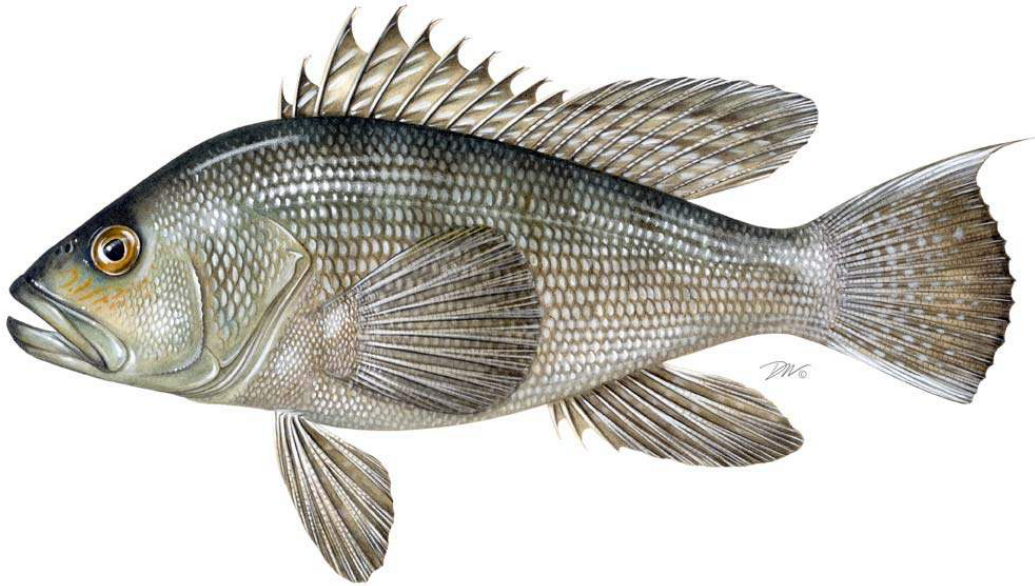


Atlantic States Marine Fisheries Commission

Proceedings of the 2013 Black Sea Bass Ageing Workshop



December 2013



Working towards healthy, self-sustaining populations for all Atlantic coast fish species or successful restoration well in progress by the year 2015

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1. Statement of Problem

In the 2011 assessment of the northern stock (Cape Hatteras to New England) of black sea bass (*Centropristis striata*), an age-based assessment model (ASAP) was considered as an alternative to the length-based model (SCALE) used in the previous assessment. Only age data from the NMFS Northeast Fisheries Science Center (NEFSC) was used to develop an age-length key for this assessment (NMFS 2012). The stock assessment peer review panel expressed concern that the age-length key was insufficient and suggested that low sample size, especially for older fish (age 4+), and resultant pooling over ages could be responsible for the inability of the model to track large cohorts through time, diminishing the impact of large recruitment events (Bell 2012). Recently, several additional labs have started ageing or plan to age black sea bass including MA DMF (2012), VIMS (2002), and RI DEM DFW (2013). These additional sources of age data could potentially supplement the NEFSC age data in future stock assessments to develop a more complete age-length key and reduce or avoid pooling over ages. However, ageing personnel from these labs have previously not convened to discuss a standardized ageing methodology and determine if consistent ageing criteria are used between labs. Ages are currently obtained from scales and both whole and sectioned otoliths, depending on the lab, and there has been no evaluation of ageing error between the labs. Additionally, ageing personnel from these labs have not met with ageing personnel from labs ageing southern stock fish (Cape Hatteras to Florida). These labs, including SC DNR and NC DMF, held a workshop in 2009 to standardize their ageing methodology and the results of their workshop could help inform ageing practices at labs ageing northern stock fish (SEDAR 2009).

Lack of a standardized methodology between labs and ageing personnel can cause interpretation error and unreliable age data for the assessment. Interpretation error can occur as systematic bias or imprecision and can vary between individual readers, ageing labs, and ageing structures. Systematic bias can cause significant error in model estimates of population parameters (Campana 2001). The peer review panel also expressed the importance of understanding the uncertainties inherent in extrapolating age data to length frequencies. Ultimately, the ASAP model was not accepted by the peer review panel for use by management. The panel suggested the assessment could be improved by investigating the reliability of black sea bass ageing, particularly of each ageing structure, and providing information on ageing precision to stock assessment scientists which can be incorporated into assessment models (Miller et al. 2011). The concerns with black sea bass age data were discussed further at a PMAFS data workshop in April 2013, prompting this workshop.

2. Workshop Goals and Objectives

The workshop was convened to address the concerns with age data identified during the stock assessment peer review. The workshop was to serve as a forum to discuss best practices for black sea bass ageing. Three goals of the workshop were:

- Investigate methodologies between labs and determine if labs are using consistent interpretation criteria so additional age data can be used to develop more complete and robust age-length keys.
- Develop recommendations for black sea bass ageing to improve age data.

- Provide information on ageing error between labs with available age data to inform future stock assessments.

3. Sample Collection and Ageing Methodology

NEFSC

Scales and otoliths have been collected since 1984 during fall and spring fishery-independent trawl surveys conducted by NMFS from New England to Cape Hatteras, NC. Approximately 350 samples are collected from each survey annually (≈ 700 total). Scales are typically collected from the commercial fishery by port samplers. Samples have been collected from the commercial fishery since 2008, with an emphasis on collecting samples from large and jumbo market size fish. A few thousand samples are collected from the commercial fishery annually. The size range of fish sampled is 4 – 60cm. One reader is currently ageing both scales and whole otoliths. Samples that the age reader considers unreliable for age determination are discarded. The NEFSC will phase out scale ages and begin providing age data only from otoliths. The reader tests precision six times a year, once following each trawl survey and each quarter of the commercial fishing season and provides the results of these tests online (<http://www.nefsc.noaa.gov/fbp/QA-QC/>). The threshold for precision testing is 80% agreement and a 5% mean CV.

MA DMF

Scales have been collected from the Resource Assessment fishery-independent trawl surveys since 2012. About 280 samples were collected in 2012 and the size range was similar to that of the NEFSC sampled fish (4 – 60 cm). Additionally, otolith collection began in 2013. Two readers age samples independently and reach a consensus age for any disagreements. MA DMF is currently ageing scales, but plans to compare otoliths to scales and continue ageing the more reliable structure.

RI DEM DFW

Scales have been collected on fishery-independent trawl surveys, at recreational fishing tournaments, and from the commercial floating fish trap fishery since 2013. The annual target number of samples is 100. Sample collection thus far has only included scales; however, otoliths may be collected in the future on fishery-independent trawl surveys and/or fish pot surveys. All samples have been archived for future ageing. A reader will be trained by the NEFSC reader prior to ageing the archived samples.

VIMS

Scales and otoliths have been collected from two fishery-independent trawl surveys, the Chesapeake Bay Multispecies Monitoring and Assessment Program (ChesMMAP) since 2002 and the NorthEast Area Monitoring and Assessment Program (NEAMAP) since 2007. VIMS aged scales and whole otoliths in past years, but has switched to ageing only sectioned otoliths. There are three readers at VIMS and the mode age for each sample is provided as the final age. If there is no mode from the initial read, the readers reread the sample and if there is still no mode, they examine the sample together and come to a consensus age. If a consensus age cannot be determined the sample is discarded. Very few samples are discarded. Precision tests are

performed within each reader (multiple reads of the same sample) and between readers. VIMS plans to provide these tests online in a similar format to that of NEFSC.

NC DMF

Otoliths are collected from commercial fisheries by port samplers and fish house samplers. Occasionally, otoliths are collected from recreational fisheries and fishery-independent surveys. Approximately 1,500 – 2,000 samples are collected annually. Whole otoliths are aged for fish up to five years old. Otoliths from fish older than five years and otoliths from younger fish that are difficult to age are sectioned and then aged. Scales have not been aged in the past.

SC DNR

Otoliths have been collected since 1972 during the Marine Monitoring, Assessment, and Prediction Program (MARMAP) fishery-independent survey. In recent years the collections made via MARMAP have been supplemented by additional collections made via the Southeast Area Monitoring, Assessment, and Prediction (SEAMAP-SA; since 2009) Reef Fish Survey and the Southeast Fishery Independent Survey (SEFIS; since 2010). This has resulted in the collection of life history samples from 61,006 (58,619 aged) black sea bass through 2012, with approximately an additional 3000 specimens collected in 2013. To obtain these samples, since 2008 SC DNR has been retaining a random sample of all black sea bass collected at known live/hard bottom monitoring stations sampled by the three programs. From 2008-2012, approximately 30% of all black sea bass were retained for age determination while in 2013 this percentage was dropped to 20%. Prior to 2008, the samples retained for annual age determination were generally collected via the use of tally sheets with the goal being to collect a certain number of black sea bass in different size classes at different latitudes. These samples were collected primarily for the construction of age-length keys. Whole otoliths are aged for fish up to five years old. Otoliths from fish older than five years and otoliths from younger fish that are difficult to age are sectioned and then aged. Scales have not been aged in the past. Two age readers independently determine ages for each sample. If there are disagreements, the readers will read the sample together and provide a consensus age. If the readers cannot determine a consensus age, the sample is discarded. Very few samples are discarded.

The labs involved in the workshop generally follow the techniques outlined in Penttila and Dery (1998) and the SEDAR workshop report to prepare age structures and for age determination. Annuli (opaque zones) are generally deposited in May – June in the northern stock (Penttila and Dery 1998). All labs ageing northern stock fish use a January 1 birth date.

Scales

Five to six scales are pressed in laminated plastic.

Whole Otoliths

Whole otoliths are immersed in water to enhance clarity and viewed distal surface up on a black background. Prior to switching to sectioned otoliths, VIMS immersed whole otoliths in ethanol. Both transmitted and reflected light are used, based on user preference, and both can be used on the same sample to assist with age determination. The otoliths can also be tilted to assist with interpretation of the edge.

Sectioned Otoliths

Whole otoliths are sectioned with an IsoMet® low speed saw. The NEFSC mounts sectioned otoliths in black dyed wax. VIMS mounts sectioned otoliths on glass slides with Crystalbond™. SC DNR and NC DMF mount sectioned otoliths on glass slides with Cytoseal™.

4. Preliminary Exchange

Prior to the 2011 stock assessment, there had not been an exchange of black sea bass ageing structures between labs ageing northern stock fish or between labs ageing northern stock fish and labs ageing southern stock fish. There have been two intra-lab black sea bass ageing structure evaluations done with conflicting results. A NEFSC comparison found no systematic bias between scales and whole otoliths and about 75% agreement between the structures (personal communication, NMFS). A VIMS comparison between readers found the highest agreement in sectioned otoliths, the lowest agreement in whole otoliths, and about 90% agreement in scales. There was about 50% agreement between structures (personal communication, VIMS).

NEFSC, VIMS, and SC DNR/NOAA NOS black sea bass age readers completed an exchange of ageing structures prior to the workshop to initiate and inform discussions on ageing interpretation criteria at the workshop. The results were used for a preliminary evaluation of precision between labs and to identify any systematic bias. NEFSC contributed 98 paired otolith (whole only) and scale samples from the same fish. VIMS contributed 100 paired otolith (whole and sectioned) and scale samples from the same fish. SC DNR contributed 100 whole otoliths and 100 sectioned otoliths. The SC DNR whole and sectioned otolith samples were representative of the general population of black sea bass that SC DNR would age with these structures. The whole otolith samples were a random sample of 100 black sea bass otoliths collected during the 2010 sampling season that were determined, based upon consensus reads, to be less than 6 years of age. The sectioned otolith samples were a random sample (from 2006-2010 sampling years) of 100 black sea bass otolith sections that, based on initial whole otolith reads by one or more readers, were thought to be greater than 5 years of age. Six readers participated in the exchange (1 from NEFSC, 3 from VIMS, 1 from SC DNR, and 1 from NOAA NOS). SC DNR/NOAA NOS readers did not age scales because they had no experience ageing black sea bass scales. The NEFSC reader had no experience ageing sectioned black sea bass otoliths. VIMS readers had recent experience ageing only sectioned otoliths. Comparisons during the workshop focused on preparation and age determination of whole and sectioned otoliths because scales are not regularly aged by two of the three labs involved in the exchange. There was also consensus that otoliths tend to be more reliable ageing structures than scales.

The primary focus of the workshop was consistency of ageing criteria used by labs ageing northern stock fish. Therefore, the exchange data was analyzed for two sets of samples, samples collected from only northern stock fish (VIMS and NEFSC samples) and a secondary set of all samples collected from northern and southern stock fish (VIMS, NEFSC, and SC DNR samples). The sample size of sectioned otoliths was smaller (N northern stock fish=89, N all samples=177) than the sample size of whole otoliths (N northern stock fish=183, N all samples=270) because NEFSC did not have sectioned otoliths for the exchange. Age data used in the analyses for labs ageing northern stock fish (VIMS and NEFSC) were the same as would be provided under lab protocol for stock assessment age data. A few samples were excluded from the analyses because they were considered unreliable for age determination by the NEFSC reader or there was not a

mode for the three VIMS readers. VIMS readers would typically reread samples with no mode, but did not do so for the exchange. SC DNR age determinations for the exchange were from only one reader, but would be a consensus age determination by two readers when provided for stock assessments.

A Bowker's test of symmetry was used to test for systematic bias with a p-value < 0.05 indicating systematic bias. There was no systematic bias detected for inter-lab comparisons of the sectioned otoliths from northern stock fish (table 1). However, systematic bias was detected for SC DNR comparisons to both NEFSC and VIMS when all sectioned otoliths were included in the analysis (table 2). There was no systematic bias for the comparison of NEFSC and VIMS reads of all sectioned otoliths (table 2). SC DNR sectioned otoliths are primarily from older fish (>5). The mean age assigned to SC DNR samples was 5.7 and the mean age assigned to samples from northern stock fish was 2.8. Detection of systematic bias and the age bias plots (figures 5 and 6) indicate that SC DNR is over ageing sectioned otoliths relative to the labs ageing northern stock fish. This bias may increase with age because systematic bias was not detected in the analysis with only sectioned otoliths from younger, northern stock fish. This bias does not have major implications for the black sea bass assessments because SC DNR will not be providing age data for the assessment of the northern stock and VIMS and NEFSC will not be providing age data for the assessment of the southern stock. Systematic bias was detected in VIMS comparisons to both NEFSC and SC DNR for the analyses with whole otoliths from northern stock fish and all whole otoliths (tables 3 and 4). Detection of systematic bias and the age bias plots (figures 7, 8, 10, and 11) indicate that VIMS is under ageing whole otoliths relative to NEFSC and SC DNR. This bias is concerning for potential use of age data from both VIMS and NEFSC in an assessment. One potential cause is experience, as VIMS has switched to ageing only sectioned otoliths in the recent past. To check for bias between the age determinations made from the structure that is used at each lab, NEFSC age determinations from whole otoliths were compared to VIMS age determinations from sectioned otoliths from the same fish. Systematic bias was not detected at a 0.05 significance level (table 5), though the p-value (0.08) could be considered marginally significant.

Precision was evaluated with exact agreement of age determinations and the mean coefficient of variation (CV). Agreement in sectioned otolith comparisons ranged from 50%-71% with a mean of 60% (tables 1 and 2). Agreement ranged from 55%-70% with a mean of 62% in whole otolith comparisons (tables 3 and 4). CVs for sectioned otolith comparisons ranged from 10.0%-24.8% with a mean of 16.0% (tables 1 and 2). CVs ranged from 8.6%-15.6% and averaged 13.3% in whole otolith comparisons (tables 3 and 4). Generally, precision was slightly better for VIMS and NEFSC comparisons. Despite systematic bias in the VIMS and NEFSC comparison of whole otoliths from northern stock fish, the precision was best for this comparison with agreement of 70% and a CV of 8.6%.

The group was cautious to make conclusive decisions on the more reliable form of otolith for ageing based on the results of this exchange.

5. Workshop Sample Evaluation

Several whole otoliths and corresponding sectioned otoliths were selected from the exchange set to be examined by workshop participants as a group. Samples with high agreement as well as samples with poor agreement during the exchange were selected. Four areas of concern were discussed in attempts to identify the sources of error in the exchange results: identifying the first annulus, differentiating between check marks and true annuli, evaluating the otolith edge type, and sample preparation.

The group examined otoliths that were assigned ages ranging from 0-2 to evaluate identification of the first annulus. A consensus age was quickly reached for otoliths that were characterized by high agreement during the exchange. A consensus age for some otoliths with low agreement during the exchange required discussion to reach and, though consensus ages were possible for all otoliths among the workshop participants, the group noted that there are otoliths with multiple potential interpretations that could be made during production ageing. The group agreed that there should generally be a hyaline zone between the core and the first annulus and that the first annulus should be continuous around the core.

Otoliths with varying hyaline and opaque zones on the edge were examined and the group discussed how to assign a fish to the correct year class based on the edge type and the date the fish was collected. SC DNR and NC DMF use an edge type classification based on the percentage of the hyaline band on the edge of the otolith (SEDAR 2009). The labs ageing northern stock fish do not use a defined classification, but are in agreement about how to age an otolith based on edge type and date collected that is synonymous with the SC DNR classification. If the hyaline band has progressed to a certain width relative to the previous hyaline zone and is collected after January 1 (calendar birth date) but before June when the annulus is typically laid down, the age class is assigned by advancing the number of annuli by one. If there is a minimal hyaline zone on the edge relative to the previous hyaline zone and it is collected during this timeframe, the age class is equal to the number of annuli. This is a judgment call that is subjective and likely to explain some imprecision. Based on examination of the otoliths and the discussion, the group agreed that each lab was using consistent criteria for evaluating the edge type.

The major issue with precision was identified as differentiating between check marks and true annuli. Similar to samples examined to identify the first annulus, a consensus age for samples with high agreement during the exchange was relatively quick to reach and much more difficult to reach for samples with low agreement during the exchange. Continuous and distinct annuli and hyaline zones were identified by workshop participants for the high agreement samples, but the alternating zones were often incomplete and merged together for the low agreement samples, making true annuli difficult to identify. The widths of the zones also were inconsistent with surrounding zones, making interpretation based on expected increments difficult. Following discussion, there were often multiple ages that could be interpreted and there was no distinct solution for addressing this issue. The group agreed that check marks are potentially the most significant source of imprecision and bias. Check marks will need to be considered on a case by case basis. The group also suggested that inexperienced readers may be more likely to count check marks and overestimate age and that this source of error may diminish with experience.

This is addressed by consensus ageing and precision checks within labs. Sex change and migration were discussed as potential sources of check marks. Further investigation of the sources of check marks and how to differentiate between check marks and true annuli is necessary. During the workshop evaluation, agreement was generally similar for whole and sectioned otoliths from the same fish. The group discussed the preparation quality of the samples and agreed that preparation by the different labs did not appear to be affecting readability and interpretation.

6. Post-Workshop Exchanges

Following the workshop, the VIMS collection of 100 whole otoliths was exchanged between NEFSC and VIMS as an additional test of precision and bias between labs ageing northern stock fish. The results were similar to the preliminary exchange of whole otoliths from northern stock fish. Systematic bias was detected (table 6) and the age bias plot (figure 14) indicates that VIMS is under ageing whole otoliths relative to NEFSC. Based on the discussions during the workshop, a potential cause of the systematic bias is different interpretation of check marks by VIMS and NEFSC. Overall agreement was 73% and the mean CV was 6.0% (table 6).

All labs ageing scales (NEFSC, RI DEM DFW, and MA DMF) are planning an exchange of scales to evaluate inter-lab precision and bias for scale age assignments. This exchange and evaluation will follow the NEFSC training of RI DEM DFW staff.

7. Workshop Recommendations

- Labs ageing northern stock fish should conduct additional exchanges and continue collaboration to determine causes and identify persistence of systematic bias in age determinations, especially for whole otoliths. Ageing structures should be further evaluated to identify if there is a more reliable structure. In the absence of a standardized ageing structure, labs should exchange paired samples of all structures used for age determinations and compare age determinations from the structure used by each lab.
- Labs can continue with current preparation methods for whole and sectioned otoliths. All sectioned otoliths should be cut to a thickness of 0.5 mm.
- Otoliths from fish older than five and otoliths that are difficult to read should be sectioned for ageing. Overgrowth of calcium in otoliths of older fish may confound age assignments and decrease precision or cause systematic bias. Sectioning will provide a comparison to the whole otolith.
- Samples with poor readability should not be excluded from age data. Readability may be a result of geographic factors and therefore excluding certain samples based on readability may result in a non-representative sample. Comments should be provided for any difficult to read samples to stock assessment scientists. VIMS recommended confidence coding for each sample with 1 being most confident about age assignment and 5 being least confident about age assignment. A similar “confidence scale” is used to assess readability for southern stock

black sea bass, in this case having a five letter scale where A is least confident and E is most confident (SEDAR 2009).

- Labs should provide data in a standardized format with raw annuli counts, year class assignments, confidence codes/quality comments, and intra-lab precision test results (sample size, mean CV, % agreement, and Bowker's p-value).
- Continue work to identify sources of check marks and determine how to differentiate check marks from true annuli. New readers should read calibration sample sets before production ageing.

8. References

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9. Tables and Figures

Table 1. Sample size (N), mean CV, agreement, and Bowker's p-value for inter-lab age comparisons from preliminary exchange of sectioned otoliths from northern stock fish.

Labs	N	CV	Agreement	Bowker's p-value
VIMS and NEFSC	89	15.5%	64%	0.17
VIMS and SC DNR	89	15.5%	71%	0.50
NEFSC and SC DNR	89	24.8%	57%	0.49

Table 2. Sample size (N), mean CV, agreement, and Bowker's p-value for inter-lab age comparisons from preliminary exchange of all sectioned otoliths combined.

Labs	N	CV	Agreement	Bowker's p-value
VIMS and NEFSC	177	10.0%	66%	0.26
VIMS and SC DNR	177	13.7%	53%	0.00*
NEFSC and SC DNR	177	16.2%	50%	0.00*

Table 3. Sample size (N), mean CV, agreement, and Bowker's p-value for inter-lab age comparisons from preliminary exchange of whole otoliths from northern stock fish.

Labs	N	CV	Agreement	Bowker's p-value
VIMS and NEFSC	183	8.6%	70%	0.00*
VIMS and SC DNR	183	14.6%	57%	0.00*
NEFSC and SC DNR	183	15.6%	62%	0.14

Table 4. Sample size (N), mean CV, agreement, and Bowker's p-value for inter-lab age comparisons from preliminary exchange of all whole otoliths combined.

Labs	N	CV	Agreement	Bowker's p-value
VIMS and NEFSC	270	11.2%	63%	0.00*
VIMS and SC DNR	270	15.5%	55%	0.00*
NEFSC and SC DNR	270	14.0%	62%	0.64

Table 5. Sample size (N), mean CV, agreement, and Bowker's p-value for comparison of NEFSC whole otolith ages to VIMS sectioned otolith ages for northern stock fish.

Labs	N	CV	Agreement	Bowker's p-value
NEFSC and VIMS	91	16.0%	58%	0.08

Table 6. Sample size (N), mean CV, agreement, and Bowker's p-value for inter-lab age comparisons from post-workshop exchange of VIMS whole otolith samples.

Labs	N	CV	Agreement	Bowker's p-value
VIMS and NEFSC	90	6.0%	73%	0.01*

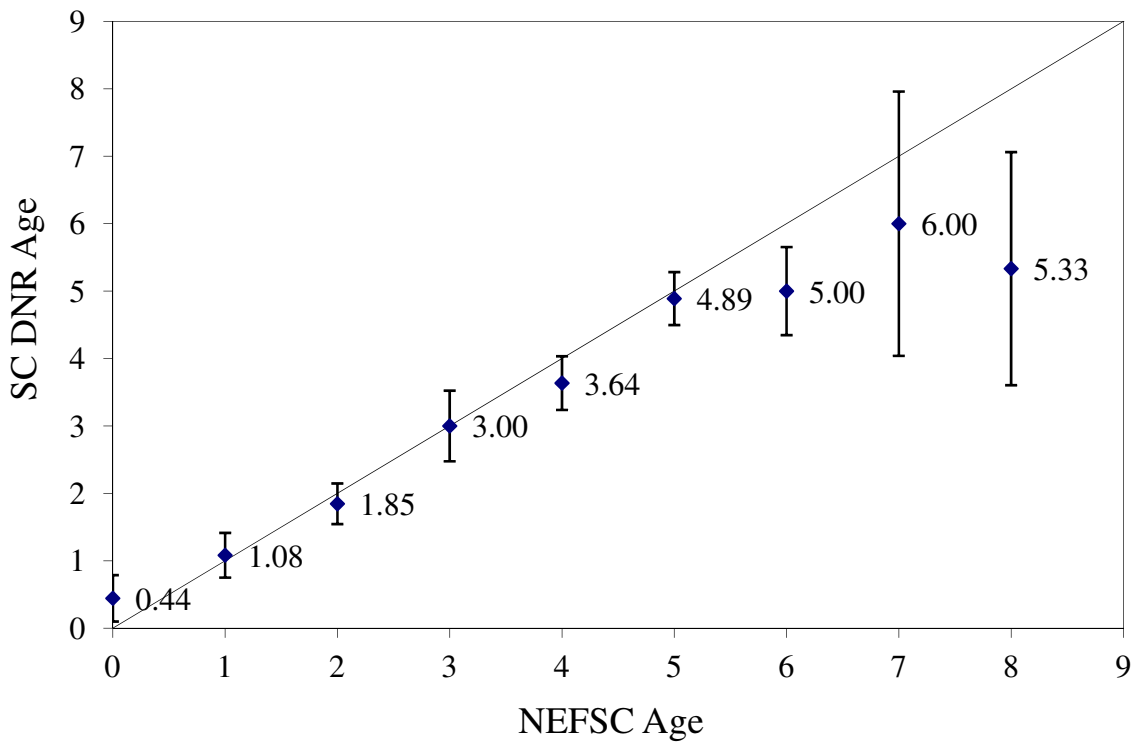


Figure 1. SC DNR ages vs. NEFSC ages from preliminary exchange of sectioned otolith samples from northern stock fish. Error bars indicate 95% confidence intervals.

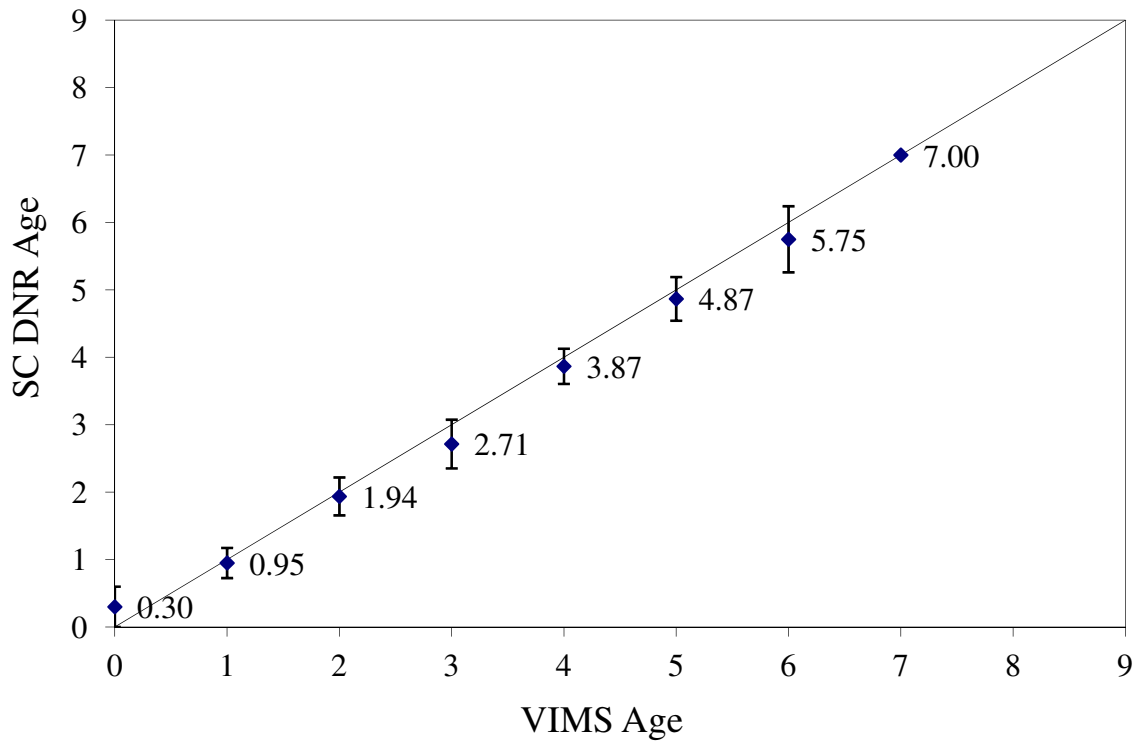


Figure 2. SC DNR ages vs. VIMS ages from preliminary exchange of sectioned otolith samples from northern stock fish. Error bars indicate 95% confidence intervals.

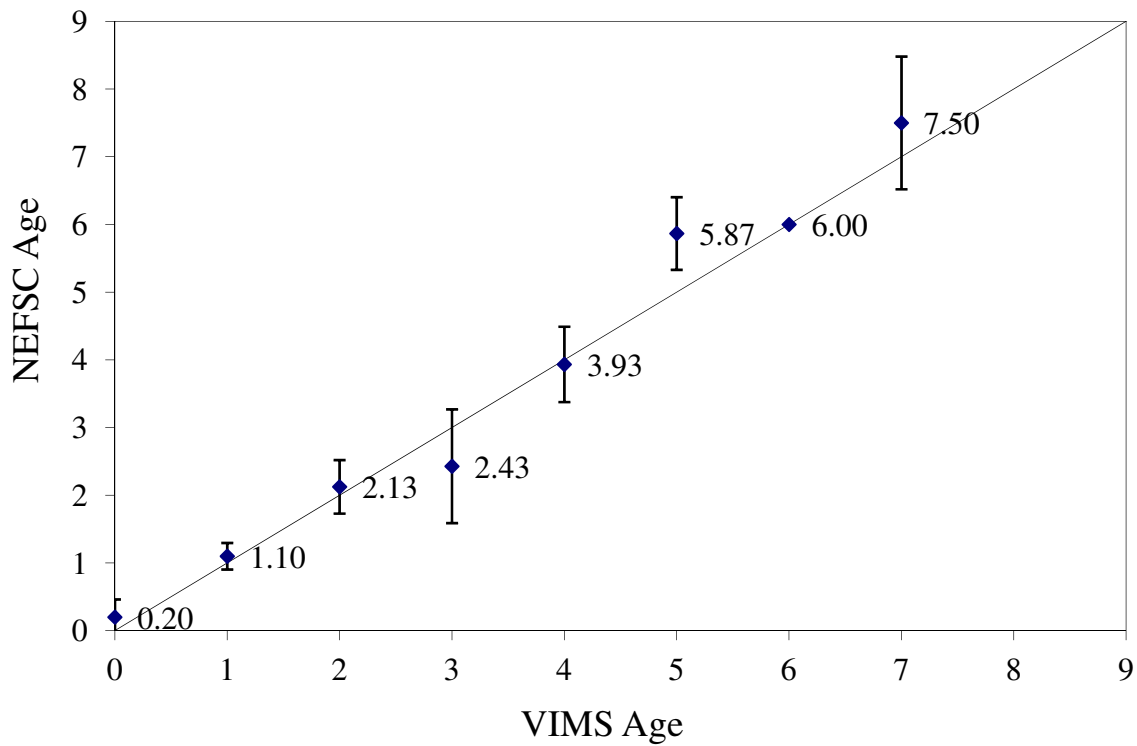


Figure 3. NEFSC ages vs. VIMS ages from preliminary exchange of sectioned otolith samples from northern stock fish. Error bars indicate 95% confidence intervals.

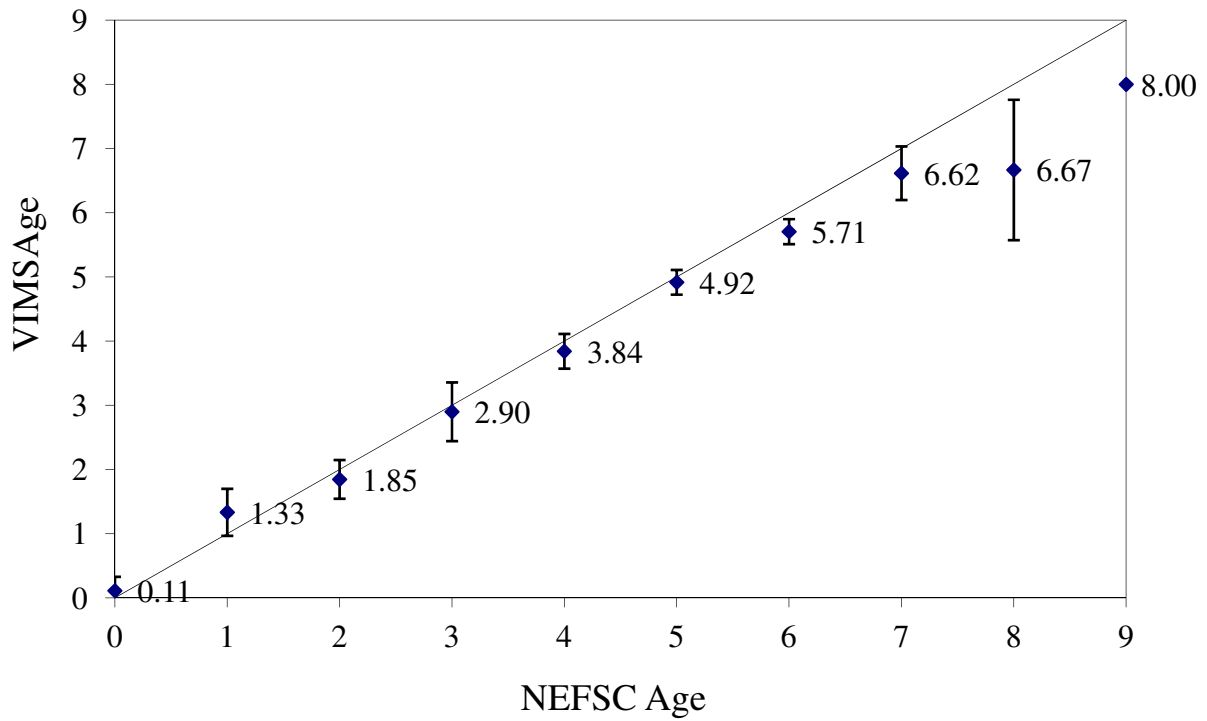


Figure 4. NEFSC ages vs. VIMS ages from preliminary exchange of all sectioned otolith samples. Error bars indicate 95% confidence intervals.

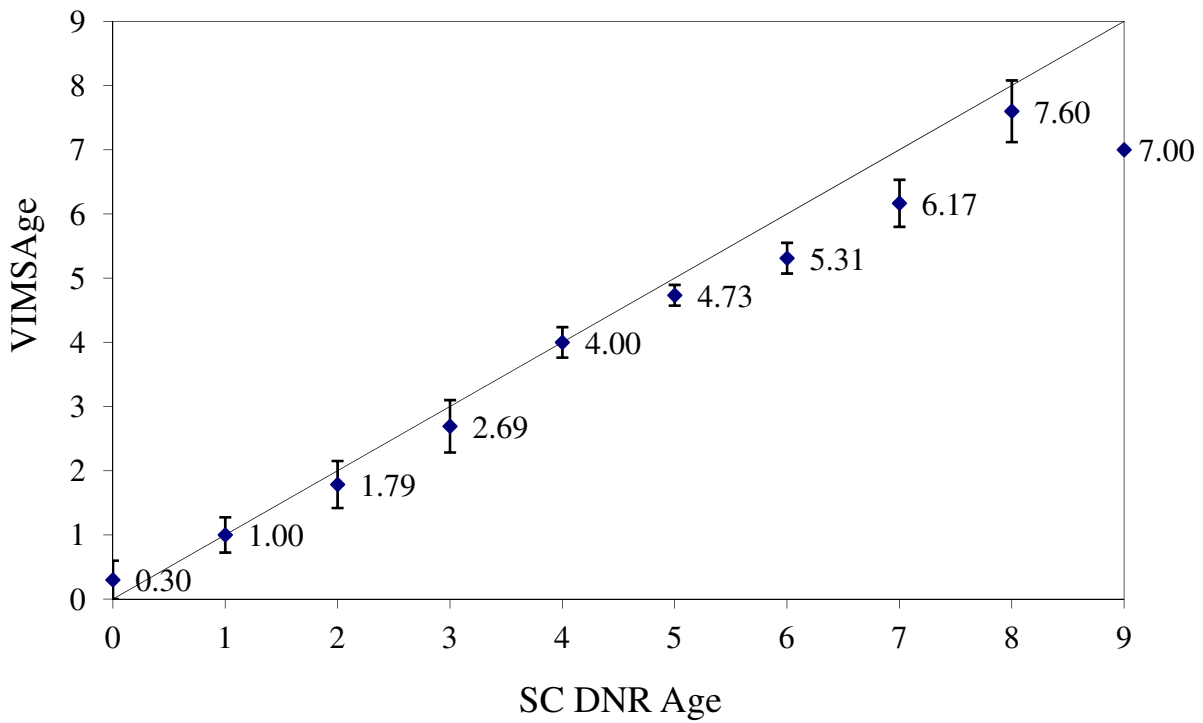


Figure 5. SC DNR ages vs. VIMS ages from preliminary exchange of all sectioned otolith samples. Error bars indicate 95% confidence intervals.

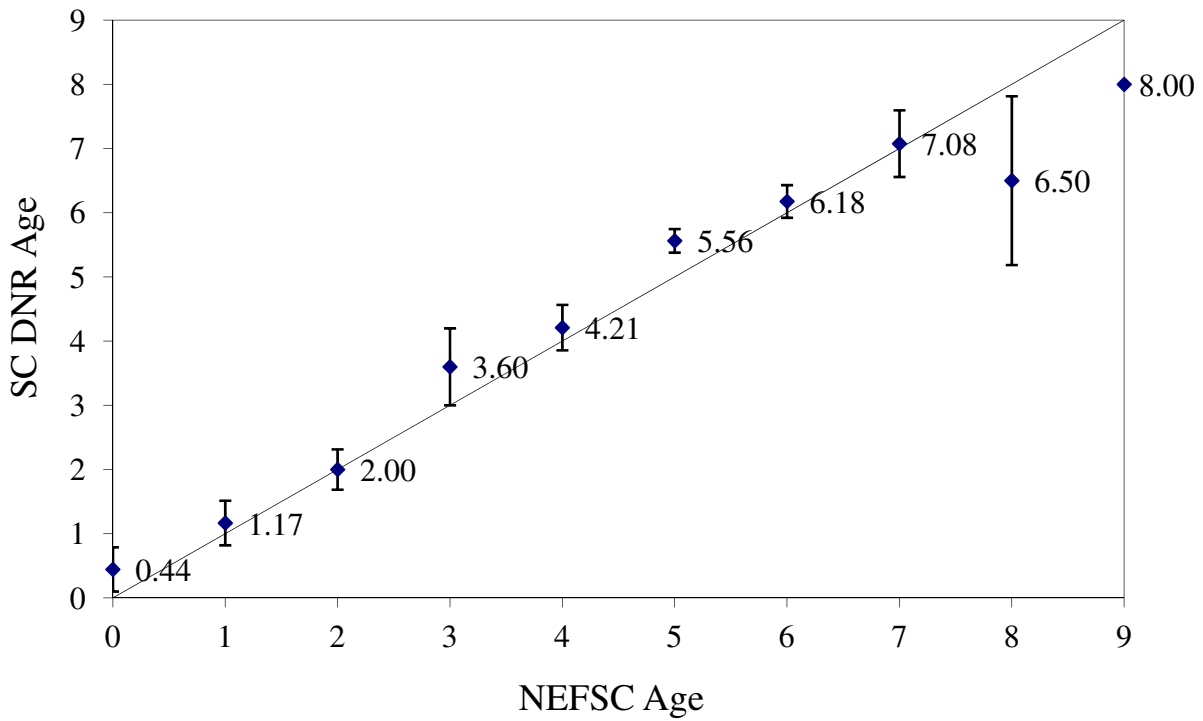


Figure 6. NEFSC ages vs. SC DNR ages from preliminary exchange of all sectioned otolith samples. Error bars indicate 95% confidence intervals.

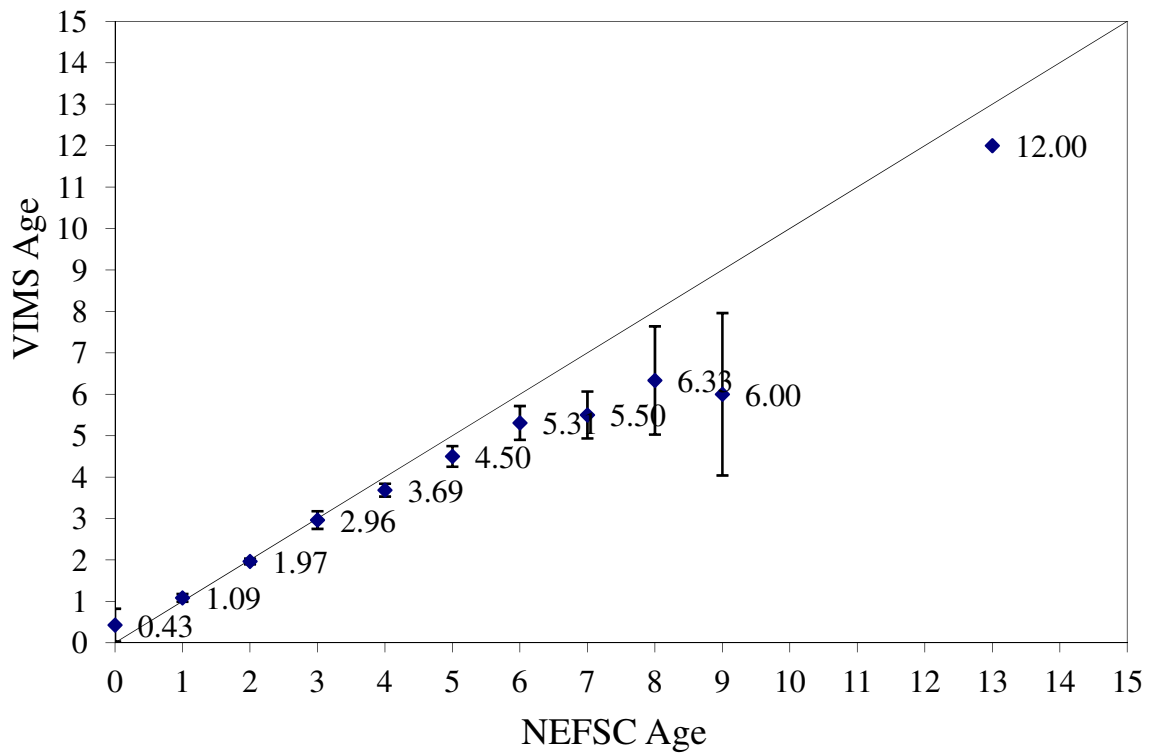


Figure 7. NEFSC ages vs. VIMS ages from preliminary exchange of whole otolith samples from northern stock fish. Error bars indicate 95% confidence intervals.

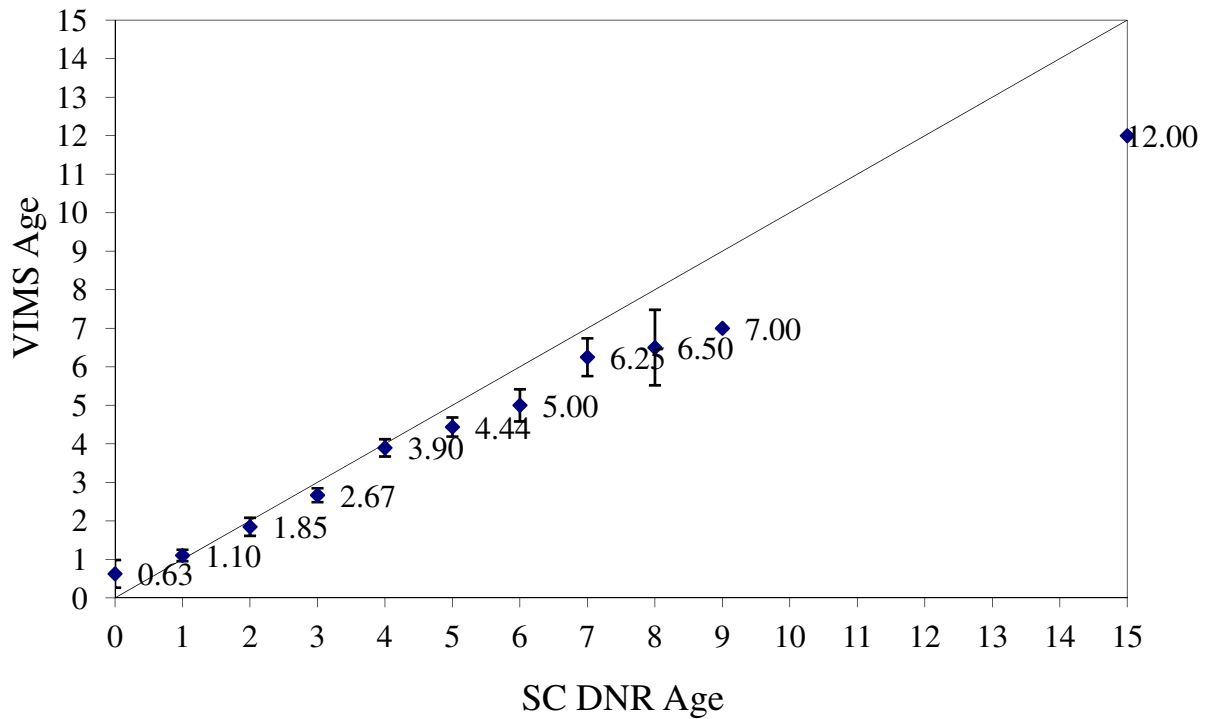


Figure 8. SC DNR ages vs. VIMS ages from preliminary exchange of whole otolith samples from northern stock fish. Error bars indicate 95% confidence intervals.

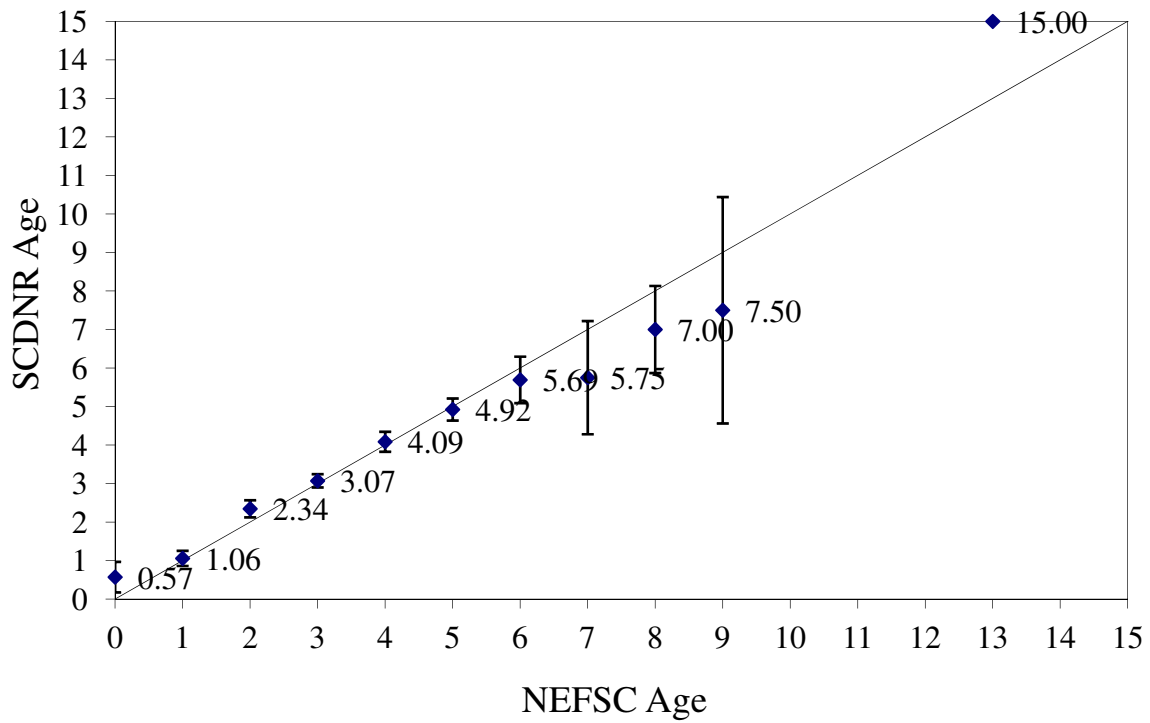


Figure 9. NEFSC ages vs. SC DNR ages from preliminary exchange of whole otolith samples from northern stock fish. Error bars indicate 95% confidence intervals.

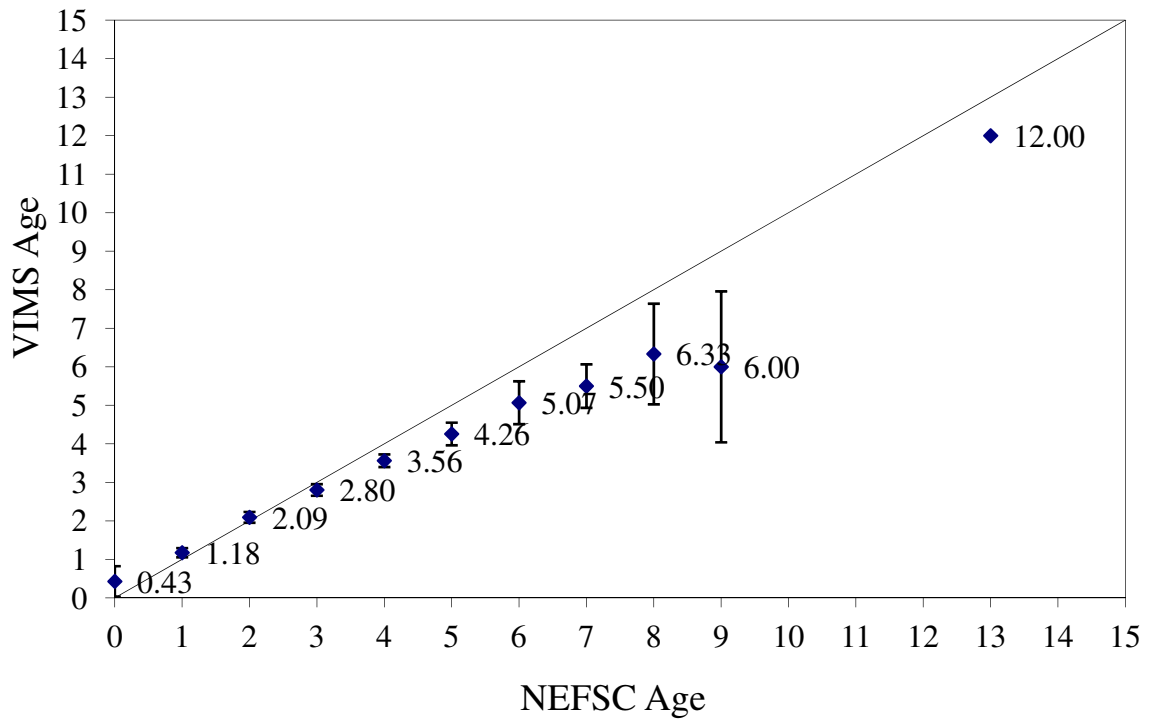


Figure 10. NEFSC ages vs. VIMS ages from preliminary exchange of all whole otolith samples. Error bars indicate 95% confidence intervals.

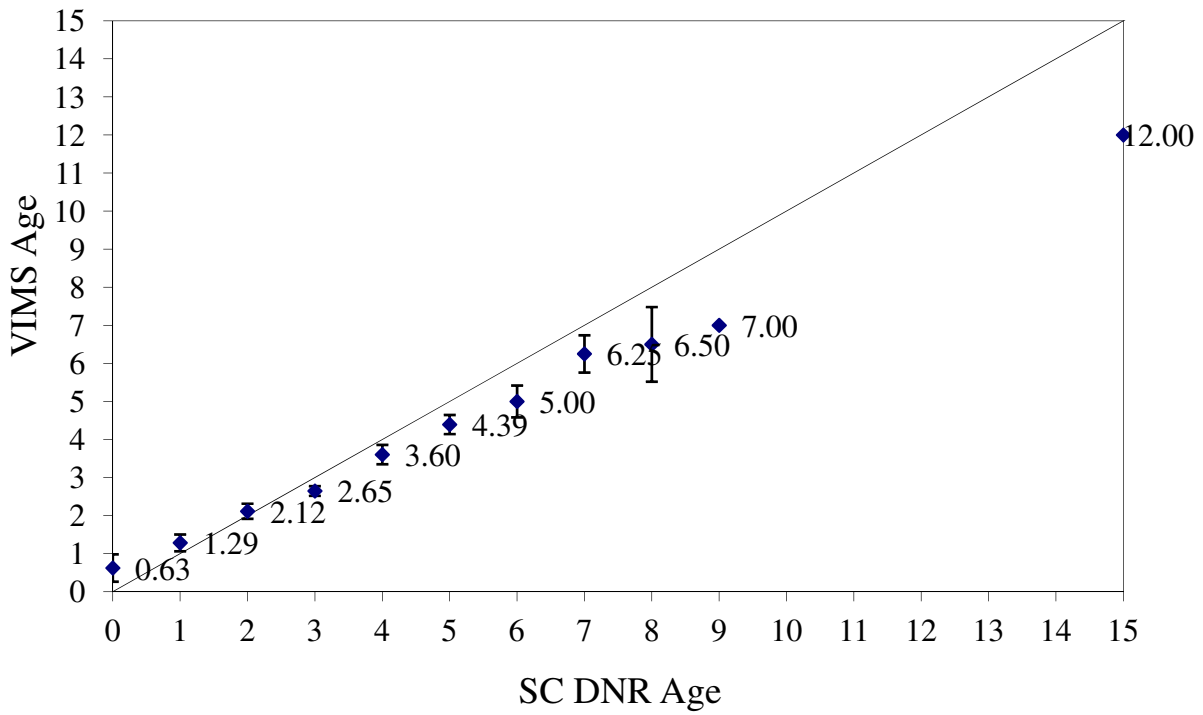


Figure 11. SC DNR ages vs. VIMS ages from preliminary exchange of all whole otolith samples. Error bars indicate 95% confidence intervals.

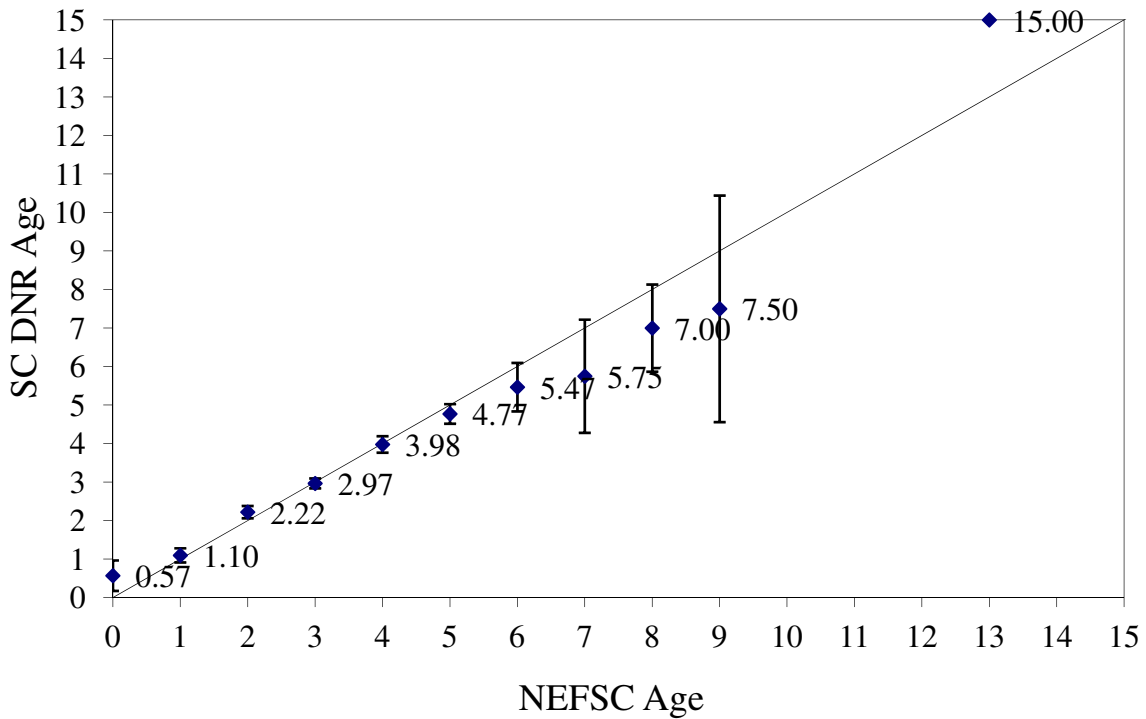


Figure 12. NEFSC ages vs. SC DNR ages from preliminary exchange of all whole otolith samples. Error bars indicate 95% confidence intervals.

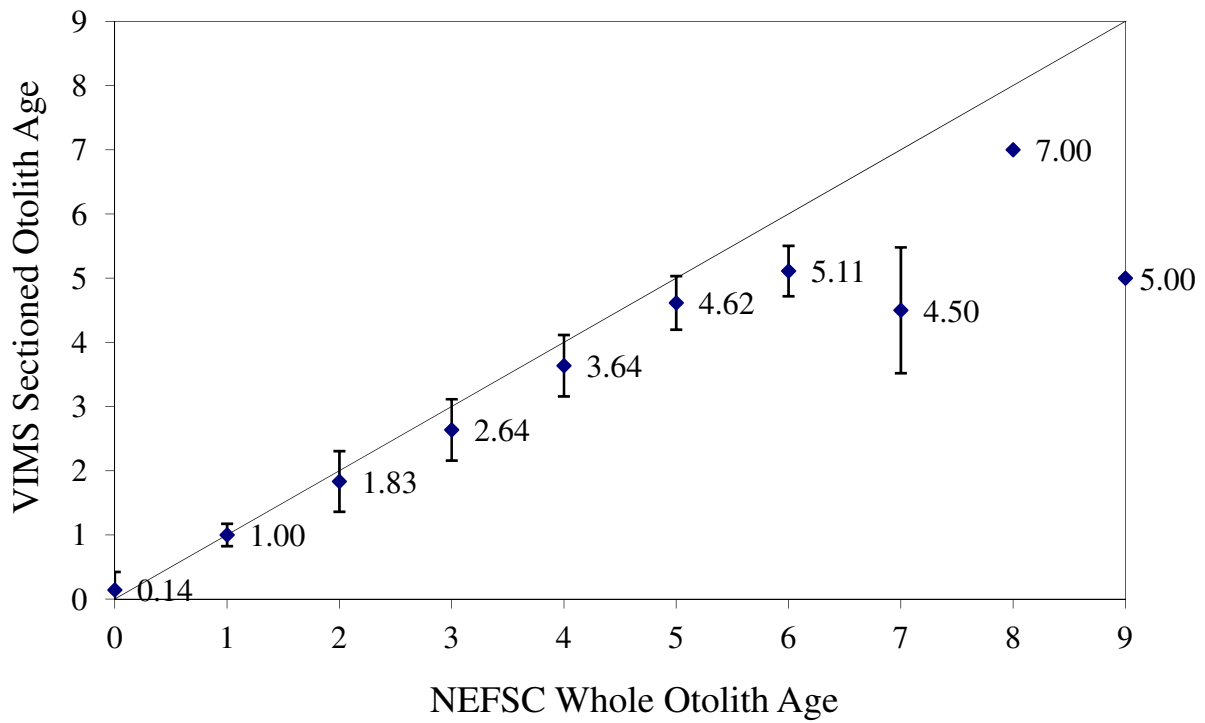


Figure 13. NEFSC whole otolith ages vs. VIMS sectioned otolith ages from preliminary exchange of samples from northern stock fish. Error bars indicate 95% confidence intervals.

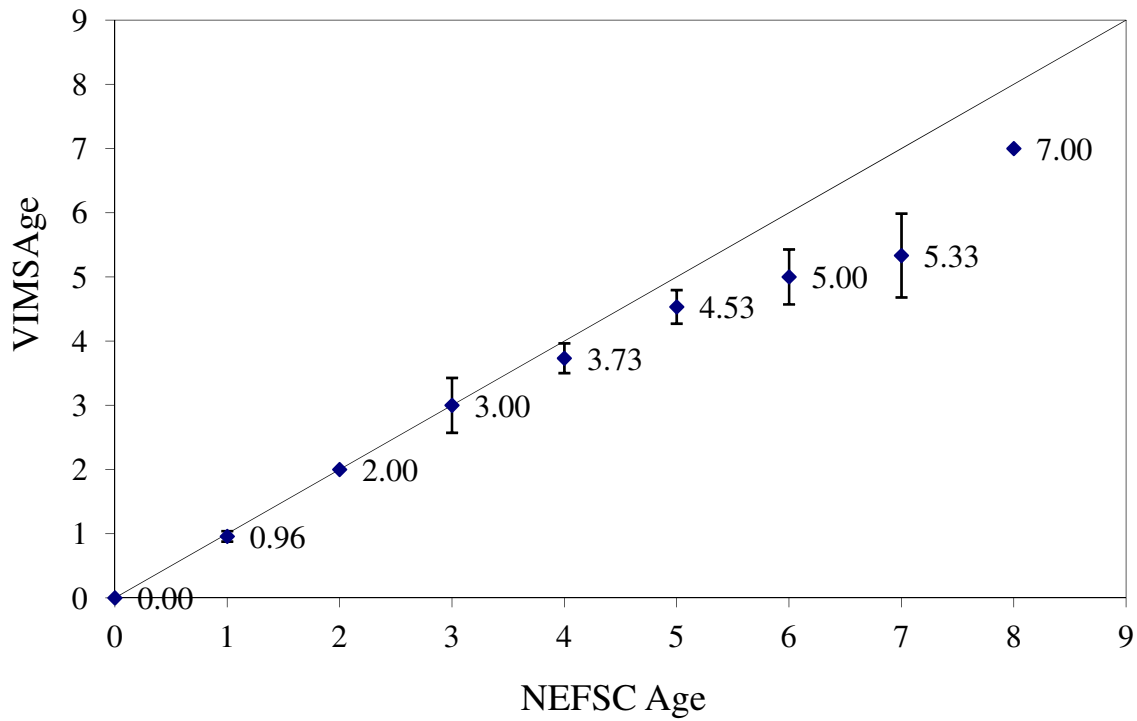


Figure 14. NEFSC ages vs. VIMS ages from post-workshop exchange of VIMS whole otolith samples. Error bars indicate 95% confidence intervals.

Appendix A: Workshop Agenda

Black Sea Bass Ageing Workshop
Atlantic States Marine Fisheries Commission
July 22-23, 2013
Massachusetts DMF – Annisquam River Marine Fisheries Field Station
30 Emerson Avenue
Gloucester, MA 01930

Agenda

Monday, July 22 (1:00 pm – 5:00 pm)

1. Welcome and Introductions
2. Workshop Goals and Objectives
3. Review Ageing Methodologies and Black Sea Bass Ageing History
4. Review Results of Scale and Otolith Exchange between VIMS, NEFSC, and SCDNR
5. Examine Scale and Otolith Samples from Exchange Collection

Tuesday, July 23 (9:00 am – 12:00 pm)

1. Develop Exchange between all Labs Ageing Black Sea Bass
2. Discuss Ageing Error Matrix Development
3. Adjourn