COASTAL RECREATIONAL FISHING LICENSE

FINAL PERFORMANCE REPORT

Recipient: Dr. Roger A. Rulifson, Institute for Coastal Science and Policy, East Carolina University, Greenville, NC 27858-4353

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Grant Award #: 2010-F-004 2F27 3207 Maturation, Fecundity, & Spawning of Striped Bass

Grant Title: Maturation, Fecundity, and Spawning Frequency of the Albemarle/Roanoke Striped Bass Stock

Grant Award Period: April 1, 2010 – June 30, 2013

Performance Reporting Period: April 1, 2010 - June 30, 2013

Project Costs: 205,750.00

Account Pool	Account	Budget	Expenditures	Encumbrances	Budget Balance
60000-Miscellaneous Salary Pool		113,153.00	20,705.40	0	92,447.60
60100-EPA Non-Teaching Salaries		0	67,794.47	0	-67,794.47
60150-EPA Faculty Salaries		0	15,888.86	0	-15,888.86
60200-SPA Staff Salaries		0	10,952.52	0	-10,952.52
61200-Benefits Budget Pool		11,314.00	12,372.33	0	-1,058.33
72000-Supplies Budget Pool		5,600.00	5,350.28	0	249.72
73000-Purchased Contractual Svcs Pool		31,423.00	33,237.30	0	-1,814.30
73200-Travel Budget Pool		13,227.00	10,950.11	0	2,276.89
73400-Current Services Pool		1,500.00	282.72	0	1,217.28
73600-Other Fixed Charges Pool		0	1,151.50	0	-1,151.50
73764-Indirect Overhead Cost		26,039.00	27,064.51	0	-1,025.51
74060-Other Educational Awards Pool		3,494.00	0	0	3,494.00
	Grand Total	205,750.00	205,750.00	0	0

Total Cumulative Expenditures: $205,750.00
Total Remaining Balance: $0

Description of Work**:**

Objectives

1. Obtain fish from the Albemarle Sound independent gillnet survey during the pre-spawn, spawning, and post-spawn period (February through May, 2009 and 2010).

2. Obtain fish from the electroshocking survey on the spawning grounds of Roanoke River during the pre-spawn, spawning, and post-spawn periods (Feb-May, 2009 and 2010).

3. Collect biological information and material from collected fish for laboratory examination.

4. Analyze biological samples to determine fecundity, age, LSI, and K factor.

5. Analyze otoliths for patterns in the microchemistry to determine residency or anadromy, and spawning frequency.

6. Analyze data to determine fecundity, GSI, and age to maturity, and develop a maturity schedule for the stock.

7. Compare results with previous maturity and fecundity estimates (e.g., Olsen and Rulifson 1992).

8. Prepare report of publishable quality for submission to fishery management agencies.

Objectives Completed

1. Obtain fish from the Albemarle Sound independent gillnet survey during the pre-spawn, spawning, and post-spawn period (February through May, 2009 and 2010).

In 2009 there were a total of n=104 fish sampled from the IGNS. By month the samples were: March n=44, April n=22, May n=38. In 2010 there were a total of n=122 fish sampled from the IGNS. By month the samples were: February n=3, March n=50, April n=39, May n=33. This makes a total of n=226 fish sampled from the IGNS during 2009-2010 (Figure 1). The length frequency was as expected for both years (Figures 2 and 3).

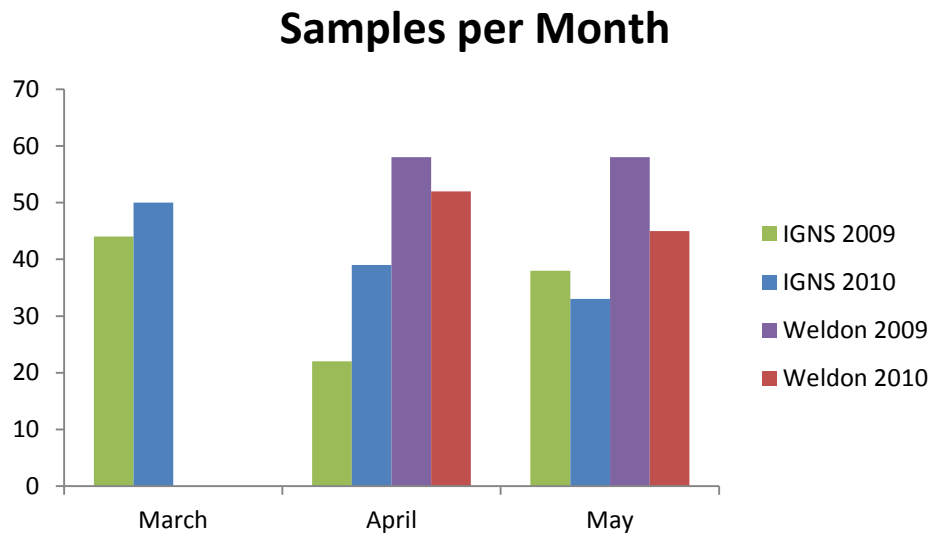

Figure 1. Samples collected from the IGNS and Weldon during the pre-spawn, spawning, and post-spawn periods from 2009-2010.

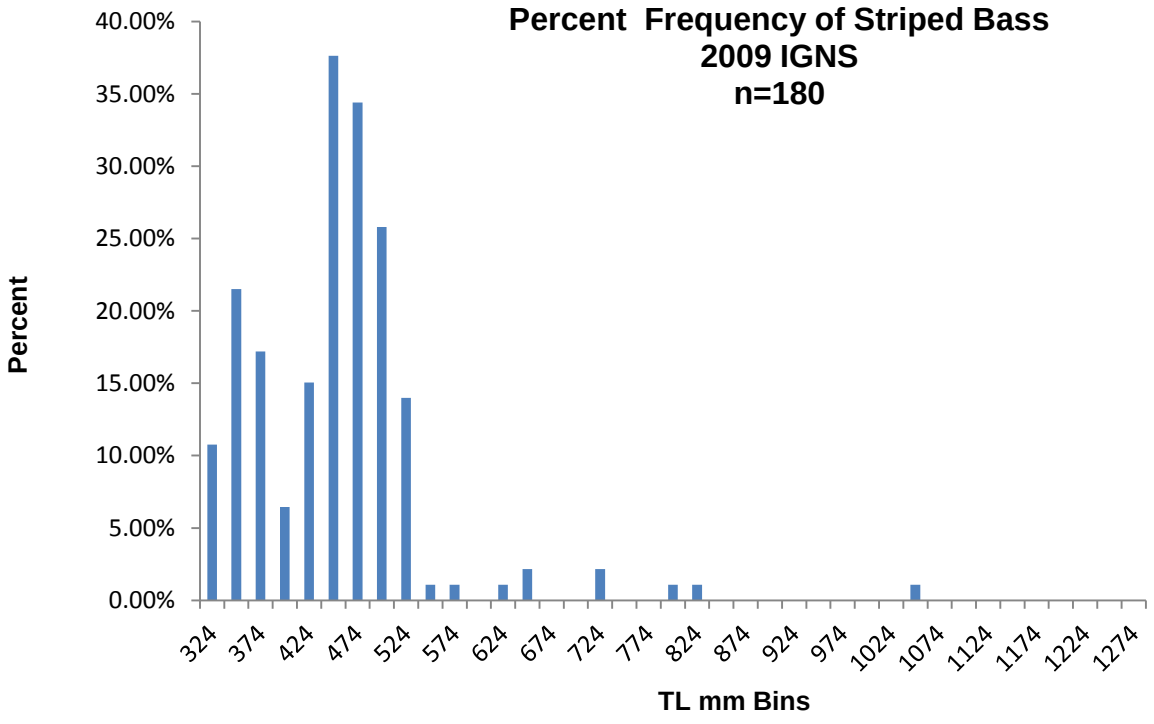

Figure 2. Length frequency analysis of fish collected from the IGNS during the 2009 sampling year (n=180).

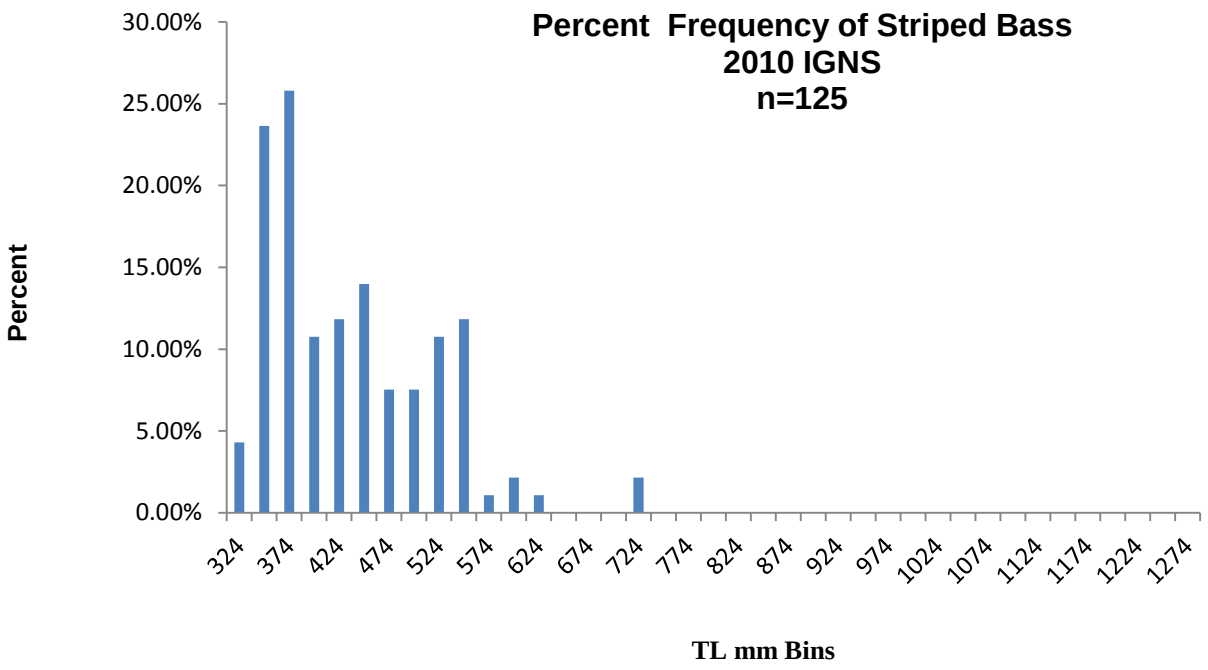

Figure 3. Length frequency analysis of fish collected from the IGNS during the 2010 sampling year (n=125).

2. Obtain fish from the electroshocking survey on the spawning grounds of Roanoke River during the pre-spawn, spawning, and post-spawn periods (April-May, 2009 and 2010).

In 2009 there were a total of n=116 fish sampled from the electroshocking survey in Weldon, NC. By month the samples were: April n=58, May n=58. In 2010 there were a total of n=97 fish sampled from the electroshocking survey in Weldon, NC. By month the samples were: April n=52, May n=45. This makes a total of n=213 fish sampled from the IGNS and Weldon during 2009-2010 (Figure 1). The length frequency was as expected for both years (Figures 4 and 5).

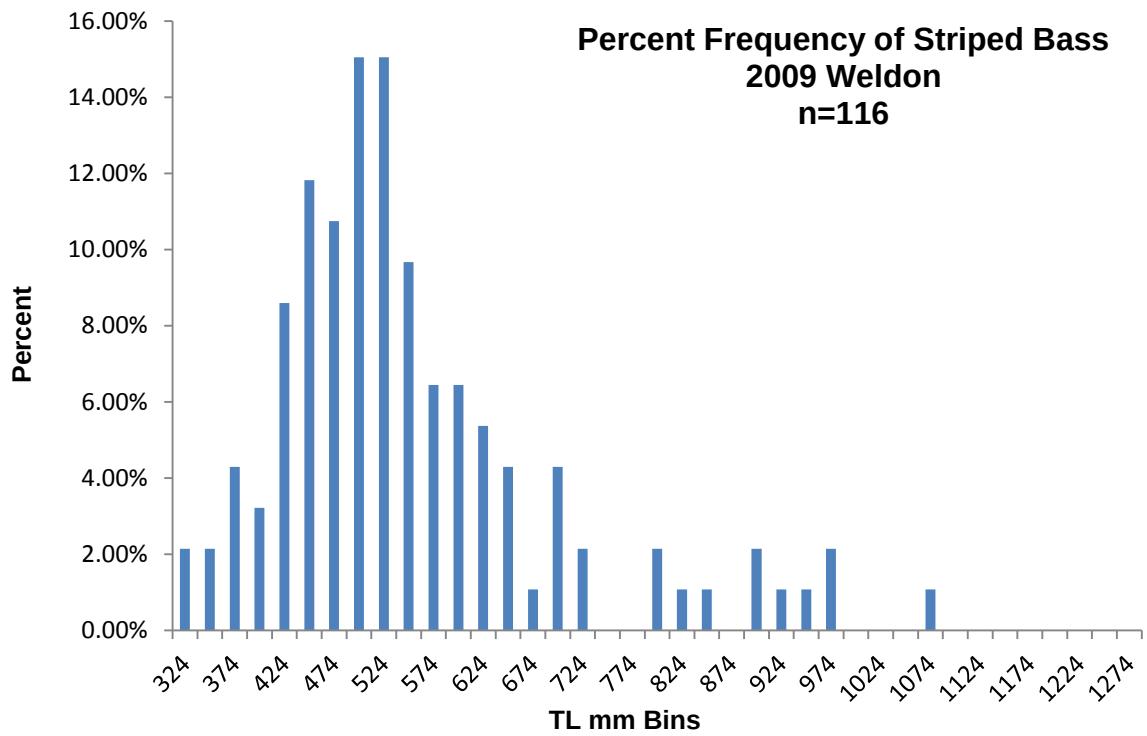

Figure 4. Length frequency analysis of fish collected from Weldon during the 2009 sampling year (n=116).

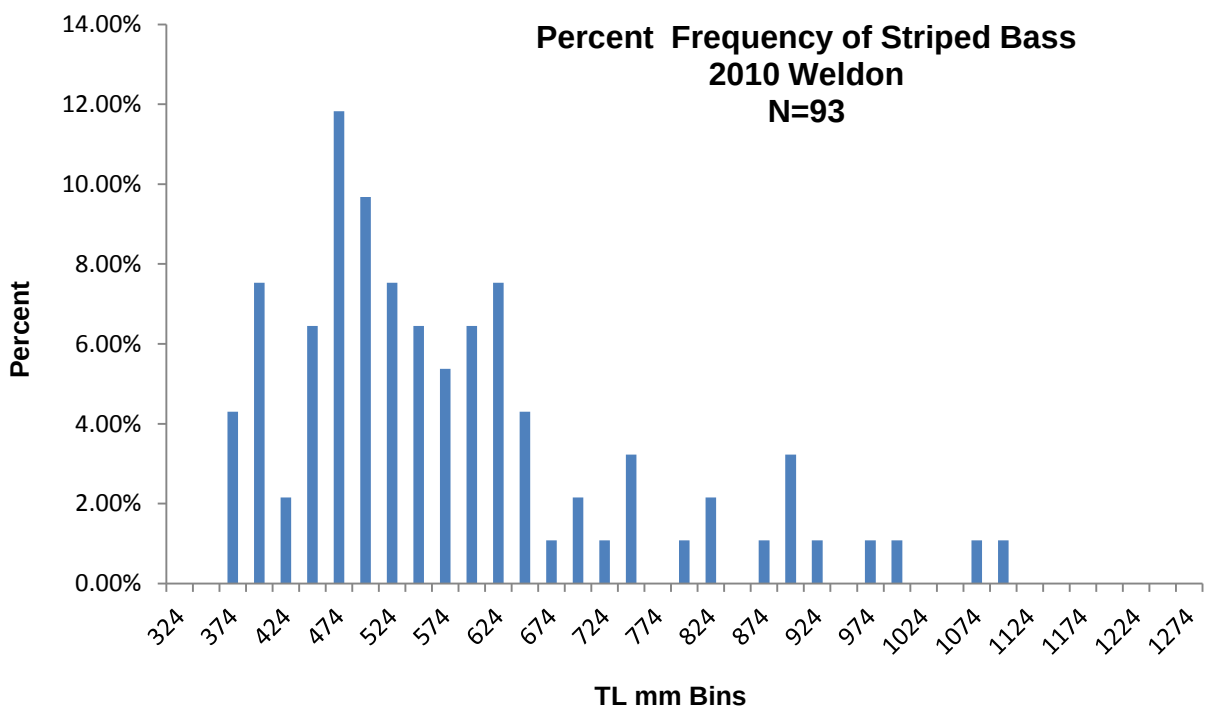

Figure 5. Length frequency analysis of fish collected from Weldon during the 2010 sampling year (n=97).

3. Collect biological information and material from collected fish for laboratory examination.

Biological information and material was collected from each fish for laboratory examination. Each fish was measured (FL and TL, mm), weighed (g), and sexed. Ovaries were removed and weighed (g); ovaries were preserved in cold 10% buffered formalin for 24-48 hours then set in 70% ethyl alcohol for histological sectioning. The middle portion of one ovary was excised and placed in 70% ethyl alcohol and shipped to Dermprep Customized Histology Services in Tampa, FL to be placed on slides for histological examination. The liver was excised and weighed (0.01 g). Scales were collected for ageing, and otoliths were removed for ageing and for otolith microchemistry.

4. Analyze biological samples to determine fecundity, age, LSI, and K factor.

Fecundity analysis was completed. Subsamples of preserved ovarian tissue were removed from each ovary, weighed individually (0.1 g), and then individual counted using digital images. The average number of eggs per gram of ovarian tissue was calculated by multiplying the average number of eggs per gram in the subsample by total ovarian weight (Nielson and Johnson 1983; Cailliet et al. 1986). Fecundity was estimated by age, TL, FL, and weight using linear regressions (Table 1). Scales (n=320) were pressed using acetate slides and a heat press and have been aged. Otoliths (n=439) were cut using a Hillquist high speed saw and have been aged. The Hillquist high speed saw was used because it is the tool that NCDMF utilizes for all of its otolith ageing samples that require cutting. The Hillquist saw cuts the identical portion of the otolith as the Buehler Isomet low speed saw, therefore the ages determined from the cut would be the same. The Hillquist saw is more efficient than the Isomet saw. Scale ages were then compared to otolith ages (Figure 6). Condition factors liversomatic index (LSI), Fulton's condition factor (K), relative weight (W_r), and gonadosomatic index (GSI) were calculated per month in 2009 and 2010 (Table 3).

Table 1. Fecundity estimated gravimetrically and using linear regressions estimating fecundity by age (yrs), weight (g), total length (TL mm), fork length (FL mm), and TL from FL regression (Olsen and Rulifson 1992).

Age	N	Gravimetric fecundity	Fecundity estimated by age	Fecundity estimated by weight	Fecundity estimated by TL	Fecundity estimated by FL	Fecundity estimated by TL from FL regression (Olsen and Rulifson 1992)
3	4	176,873	52,705	205,606	101,901	101,486	176,873
4	38	231,122	216,292	262,849	216,219	215,657	241,134
5	22	310,942	379,880	368,166	390,935	397,950	296,417
6	2	381,998	543,468	364,553	414,200	419,747	381,998
7	0						
8	3	1,296,463	870,643	1,055,734	1,296,175	1,354,706	1,296,463
9	3	854,930	1,034,231	639,701	887,439	912,270	854,930
10	4	1,207,771	1,197,818	1,275,792	1,520,660	1,450,706	1,104,918
11	1	1,570,737	1,391,996	1,370,618	1,730,456	1,763,750	1,570,737
12	0						
13	2	2,833,090	2,423,346	2,914,937	2,441,272	2,492,100	2,833,090
14	0						
15	0						
16	1	3,163,130	3,556,701	3,068,466	2,515,994	2,594,361	3,163,130

Table 2. - Number of female striped bass samples aged using otoliths (n=414).

	Ages													
	2	3	4	5	6	7	8	9	10	11	12	15	16	Total
Number of Females	70	78	127	68	15	8	12	17	10	3	2	3	1	414

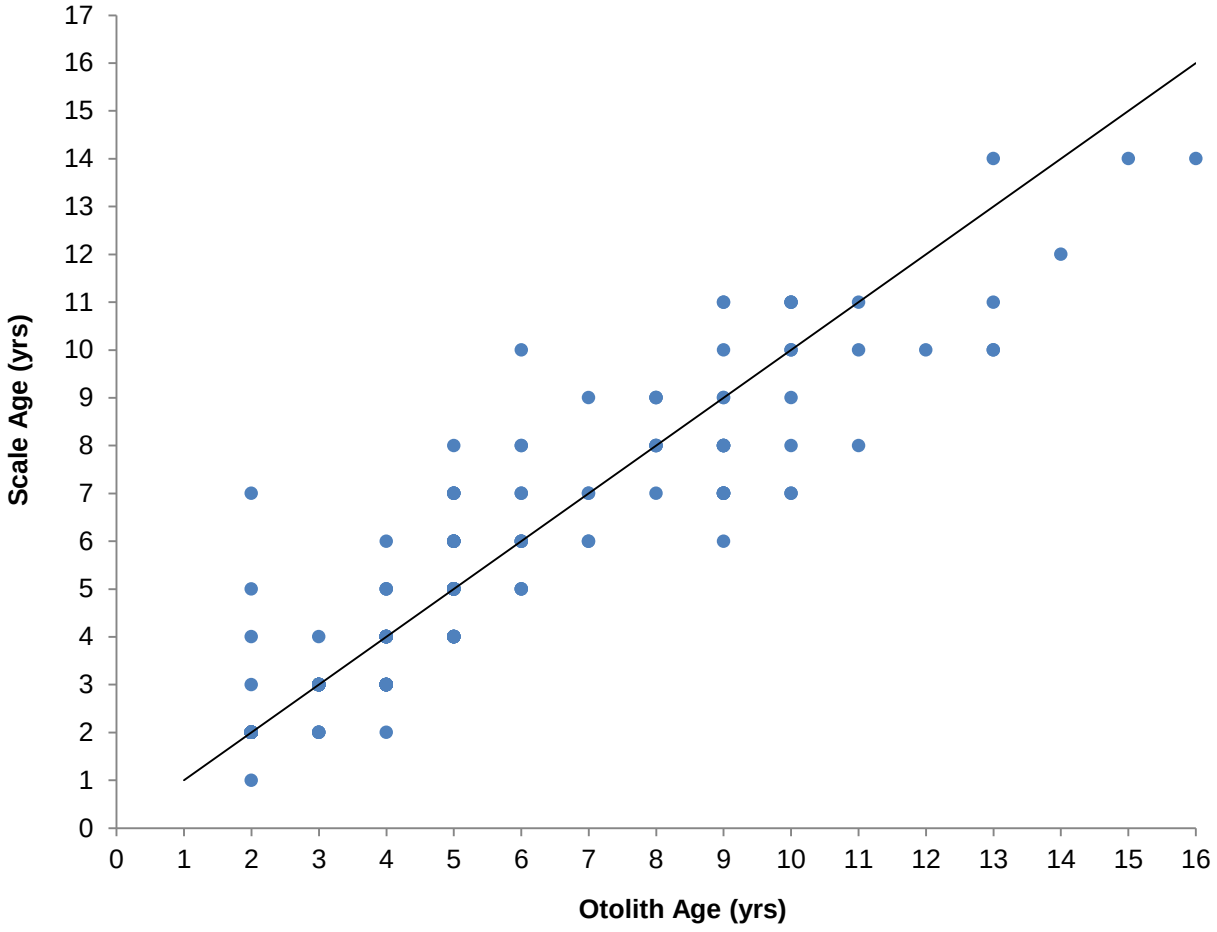

Figure 6. Scale age plotted against otolith age (n=320) to compare age determinations from each ageing structure.

Table 3. Mean, standard error, minimum, and maximum values for condition factors W_r (a), LSI (b), GSI (c), and K (d) for Albemarle/Roanoke striped bass from 2009 and 2010.

a)

	Relative weight (W_r)					
	2009			2010		
	MAR	APR	MAY	MAR	APR	MAY
N	44	79	87	53	86	86
Mean	85.20	90.44	86.01	93.15	89.13	81.8
SE	0.78	1.00	1.17	1.00	2.46	1.94
Min	74.69	41.77	69.72	79.01	56.60	0
Max	98.83	111.57	142.40	119.75	279.44	172.10

b)

	Liversomatic index (LSI)					
	2009			2010		
	MAR	APR	MAY	MAR	APR	MAY
N	44	79	87	50	84	75
Mean	1.48	1.44	1.26	2.19	1.55	1.40
SE	0.05	0.05	0.05	0.09	0.04	0.09
Min	0.84	0.22	0.12	1.08	0.58	0.67
Max	2.37	3.52	3.66	4.15	2.63	7.19

c)

	Gonadosomatic index (GSI)					
	2009			2010		
	MAR	APR	MAY	MAR	APR	MAY
N	44	79	82	50	82	75
Mean	1.65	8.54	5.95	1.66	6.83	5.76
SE	0.23	2.34	0.59	0.25	0.58	0.85
Min	0.20	0.26	0.14	0.26	0.28	0.07
Max	6.44	187	19.13	6.41	18.39	40.87

d)

	Fulton's condition factor (K)					
	2009			2010		
	MAR	APR	MAY	MAR	APR	MAY
N	44	79	87	53	86	85
Mean	1.06	1.13	1.07	1.16	1.11	1.03
SE	0.01	0.01	0.01	0.01	0.03	0.02
Min	0.93	0.52	0.87	0.98	0.70	0.40
Max	1.23	1.40	1.77	1.49	3.48	2.14

5. Analyze otoliths for patterns in the microchemistry to determine residency or anadromy, and spawning frequency. Also, examine environmental parameters and trace elements in Albemarle Sound habitats.

Otolith microchemistry has increasingly been used in fisheries research as a natural tag to study migration behavior (Campana 1999). Otoliths are calcified structures located in the inner ear providing balance and hearing to all teleost fishes. They are also the first calcified structure found during early development (Campana and Neilson 1985). Current research shows that striped bass otoliths begin to form before the larva hatches (B. Elking, East Carolina University, personal communication). Trace elemental composition of fish otoliths rely on the premise that elemental concentrations in the ambient water are incorporated into the protein and calcium carbonate matrix (Campana 1999). The otolith is metabolically inert, which means that the elements in the water are represented in the endolymphatic fluid and incorporated into the otolith. Therefore, the ambient water chemistry that the fish encounters its entire life is deposited and recorded in the otolith (Bath et al. 2000; Elsdon and Gillanders 2003; Kraus and Secor 2004).

The fundamental basis of otolith microchemistry is reliant on knowing the spatial and temporal variation of water chemistry. Surface water chemistry can be used to discriminate between different water bodies because typically each system has different types and abundance of elements (Dorval et al. 2007; Elsdon and Gillanders 2003). Previous research has shown that the RRMA/ASMA has stable patterns of water chemistry over time and temporal variations have been relatively short (Mohan 2009; Zapf 2012). However, collecting water samples over several seasons and years is essential to verify stable conditions. Consistency in elemental chemistry between rivers within the Albemarle Sound watershed will allow for a comparison of fish from multiple year classes to determine natal origins and movement.

Water Quality

Water samples (n=420) were analyzed to identify whether watersheds have elemental signatures and if so, determine the trace elements that make each of these river systems unique. Eight river systems were designated as sampling sites: North River, Pasquotank River, Little River, Perquimans River, Chowan River, Roanoke River, Scuppernong River and Alligator River. Sub-surface and deep (0.5 m above bottom) water samples were collected upstream and downstream of each river using a peristaltic pump and filtered to remove particulate matter. Environmental data were collected at each site: Air and water temperature (C°) water clarity (m), dissolved oxygen (DO) (mg/l), percent saturation (%), salinity (ppt), conductivity (µs), temperature corrected conductivity (µs), and pH.

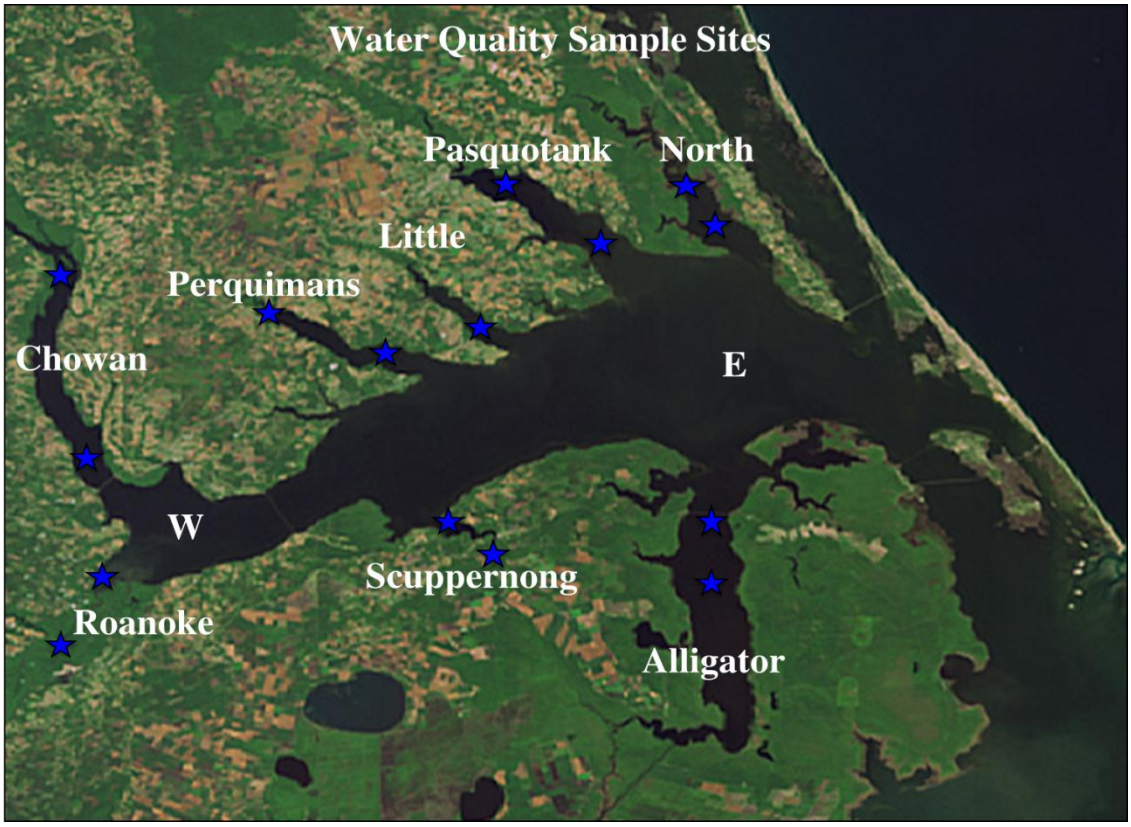

Figure 7. Map of study site showing upstream (B) and downstream (A) collection sites for each river system. This plot is accurate for locations of sites for figures 15 through 20.

During the study period, salinities ranged from 0 to 5.5 parts per thousand (ppt). In the eastern portion of the Albemarle Sound where salinity is the highest, the Alligator and North rivers exhibited salinity levels ranging from 2.4 to 5.5 ppt (Table 4). In comparison, salinities in the western portion of the sound in the Roanoke and Chowan rivers ranged from 0 to 1.3 ppt (Table 4). The Albemarle Sound exhibited salinity levels that were inversely related to river flow. As river flow increases the discharge is high enough (long-term annual average of 8,900 ft3/s) to push saline water out of the system and decrease surface salinities (Giese et al. 1985). Therefore, high flow periods during the early spring resulted in generally lower salinity values (Figure 8). There was more fluctuation in salinity values during the fall and the winter produced the highest mean values of salinity during the reporting period (Figure 8). There was also an expected difference between Site A and Site B salinities with the mouth of the rivers exhibiting a mean of 2.76 ppt and the upriver sites with a mean of 2.01 ppt (Figure 9).

Table 4. N, Mean, standard deviation, minimum, maximum, range, and standard error for salinity values (ppt) at collection sites for the surface and bottom in the Albemarle Sound from April 2011 to July 2012.

	Salinity (PPT) (surface)							Salinity (PPT) (bottom)						
River	N	Mean	Std Dev	Min	Max	Range	Std Err	N	Mean	Std Dev	Min	Max	Range	Std Err
Alli	22	4.5	0.5	3.3	5.3	2	0.11	7	4.67	0.38	4.2	5.2	1	0.14
Chow	10	0.51	0.58	0	1.3	1.3	0.18	4	0.1	0.08	0	0.2	0.2	0.04
Little	7	3.54	0.8	2.6	4.6	2	0.3	4	3	0.59	2.2	3.6	1.4	0.29
North	15	3.73	0.9	2.4	5.5	3.1	0.23	5	3.56	0.59	3.2	4.6	1.4	0.26
Pasq	15	2.79	1.66	0.07	5	4.93	0.43	4	3.3	1.41	1.4	4.8	3.4	0.7
Perq	17	1.83	0.98	0.1	3.1	3	0.24	6	1.47	1.06	0.2	2.7	2.5	0.43
Roan	17	0.10	0	0.1	0.1	0	0	4	0.1	0	0.1	0.1	0	0
Scup	20	1.79	0.99	0.1	3.1	3	0.22	4	1.85	0.17	1.6	2	0.4	0.09

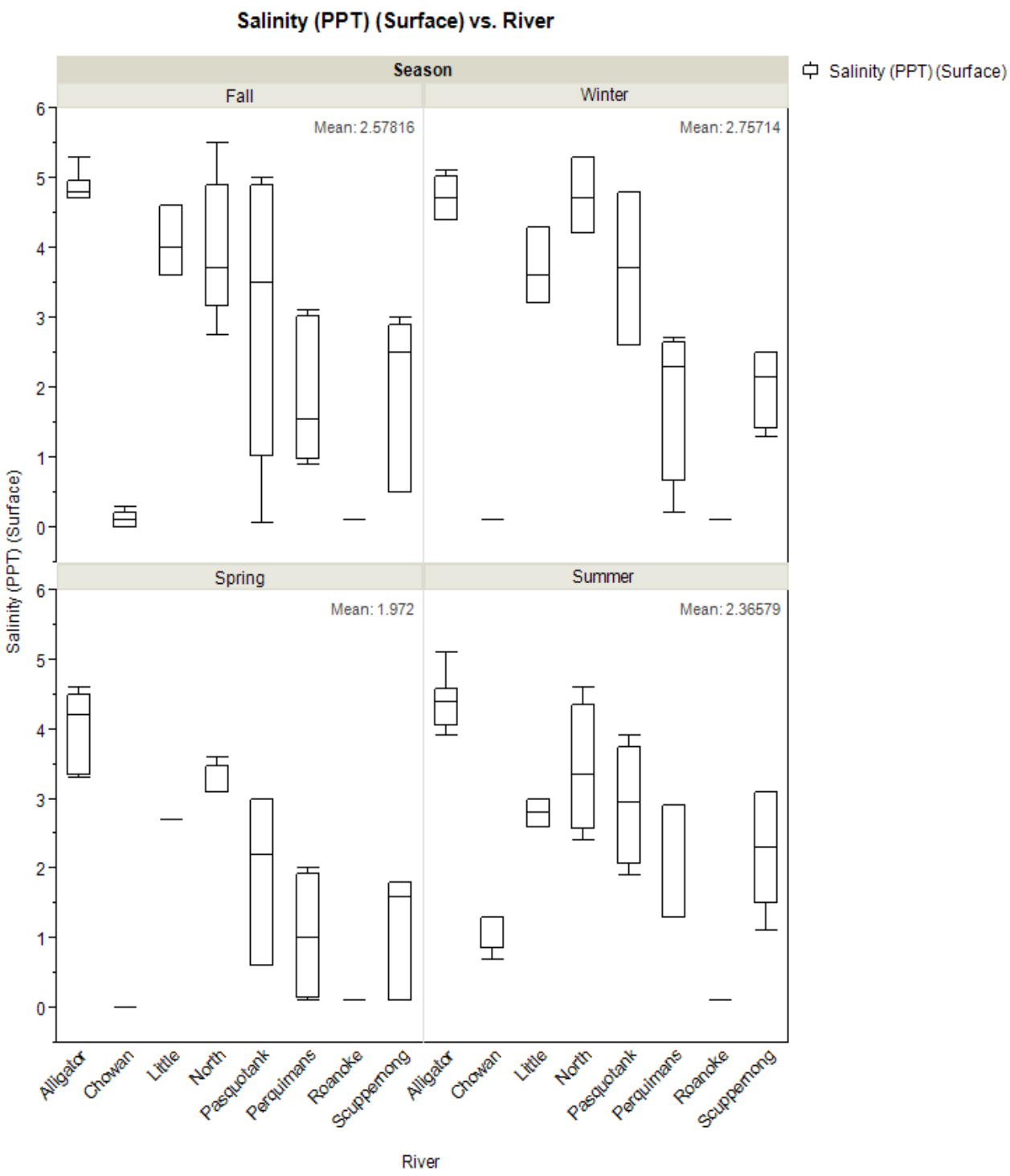

Figure 8. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar) of surface salinity (ppt) by river and season from April 2011 to July 2012.

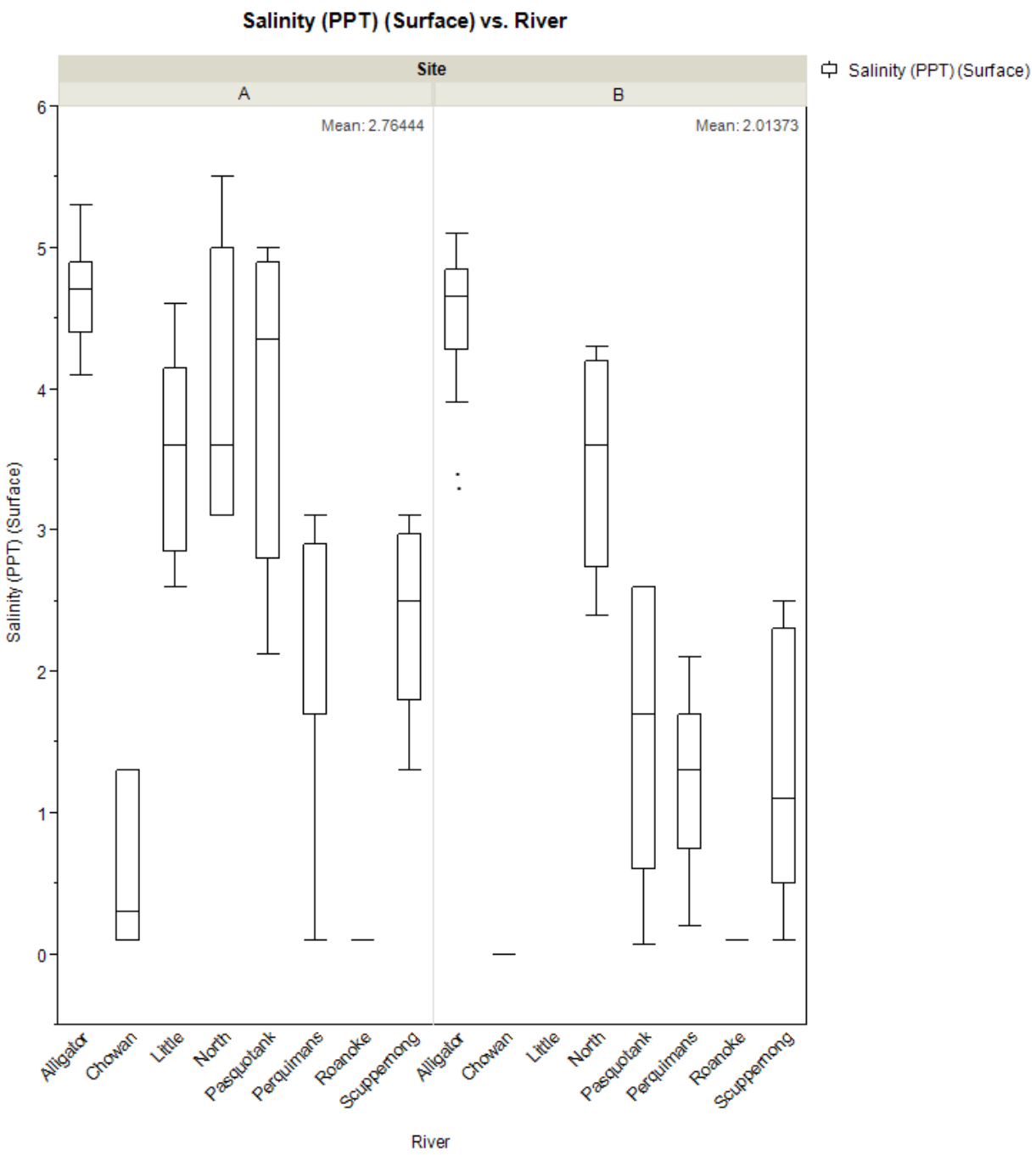

Figure 9. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar) of surface salinity (ppt) by river and site from April 2011 to July 2012. Site A indicates samples collected at the mouth of the rivers and Site B is upstream sample sites.

Observed seasonal temperature fluctuations had minimal variation among sample sites. The fall season exhibited the greatest fluctuation in temperature with a range from 27°C dropping to 8.8 °C (Figure 10). The mean seasonal temperature range

was 17.6 °C for the fall, 9.16 °C for the winter, 19 °C for the spring, and 29.1 °C during the summer period of record. The maximum observed seasonal range was 33.7 °C (Pasquotank) in the summer, and the minimum was 7 °C (North) in the winter (Figure 10).

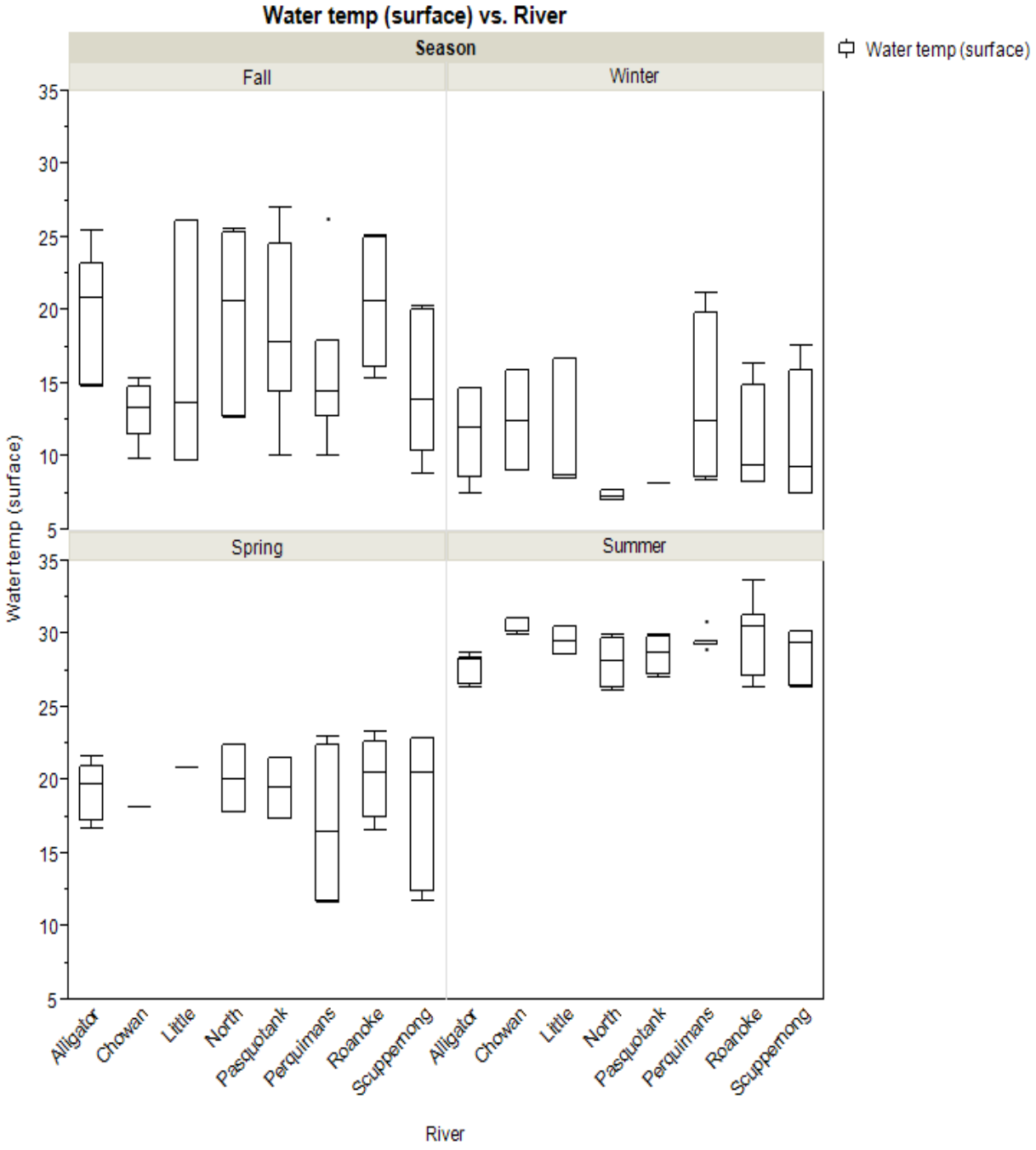

Figure 10. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar)of seasonal comparison of surface water temperature (C°) by river from April 2011 to July 2012.

As might be expected, the daily range in dissolved oxygen typically was higher during the winter months than during the rest of the year. Dissolved oxygen concentrations were higher during the early spring period than during the summer period (Figure 11). They also were generally higher in the Little River (mean 9.38 mg/L) located near the east-west midpoint of Albemarle Sound, and the North River (mean 9.40 mg/L) located in the north-west portion of the sound (Figure 11). Less fluctuation in dissolved oxygen values was observed during the winter period, but the fall period had the highest amount of variation with a minimum of 0.03 (mg/L) to a maximum of 11.5 (mg/L) (Figure 11). A comparison of dissolved oxygen levels between surface and bottom showed the mean values at surface (7.87 mg/L) to be slightly lower than values at the bottom (8.09 mg/L) (Figure 12). Additionally, the Perquimans River varied significantly ($p=0.0006$) from the other sites with a mean bottom dissolved oxygen value of 3.71 (mg/L) (Figure 12).

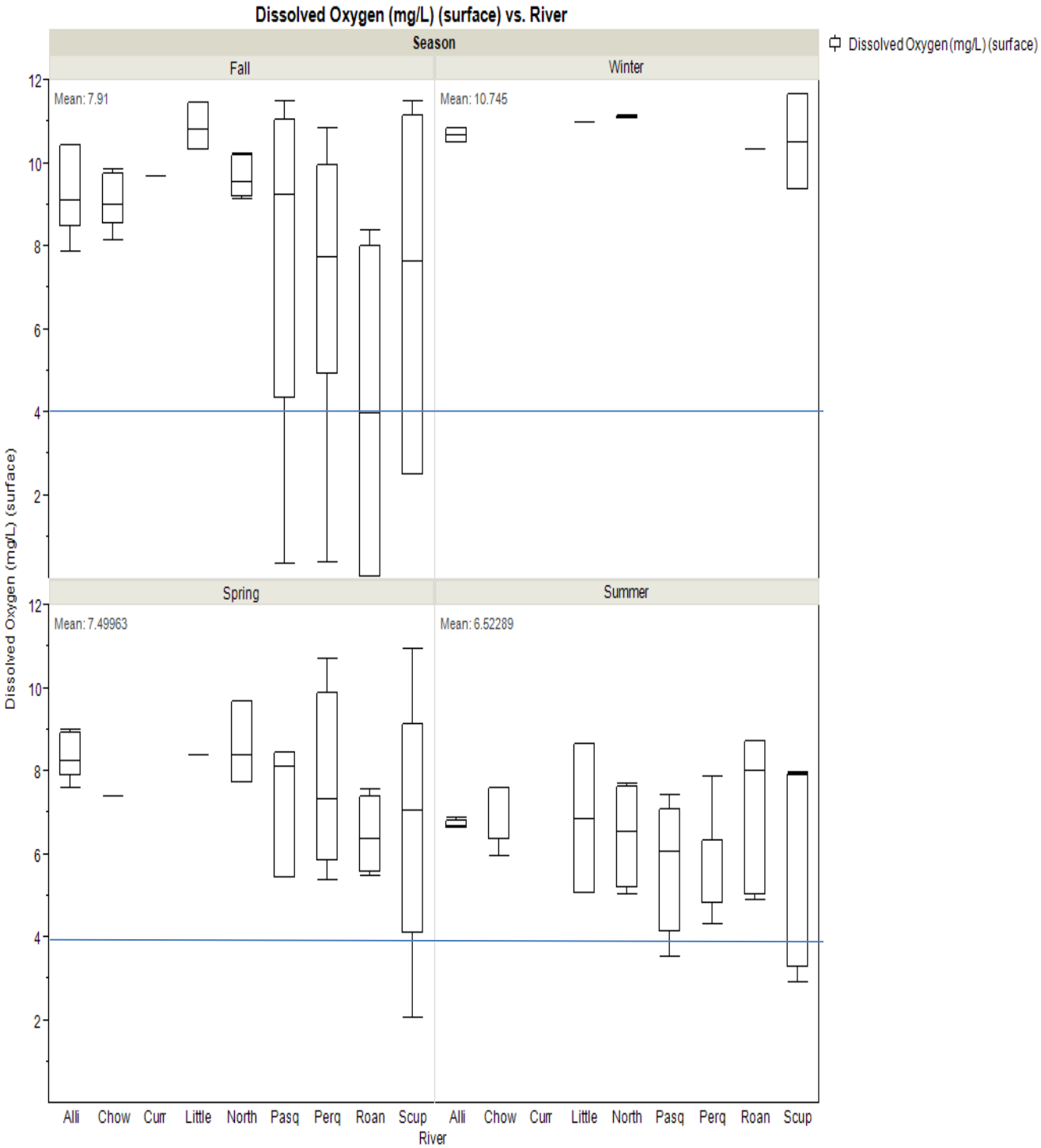

Figure 11. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar) of dissolved oxygen (mg/L) from the surface by season of each river system from April 2011 to July 2012.

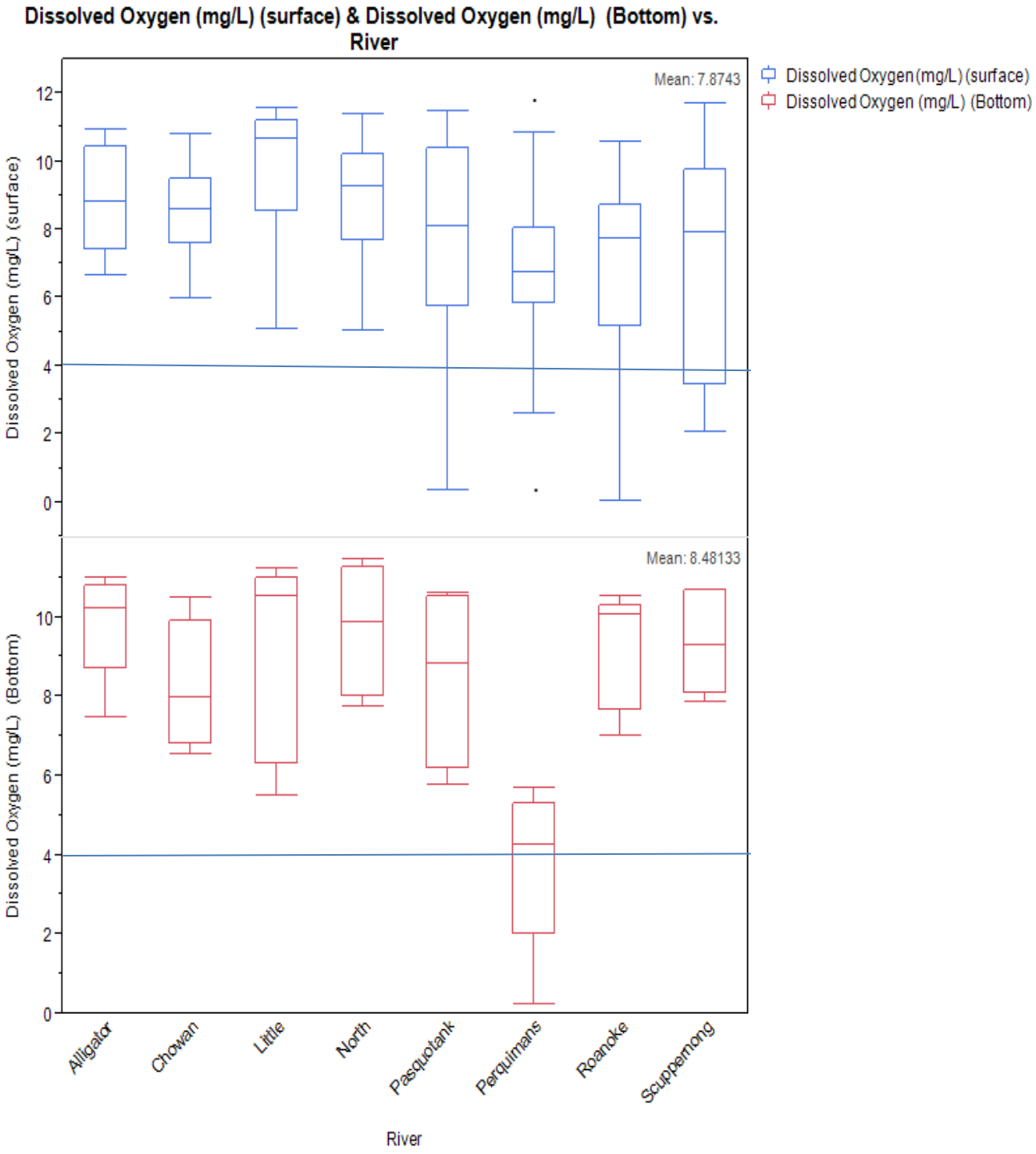

Figure 12. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar) of dissolved oxygen (mg/L) from the surface and bottom of each river system from April 2011 to July 2012.

Water Chemistry

Trace elements of Sr:Ca, Ba:Ca, Mg:Ca, and Mn:Ca, were examined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) to measure dissolved elemental concentrations in water samples. Water samples (n=420) were collected and analyzed to identify whether watersheds have elemental signatures, and if so determine the trace elements that make each of these river systems unique. The following maps provide a visual representation of seasonal elemental concentrations collected from sample sites.

Results show that the Mg:Ca ratio is a principal element used to establish a chemical signature in this watershed. Descriptive statistics provided a mean of 2.6663 ppm, minimum of 0.3646 ppm, maximum of 4.1600 ppm, standard deviation of 1.2164 and range of 3.7954. Mg:Ca was significantly higher in the western portion of the sound compared to the eastern portion. A strong positive relationship exists between salinity and Mg:Ca indicating Mg may follow a similar pattern to Sr in this system. This pattern is consistent with an earlier study sampling in similar locations (Mohan et al. 2012) and also in the Chesapeake Bay (Dorval et al. 2005). The Chowan River and Roanoke River systems indicate low Mg levels (<1 ppm) located in the western portion of the ASMA. The Alligator River and North River have high levels of magnesium (>3.5 ppm) and are geographically located in the eastern portion of the ASMA (Figure 13).

The seasonal comparison of Mg:Ca is consistent with the positive relationship between salinity and Mg:Ca concentrations. A slight increase in salinity during the winter months, particularly in the western portions of the sound, also correlated with an increase in Mg:Ca concentrations (Figure 13). Our research indicates that watersheds vary greatly in Mg:Ca however, direct comparisons to otoliths have been inconclusive in previous studies (Campana 1999; Dorval et al. 2007; Mohan et al. 2012).

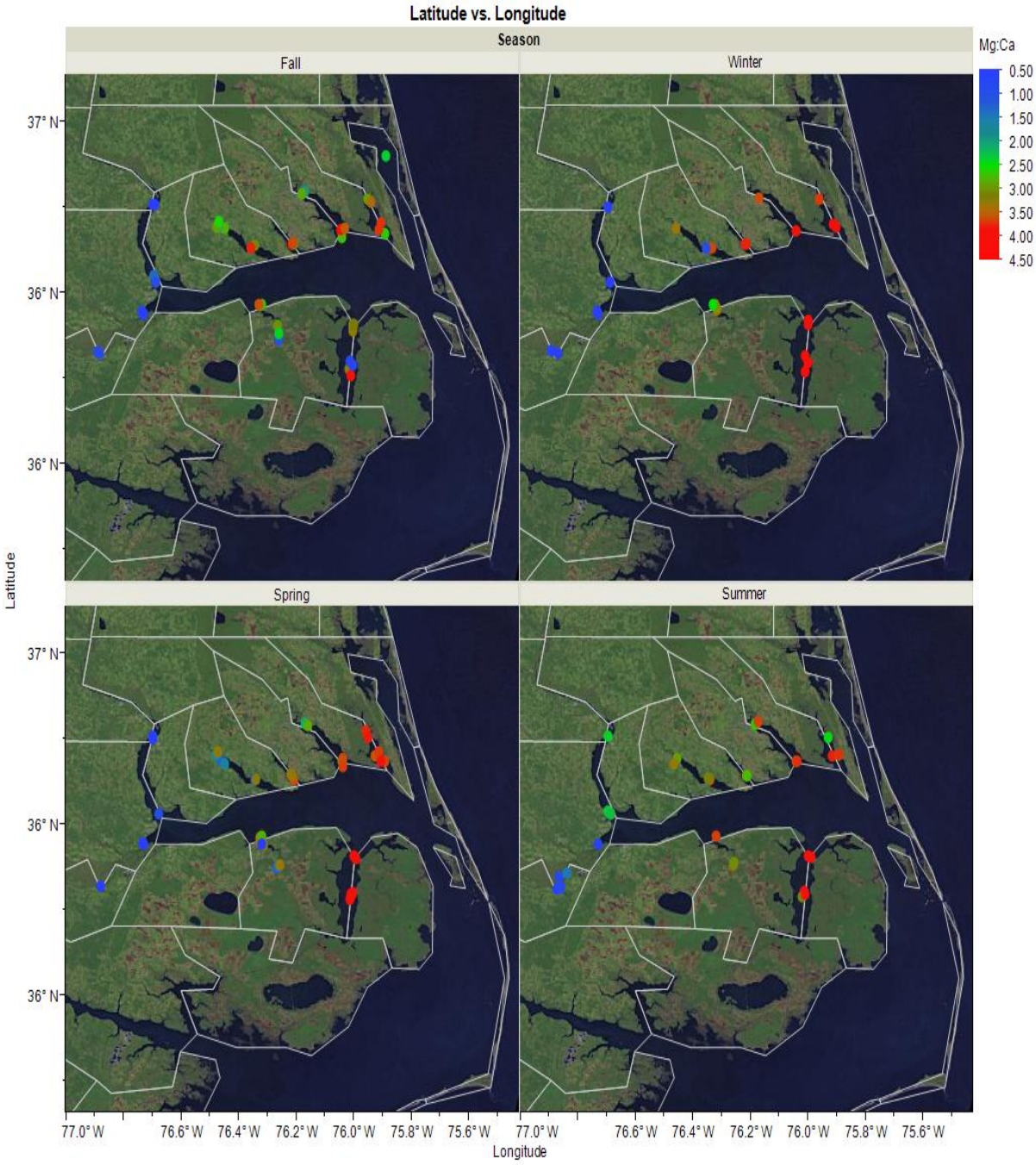

Figure 13. Seasonal comparison of mean values of dissolved concentrations of Mg:Ca in water samples (n=320) of each river system sampled from April 2011 to July 2012.

Sr is consistently used in otolith microchemical analysis because of its direct positive correlation with salinity. Salinity in the Albemarle Sound is low and can vary greatly based on wind and rainfall. The eastern Albemarle Sound generally has higher salinity than the western sound (Mohan et al. 2012; Zapf 2012). In general,

results from our study followed an expected pattern; the eastern portion of the sound with higher salinity (Alligator River) had higher Sr:Ca (Figure 14). In contrast, the western portion of the sound (Chowan and Roanoke Rivers) had lower Sr:Ca (Figure 15). Sr:Ca results indicated a mean of 0.0157 ppm, a minimum of 0.0081 ppm, maximum of 0.0245 ppm, standard deviation of 0.003, and range of 0.0164 (Figure 15).

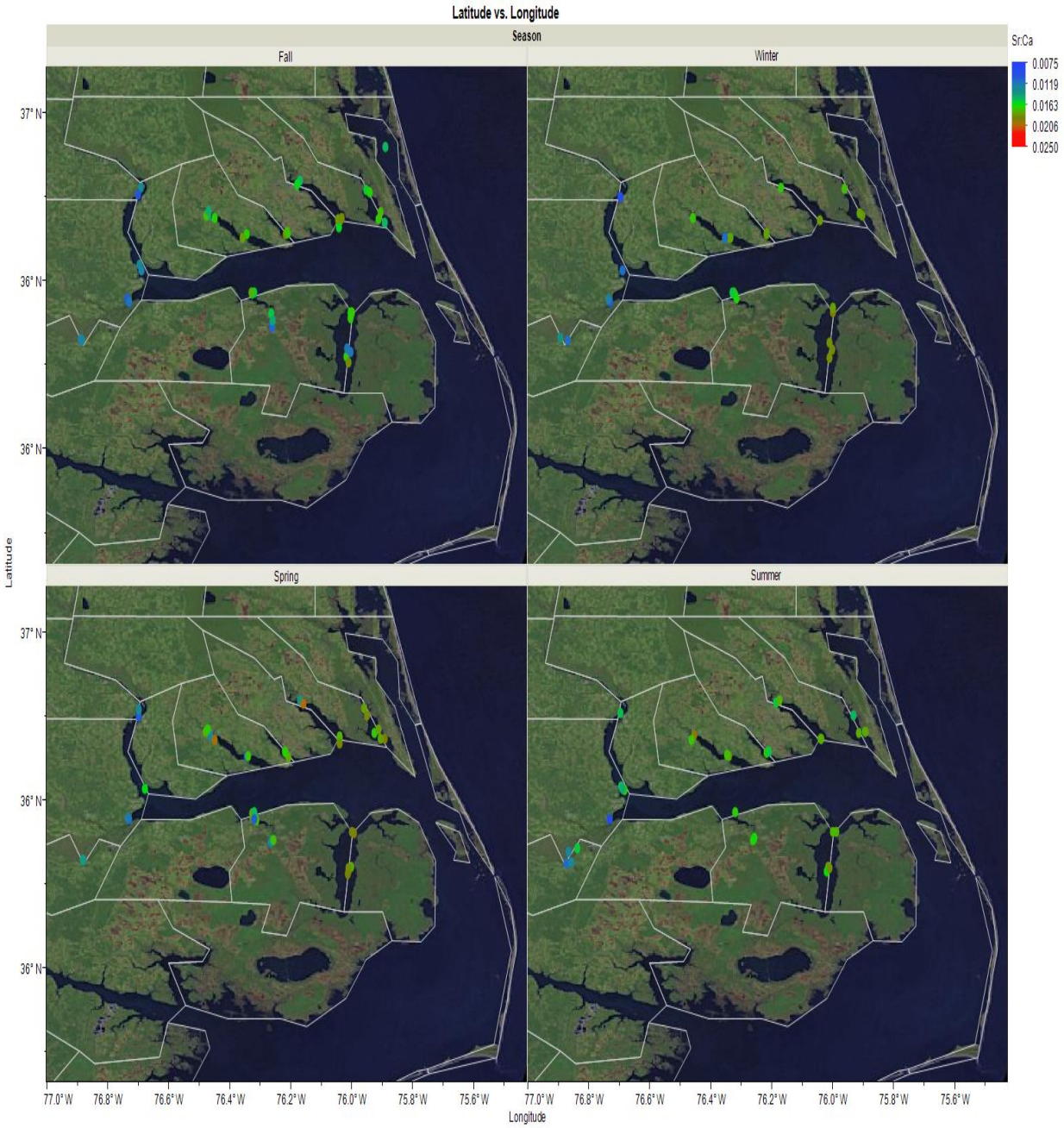

Figure 14. Seasonal comparison of mean values of dissolved concentrations of Sr:Ca in water samples (n=320) of each river system sampled from April 2011 to July 2012.

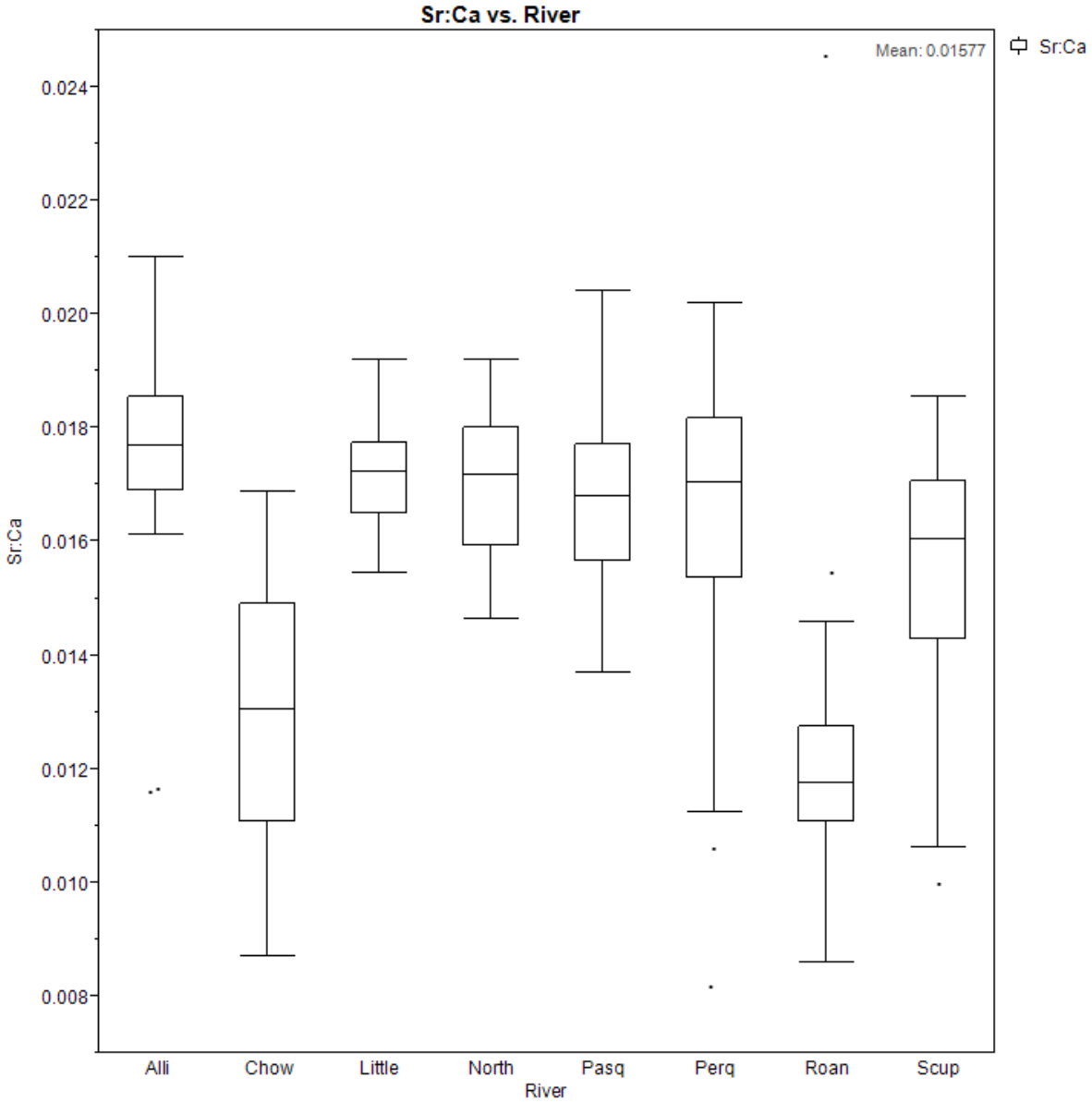

Figure 15. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar) of Sr:Ca in water samples (n=320) of each river system sampled from April 2011 to July 2012.

Ba:Ca ratios are expected to exhibit a negative relationship with salinity (Guay and Falkner 1998). Our study followed this trend with a negative relationship between salinity and Ba:Ca (Figure 16). Results indicated a mean value of 0.0056 ppm, minimum of 0, maximum of 0.0596, standard deviation of 0.0079, and range of 0.0596 (Figure 17). Ba:Ca ratio showed spatial and temporal variation among the freshwater sample sites (Figure 16). As expected, the lowest Ba:Ca ratios were found in the Alligator and North River (Figure 17). These results correspond with previous studies in the same study area (Mohan et al. 2012; Zapf 2012). The

maximum levels of Ba:Ca occurred in the fall and summer of upstream sites in the Roanoke and Chowan River (Figure 17). These two systems also had significant variation ($p < .0001$) compared to the other sites. These findings are consistent with results from other studies that show significant variation in Ba:Ca ratios in freshwater systems (Wells et al. 2003; Limburg and Seigel 2006; Humston et al. 2010, Dobbs 2013). The Ba:Ca spatial variation makes it a reliable element in determining chemical signatures of natal origin in otoliths.

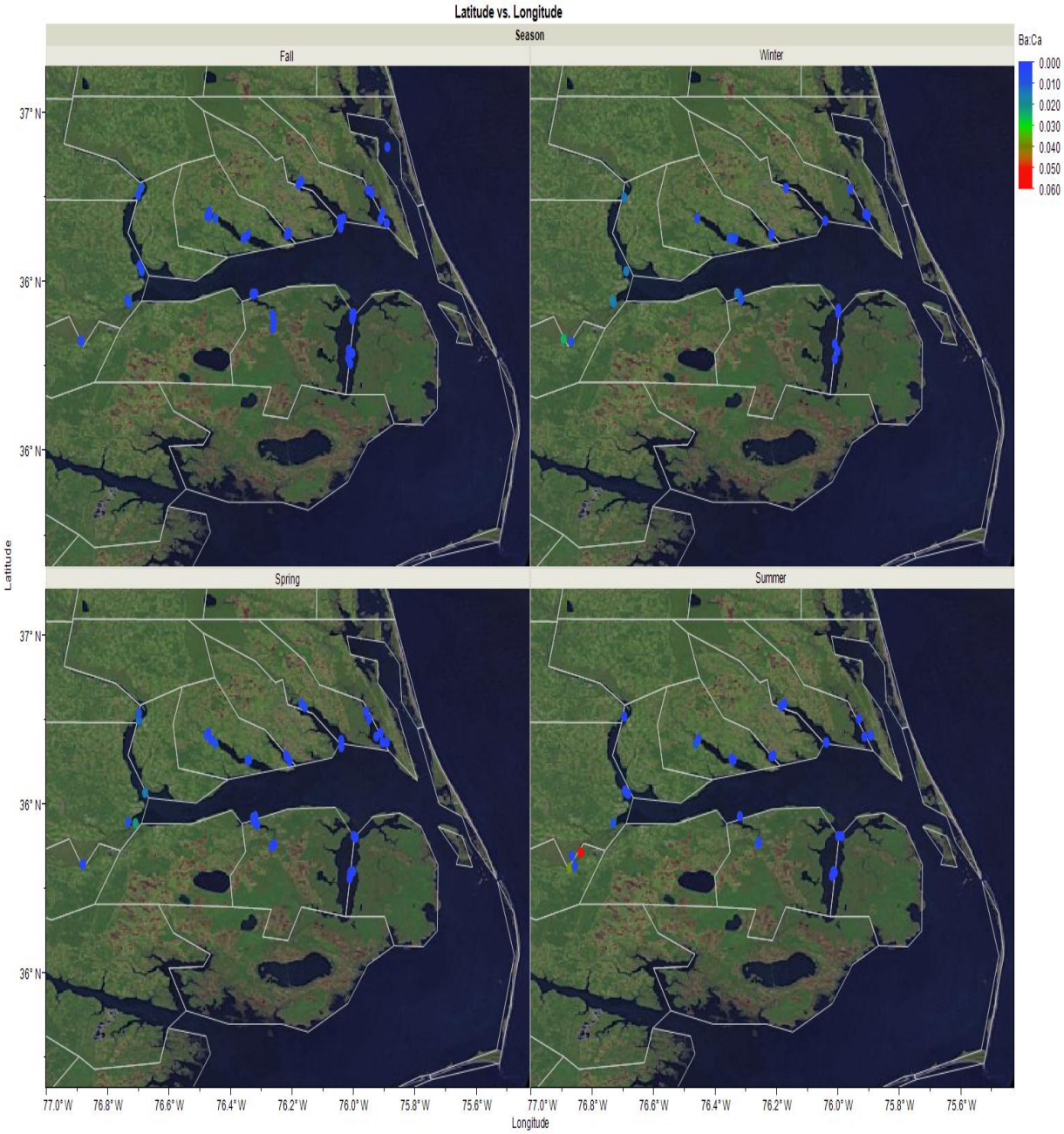

Figure 16. Seasonal comparison of mean values of dissolved concentrations of Ba:Ca in water samples (n=320) of each river system sampled from April 2011 to July 2012.

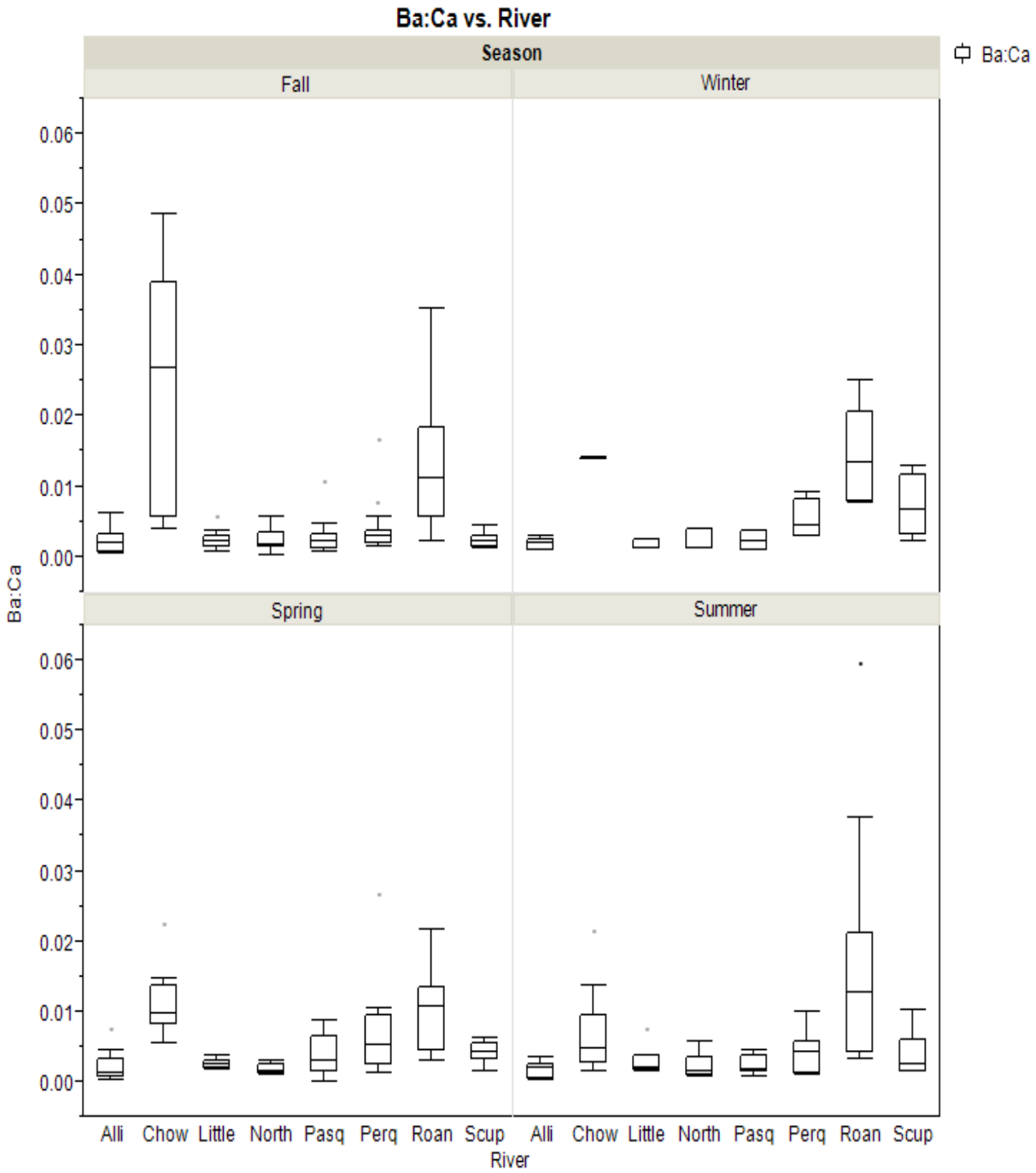

Figure 17. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar) of Ba:Ca in water samples (n=320) of each river system sampled from April 2011 to July 2012.

Dissolved concentrations of Mn:Ca has been associated with anoxic conditions caused by periods of low dissolved oxygen (Sundby et al. 1986; Laslett 1995). Our results follow the relationship between dissolved oxygen and Mn:Ca ratios. Hypoxic events occurred in the Albemarle Sound following Hurricane Irene in late August 2011. We believe that these large rainfalls resulted in Mn being released from the sediments at the upstream and downstream sites of the Roanoke River in early September 2011 (Figure 18). Regardless of the proven relationship between hypoxia and Mn, this study found a weak negative relationship between Mn:Ca concentrations and geographic location (Figure 18).

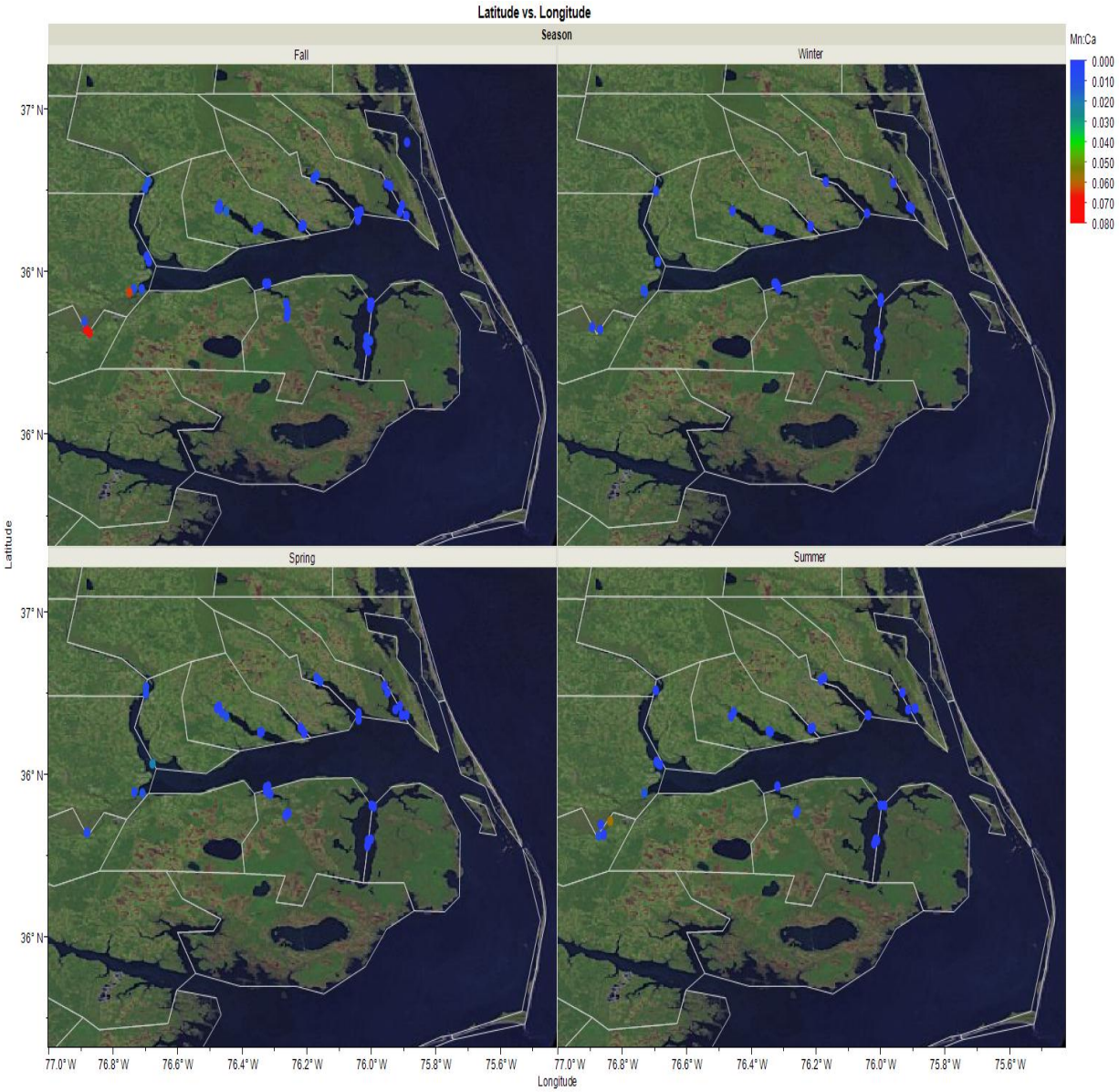

Figure 18. Seasonal comparison of mean values of dissolved concentrations of Mn:Ca in water samples (n=320) of each river system sampled from April 2011 to July 2012.

Ca concentrations are not typically evaluated in otolith microchemistry studies because it is assumed that otoliths are aragonite ($CaCO^3$) and therefore relatively constant. However further evaluation was necessary to ensure ambient concentrations would not affect prediction of salinity levels based on Sr:Ca ratios. Our study found results that support similar findings in previous studies (Mohan 2009, Zapf 2012). Ca is typically correlated with salinity; however, both previous studies supported evidence that the Albemarle Sound/Roanoke River (A/R) does not follow that correlation. The current study found maximum Ca concentrations to be 48.9690 in the Alligator River to minimum concentrations at 7.4730 in the Roanoke (Figure 19). A one-way ANOVA was used to examine differences in Ca levels between sample sites. When significant differences were detected, Tukey-Kramer all pairs test was used to examine which locations differed significantly. The significant difference ($p < .0001$) between the eastern and western portions of the sound could greatly affect Sr uptake and therefore result in unusually high Sr levels in the otolith (Figure 19). Mohan (2009) found that high ambient concentrations of Ca resulted in max Sr:Ca ratios of 21.0 ppt salinity that was actually only 5 ppt. Findings suggest that Sr may only be useful for determining movement from oligohaline to mesohaline environments.

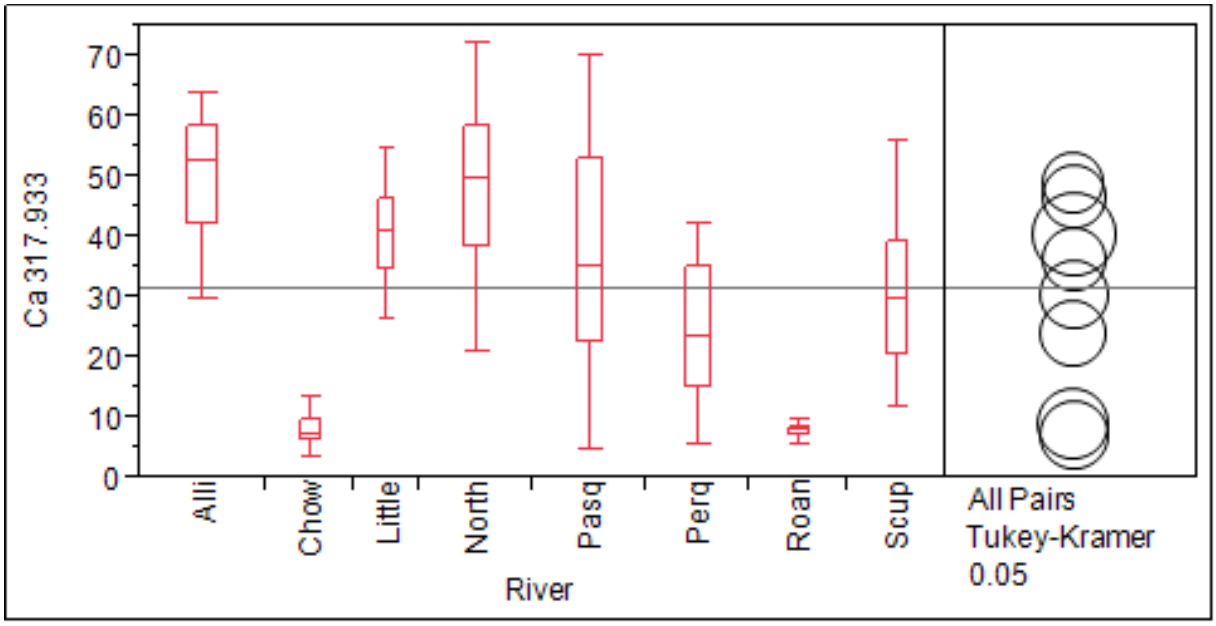

Figure 19. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar) with grand mean and comparison circles of values of Ca concentrations in water samples (n=320) of each river system sampled from April 2011 to July 2012. Additional analysis using Tukey-Kramer all pairs analysis showing significant differences in the Roanoke River and Chowan River.

Finally, quadratic discriminant function analysis (QDFA) was used to determine if elemental ratios could be used to classify water samples to river of collection. Upriver

sample sites (Site B) were chosen for this analysis because they are known nursery areas and provide more accurate classification rates when determining chemical signatures of river systems. As expected, geographical locations of sample sites had similar elements and were grouped accordingly in the canonical discriminant function plot (Figure 20). A QDFA analysis showed 28 to 86% of water samples being correctly classified by elemental ratios to rivers of collection (Table 5).

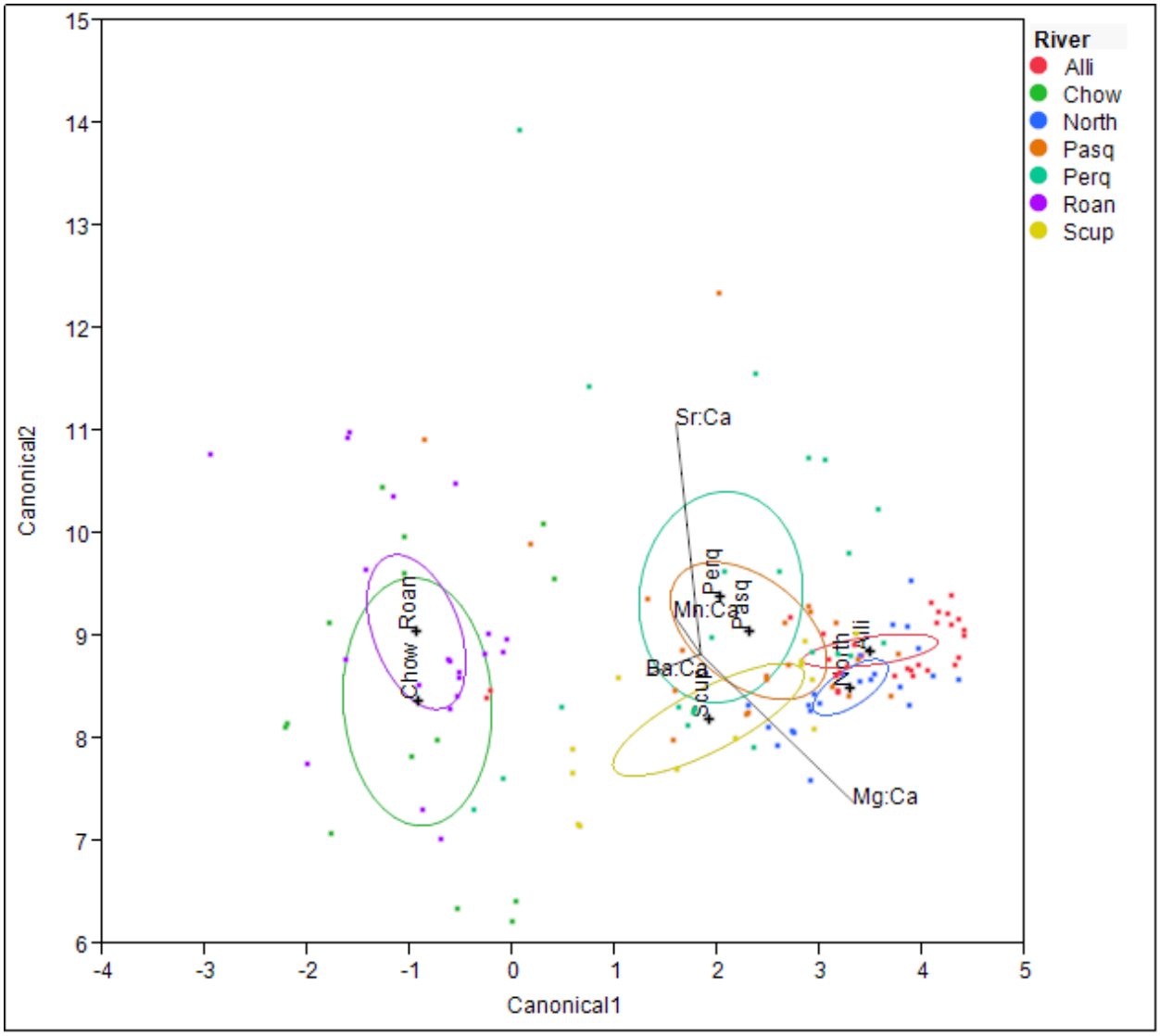

Figure 20. Canonical Discriminant Function Plot of water chemistry signatures in upstream sample sites (Site B) using Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca ratios in water samples (n=143) collected from April 2011 to July 2012. Group circles represent 95% confidence limit and element lines represent the grand multivariate mean projected in canonical space from the center.

Table 5. – Results of quadratic discriminant function analysis used to classify water samples (n=143) at upstream sample sites (Site B) to river of collection.

Location	N	ALLI	CHOW	NORTH	PASQ	PERQ	ROAN	SCUP	% Correct
ALLI	29	16	0	10	1	0	2	0	55.1
CHOW	14	0	12	0	2	0	0	0	85.7
NORTH	23	2	0	20	0	0	0	1	86.9
PASQ	21	2	0	6	6	2	0	5	28.5
PERQ	22	3	2	0	2	9	0	6	40.9
ROAN	21	0	6	0	0	0	15	0	71.4
SCUP	13	2	0	1	2	1	0	7	53.8

Otolith Microchemistry

Otoliths (n=246) were sent to the University of Manitoba, Winnipeg, Canada for microchemical analysis using laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The otoliths were sectioned using a diamond blade Isomet saw (Buehler 646) into 2 mm thick dorso-ventral transverse sections. A 15 µm beam with a 2 μms^{-1} scan speed was used to scan the otolith beginning before the nucleus, through the core, and continuing along the long axis until reaching the outer edge of otolith growth. Elements quantified during the scan included: Ca, Mg, Sr, Ba, Mn, Cu, Zn, and Pb. These elements were examined to determine spawning frequency, maternal contribution, movement at various stages of life, and wild versus hatchery fish.

Spawning frequency

Spawning frequency was unable to be determined in our study. While evidence of spawning migration can be observed using otolith microchemistry, many assumptions must be made when interpreting results. Spawning migration occurs during short time periods, often too short for concentration variation within the otolith to be resolved. As a result, a Type II error occurs when analyzing results because the variation of the Sr signal in the fish has little time to equilibrate or exhibit disparity between water bodies (Secor and Piccoli 2007).

Maternal contribution

Analyses were completed to determine maternal contribution and migratory behavior within the first year of life (age 0-1) by examining levels of Sr at the focus of the otolith.

The primordium (core) is thought to be the center of accretion and therefore corresponds to the larval stage. Examining the core can also provide researchers with the elemental concentrations of the maternal contribution region. As a result, elemental concentrations near the core reflect the chemistry of the egg, indicating the Sr uptake of the mother during egg development (Volk et al. 2000). Females that overwinter in the ocean before quickly migrating upriver to spawn should carry a more saline yolk sac. Otolith formation begins prior to hatching (B. Elking, East Carolina University, personal communication) and therefore precludes elemental deposition from any source other than the mother. The yolk-sac larvae rely on the egg for nourishment and otolith chemistry is influenced by the egg material until the larvae begin feeding (Volk et al. 2000). Sr levels observed in the maternal contribution region were evaluated to determine if the mother was resident (<4000 ppm) or anadromous (>4000 ppm) (Table 6).

Movement: First year

Sr is the most widely used element for otolith elemental analysis. It is deposited in the otolith in similar proportion to the ambient water. It varies predictably along a salinity gradient, with Sr:Ca positively related to salinity (Secor and Piccoli 2007). Otolith chemistry is used to interpret the migration history of freshwater and diadromous species based on their Sr:Ca profiles. Otoliths Sr:Ca ratios (ppm) were examined for elemental signature from the core through the first year of life to determine movement during the juvenile stage (Campana et al. 1994). Identifying movement during the juvenile stage is necessary in understanding the status of the stock and designing effective management strategies. Migratory behaviors based on strontium levels suggest that overall, 85% of striped bass exhibited signs of anadromy within the first year of life (Table 6). This is comparable to Patrick (2010), who found that a majority (79%) of young-of-year (YOY) migrated into marine waters.

Movement: Mature fish

Trace levels of Sr were examined to further evaluate movement in mature fish (age ≥ 3) by determining if A/R stock fish were resident or anadromous. A threshold of 4000 (ppm) Sr was used based on a review of Sr:Ca ratios and estimated salinity values from Secor et al. (1995). The equation used for estimating salinity relies on Sr:Ca ratios and is as follows:

Estimated Salinity = 40.302 (1 + 56.337 EXP-1523.31*Sr:Ca Ratio)-1

The conversion equation provided ranges determined by Morris et al. (2005) to equate to 0 – 11 ppt (< 2,000 ppm), 11 - 35 ppt (2,000 – 4,000 ppm), and > 35 ppt (> 4,000 ppm). This conversion equation provides the foundation for estimates of marine migration. The estimation is determined from salinities exceeding 35 ppt so additional elemental analysis will be conducted to further elucidate habitat profiles. Additional precautions were taken to ensure the maternal contribution was not a factor in determining migration patterns. Secor and Dean (1992) described that a larval striped

bass standard length (SL) to otolith diameter can be establish with the following equation:

$$SL=4.160 + (0.0239* \text{otolith diameter})$$

where the otolith diameter is in microns (µm) and SL is in millimeters (mm). Using this equation and research from Mohan (2009), Dobbs (unpublished thesis), and Elking (unpublished thesis) it was determined that the first 60 µm from the otolith focus should not be included to ensure that no maternal contribution material was included in the analyses.

Examination of migratory movements of striped bass at maturity (age ≥ 3) revealed that 17% of fish were resident and 83% were migratory (Table 6). Again, these results are comparable to Patrick (2010) with 23% determined resident and 77% migratory.

Wild versus hatchery

Further elucidation of behavior observed during the first year of life (beyond the 60 µm determined as maternal contribution) revealed evidence of hatchery reared fish. While evaluating migratory behaviors for YOY, some fish exhibited extremely high Sr concentrations (>6000 ppm) followed by a precipitous decline. Examination of the water chemistry from the Edenton National Fish Hatchery (ENFH) revealed a high (0.026) Sr:Ca ratio (Dobbs, unpublished thesis). In comparison, seawater has low variability with a Sr:Ca ratio of 0.0185 (Quinby-Hunt and Turehian 1983) and Woods (2000) found the Castle Hayne Aquifer to vary between 0.005 and 0.1 Sr:Ca. Additionally, water chemistry results from the Albemarle Sound/Roanoke River indicated a mean of 0.0157 with a minimum of 0.0081 and a maximum of 0.0245. Therefore, the well water consists of Sr:Ca ratios higher than the marine environment and the study sites, but within the range reported by Woods (2000). Additional information resulting from elemental data analysis conducted in the Central Southern Management Area (CSMA) also revealed a hatchery signal (Dobbs, unpublished thesis). Data analysis in our study supports that fish are being naturally tagged as a result of the Upper Castle Hayne Aquifer being used as the well water source for pond rearing.

The subsequent data gathered from water chemistry provided researchers with an inadvertent yet distinctive otolith chemical signature found in hatchery reared fish. Juveniles were evaluated for chemical signatures beyond maternal input and within the first year of life for Sr levels exceeding 6000 ppm. Sr levels >6000 ppm were chosen to eliminate the possibility of high Sr:Ca ratios in the otolith developed during naturally occurring upwelling events. Additional precautionary procedures were taken to establish that such levels must be reached a minimum of 10 times to eliminate false positives resulting from mechanical error. Using these criteria, results indicated that 77% of fish sampled in the A/R stock were wild and 23% were hatchery fish (Table 6).

Table 6. Characterizing levels of Sr to determine maternal contribution of resident versus anadromous mothers, movement within the first year (age 0-1) (constant, sprinter, or stager), mature fish (resident vs. migratory), and wild versus hatchery fish (Age 0-1 and beyond maternal contribution) for female striped bass (n=246) collected from A/R stock from 2009 to 2010.

Maternal Contribution	Age 0-1 (<60 microns)	N	%
	Resident (<4,000 ppm)	201	81.71
	Anadromous (>4,000 ppm)	45	18.29
Movement: First Year	Age 0-1 (>60 microns)		
	Constant (within a 2,000 ppm level)	10	4.07
	Sprinter (>4,000 ppm)	209	84.96
	Stager (2,000 ppm<Sr<4,000 ppm)	27	10.98
	Total	246	100
Movement: Mature Fish	Age $\geq$ 3		
	Resident (<4,000 ppm)	41	16.67
	Anadromous (>4,000 ppm)	205	83.33
	Total	246	100
Wild vs. Hatchery	Age 0-1 (>60 microns)		
	Wild (<6,000 ppm)	215	77
	Hatchery (>6,000 ppm)	31	23

Once the hatchery fish were determined, further comparison of elemental analysis was completed to establish additional elements indicative of hatchery reared fish. Analysis of means was completed to ascertain if elements found in hatchery reared fish were significantly different. Results indicated that Sr, Ba, and Pb were all significantly different between non-hatchery and hatchery fish (Table 7).

Table 7. Mean, minimum, maximum, standard deviation, and p value of each element in otoliths of A/R stock fish collected (n=246) from 2009 to 2010 comparing non-hatchery to hatchery fish.

Elements	**Non-Hatchery**				**Hatchery**				
	Mean	**Min**	**Max**	**Std Dev**	**Mean**	**Min**	**Max**	**Std Dev**	**p**
Mg26	23.21	4.52	59.73	7.75	21.86	11.03	50.71	8.22	0.3946
Mn55	12.46	-0.75	63.48	9.33	10.65	2.60	32.69	6.82	0.1969
Cu65	0.13	-4.00	2.35	0.41	0.23	-1.21	4.25	0.79	0.5062
Zn66	0.18	-3.50	1.52	0.56	0.04	-2.13	0.71	0.55	0.1854
Sr88	2786	814.60	4100	667.1	3988	2059	6513	924.3	<0.0001*
Ba138	36.65	9.81	105.8	16.42	81.48	24.55	312.3	62.01	<0.0004*
Pb208	0.04	-0.27	1.85	0.17	0.01	-0.04	0.05	0.02	0.0140*

When significant differences were detected a one way Kruskal-Wallis test was used to further evaluate mean element score comparisons from non-hatchery to hatchery fish (Table 7 and Figures 21, 22, 23). This analysis was employed to test the null hypothesis that hatchery fish would not be determined by elemental analysis. The null hypothesis was not supported according to our results. Hatchery fish had higher levels of Sr (65%) than non-hatchery fish (35%) $\chi2(1, N = 246) = 47.86$, $p < 0.0001$, 95% CI [852.15, 1551.32] (Figure 21). Ba showed hatchery fish had higher levels (63%) than non-hatchery fish (37%) $\chi2(1, N = 246) = 31.73$, $p < 0.0001$, 95% CI [21.99, 67.67] (Figure 22). Finally, analysis indicated that non-hatchery fish had more Pb (56%) than hatchery fish (43%) $\chi2(1, N = 246) = 4.82$, $p < 0.0281$, 95% CI [-0.05, -0.0061] (Figure 23).

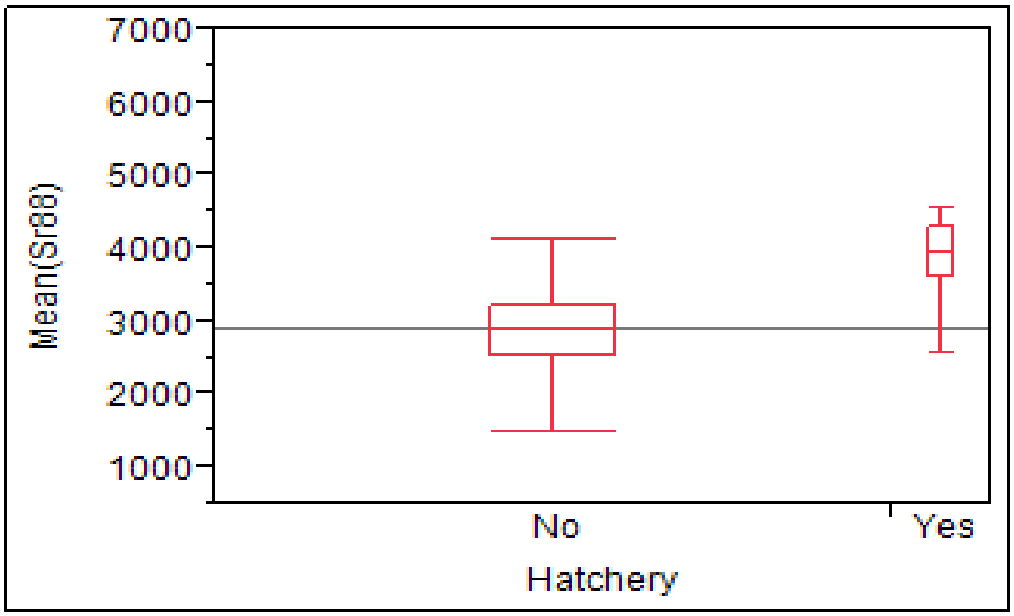

Figure 21. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar) of mean values of Sr concentrations in otoliths (n=246) comparing non-hatchery (n=215) to hatchery (n=31) fish collected from A/R stock from 2009 to 2010.

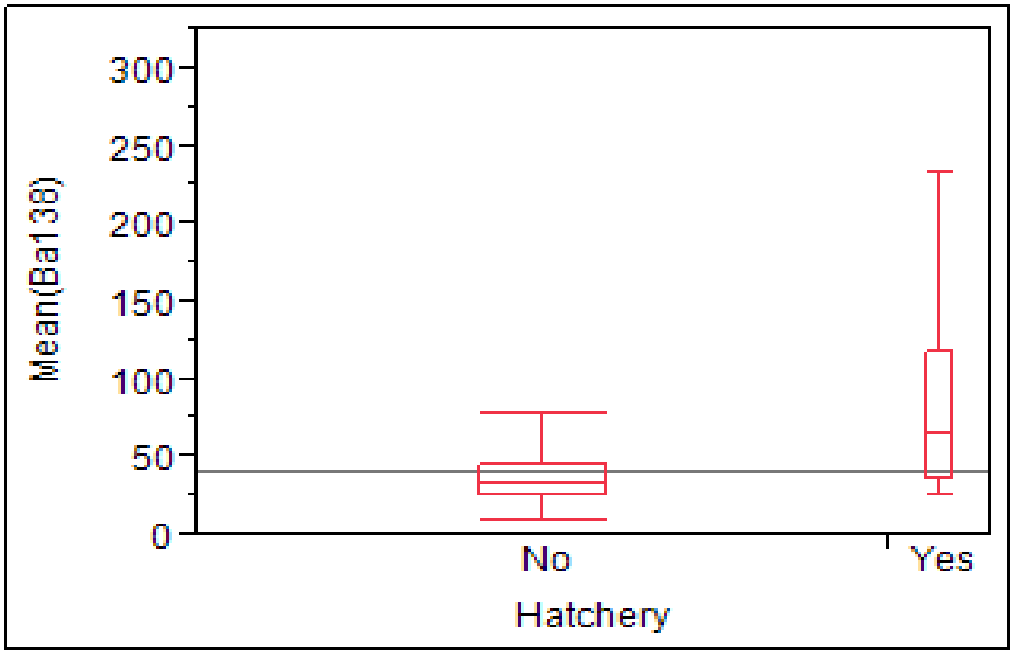

Figure 22. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar) of mean values of Ba concentrations in otoliths (n=246) comparing non-hatchery (n=215) to hatchery (n=31) fish collected from A/R stock from 2009 to 2010.

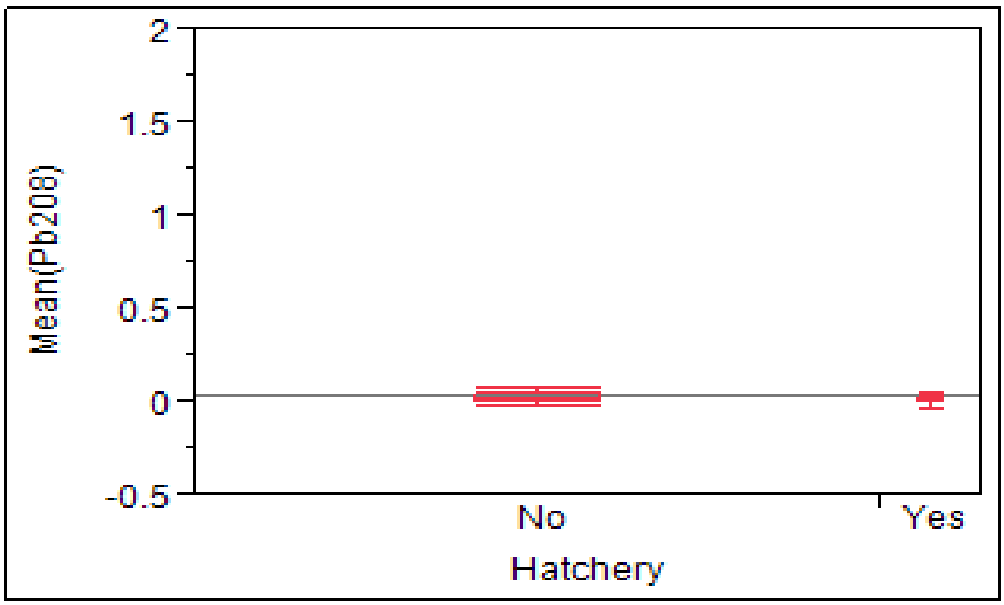

Figure 23. Box plots showing the median (horizontal line within box), 25th percentile (lower edge of box), 10th percentile (lower t-bar) and 90th percentile (upper t-bar) of mean values of Pb concentrations in otoliths (n=246) comparing non-hatchery (n=215) to hatchery (n=31) fish collected from A/R stock from 2009 to 2010.

6. Analyze data to determine fecundity, GSI, and age to maturity, and develop a maturity schedule for the stock.

Ovarian tissue samples were prepared and affixed to slides for histological examination. Fish were considered sexually mature in the developing through regenerating stages, with mature female striped bass found as young as age-3 (28.6%); 96.8% were mature at age 4, and were 100% mature by age-5 (Table 8). To determine the portion of these fish that were thought to be spawning or about to spawn, the total number of fish in the "spawning capable maturity phase and greater" was calculated: only 16.1% of age-3 fish were spawning imminently, 83.2% of age-4, and 95.6% of age-5 with all fish spawning capable by age-6 (Table 8).

We determined that striped bass were total spawners with determinate fecundity. Of the 453 female striped bass sampled, 186 were found to be immature, regenerating, regressing, or the sample was unusable. From the 267 individuals that could be used for fecundity analysis, 97 fish were subsampled for fecundity across age classes and between sampling areas. Subsamples that had a CV$\geq$10%, no age assigned, or missing other biological parameters were removed before analyses (n=17). Potential fecundity was estimated gravimetrically from the subsample (n=80) with fish ranging in age from 3 through 16 years (Table 1). Fecundity increased approximately 50,000 to 100,000 eggs per year for fish $\leq$6 years old and 150,000 to 250,000 for fish >6 years old. The relationship between fecundity and age was approximately linear (r^2=0.86), but variable (Figure 24). Potential annual fecundity, estimated gravimetrically, ranged from 176,873 eggs for age-3 females (n=4) to 3,163,130 eggs for a single age-16 female.

The average number of eggs per gram of ovarian tissue decreased with age (Figure 25).

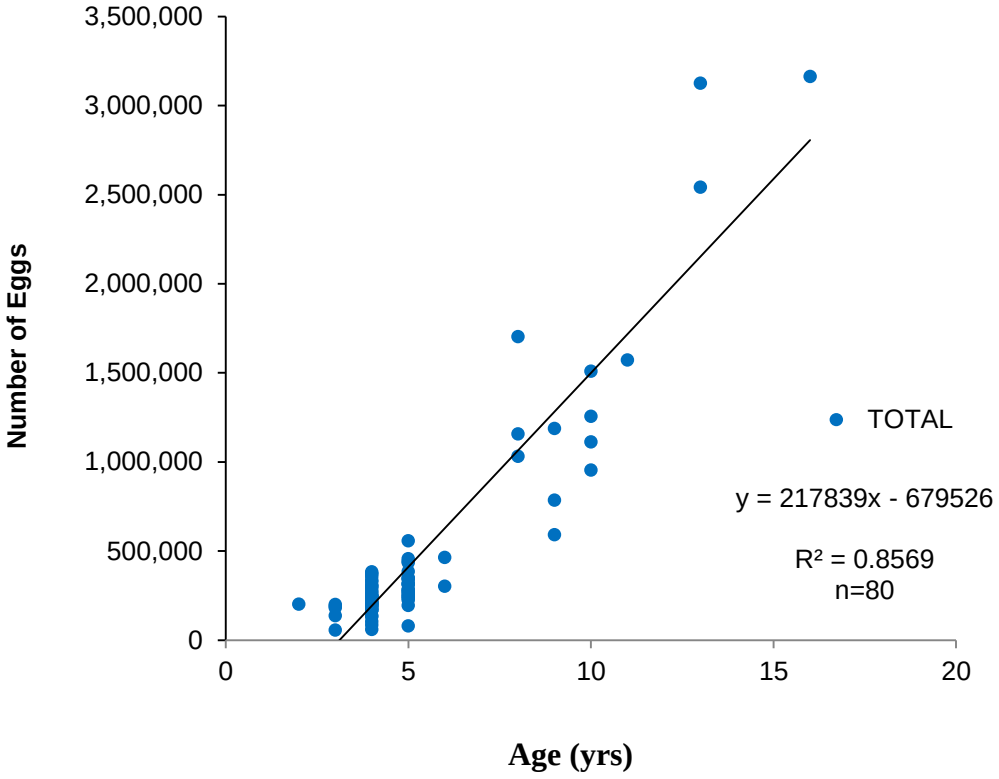

Figure 24. Total number of eggs by age counted gravimetrically from Albemarle/Roanoke stock striped bass (n=80) from 2009 and 2010.

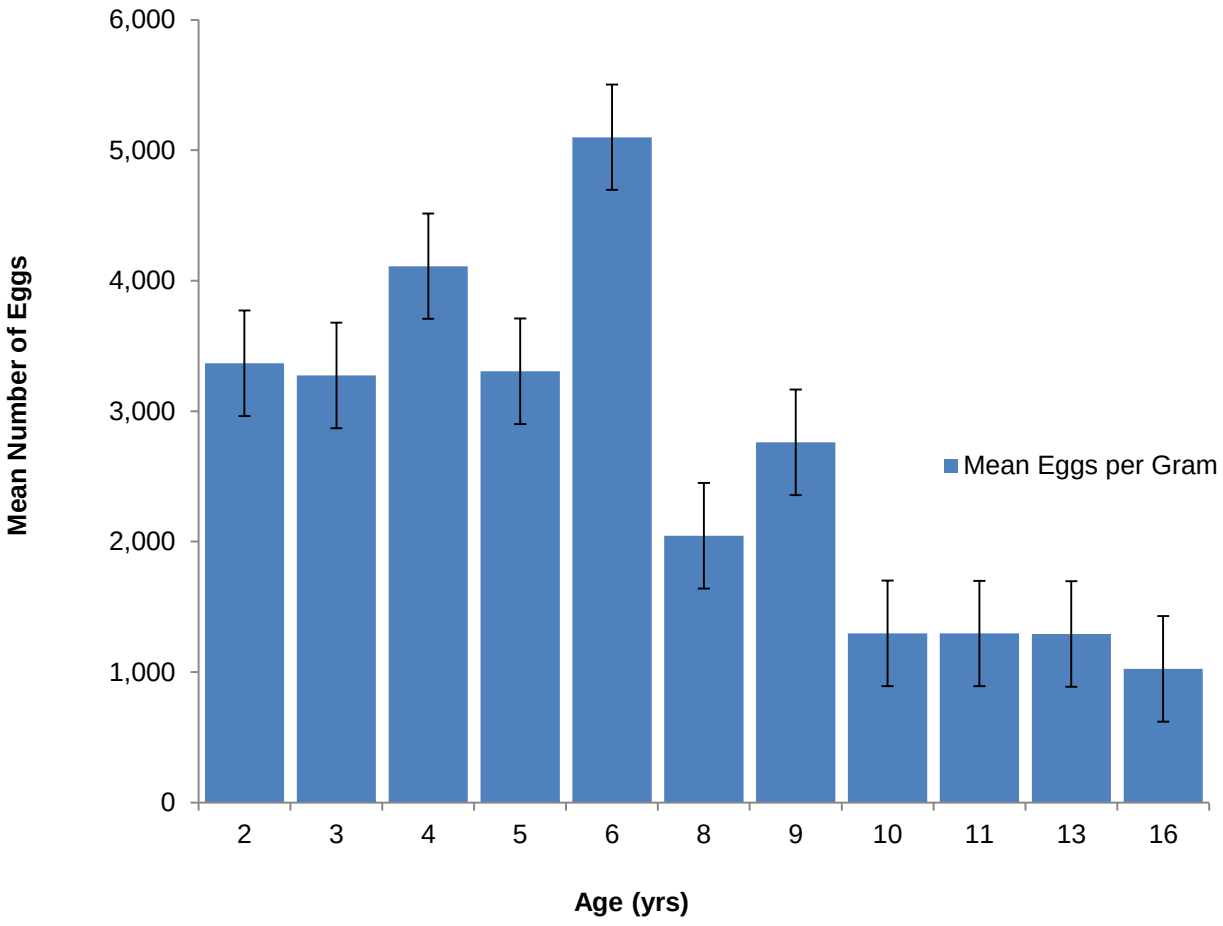

Figure 25. Mean eggs per gram of ovarian tissue counted gravimetrically for 80 Albemarle/Roanoke stock striped bass by age from 2009 and 2010.

Table 8. Percent age-at-maturity for female striped bass (n=405) from the Albemarle/Roanoke stock. Total mature equals all stages except immature (n=275); Total spawning equals all stages except immature and developing (n=248).

	Age														
Maturity Stage	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Immature	98.8	73.2	3.2												
Developing		12.5	13.6	4.5											
Spawning Capable		10.7	65.6	68.7	35.7	40.0	33.3	16.7	10.0						
Actively Spawning		3.6	8.0	17.9	50.0	20.0	66.7	50.0	70.0	66.7		100.0	100.0		100.0
Regressing			8.8	9.0	14.3	40.0		22.2	20.0	33.3	100.0			100.0	
Regenerating			0.8					11.1							
Total Sample	86	56	125	67	14	5	6	18	10	3	1	4	1	1	1
Total Mature	0	28.6	96.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Total Spawning	0	16.1	83.2	95.6	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

7. Compare results with previous maturity and fecundity estimates (e.g., Olsen and Rulifson 1992).

The percentage of oocytes showing signs of secondary growth (developing) and meeting the minimum oocyte diameter criteria (10-40%) used by Olsen and Rulifson (1992) was calculated for 29 fish (Table 9). Each methodology used by Olsen and Rulifson (1992) was followed and the percent of oocytes mature by age were compared (Table 10). Olsen and Rulifson (1992) found that assigning maturity using a 20% cutoff of minimum oocyte diameters was accurate for striped bass, and that age-3 fish were 44% mature. Using the same criterion, the fish examined in this study were only 17.9% mature at age-3, and age-4 fish were 96.8% mature compared to 92.9% found by Olsen and Rulifson (1992) (Table 10). Both studies had 100% maturity by age-6 and when comparing methods, all the developing fish (n=29) were considered mature while Olsen and Rulifson (1992) would have only considered 24 fish (82.7%) mature using their criterion (Table 10). Fecundity estimated by Olsen and Rulifson (1992) was higher by age, TL, observed FL, and estimated TL compared to this study (Table 11).

Table 9. Maturation criteria and percent mature by age comparison of Olsen and Rulifson (1992) using percent of minimum, mean oocyte diameter compared to current study using secondary growth characteristics. Current percentages based only on fish determined to be developing.

	Maturation Criteria								
	10%		20%		30%		40%		Secondary Growth
Age	Olsen	Current	Olsen	Current	Olsen	Current	Olsen	Current	Current
3	48.0	19.3	44.0	17.9	37.0	9.0	22.0	9.0	28.6
4	94.0	96.8	92.9	96.8	92.0	96.8	67.0	96.8	96.8
5	95.0	100.0	95.0	100.0	92.0	100.0	71.0	100.0	100.0
6	100.0	100.0	100.0	100.0	100	100.0	76.0	100.0	100.0

Table 10. Percent of mature oocytes per Albemarle/Roanoke stock fish (n=29) determined to be developing using oocyte diameter criterion from Merriman (1941), Lewis (1962), Specker et al. (1987) and Olsen and Rulifson (1992) for striped bass.

		Oocyte diameter criterion			
		Merriman (1941)	Lewis (1962)	Specker et al. (1987)	Olsen and Rulifson (1992)
Age	Measured	0.15 mm (%)	0.16 mm (%)	0.216 mm (%)	0.175 mm (%)
3	86	47	26	5	13
3	111	11	8	3	5
3	110	78	66	33	56
3	54	87	66	22	48
3	102	25	11	0	5
3	78	45	17	0	5
3	101	63	44	0	13
4	97	96	92	10	89
4	89	71	57	18	40
4	82	100	95	56	84
4	52	98	98	94	98
4	51	92	88	6	63
4	53	98	98	68	92
4	72	83	68	22	49
4	105	100	100	96	99
4	51	100	100	100	100
4	76	97	92	46	83
4	78	96	95	62	83
4	88	100	100	98	100
4	50	100	100	86	100
4	81	100	100	89	100
4	94	100	100	94	100
4	95	100	100	95	100
4	139	83	74	16	60
5	52	100	100	98	100
5	50	100	100	88	100
5	50	100	100	96	100
10	105	100	100	100	100
14	29	100	100	100	100

Table 11. Comparison of fecundity of Albemarle/Roanoke stock estimated gravimetrically (n=80) and using linear regressions estimating fecundity by age (yrs), weight (g), total length (TL mm), fork length (FL mm), and TL from FL regression (Olsen and Rulifson 1992) from current study and from Olsen and Rulifson (1992).

		Current				Olsen and Rulifson (1992)				
Age	N	Gravimetric fecundity	Fecundity estimated by age	Fecundity estimated by weight	Fecundity estimated by TL from FL regression (Olsen and Rulifson 1992)	N	Gravimetric fecundity	Fecundity estimated by age	Fecundity estimated by weight	Fecundity estimated by FL
3	4	176,873	52,705	205,606	176,873	13	180,929	195,588	245,212	211,007
4	38	231,122	216,292	262,849	241,134	15	413,440	364,532	412,984	416,920
5	22	310,942	379,880	368,166	296,417	15	509,817	533,477	494,768	511,302
6	2	381,998	543,468	364,553	381,998	10	693,130	702,422	612,693	620,232
7	0					3	1,184,410	871,366	901,087	931,840
8	3	1,296,463	870,643	1,055,734	1,296,463	3	1,018,675	1,040,311	1,164,059	1,138,416
9	3	854,930	1,034,231	639,701	854,930	0				
10	4	1,207,771	1,197,818	1,275,792	1,104,918	1	2,158,868	1,378,200	1,830,527	1,464,718
11	1	1,570,737	1,391,996	1,370,618	1,570,737	0				
12	0					0				
13	2	2,833,090	2,423,346	2,914,937	2,833,090	0				
14	0					0				
15	0					0				
16	1	3,163,130	3,556,701	3,068,466	3,163,130	1	4,938,991	2,391,869	4,424,102	2,778,514

8. Prepare report of publishable quality for submission to fishery management agencies.

The final report is complete.

Deviations

All planned activities have been completed to date. Otoliths were cut using a Hillquist high speed saw rather than the Buehler Isomet low speed saw as specified in the grant. Digital images were used for fecundity estimates instead of a dissecting microscope because the samples can be completed more efficiently and images can be saved for future observations. The digital images were manually counted using Image Pro Plus 5.1 software.

Additional Guidance:

Progress

- All samples have been collected and categorized.
- Ovarian tissue samples were prepared and maturity analysis was completed.
- Subsamples of ovarian tissue were extracted from each ovary and fecundity analysis was completed.
- Otoliths were sent for microchemical analysis and data analysis was completed to determine maternal input, migration patterns, and wild versus hatchery fish.
- Otoliths and scales have been aged.
- Condition factors (GSI, LSI, and K) were calculated.
- All data was compiled and sorted using SAS 9.2, JMP Pro 10 statistical software and Microsoft Excel.

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APPENDIX A: ELEMENTAL PROFILES OF NON-HATCHERY VERSUS HATCHERY FISH

See attachment.

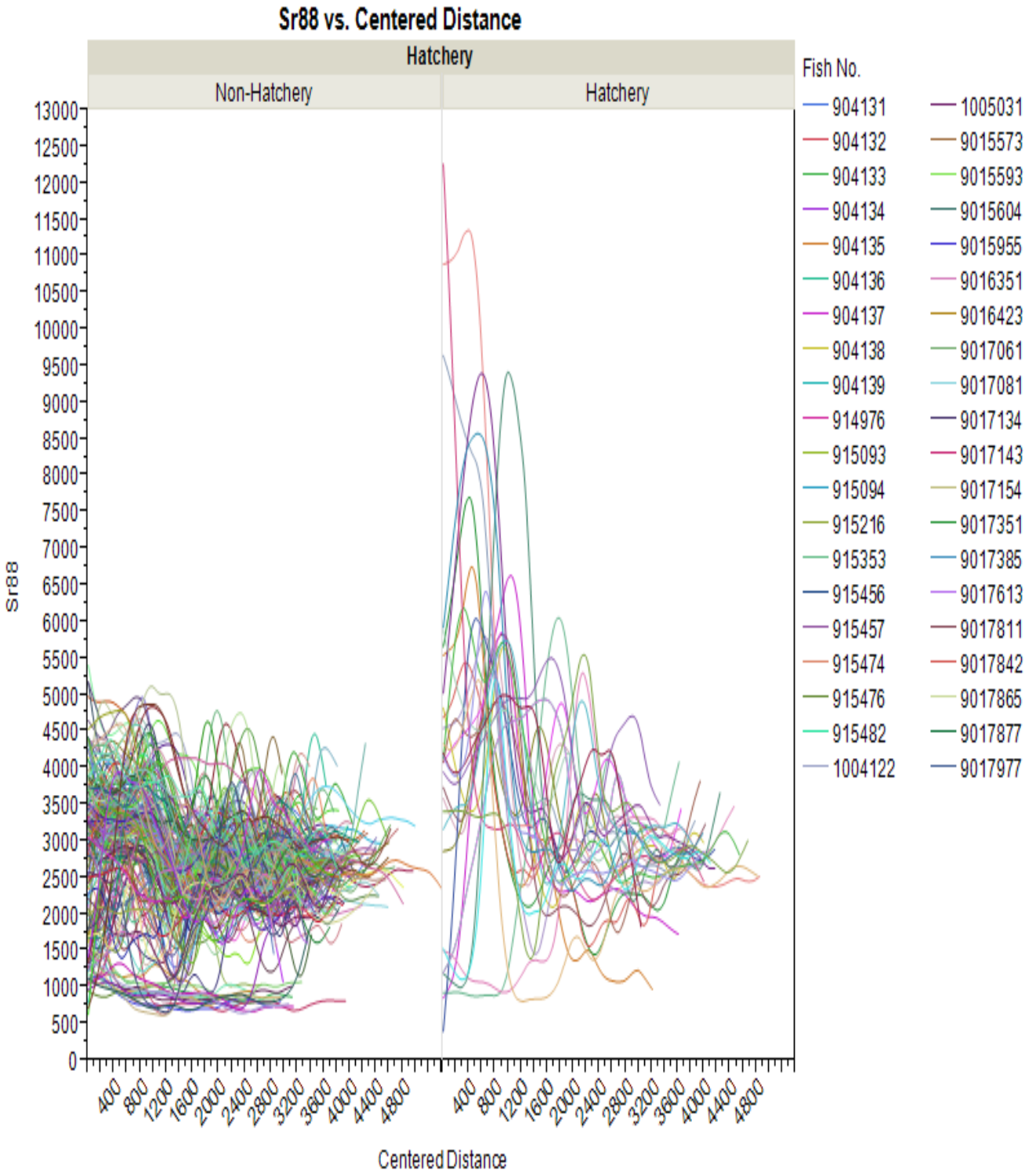

Figure 26. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) comparing non-hatchery (n=215) to hatchery (n=31) fish collected from A/R stock from 2009 to 2010.

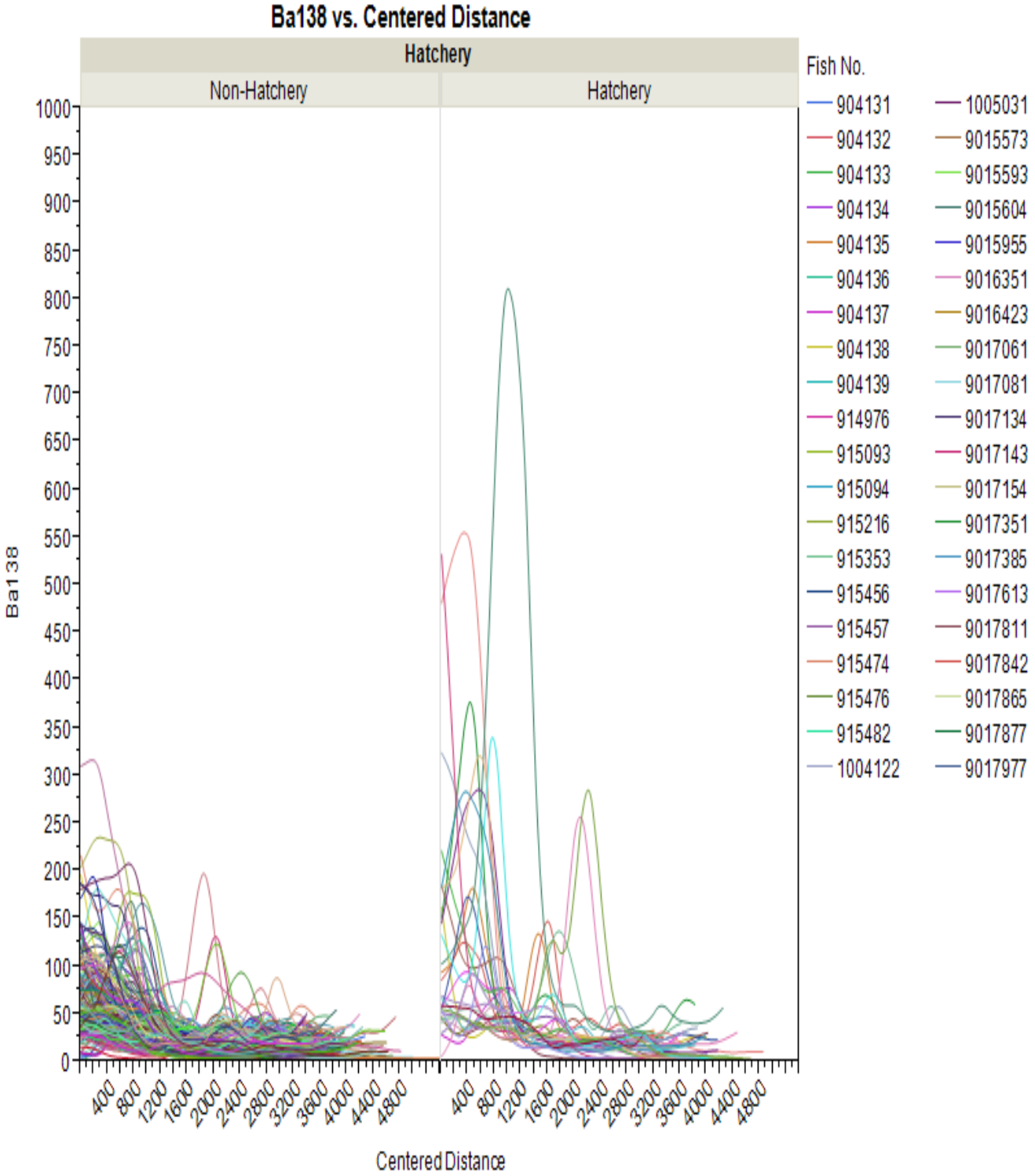

Figure 27. Reported values of Ba with distance across the otolith (X axis) and Ba values (ppm) (Y axis) in otoliths (n=246) comparing non-hatchery (n=215) to hatchery (n=31) fish collected from A/R stock from 2009 to 2010.

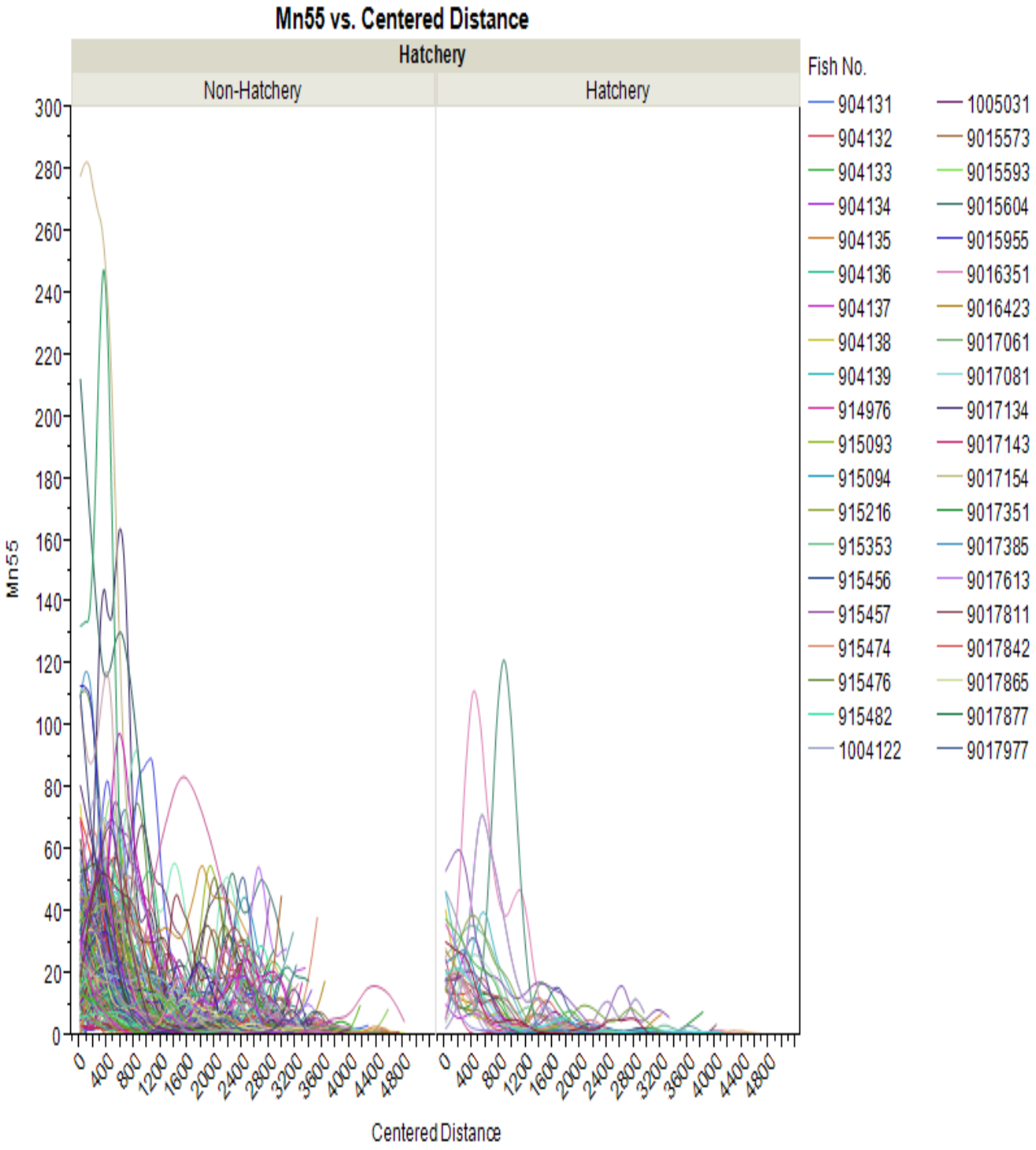

Figure 28. Reported values of Mn with distance across the otolith (X axis) and Mn values (ppm) (Y axis) in otoliths (n=246) comparing non-hatchery (n=215) to hatchery (n=31) fish collected from A/R stock from 2009 to 2010.

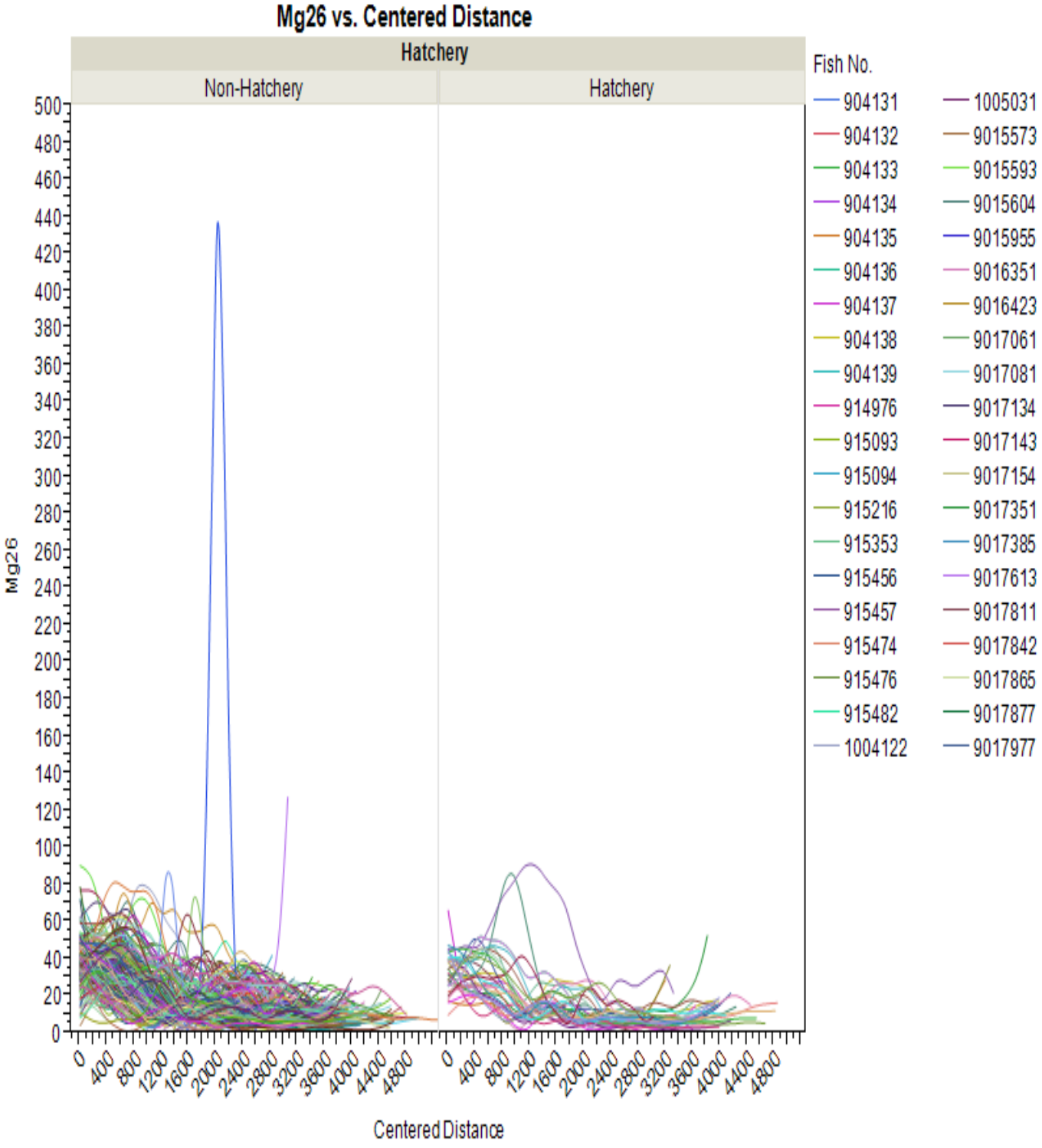

Figure 29. Reported values of Mg with distance across the otolith (X axis) and Mg values (ppm) (Y axis) in otoliths (n=246) comparing non-hatchery (n=215) to hatchery (n=31) fish collected from A/R stock from 2009 to 2010.

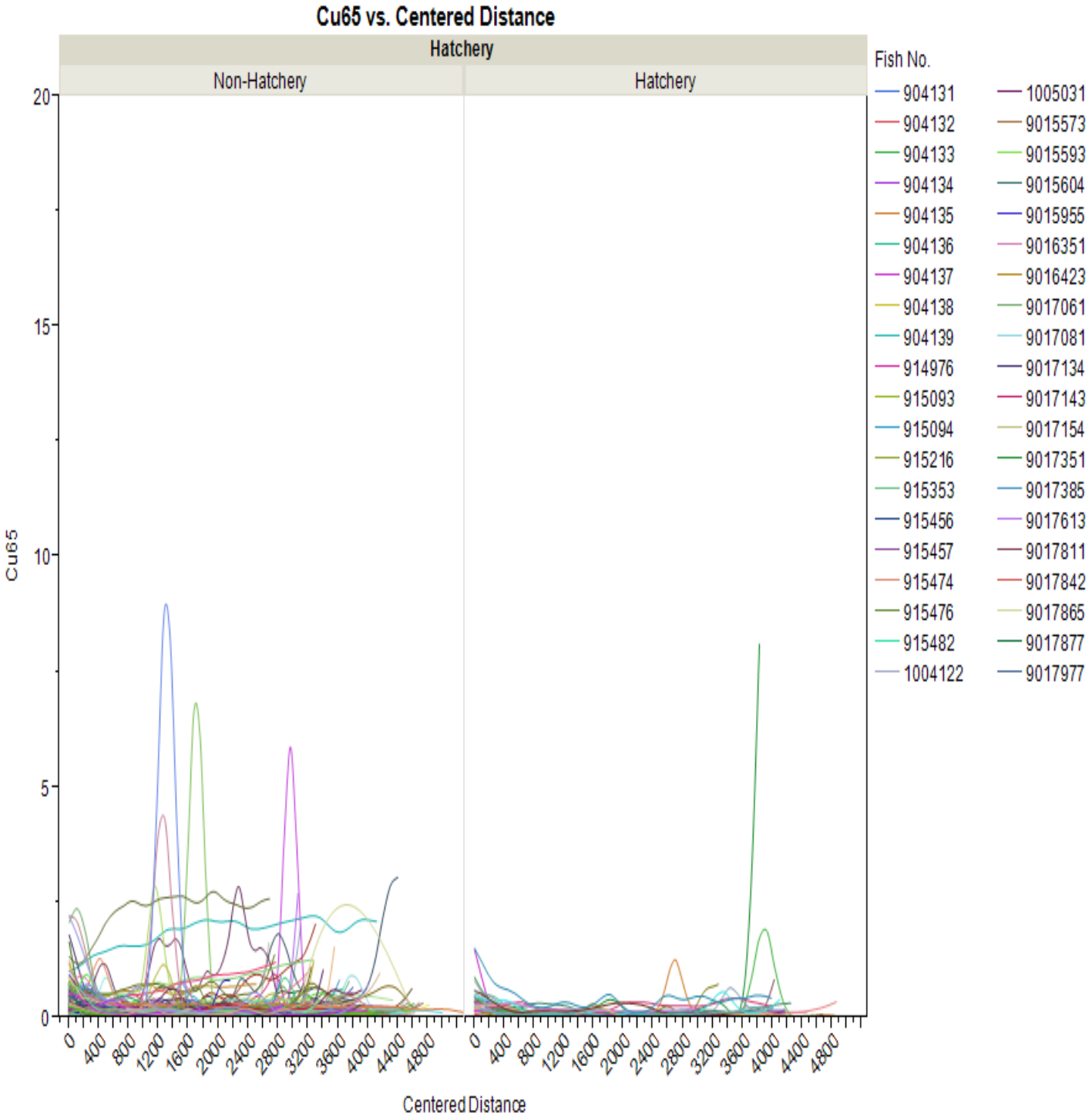

Figure 30. Reported values of Cu with distance across the otolith (X axis) and Cu values (ppm) (Y axis) in otoliths (n=246) comparing non-hatchery (n=215) to hatchery (n=31) fish collected from A/R stock from 2009 to 2010.

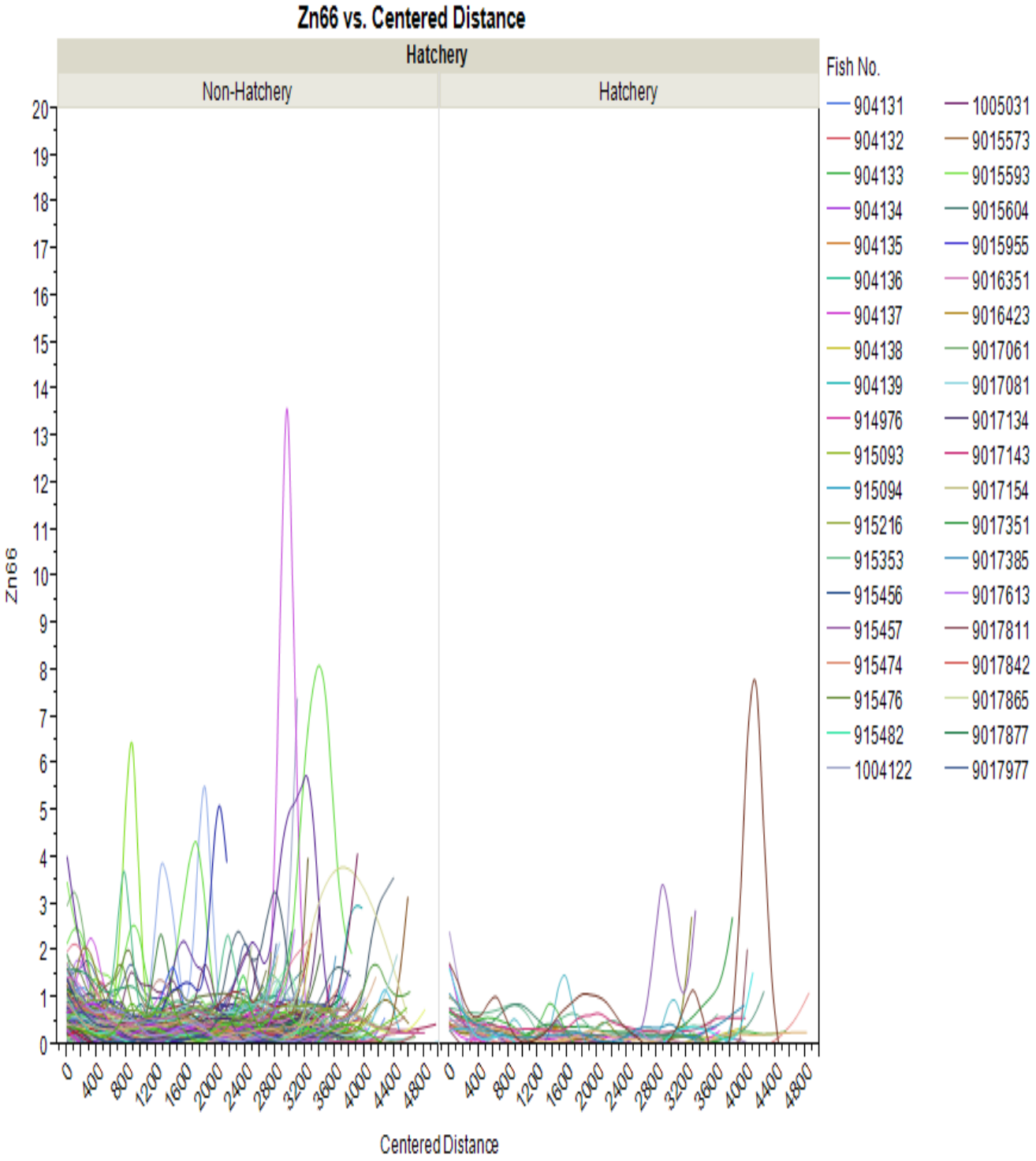

Figure 31. Reported values of Zn with distance across the otolith (X axis) and Zn values (ppm) (Y axis) in otoliths (n=246) comparing non-hatchery (n=215) to hatchery (n=31) fish collected from A/R stock from 2009 to 2010.

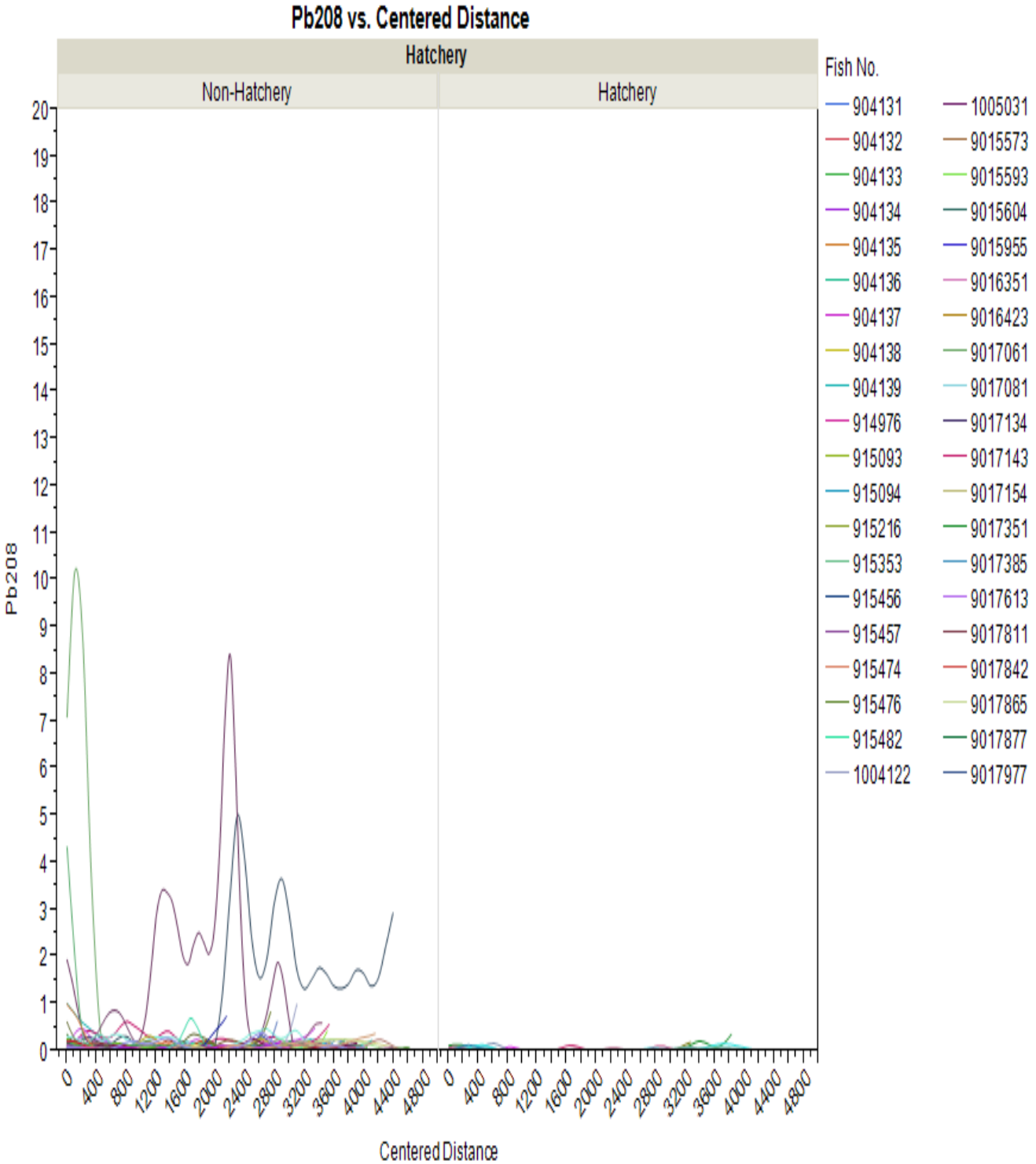

Figure 32. Reported values of Pb with distance across the otolith (X axis) and Pb values (ppm) (Y axis) in otoliths (n=246) comparing non-hatchery (n=215) to hatchery (n=31) fish collected from A/R stock from 2009 to 2010.

APPENDIX B: STRONTIUM PROFILES OF STRIPED BASS EXAMINED BY YEAR CLASS (2-16 YEARS OF AGE)

See attachment.

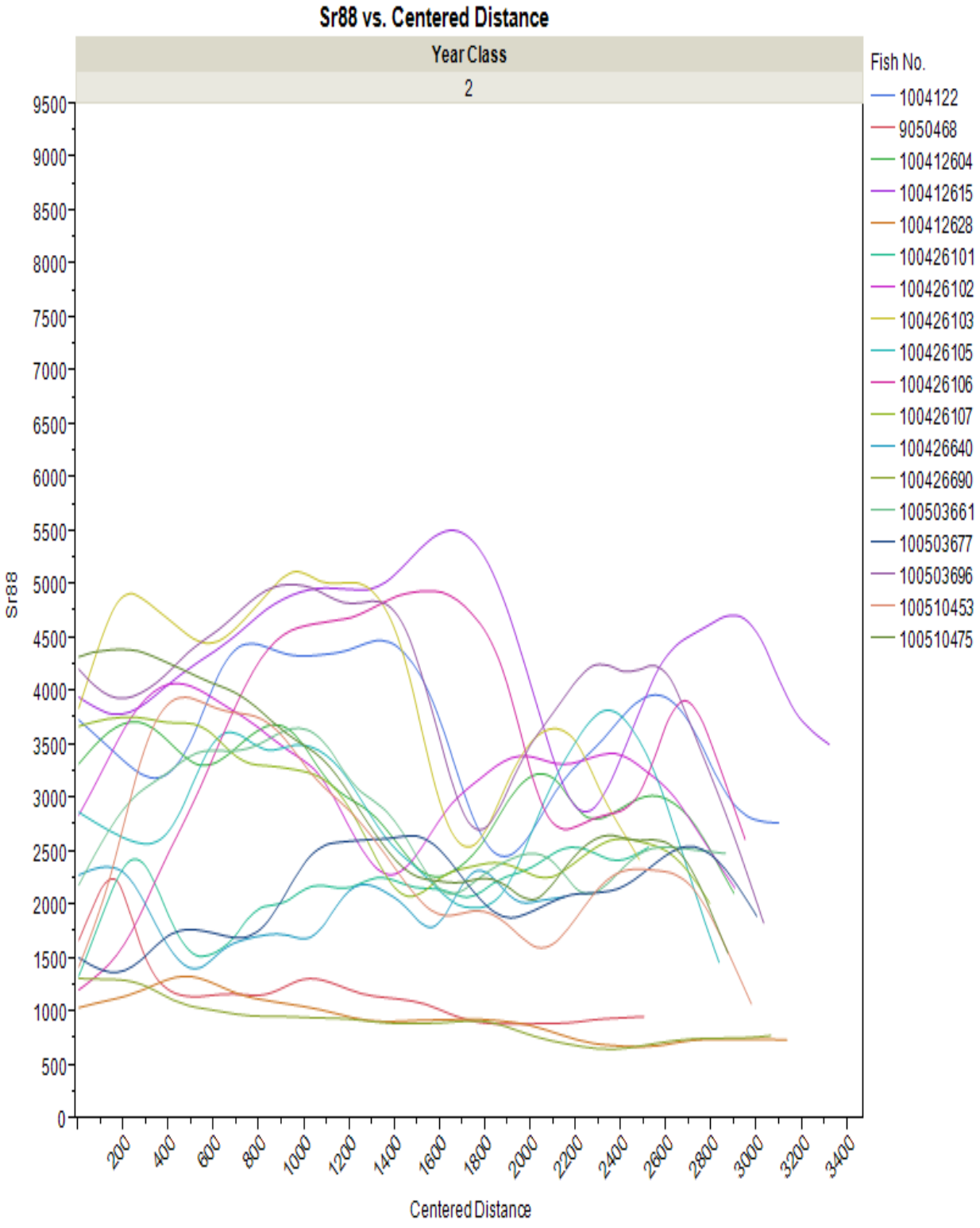

Figure 33. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (2 year olds) collected from A/R stock from 2009 to 2010.

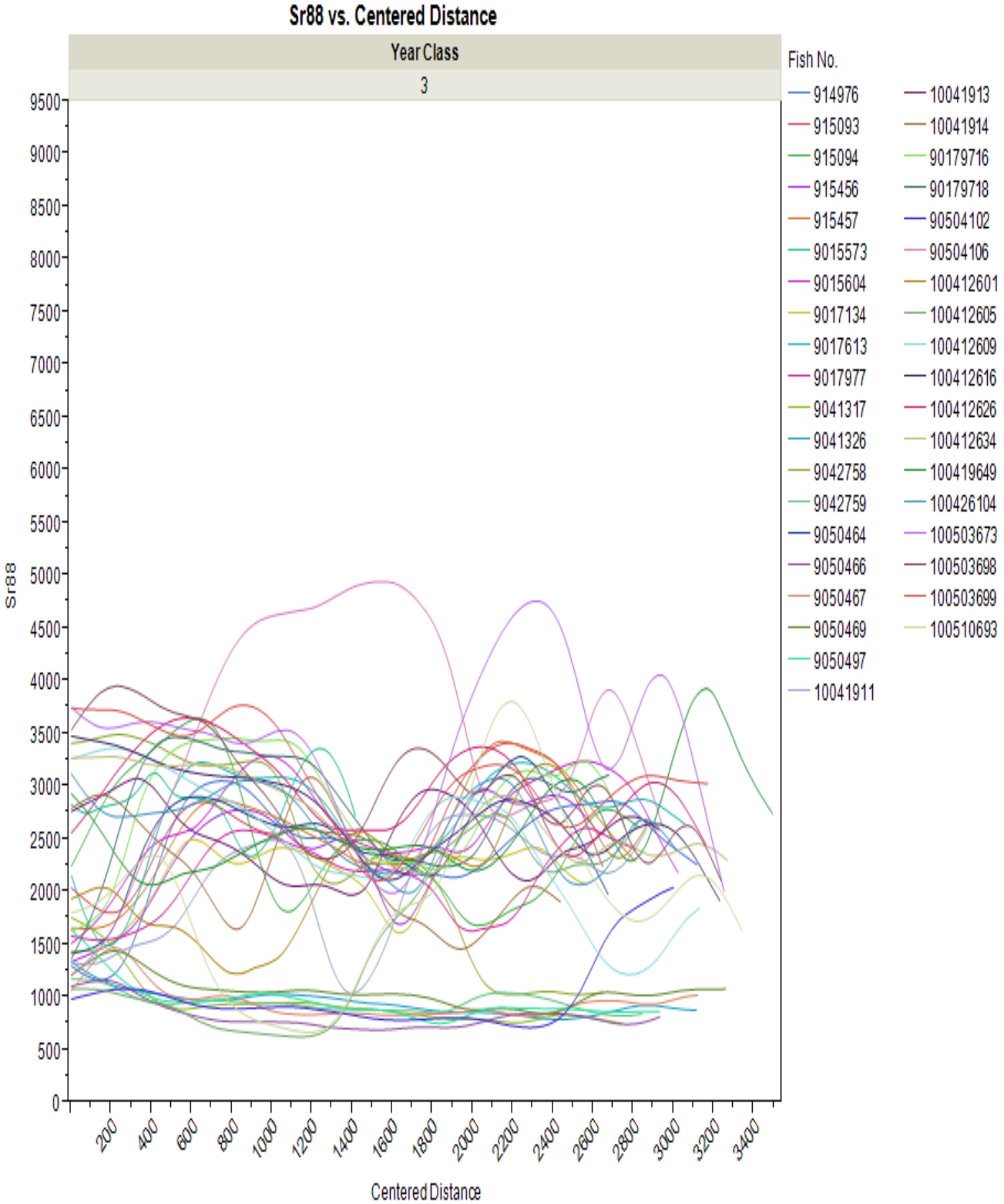

Figure 34. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (3 year olds) collected from A/R stock from 2009 to 2010.

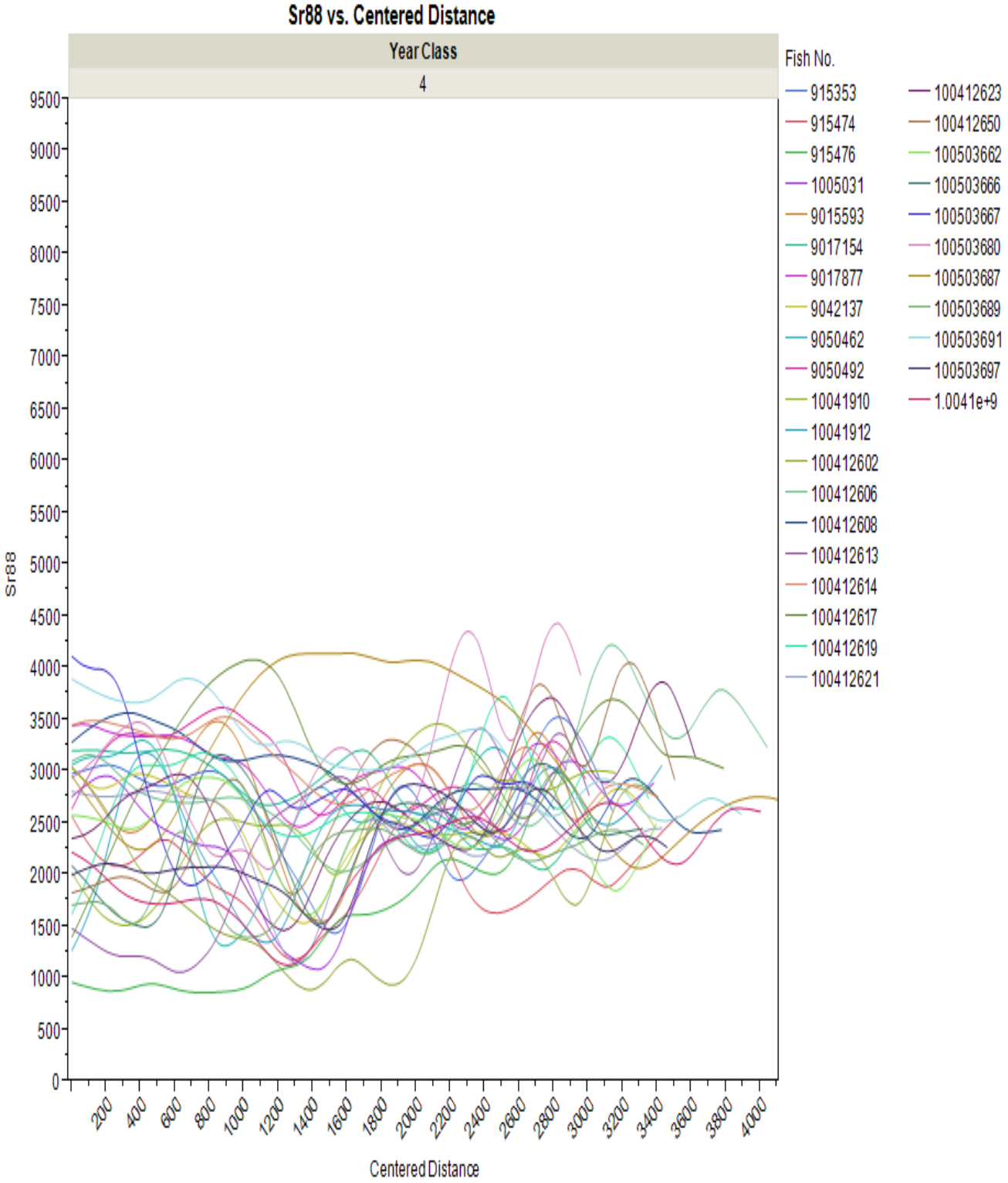

Figure 35. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (4 year olds) collected from A/R stock from 2009 to 2010.

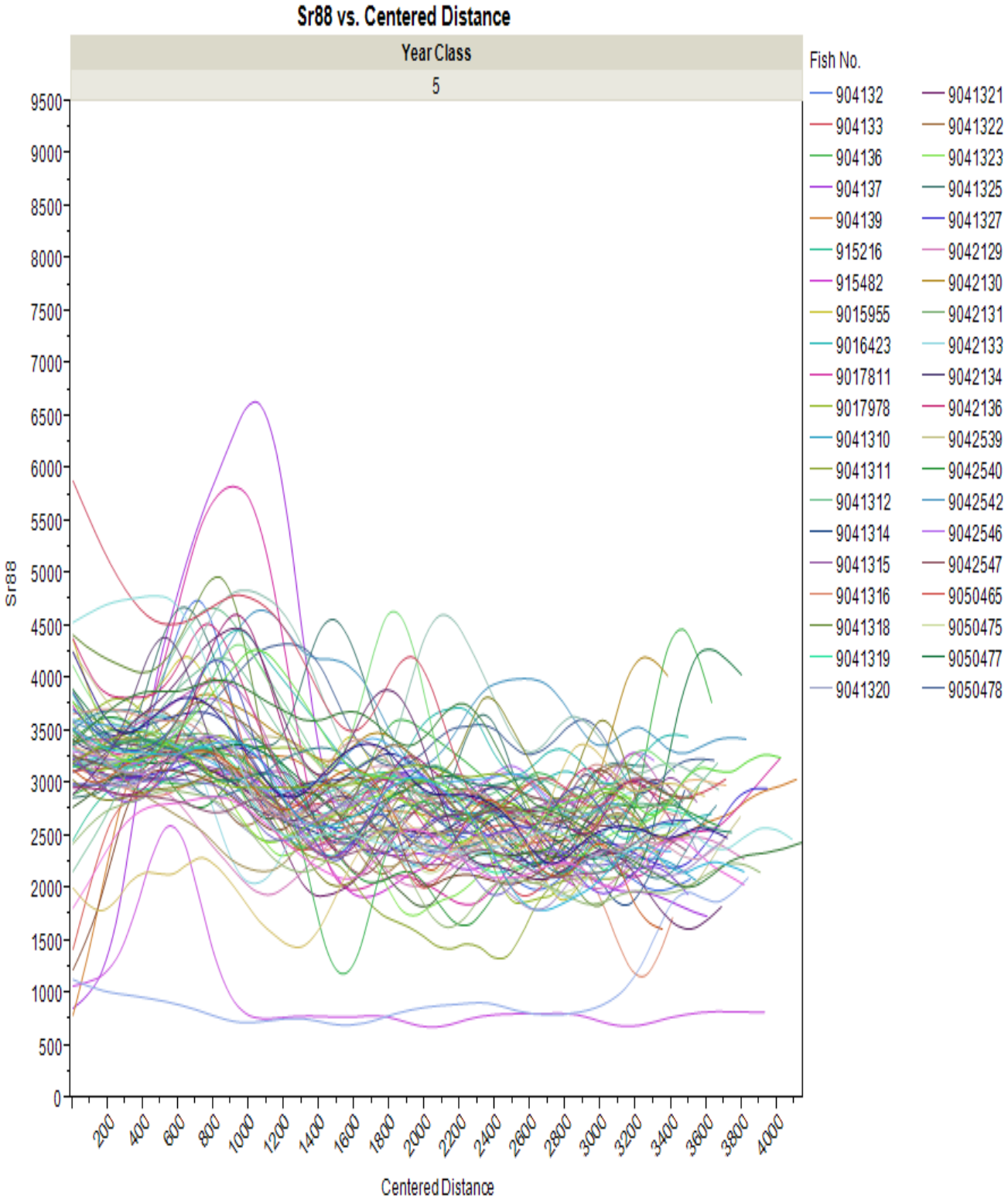

Figure 36. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (5 year olds) collected from A/R stock from 2009 to 2010.

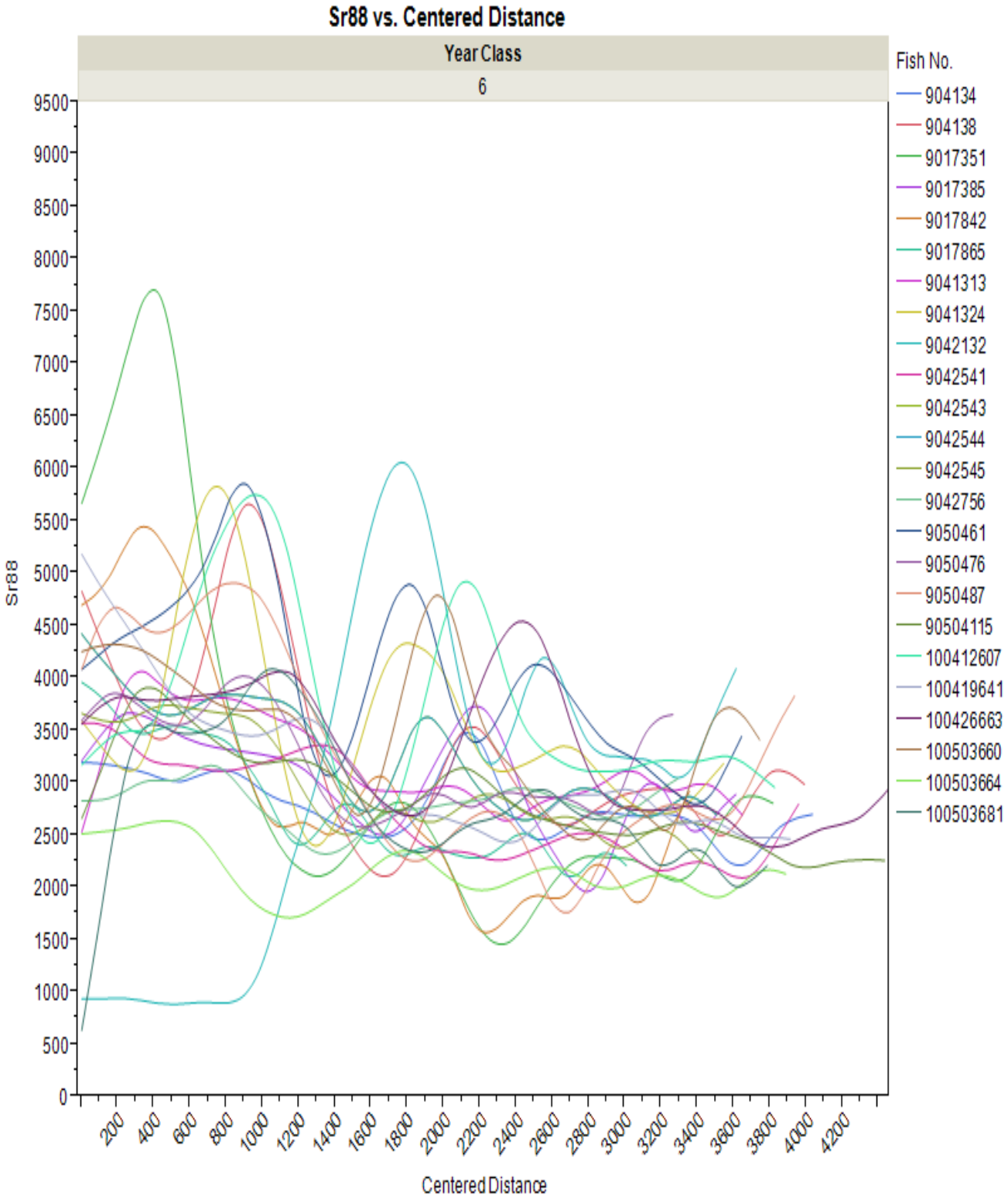

Figure 37. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (6 year olds) collected from A/R stock from 2009 to 2010.

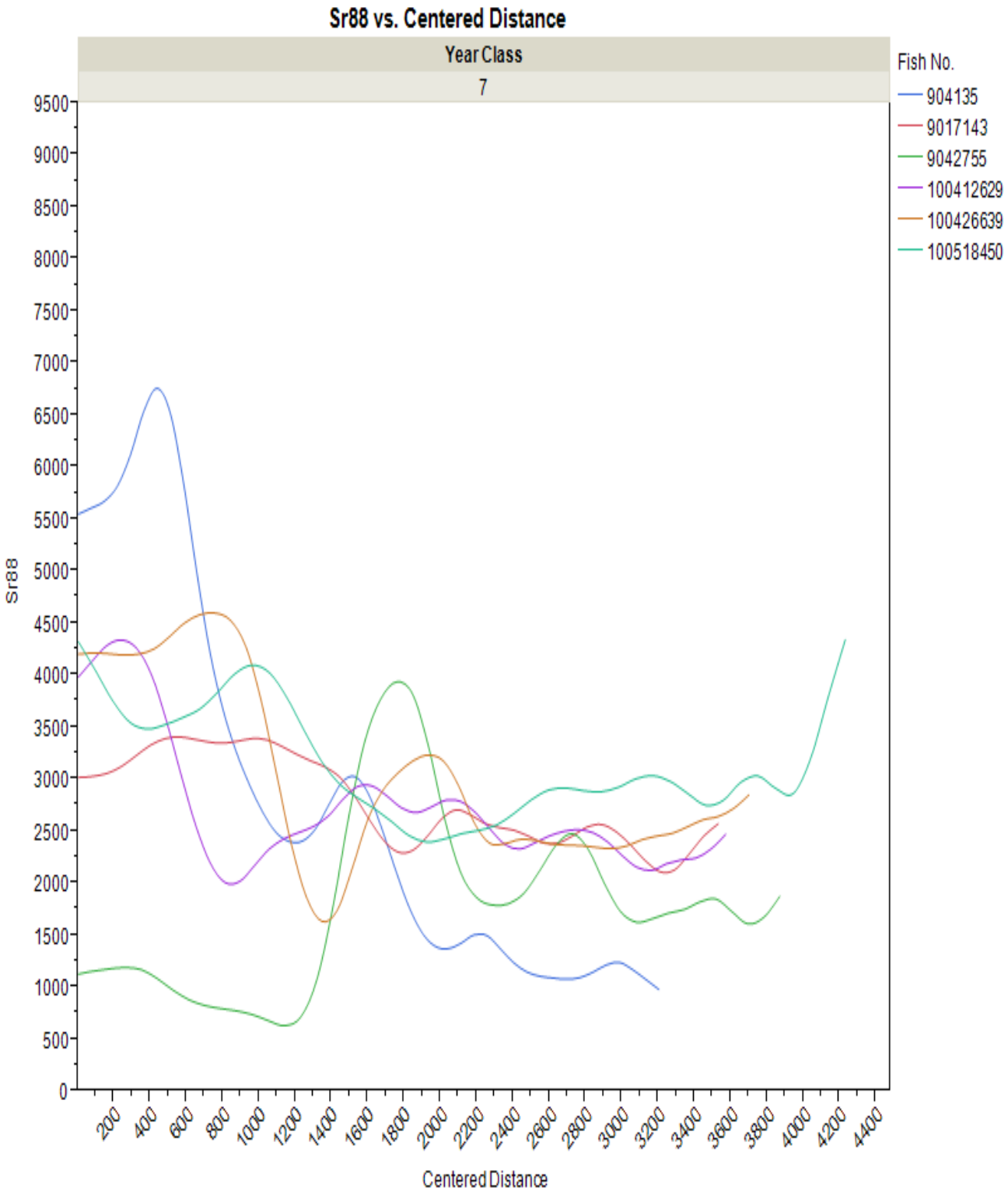

Figure 38. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (7 year olds) collected from A/R stock from 2009 to 2010.

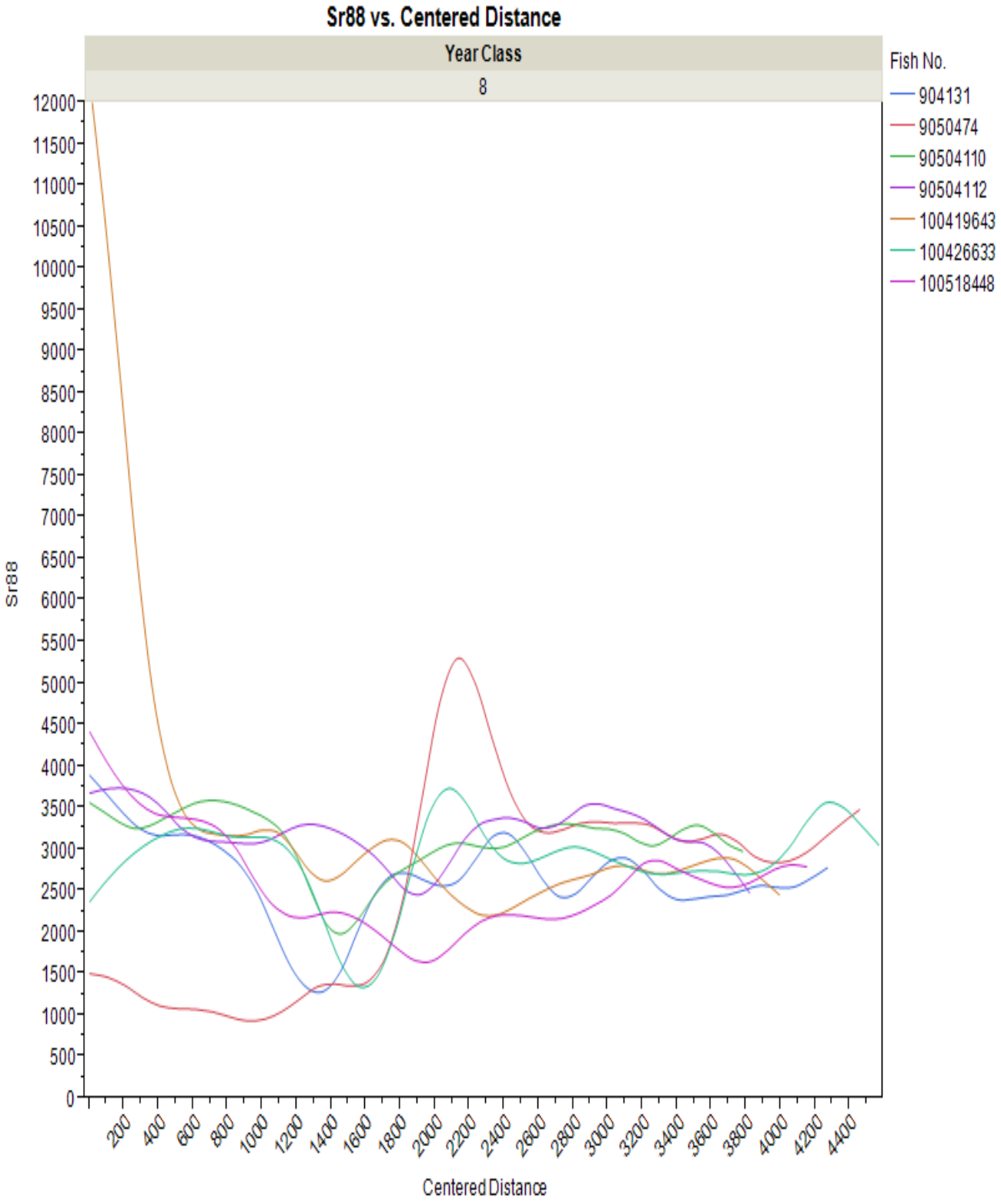

Figure 39. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (8 year olds) collected from A/R stock from 2009 to 2010.

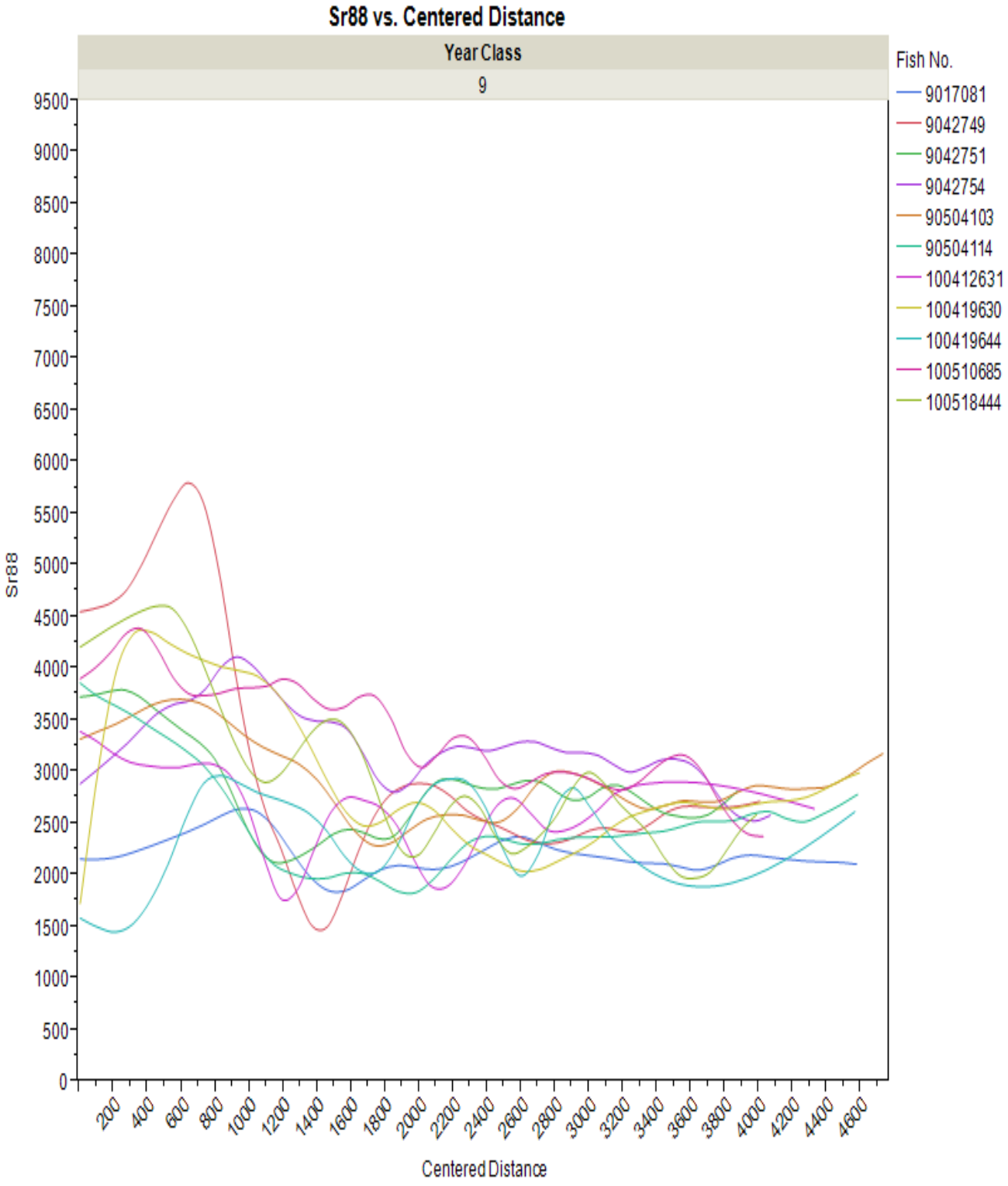

Figure 40. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (9 year olds) collected from A/R stock from 2009 to 2010.

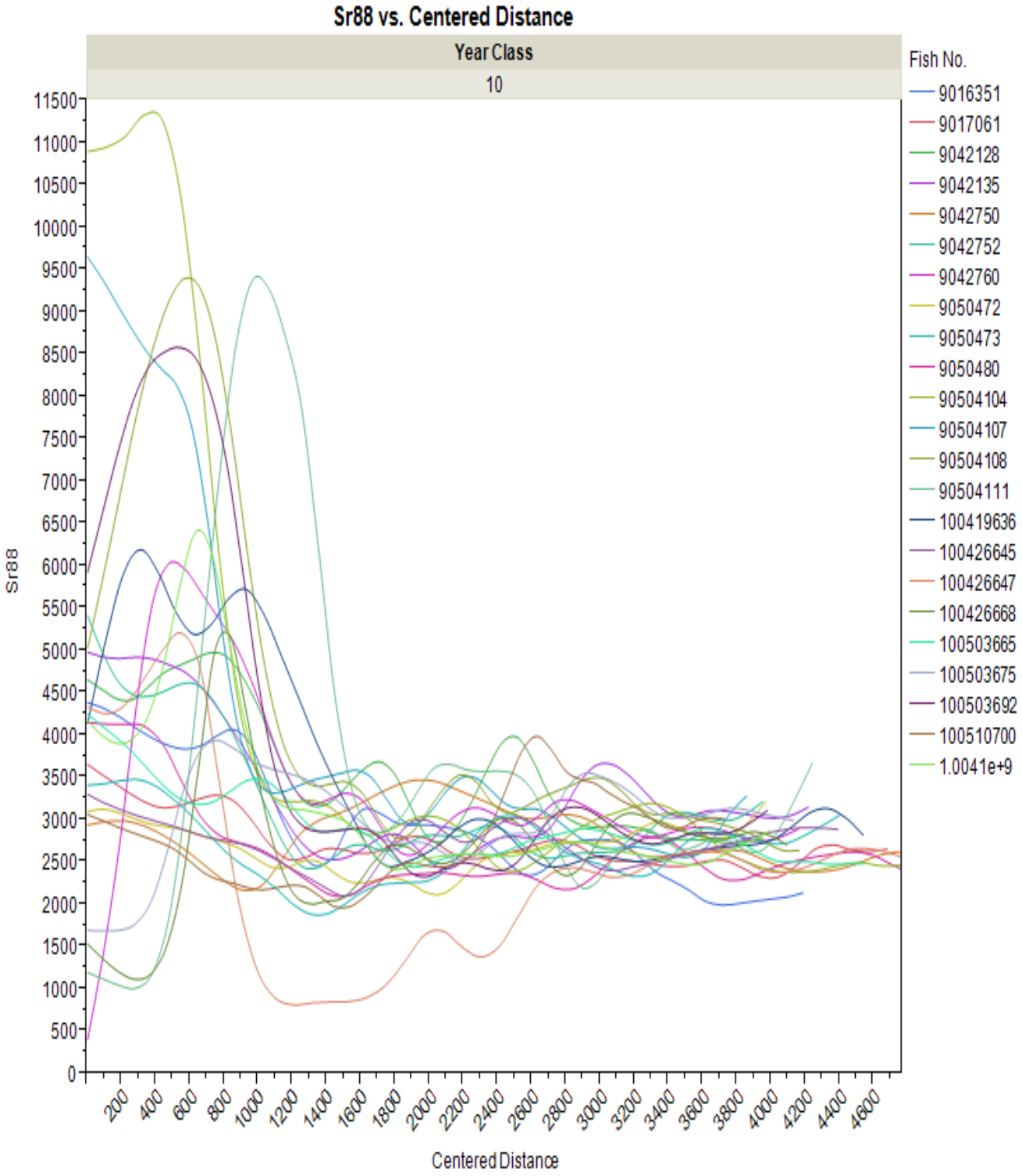

Figure 41. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (10 year olds) collected from A/R stock from 2009 to 2010.

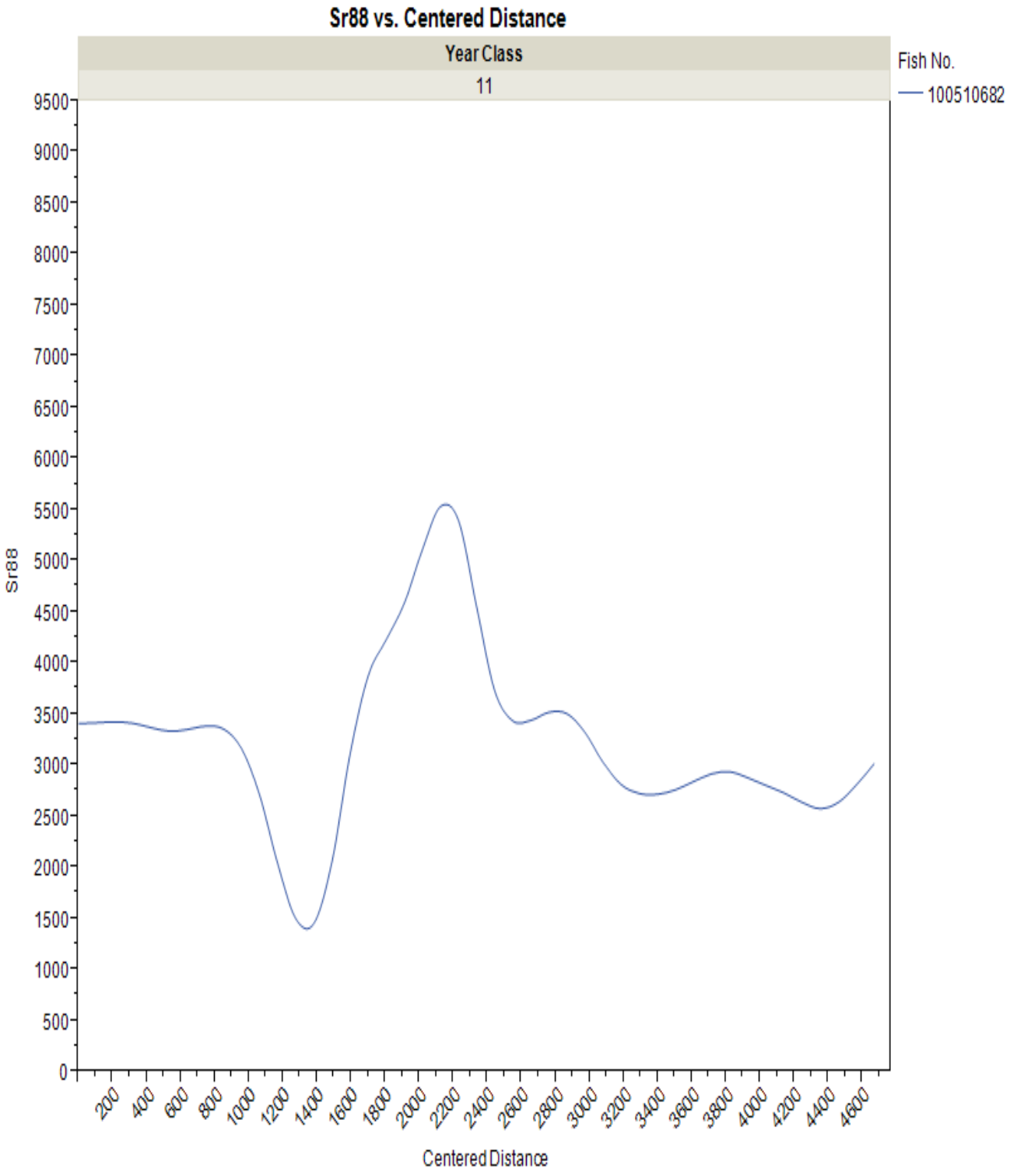

Figure 42. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (11 year old) collected from A/R stock from 2009 to 2010.

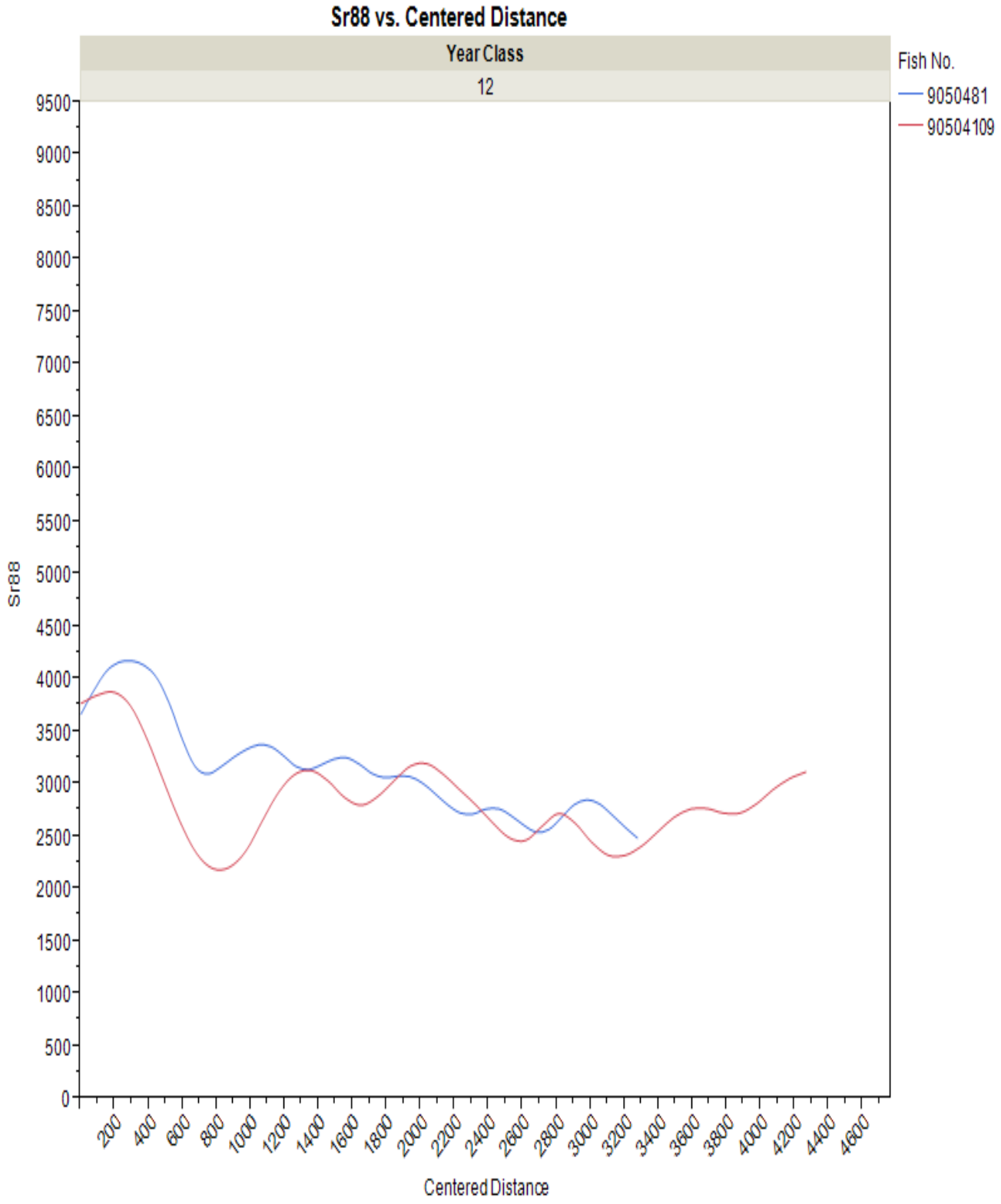

Figure 43. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (12 year olds) collected from A/R stock from 2009 to 2010.

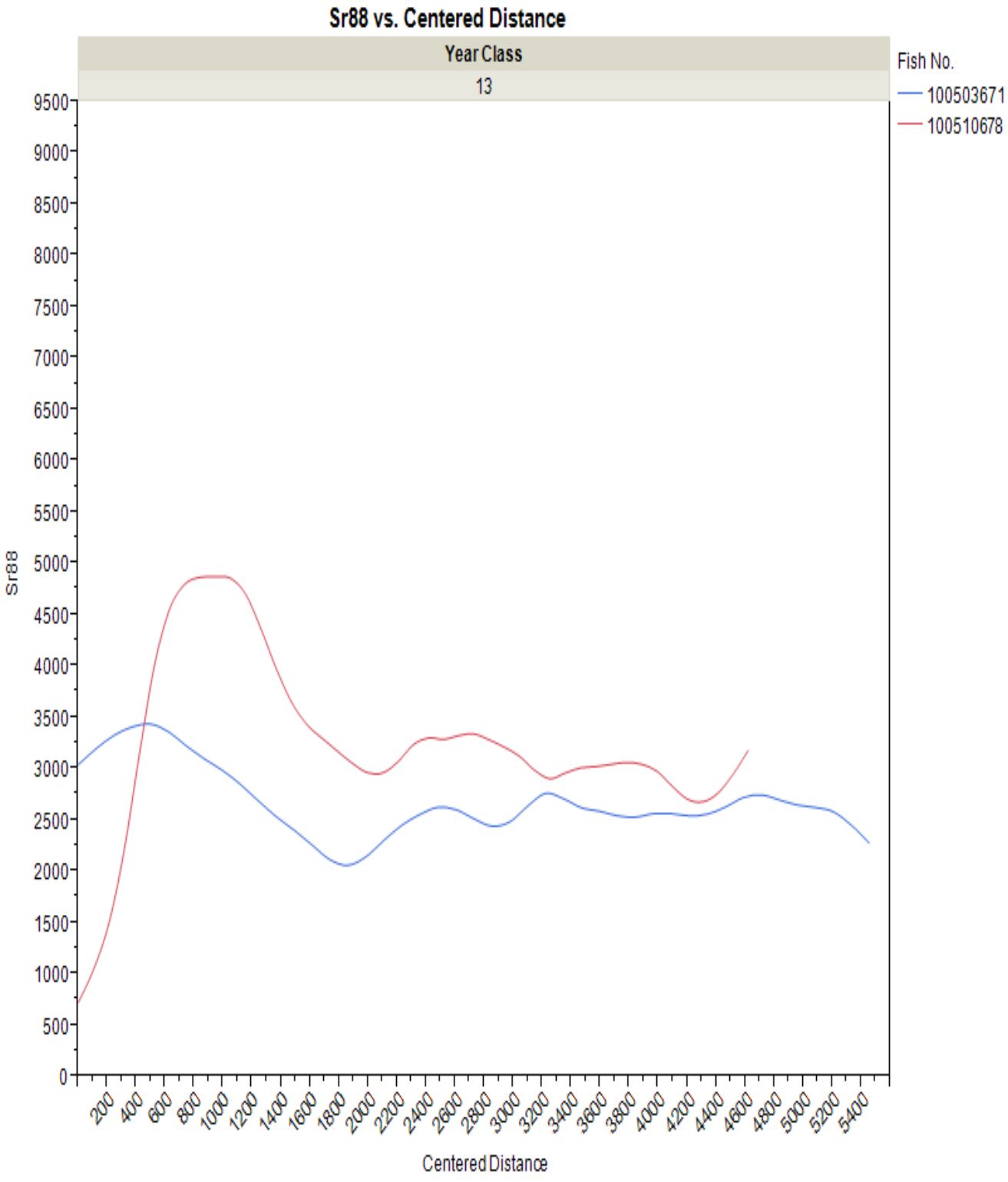

Figure 44. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (13 year olds) collected from A/R stock from 2009 to 2010.

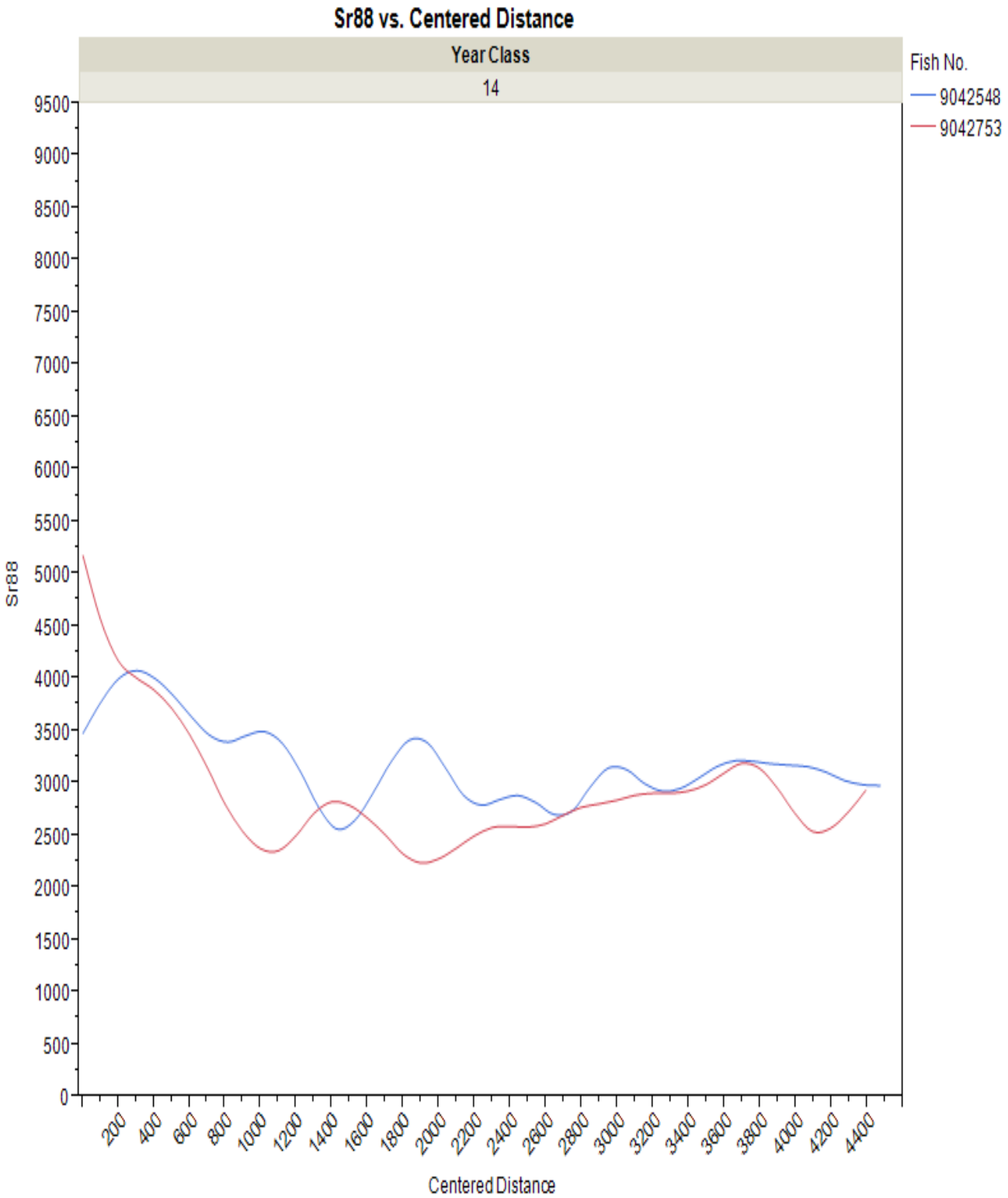

Figure 45. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (14 year olds) collected from A/R stock from 2009 to 2010.

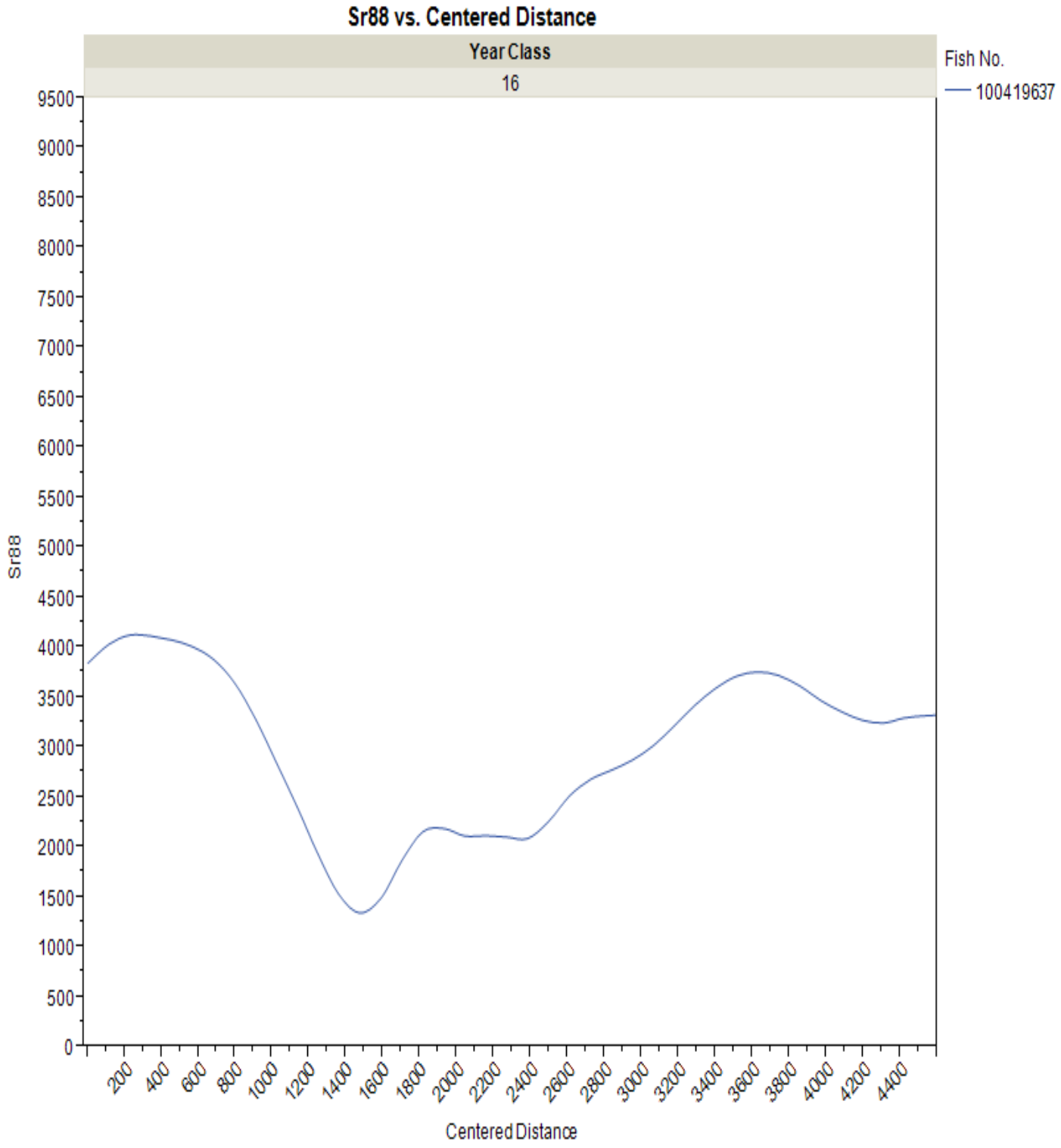

Figure 46. Reported values of Sr with distance across the otolith (X axis) and Sr values (ppm) (Y axis) in otoliths (n=246) by year class (16 year old) collected from A/R stock from 2009 to 2010.