workshop, the group made the following recommendations to standardize and improve ageing practices.

- Scale samples should be collected from fishery-independent surveys, with an emphasis on surveys that sample outside the range of the fisheries and surveys used to develop indices of abundance in SEDAR 40.
- All jurisdictions should follow the NOAA-Beaufort Lab protocol for processing scale age samples (Appendix A). If a jurisdiction does not have the capabilities to age Atlantic menhaden scale samples and will continue to send samples to the NOAA-Beaufort Lab, samples should be processed according to the protocol before sending.

- Blunt edges of scalpels should be used to scrape as much slime and debris from the scale sampling area of the fish before taking the sample.
- Jurisdictions should begin production ageing of Atlantic menhaden scale samples collected in their sampling programs.
- All jurisdictions submitting age data should provide annuli counts and final year class ages.
- An annual subsample of age samples should be sent to the NOAA-Beaufort Lab for ageing and comparison once jurisdictions begin submitting age data.
- Paired otolith and scale samples from the same fish should be evaluated for interchangeability and appropriate precision levels.
- Otoliths should be collected with scale samples as potential assistance for age determination from poor quality/illegible scale samples. A scale-otolith comparison will be necessary before using otoliths as supplemental ageing structures.
- Only count rings that can be identified as continuous around the anterior and lateral fields. If a ring is difficult to see, distances between previous annuli can be used for guidance in identifying true annuli. The distance between two annuli should be approximately half the distance between the previous two annuli.
- Complete a marginal increment analysis to assist in edge code classification.
- Continue analyses of distance to first annulus and marginal increments in standard units (e.g., mm or proportion of distance between earlier annuli), with considerations of regional effects.

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Tables

Table 1. Length frequency, capture month, and landing port or state of Atlantic menhaden in the reference set.

		Month	Count		
Fork Length (mm)	Count	Jan	0	Port	Count
120-140	2	Feb	0		
140-160	2	Mar	3		
160-180	2	Apr	3		
180-200	4	May	8		
200-220	18	Jun	9		
220-240	9	Jul	11		
240-260	21	Aug	4	Reedville	74
260-280	27	Sep	15	MD	12
280-300	12	Oct	0	NJ	11
300-320	1	Nov	43	RI/MA	1
		Dec	2		

Table 2. Symmetry test p-values for inter-lab age comparisons (n=98) using Bowker's (1948) test of symmetry (above diagonal) and Evans and Hoenig's (1998) test of symmetry (below diagonal). Significant p-values ($\alpha < 0.05$) are indicated with asterisks and red highlight. Ethel Hall (EAH) is the current reader of menhaden scales at the NOAA-Beaufort Lab and Jennifer Potts (JCP) is the future reader of menhaden scales at the NOAA-Beaufort Lab.

Lab	Beaufort-EAH	NC	ODU	VIMS	MD	DE	NJ	NY	RI	MA	CT	Beaufort-JCP
Beaufort-EAH		0.253	0.576	0.053	0.001 *	0.124	0.613	0.515	0.38	0.172	0.019 *	0.766
NC	0.18		0.267	0.166	0.001 *	0.406	0.506	0.172	0.513	0.494	0.000 *	0.658
ODU	0.976	0.178		0.016 *	0.003 *	0.507	0.182	0.952	0.164	0.065	0.012 *	0.525
VIMS	0.134	1	0.221		0.000 *	0.119	0.132	0.013 *	0.377	0.506	0.000 *	0.05
MD	0.001 *	0.000 *	0.000 *	0.000 *		0.005 *	0.000 *	0.003 *	0.001 *	0.000 *	0.129	0.001 *
DE	0.336	0.317	0.746	0.45	0.001 *		0.48	0.363	0.46	0.482	0.01 *	0.453
NJ	1	0.201	0.827	0.109	0.000 *	0.853		0.397	0.705	0.731	0.005 *	0.675
NY	0.782	0.108	1	0.144	0.000 *	0.67	0.819		0.171	0.086	0.005 *	0.608
RI	0.67	0.465	0.607	0.414	0.001 *	0.876	0.683	0.577		0.675	0.006 *	0.46
MA	0.655	0.433	0.695	0.371	0.000 *	0.857	0.655	0.564	0.808		0.004 *	0.406
CT	0.000 *	0.000 *	0.000 *	0.000 *	0.117	0.000 *	0.000 *	0.000 *	0.001 *	0.000 *		0.006 *
Beaufort-JCP	1	0.251	0.974	0.109	0.000 *	0.847	0.739	0.796	0.683	0.819	0.000 *	

Table 3. Percent exact agreement (above diagonal) and percent agreement within one year (below diagonal) of inter-lab age comparisons (n=98). Exact agreement less than 80% and agreement within one year less than 95% is highlighted in red. Ethel Hall (EAH) is the current reader of menhaden scales at the NOAA-Beaufort Lab and Jennifer Potts (JCP) is the future reader of menhaden scales at the NOAA-Beaufort Lab.

Lab	Beaufort-EAH	NC	ODU	VIMS	MD	DE	NJ	NY	RI	MA	CT	Beaufort-JCP
Beaufort-EAH	100	79.6	76.5	83.7	65.3	75.5	85.7	86.7	77.6	79.6	69.4	91.8
NC	100.0	100	72.4	75.5	60.2	74.5	77.6	80.6	69.4	73.5	67.3	80.6
ODU	98.0	100.0	100	75.5	64.3	61.2	78.6	77.6	69.4	73.5	68.4	78.6
VIMS	100.0	100.0	99.0	100	59.2	71.4	85.7	76.5	75.5	79.6	65.3	85.7
MD	93.9	93.9	95.9	91.8	100	60.2	60.2	61.2	58.2	59.2	64.3	63.3
DE	98.0	99.0	99.0	100.0	93.9	100	70.4	77.6	58.2	68.4	58.2	72.4
NJ	100.0	100.0	100.0	100.0	96.9	99.0	100	80.6	75.5	79.6	70.4	90.8
NY	100.0	100.0	100.0	100.0	96.9	99.0	100.0	100	70.4	72.4	70.4	84.7
RI	99.0	99.0	95.9	99.0	92.9	100.0	99.0	99.0	100	82.7	64.3	75.5
MA	100.0	100.0	99.0	100.0	93.9	99.0	100.0	100.0	99.0	100	65.3	80.6
CT	91.8	90.8	93.9	91.8	95.9	91.8	89.8	92.9	90.8	90.8	100	74.5
Beaufort-JCP	100.0	99.0	98.0	100.0	93.9	99.0	99.0	100.0	99.0	99.0	90.8	100.0

Table 4. Mean CVs of inter-lab age comparisons (n=98). Mean CVs greater than 5% are highlighted in red. Ethel Hall (EAH) is the current reader of menhaden scales at the NOAA-Beaufort Lab and Jennifer Potts (JCP) is the future reader of menhaden scales at the NOAA-Beaufort Lab.

Lab	NC	ODU	VIMS	MD	DE	NJ	NY	RI	MA	CT	Beaufort-JCP
Beaufort-EAH	6.3	7.5	4.9	11.2	8.3	3.7	4.4	8.4	6.2	9.6	2.1
NC	-	8.6	8.2	13.4	9.2	6.9	6.8	11.6	8.8	10.5	6.2
ODU	-	-	8.0	11.0	12.6	6.8	7.3	11.6	8.7	10.6	7.1
VIMS	-	-	-	13.0	9.8	4.3	7.7	9.2	6.8	11.4	4.4
MD	-	-	-	-	12.6	11.9	11.9	14.6	13.0	10.5	11.5
DE	-	-	-	-	-	9.5	7.4	14.9	10.8	14.0	8.8
NJ	-	-	-	-	-	-	6.0	9.4	6.4	9.8	2.7
NY	-	-	-	-	-	-	-	11.2	9.0	9.7	4.9
RI	-	-	-	-	-	-	-	-	6.6	13.7	9.1
MA	-	-	-	-	-	-	-	-	-	11.9	6.3
CT	-	-	-	-	-	-	-	-	-	-	8.6

Table 5. Marginal increment analysis of Atlantic menhaden scales collected from fishery-dependent sampling in 2013 and aged by the NOAA Beaufort Laboratory. Units are Eberbach units.

Month Collected	N	Mean	Standard Deviation	Minimum	Maximum
May	6	35.33	17.90	4	59
June	140	18.85	5.72	6	45
July	199	33.58	8.04	14	61
August	266	40.59	8.73	21	81
September	173	44.65	7.42	25	69
October	83	47.67	10.61	18	90
November	30	50.10	10.39	32	78
December	5	43.20	7.76	35	56

Table 6. Distribution of the distance (Eberbach units) between the scale focus and first annulus for Atlantic menhaden scales collected from fishery-dependent sampling in 2013 and aged by the NOAA Beaufort Laboratory.

N	Mean	Standard Deviation	Minimum	Maximum
2222	113.14	18.56	55	162

Figures

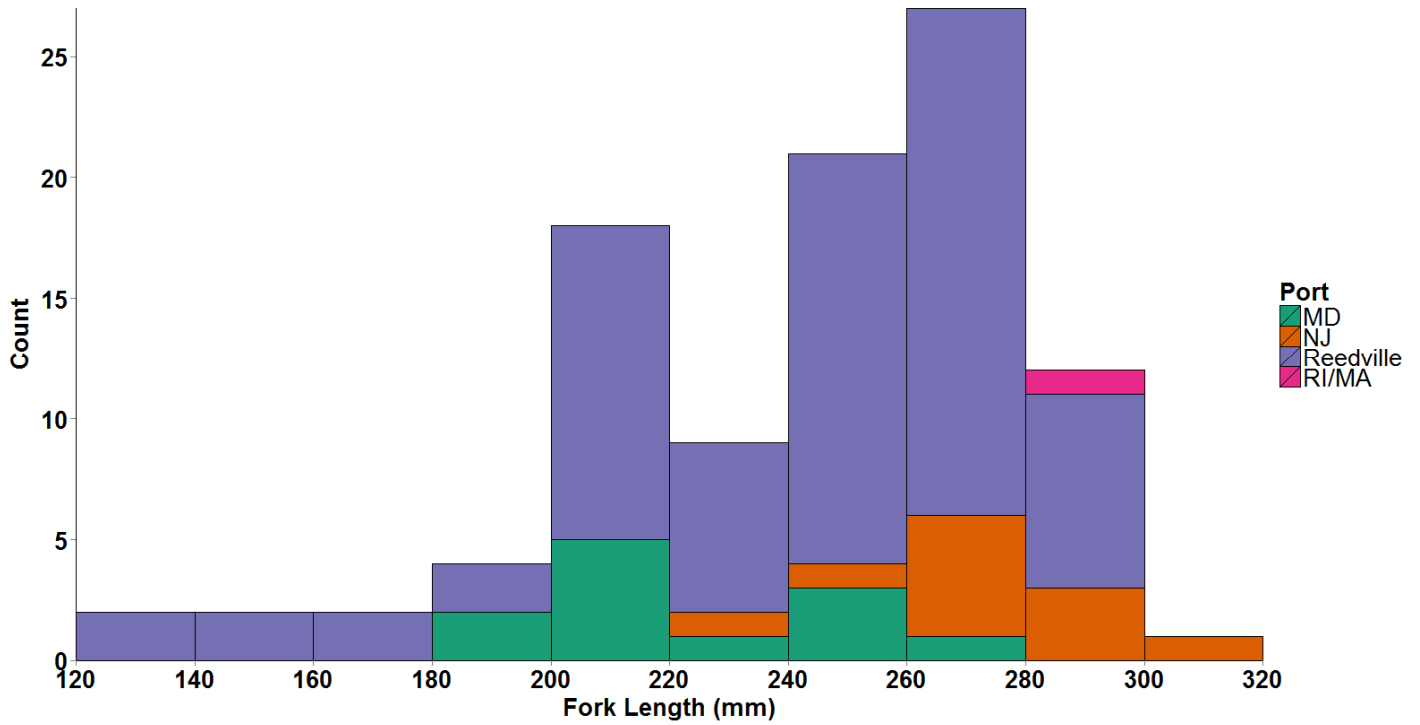


Figure 1. Length frequency of Atlantic menhaden in the reference set by port collected.

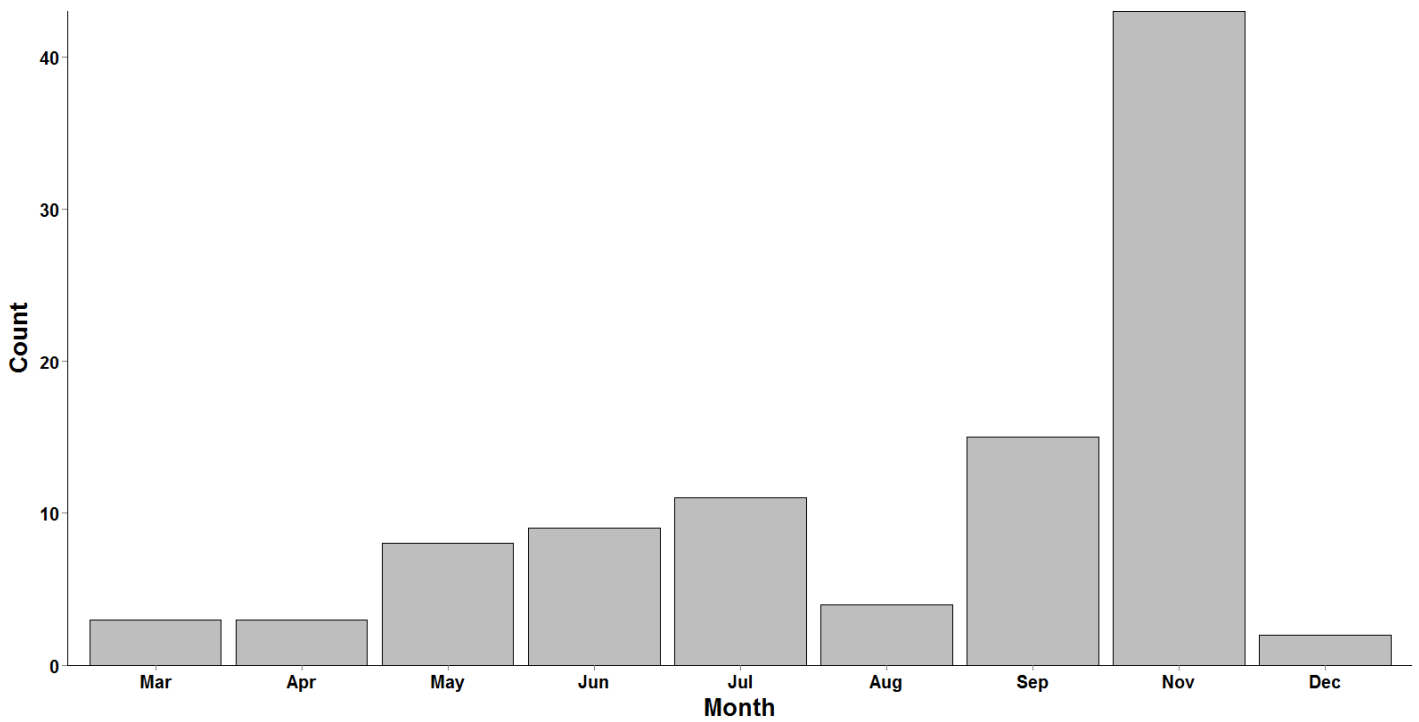
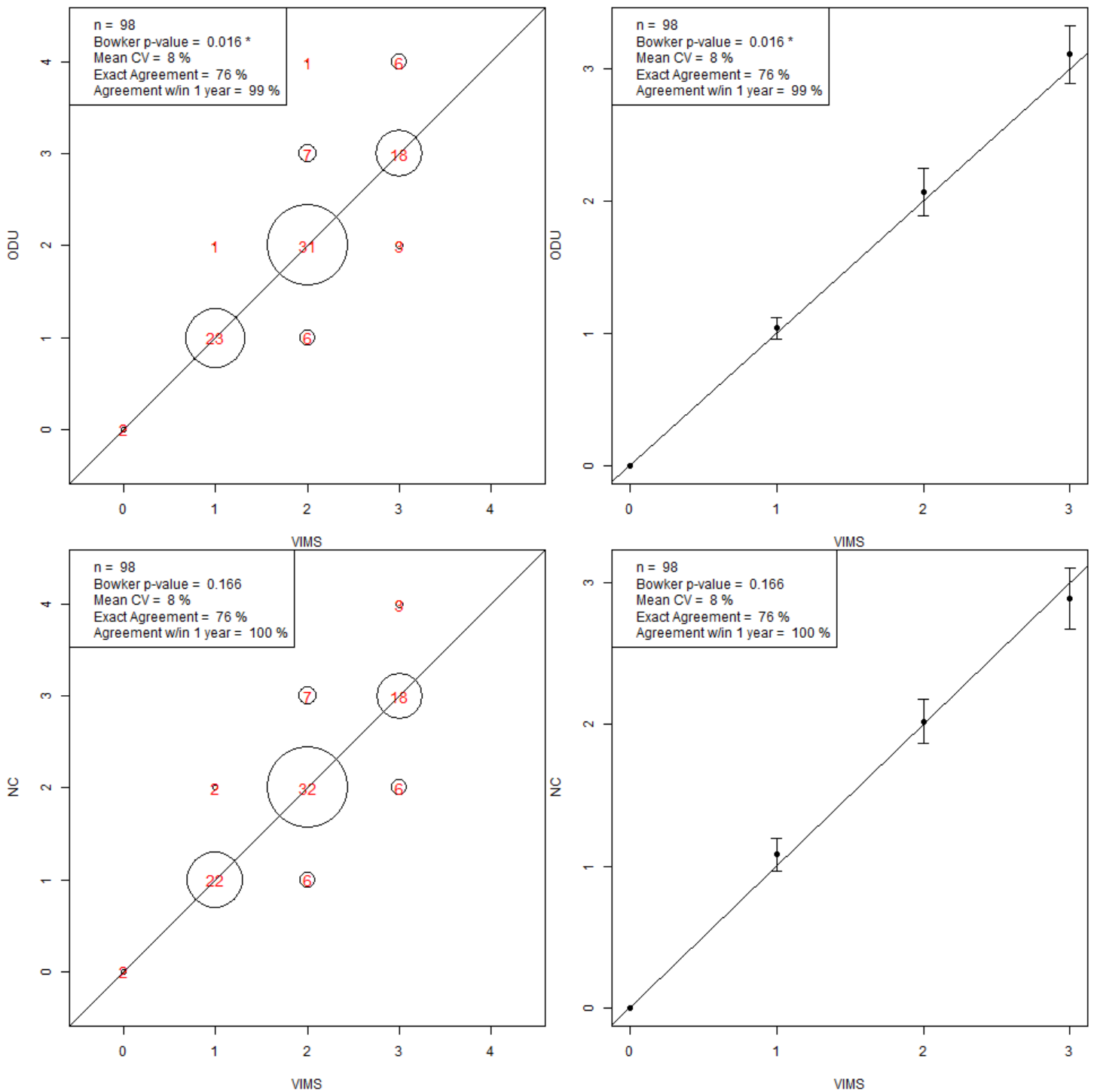
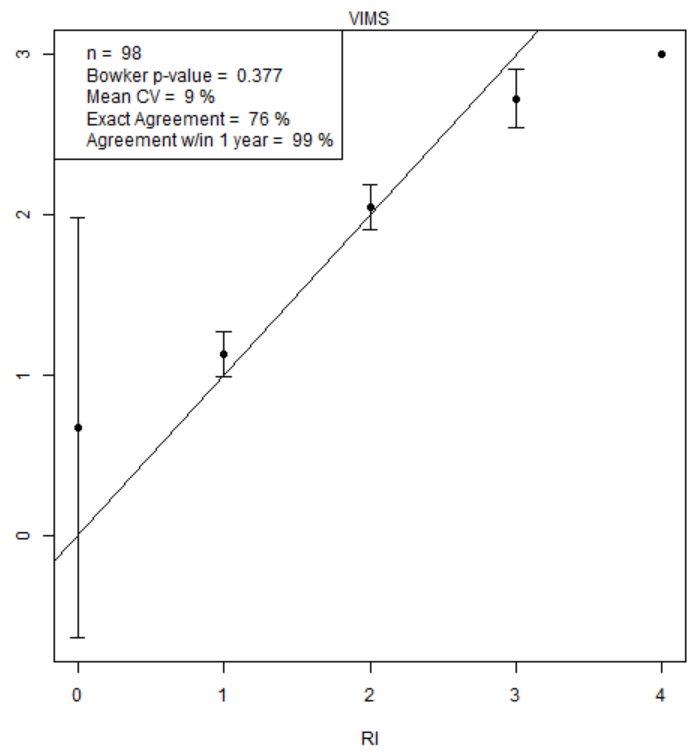
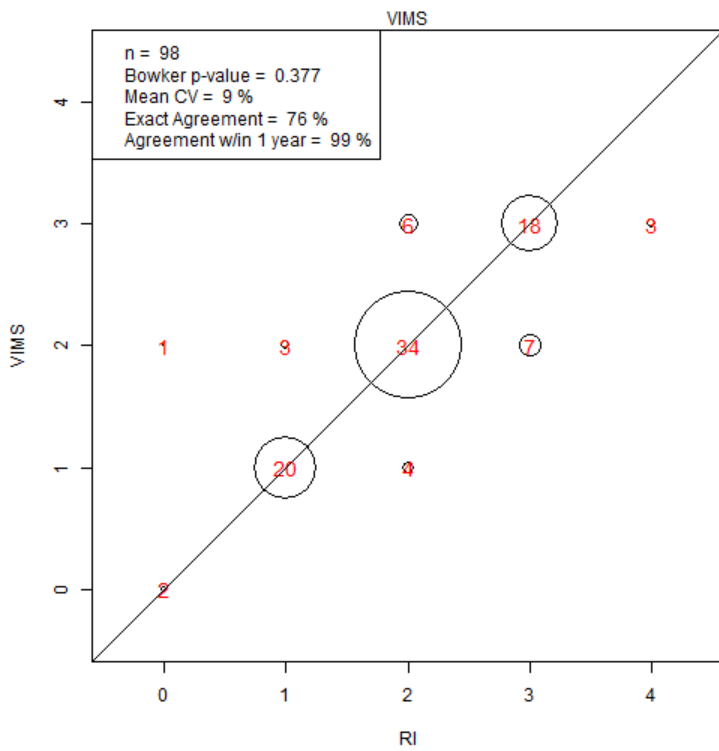
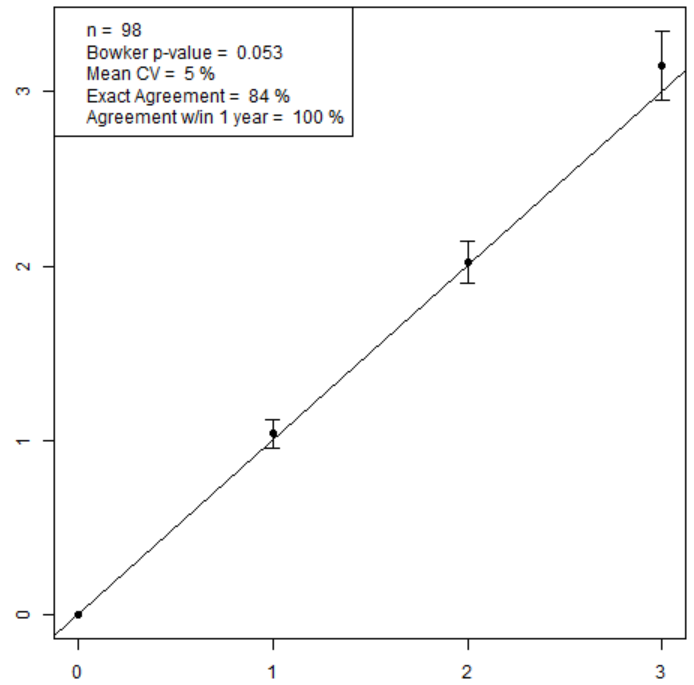
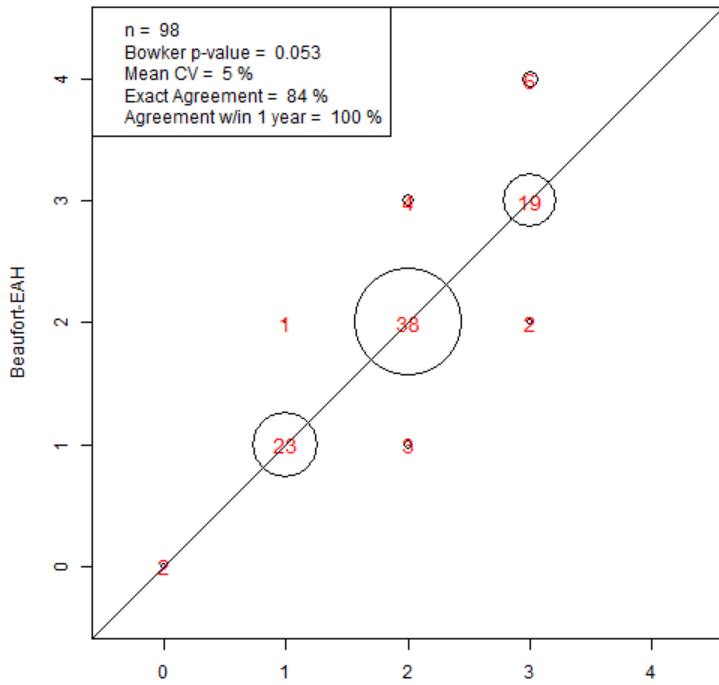
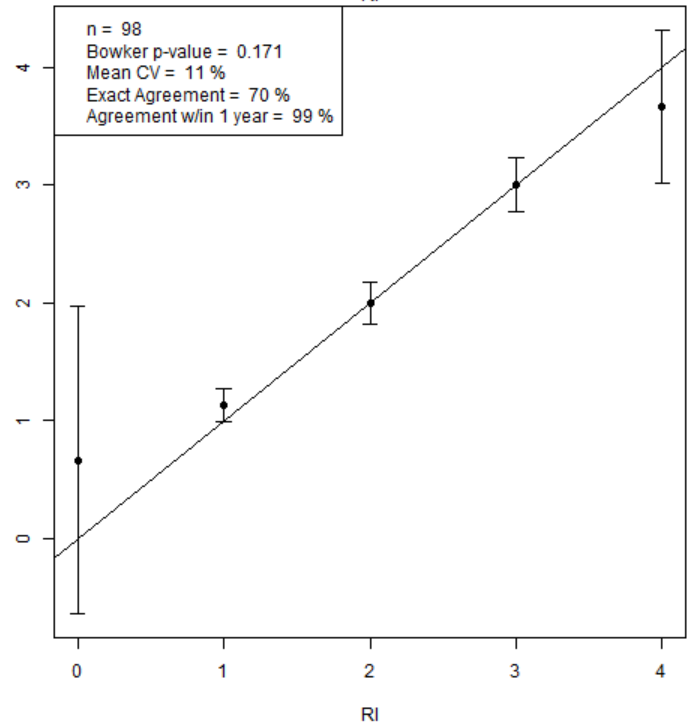
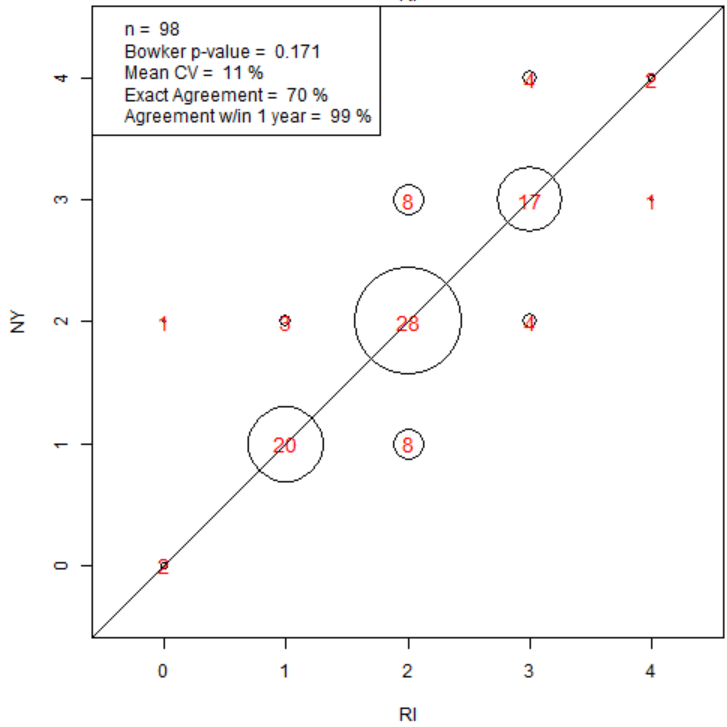
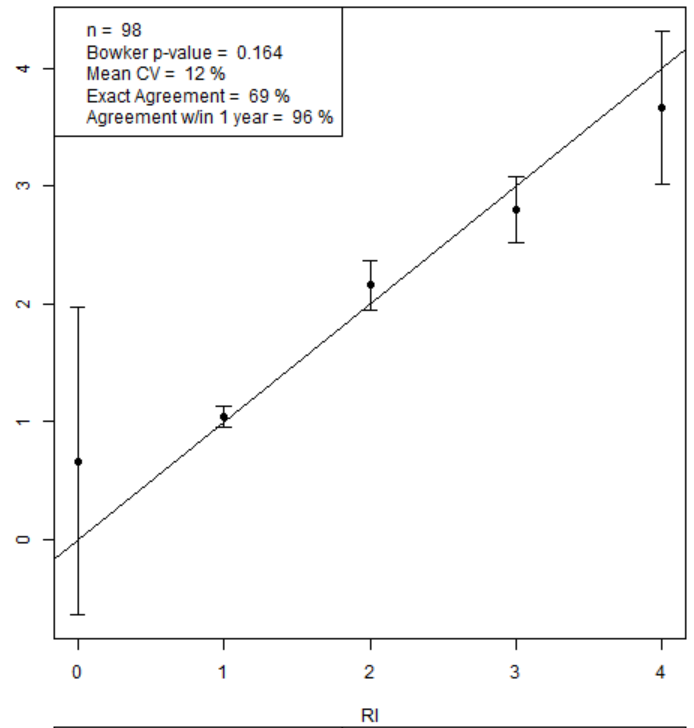
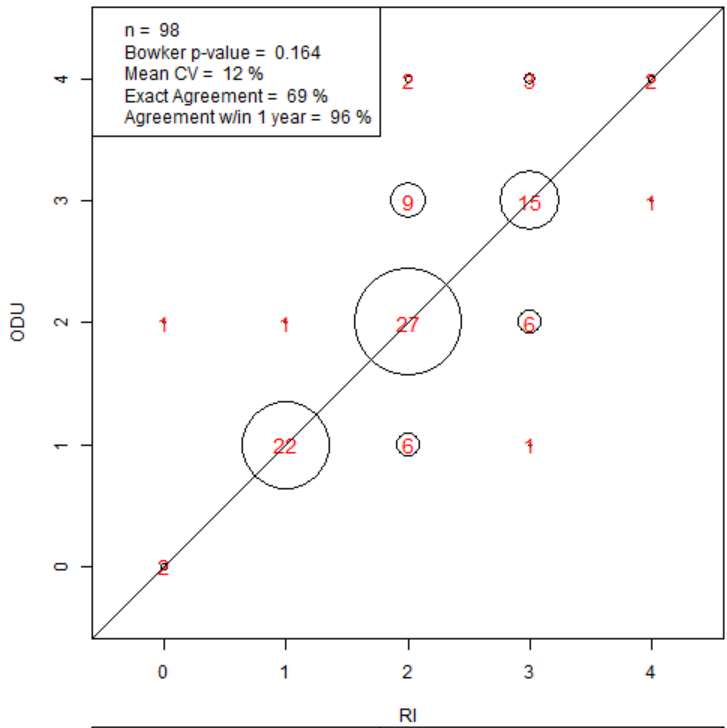


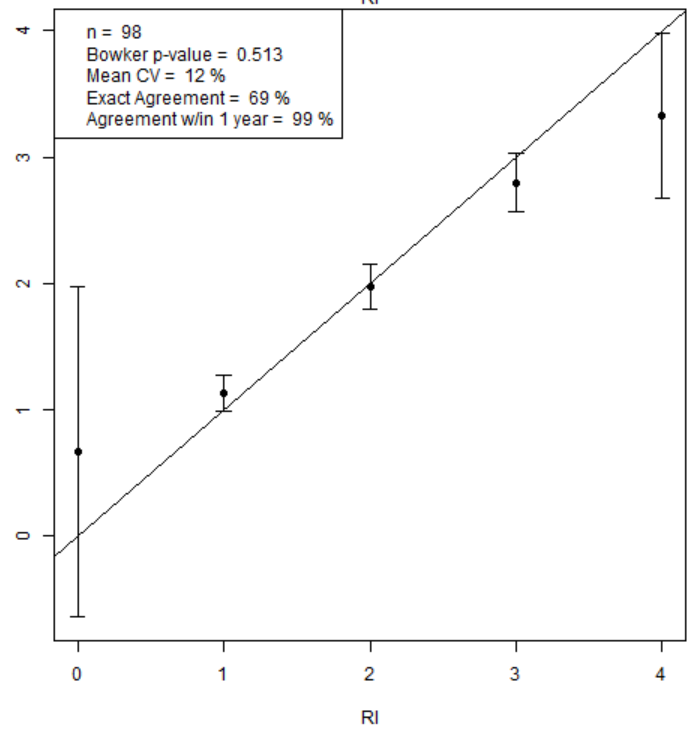
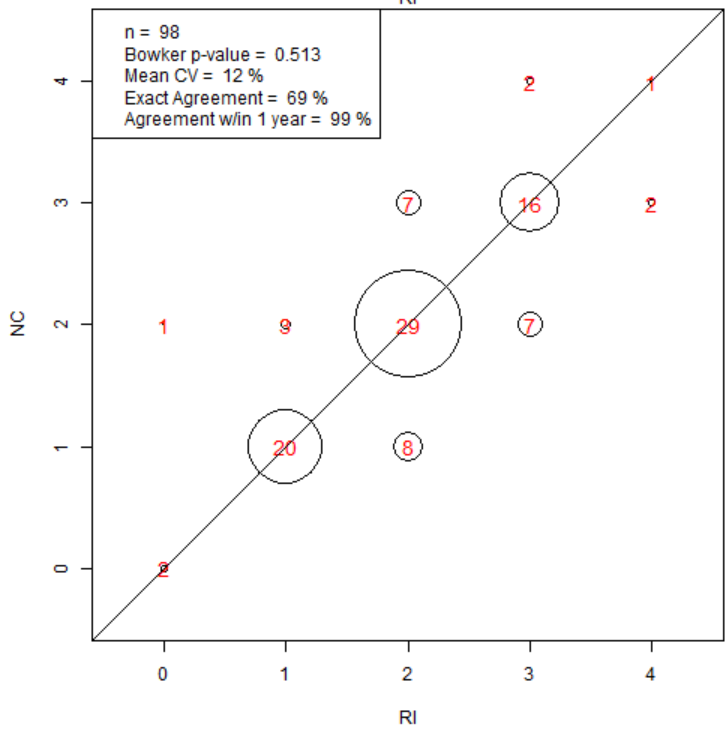
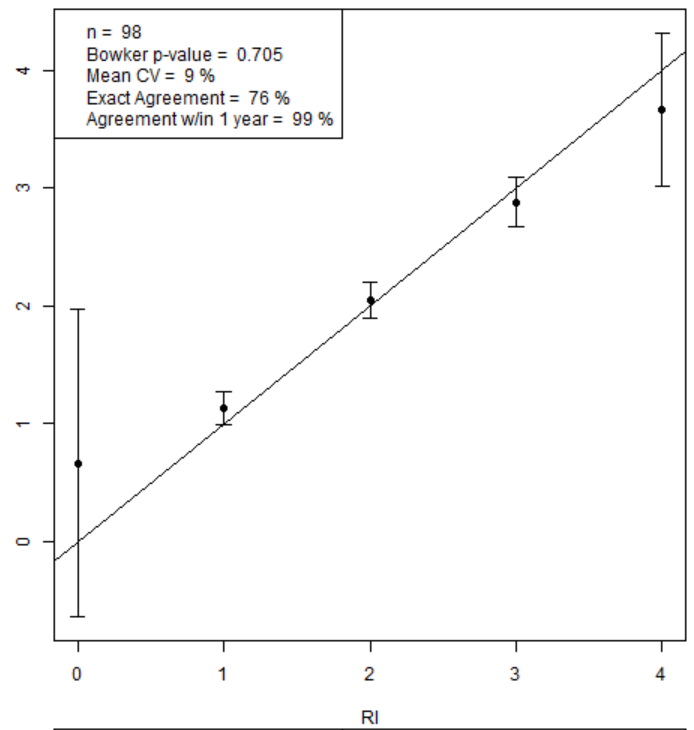
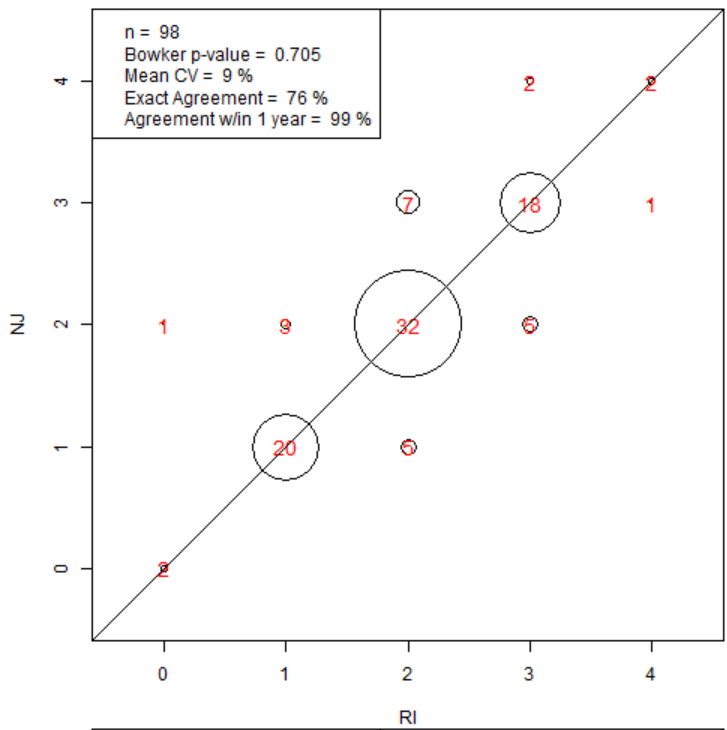
Figure 2. Capture month of Atlantic menhaden in the reference set.

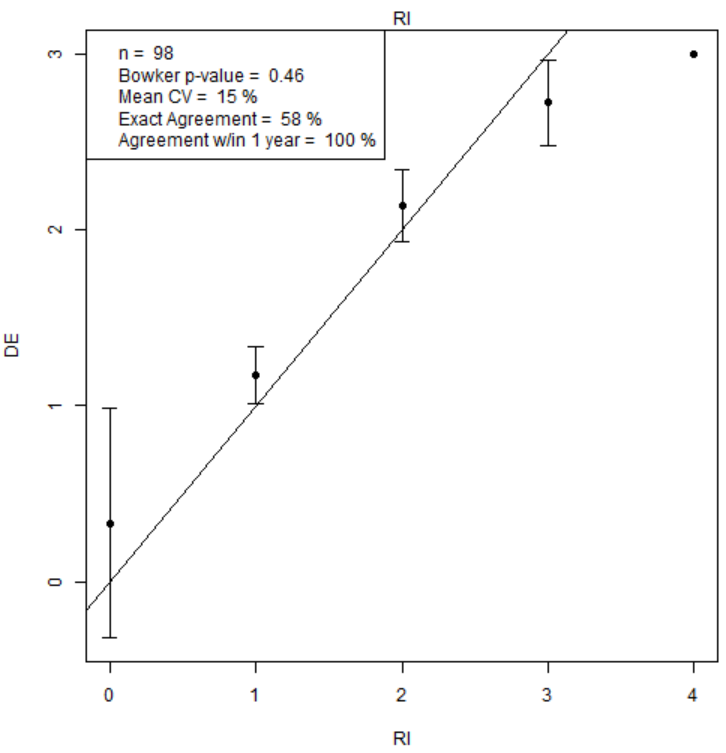
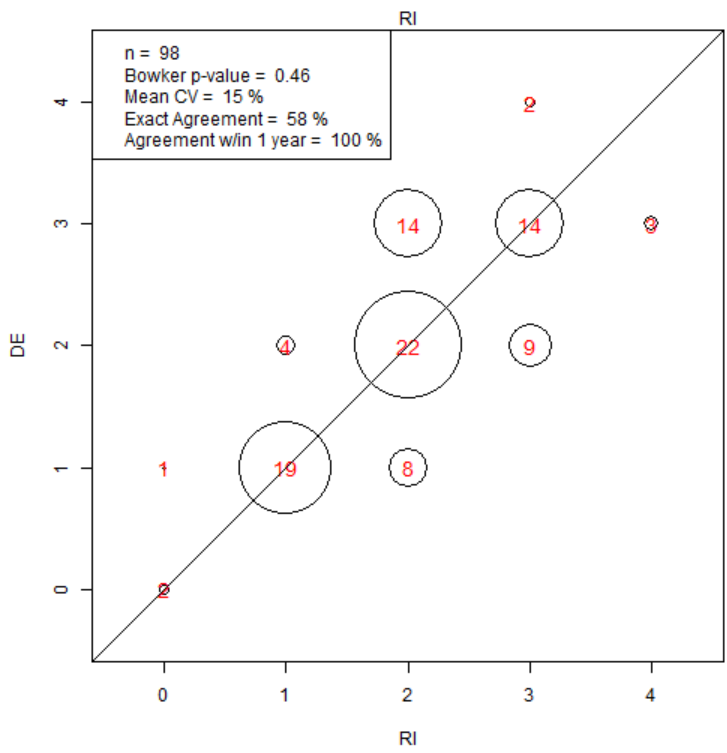
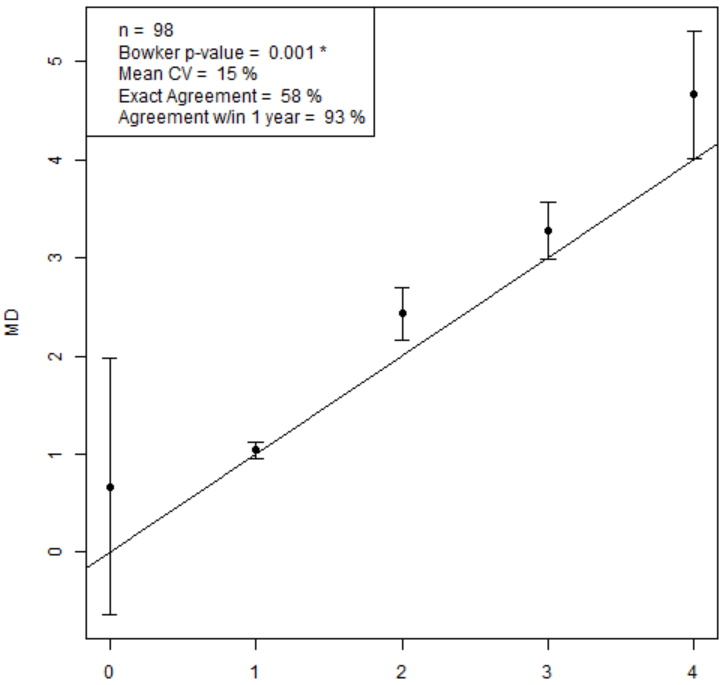
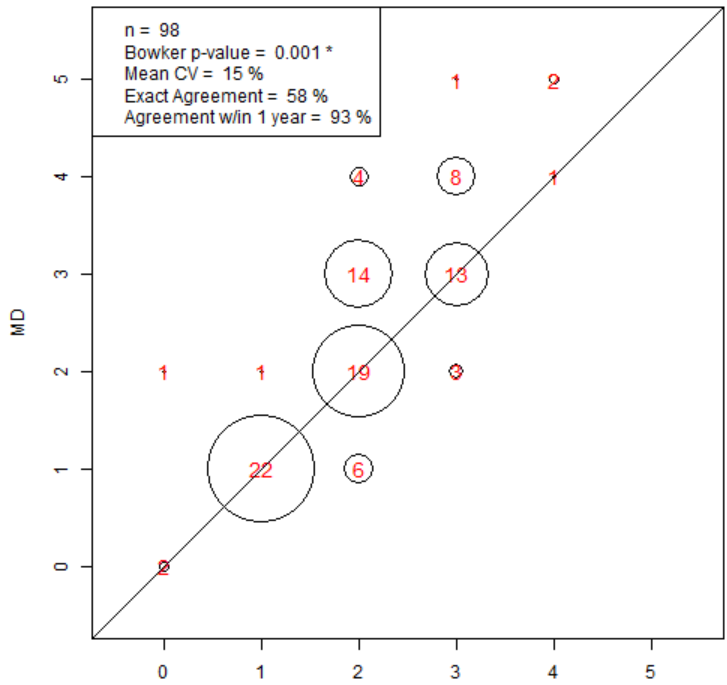


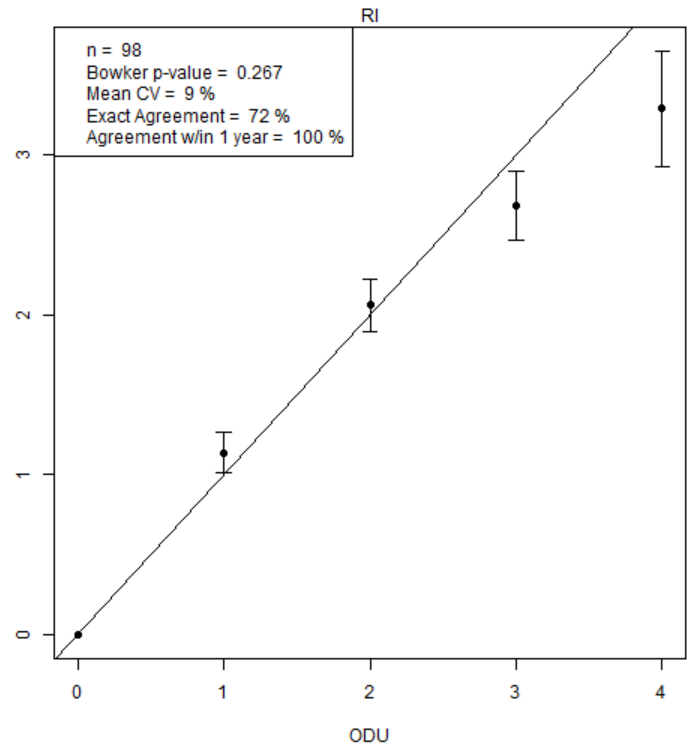
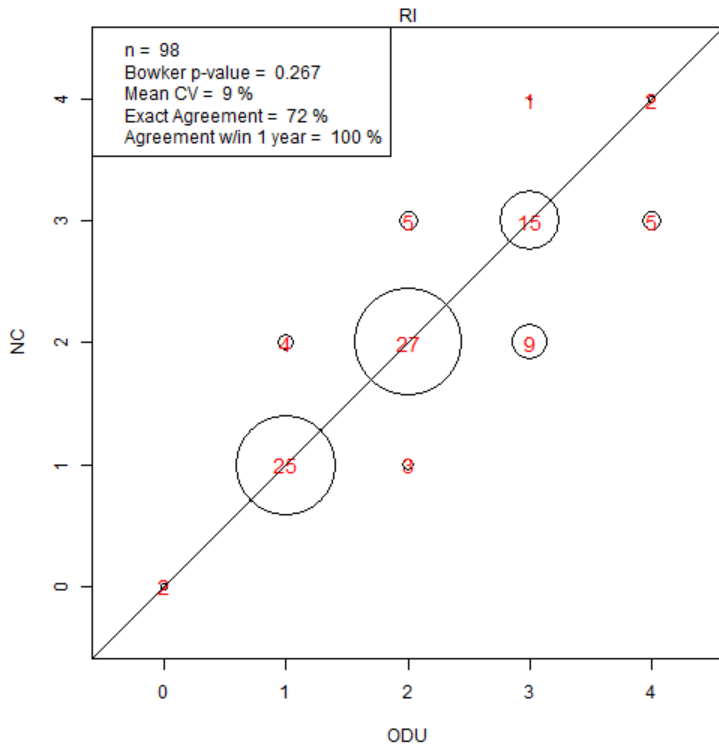
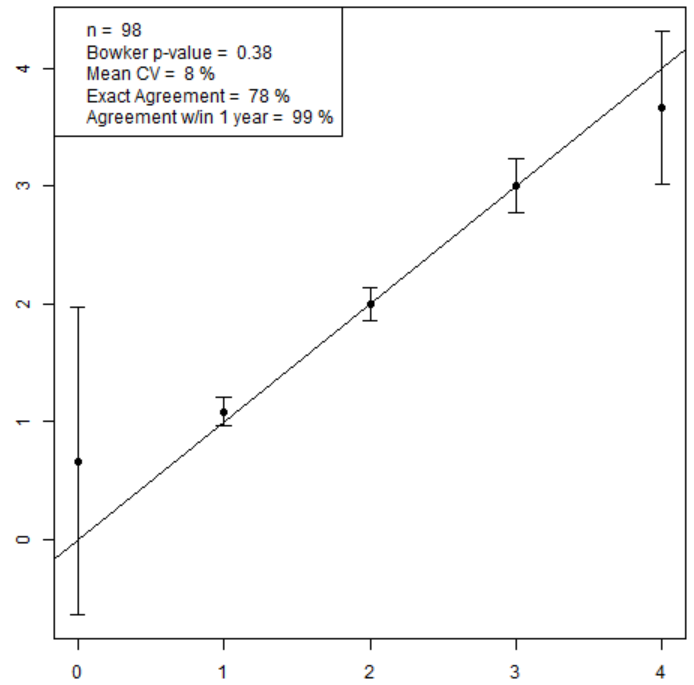
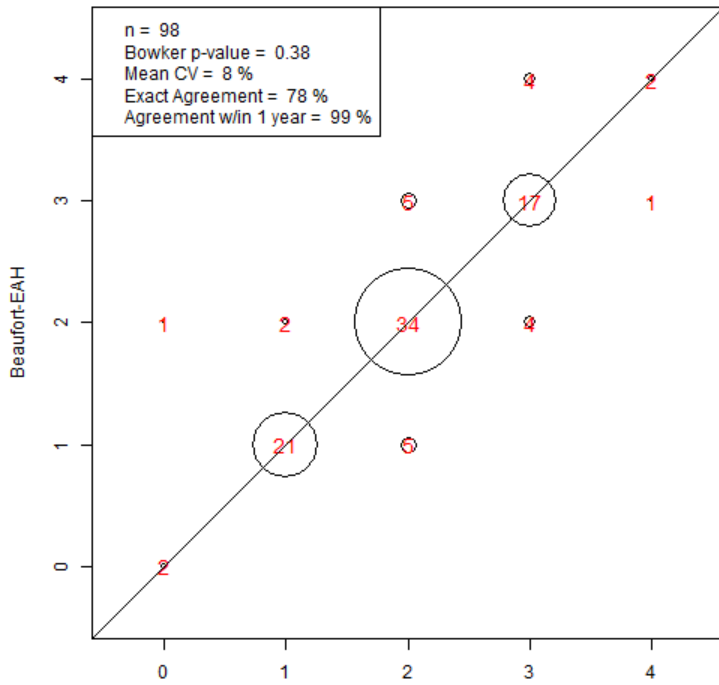
Figures 3-88. Age frequency and age bias plots for inter-lab age comparisons. Significant p-values ($\alpha < 0.05$) from a Bowker's test of symmetry indicating systematic differences between labs are noted with asterisks. Ethel Hall (EAH) is the current reader of menhaden scales at the NOAA-Beaufort Lab and Jennifer Potts (JCP) is the future reader of menhaden scales at the NOAA-Beaufort Lab.

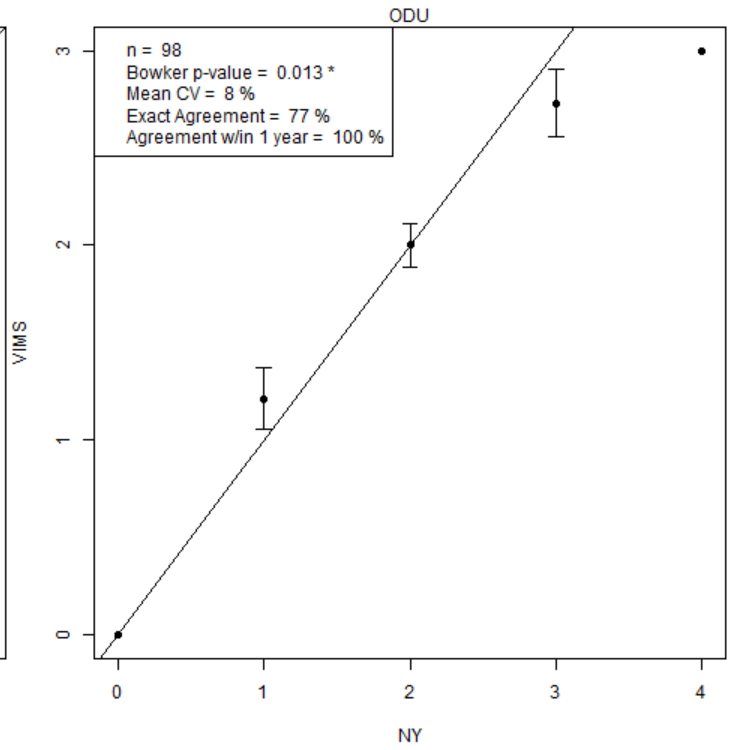
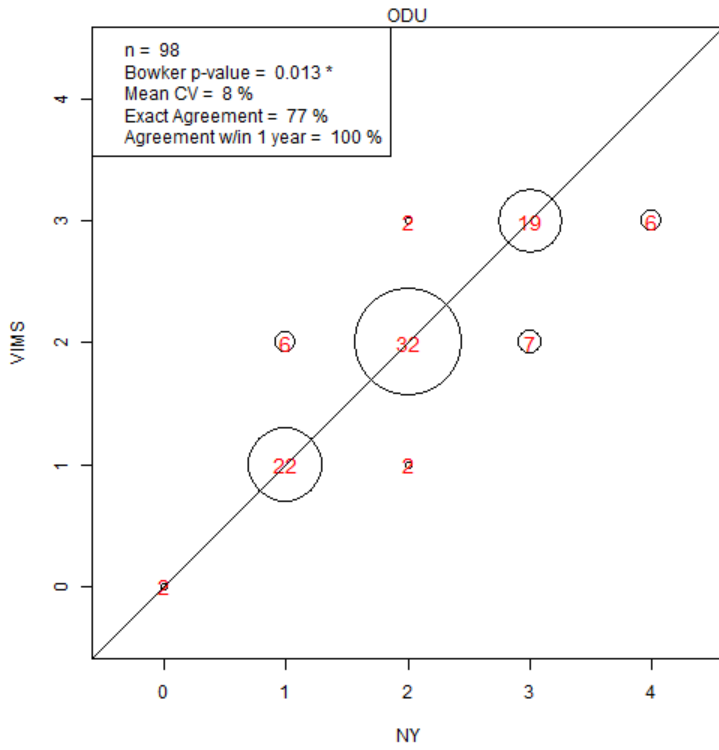
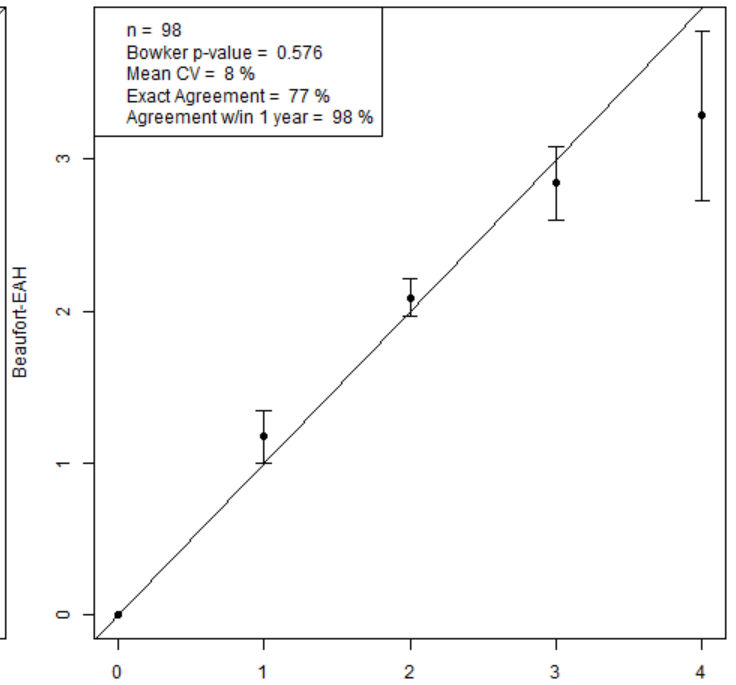
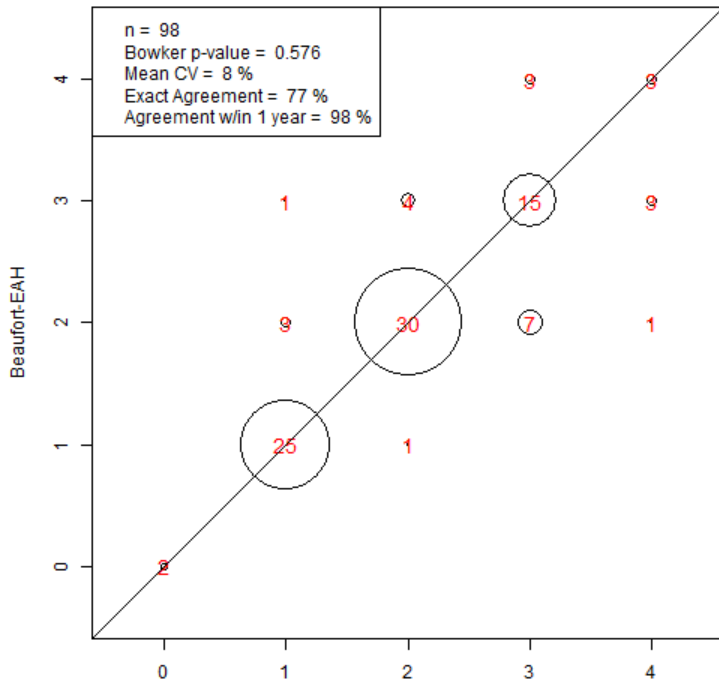


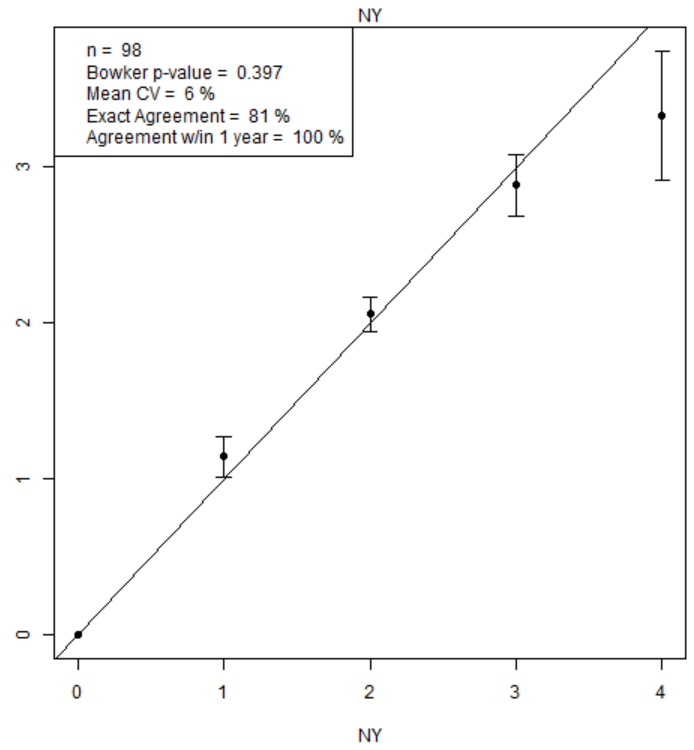
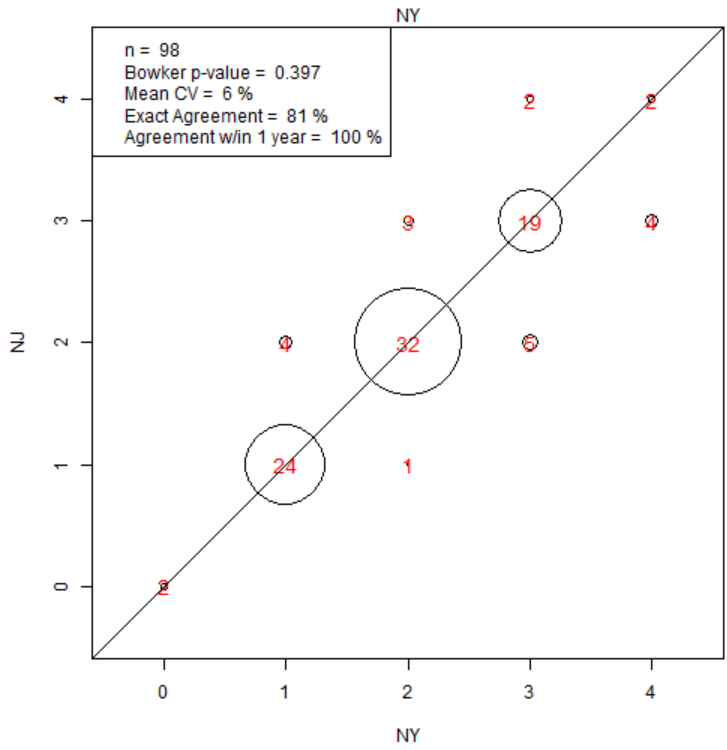
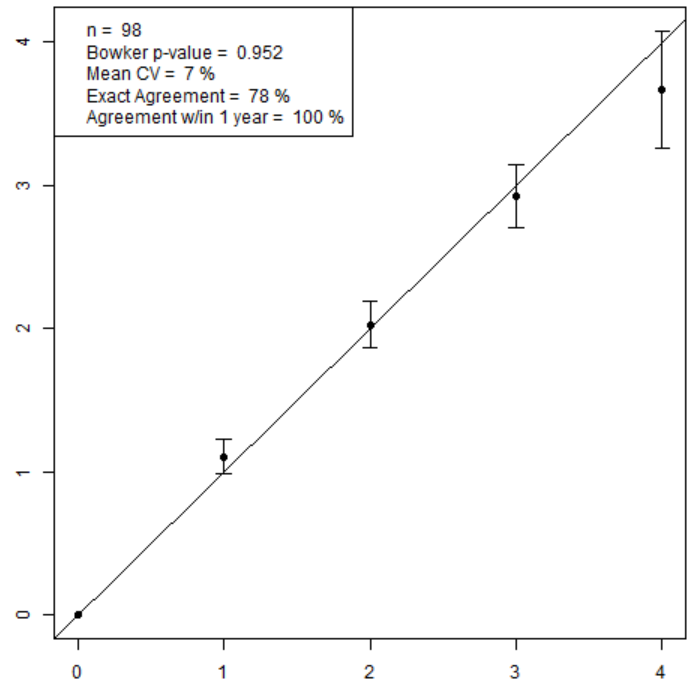
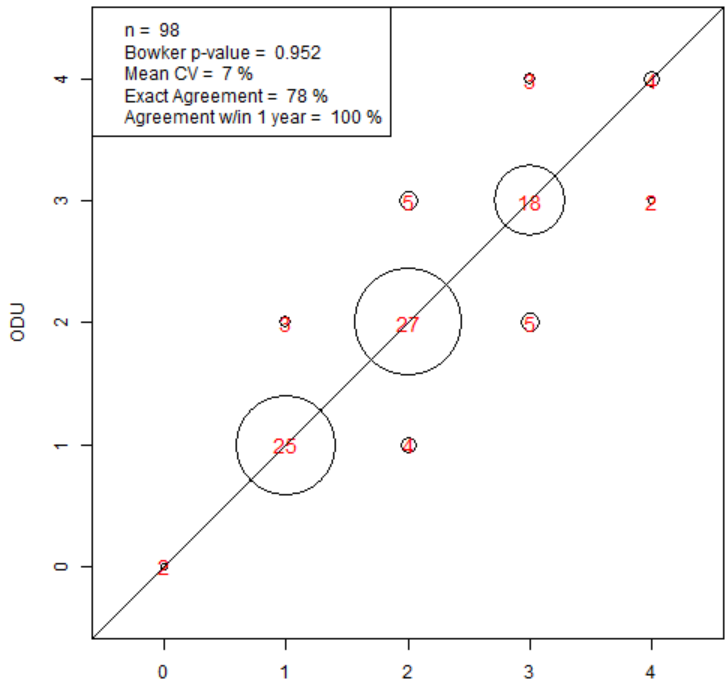


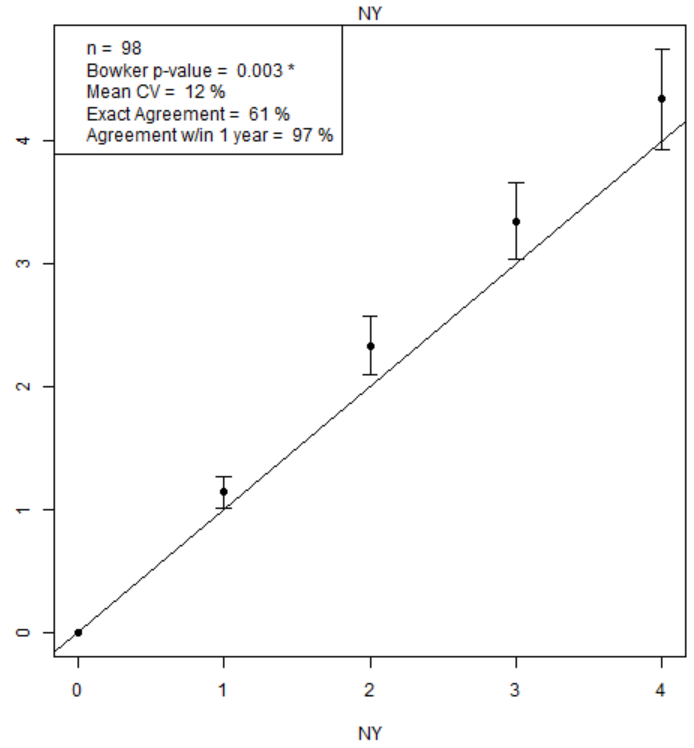
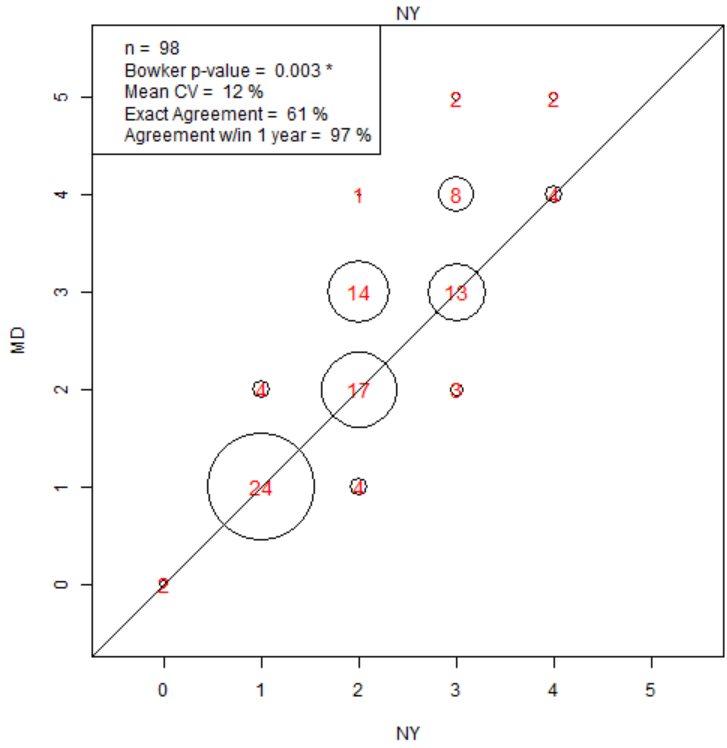
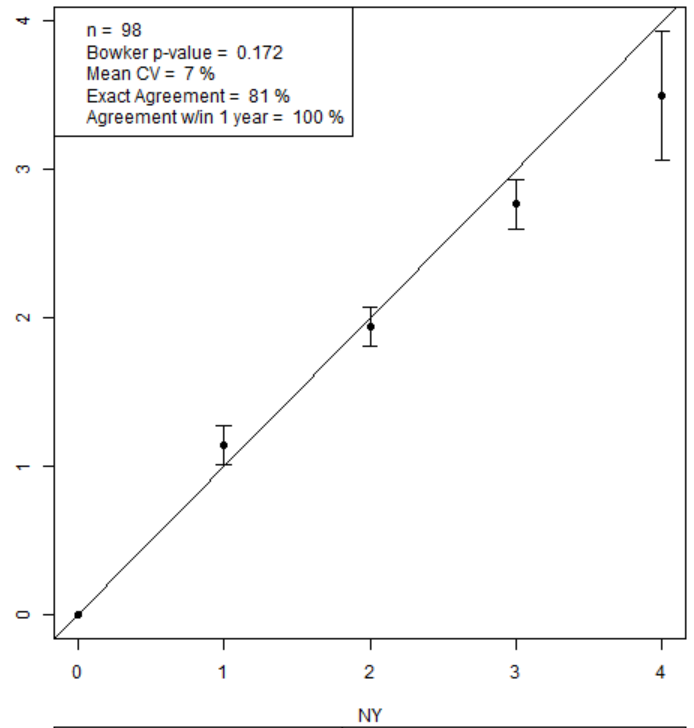
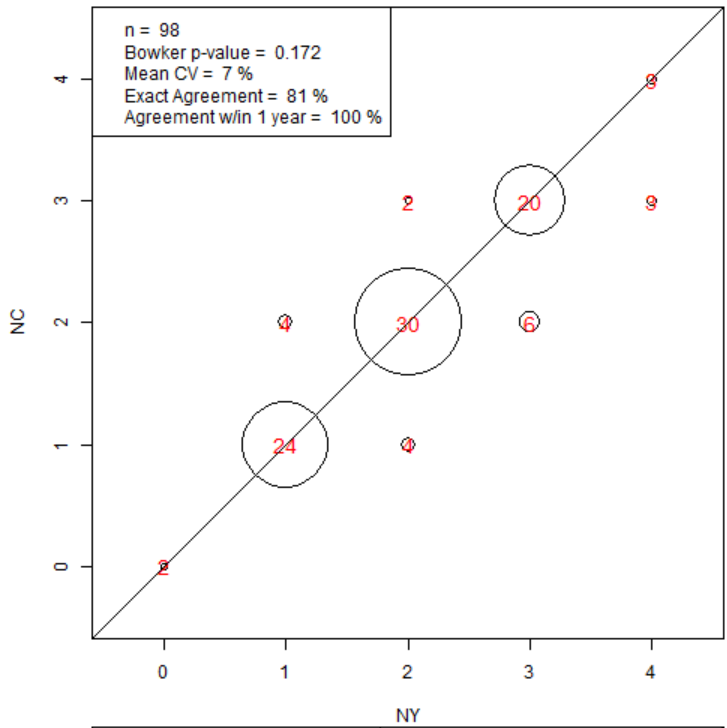


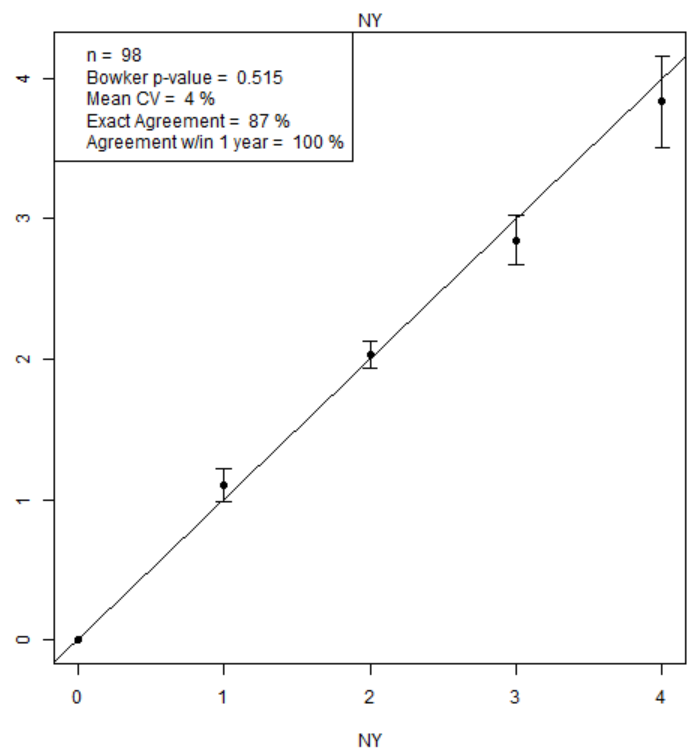
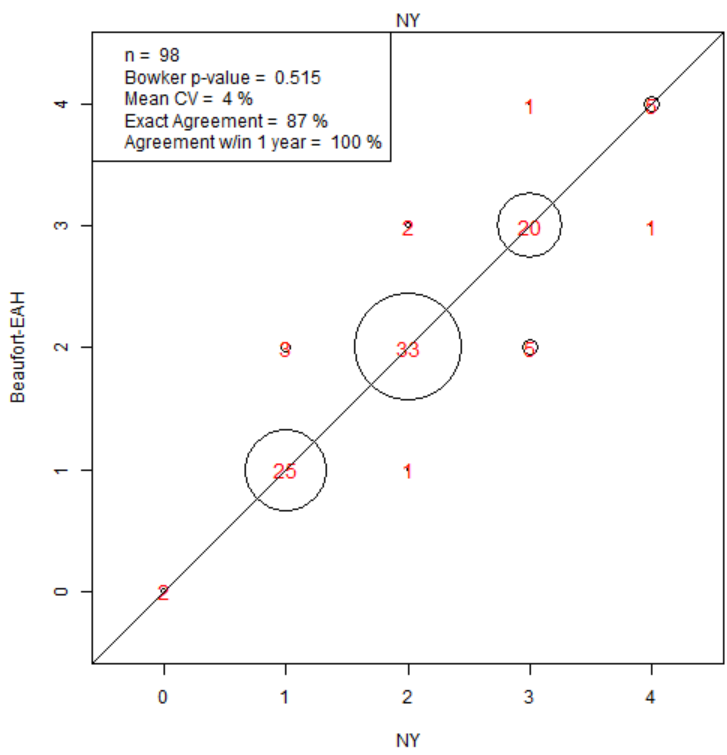
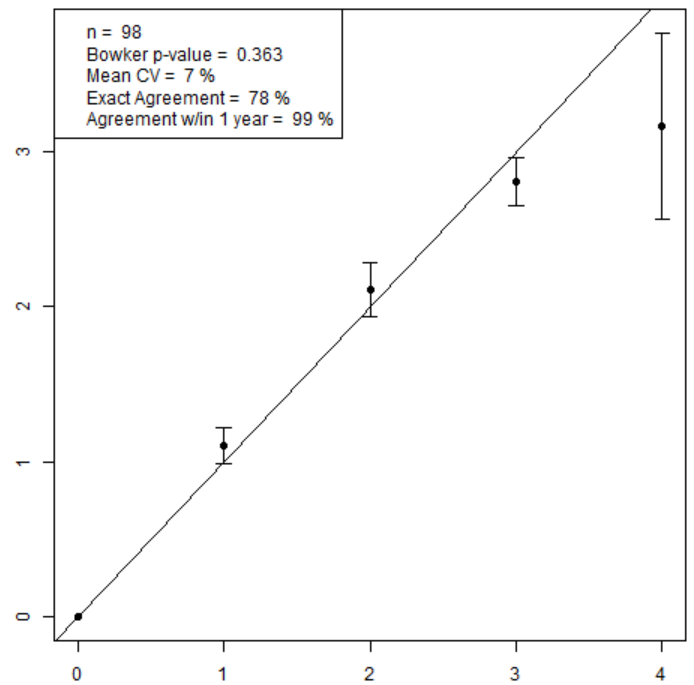
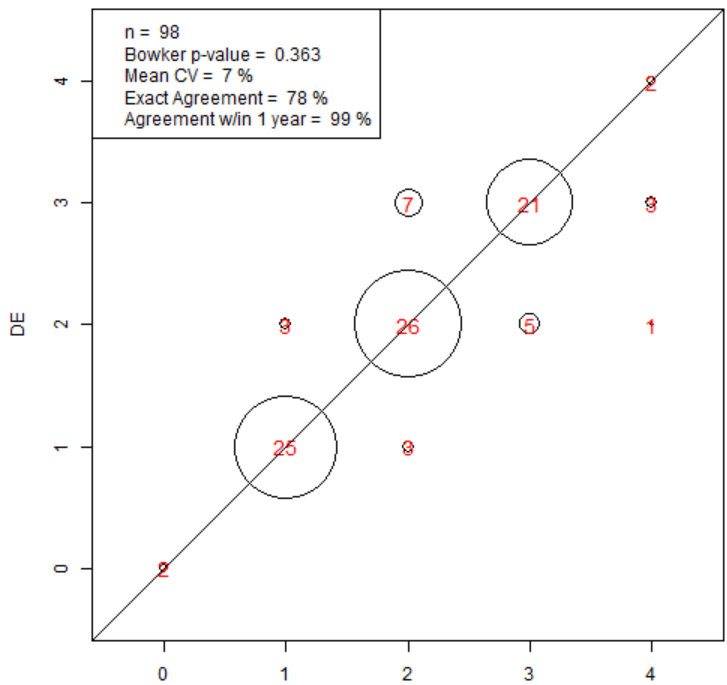


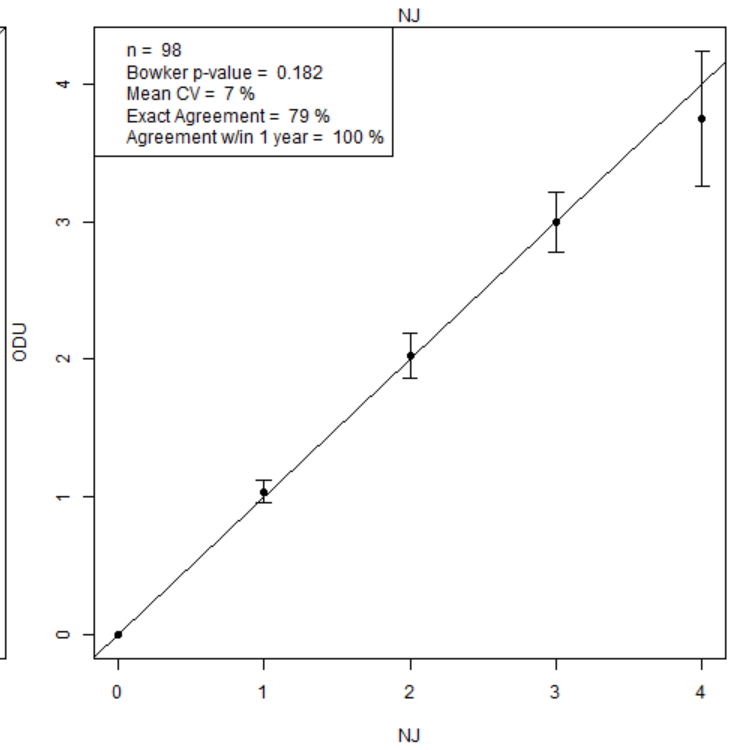
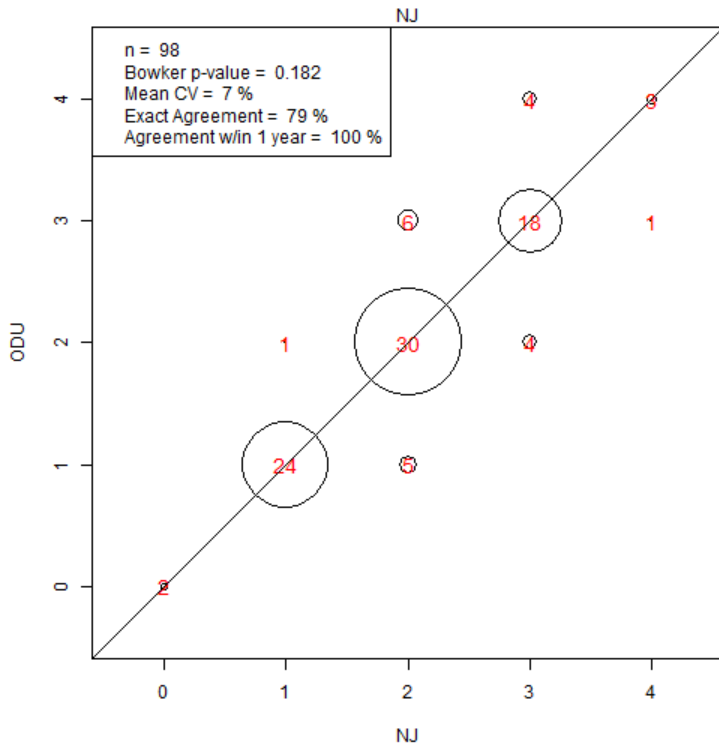
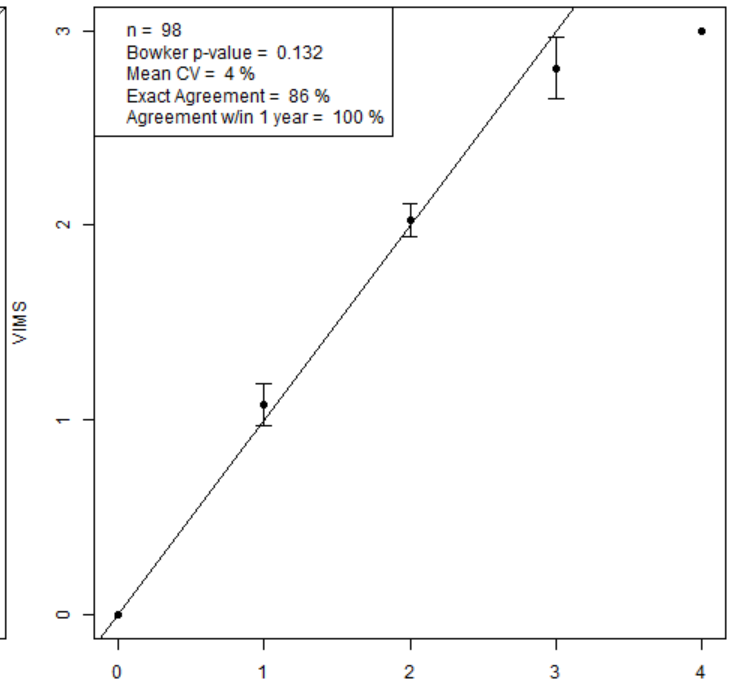
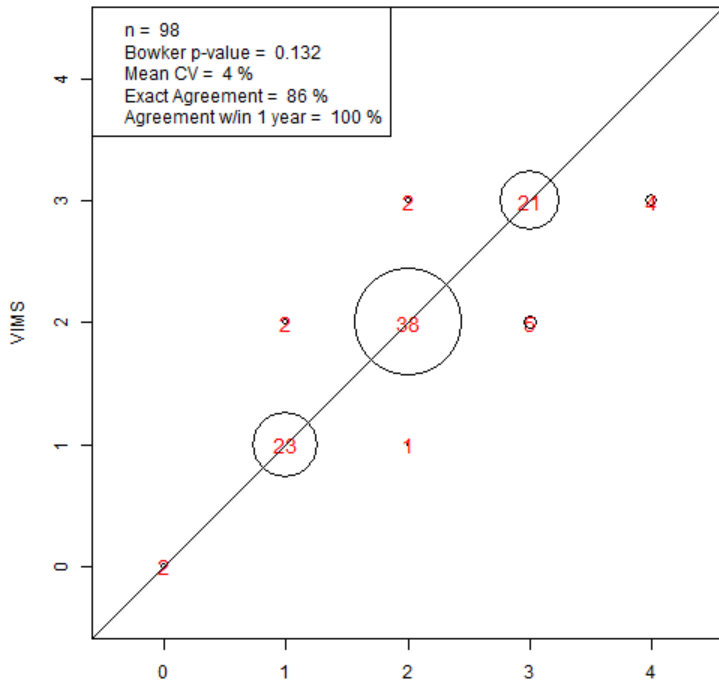


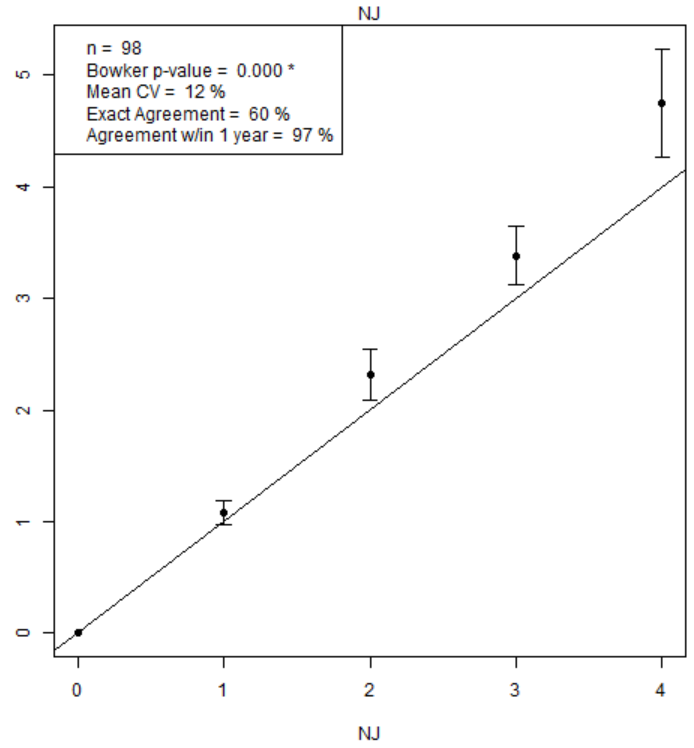
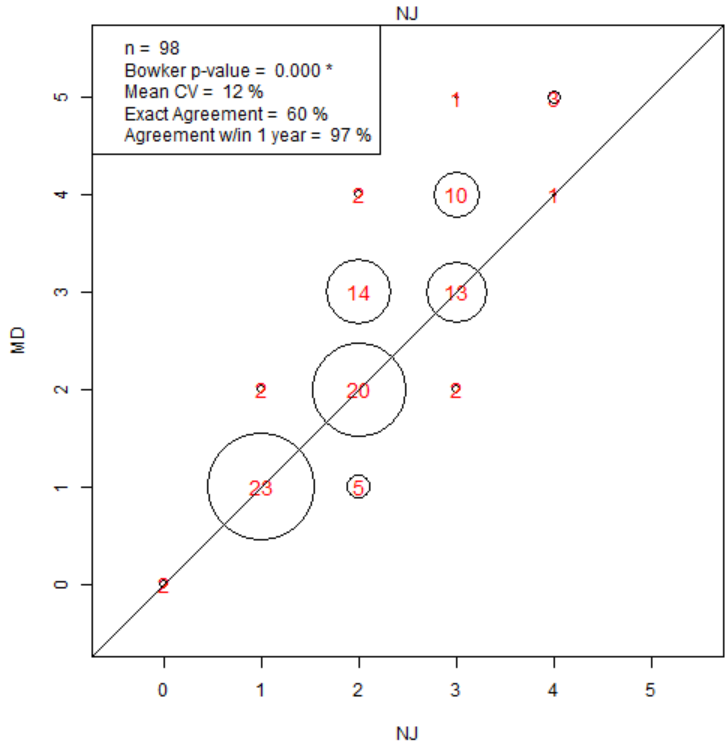
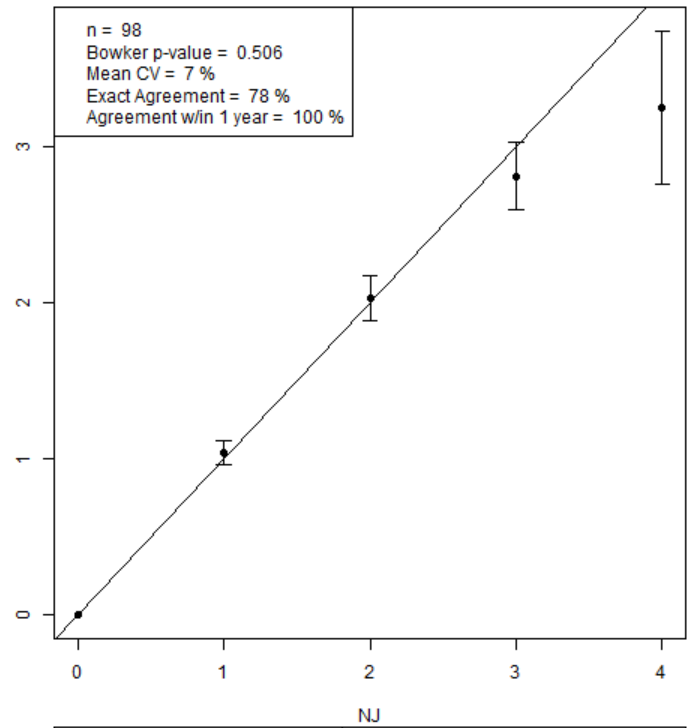
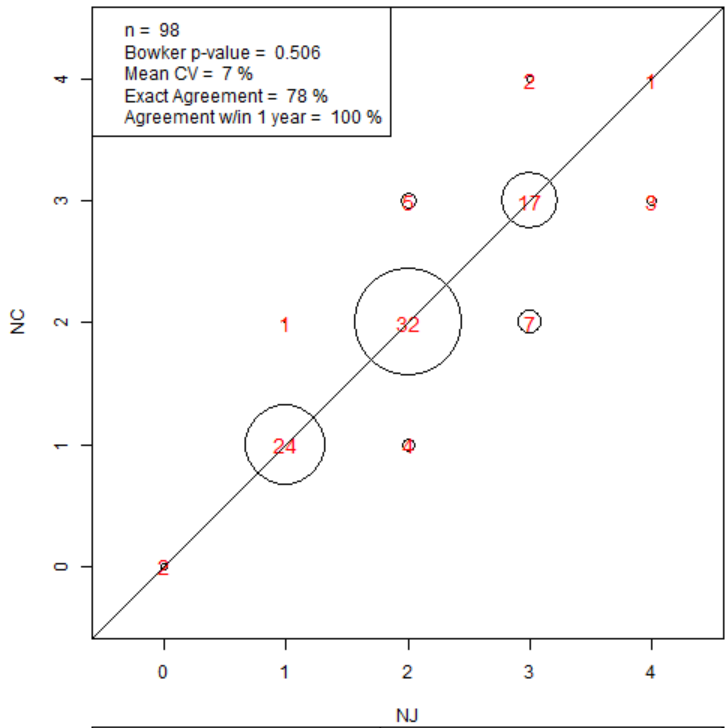


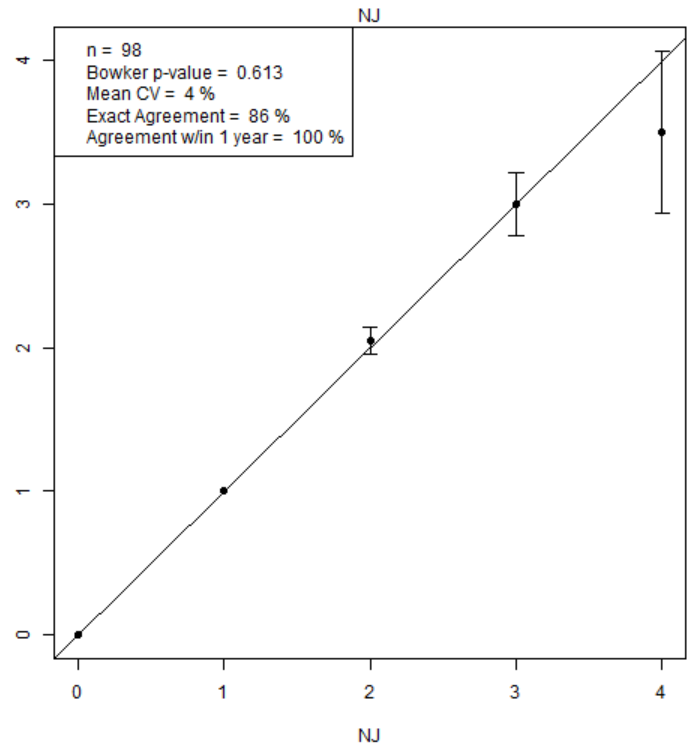
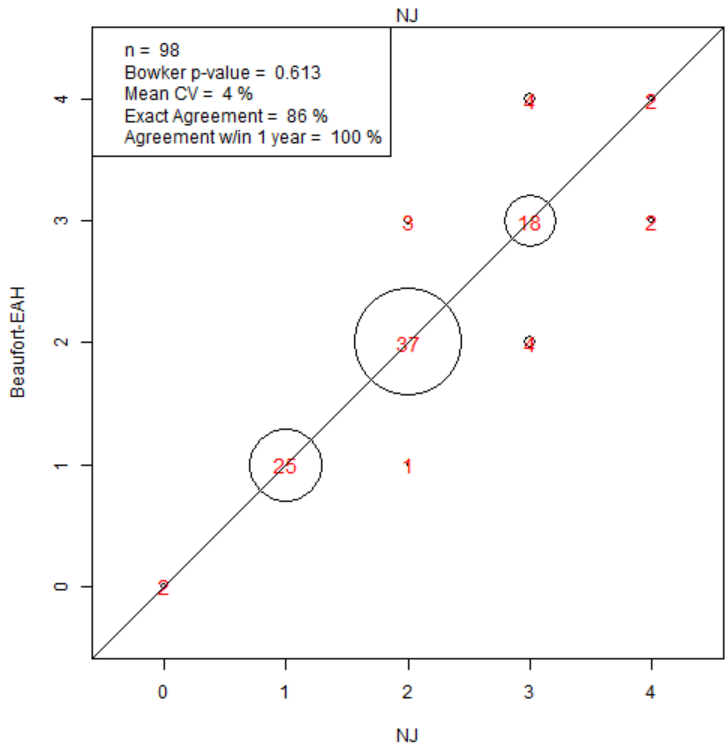
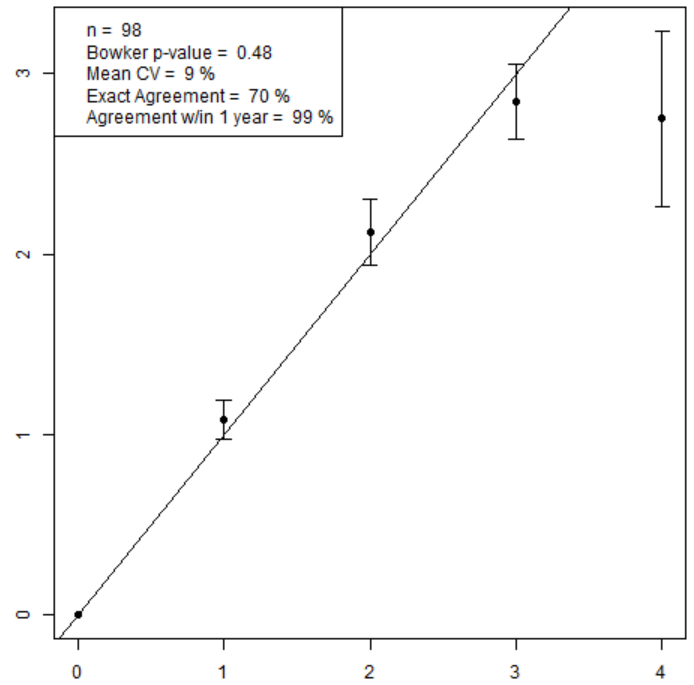
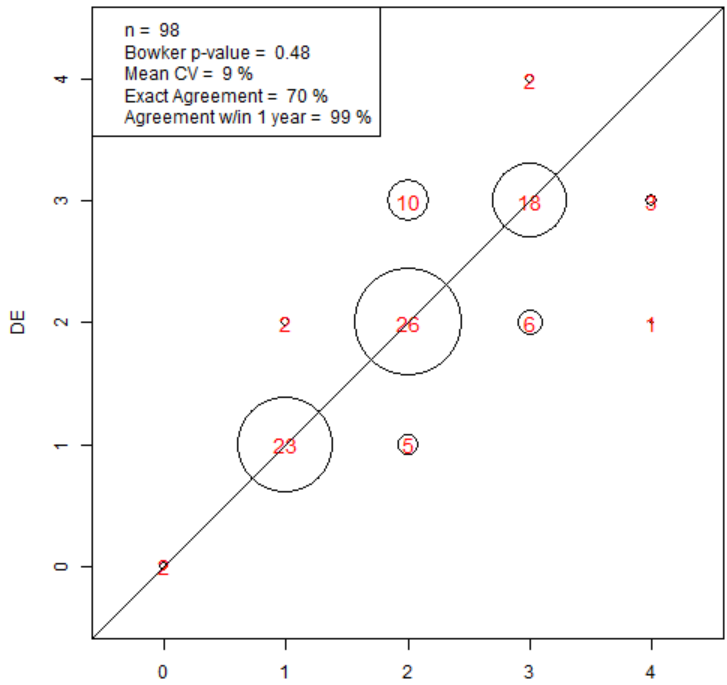


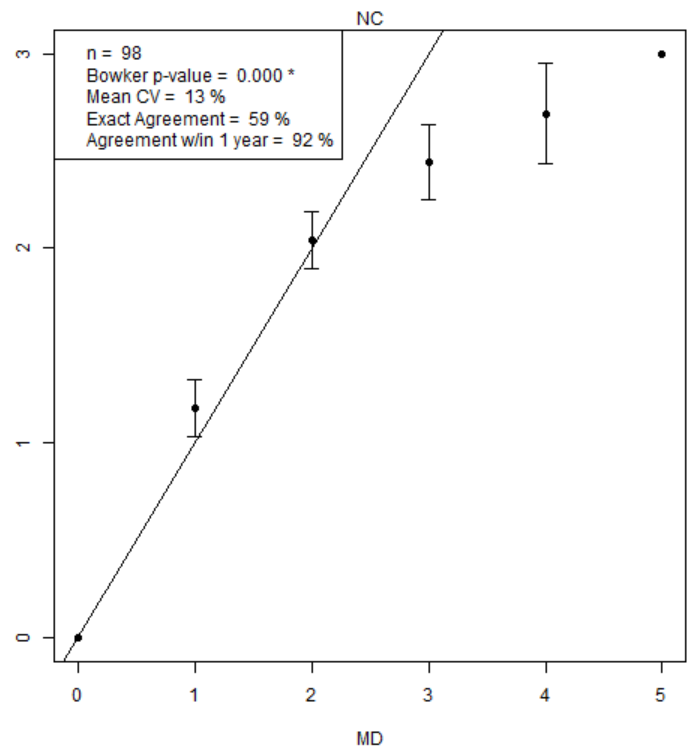
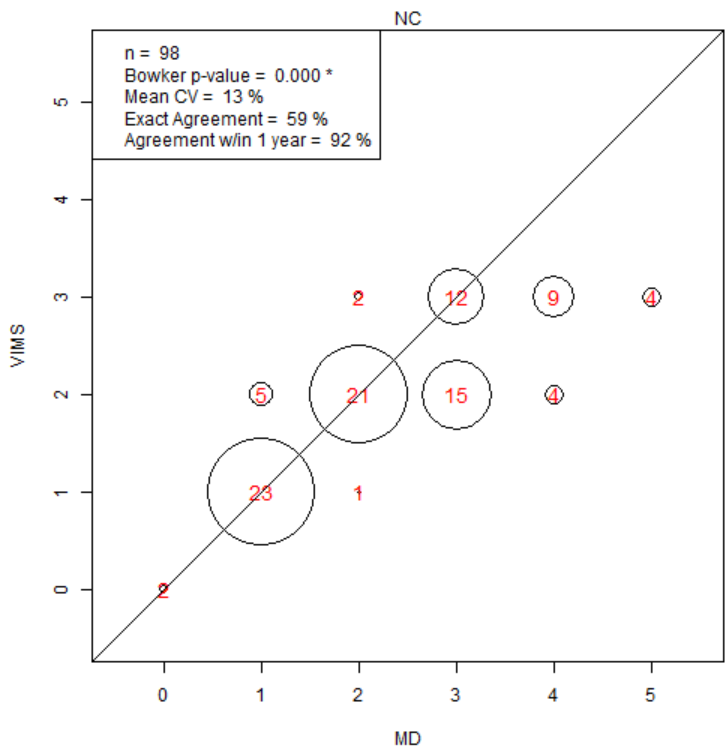
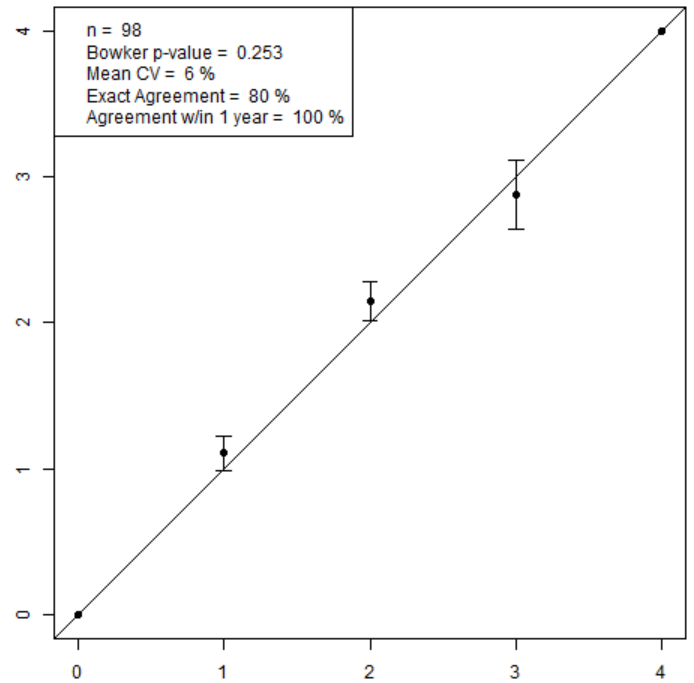
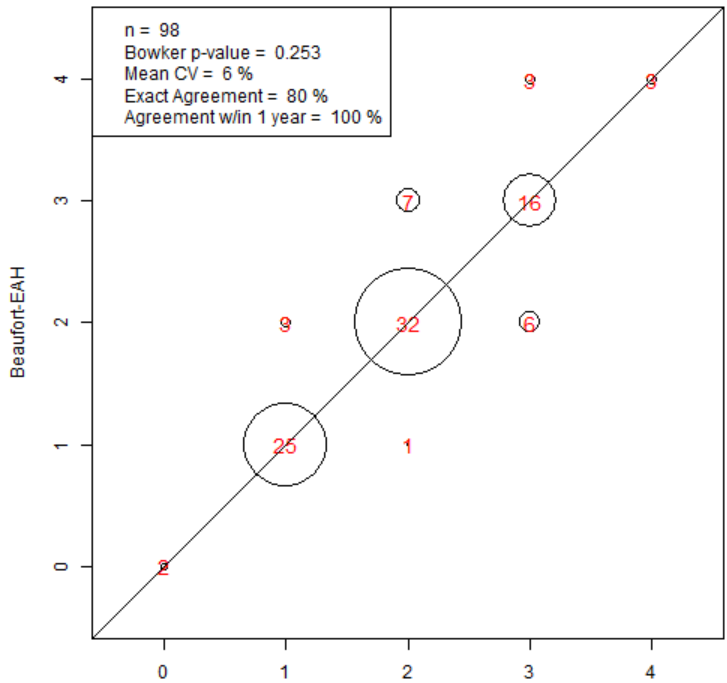


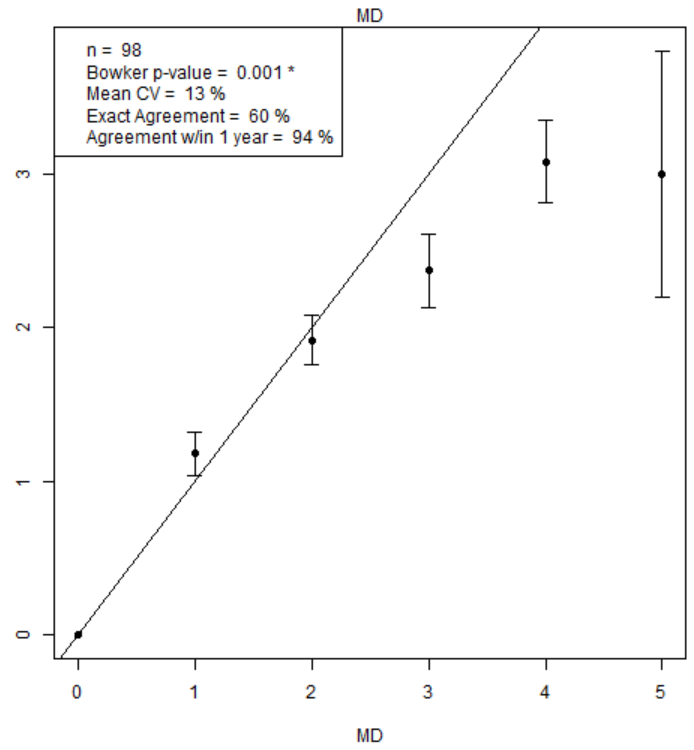
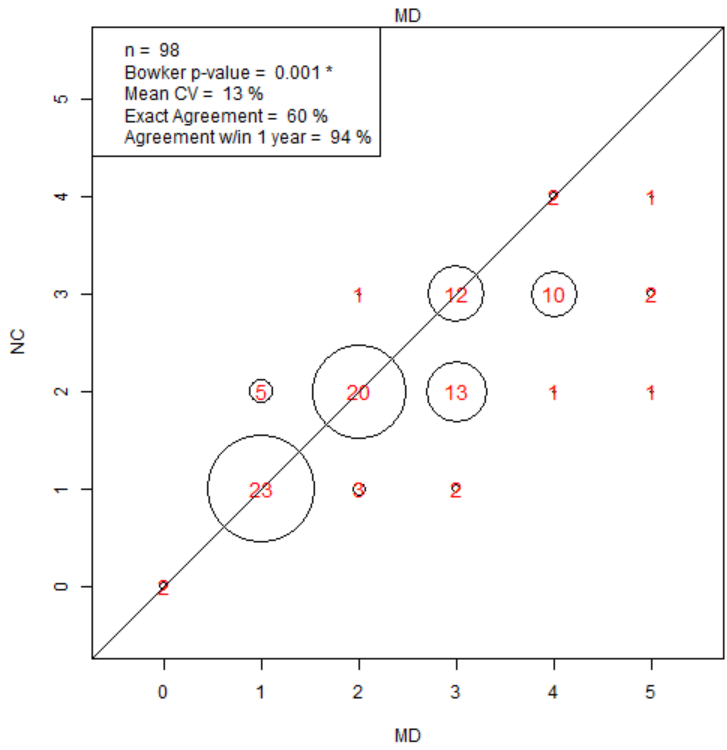
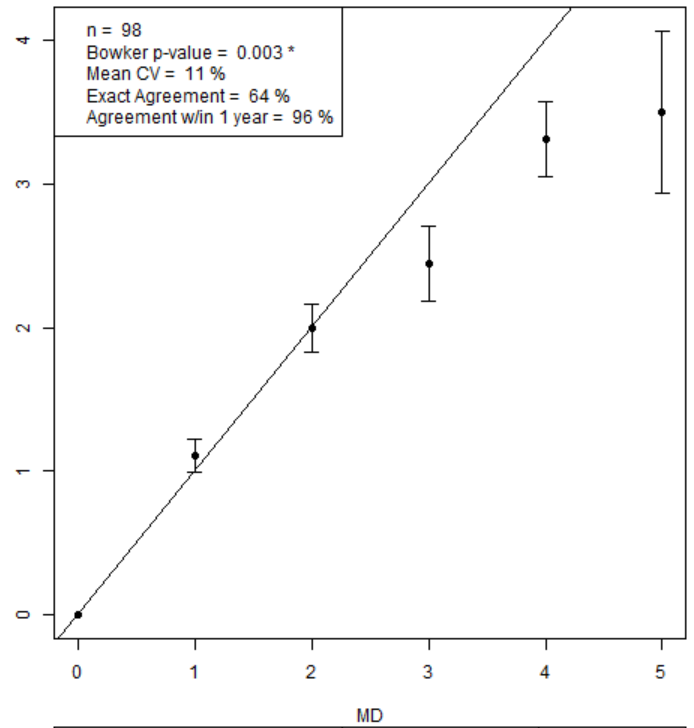
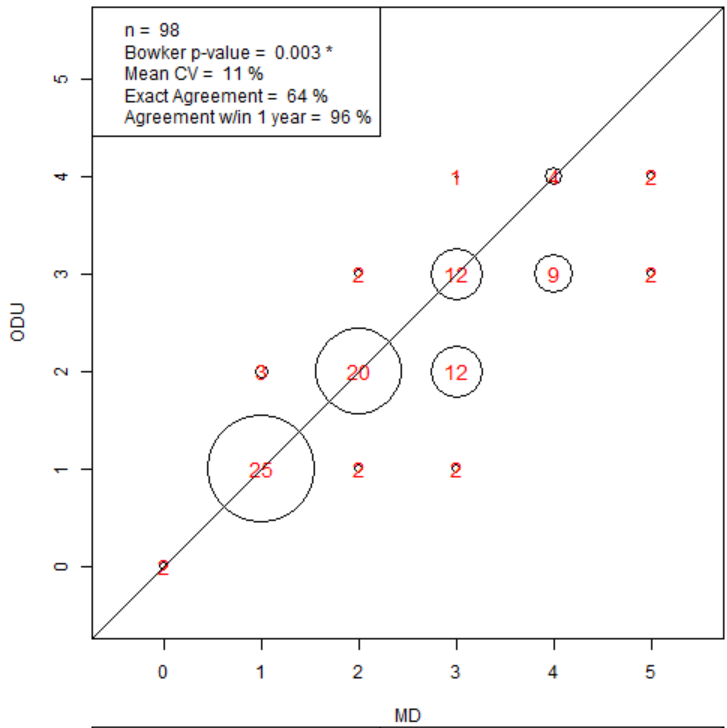


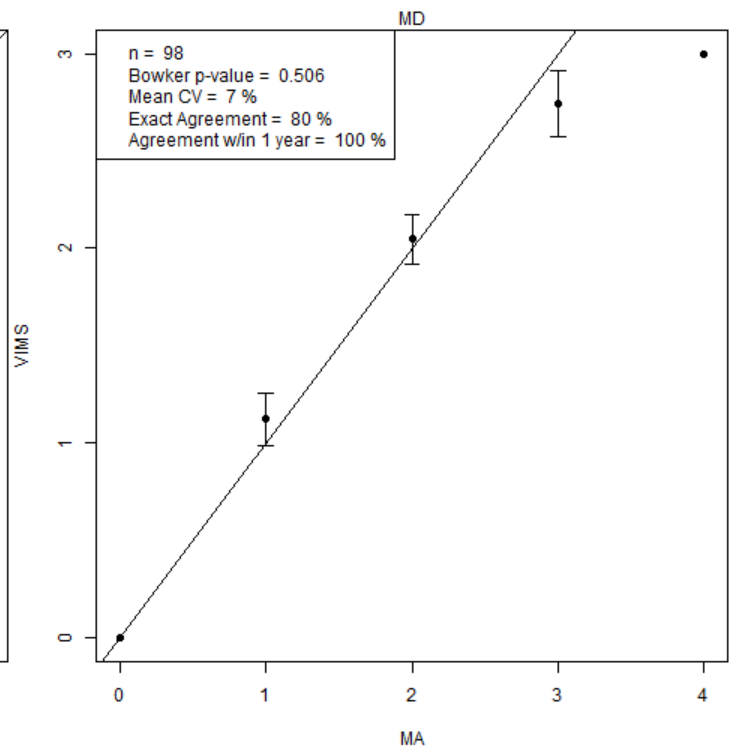
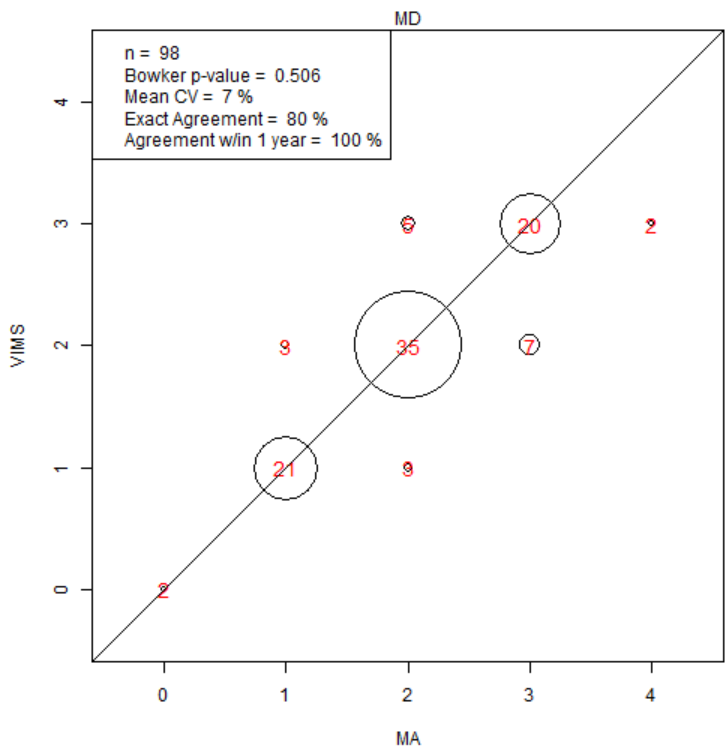
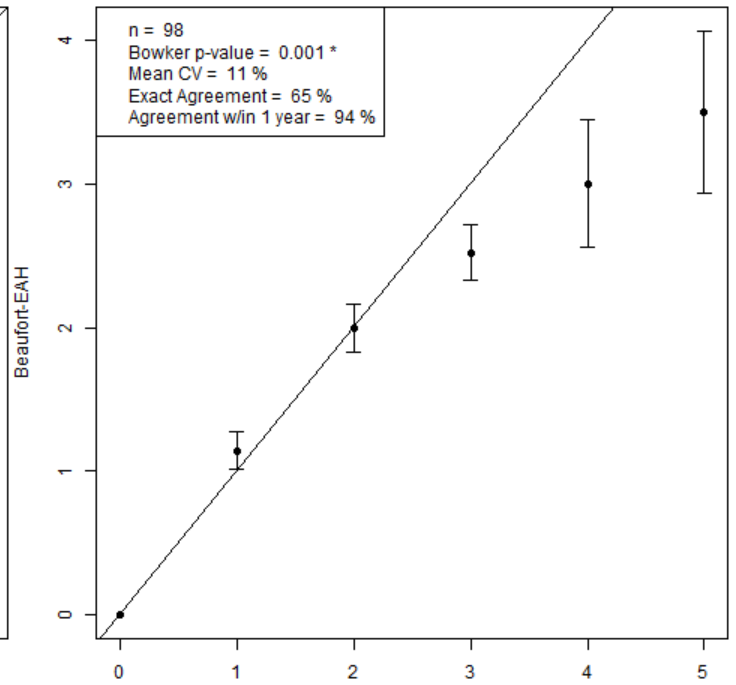
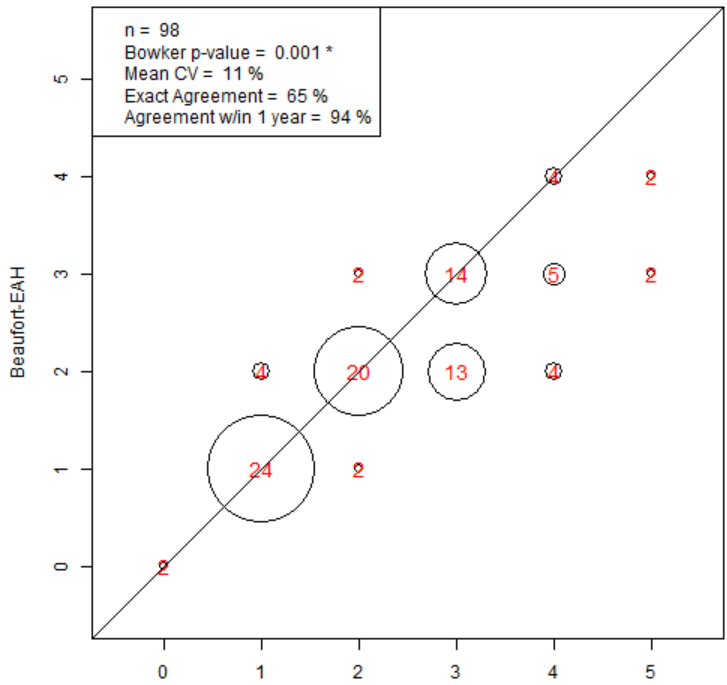


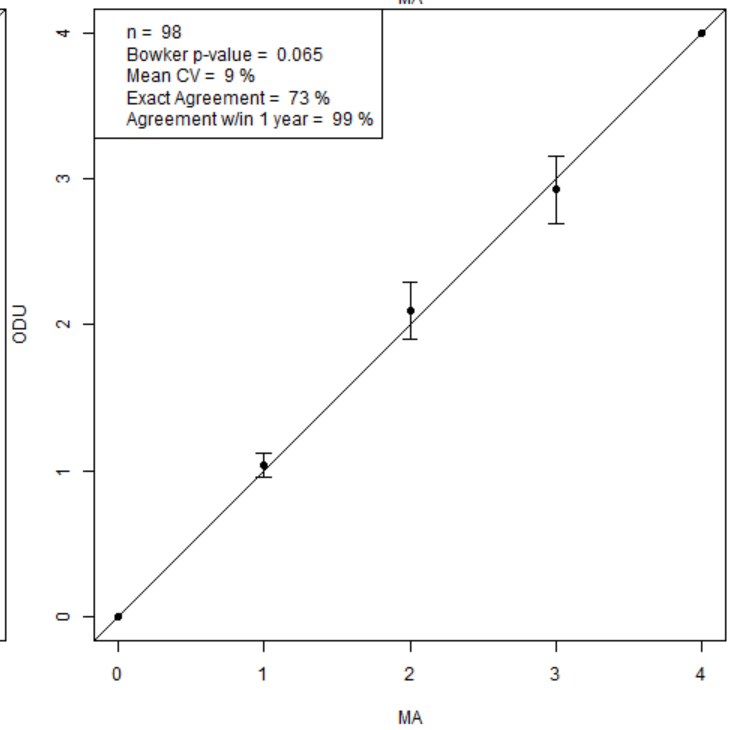
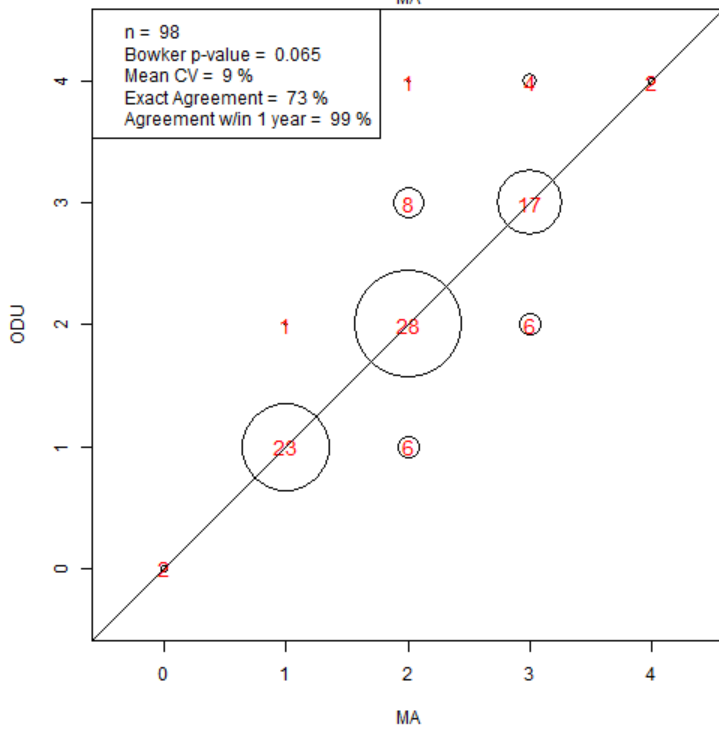
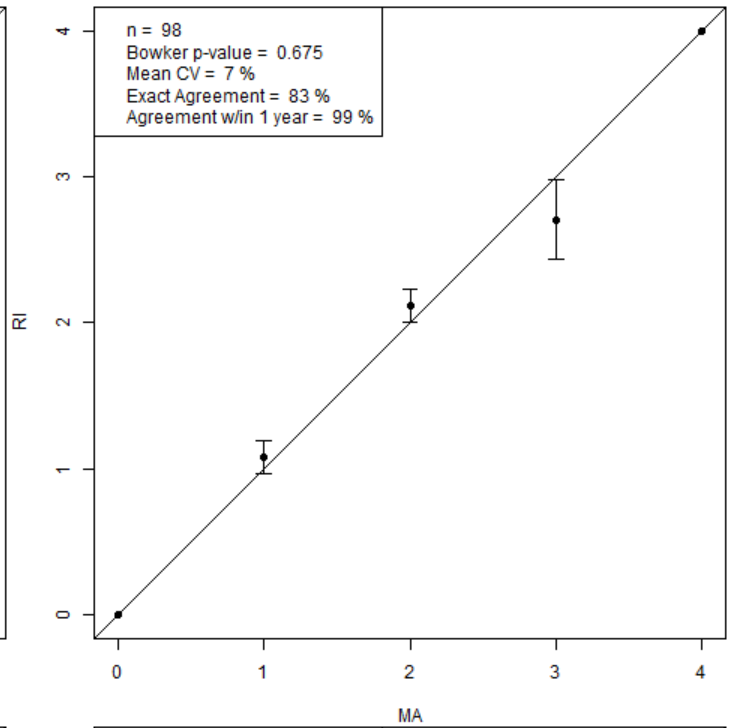
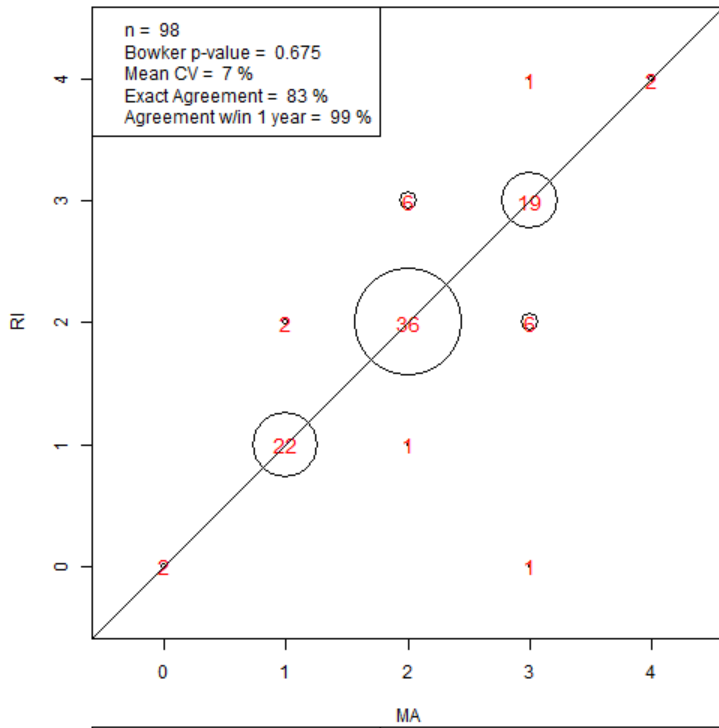


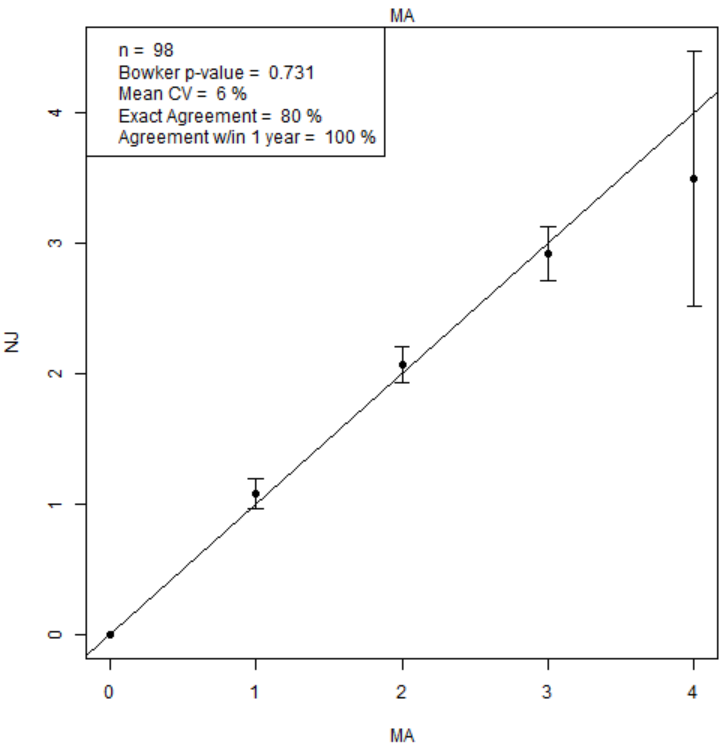
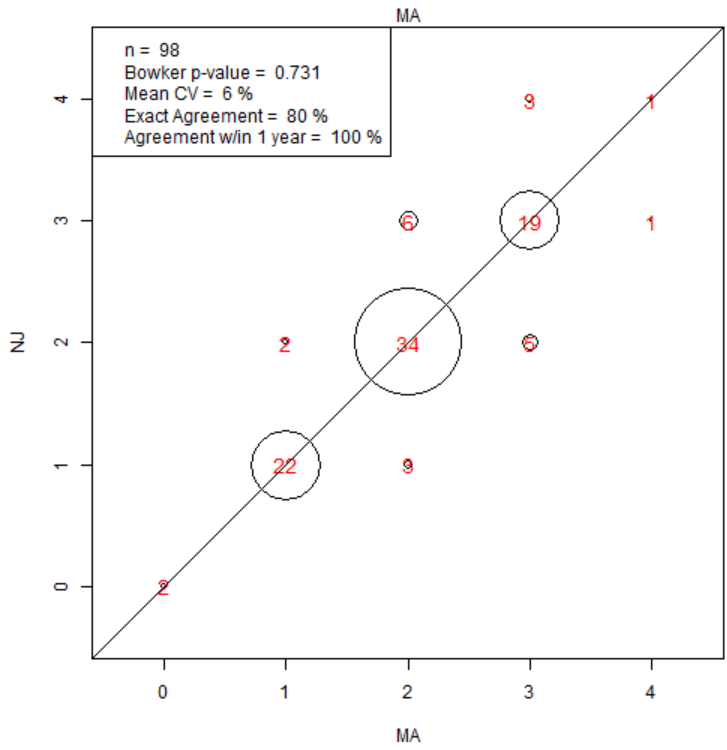
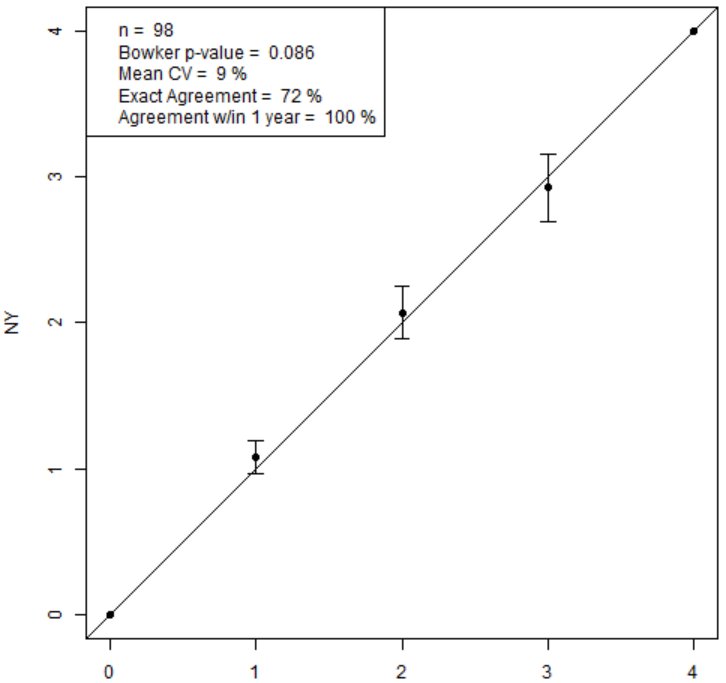
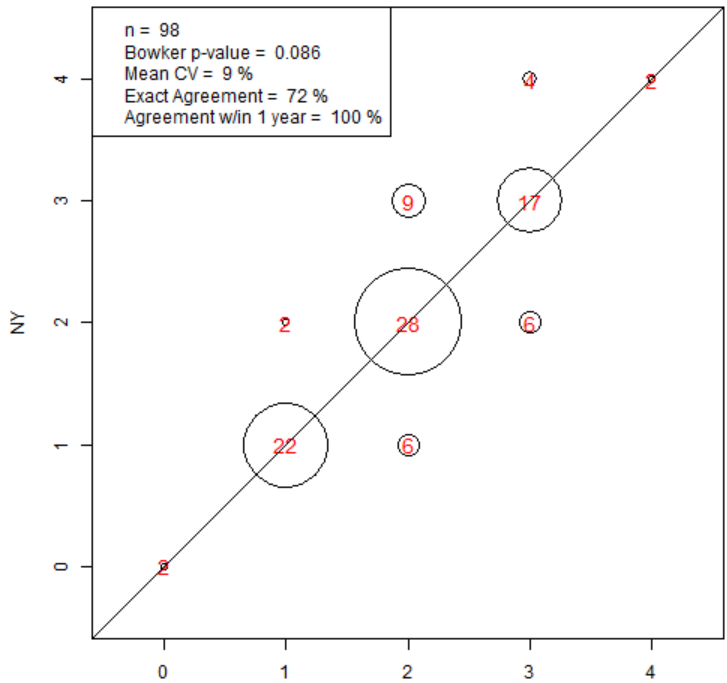


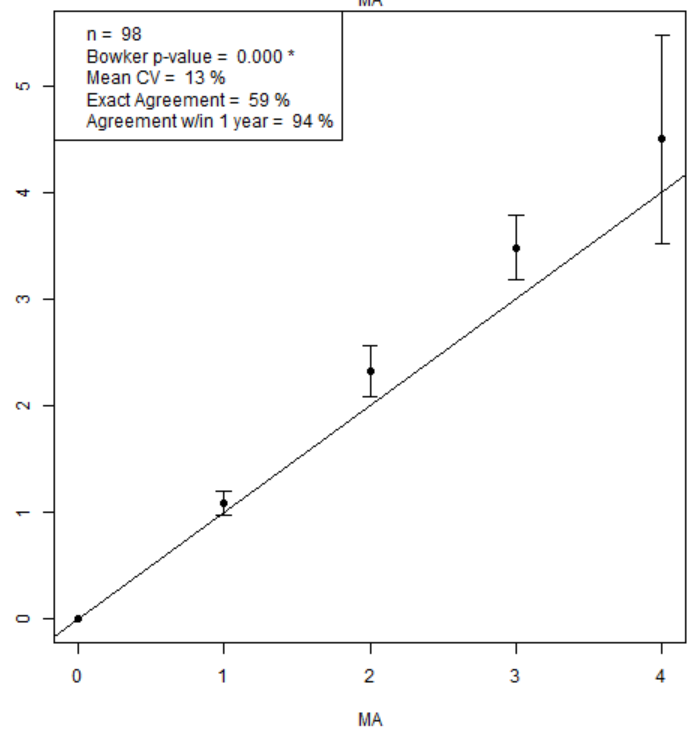
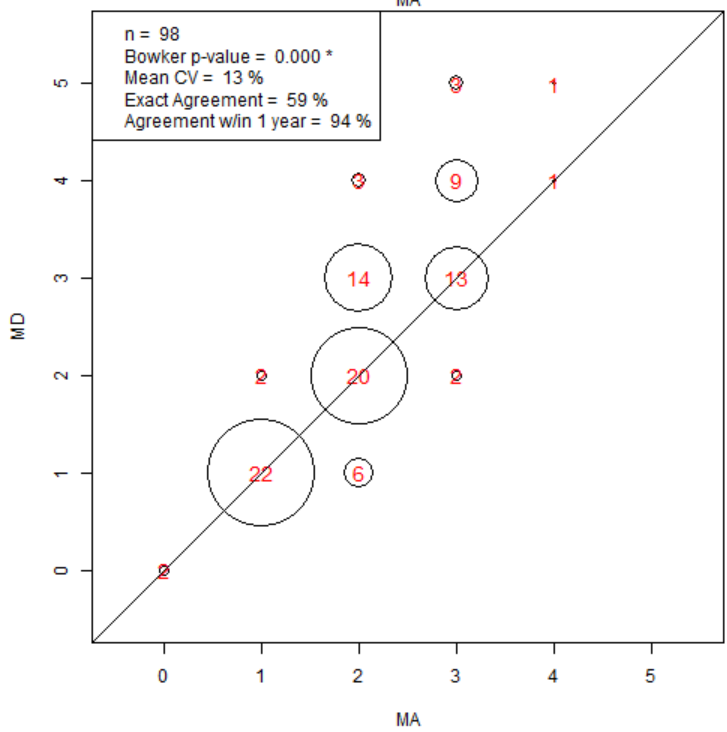
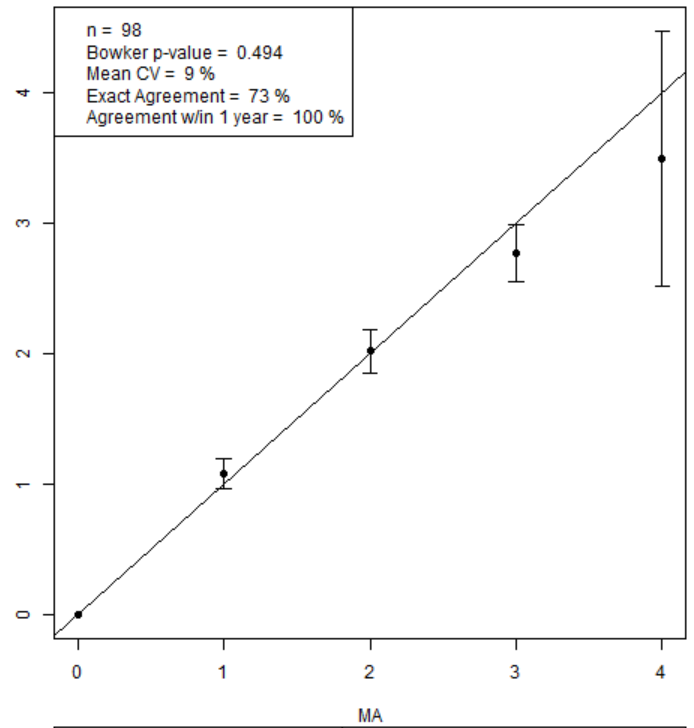
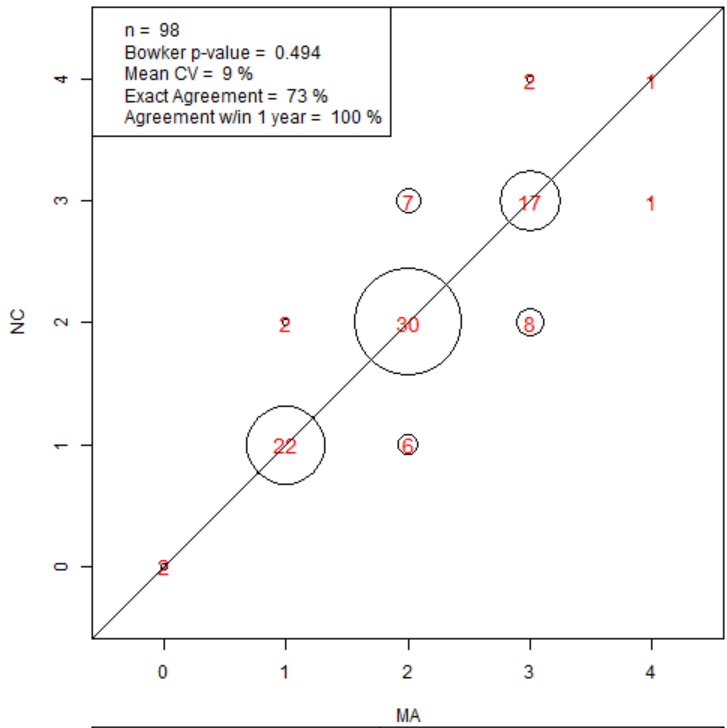


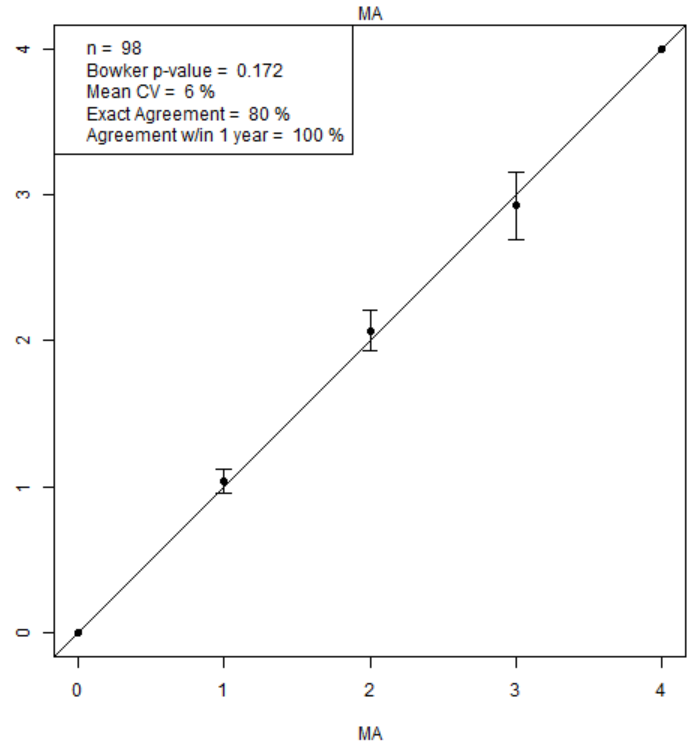
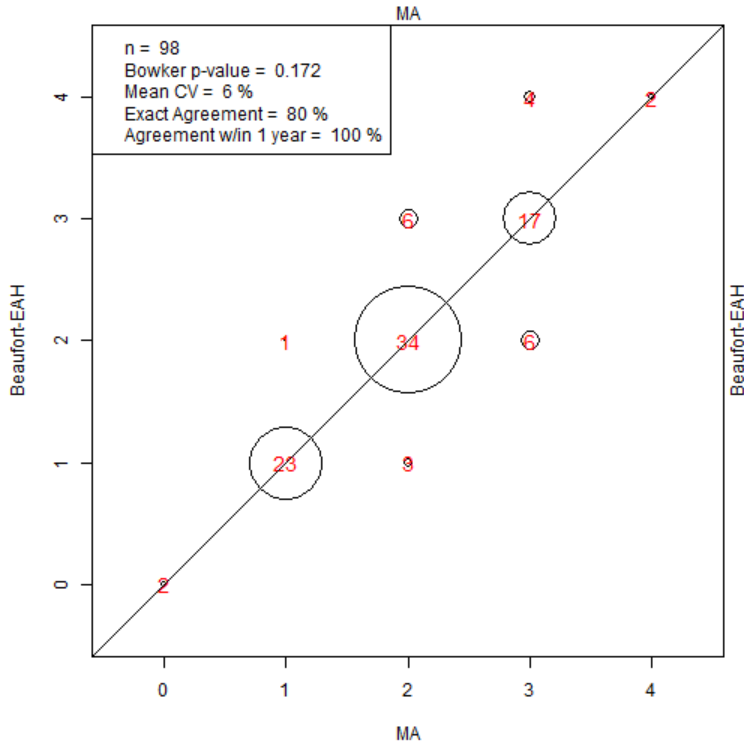
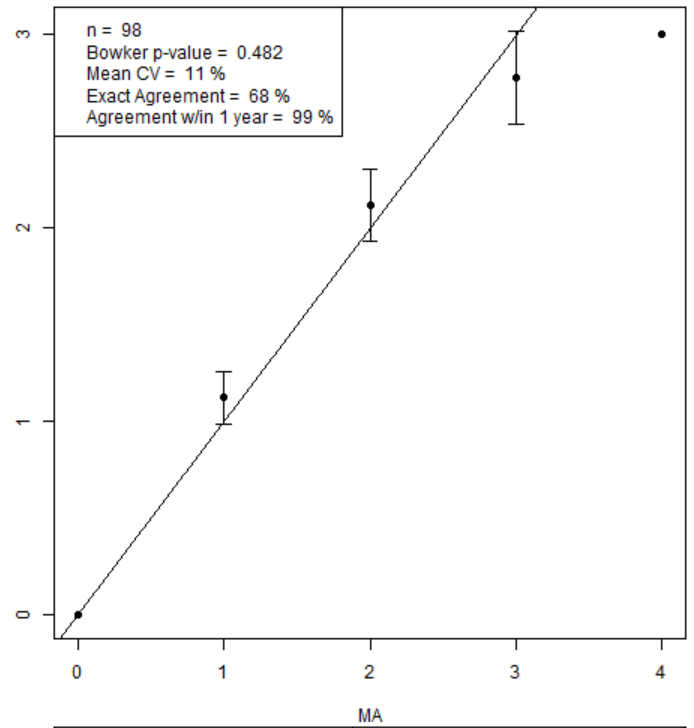
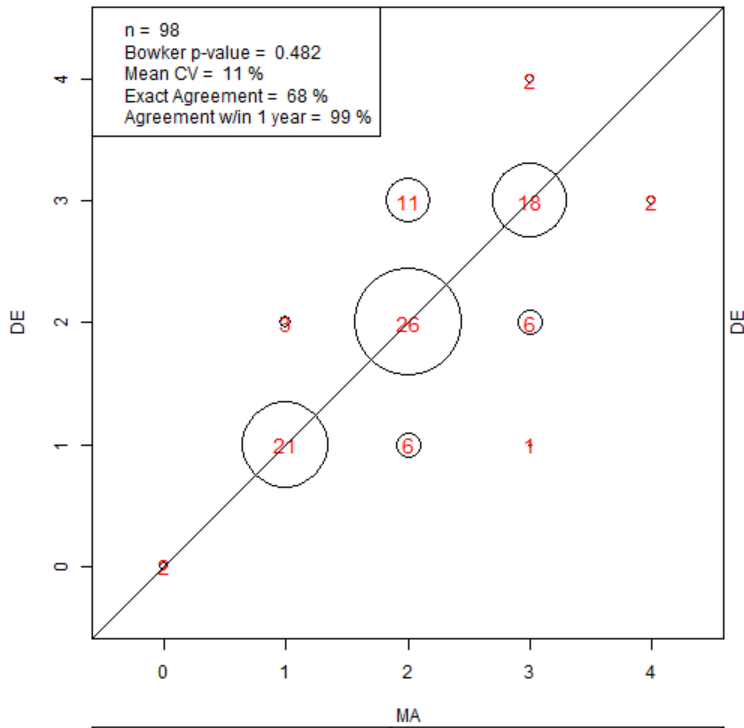


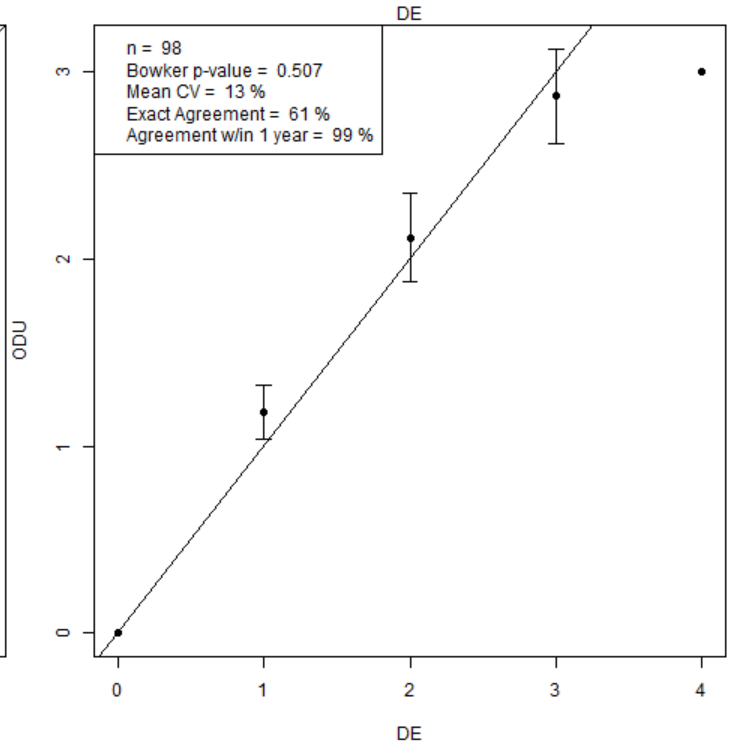
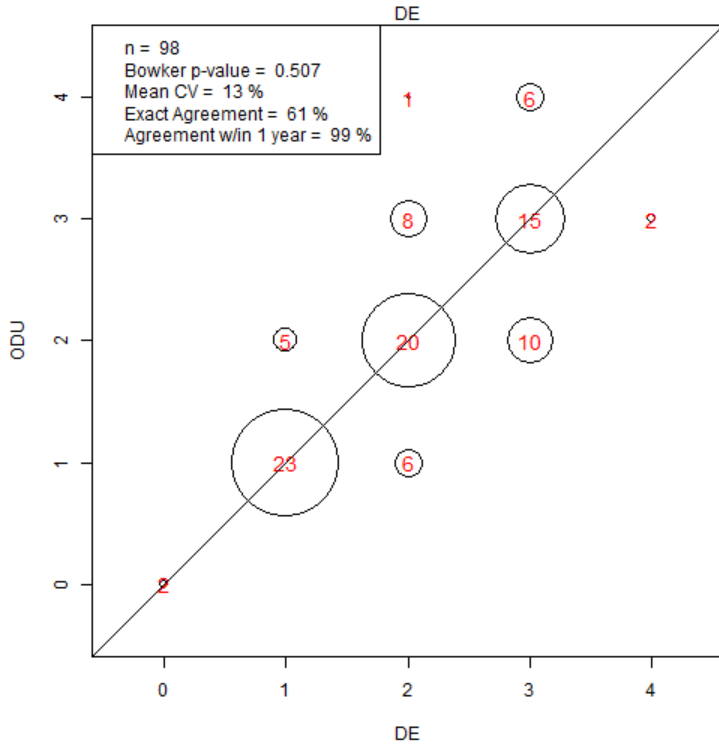
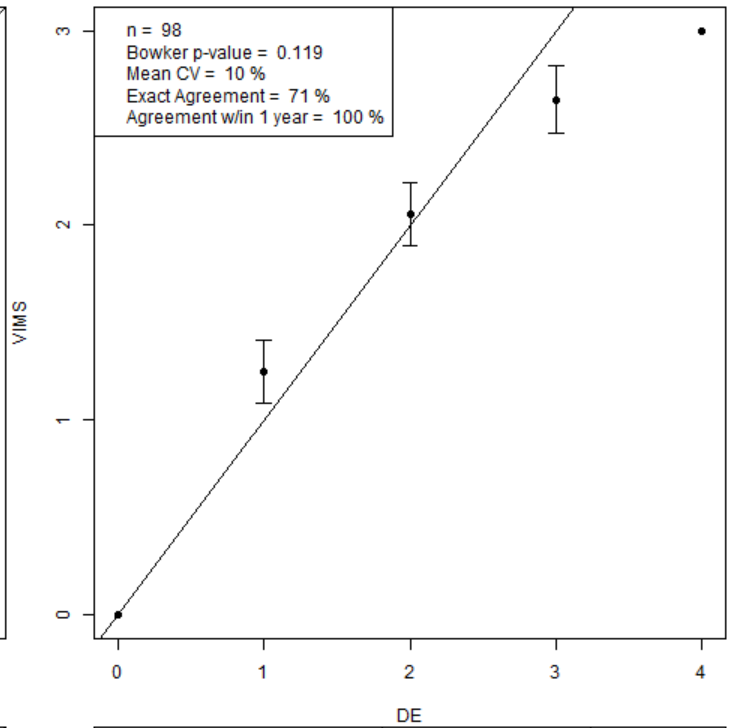
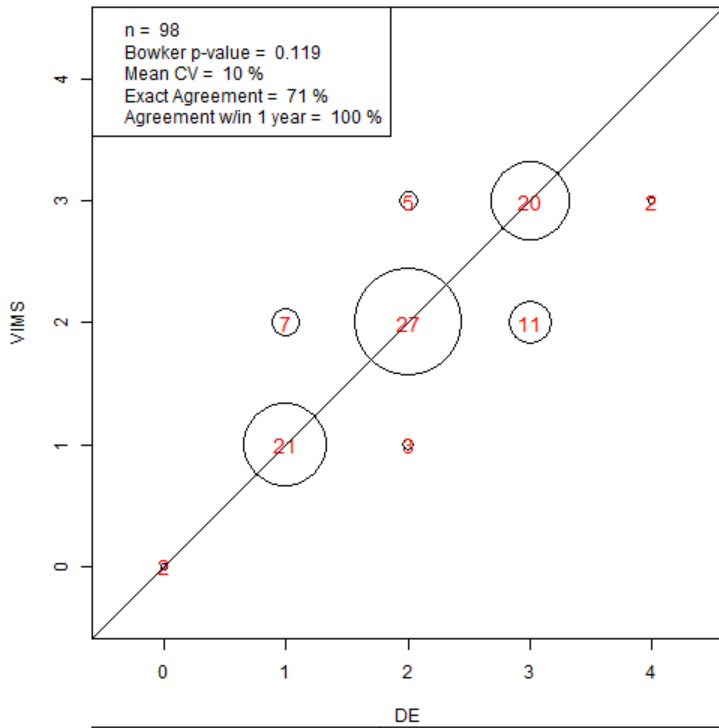


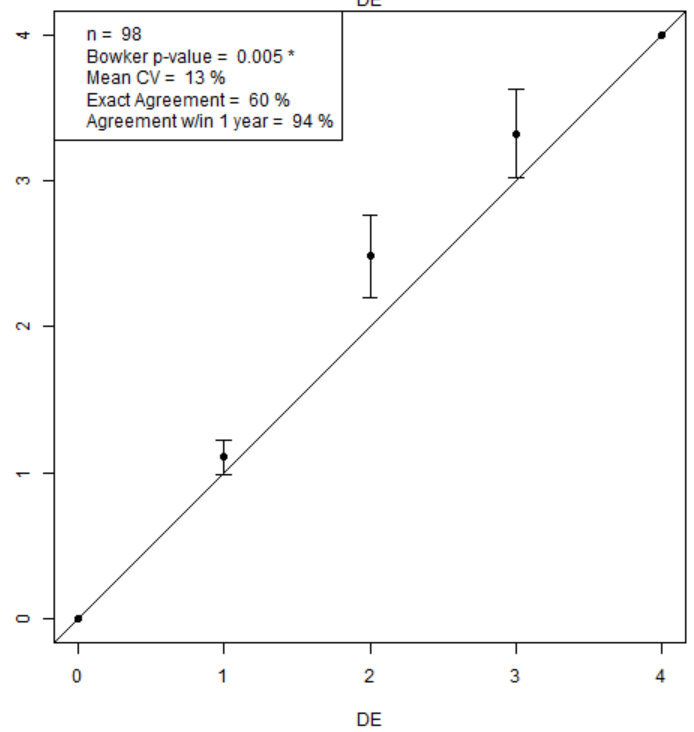
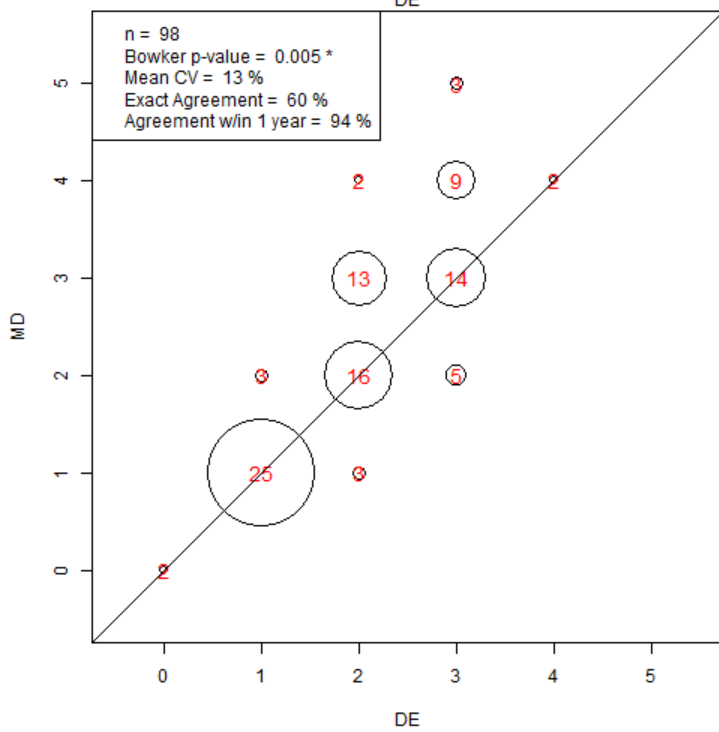
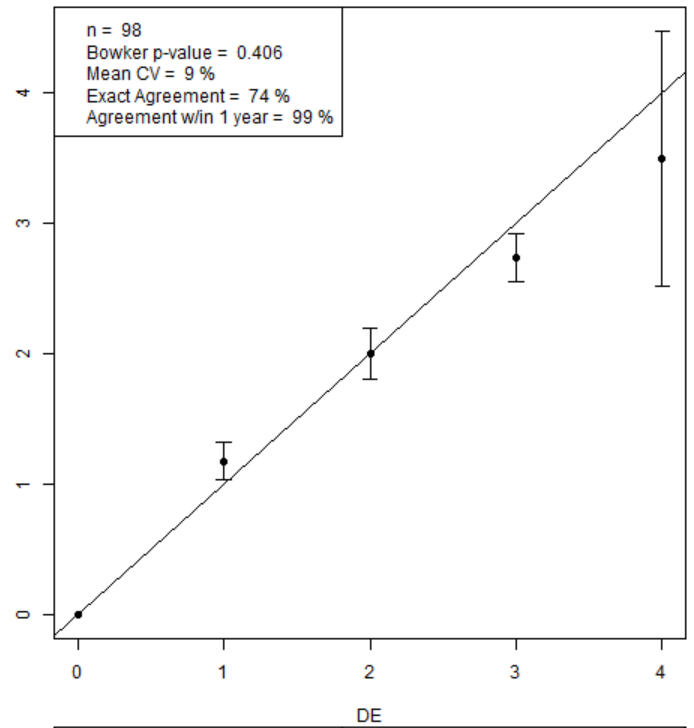
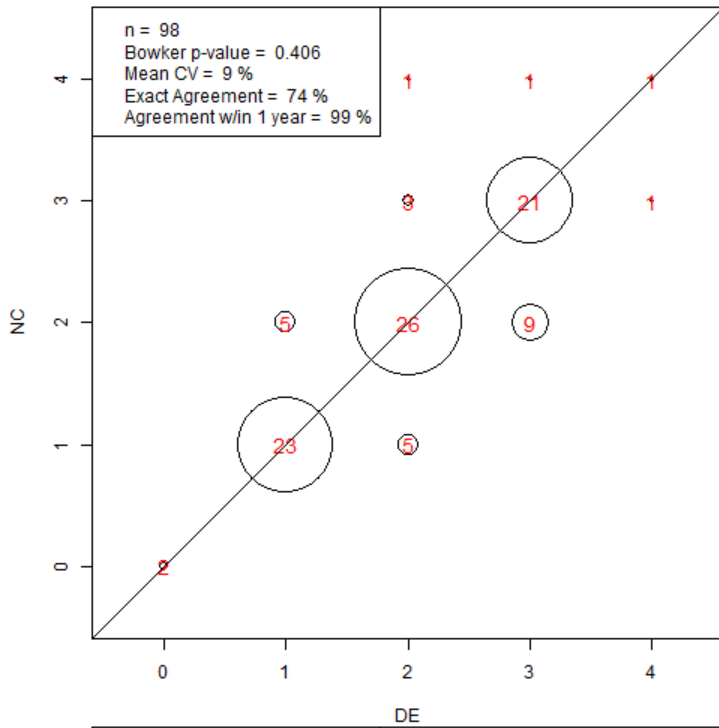


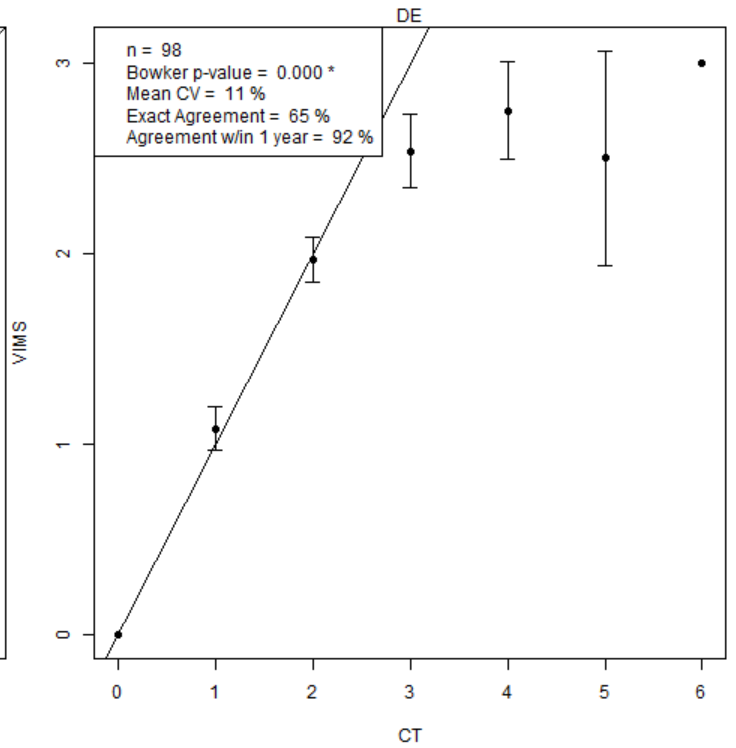
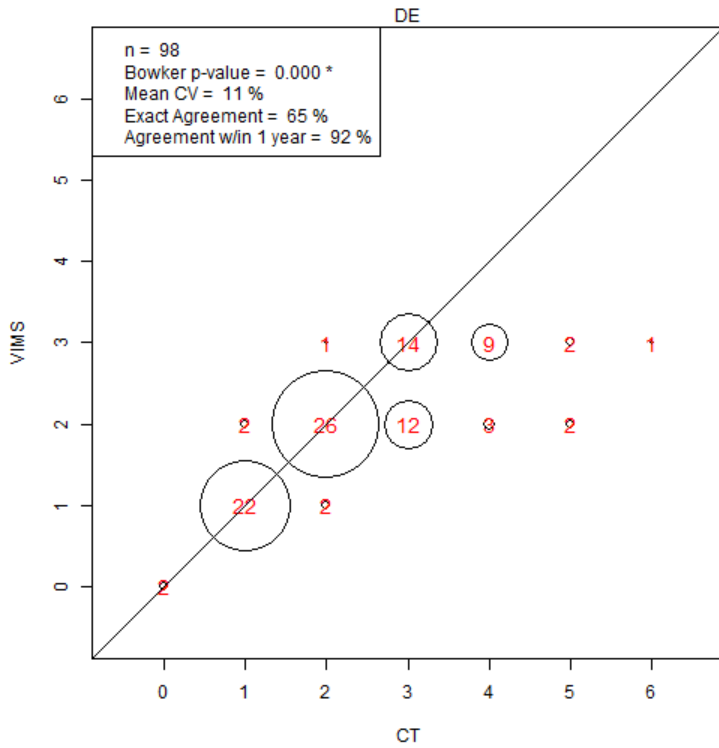
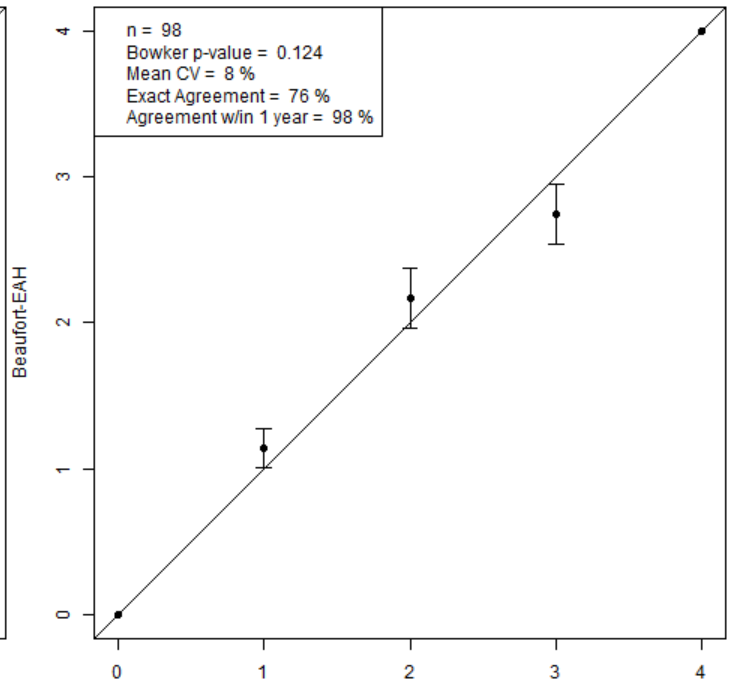
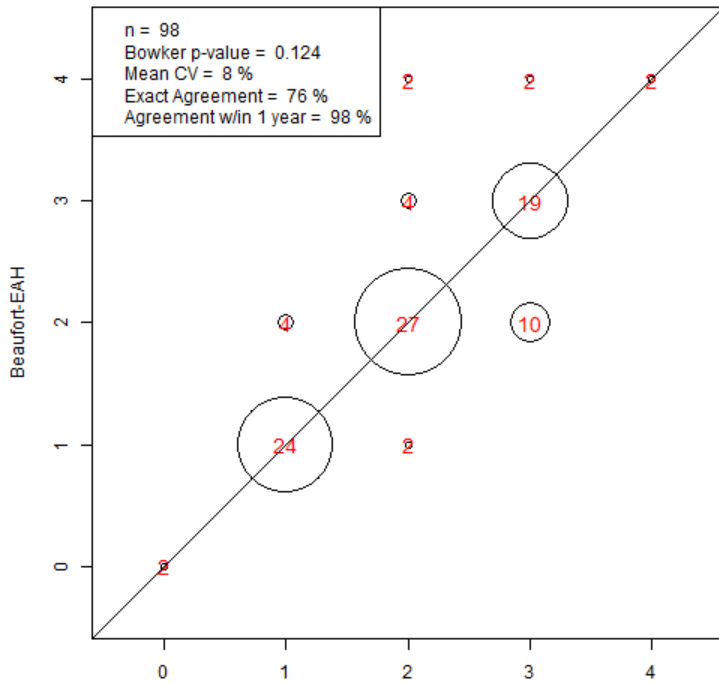


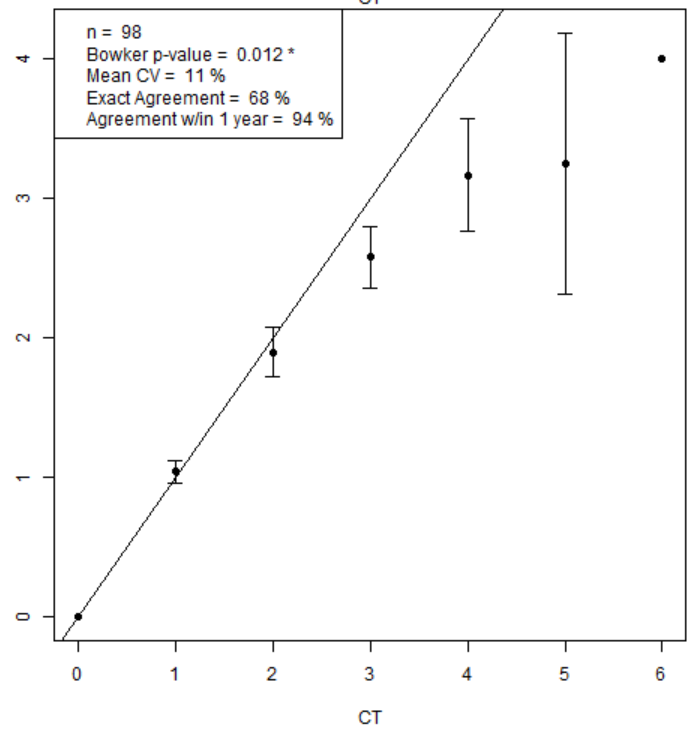
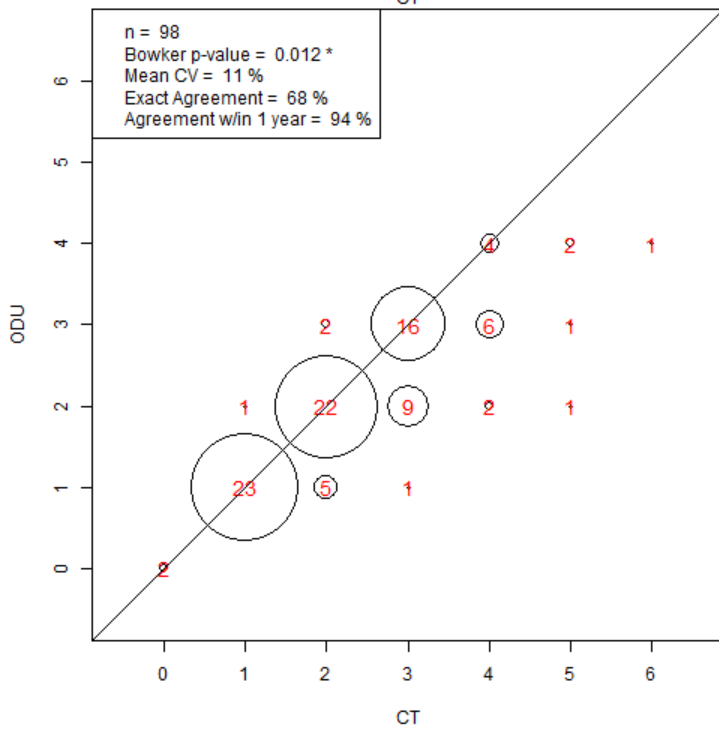
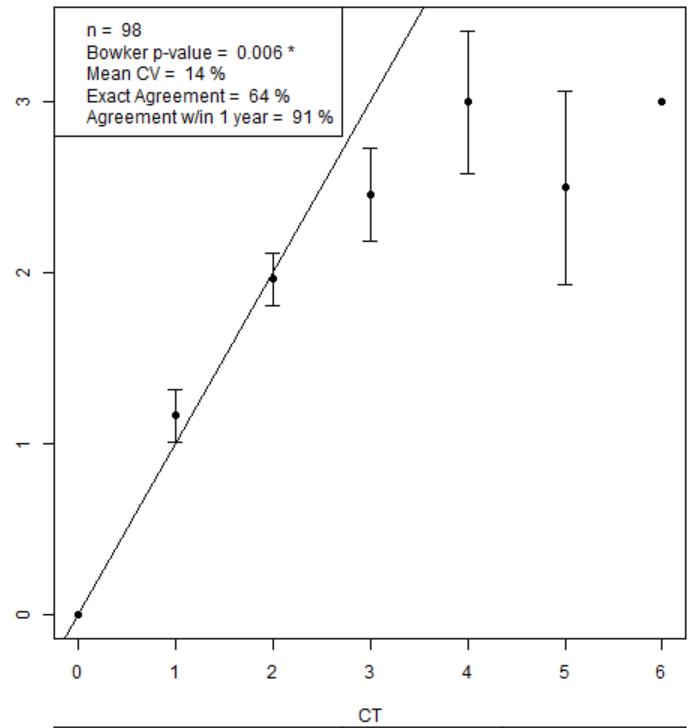
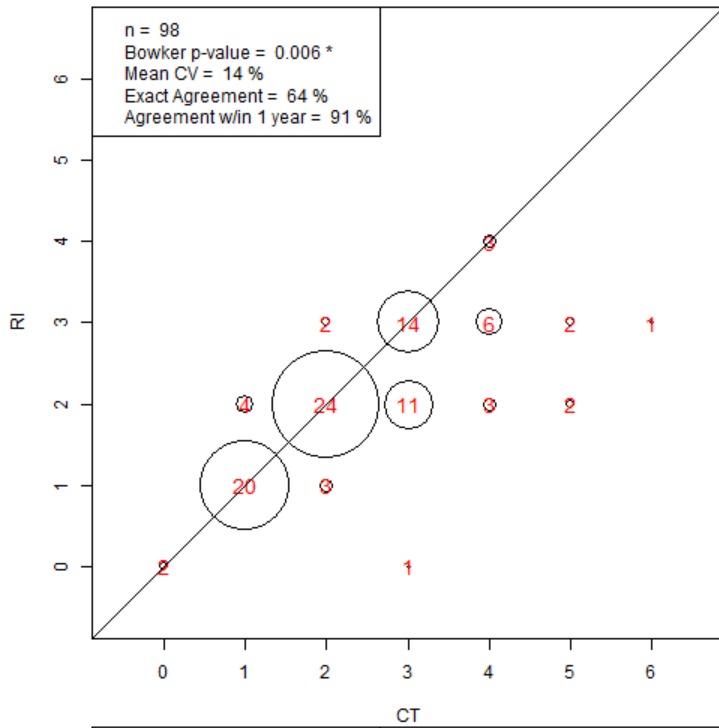


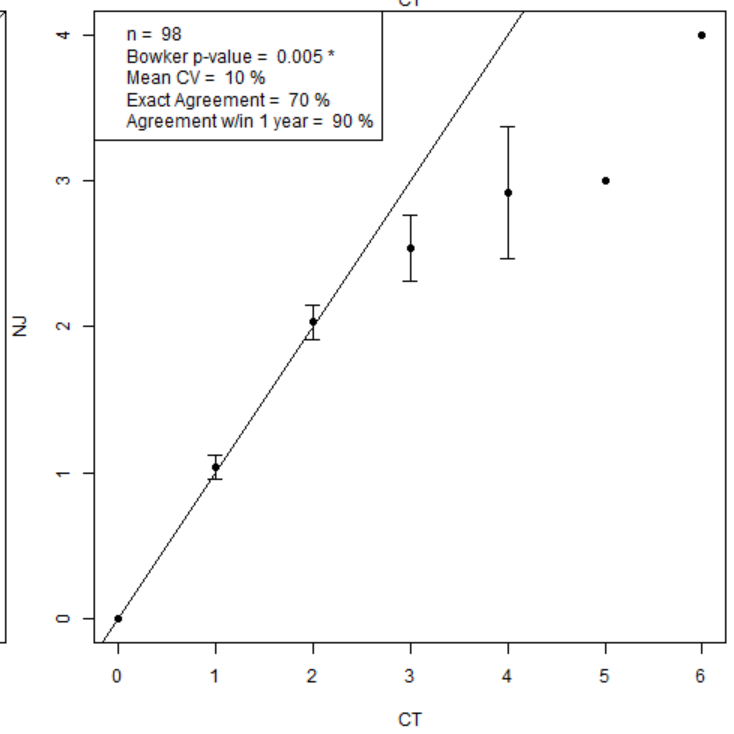
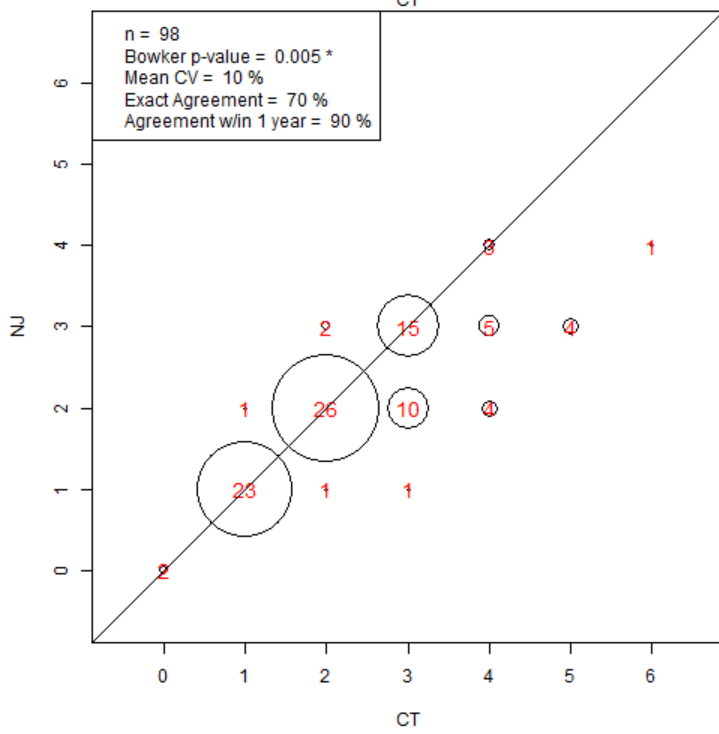
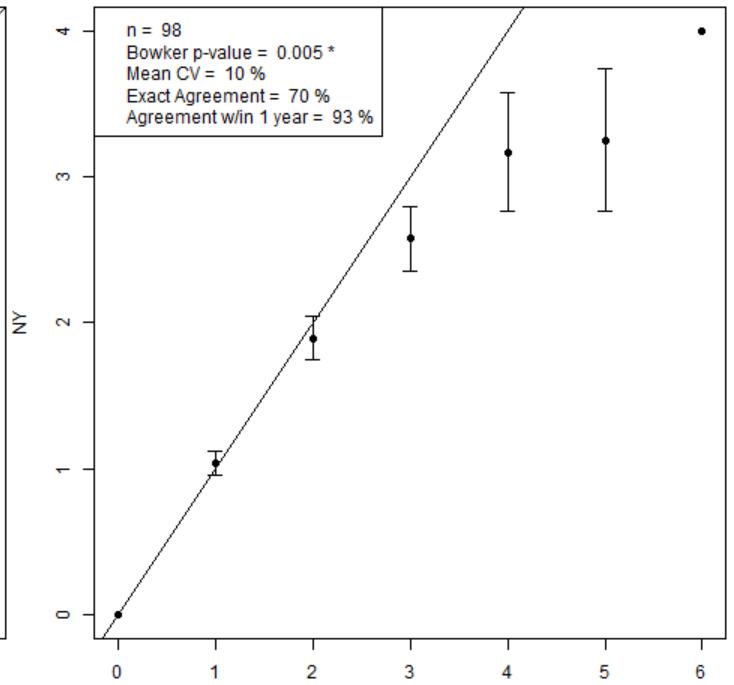
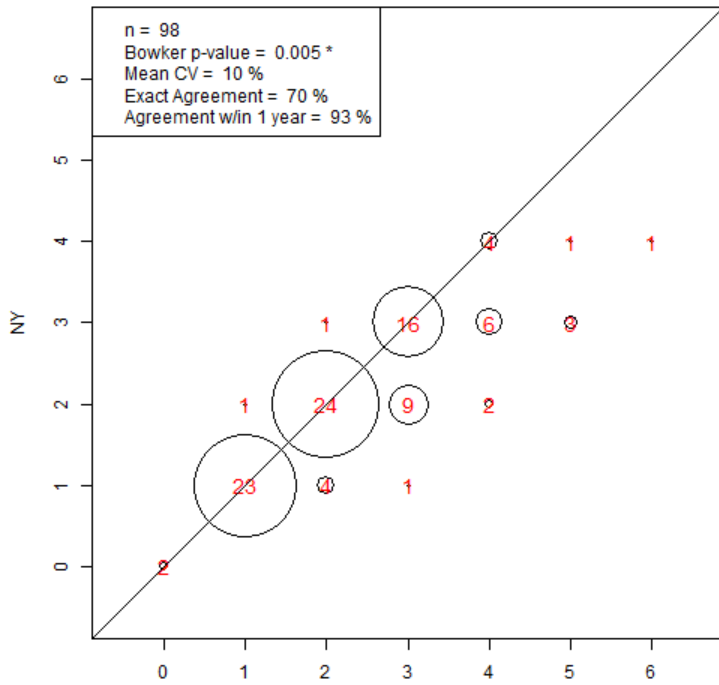


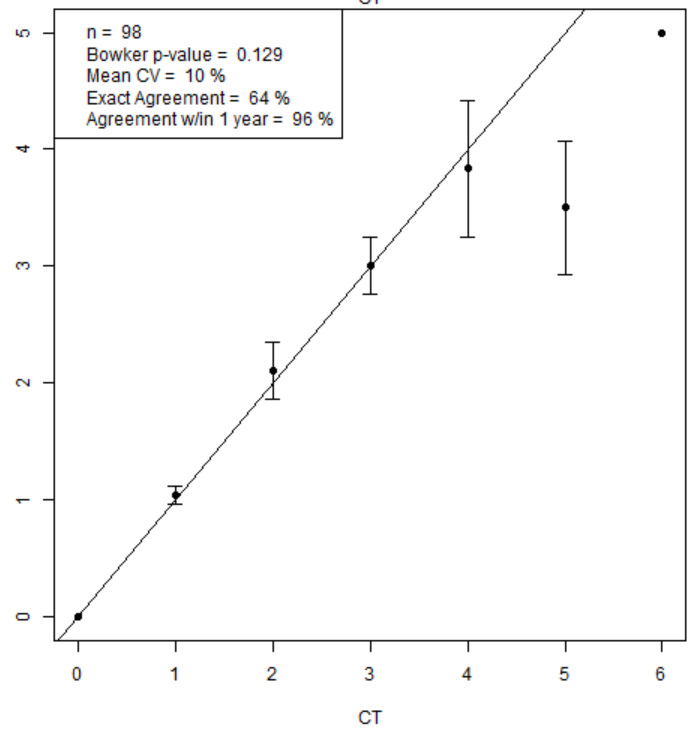
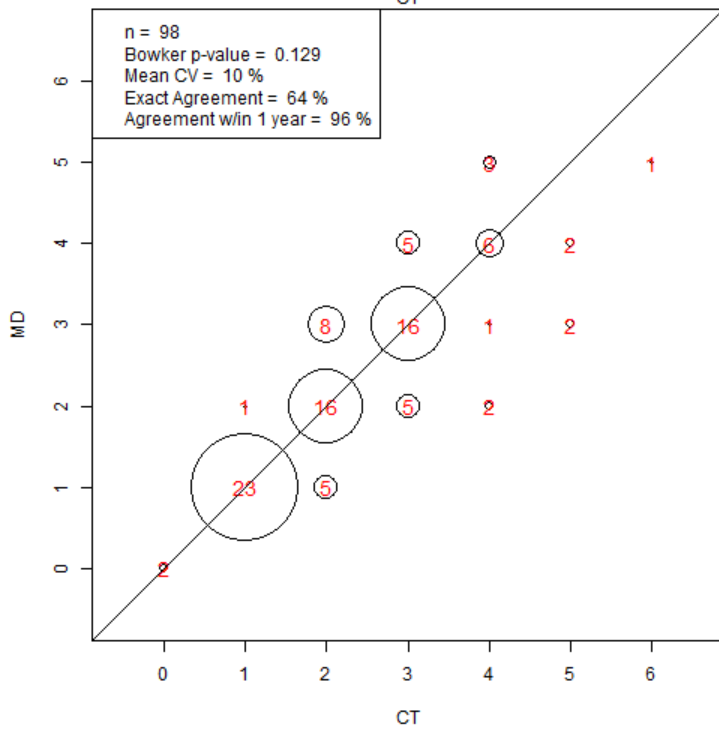
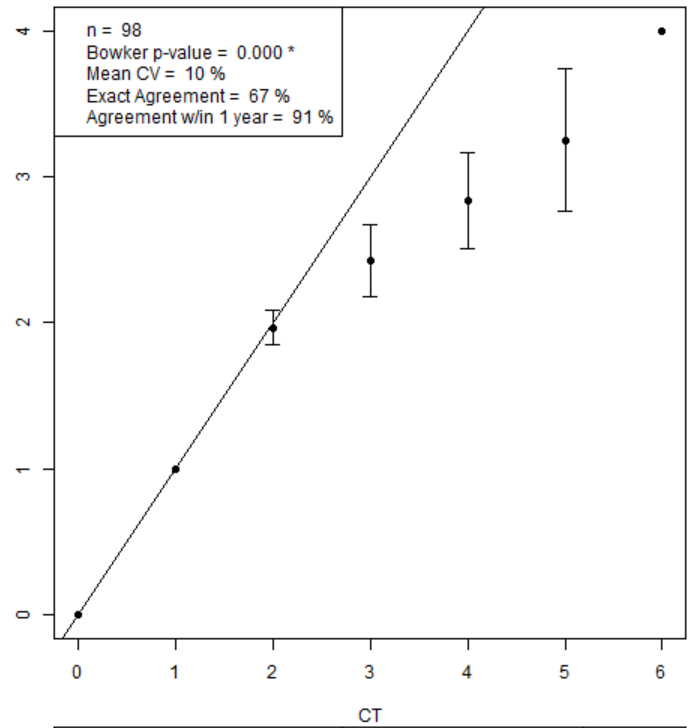
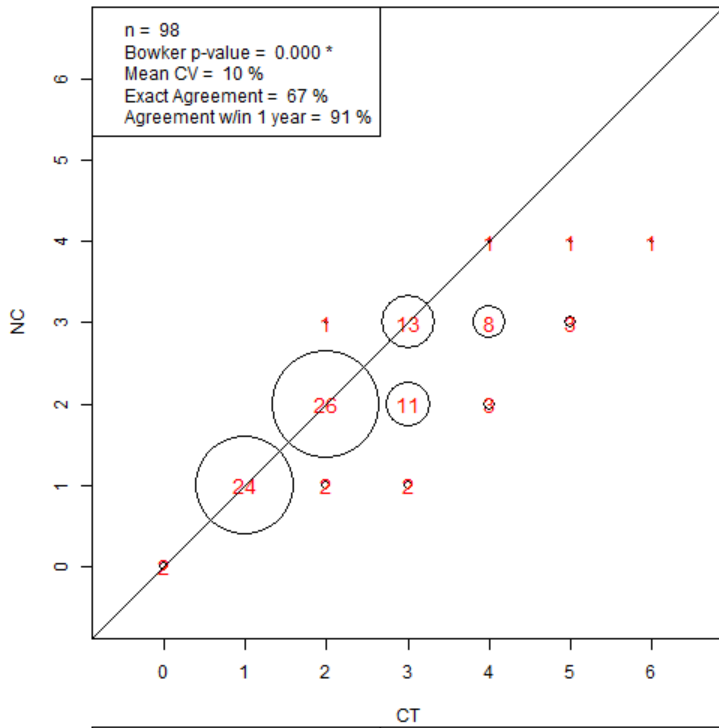


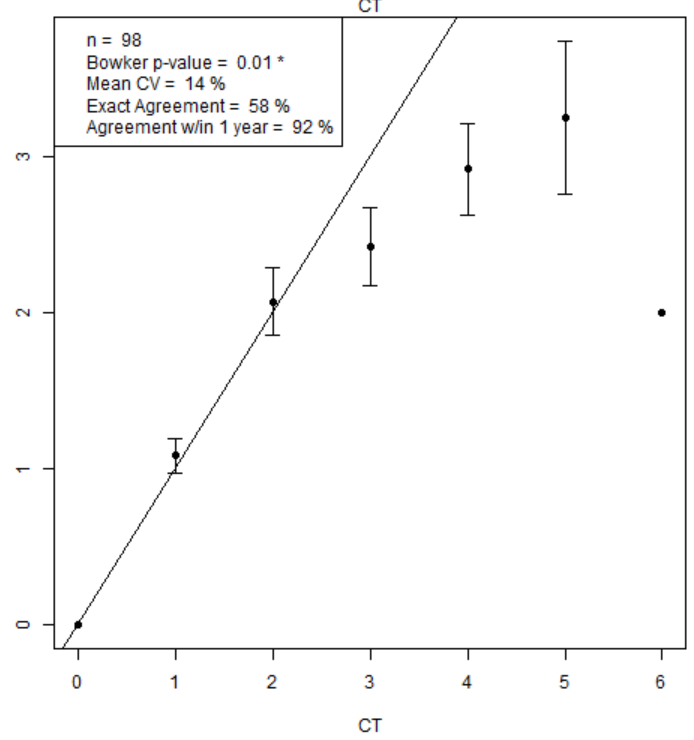
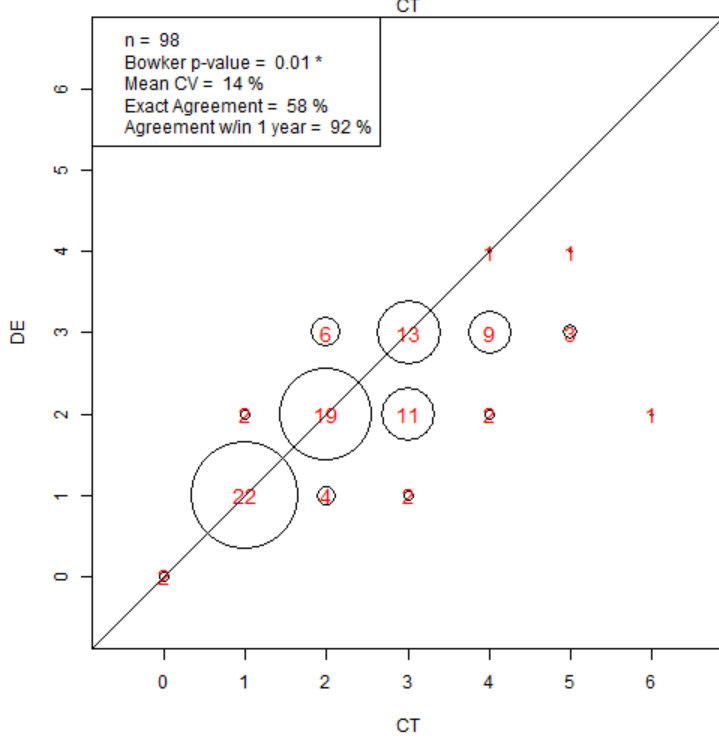
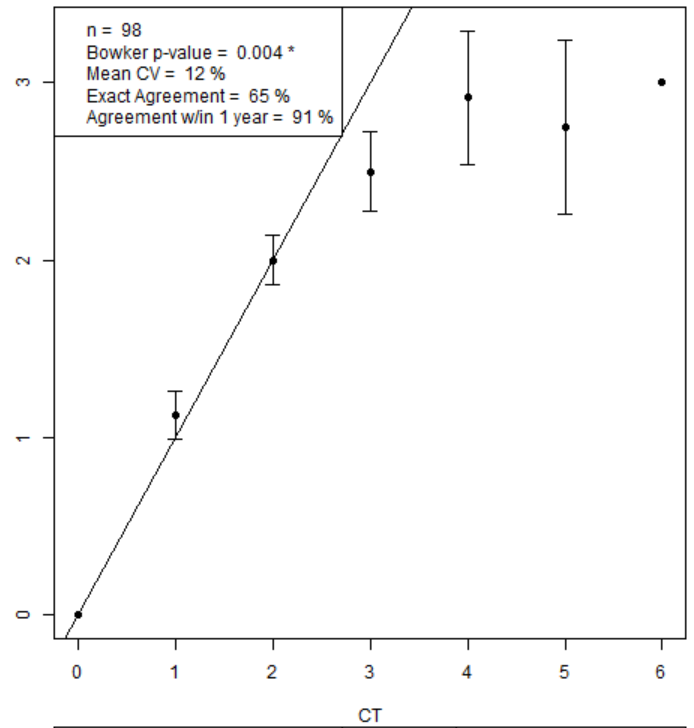
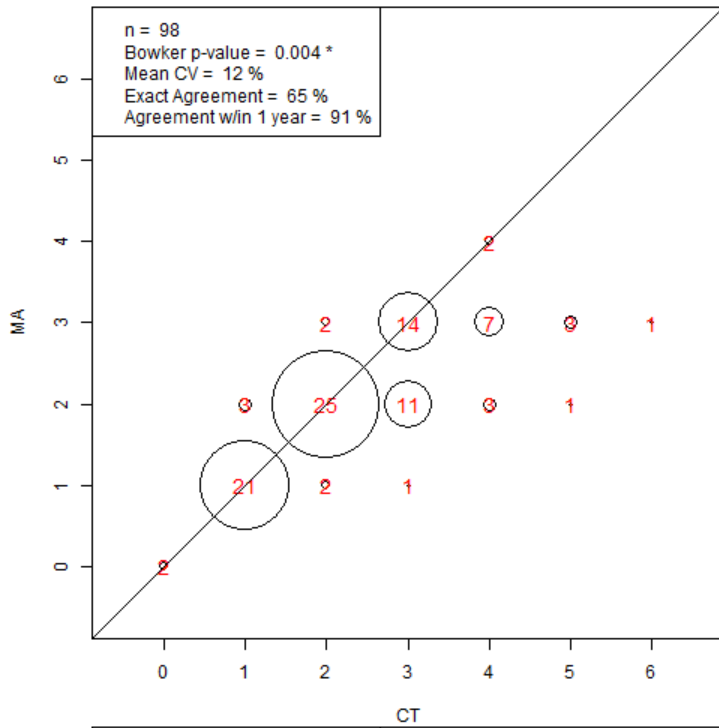


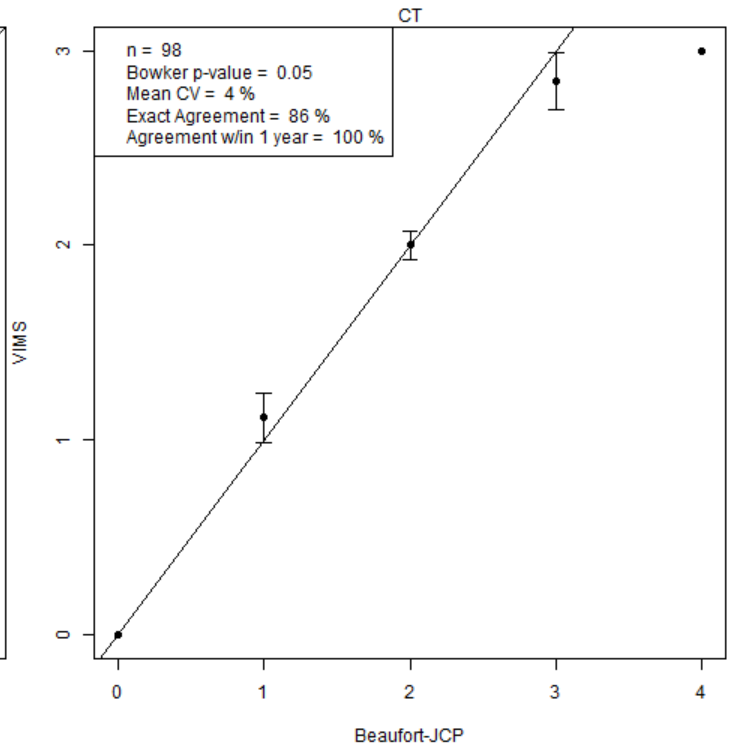
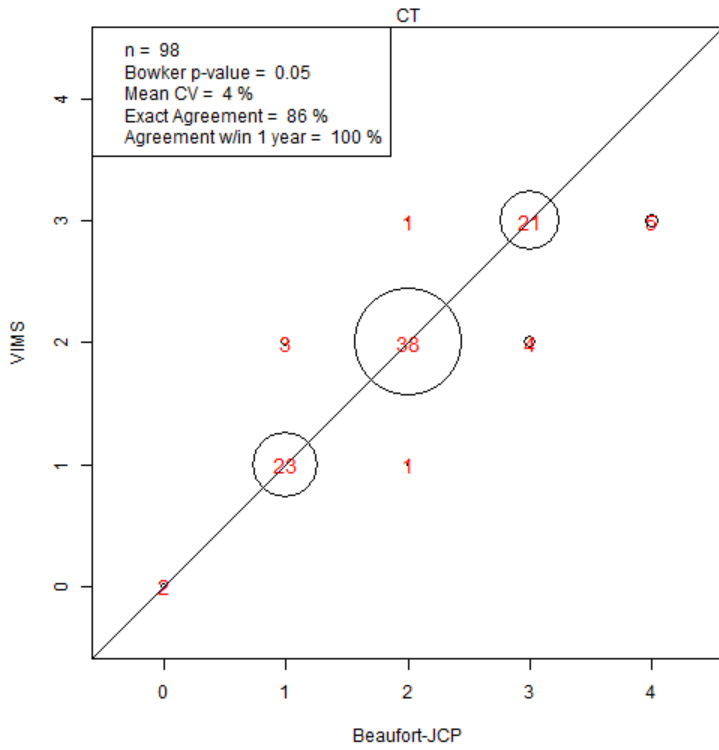
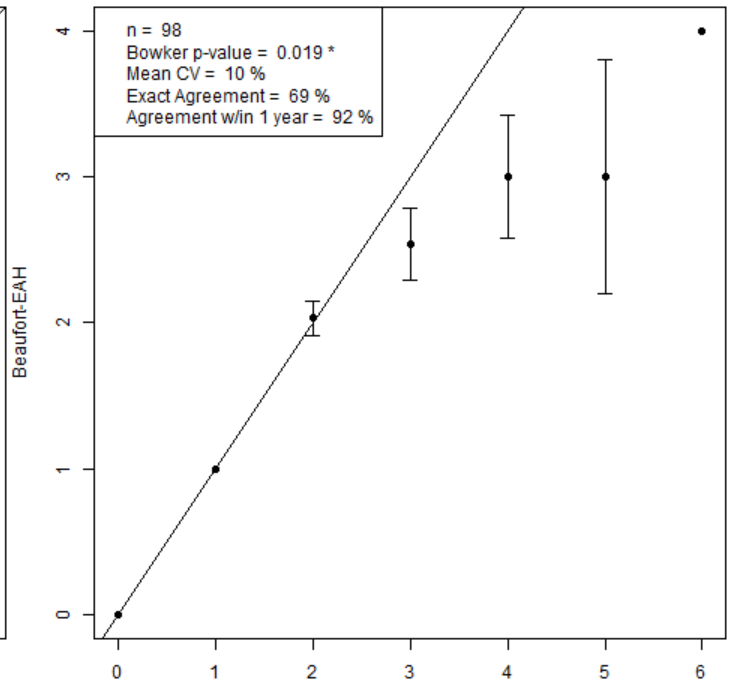
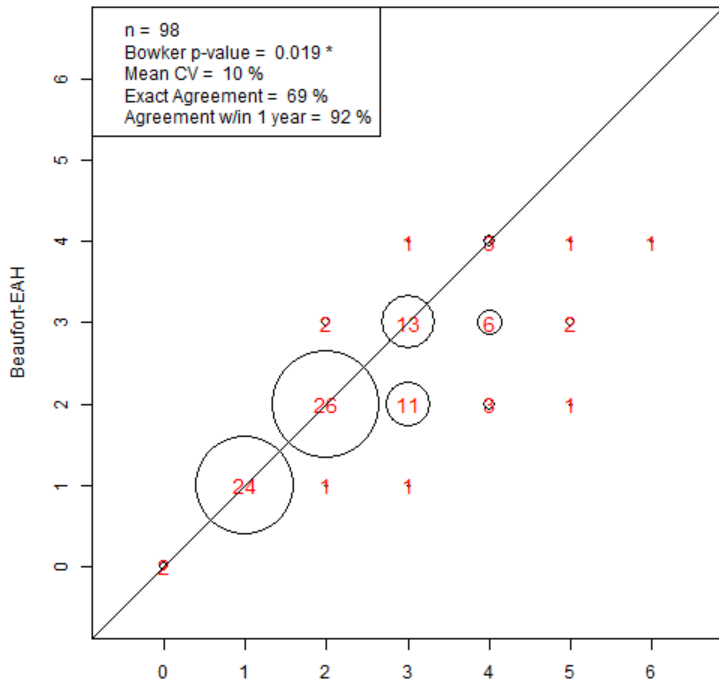


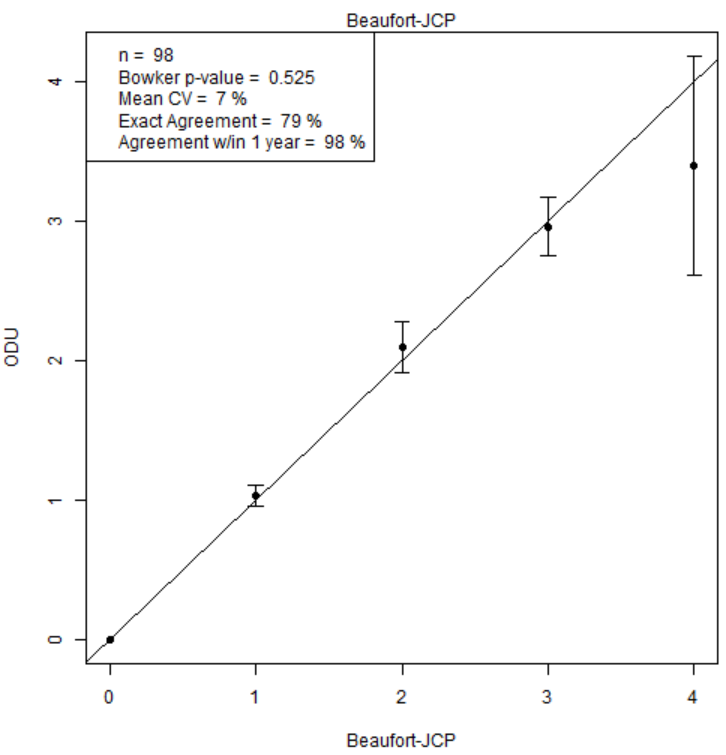
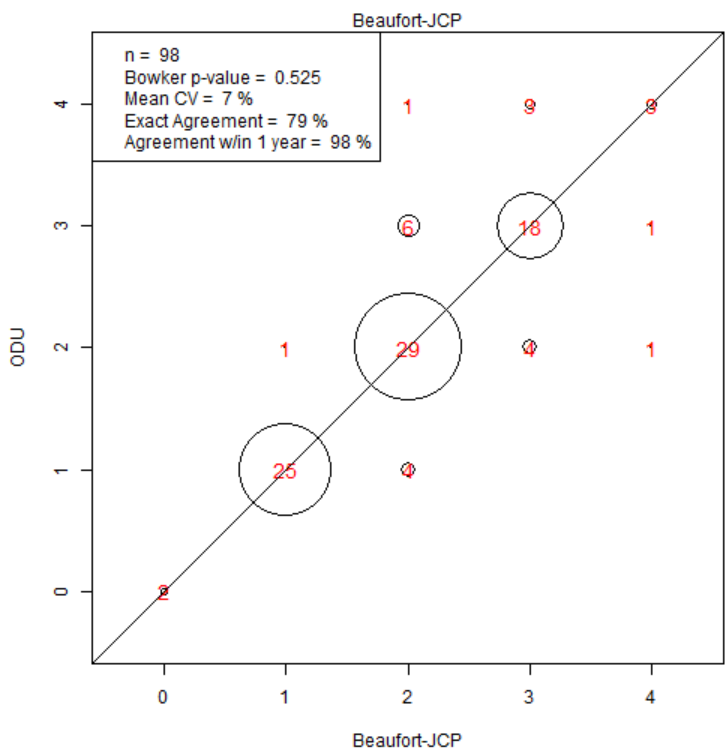
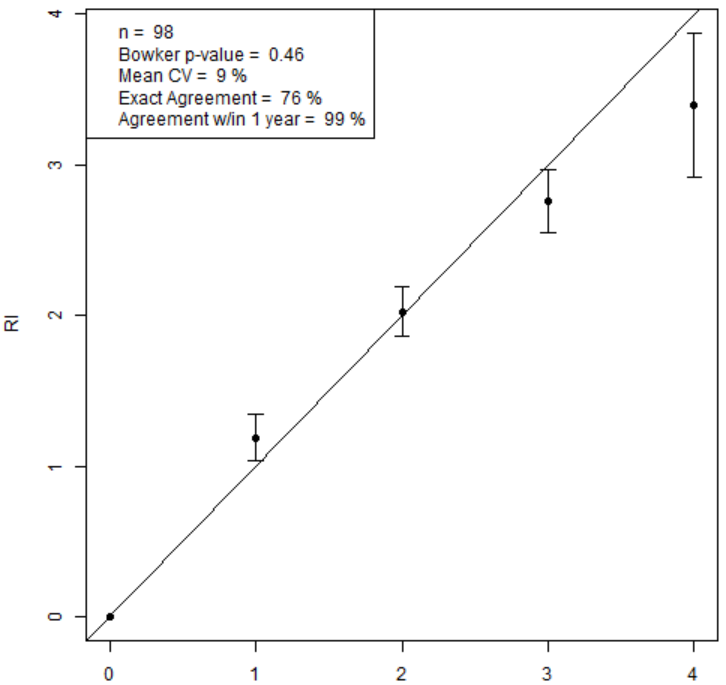
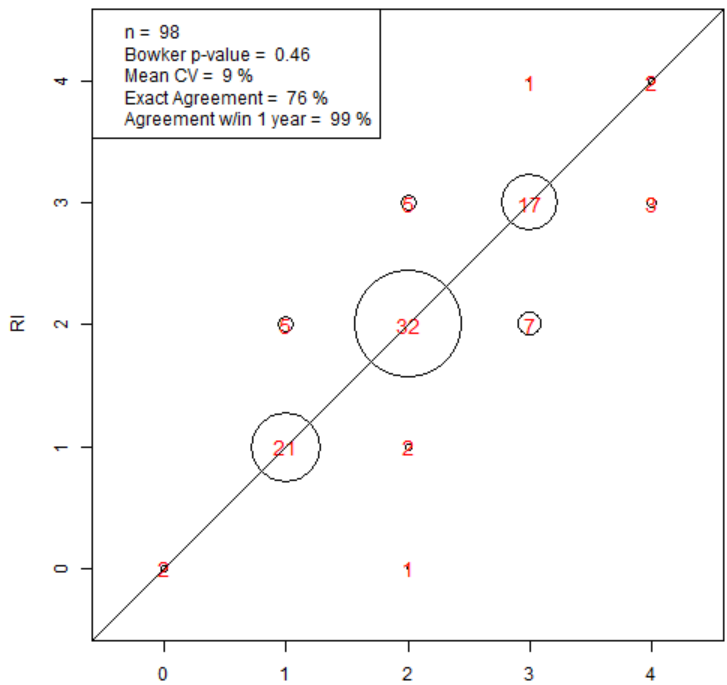


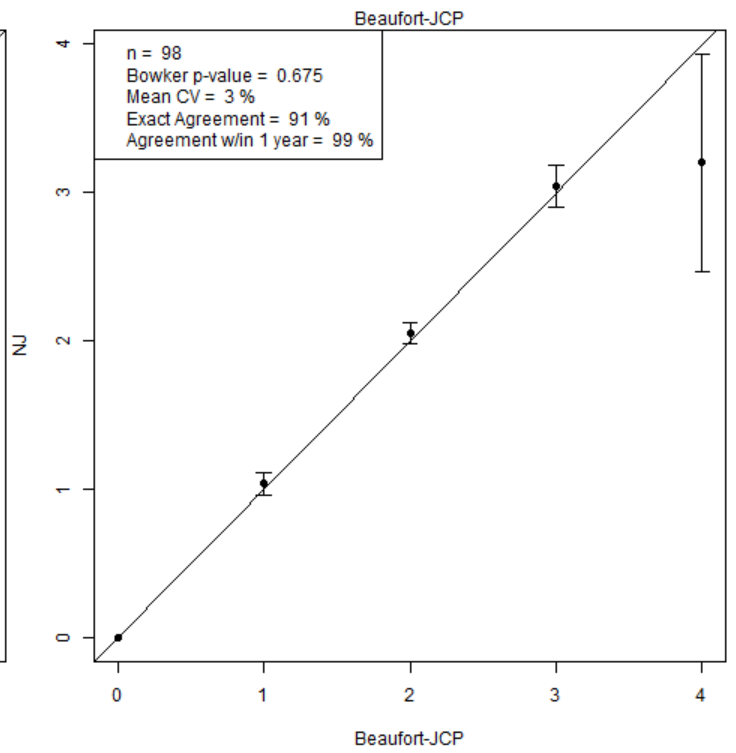
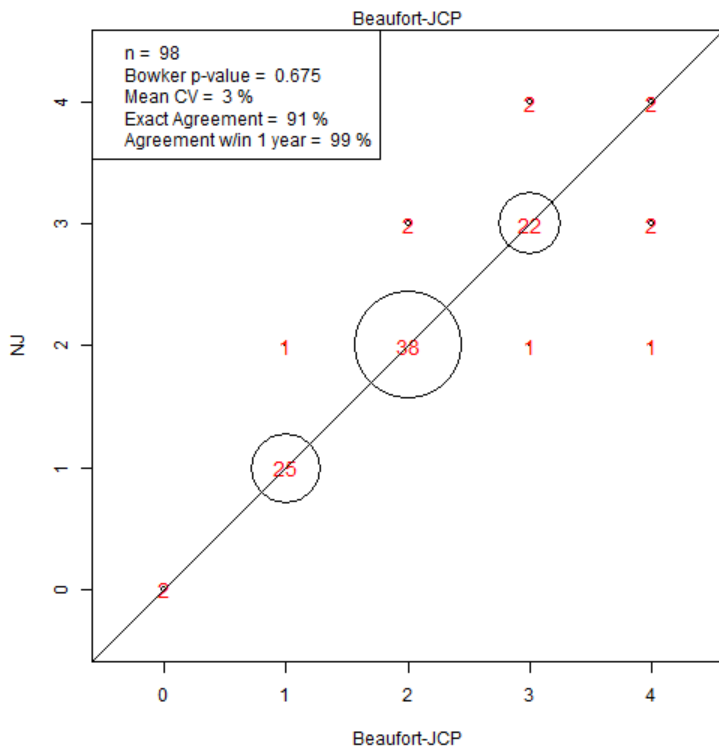
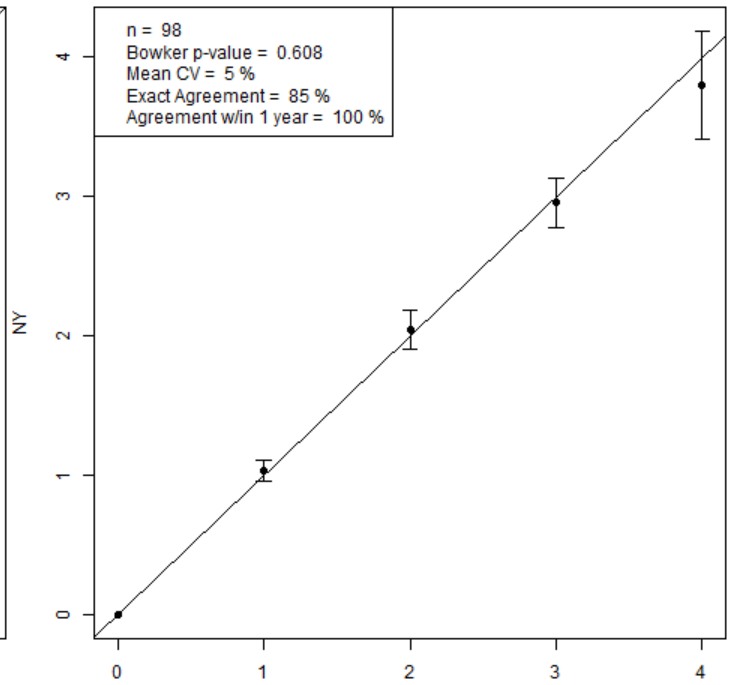
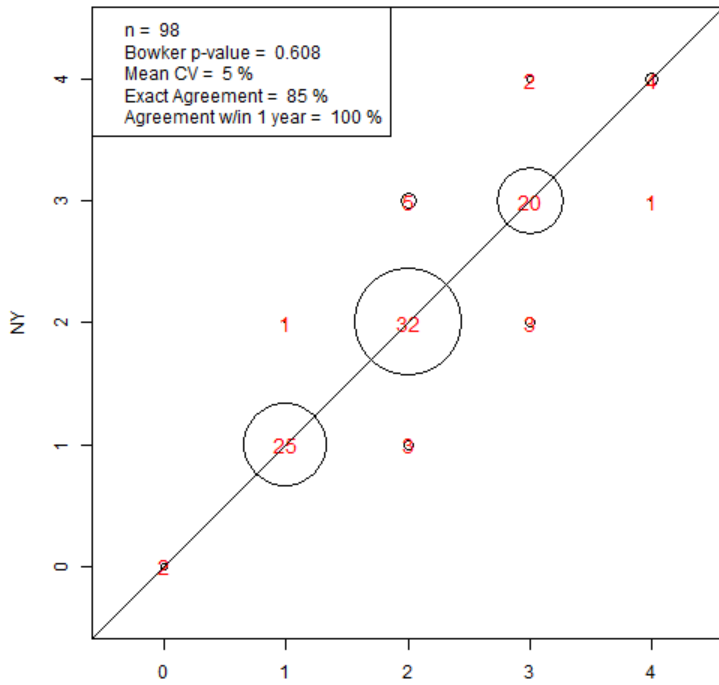


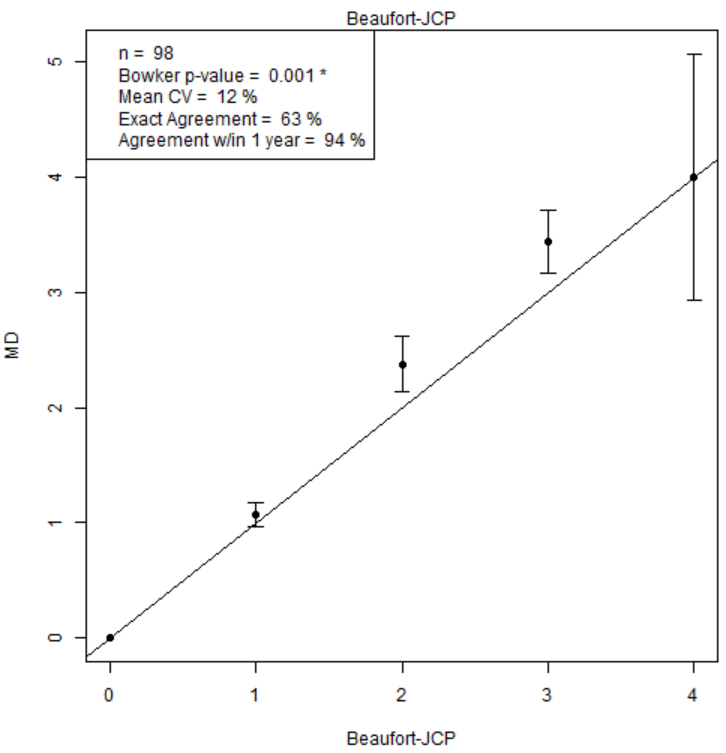
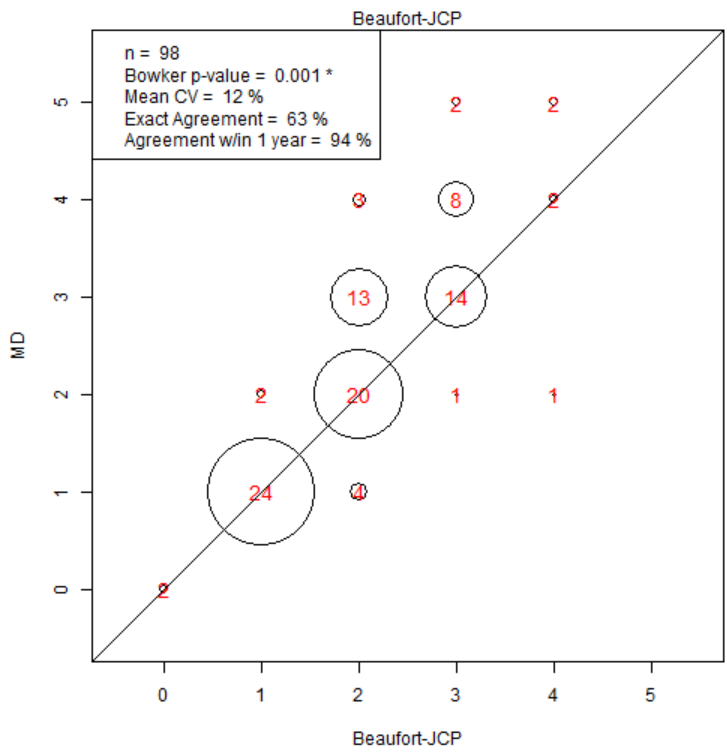
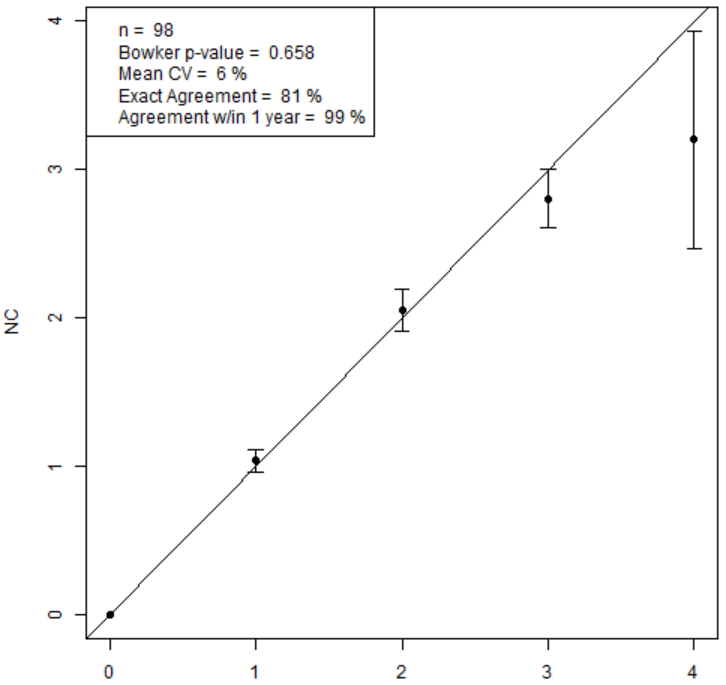
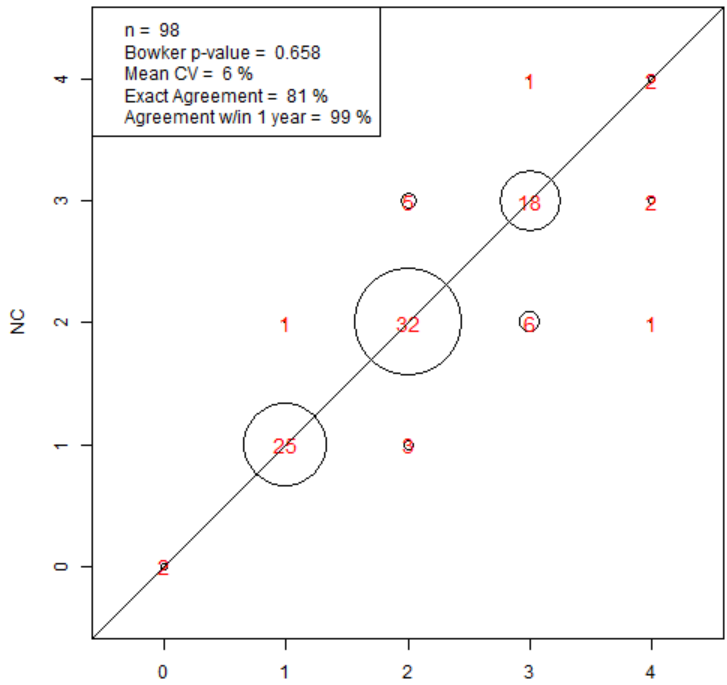


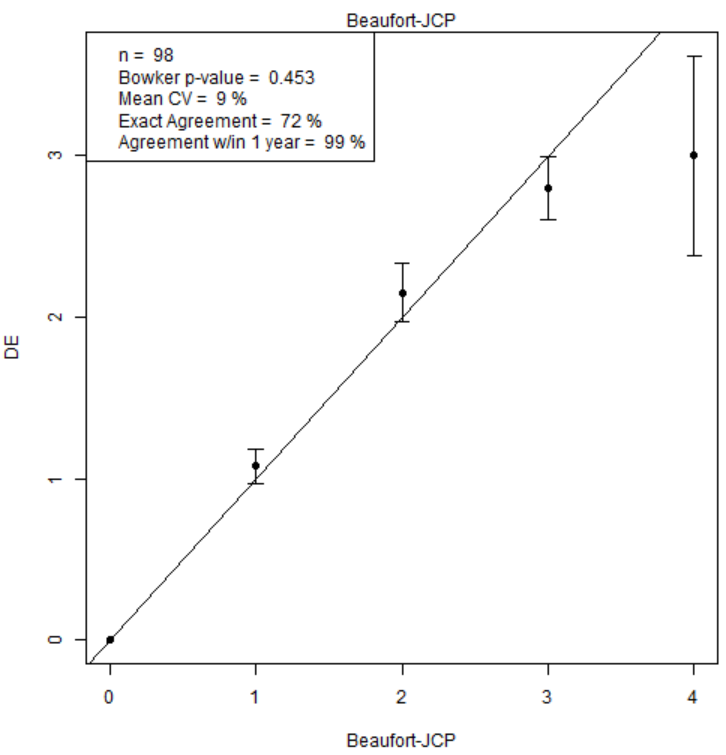
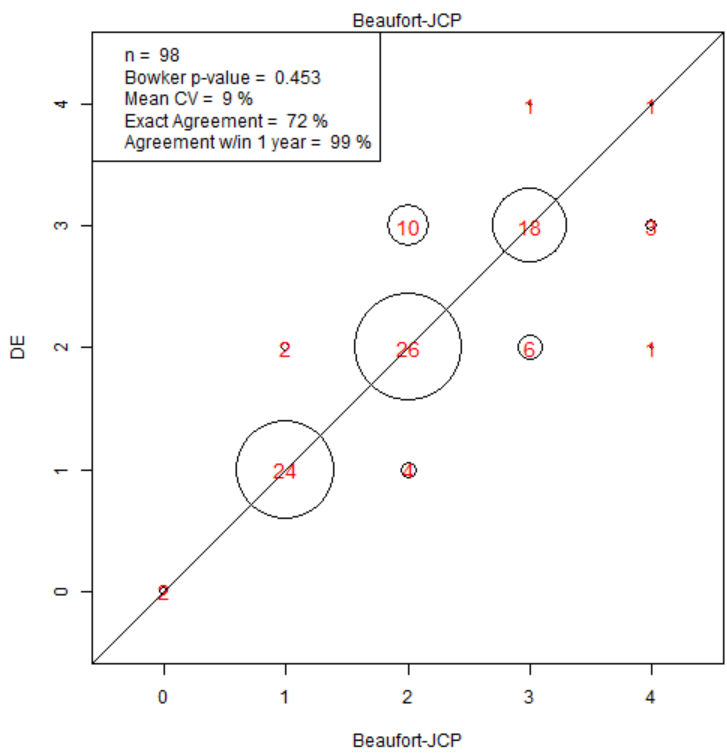
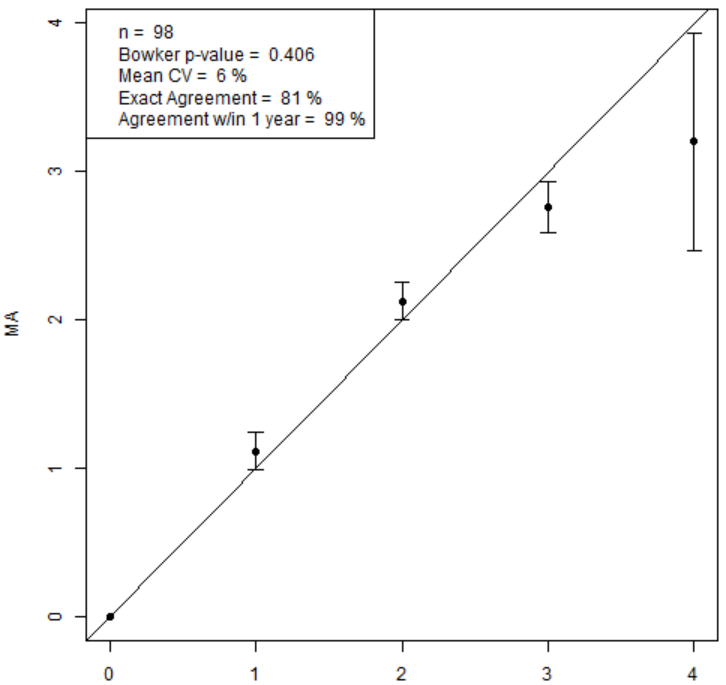
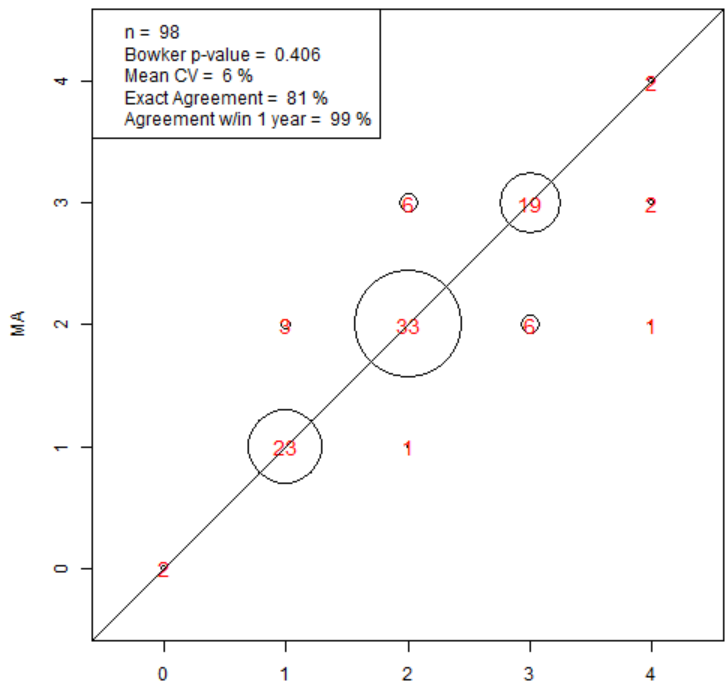


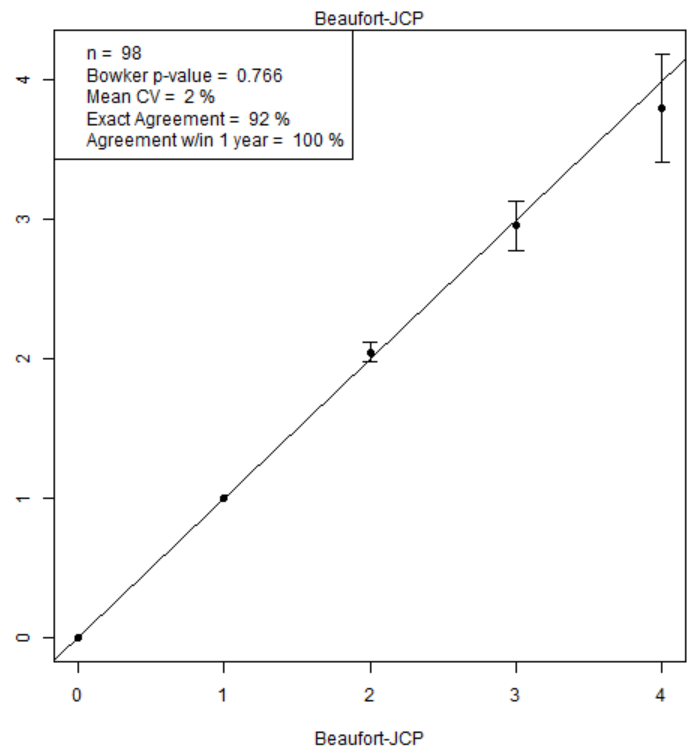
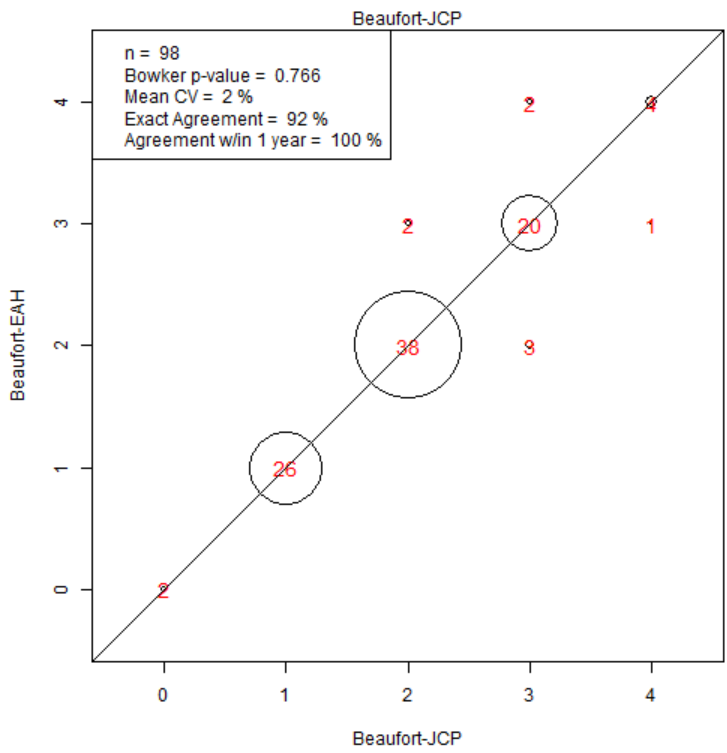
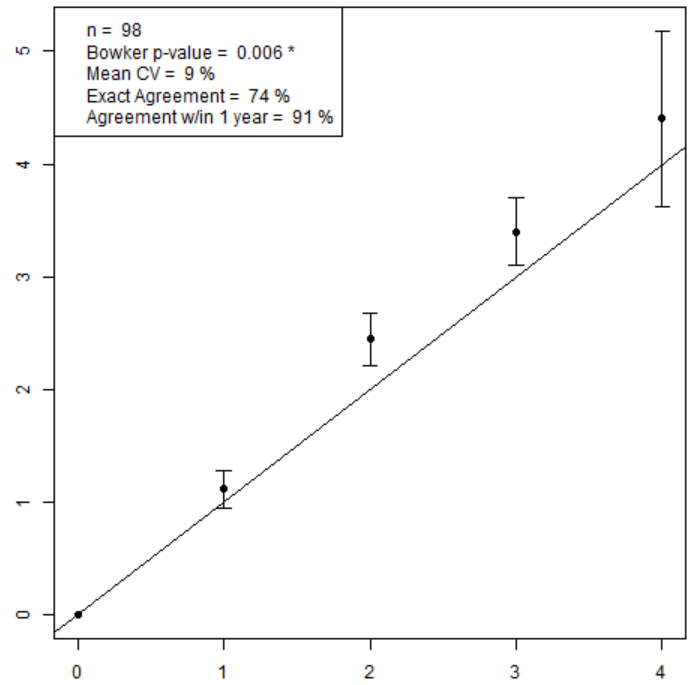
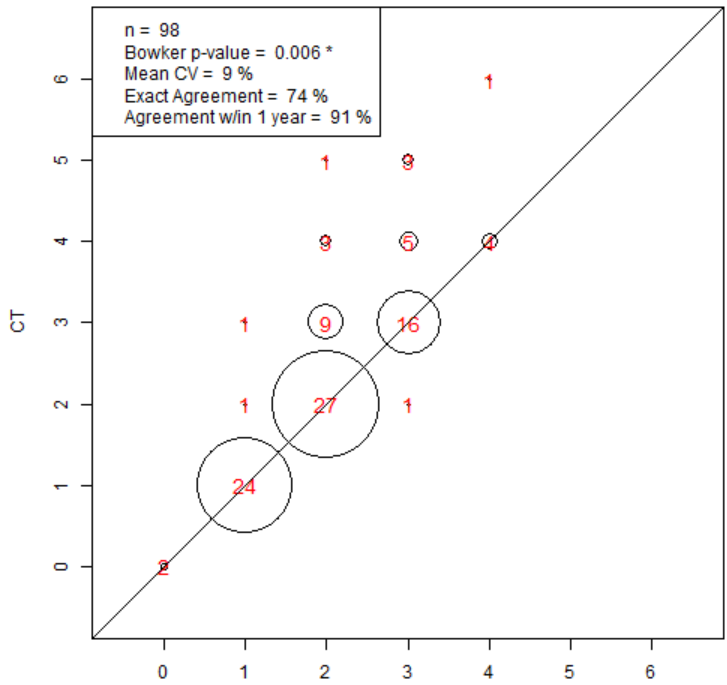












Appendix A: NOAA-Beaufort Atlantic Menhaden: Port Sampling and Scale Processing Protocols

- A. **Background:** Biological sampling for the menhaden from the reduction purse-seine fishery has been conducted on the Atlantic coast since 1955. It is based on a two-stage cluster design and it is conducted over the range of the fishery, both temporally and geographically. Port agents randomly select purse-seine vessels and at dockside retrieve a bucket of fish (first cluster) from the top of the vessel's fish hold (Figure 1). The sample is assumed to represent fish from the last purse-seine set of the day or trip, not the entire boat load. The agent ascertains from the crew the location and date of the last set. From the bucket the agent randomly selects ten fish (second cluster; Figure 2), which are measured (fork length in mm), weighed (grams), and the scales are removed for ageing. As background, by the late 1960s staff biologists determined that menhaden tend to school by size and age, and that variability in age and size was greater among vessels than within vessels. Prior to 1970 the number of fish sampled in the second cluster was 20. After 1970 the number of fish sampled in the second cluster was reduced to 10 fish in an effort to increase sampling of more purse-seine vessels.



Figure 1. Port agent acquires sample (first cluster) from the top of the fish hold on a reduction purse-seine vessel using a dip net; alternately, a bucket is sometimes used.



Figure 2. From the dip net or bucket of fish (first cluster), ten menhaden are randomly chosen (second cluster) to be measured (fork length in mm), weighed (in grams), and a scale patch removed for ageing.

In the mid-1990s landings of menhaden for bait by purse-seine became a growing portion of the total coastwide landings. In order to better characterize the menhaden bait fishery for size and age composition of the catch, federal and state port agents (primarily in NJ, RI, MA, and ME) were asked to sample purse-seine-for-bait vessels using sampling protocols similar to those for the reduction fishery, that is, using the two-stage cluster design (above).

- B. **Processing a 10-Fish Sample:** Essential equipment to process menhaden samples includes a measuring board graded in mm, a balance (preferably electronic and reading to the nearest g), wooden block to hold glass vials for the scale samples (see below), blunt scalpel (Fisher Scientific Cat. # 08920-B), and a 12-16 oz wash bottle. Roughly, a 5-in x 5-in wooden block (cut from 2-in lumber) is used to accommodate the glass vials that will hold the scale samples. The wooden blocks have four rows of five holes drilled into them; each two rows are separated by a space (Figures 3 and 4). Holes are drilled with a drill press using a 5/8" Forstner bit. One dram glass vials (Fisher Sci. Cat. # 03-339-30C [15.00 x 45 mm]) fit nicely into these holes.

After the ten-fish sample is secured, the fish are lined-up on a bench top with heads pointing away from the processor and #1 fish to the left and #10 fish to the right (Figure 2). The wash bottle is filled with water and a few drops of dish washing detergent. Vials are placed in the top two rows of five holes in the wooden block. Each vial is filled about 2/3 full with water from the wash bottle. Vials will hold scale samples and the detergent helps cut through slime and mucus left on the scales.

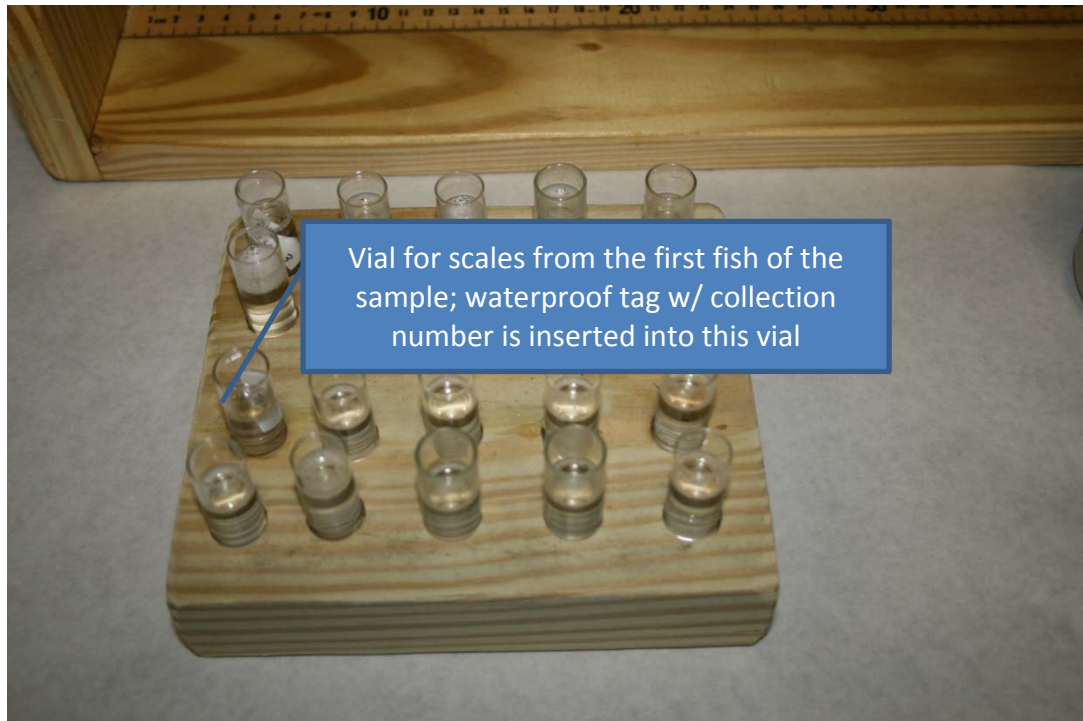


Figure 3. Wooden vial block that will hold two 10-fish samples of scales.

In NMFS protocols, each 10-fish sample is termed a collection and given a unique 4-digit collection number, which is written on a small piece of waterproof paper and inserted into the vial at the top and left of the double rows of five vials (Figure 3); by convention, this will be vial #1 for the first fish in the collection (or sample). The four vials to the right of the first vial will be for scales from fish #,2, #3, #4, and #5, respectively; the row of vials below the first row will be for scales from fish #6 - #10.

The first fish of the sample is measured for FL (Figure 4; snout snubbed to the start of the measuring board and measurement made to the longest central rays in the fork of the tail) and weighed (g). Then, a scale patch is removed from about midway down on the left flank (near the lateral line) and below the insertion of the dorsal fin (Figure 5).



Figure 4. Fork length measurement taken in mm.



Figure 5. Atlantic menhaden showing area (yellow box) from which scales for ageing should be removed.

Scales in this vicinity are preferred for ageing as they are symmetrical and generally uniform in shape. Initially, run the edge of the scalpel blade in several quick motions from head to tail across the scales to be removed; this removes a majority of slime, debris, and mucus from the scale sample; wipe the scalpel with a paper towel to remove slime. Insert the scalpel blade under a row of scales and move it forward, lifting a couple of rows of scales upward in the process (Figure 6). Place the thumb down on the scale patch and scalpel, lift the patch from the body (Figure 7) (it should have about 20-25 scales), and place it into vial #1. Measure, weigh, and remove a scale patch from fish #2, etc. Scales placed in the vials with water may be kept in a refrigerator up to several days before the mounting procedure.



Figure 6. Scalpel is inserted below a row of scales and moved forward slightly to raise several rows of scales from the skin; “pin” the patch of scales between the thumb and scalpel and remove the scale patch from the flank of the fish.



Figure 7. Scale patch on the scalpel before it is placed in the vial.

- C. **Mounting Menhaden Scales:** Menhaden scales are mounted, or sandwiched, between two microscope slides. The bottom slide is blank. Six Atlantic menhaden scales are placed in two rows of three each with the scale pectinations in the “up” position (Figure 8). On top of the scales and the bottom slide is placed a cover microscope slide which has a stick-on label on the left side with a unique specimen number. The two slides are held in place with two

small strips of transparent tape; be careful not to cover any of the scales with overlapping tape (Figure 8).



Figure 8. Mounted menhaden scales between two microscope slides.

Mounted Atlantic menhaden scales are viewed on an Eberbach macro-projector at 48x magnification (Figure 9). Age rings on Atlantic menhaden scales are defined as compressions or interruptions of uniformly spaced circuli in the anterior field of the scale, which are continuous through the lateral fields. Under transmitted light age rings form narrow, continuous, dark bands roughly paralleling the lateral and anterior margins of the scale. A focus is arbitrarily chosen near the center of the posterior field at the base of the circuli. Straight-line measurements are made from the focus to successive scale rings and the scale edge.

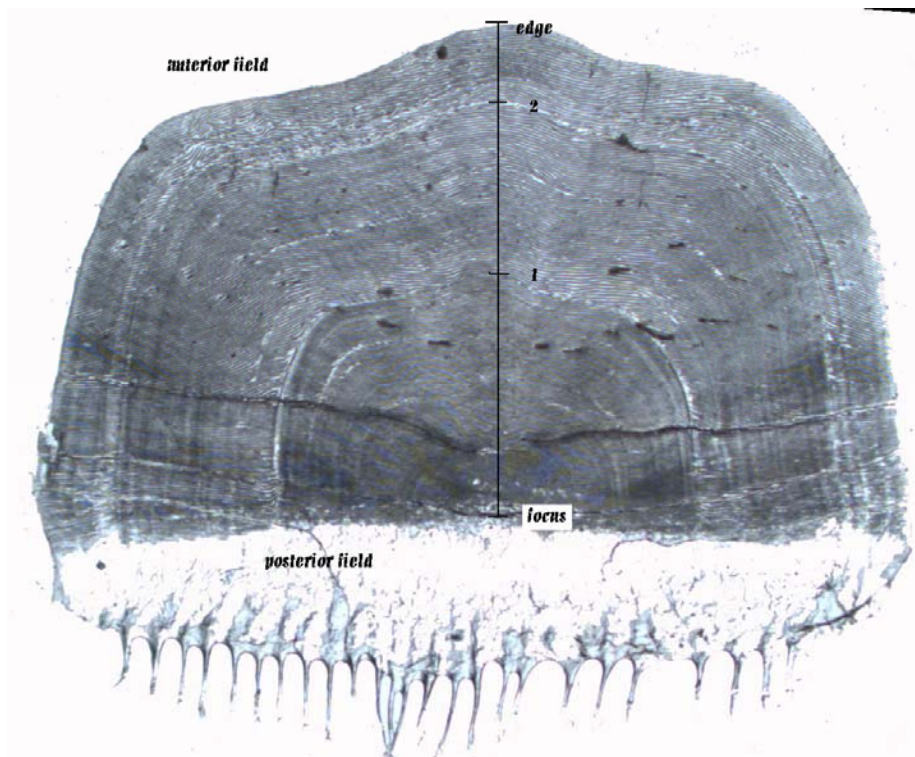


Figure 9. Menhaden scale as viewed with an Eberbach macroprojector.

Often, scale samples from menhaden-for-bait landings come to us from state port agents. The scales are frequently stored dry in coin envelopes and mailed to the Beaufort Lab. We have had good success soaking these dried scales in water, then mounting them between slides. In particular, we place the dried scale patch overnight in a small Petri dish with a 10% solution of hydrogen peroxide and water. The next day the scales are usually pliable and ready for mounting.

Disclaimer: Mention of source for supplies and equipment does not convey NOAA endorsement of the given products.

Joseph W. Smith, NMFS Beaufort, 5/29/2015

Appendix B: Atlantic Menhaden Reference Set Instructions

Atlantic Menhaden

Reference Set of Scales

-

Introduction

Atlantic States Marine Fisheries
Commission
and
NOAA Beaufort Lab

Preface

The purpose of these instructions is to:

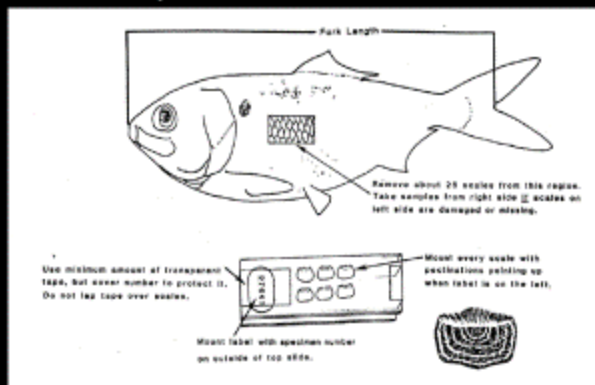
1. serve as a basic training guide for those beginning to age Atlantic menhaden using scales
2. increase precision among readers in the interpretation of annual marks on the scales, and
3. provide accurate age structure of Atlantic menhaden collected by the states in fishery-independent surveys.

These instructions were adapted from a PowerPoint developed by the Gulf States Marine Fisheries Commission for training staff from various Gulf states in the methods of ageing gulf menhaden scales. GSMFC's contributions are appreciated and acknowledged.

Introduction

Historically, scales have been used to age Atlantic menhaden; the seminal paper on ageing Atlantic menhaden was published by June and Roithmayr (1960, Fish. Bull. 171[60]: 323-342); a pdf of this article will be provided to you.

NMFS port sampling protocols call for port agents to mount six scales per fish. A patch of scales is removed from the flank (mid-body) below the insertion of the dorsal fin and in the vicinity of the lateral line.



Some caveats about “reading” scales

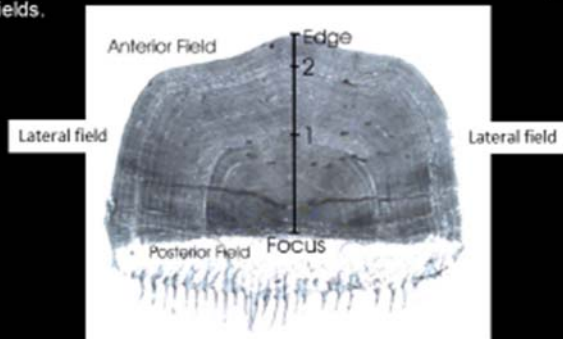
At NMFS Beaufort, menhaden scales are read using an Eberbach macroprojector; distances to annuli and scale edge are measured and recorded by a sonic digitizer (Graybar Co.)

Not all state labs are equipped w/ Eberbach machines; presumably most state labs will use compound/dissecting microscopes or microfiche machines to read menhaden scales.

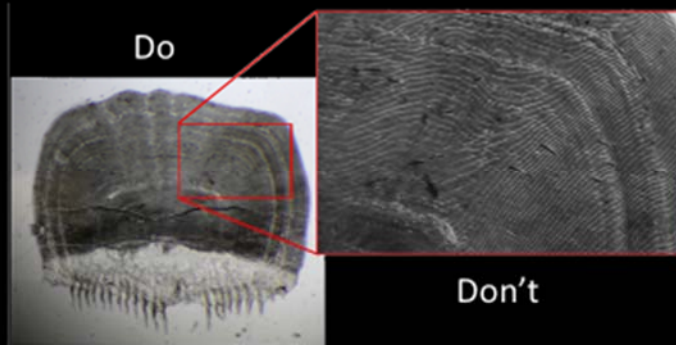
Whatever the device used, the following criteria are guidelines for distinguishing annuli on menhaden scales.



- General criteria for valid annuli are compressions (or crowding) in the normal pattern of circuli in the anterior field of the scale which are continuous with "cutting over" zones in the lateral fields; the latter features are most important in defining valid annuli.
- Regenerated scales are common on menhaden. The circuli on regenerated scales often appear as a thumb print that has been skewed or smeared to the left or right. These are coded by NMFS as illegible.
- A small proportion (usually <10% per given sampling year) of menhaden scales lack well-defined annuli; these are usually coded also as illegible.
- Accessory rings may appear between valid annuli; accessory rings often show strong compression of circuli in the anterior field of the scale, but lack the strong "cutting over" in the lateral fields.



Under transmitted light, annuli form narrow, continuous, bands roughly paralleling the lateral and anterior margins of the scale; these tend to become less obvious with increased magnification. It is recommended that the scales be viewed at a relatively low magnification.



When using a microscope the reader can quickly adjust the focus, shift magnification, adjust light levels and view other scales to locate or confirm annuli. However, unlike otolith sections, flipping and tilting scale mounts do not generally improve readability.

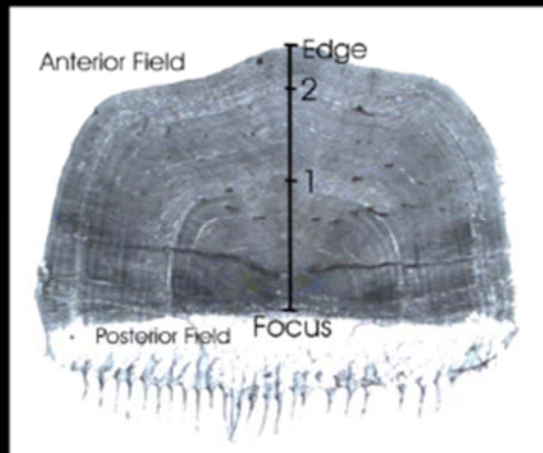
Measurements

The focus is arbitrarily chosen near the center of the posterior field at the base of the scale and measurements are made along a straight line "up" toward the center of the anterior edge.

The annuli are generally measured to the proximal or leading edge of the annual mark.

A spreadsheet is to be provided with information about the reference set of slides (n = 100) and specimens from which they were taken, e.g., age assigned by Beaufort reader, specimen FL (in mm), weight (g), date and port of capture, and distances from scale focus to successive annuli and scale edge. The latter measurements were made on the NMFS Eberbach macroproject and are in mm. Obviously, state labs will have several different scale viewing systems; measurements to successive annuli are provided on the spreadsheet so that state personnel might have an idea of the proportional distance(s) to each annulus

Scale Morphology



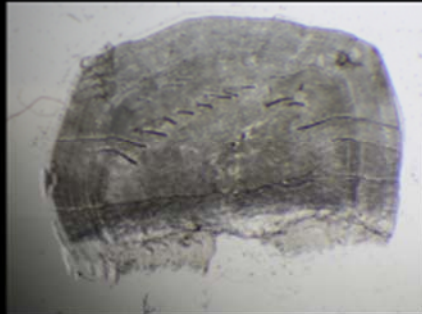
Scale from an age-2 menhaden, showing the focus, scale edge, and first and second annuli.

For the reference set, each fish scale which was used for determining the "Beaufort age" is designated in the Excel spreadsheet. The scale numbering convention for an individual slide is as follows:

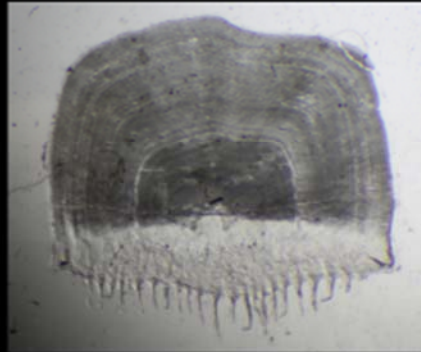


Port #5
Slide #35179
Scale #2

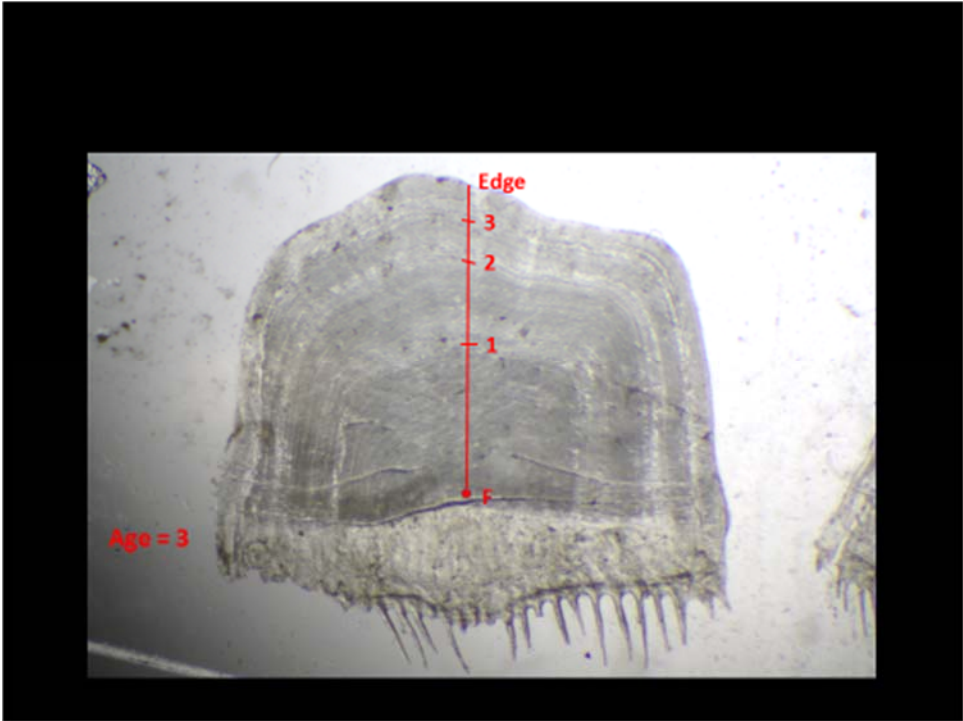
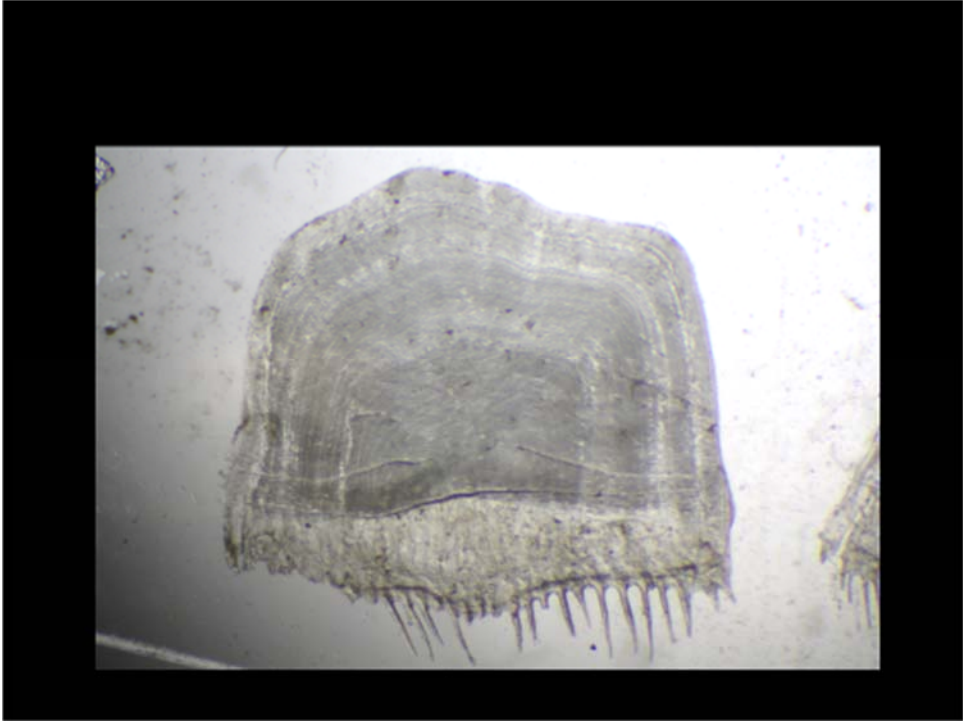
What to Expect



Illegible, some regeneration



Good



Assigning Age Class

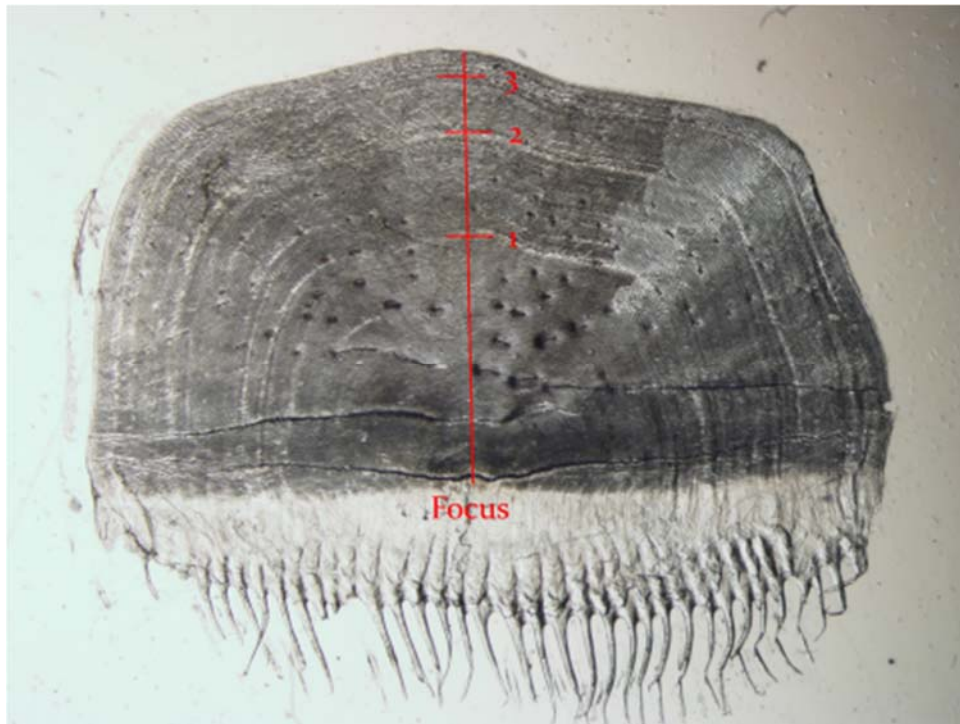
- The assumed birthdate for Atlantic menhaden is March 1
- Annuli deposition generally occurs from March – May
- See GSMFC manual (section 4) for guidance on assigning age class based on number of annuli, date of collection, and growth on scale edge
 - <http://www.gsmfc.org/publications/GSMFC%20Number%20167.pdf>

Appendix C: Atlantic Menhaden Ageing Workshop Sample Evaluation

ASMFC Atlantic Menhaden Ageing Workshop Sample Evaluation

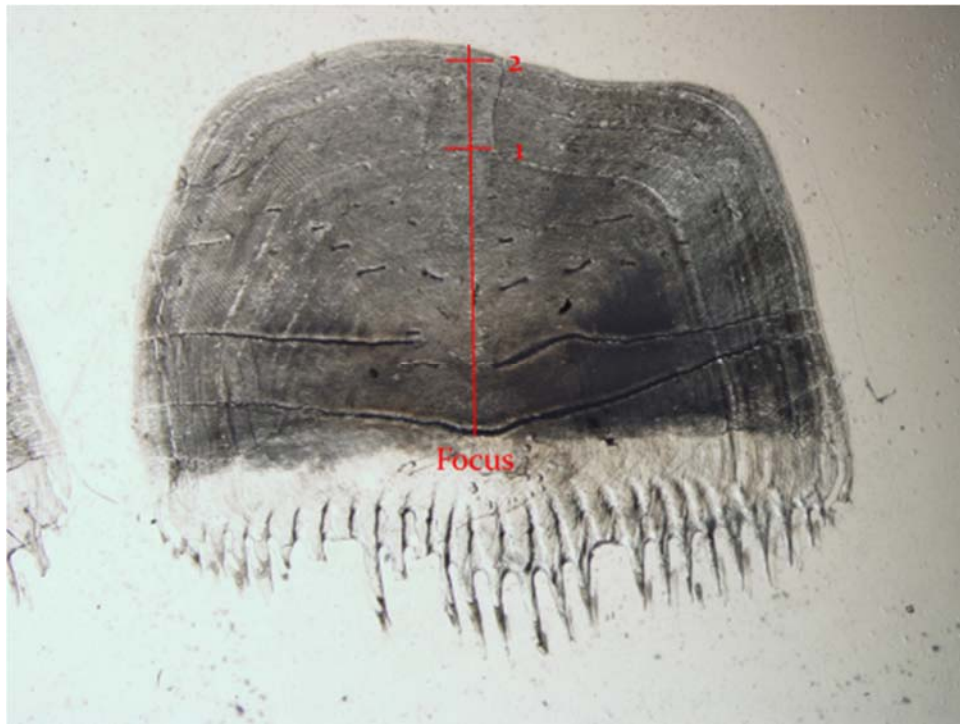
Sample 43867

- 272 mm fork length
- Collected 9/3/2013 in Reedville, VA
- Workshop consensus read reason
 - Perfect agreement during the exchange.
- Exchange statistics
 - mean age = 3.0, sd = 0.00
- Workshop consensus age
 - 3



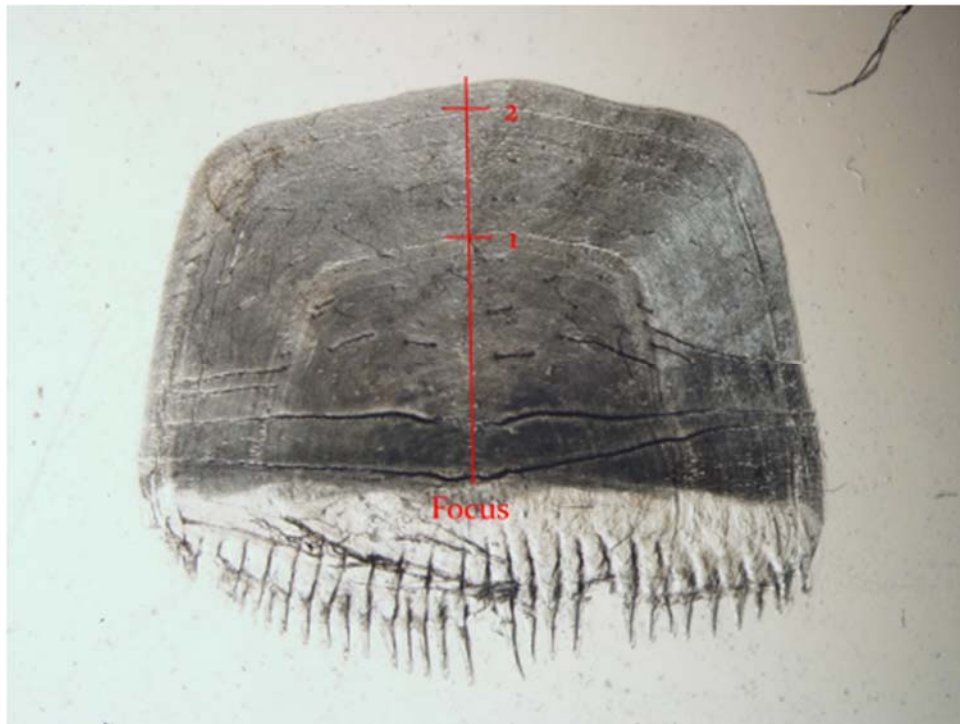
Sample 45133

- 243 mm fork length
- Collected 5/19/2014 in Reedville, VA
- Workshop consensus read reason
 - Perfect agreement during the exchange.
- Exchange statistics
 - mean age = 2.0, sd = 0.00
- Workshop consensus age
 - 2



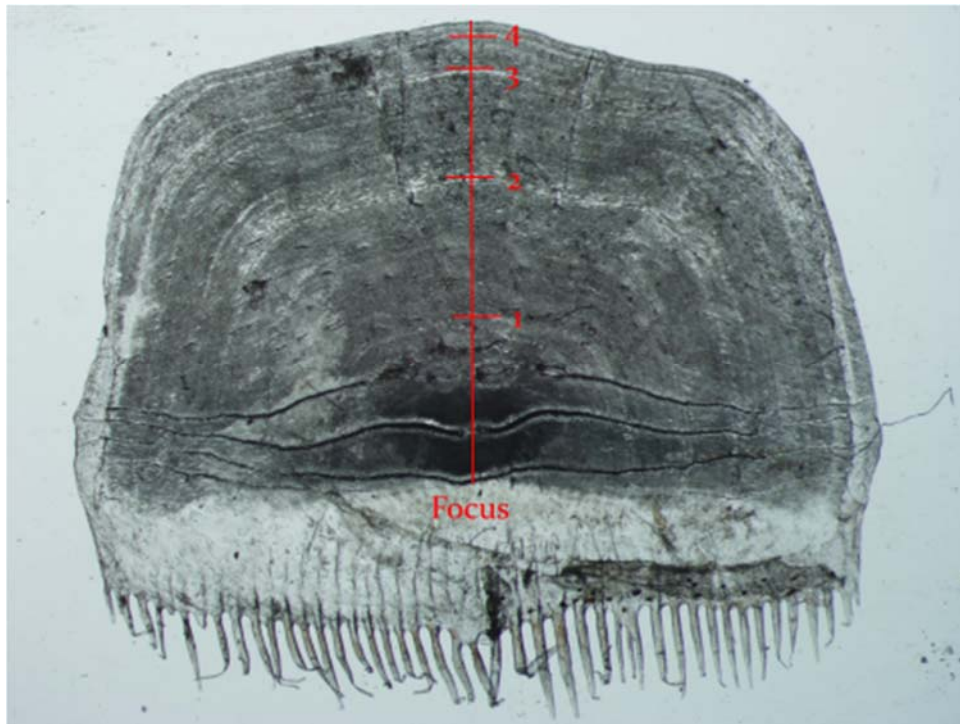
Sample 45374

- 225 mm fork length
- Collected 6/3/2014 in Reedville, VA
- Workshop consensus read reason
 - Perfect agreement during the exchange.
- Exchange statistics
 - mean age = 2.0, sd = 0.00
- Workshop consensus age
 - 2



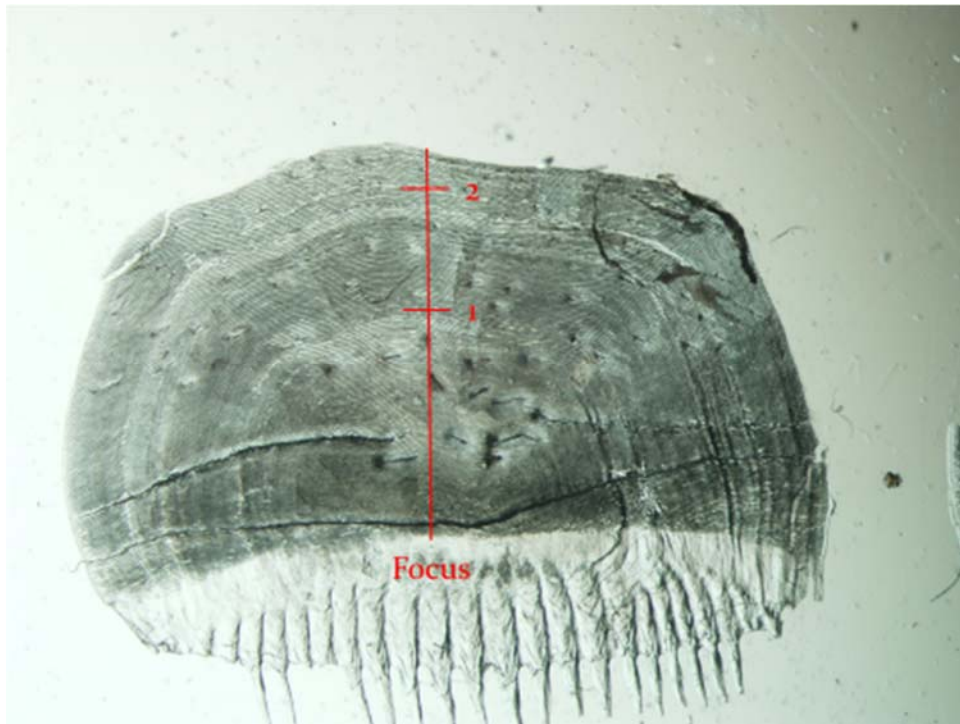
Sample 22600

- 290 mm fork length
- Collected 5/20/2013 in RI/MA
- Workshop consensus read reason
 - The ages assigned during the exchange were split 50/50 between ages 3 and 4.
- Workshop consensus read comments
 - The fish was caught in the spring and there was little growth between the fourth annulus and the scale edge.
- Exchange statistics
 - mean age = 3.5, sd = 0.52
- Workshop consensus age
 - 4



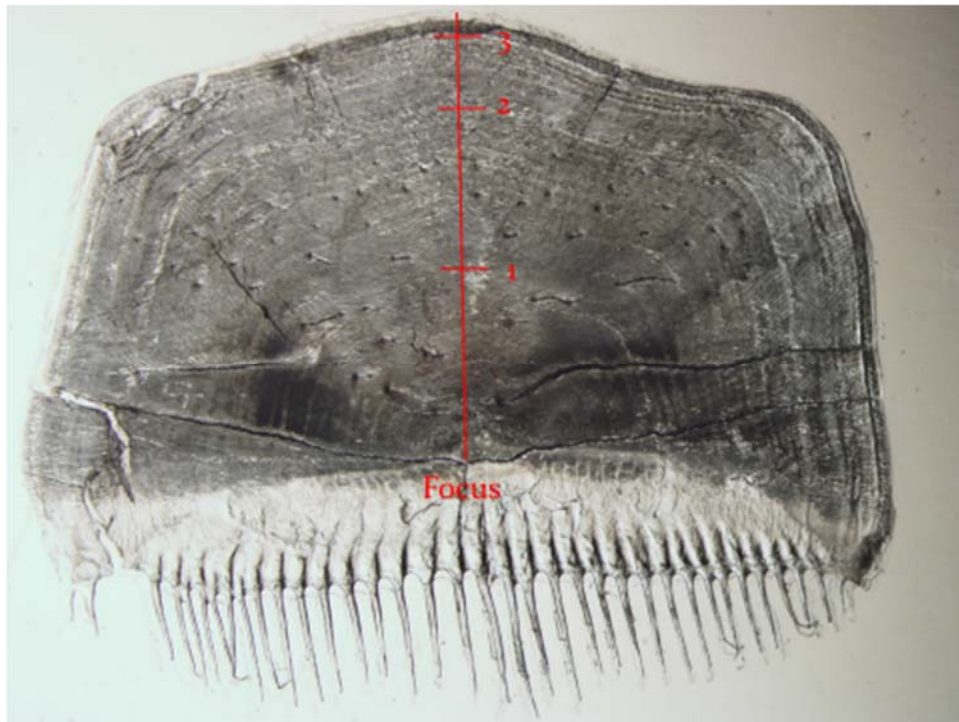
Sample 43434

- 201 mm fork length
- Collected 8/2/2013 in Reedville, VA
- Workshop consensus read reason
 - The ages assigned during the exchange were split between ages 1 and 2.
- Workshop consensus read comments
 - This scale is difficult to read.
- Exchange statistics
 - mean age = 1.6, sd = 0.51
- Workshop consensus age
 - 2



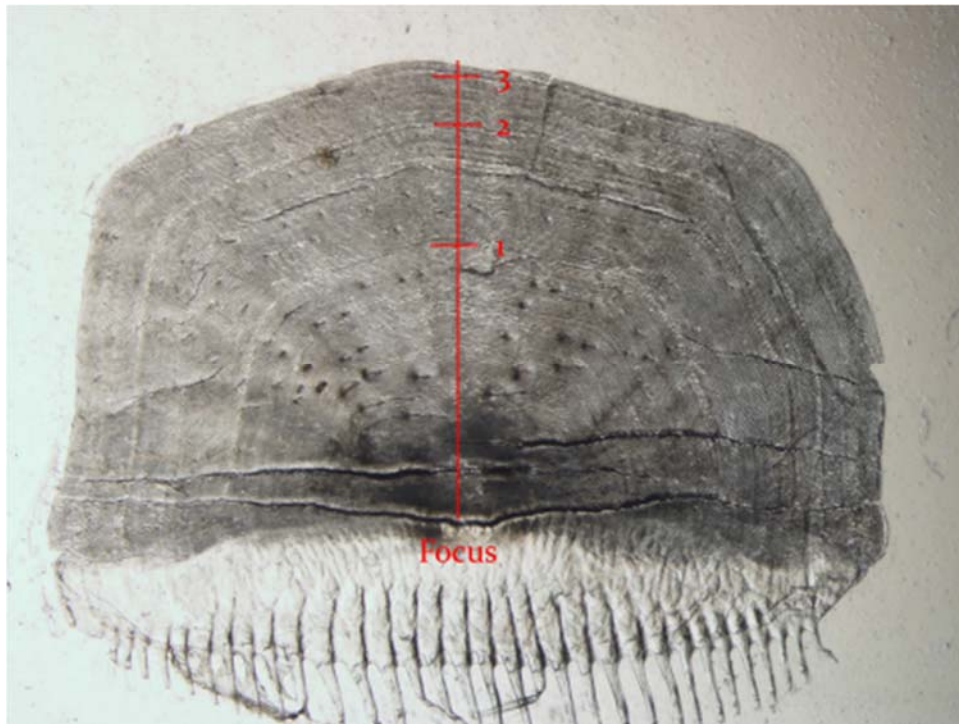
Sample 42773

- 271 mm fork length
- Collected 6/24/2013 in Reedville, VA
- Workshop consensus read reason
 - There is a small distance to the first annulus.
- Workshop consensus read comments
 - This scale is difficult to read.
- Exchange statistics
 - mean age = 3.0, sd = 0.74
- Workshop consensus age
 - 3



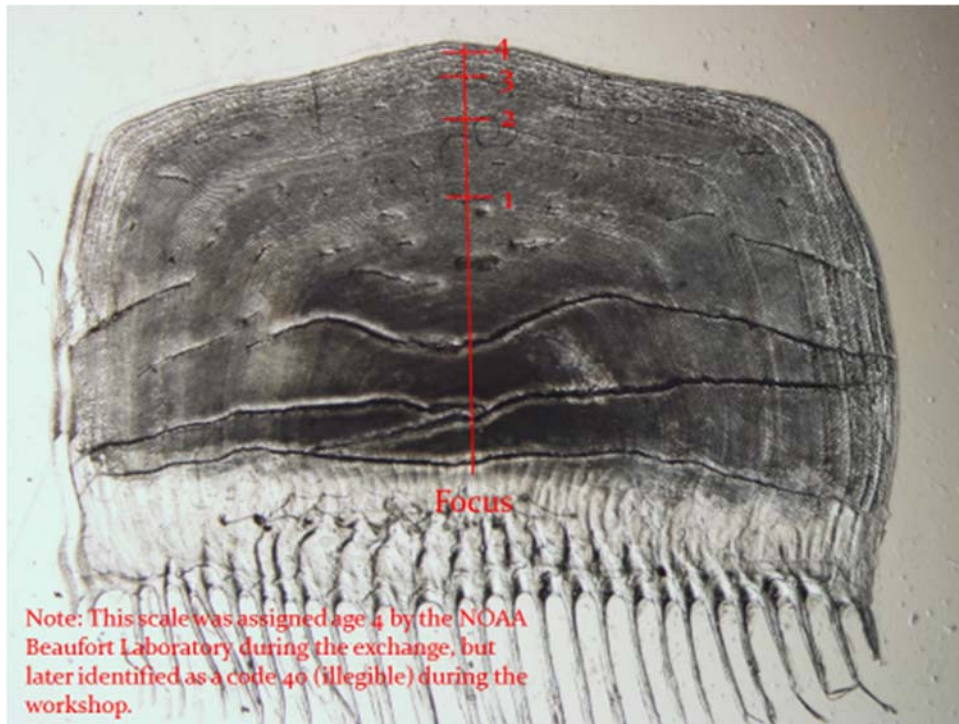
Sample 13969

- 278 mm fork length
- Collected 11/6/2013 in NJ
- Workshop consensus read reason
 - The ages assigned during the exchange were split between ages 2 and 3.
 - There is a small distance to the first annulus.
- Workshop consensus read comments
 - Group could not come to a consensus age on the dissecting scope, but did agree on an age on the Eberbach.
 - A poor quality margin may have led to age 2 assignments during the exchange.
 - There is a regenerated scale in the sample.
- Exchange statistics
 - mean age = 2.8, sd = 0.75
- Workshop consensus age
 - 3



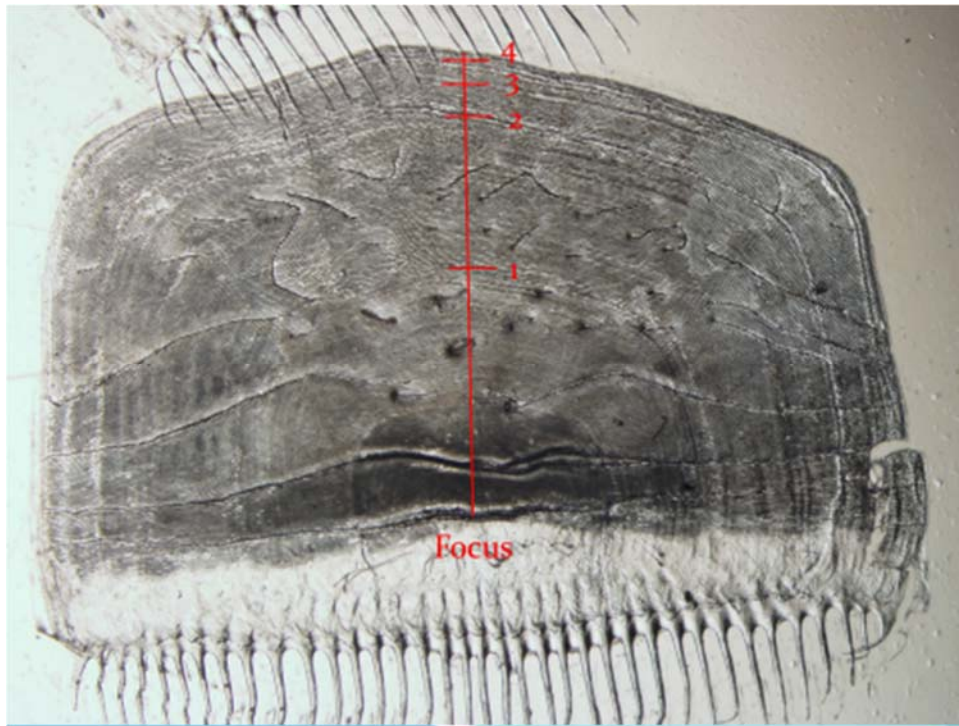
Sample 44816

- 281 mm fork length
- Collected 11/18/2013 in Reedville, VA
- Workshop consensus read reason
 - There was poor agreement with a wide range of age assignments during the exchange.
- Workshop consensus read comments
 - The sample was illegible on both the dissecting scope and Eberbach.
 - An otolith may have helped age assignment.
- Exchange statistics
 - mean age = 3.8, sd = 1.03
- Workshop consensus age
 - No consensus age (Beaufort classification of 40).



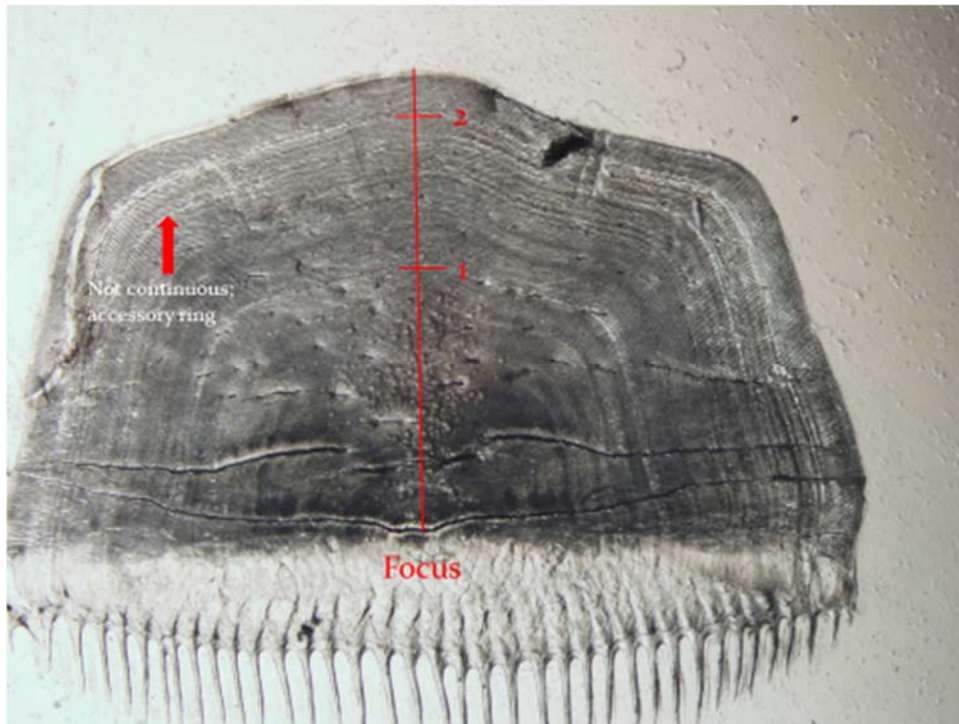
Sample 44811

- 275 mm fork length
- Collected 11/18/2013 in Reedville, VA
- Workshop consensus read reason
 - The ages assigned during the exchange were split between ages 3 and 4.
- Workshop consensus read comments
 - The group could not come to a consensus age on the dissecting scope and suggested it was a 40. The group was more confident with a consensus age when viewed on the Eberbach.
- Exchange statistics
 - mean age = 3.6, sd = 0.67
- Workshop consensus age
 - 4



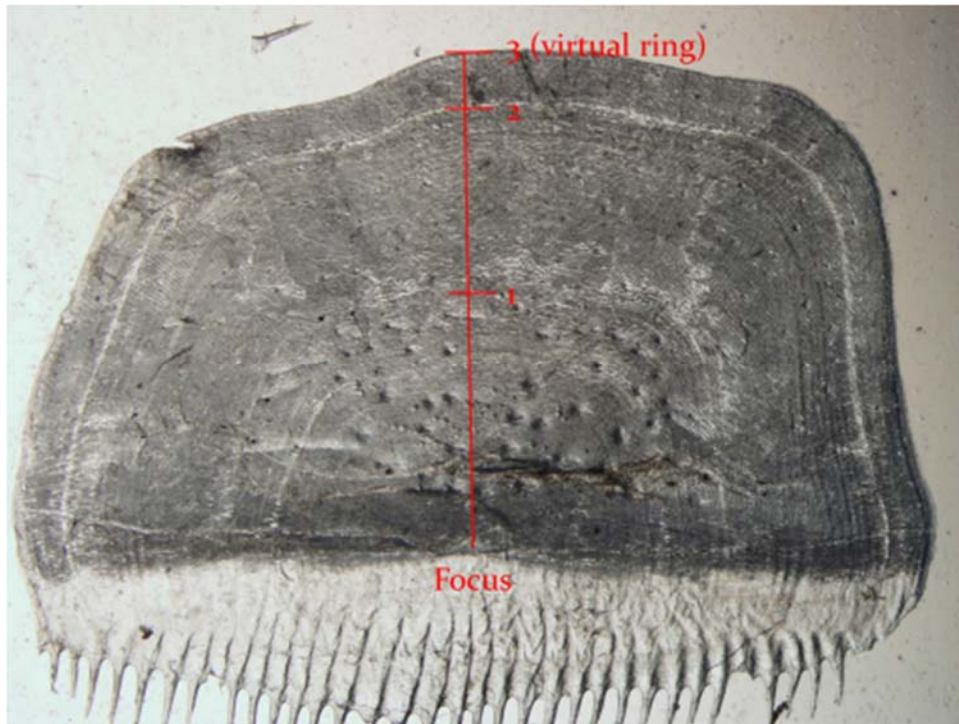
Sample 44780

- 272 mm fork length
- Collected 11/15/2013 in Reedville, VA
- Workshop consensus read reason
 - The ages assigned during the exchange were split between ages 2 and 3.
 - MD DNR and CT DEEP both aged the sample two years older than Ethel.
- Workshop consensus read comments
 - There are some accessory rings that are not continuous around the anterior and lateral fields. Some exchange participants likely counted the false rings in the exchange.
 - The distances between the rings could be used to justify accessory rings. The distances between the true annuli and accessory rings don't make since based on the one half subsequent growth rule of thumb.
- Exchange statistics
 - mean age = 2.7, sd = 0.78
- Workshop consensus age
 - 2



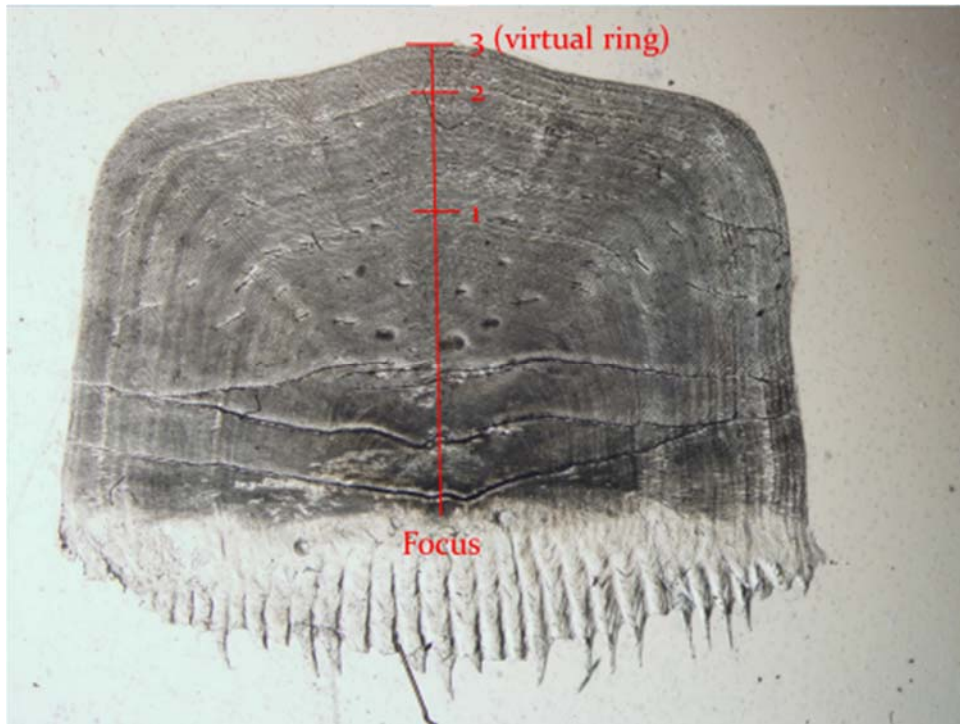
Sample 14041

- 285 mm fork length
- Collected 5/7/2013 in NJ
- Workshop consensus read reason
 - The ages assigned during the exchange were split between ages 2 and 3.
- Workshop consensus read comments
 - A virtual ring should be counted for this fish due to the distance between the last annulus and the edge (growth) and the capture date.
- Exchange statistics
 - mean age = 2.7, sd = 0.49
- Workshop consensus age
 - 3



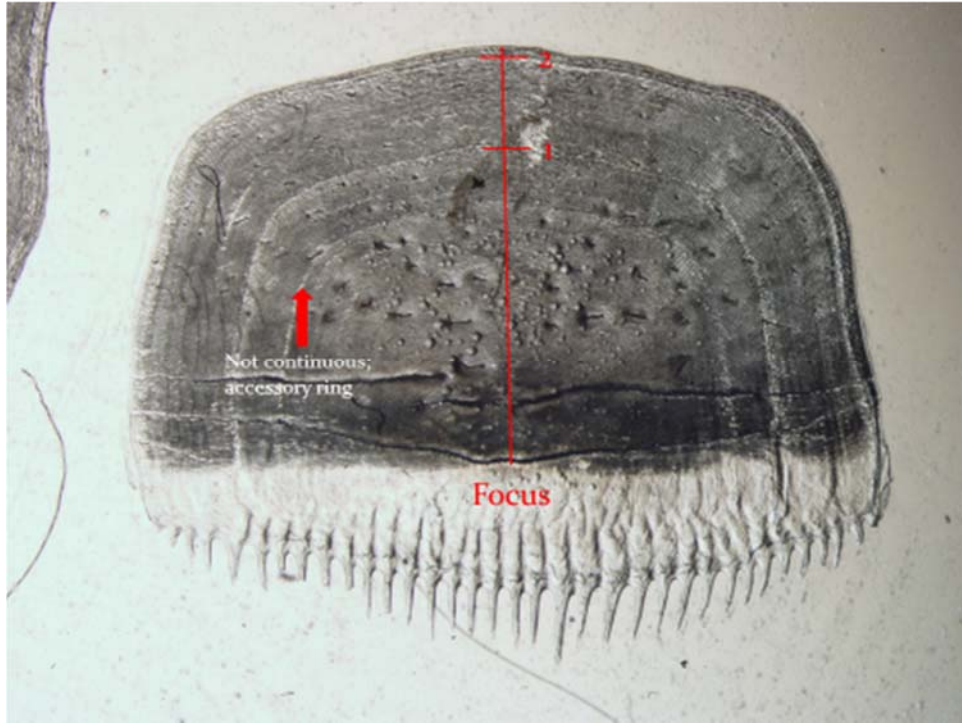
Sample 45030

- 253 mm fork length
- Collected 5/15/2013 in MD
- Workshop consensus read reason
 - The ages assigned during the exchange were split between ages 2 and 3.
 - MD DNR and CT DEEP both aged the sample two years older than Ethel.
- Workshop consensus read comments
 - A virtual ring should be counted for this fish due to the distance between the last annulus and the edge (growth) and the capture date.
- Exchange statistics
 - mean age = 2.7, sd = 0.78
- Workshop consensus age
 - 3



Sample 45022

- 253 mm fork length
- Collected 5/15/2013 in MD
- Workshop consensus read reason
 - The ages assigned during the exchange were split between ages 2 and 3.
 - MD DNR and CT DEEP both aged the sample two years older than Ethel.
- Workshop consensus read comments
 - A virtual ring should be counted for this fish due to the distance between the last annulus and the edge (growth) and the capture date.
 - An accessory ring was identified on the scale in this sample.
- Exchange statistics
 - mean age = 2.3, sd = 0.49
- Workshop consensus age
 - 2



Appendix D:Atlantic Menhaden Ageing Workshop Agenda

Atlantic Menhaden Ageing Workshop
Atlantic States Marine Fisheries Commission
March 9-10, 2015
NOAA Beaufort Lab
101 Pivers Island Road
Beaufort, NC

Monday, March 9 (1:00 pm – 5:00 pm)

1. Welcome and Introductions
2. Workshop Goals and Objectives
3. Review Lab Ageing Practices (Sample Collection, Sample Preparation, Age Determination)
4. Review Results of Reference Set Exchange
5. Reference Set Sample Evaluation

Tuesday, March 10 (9:00 am – 12:00 pm)

1. Continue Reference Set Sample Evaluation
2. Next Steps and Recommendations
3. Adjourn