PWD Comments on Draft EPA Rulemaking

Technical Comments on the Evidence for Hypoxia as a Stressor on Atlantic Sturgeon in the Delaware River

February 20, 2024

Michael S. Regan
Environmental Protection Agency
Office of Water
1200 Pennsylvania Avenue NW
Washington, DC 20460

Re: Water Quality Standards to Protect Aquatic Life in the Delaware River
Docket ID EPA–HQ–OW–2023–0222

Dear Administrator Regan,

The Philadelphia Water Department (PWD) appreciates the opportunity to submit comments on EPA's Proposed Water Quality Standards to Protect Aquatic Life in the Delaware River dated December 21, 2023, Docket ID No. EPA–HQ–OW–2023–0222. PWD has reviewed the rule proposing federal water quality standards for protection of aquatic life in zones 3, 4, and upper zone 5 of the Delaware River. We have also reviewed the supporting document entitled *Technical Support Document for the Proposed Rule: Water Quality Standards to Protect Aquatic Life in the Delaware River*, ("TSD") which describes a fish cohort model developed by EPA for the purpose of developing DO criteria protective of juvenile Atlantic sturgeon.

PWD found the TSD to be deficient in failing to utilize the best scientific information available on Atlantic sturgeon for the Delaware River. PWD is providing along with our comments a compilation of data for observations of more than 5000 juvenile sturgeon from the Delaware River collected by the Delaware Department of Natural Resources and Environmental Control Division of Fish and Wildlife (DNREC) and Environmental Resources Consulting (ERC) under contract to the U.S. Army Corps of Engineers. We are also providing detailed comments regarding the evidence for hypoxia as a stressor on Atlantic sturgeon in the Delaware River.

If EPA has any questions regarding PWD's comments and the accompanying analysis and data set, please contact Jason Cruz (jason.cruz@phila.gov).

Sincerely,

Randy E. Hayman

Commissioner and Chief Executive Officer

Philadelphia Water Department

Attachments
Microsoft Excel file containing sturgeon records from Delaware River compiled by PWD.

Technical Comments on the Evidence for Hypoxia as a Stressor on Atlantic Sturgeon in the Delaware River

1.) PWD compiled and analyzed more than 5,000 recent juvenile sturgeon collection records from the Delaware River to test various hypotheses regarding the effects of hypoxia on Atlantic sturgeon. These data have not been fully used by EPA in its analysis underlying the proposed rule.

PWD's technical comments herein provide detailed information on PWD's analysis of hypoxia as a stressor on the Delaware River population of Atlantic sturgeon. The conclusions suggest that more research using the entire available data set is critical to understand the necessary levels of DO to protect Atlantic sturgeon spawning and juvenile growth in the Delaware River, especially given the potential enormous costs of treatment plant modifications changes to meet more stringent ammonia effluent limitations and the substantial and widespread economic impact of the proposed rule. In PWD's view, the question of whether – and to what extent – hypoxia is affecting spawning and growth of juvenile Atlantic sturgeon can be informed by formulating and testing hypotheses scientifically using factual information. PWD has repeatedly urged DRBC and EPA to consider the available data and scientific information on actual fish spawning in the Delaware River when evaluating the need for higher DO levels (PWD 2023).

2.) Total length measurements of young-of-year (YOY) Delaware River Atlantic sturgeon were found to be within the expected range for the species, consistent with normal healthy growth given the thermal regime of the Delaware River.

PWD compiled total length (TL) measurements for 4,593 YOY (*i.e.*, < 500mm) Atlantic sturgeon collected from the Delaware River. Overall, fish collected during 2009-2022 had average TL 345mm, or 13.5 inches. Expected annual variability was observed in YOY total lengths, however the number of fish captured and measured varied over two orders of magnitude (Table 1). For the YOY cohort years 2015-2018 when samples were collected by both the Delaware Department of Natural Resources and Environmental Control (DNREC) and Environmental Research and Consulting, Inc. (ERC), the ERC fish collected in winter had slightly longer TL, suggesting that fish continued to grow, albeit more slowly, during fall and winter when temperatures are not ideal for growth. Given the location of the Delaware River between the spawning populations of Atlantic sturgeon in the Chesapeake Bay and Hudson River, the average overall YOY total length and annual ranges from 2009-2022 were within the expected range reported by Markin and Secor (2020) based on values from literature review, a laboratory experiment simulating the thermal regime of different sturgeon habitats, and a simple fish growth model based on accumulated growing degree days (GDD; Figure 1). There was no evidence from the TL analysis for the years 2009-2022 that the Delaware River Atlantic sturgeon population had smaller fish than would be expected for the Delaware River's position along a latitude and temperature gradient of spawning Atlantic sturgeon populations.

Table 1.) Annual average total length measurements for Delaware River YOY Atlantic sturgeon collected by DNREC and ERC 2009-2022

Year	Total YOY Captured and Measured	Mean TL (mm)	Sample Size, DNREC	Mean TL, DNREC (mm)	Sample Size, ERC	Mean TL, ERC (mm)
2009	23	287	23	287	-	-
2011	49	290	49	290	-	-
2013	29	368	-	-	27	372
2014	47	315	47	315	-	-
2015	271	370	15	353	256	370
2016	97	330	18	326	79	330
2017	2396	338	127	324	2269	339
2018	1528	358	221	335	1307	362
2019	5	347	5	347	-	-
2020	20	326	20	326	-	-
2021	105	329	105	329	-	-
2022	15	384	15	384	-	-

NOTE: number of samples may not match exactly between all total length and weight tables. Some samples may have lacked total length or weight.

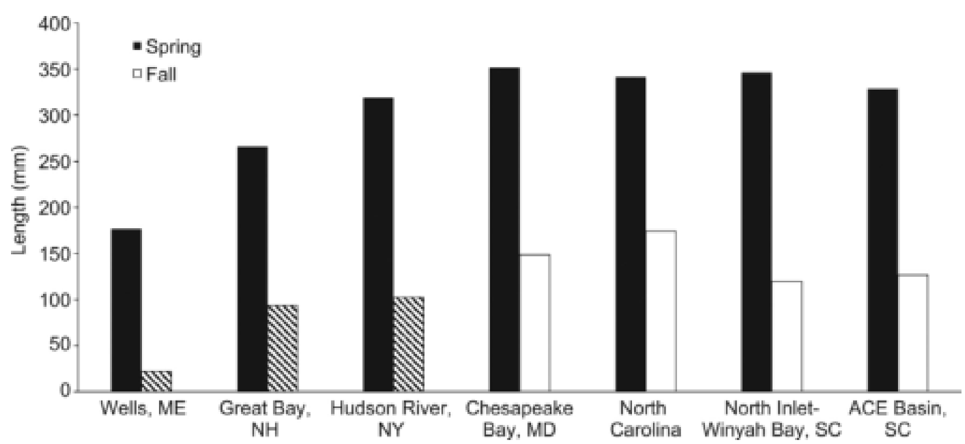

Figure 6

Total lengths of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) that spawned in spring and fall at the onset of winter (1 January), by the thermal regimes to which they were exposed across a latitudinal range. Water temperature data used for thermal regimes were collected from National Estuarine Research Reserve System sites from Maine to South Carolina during 2008–2012. Open bars represent the cohort that spawns in fall, and black bars represent the cohort that spawns in spring. The bars with diagonal lines indicate a hypothetical scenario of spawning occurring in fall in those locations. The southernmost site is the Ashepoo–Combahee–Edisto Basin (ACE Basin) in South Carolina.

Figure 1.) Total lengths of Atlantic sturgeon from seven spawning rivers along the U.S. east coast arranged along a latitudinal gradient (reproduced from Figure 6 in Markin and Secor 2020).

3.) Delaware River Atlantic sturgeon consistently exhibit growth rates expected for the species under existing DO conditions based on length-weight regression models.

PWD analyzed the relationship between total length and weight of Atlantic sturgeon collected from the Delaware River using a standard fisheries science method of linear regression of weight vs length ("L-W" regression). Log-transformed values log(weight) and log(total length) are typically used due to the relationship between length and weight being non-linear (Le Cren 1951). Since the increase in length is on a one-dimensional linear scale, while the increase in weight is related to the increase in volume (*i.e.*, cross-sectional area of the fish extending, enclosing space in three dimensions), a L-W regression slope parameter (*b*) value of 3 indicates *isometric growth*, in which the relationship between weight and length remains constant as the fish gets larger. L-W slope parameter *b* values less than or greater than 3 indicate *allometric growth*, or the condition when the overall relationship between fish weight and fish length changes as length increases. L-W slope parameter *b* values less than 3 indicate that the fish tends to get leaner as it grows longer, while slope parameter *b* values greater than 3 indicate that the fish is becoming "plumper" as length increases (Figure 2). L-W regression slope *b* parameter values greater than 3 are usually interpreted as a positive measure of fish population health and well-being (Blackwell *et al*. 2000, Hillborn and Walters 2001, Ogle 2013).

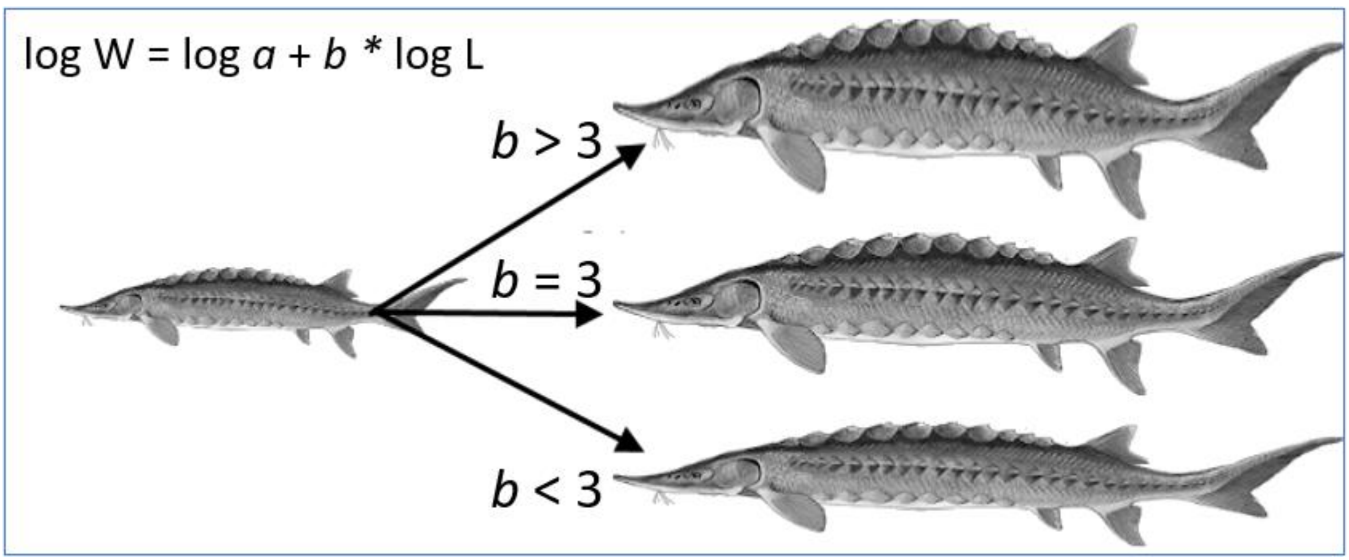

Figure 2.) Conceptual diagram of isometric (*b* = 3) and allometric (*b* < 3 or *b* > 3) growth in Atlantic sturgeon

The overall result of L-W regression for all observed Delaware River Atlantic sturgeon collected between 2009 and 2022 with valid length and weight measurements had L-W regression slope *b* parameter 3.16, which was within the range of observed values for four Distinct Population Segments (DPS) of Atlantic sturgeon in the Atlantic States Marine Fisheries Commission (ASMFC) 2017 Atlantic Sturgeon Benchmark Stock Assessment and Peer Review Report (ASMFC 2017) for Atlantic sturgeon (Figure 3 Table 2). The slope parameter *b* estimate for all observed Delaware River fish was statistically significantly higher than 3 indicating healthy, positive allometric growth (Student's t-test p <2.23e-139) with 95% confidence limits for the *b* estimate 3.14 to 3.17 (Table 3).

The Delaware River sample predominantly consisted of YOY and yearling sturgeon, with very few fish >800mm. Although the L-W regression had a good fit to the data (adjusted R^2 value 0.98, $p < 0.000000001$), young sturgeon grow more rapidly than adults, so the comparison to parameter values computed for samples from a larger range of fish sizes, including mature adults, may not be appropriate. The main conclusion from the L-W length regression for all Delaware River data was that there is very strong evidence that the Delaware fish experience allometric growth during the first two years of life, becoming "plumper" as they grow longer, consistent with a healthy population growing in the normal range of growth for the species.

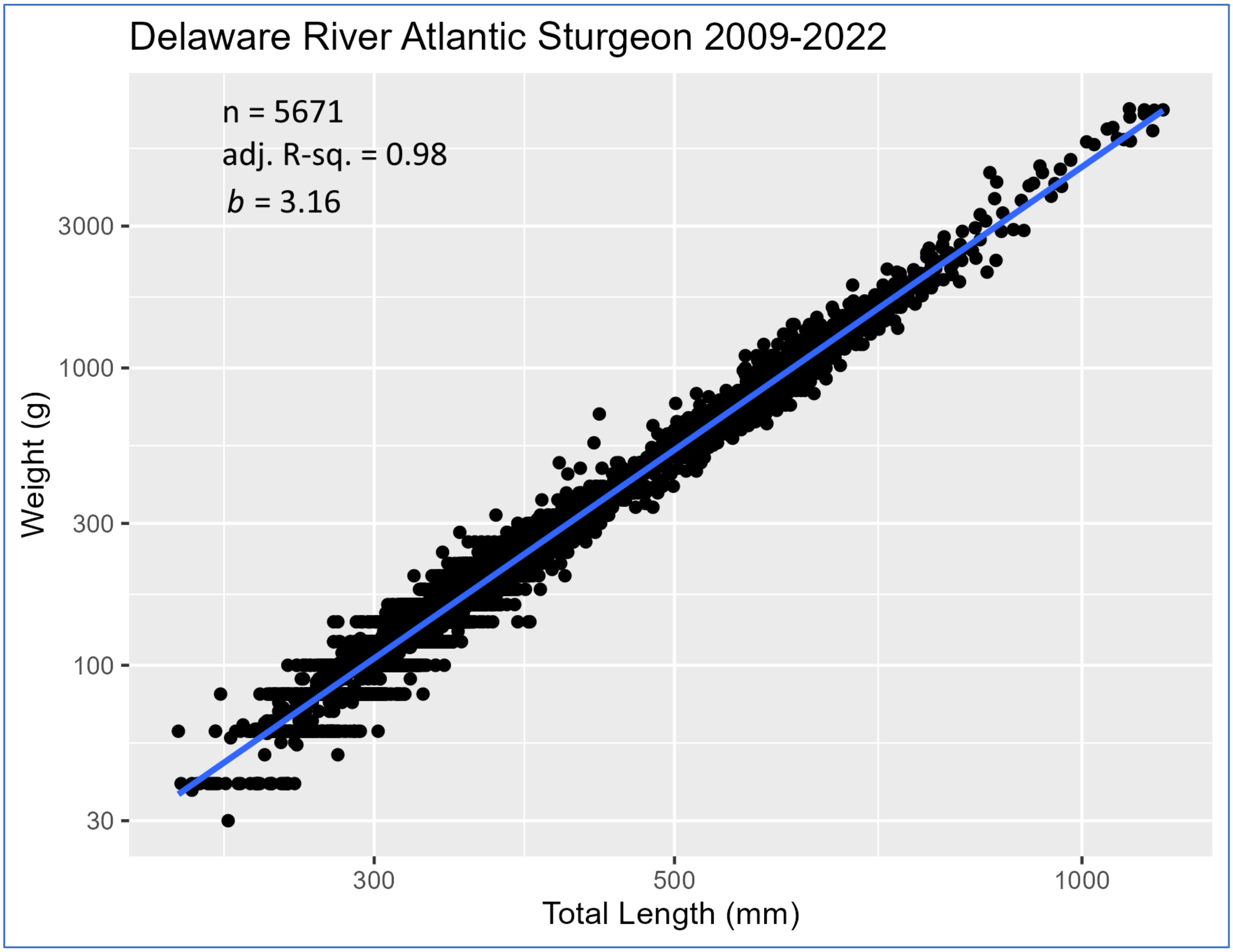

Figure 3.) Weight and Length of Delaware River Atlantic sturgeon collected 2009-2022 with L-W linear regression line and fit statistics.

Table 2.) L-W regression parameter estimates for four Atlantic sturgeon Discrete Population Segments. Reproduced from Table 2 in ASMFC 2017 Atlantic Sturgeon Benchmark Stock Assessment and Peer Review Report.

Table 2. Parameters estimates and associated standard errors of the total length (cm)-weight (g) relationship for Atlantic sturgeon by DPS.

DPS	n	a	SE[a]	b	SE[b]
All	6,304	0.00513	0.000286	3.05	0.0107
Gulf of Maine	618	0.0119	0.00211	2.85	0.0354
New York Bight	735	0.0235	0.00428	2.76	0.0345
Chesapeake Bay	190	0.00549	0.00306	3.06	0.109
Carolina	4,761	0.00186	0.000119	3.25	0.0129

Table 3.) Student's t-test for allometric growth, slope parameter *b* estimate based on L-W regression analysis for all Delaware River Atlantic sturgeon

term	Null Hypothesis (H_0) Value	Estimate	SE	t-statistic	df	p-value	95% conf low	95% conf high
2	3	3.16	0.006	25.85	5669	2.23E-139	3.15	3.17

PWD also analyzed L-W relationships for Atlantic sturgeon YOY (< 500mm) fish only (Table 4, Figure 4) and for both the complete data set and the YOY subset of the data on an annual basis (Tables 5 and 6; Figures 6 and 7). The L-W regression slope *b* parameter estimate for the YOY subset of the 2009-2022 data was 3.19 and also determined to be statistically significantly greater than 3, indicating healthy allometric growth (Student's t-test, p <4.92e-42, Table 4).

Table 4) Student's t-test for allometric growth, slope parameter *b* estimate based on L-W regression analysis for YOY Delaware River Atlantic sturgeon.

term	H_0 Value	Estimate	SE	t-statistic	df	p-value	95% conf low	95% conf high
2	3	3.19	0.013	13.72	4589	4.92E-42	3.16	3.22

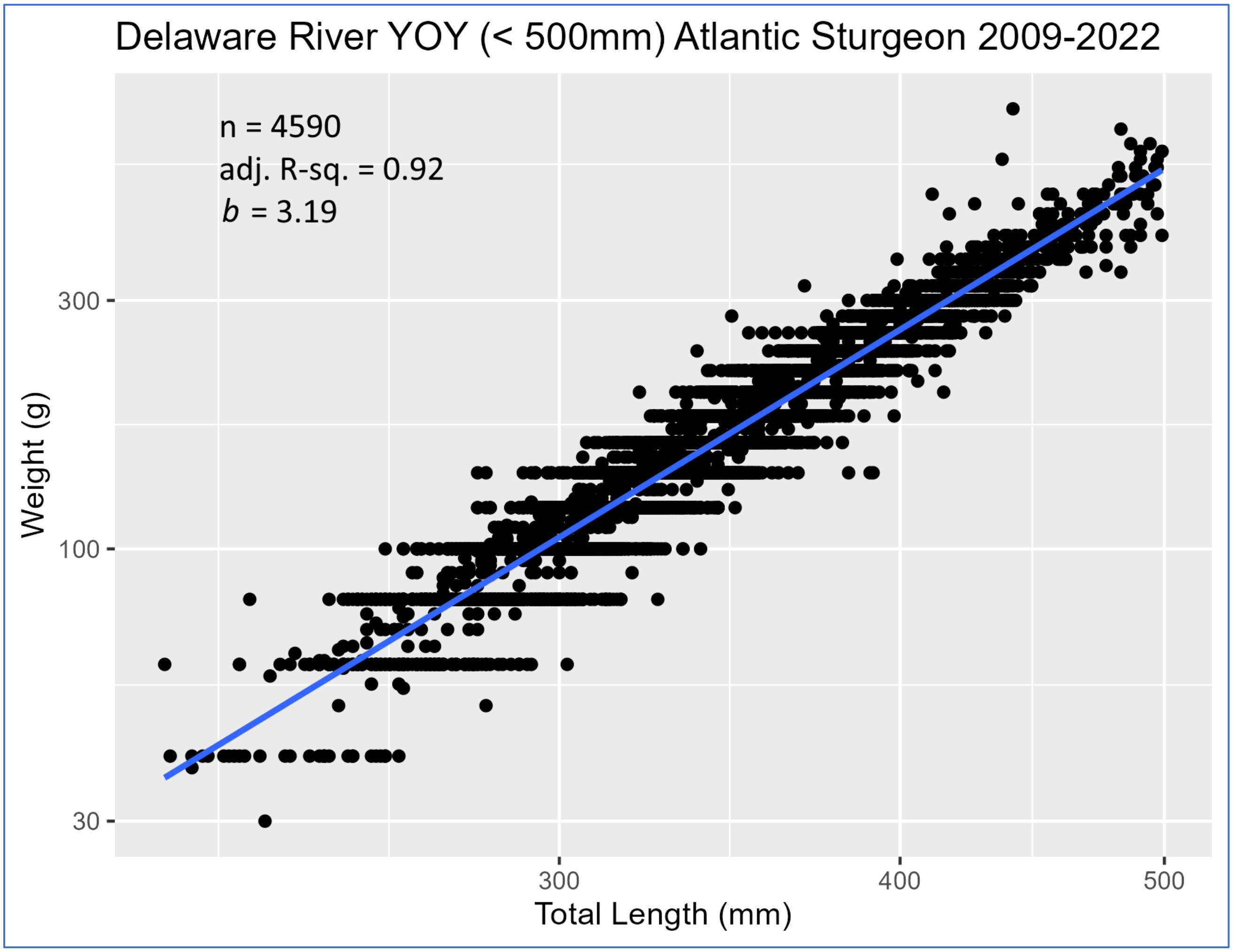

Figure 4.) Weight and Length of YOY (<500mm) Delaware River Atlantic sturgeon collected 2009-2022 with L-W linear regression line and fit statistics

As with the complete 2009-2022 and 2009-2022 YOY subset data sets, L-W regression slope *b* parameter values estimated on an annual basis were greater than 3, indicating positive allometric growth. Only two of 25 annual data sets (*i.e.*, the full 2022 and YOY subset 2013 data sets) had slope parameter *b* estimates less than 3 (Tables 5 and 6. Regression models for annual data sets using the complete range of length and weight were fit very well with the linear models, with adjusted r^2 values from 0.95 to 0.99. More variability was observed in linear model fits for the smaller annual data sets for YOY sturgeon, which also exhibited more heteroscedasticity; smaller fish were affected by lack of precision in weight measurements (See comment 4, below). Goodness-of-fit adjusted r^2 values for the YOY annual data sets ranged from 0.82 to 0.97. All observed fish data model fits were significant at an alpha value of $p < 0.001$. Standard errors of the slope parameter estimates varied as expected, with higher error associated with smaller sample sizes (Tables 5 and 6).

Table 5) Annual L-W regression statistics for all Delaware River Atlantic sturgeon records with total length and weight measurements 2009-2022.

Combined cohort year	n	slope	SE	r^2	p-value
2009	52	3.28	0.04	0.99	8.27E-57
2011	52	3.23	0.04	0.99	5.04E-57
2012*	22	3.29	0.07	0.99	6.30E-22
2013	43	3.14	0.07	0.98	3.06E-37
2014	50	3.28	0.12	0.94	1.15E-30
2015	746	3.20	0.01	0.98	0
2016	355	3.04	0.02	0.99	0
2017	2547	3.16	0.01	0.96	0
2018	1571	3.06	0.02	0.94	0
2019	13	3.18	0.07	0.99	3.71E-14
2020	66	3.11	0.04	0.99	2.04E-62
2021	104	3.07	0.08	0.93	5.36E-62
2022	44	2.99	0.08	0.97	3.57E-34

* DNREC 2012 records mostly from summer 2012, transcribed from Fisher 2015 Appendix A Table 5.1

Table 6.) Annual L-W regression statistics for Delaware River Atlantic sturgeon, YOY (< 500mm) only.

Combined cohort year	n	slope	SE	r^2	p-value
2009	23	3.12	0.32	0.82	3.40E-09
2011	49	3.14	0.08	0.97	7.95E-37
2013	29	2.74	0.20	0.87	1.38E-13
2014	47	3.18	0.21	0.84	2.75E-19
2015	271	3.43	0.06	0.92	2.14E-150
2016	103	3.02	0.07	0.95	1.10E-69
2017	2396	3.20	0.02	0.92	0
2018	1528	3.05	0.02	0.91	0
2019*	5	3.21	0.14	0.99	0.000171
2020	20	3.05	0.13	0.97	4.95E-15
2021	105	3.07	0.08	0.93	5.36E-62
2022	15	3.02	0.14	0.97	9.68E-12

* 2019 is included for completeness, but had only 5 YOY records with total length and weight

The slope *b* parameter estimate for YOY sturgeon for 2015 was investigated as an outlier, as the value of 3.43 was substantially larger than estimated for other YOY subsets of the data. The 2015 DNREC data had been obtained later in the data analysis workflow than the 2009-2014 or 2016-2022 due to a miscommunication from PWD to DNREC. PWD reviewed the data and concluded that although the linear model fit R^2 value was somewhat lower than other YOY fits and there appeared to be some outliers there was no reason to reject the 2015 YOY slope parameter estimate. The year 2018 was also checked as the slope parameter *b* value estimates for 2018 were lower than other years in which ERC collected substantially larger data sets. Hydrologic conditions during 2018 were also somewhat unusual with relatively high discharge throughout spring, an early summer dry period, and consistently high discharge from August through the remainder of 2018 (Figure 4).

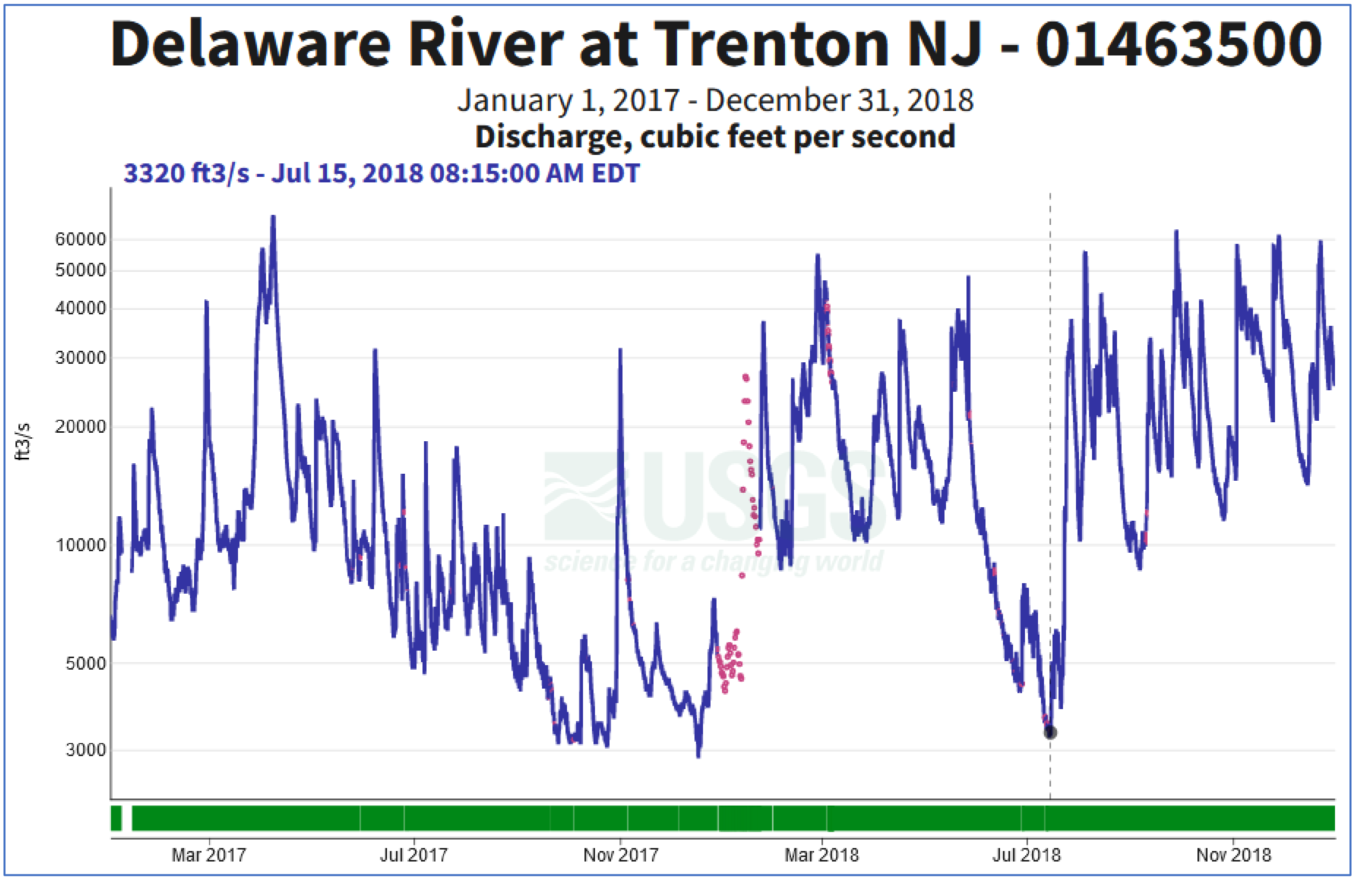

Figure 4.) Discharge for USGS Station 01463500 Delaware River at Trenton, NJ 2017-2018.

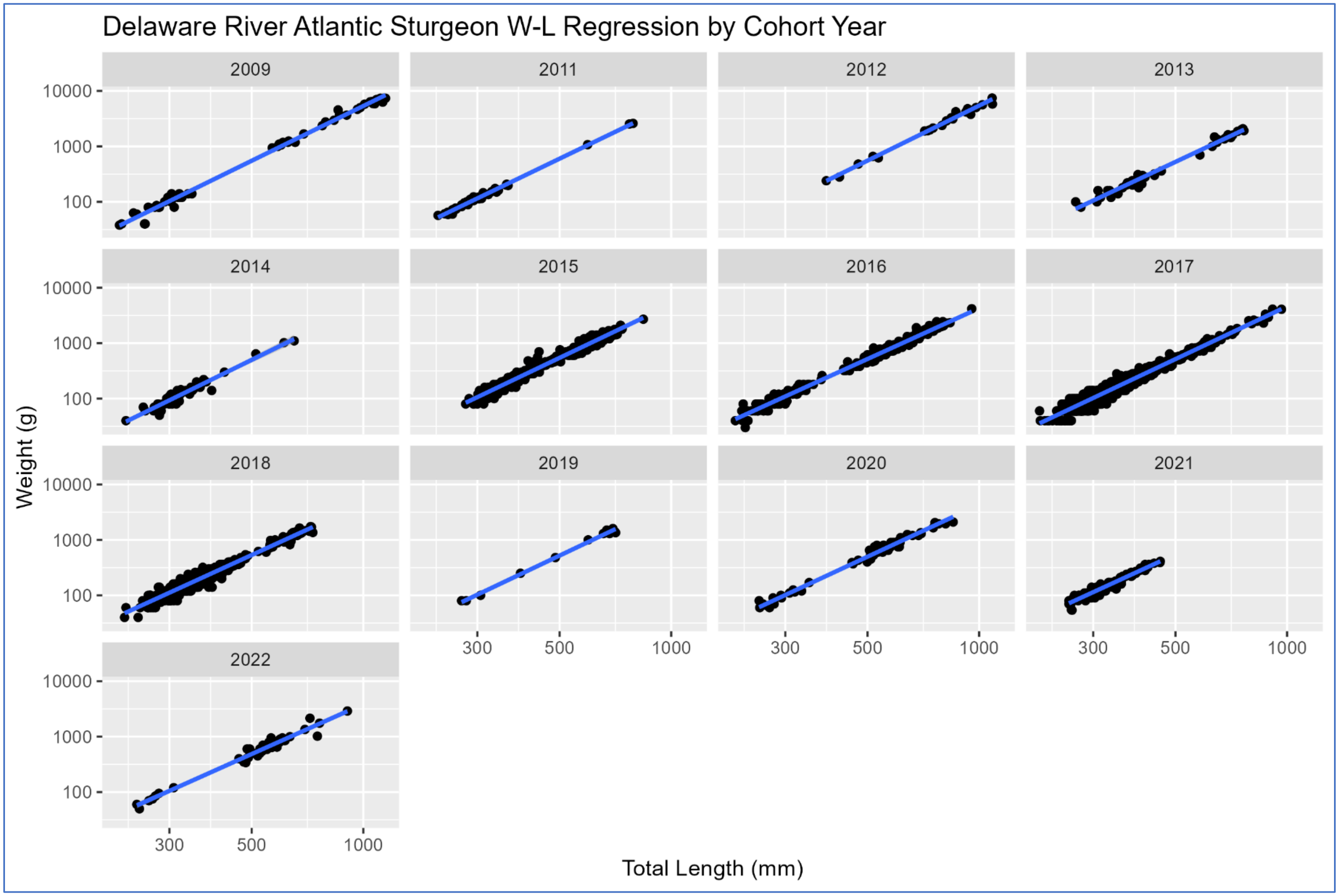

Figure 6.) Weight and Length of Delaware River Atlantic sturgeon collected 2009-2022 with L-W linear regressions by cohort year.

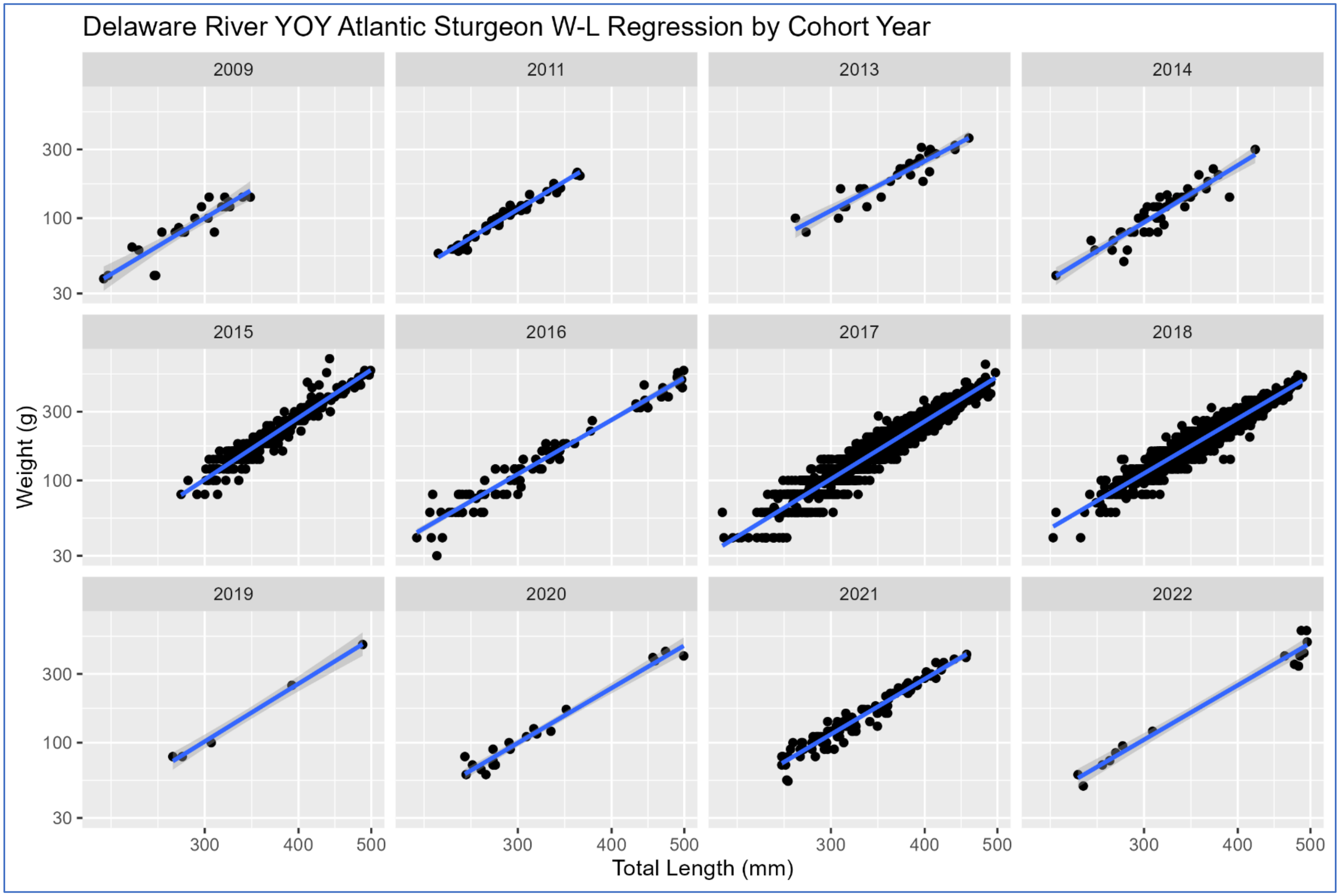

Figure 7.) Weight and Length of YOY (<500mm) Delaware River Atlantic sturgeon collected 2009-2022 with L-W linear regression by YOY cohort year. 2012 not shown; 2019 (n = 5) is included for continuity.

4.) Weight measurements for Delaware River Atlantic sturgeon were found to be within the appropriate range for the species; YOY weight was consistently and strongly underpredicted by EPA's fish cohort model.

Measured weights of Atlantic sturgeon collected from the Delaware River were subject to substantial measurement error and lack of precision when compared to length measurements, which were very precise (*i.e.*, nearest 1mm). Likely owing to the logistical constraints of measuring wet weight of fish with a very large range of sizes aboard moving vessels, sturgeon weights reported for the Delaware River by DNREC and ERC were recorded to the nearest 5g or 20g, respectively (Figure 8.) Imprecise weight measurements more strongly affected small fish, as the weight increments represented a greater proportion of body size. ERC weights reported in final reports did not match the weight precision values described in Materials and Methods, but PWD was unable to resolve this discrepancy. If EPA and/or NMFS are able to obtain the raw data from ERC for QAQC purposes, the more precise weight measurements described in report methods may be available.

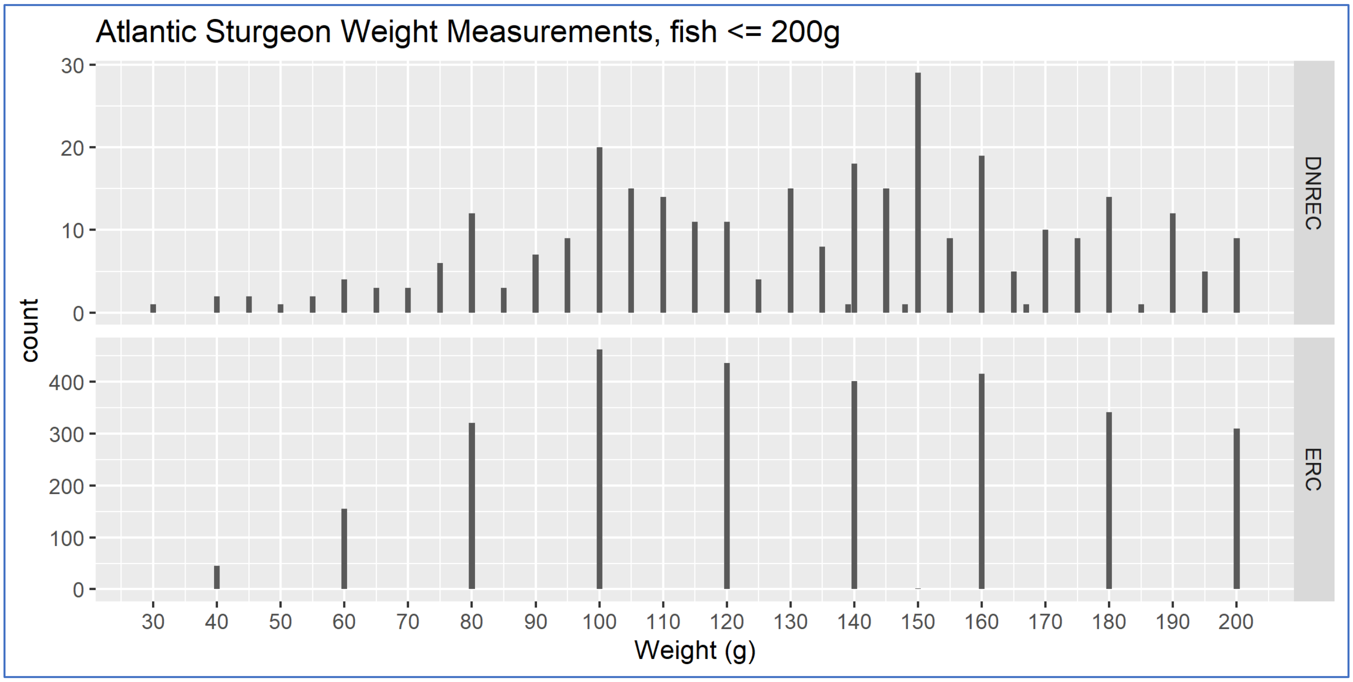

Figure 8.) Example of imprecise weight increments from measurements of YOY and juvenile sturgeon collected by DNREC and ERC, fish less than or equal to 200g only.

The average weight of 4,590 YOY (*i.e.*, <500mm) Delaware River Atlantic sturgeon was 178g, while the median value was 160g. Annual variability was observed in weight measurements, however total annual sample sizes for YOY varied over two orders of magnitude (Table 7). Similar to the total length analysis, for the YOY cohort years 2015-2018 when samples were collected by both DNREC and ERC, the ERC fish collected in winter had slightly higher weight in three of the four years monitored, suggesting that fish continued to grow, albeit more slowly, during fall and winter when temperatures are not ideal for growth.

Table 7.) Annual average weight measurements for Delaware River YOY Atlantic sturgeon collected by DNREC and ERC 2009-2022

Cohort year	n	Average weight (g)	n DNREC	DNREC avg wt. (g)	n ERC	ERC avg wt. (g)
2009	23	93	23	93	-	-
2011	49	107	49	107	-	-
2013	27	213	-	-	27	213
2014	47	116	47	116	-	-
2015	271	225	15	214	256	225
2016	102	180	20	185	82	179
2017	2396	162	127	139	2269	163
2018	1528	201	221	160	1307	208
2019	5	198	5	198	-	-
2020	20	153	20	153	-	-
2021	105	163	105	163	-	-
2022	15	278	15	278	-	-

NOTE: number of samples may not match between total length and weight tables. Some samples may have lacked total length or weight

In EPA's Technical Support Document (TSD) for the proposed rule (EPA 2023a), EPA's fish cohort model-predicted growth rates were plotted against a subset of DNREC data (TSD Figure 6) for 72 fish that were marked and recaptured. EPA made the model code and data sets used in the fish cohort model available in a GitHub repository (EPA 2023b), allowing PWD to obtain the EPA results for comparison against the full set of DNREC and ERC data (Figure 9).

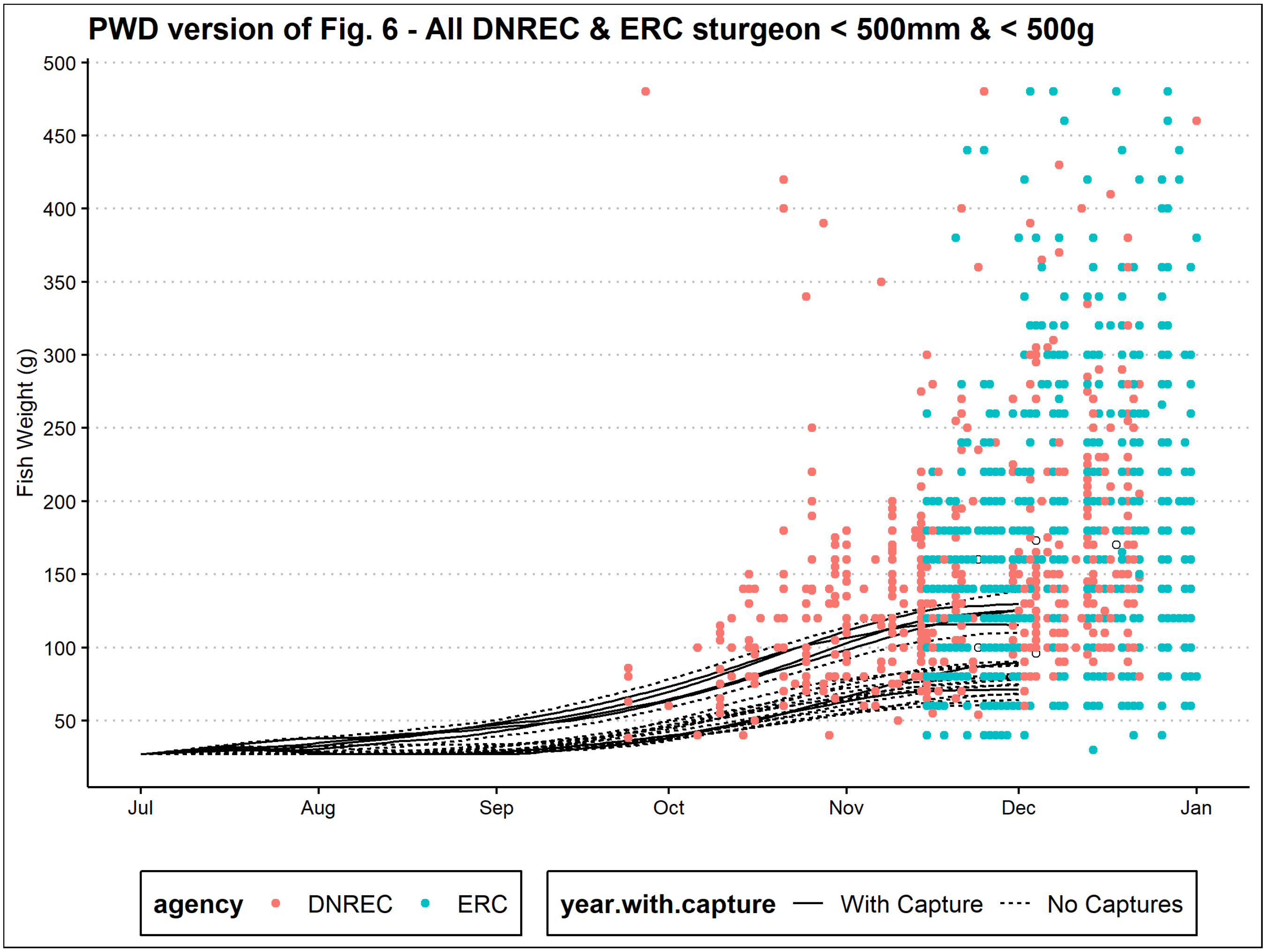

Figure 9.) EPA-modeled growth of Atlantic sturgeon cohorts from 2002-2021 compared to observed weight of YOY Atlantic sturgeon collected by DNREC and ERC 2009-2022.

Annual fish cohorts were modeled by EPA through December 1st of each year. Only DNREC data were available for the cohort years 2009, 2011, 2014, and 2020-2022, and DNREC data did not extend beyond December 1st, facilitating a direct comparison of observed data to the modeled fish cohort results (Figure 10). EPA's fish cohort model achieved a reasonably good fit for the center of the distribution of observed data for 2009 and 2014, but underpredicted the observed data for 2011 and 2021. The years 2020 and 2022 had relatively few observed data points for YOY sturgeon and appeared to have a bimodal distribution in weight. EPA's model was a reasonably good fit for the weight measurements with a grouping around 100g for these years. If the smaller fish are assumed to represent a relatively low weight YOY cohort and the heavier fish a yearling (or possibly fall-spawned, different cohort), the model fit the observed data reasonably well for 2020 and 2022. With such small sample sizes, it is not possible to determine if the fish weights came from a bimodal distribution with two different cohorts or were just the result of collecting a random sample from a continuous distribution that happened to have a cluster of small and large values.

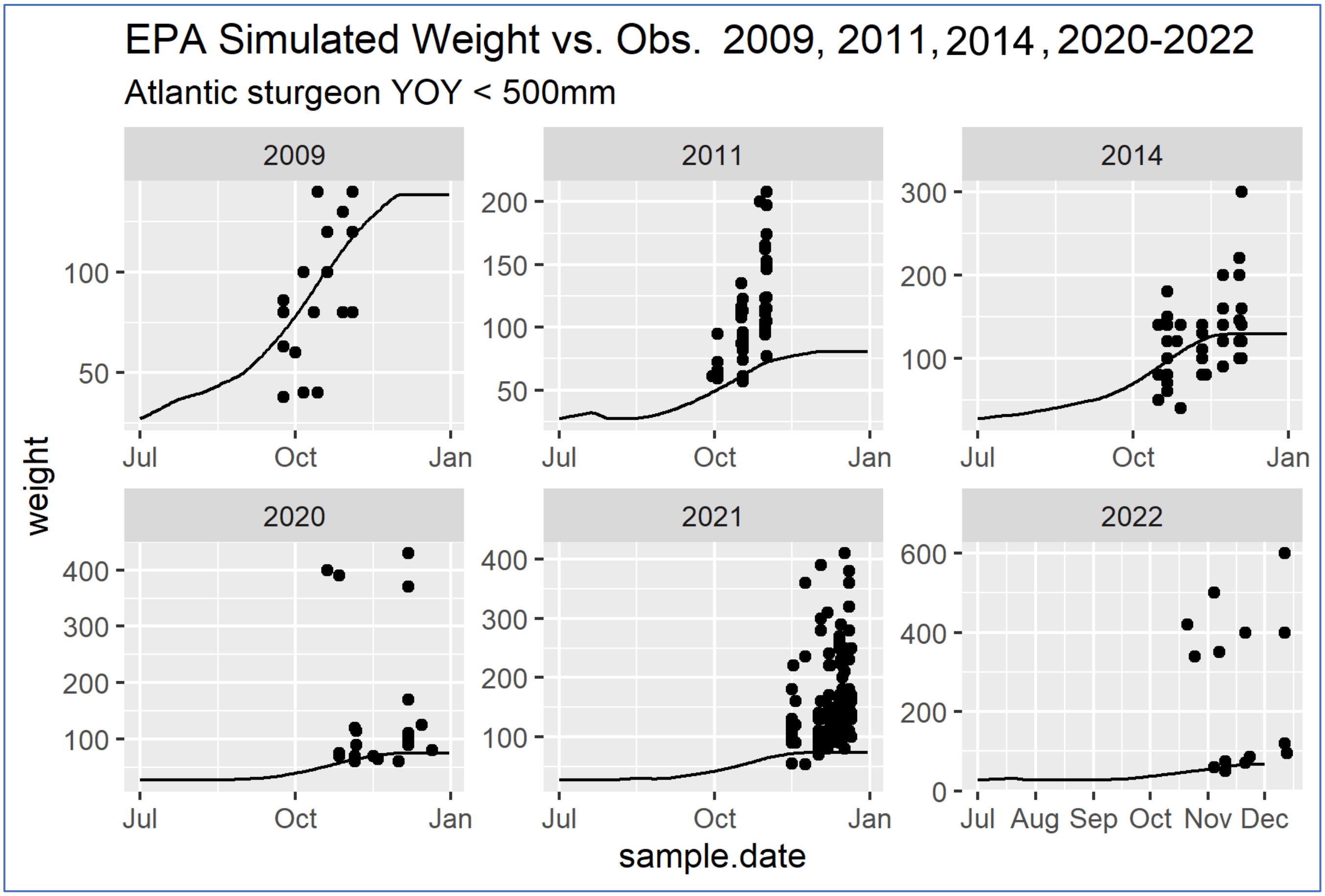

Figure 10.) EPA modeled growth of Atlantic sturgeon cohorts (black line) compared to observed weight of YOY Atlantic sturgeon collected by DNREC and ERC 2009, 2011, 2014, and 2020-2022.

Most of the ERC sturgeon data were collected in winter, in the next calendar year after spawning was assumed to occur, so PWD extended the x-axis of plots of the observed fish weight to include additional days for analysis. Annual fish cohorts were modeled by EPA through December 1st of each year, but to facilitate visual interpretation, PWD extrapolated the final value for modeled fish weight from December 1st through March 31st to represent no additional growth. PWD recognizes that EPA's model may have predicted additional growth or reduced growth, so the horizontal lines in figures 11 through 14 are presented only as a reference for the modeled fish weight on December 1st and not intended to represent EPA model results.

When compared to the generally larger data sets for the combined data from DNREC and ERC for the cohort years 2015-2018, the final December 1st value for EPA fish cohort model generally strongly underpredicted the observed data for 2015, 2017 and 2018 – years with the most available data on observed fish weight. The year 2016 was a less extreme example of the bimodal distribution in weight values seen in 2020 and 2022, although the data distribution was more continuous. The final model value from December 1st of approximately 100g was within the center of the range of the grouping of lower fish weight. Again, it is possible that the other fish less than 500mm in length may have corresponded to a yearling 2014 cohort or other (*e.g.*, fall spawning) cohort.

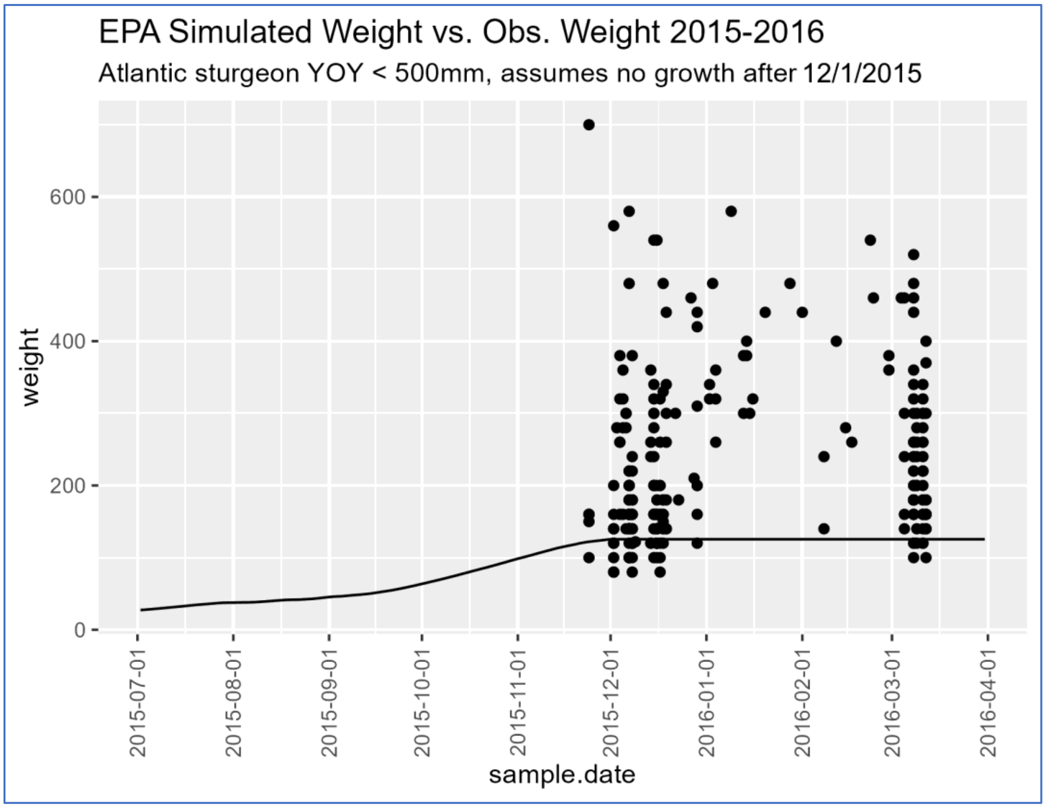

Figure 11.) EPA fish cohort model simulated weight (black line) vs. observed DNREC and ERC data for YOY Atlantic sturgeon collected 2015-2016

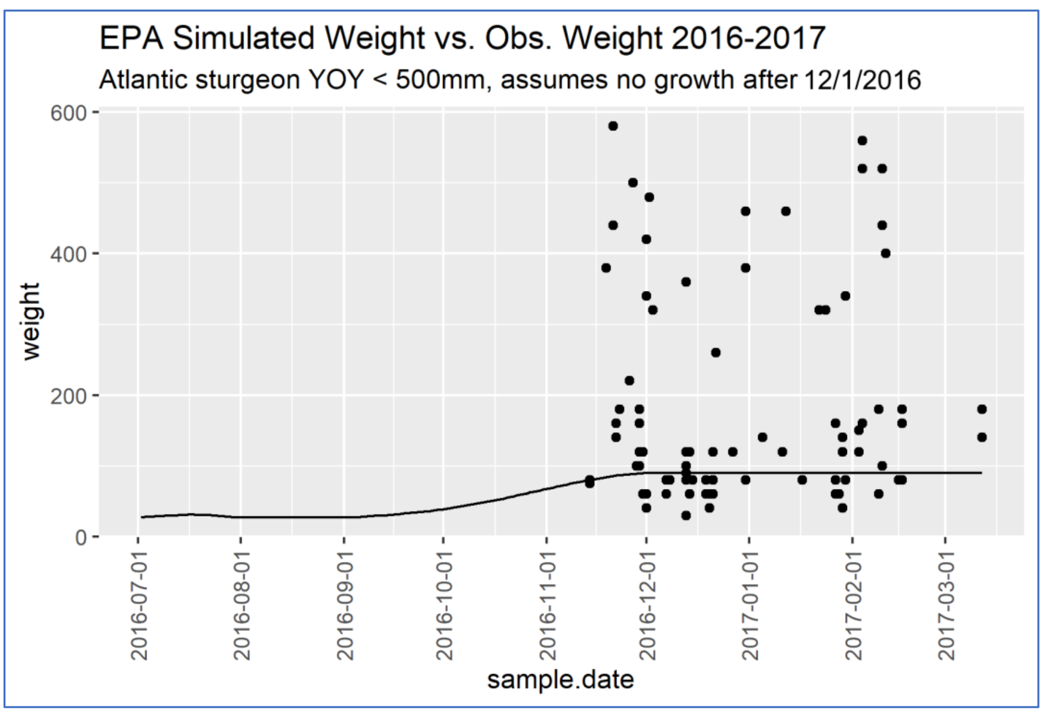

Figure 12.) EPA fish cohort model simulated weight (black line) vs. observed DNREC and ERC data for YOY Atlantic sturgeon collected 2016-2017

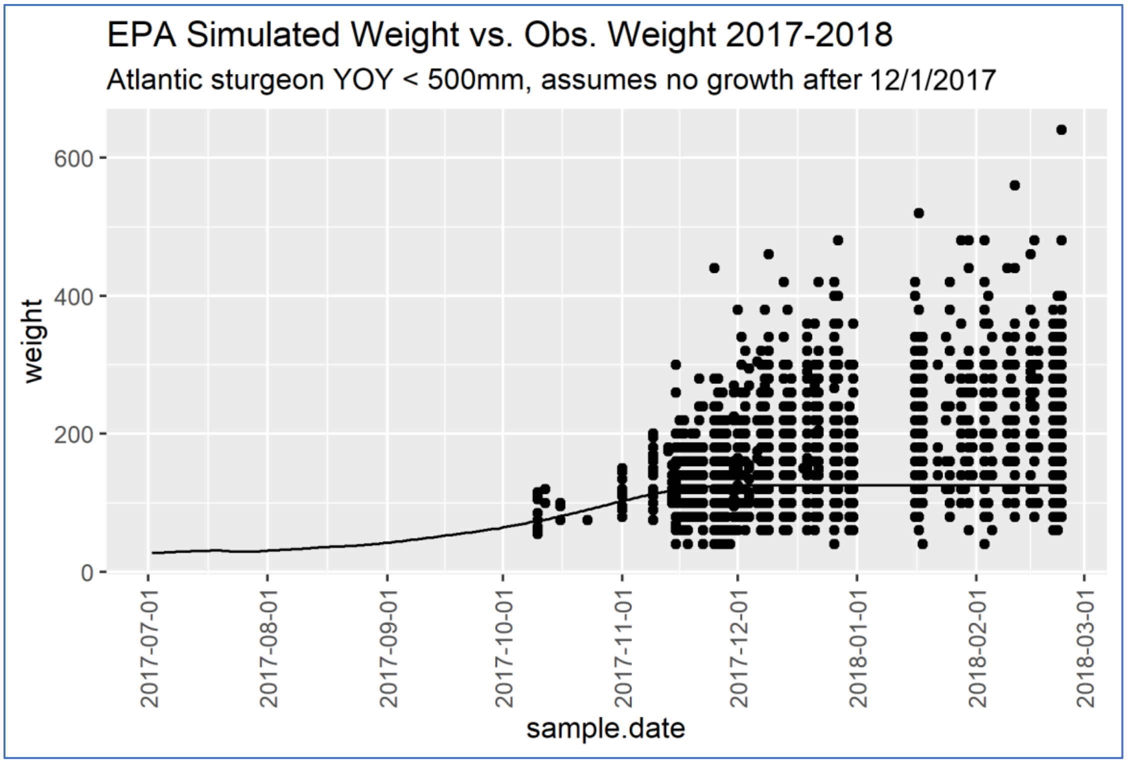

Figure 13.) EPA fish cohort model simulated weight (black line) vs. observed DNREC and ERC data for YOY Atlantic sturgeon collected 2017-2018

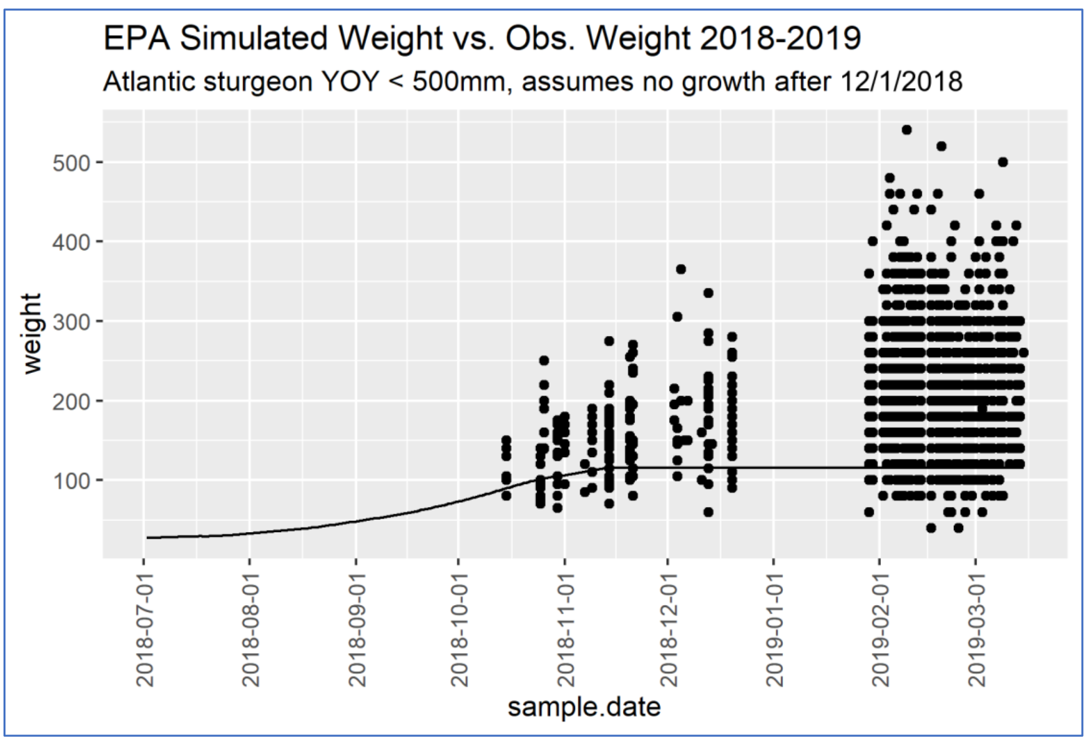

Figure 14.) EPA fish cohort model simulated weight (black line) vs. observed DNREC and ERC data for YOY Atlantic sturgeon collected 2018-2019

5.) Atlantic sturgeon growth rates simulated by EPA's fish cohort model are much lower than growth rate estimates calculated from observed data in the Delaware River.

As described above, EPA provided R code and input data sets for the fish cohort model, allowing PWD to compare modeled growth rates with growth rate estimates for observed data. PWD estimated growth rates for observed data using two different methods:

1.) Assume fish were 27g and 100mm on July 1st and calculate linear growth rates from observed data

2.) Assume fish spawned May 1st as suggested by literature on Delaware River Atlantic sturgeon

For method 2 PWD assumed that fish were 0.1g on the hatch date of May 1st. For analyzing growth in length for scenario 1, PWD assumed that fish were 100mm long on July 1st based on a linear regression model of log(weight) - log(length) and EPA's assumption that fish were 27g on July 1st. The linear regression model had adjusted r^2 value of 0.978 (See Figure 3 and further discussion in Comment 3). Additionally, for each of the two growth calculation methods, PWD calculated growth for fish collected in winter by ERC either directly as observed based on the collection date, or with an alternative "capped" scenario where the observed fish length or weight was assumed to have been achieved by January 1st. Combining the two methods of assumptions for starting conditions with the alternative methods of accounting for winter growth resulted in four different scenarios that were evaluated for changes in weight and length over time.

Table 8.) Estimated change in total length of Atlantic sturgeon based on observed data and four scenarios of starting length and growth period duration.

YOY Cohort Year	Growth estimate July 1st (mm/d)	Growth estimate May 1st (mm/d)	Growth estimate July 1st capped* (mm/d)	Growth estimate May 1st capped* (mm/d)
2009	1.72	1.69	1.72	1.69
2011	1.70	1.68	1.70	1.68
2013	1.17	1.26	1.51	1.54
2014	1.68	1.66	1.68	1.66
2015	1.45	1.49	1.58	1.60
2016	1.29	1.37	1.38	1.44
2017	1.42	1.47	1.48	1.52
2018	1.21	1.30	1.46	1.51
2019	1.75	1.72	1.75	1.72
2020	1.62	1.62	1.62	1.62
2021	1.43	1.49	1.43	1.49
2022	2.05	1.91	2.05	1.91

* NOTE: "capped" scenarios assume that fish collected in winter reached maximum TL value on Jan 1st.

Table 9.) Estimated change in weight of Atlantic sturgeon based on observed data and four scenarios of starting weight and growth period duration.

YOY Cohort year	Growth estimate July 1st (g/d)	Growth estimate May 1st (g/d)	Growth estimate July 1st capped* (g/d)	Growth estimate May 1st capped* (g/d)
2009	0.59	0.54	0.59	0.54
2011	0.70	0.62	0.70	0.62
2013	0.77	0.70	1.01	0.87
2014	0.68	0.60	0.68	0.60
2015	1.05	0.89	1.15	0.97
2016	0.87	0.76	0.93	0.80
2017	0.77	0.69	0.82	0.72
2018	0.80	0.72	0.98	0.84
2019	1.19	0.97	1.19	0.97
2020	0.91	0.76	0.91	0.76
2021	0.84	0.73	0.84	0.73
2022	1.79	1.37	1.79	1.37

* NOTE: "cap" scenarios assume that fish collected in winter reached maximum value on Jan 1st.

Mean growth in length was greater than 1mm/day for all the scenarios tested (Table 8), which provides solid evidence that sturgeon are growing in the Delaware River. PWD recognizes that sturgeon growth is likely non-linear. However, due to the relatively short window of length measurements during the growing season and inadequate 60-day comment period allowed by EPA for review of the proposed rule PWD, was unable to fully investigate more appropriate specific growth rates as a function of body size.

Estimated growth based on change in weight was much more variable than observed for length measurements, likely due to the lack of precision and measurement error for weight of small specimens (Figure 8, Table 9). Most cohort years experienced growth in weight by more than 0.5g/day, with estimates for some cohort years closer to 1g/day. As with length growth estimates, PWD recognizes that sturgeon growth in weight is likely highly non-linear. PWD was unable to fully investigate more appropriate specific growth rates as a function of body size. EPA's initial weight estimate of 27g for fish in the hypothetical cohort on July 1st is likely conservatively high. Assuming spawning occurs in April or May, actual reported growth rates for larval Atlantic sturgeon spawned and grown in laboratory conditions suggest that sturgeon would be closer to 5-10g at 60 days post-hatch (Secor and Gunderson 1998). More consistent reporting of age, length, and weight would be beneficial, as many studies only reported one or two of these factors rather than all three consistently.

6.) Empirical growth rate estimates calculated from observed data on marked and recaptured fish were highly variable, but otherwise generally corroborated growth rate estimates from observed data.

PWD estimated that there were approximately 230 instances in which a fish tagged by DNREC or ERC was recaptured and measured a second time, allowing for calculation of empirical growth rates. Most recapture intervals involved a very short time "at large" and were observed during the fall and winter when growth rates tend to be slower overall. As mentioned previously, weight measurements for small fish in the ERC data set lacked precision, typically having been measured in 20g increments. Negative growth is feasible for both weight and length, but length was measured more precisely and conceptually it is less likely for fish to lose appreciable length. Negative growth (*i.e.*, weight loss) would tend to be more likely to occur in the colder fall and winter.

Based on breaks in the data set, PWD estimated that rates > 2mm/d or 2g/d, may be erroneous whether positive or negative. Approximately 16% of the calculated empirical growth rates appeared to be spurious high outliers, with positive growth rates in total length greater than 2mm/day or weight increase greater than 2g/day. (Table 10). Approximately 13% of the data appeared to be spurious low outliers, with negative growth rates exceeding -2mm/d or -2g/d. The central tendency of the growth data set was relatively well constrained and generally corroborated the finding from seasonal growth rate calculation estimates that positive growth continued to occur outside the summer growing season (Figure 15).

Table 10.) Summary of potential spurious outliers from empirical growth estimates based on mark-recapture data

Total recaptures	n, change in TL >2mm/d	proportion, change in TL >2mm/d	n, change in TL < -2 mm/d	proportion, change in TL <-2mm/d	n, change in W >2g/d	proportion, change in W >2g/d	n, change in W < -2 g/d	proportion, change in W <-2mm/d
234	9	0.04	10	0.04	29	0.12	20	0.09

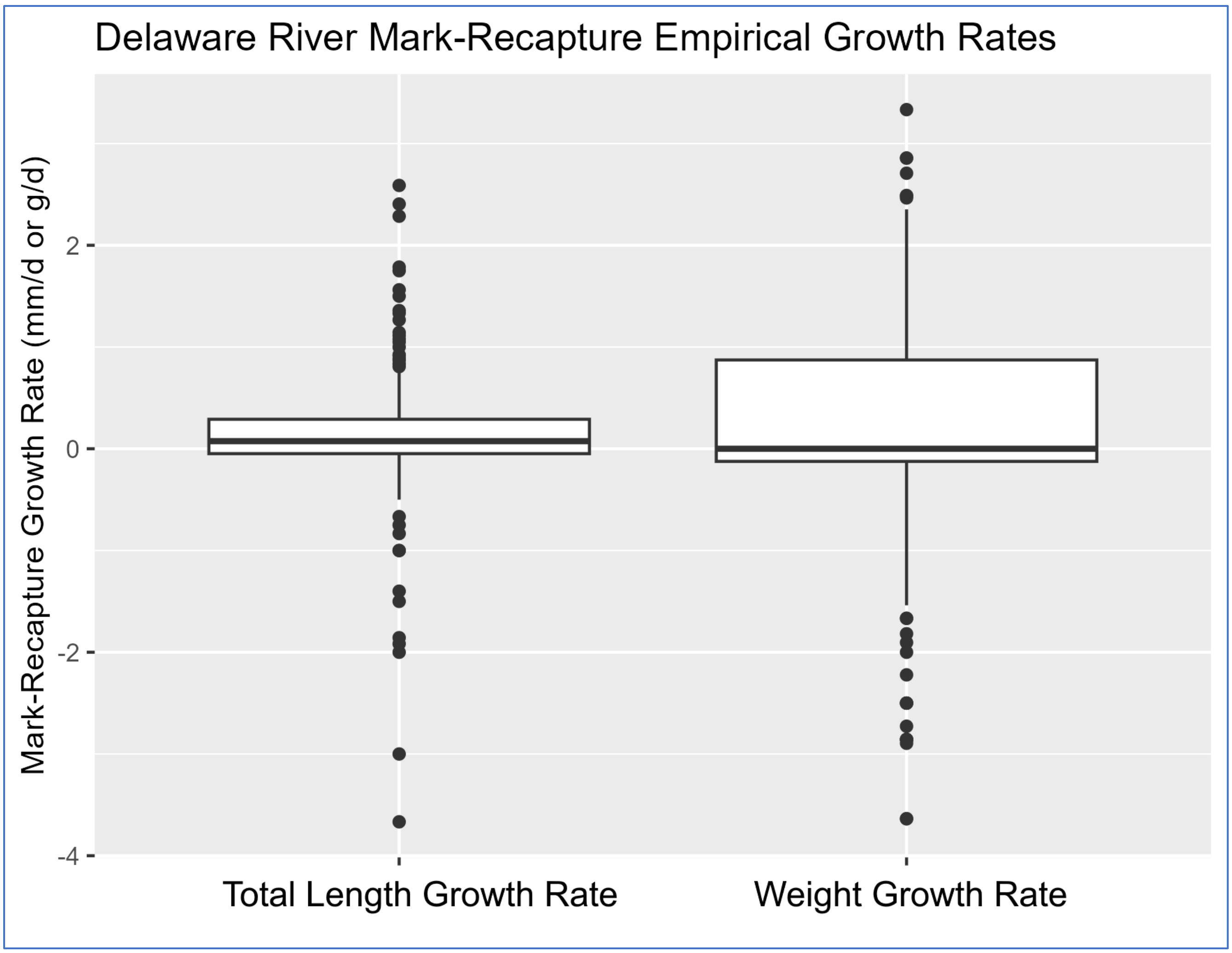

Figure 15.) Delaware River empirical growth rates from observed mark-recapture data

Only 12 marked and recaptured fish were at large 300days or more, representing nearly a full year of growth for yearling or older sturgeon (Figure 16). With longer time at large and certainty that the mark-recapture period included at least one complete growing season, these estimates of empirical growth rate are potentially more reliable, although they may still include a substantial period of fall and winter growth. One of the 12 mark-recapture records over 300 days at large is believed to be an error as the mark-recapture data suggested that the fish lost 82mm in length and 140g in weight over 363 days with condition factor remaining relatively constant at K_{TL} = 0.40.

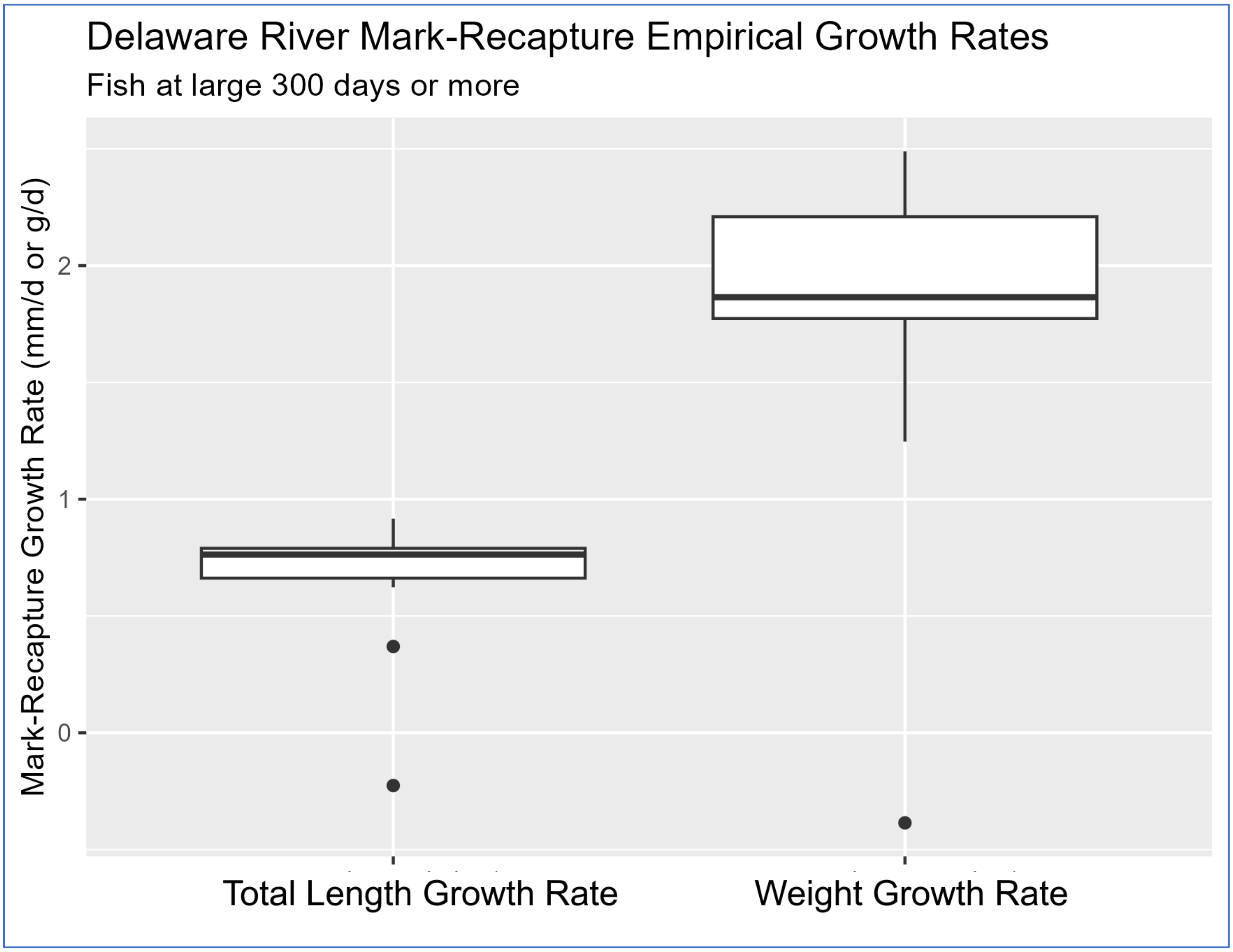

Figure 16.) Delaware River empirical growth rates from observed mark-recapture data, fish at large 300 days or more

7.) EPA's fish cohort model substantially underpredicts production for hypothetical cohorts of YOY Atlantic sturgeon as measured by change in biomass.

PWD compared the biomass estimates from EPA's fish cohort model against the total mass of observed YOY sturgeon in 2009-2022. EPA's fish cohort model includes two primary mechanisms by which biomass may change – mortality and growth. The cohort is limited to 10,000 hypothetical individuals, which decreases due to mortality on a daily time step and cannot increase. Growth, as implemented in the model, can be negative or positive. The ultimate measure of production is expressed as a Habitat Suitability Index, which indicates whether there would be a net increase or decrease in fish biomass for a given cohort year. Because the observed data for each cohort year come from a population of unknown size, direct comparison of the biomass of the hypothetical cohort and observed data is inappropriate. It is possible, however, to evaluate whether EPA's fish cohort model predicted an increase or decrease in biomass for each year along with the observed biomass for the sample from the unknown population. The biomass sample from fish that were collected is obviously an under-estimate of the true population biomass.

Table 11.) EPA HSI (change in biomass) compared to observed biomass of observed population sample for YOY Atlantic sturgeon by cohort year

YOY Cohort year	EPA TSD HSI	Observed YOY biomass(g)*
2009	0.45	2,137
2011	-0.30	52,59.3
2013	0.17	6,030
2014	-0.01	5,455
2015	0.22	60,862
2016	-0.48	18,995
2017	0.27	387,855
2018	0.56	307,069
2019	-0.96	990
2020	-1.92	3,060
2021	-1.61	16,964
2022	-1.04	4,165

* Note: Biomass only for YOY fish < 500mm, not all fish collected

EPA's fish cohort model predicted a negative net change in biomass for observed DO and temperature conditions at Chester in seven of the twelve years with adequate observed data (Table 11). The HSI model did predict the largest net increase in biomass for 2018, which was the second largest observed biomass from fish actually collected. Due to differences in sampling effort, biomass totals are not comparable from year to year. The primary conclusion from the biomass analysis is that EPA's HSI predicts a net decrease in biomass for several years that in fact had strong evidence of spawning, growth and a net increase in biomass.

8.) PWD compared several measures of observed fish growth and condition to DO statistics and found no statistically significant correlations that would indicate DO levels are adversely affecting sturgeon

PWD investigated the hypothesis that hypoxia in the Delaware River adversely affected sturgeon by comparing several DO statistics (Table 12) to measures of observed fish growth and condition for data observed 2009-2022 (Table 13). Before performing statistical tests, PWD evaluated whether the DO and sturgeon response metrics had a relatively large enough range, such that practical interpretations of the data would be feasible and appropriate. For example, if all the DO measurements were relatively very high or very low from year to year with no years of observed variability in DO conditions, there might be not enough available data over the range of DO exposures to both low and high DO for any correlation to be apparent in the correlation analysis.

Table 12.) Key to abbreviations used for DO statistics observed for Chester USGS station 2009-2022

Abbreviation	Description
crit.1.sat	critical season 1st percentile DO saturation
crit.2.sat	critical season 2nd percentile DO saturation
crit.5.sat	critical season 5th percentile DO saturation
crit.10.sat	critical season 10th percentile DO saturation
crit.mean.sat	critical season mean DO saturation
crit.med.sat	critical season median DO saturation
crit.min.sat	critical season minimum DO saturation
crit.pct.50	critical season % of DO > 50% saturation
crit.pct.60	critical season % of DO > 60% saturation
crit.pct.70	critical season % of DO > 70% saturation
grow.1.sat	growing season 1st percentile DO saturation
grow.2.sat	growing season 2nd percentile DO saturation
grow.5.sat	growing season 5th percentile DO saturation
grow.10.sat	growing season 10th percentile DO saturation
grow.mean.sat	growing season mean DO saturation
grow.med.sat	growing season median DO saturation
grow.min.sat	growing season minimum DO saturation
grow.pct.50	growing season % of DO > 50% saturation
grow.pct.60	growing season % of DO > 60% saturation
grow.pct.70	growing season % of DO > 70% saturation

Table 13.) Key to abbreviations used for sturgeon growth and condition metrics

Abbreviation	Description
YOY.grow.5.1.TL	YOY growth in length from May 1st
YOY.grow.5.1.TL.cap*	YOY growth in length from May 1st, winter growth to Jan 1st
YOY.grow.5.1.W	YOY growth in weight from May 1st
YOY.grow.5.1.W.cap*	YOY growth in weight from May 1st, winter growth to Jan 1st
YOY.grow.7.1.TL	YOY growth in length from 100mm July 1st
YOY.grow.7.1.TL.cap*	YOY growth in length from 100mm July 1st, winter growth to Jan 1st
YOY.grow.7.1.W	YOY growth in weight from 27g July 1st
YOY.grow.7.1.W.cap*	YOY growth in weight from 27g July 1st, winter growth to Jan 1st
YOY.mean.TL	YOY mean total length in mm
YOY.mean.W	YOY mean total weight in g
mean.K.YOY	YOY mean condition factor K
mean.K.all	mean condition factor K for all fish collected
slope.b.YOY	slope parameter b for L-W regression, YOY only
slope.b.all	slope parameter b for L-W regression, all fish collected

* See comment 5 for description of “capped” scenarios for fish growth

Some independent DO statistic metrics exhibited relatively large ranges, with expected higher variability in higher percentiles, such as the percent of DO values above 70% or 60% saturation. Conversely, there was little variability in the percent of data above 50% saturation, as 50% DO saturation was almost always achieved. Measurements that correspond to the central tendency of the data had a moderate to low amount of variability (Figure 17). There was relatively good coverage of the range in DO variability between 50-60%, which likely represents the range of suitable DO conditions based on the consistent propagation and normal growth that was observed each year. Notably, other than the DO minima, which may represent a very transient condition, there was no substantial or extended exposure to DO saturation levels lower than 50% saturation.

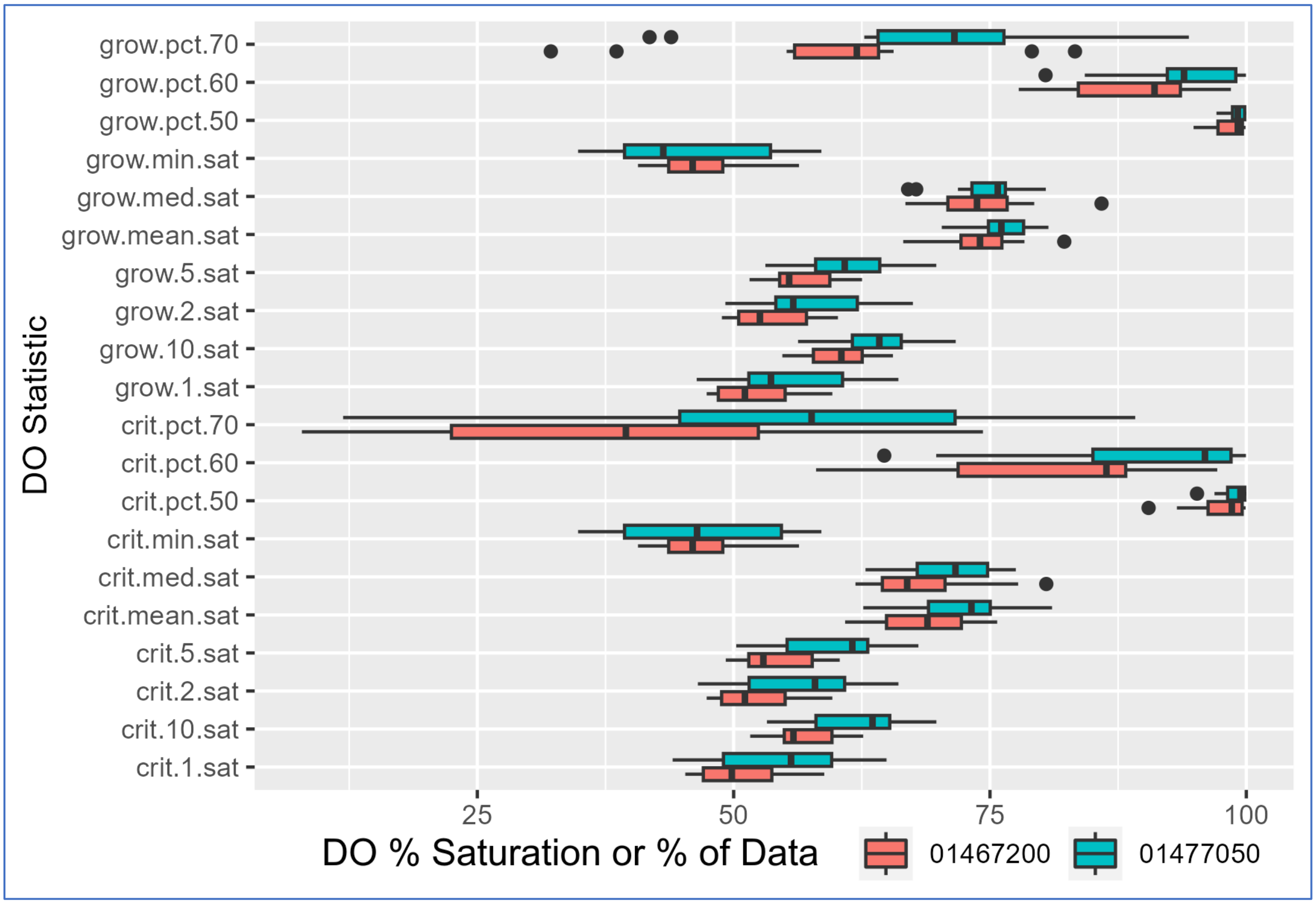

Figure 17.) Variability in DO condition metrics 2009-2022 for USGS Monitoring stations at Penn's Landing (01467200) and Chester, PA (01477050) in two seasonal periods

Outliers were observed for very high YOY growth rates in the year 2012 in which DNREC sampled mostly larger fish during the summer (Table 5). The year 2012 was excluded from the final correlation analysis as being unrepresentative compared to the rest of the data set, especially as the sample size for YOY was so small (n = 3), growth metrics were high outliers, and DO metrics were in the low range. To ensure that excluding 2012 did not bias the results, PWD performed correlation tests both with and without 2012 and did not find substantial differences in the results. The year 2009 was also an outlier with relatively high growth rates observed for YOY sturgeon. Although some 2009 samples were collected relatively early in the season, which would tend to increase estimates of growth rate calculated from fixed start dates, and theoretically the growth rate period observed would encapsulate a greater proportion of the most optimal warm growing conditions compared to fall and winter samples, PWD examined the growth rates and did not find sufficient justification to remove 2009 data from the correlation analysis.

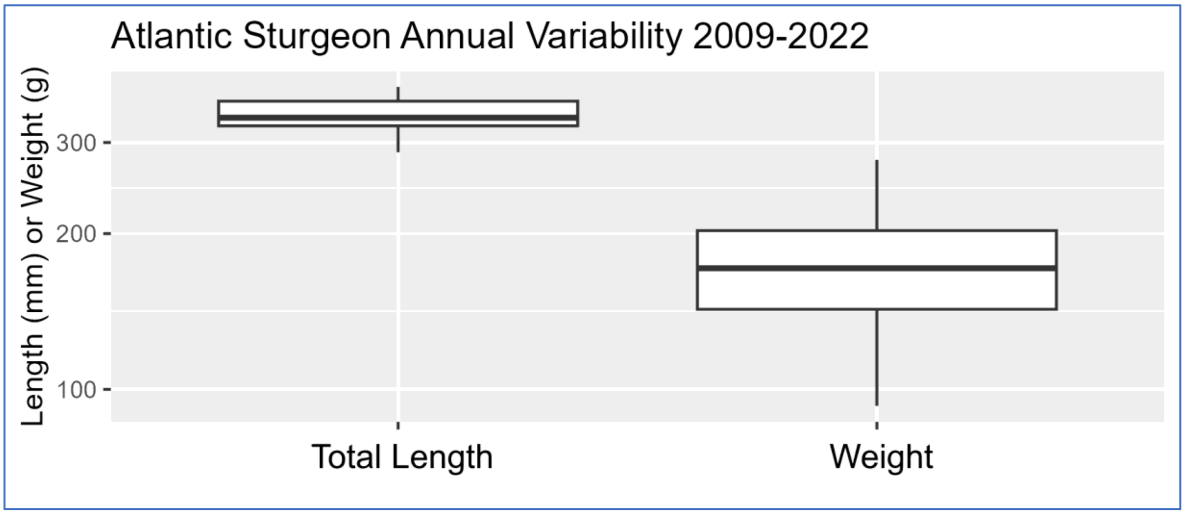

Figure 18.) Observed variability in YOY sturgeon total length and weight metrics used in DO-sturgeon growth correlation analysis

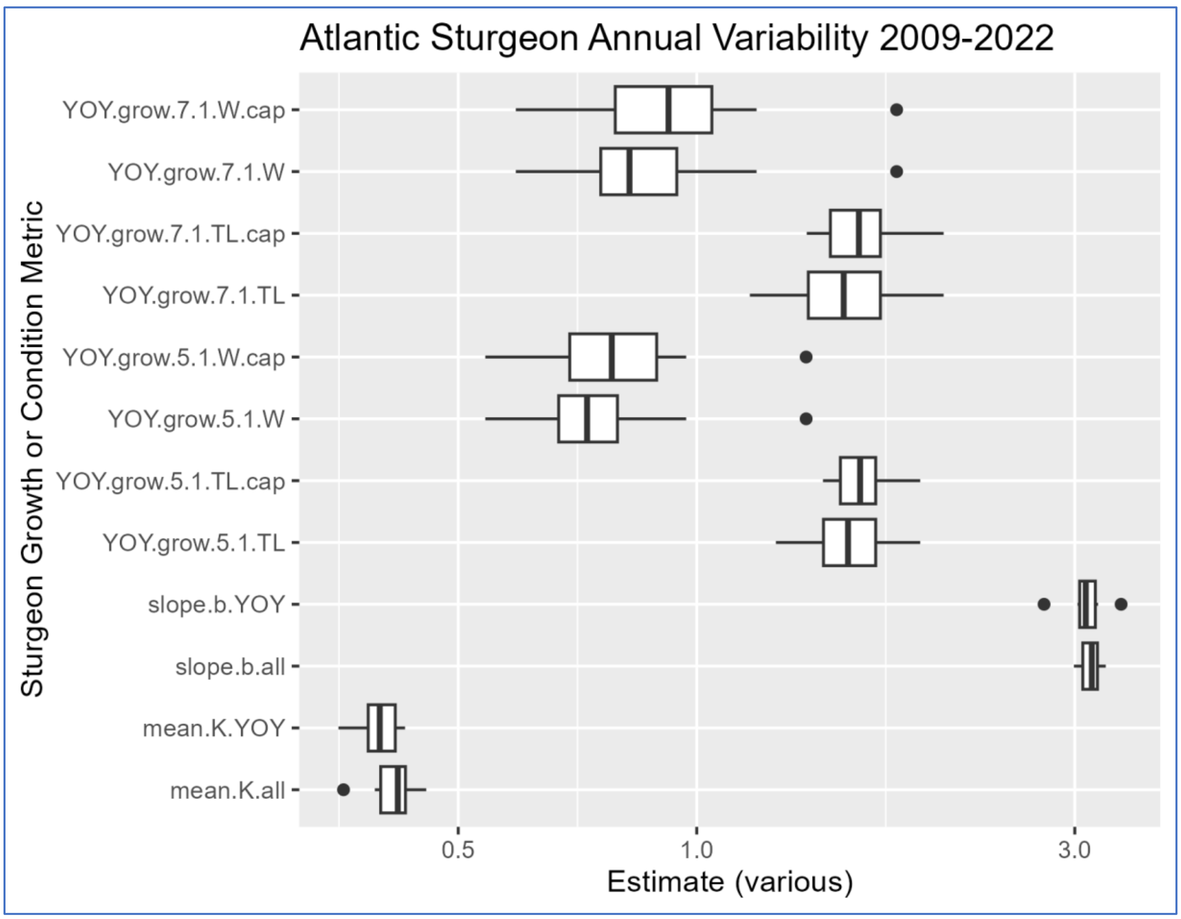

Figure 19.) Observed variability in sturgeon growth and condition metrics used in DO-sturgeon growth correlation analysis

PWD compared fish growth to different DO summary statistics for every year that large enough samples of fish were collected in a factorial design as shown in Table 14. The slope parameter b values from L-W regressions and mean condition factor K were computed separately for groups of all sturgeon collected in each combined cohort year and a subset of YOY sturgeon collected in each cohort year for comparison against DO statistics. Sturgeon response variables that were related to growth (*i.e.*, mean length, mean weight, growth as change in length, and growth as change in weight) were only evaluated for YOY. Overall, the factorial design resulted in 560 separate combinations of the factors DO Variable, USGS station, season, sturgeon response variable, and data set type (YOY or all data) (Table 14). Correlation was assessed using the nonparametric Spearman rank correlation coefficient, as it was not expected that the response between DO and a sturgeon response would be linear. The Spearman rank correlation coefficient evaluates whether the relationship between the two variables (here DO and sturgeon condition or growth) exhibits a monotonic trend.

Table 14) Conceptual factorial design of comparisons between DO statistics and measures of sturgeon growth and condition

Independent DO Variable		**USGS Station**		**Season**		**Dependent Variable**		**Data Set**
Minimum	X	Penn's Landing 01467200	X	Growing: May - Oct	X	Slope *b* parameter	X	YOY
1st percentile		Chester 01477050		Critical: Jul - Sep		Mean condition factor K		All data
2nd percentile						Mean length*		
5th percentile						Mean weight*		
10th percentile						Growth in length*		
Mean						Growth in weight*		
Median								
Percent > 50%								
Percent > 60%								
Percent > 70%								

* Denotes sturgeon growth parameters that were only evaluated for YOY.

With 560 comparisons made, it was expected that spurious results would occur due to random chance. Ordinarily when making a large number of comparisons, p values should be adjusted to take into account the likelihood of false positives, or the "family-wise error rate". With only 12 years of data, the statistical power of the correlation test to reject the null hypothesis that the correlation between the DO and sturgeon metrics is different from zero was also limited. Adjustments to the alpha level from the typical 0.05 to 0.1 and specification of the test as a one-sided test with the alternative hypothesis being "greater", (*i.e.*, positive) correlation would potentially help address the sample size issue, but relaxing the alpha value is inappropriate in the context of performing hundreds of tests.

With p values unadjusted for multiple comparisons, nine (<2%) of the 560 comparison correlation tests were significant at p <0.05, however these results were for spurious strong negative correlation between DO and sturgeon growth metrics at the Penn's Landing USGS station (Table 15). Only four (<1%) of the 560 correlation tests associated with positive correlation coefficients would have been statistically significant at p < 0.1 (Table 15).

Table 15.) Summary of DO-sturgeon metric correlation tests with significant negative correlation at p <0.05 or positive correlation test significant at p <0.1

USGS station	DO Statistic	Sturgeon metric	cor (rho)	statistic	p-value	method
1467200	crit.med.sat	YOY.grow.5.1.W	-0.75	384	0.0119	Spearman
1467200	crit.med.sat	YOY.grow.7.1.W	-0.75	384	0.0119	Spearman
1467200	crit.mean.sat	YOY.grow.5.1.W	-0.69	372	0.0231	Spearman
1467200	crit.mean.sat	YOY.grow.7.1.W	-0.69	372	0.0231	Spearman
1467200	crit.pct.70	YOY.grow.5.1.W	-0.65	362	0.037	Spearman
1467200	crit.pct.70	YOY.grow.7.1.W	-0.65	362	0.037	Spearman
1467200	grow.pct.70	YOY.grow.5.1.W	-0.65	362	0.037	Spearman
1467200	grow.pct.70	YOY.grow.7.1.W	-0.65	362	0.037	Spearman
1477050	crit.10.sat	YOY.grow.5.1.TL	-0.65	362	0.037	Spearman
Positive Correlations at p < 0.1						
1467200	crit.mean.sat	slope.b.all	0.57	94	0.0706	Spearman
1467200	crit.pct.50	slope.b.YOY	0.55	98	0.0816	Spearman
1467200	crit.min.sat	slope.b.YOY	0.53	102.7332	0.0913	Spearman
1467200	grow.min.sat	slope.b.YOY	0.53	102.7332	0.0913	Spearman

Approximately 35% (197 of 560) of the comparisons had a positive correlation coefficient, while 63% were negative (353 of 560; percentages do not exactly add to 100% because 10 correlation tests had correlation coefficient zero). The overall distribution of correlation coefficients and p values demonstrated that there was no statistically significant evidence for the hypothesis that DO levels in the Delaware River were correlated with the observed growth and condition metrics over the years 2009-2022 that were studied (Figure 20).

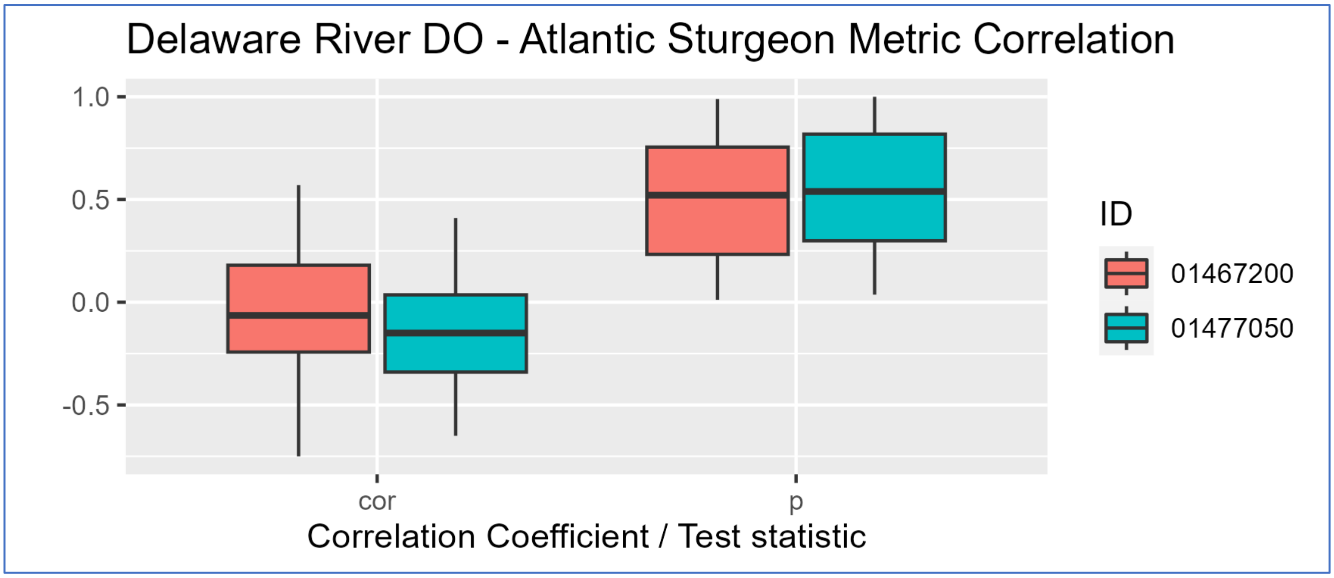

Figure 20.) Correlation coefficients and correlation test p-values for 560 Delaware River DO statistic vs Atlantic sturgeon growth and condition correlation comparisons 2009-2022 for USGS stations at Penn's Landing (01467200) and Chester, PA (01477050).

The strongest observed positive correlation between a DO statistic and a sturgeon growth response was for the comparison between the critical season (Jul-Sep) mean percent DO saturation with the slope parameter *b* of the L-W regression for all fish, with Spearman correlation coefficient 0.57 (Table 16, Figure 21). Three other DO statistics (critical season percent of DO values above 50% saturation, critical season minimum DO saturation, and growing season minimum DO saturation) also had positive correlations with the slope parameter *b* for YOY sturgeon, with Spearman correlation coefficients 0.53-0.55 (Table 17, Figure 22). Many other DO statistic comparisons for the slope parameter *b* of the L-W regression for all fish or for YOY sturgeon were not as strongly correlated (Tables 16 & 17, Figures 21 & 22). Additional plots of the correlation between DO statistics and Atlantic sturgeon growth and condition are presented in Appendix A Figures 1 through 12.

Table 16.) Summary Statistics for Spearman correlation tests for Delaware River seasonal DO statistics and Atlantic sturgeon L-W regression slope parameter *b*, 2009-2022

	1467200			1477050		
DO Statistic	**cor (rho)**	**statistic**	**p-value**	**cor (rho)**	**statistic**	**p-value**
crit.1.sat	0.11	196	0.755	-0.18	260	0.595
crit.10.sat	0.36	140	0.273	-0.082	238	0.818
crit.2.sat	0.1	198	0.776	-0.15	252	0.673
crit.5.sat	0.13	192	0.714	-0.16	256	0.634
crit.mean.sat	0.57	94	0.0706	0.14	190	0.694
crit.med.sat	0.42	128	0.203	0.027	214	0.946
crit.min.sat	0.087	201	0.8	0	220	1
crit.pct.50	0.23	170	0.503	-0.24	272	0.485
crit.pct.60	0.46	118	0.154	-0.055	232	0.881
crit.pct.70	0.5	110	0.121	-0.0091	222	0.989
grow.1.sat	0.12	194	0.734	-0.2	264	0.558
grow.10.sat	0.41	130	0.214	-0.018	224	0.968
grow.2.sat	0.19	178	0.576	-0.2	264	0.558
grow.5.sat	0.37	139	0.264	-0.0091	222	0.989
grow.mean.sat	0.33	148	0.327	0.32	150	0.341
grow.med.sat	0.27	160	0.418	0.045	210	0.903
grow.min.sat	0.087	201	0.8	-0.027	226	0.946
grow.pct.50	0.17	182	0.614	-0.39	306	0.237
grow.pct.60	0.45	120	0.163	-0.082	238	0.818
grow.pct.70	0.43	126	0.193	0.045	210	0.903

Table 17.) Summary Statistics for Spearman correlation tests for Delaware River seasonal DO statistics and YOY Atlantic sturgeon L-W regression slope parameter *b*, 2009-2022

	Penn's Landing (1467200)			Chester (1477050)		
DO Statistic	**cor (rho)**	**statistic**	**p-value**	**cor (rho)**	**statistic**	**p-value**
crit.1.sat	0.43	126	0.193	-0.18	260	0.595
crit.10.sat	0.49	112	0.129	-0.064	234	0.86
crit.2.sat	0.44	124	0.183	-0.2	264	0.558
crit.5.sat	0.46	118	0.154	-0.15	254	0.654
crit.mean.sat	0.34	146	0.313	0.018	216	0.968
crit.med.sat	0.24	168	0.485	-0.13	248	0.714
crit.min.sat	0.53	102.7332	0.0913	-0.091	240	0.797
crit.pct.50	0.55	98	0.0816	-0.27	280	0.418
crit.pct.60	0.53	104	0.1	-0.15	252	0.673
crit.pct.70	0.32	150	0.341	-0.15	254	0.654
grow.1.sat	0.47	116	0.146	-0.29	284	0.386
grow.10.sat	0.38	136	0.248	-0.045	230	0.903
grow.2.sat	0.52	106	0.107	-0.29	284	0.386
grow.5.sat	0.5	109.7491	0.116	-0.082	238	0.818
grow.mean.sat	0.25	164	0.451	0.34	146	0.313
grow.med.sat	0.027	214	0.946	0.15	188	0.673
grow.min.sat	0.53	102.7332	0.0913	-0.073	236	0.839
grow.pct.50	0.48	114	0.137	-0.35	296	0.299
grow.pct.60	0.51	108	0.114	-0.26	278	0.435
grow.pct.70	0.17	182	0.614	0.018	216	0.968

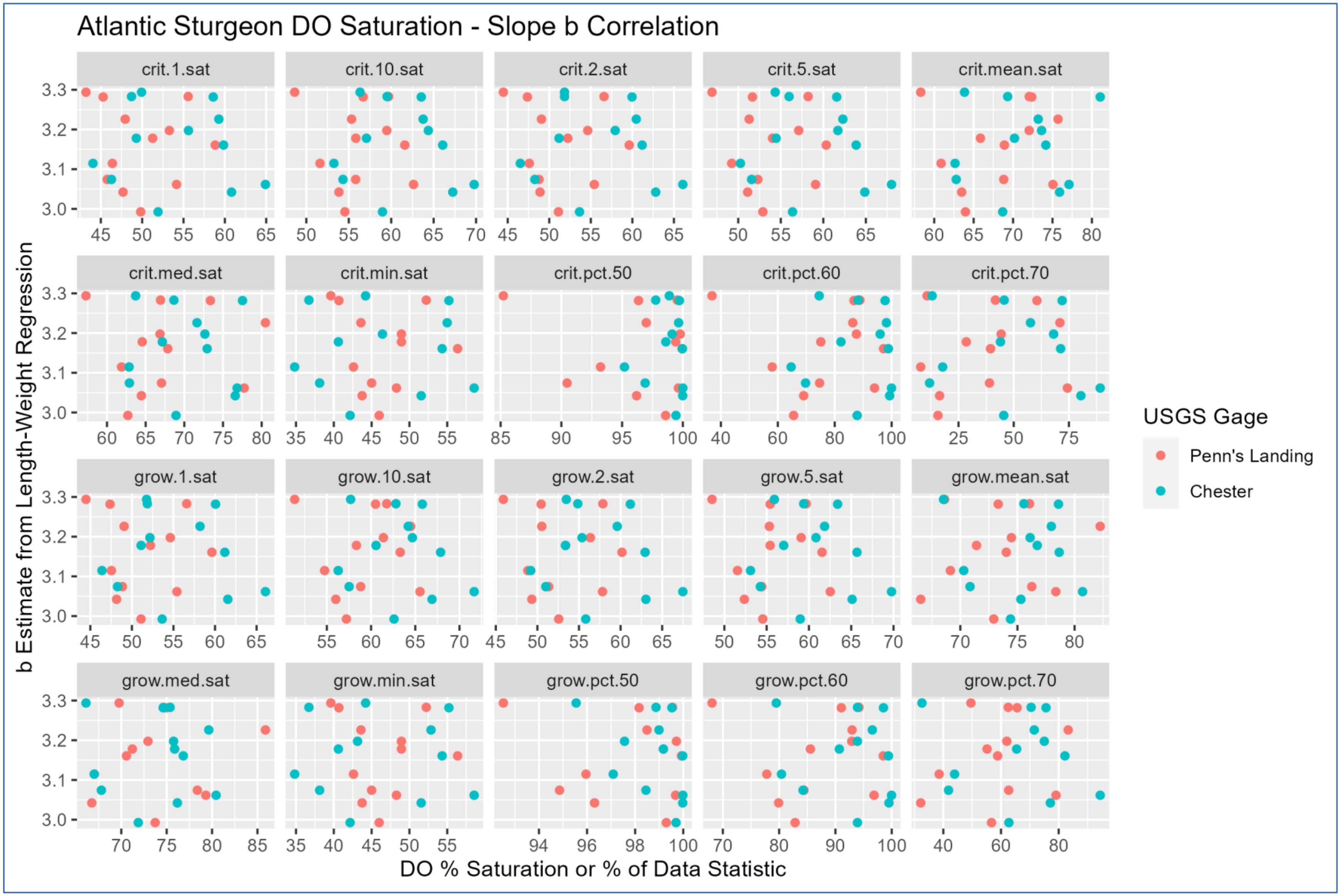

Figure 21.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon L-W regression slope parameter *b*, 2009-2022

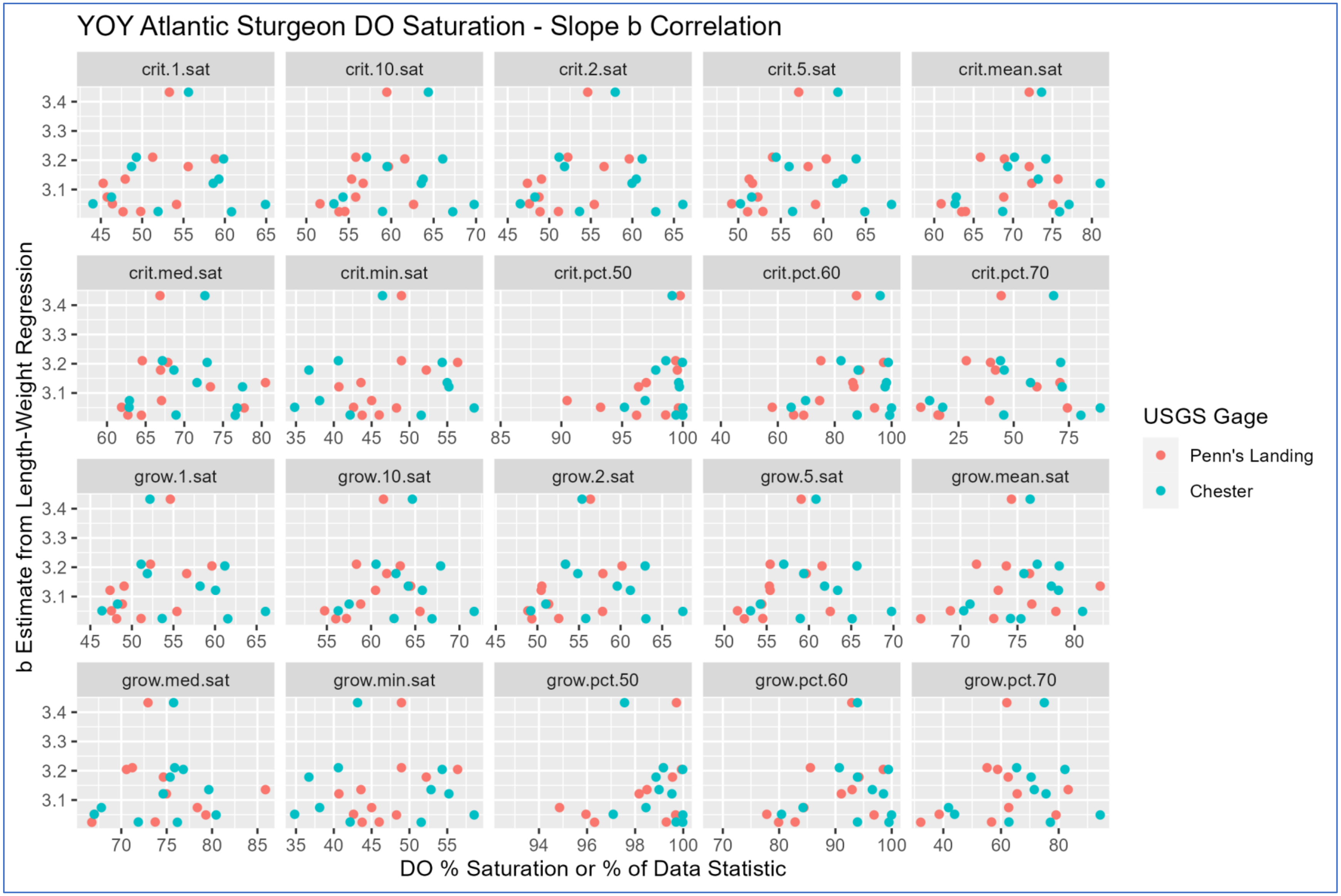

Figure 22.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon L-W regression slope parameter *b*, 2009-2022

9.) Many growth and condition characteristics of the Delaware River Atlantic sturgeon population are similar or compare favorably to those for Atlantic sturgeon collected in the Hudson River, which typically has higher DO levels than the Delaware River and is considered attaining water quality standards for DO.

PWD compiled and analyzed more than 5,000 recent juvenile sturgeon collection records from the New York Hudson River. The Hudson River supports reproduction of both Atlantic and shortnose sturgeons. The Hudson River stock has been described as the largest extant group of Atlantic sturgeon, not only in the New York Bight Distinct Population Segment, but in the entire U.S. PWD obtained Hudson River sturgeon data from the New York State Department of Environmental Conservation (NYSDEC) Gillnet Juvenile Relative Abundance Survey (2003-2022) and Hudson River Generators Fall Shoals Survey (FSS) Annual Reports from 2001-2020. CPUE from the NYSDEC relative abundance survey has varied over the past two decades (Figure 22). DO levels measured in the Hudson River at Haverstraw and Newburgh Bays are consistently greater than 5mg/L (Sweka *et al.* 2007) and have remained relatively constant over the past 40-50 years (TI 1976).

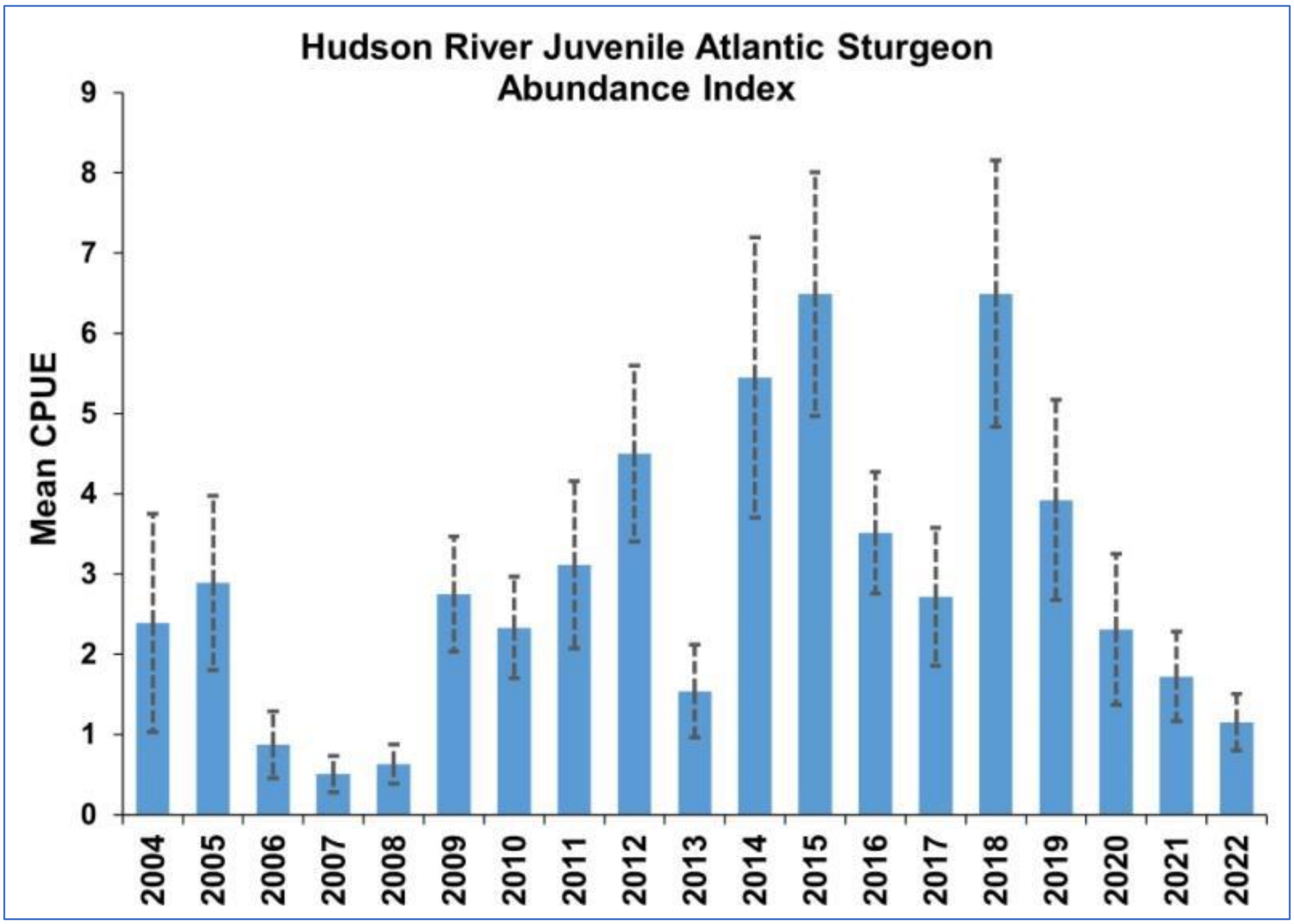

Figure 22.) NYSDEC Hudson River Juvenile Atlantic Sturgeon Abundance index 2004-2022. (https://dec.ny.gov/nature/animals-fish-plants/hudson-delaware-marine-fisheries/atlantic-sturgeon)

There was a significant difference in fish growth observed by comparing the slope *b* estimates from log(weight)-log(length) linear regression models for Delaware and Hudson River fish, with the Delaware River having a steeper slope when all fish from both river systems were included in the analysis (p = 0.001, Figure 23, Table 17). The difference in slopes was small, but with such large sample sizes (11,083 fish total) even small differences can be detected. As the Hudson River program primarily sampled juvenile sturgeons greater than ~500 mm, PWD also compared the growth patterns of only yearling or larger fish (> 500 mm) between the two systems, finding no significant differences (Figure 24, Table 18).

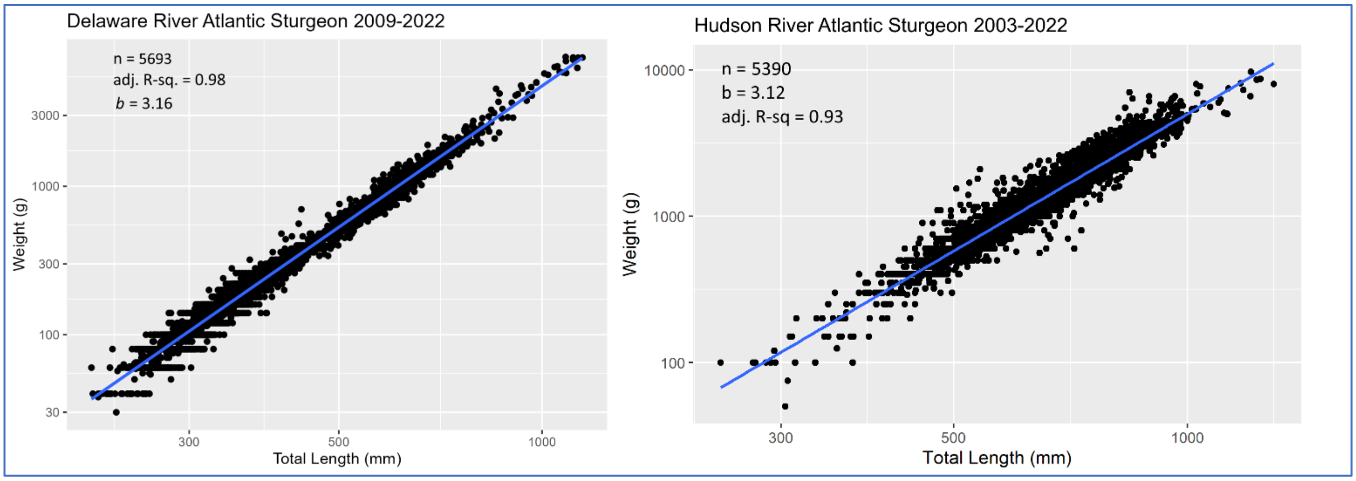

Figure 23.) Weight-Length Relationships for Atlantic sturgeon collected from the Delaware River (n = 5693) and Hudson River (n = 5390).

Table 17.) ANOVA results for L-W regression slope *b* estimates for Delaware and Hudson River Atlantic sturgeon using all fish in data set

Effect	DFn	DFd	F Statistic	p-value	p<.05	Effect size (GES)
log.tl	1	10972	288256.4	0	*	0.963
river	1	10972	380.115	2.99E-83	*	3.30E-02
log.tl:river	1	10972	10.443	0.001	*	0.000951

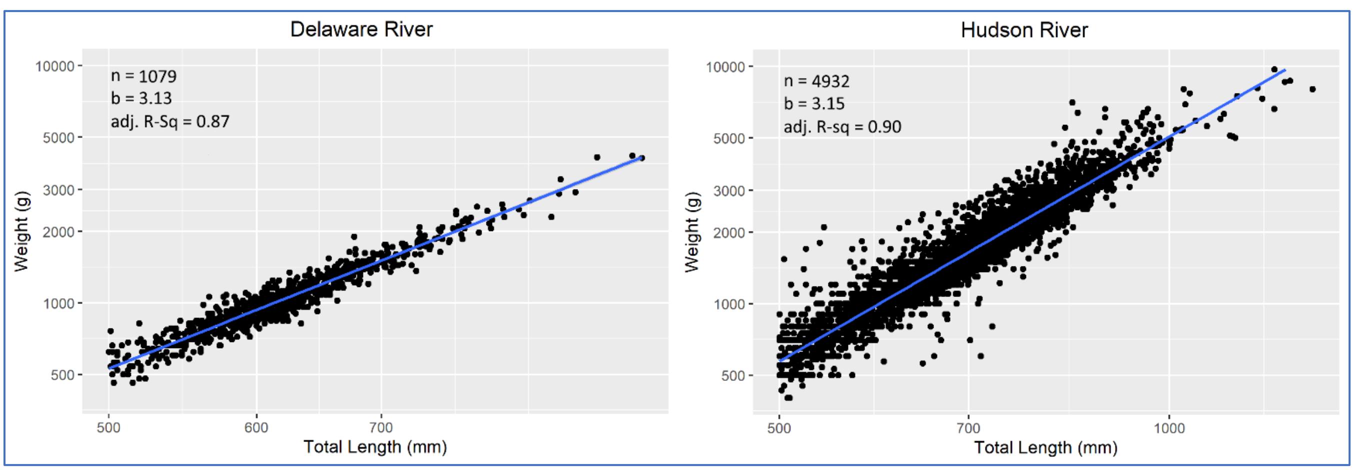

Figure 24.) Weight-Length Relationships for Yearling (500 mm) or larger Atlantic sturgeon collected from the Delaware River (n = 1079) and Hudson River (n = 4932).

Table 18.) ANOVA results for L-W regression slope *b* estimates for yearling (>500mm) Delaware and Hudson River Atlantic sturgeon

Effect	DFn	DFd	F Statistic	p-value	p<.05	Effect size (GES)
log.tl	1	5951	57876.7	0	*	0.907
river	1	5951	238.42	9.08E-53	*	0.039
log.tl:river	1	5951	3.156	0.076		0.00053

Table 19.) ANOVA results for L-W regression slope b estimates for YOY Delaware and Hudson River Atlantic sturgeon

Effect	DFn	DFd	F Statistic	p-value	p<.05	Effect size (GES)
log.tl	1	5001	49282.47	0	*	0.908
river	1	5001	148.065	1.36E-33	*	2.90E-02
log.tl:river	1	5001	0.48	0.488		9.60E-05

When compared on an annual basis for all size fish, The Delaware River had significantly higher slope parameter b estimate values than the Hudson River (Wilcox test p= 0.004, Table 20, Figure 25). The test for YOY fish did not find a statistically significant difference between the Delaware and Hudson Rivers (Table 21 Figure 25)

Table 20.) Summary of Wilcox test for L-W regression slope *b* parameter estimates for Delaware and Hudson River Atlantic sturgeon using all fish in the data set

estimate	group1	group2	n1	n2	statistic	p	conf.low	conf.high	method	alternative
0.13	Delaware	Hudson	12	20	193	0.004	0.04	0.21	Wilcoxon	two.sided

Table 21.) Summary of Wilcox test for L-W regression slope *b* parameter estimates for YOY Atlantic sturgeon from the Delaware and Hudson Rivers

estimate	group1	group2	n1	n2	statistic	P	conf.low	conf.high	method	alternative
0.09	Delaware	Hudson	12	20	165	0.08	-0.02	0.22	Wilcoxon	two.sided

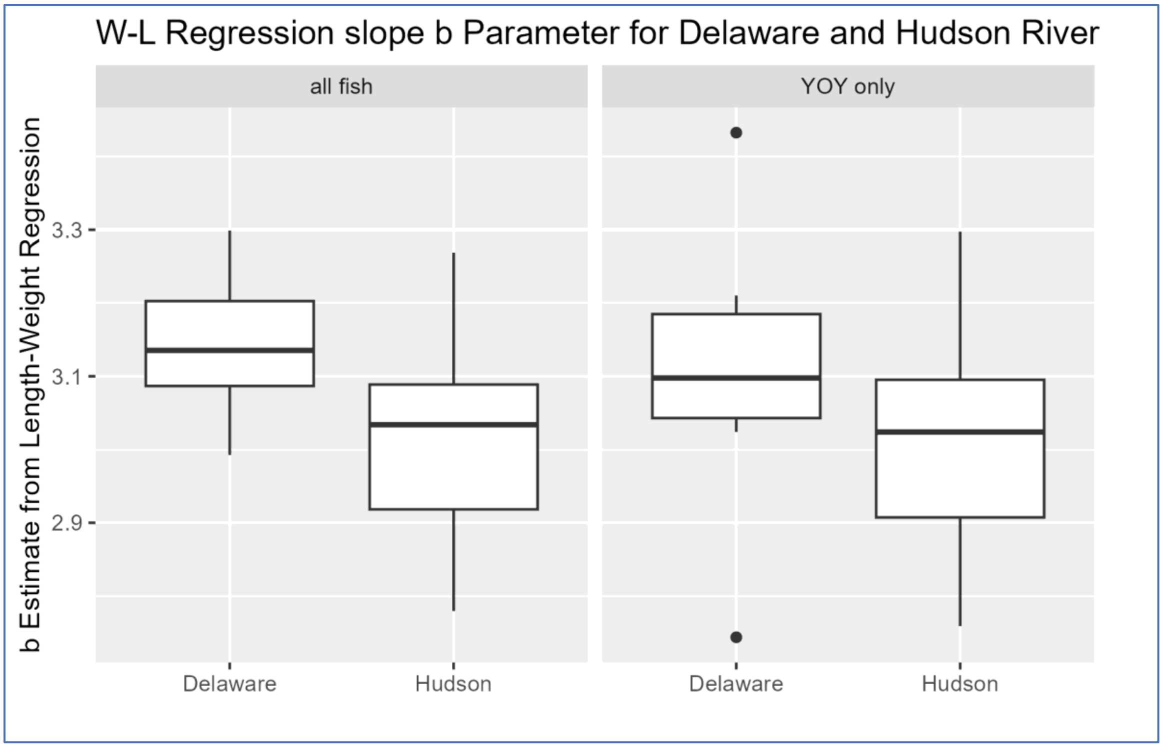

Figure 25.) Comparison of annual L-W regression slope parameter *b* estimates from Delaware and Hudson Rivers.

__Summary__

PWD compiled and evaluated several lines of evidence for the strength of the association between hypoxia and measures of Atlantic sturgeon condition and health in the Delaware River and made the following conclusions:

- Delaware River Atlantic sturgeon collected 2009-2022 showed no evidence of reduced growth (size) that would indicate poor health. Observed length for each YOY cohort was in the range of expected values, consistent with the Delaware River's latitude and other spawning populations on the Atlantic coast.
- Inferred growth rates based on estimated spawning date are in the expected range and are corroborated by observed mark-recapture empirical growth rates.
- YOY Atlantic sturgeon consistently attain lengths of more than 300mm (345mm or 13.5 inches on average) due to apparent rapid growth during the summer juvenile development period. This level of growth was observed every year 2009-2022 for which there was adequate sampling effort. Mean length of YOY was not correlated with any statistics of DO conditions.
- The full data set, all YOY, and 23 of 25 annual data sets had positive allometric growth ($b > 3$). The annual slope parameter b estimates ($n = 12$) were not significantly correlated with DO conditions.
- Despite performing 560 separate correlation tests across a large range of observed DO statistics and sturgeon metrics, PWD found no statistically significant evidence for correlation between DO and sturgeon growth or condition.
- Measures of growth and condition are similar between the Delaware and Hudson Rivers. The Hudson River has favorable DO and exhibits interannual variability in sturgeon abundance.

Based on the strength of these six lines of evidence and the lack of any substantial evidence to the contrary, PWD concludes that hypoxia was not an important stressor to Atlantic sturgeon in 2009-2022 as evaluated with the best available scientific data. Existing DO levels supported sturgeon propagation and growth.

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Appendix A: Correlation Analyses for Observed DO Conditions and Atlantic Sturgeon Measures of Growth and Condition

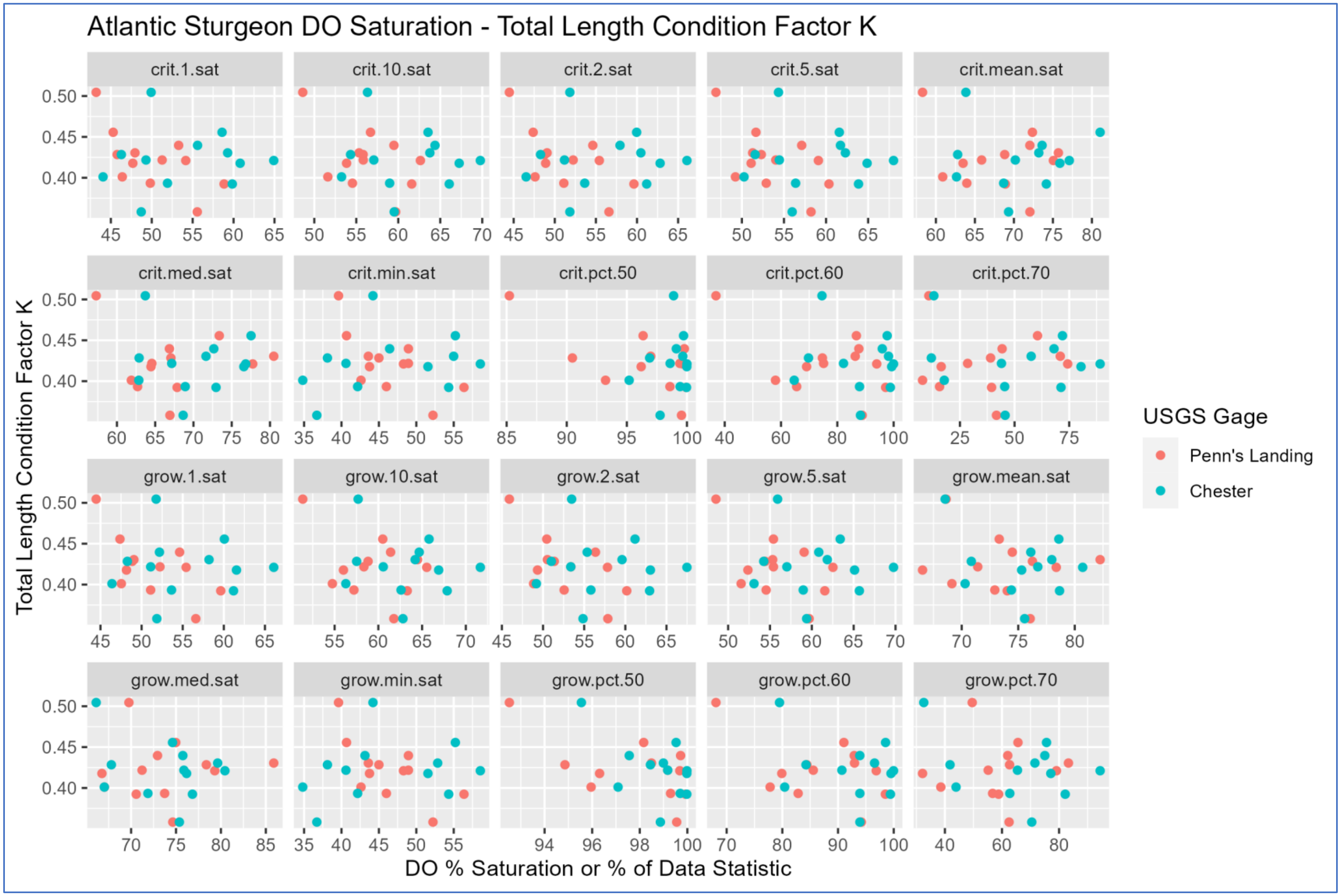

Figure 1.) Correlation for Delaware River seasonal DO statistics and Atlantic sturgeon total length condition factor K, 2009-2022

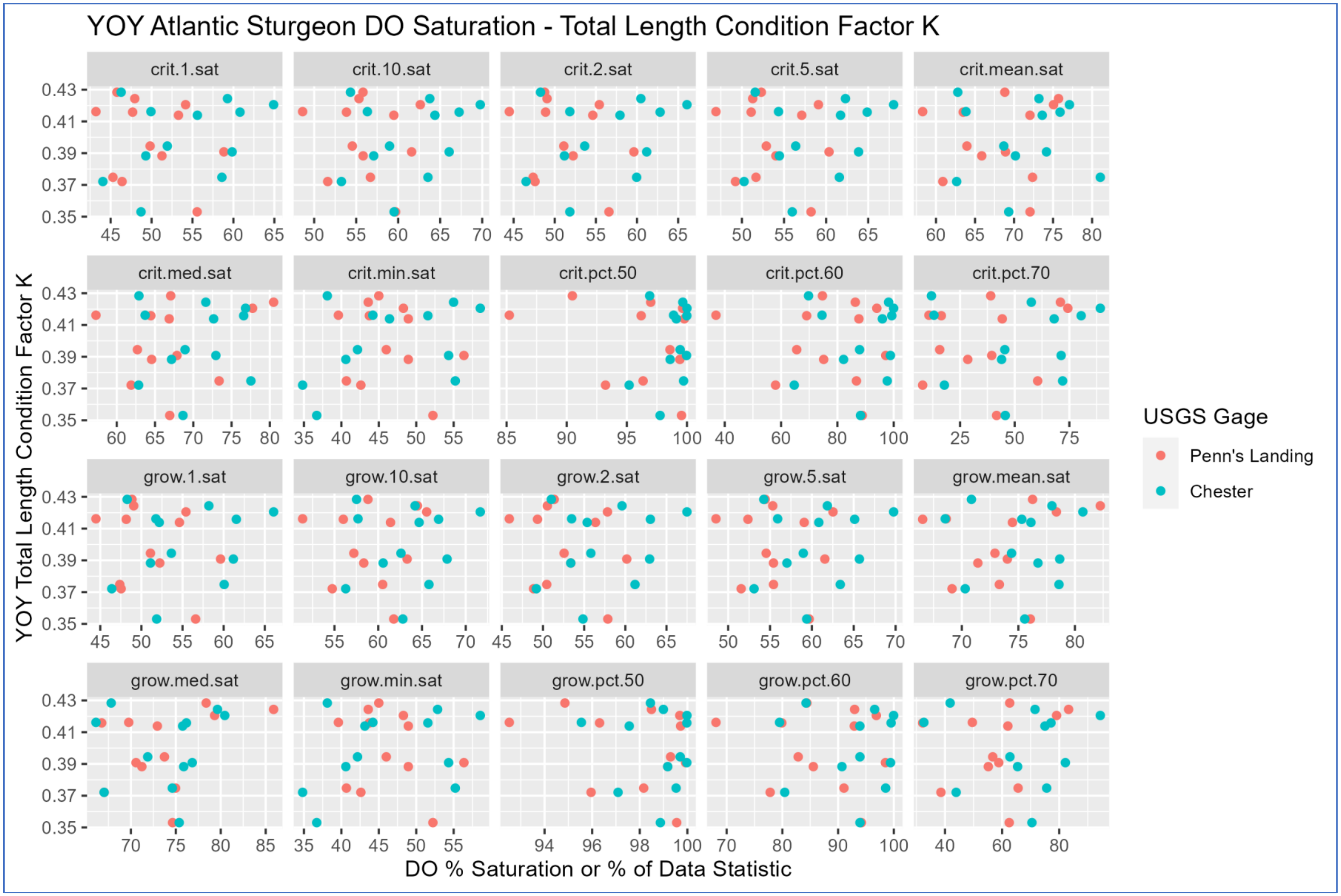

Figure 2.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon total length condition factor K, 2009-2022

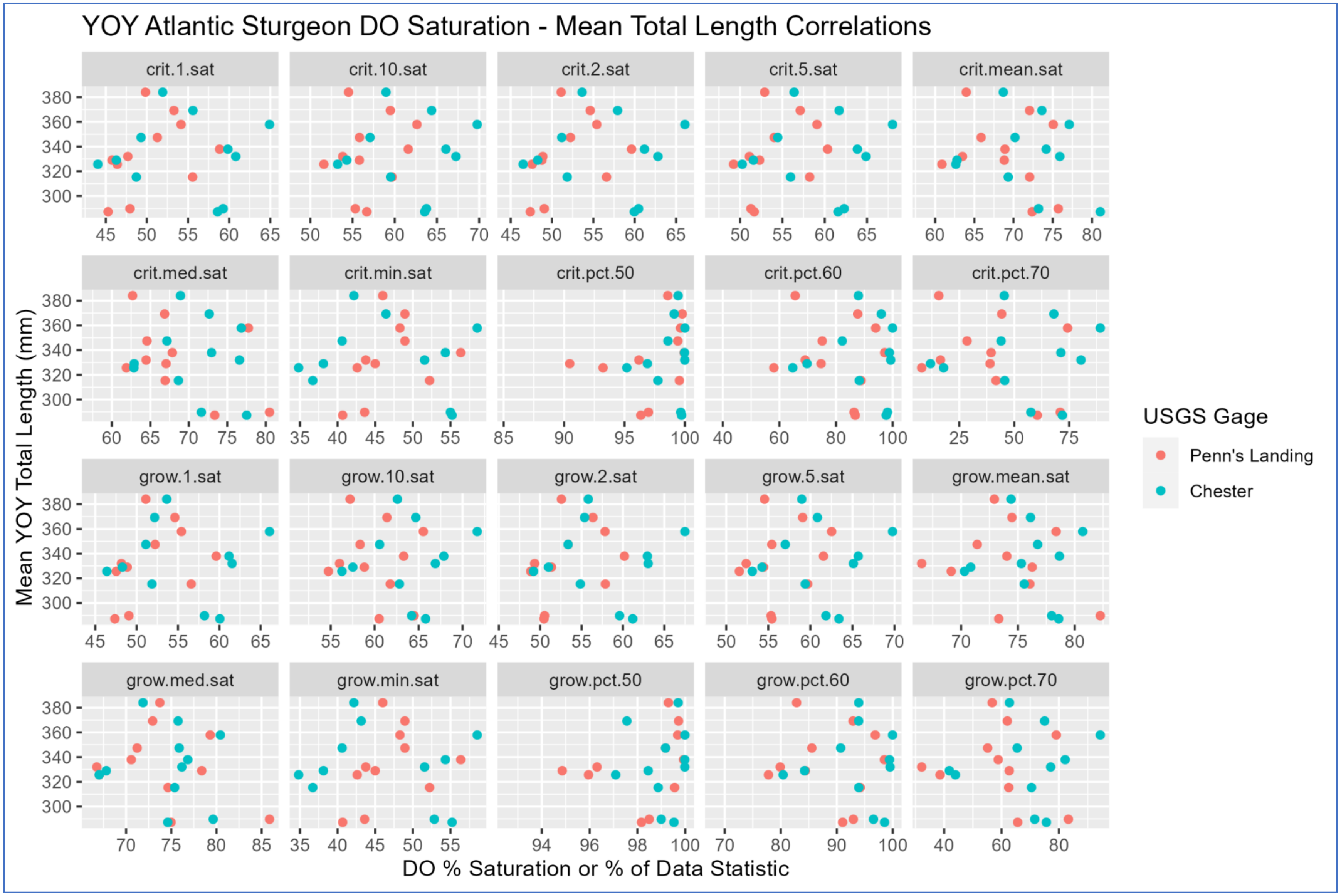

Figure 3.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon mean total length, 2009-2022

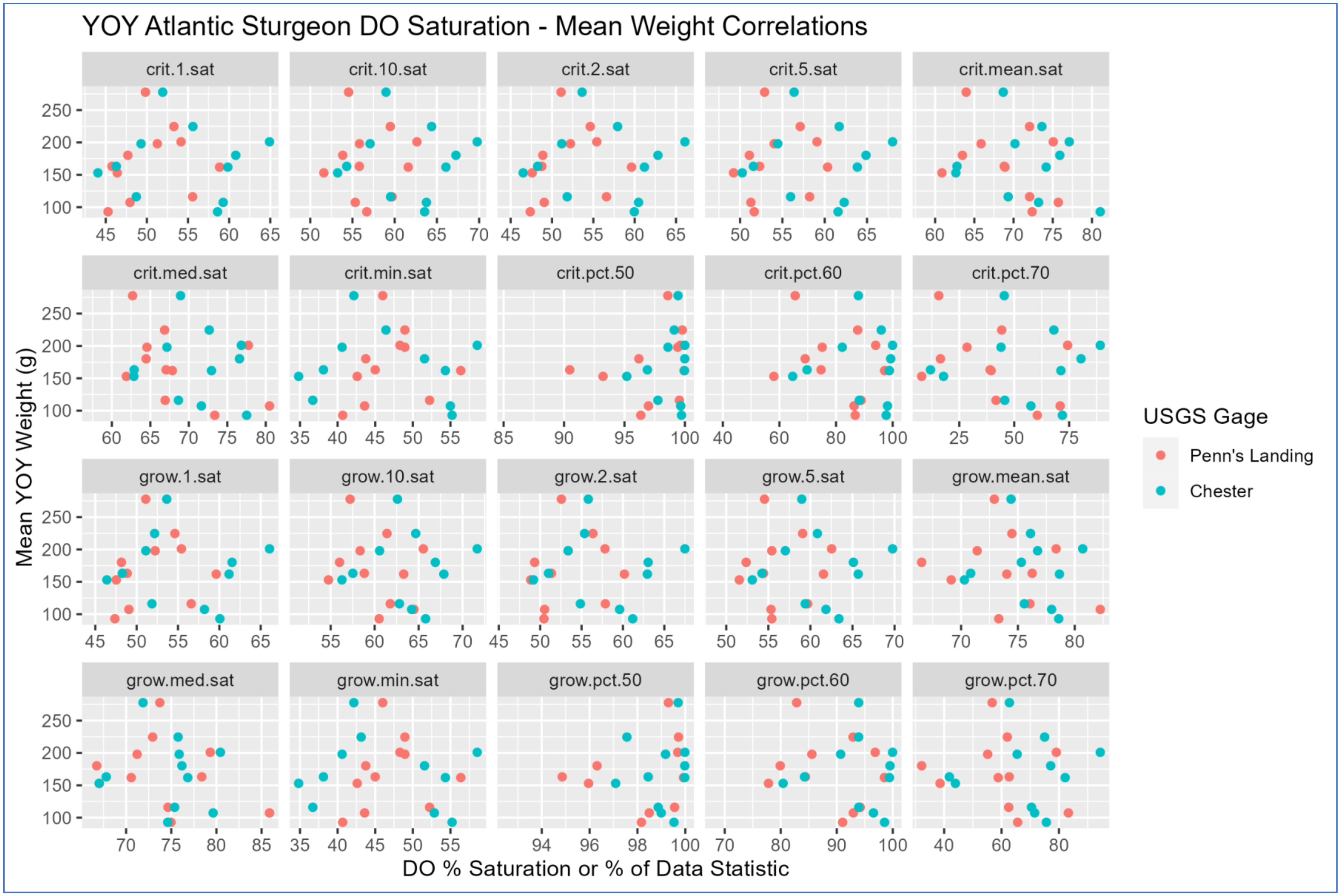

Figure 4.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon mean weight, 2009-2022

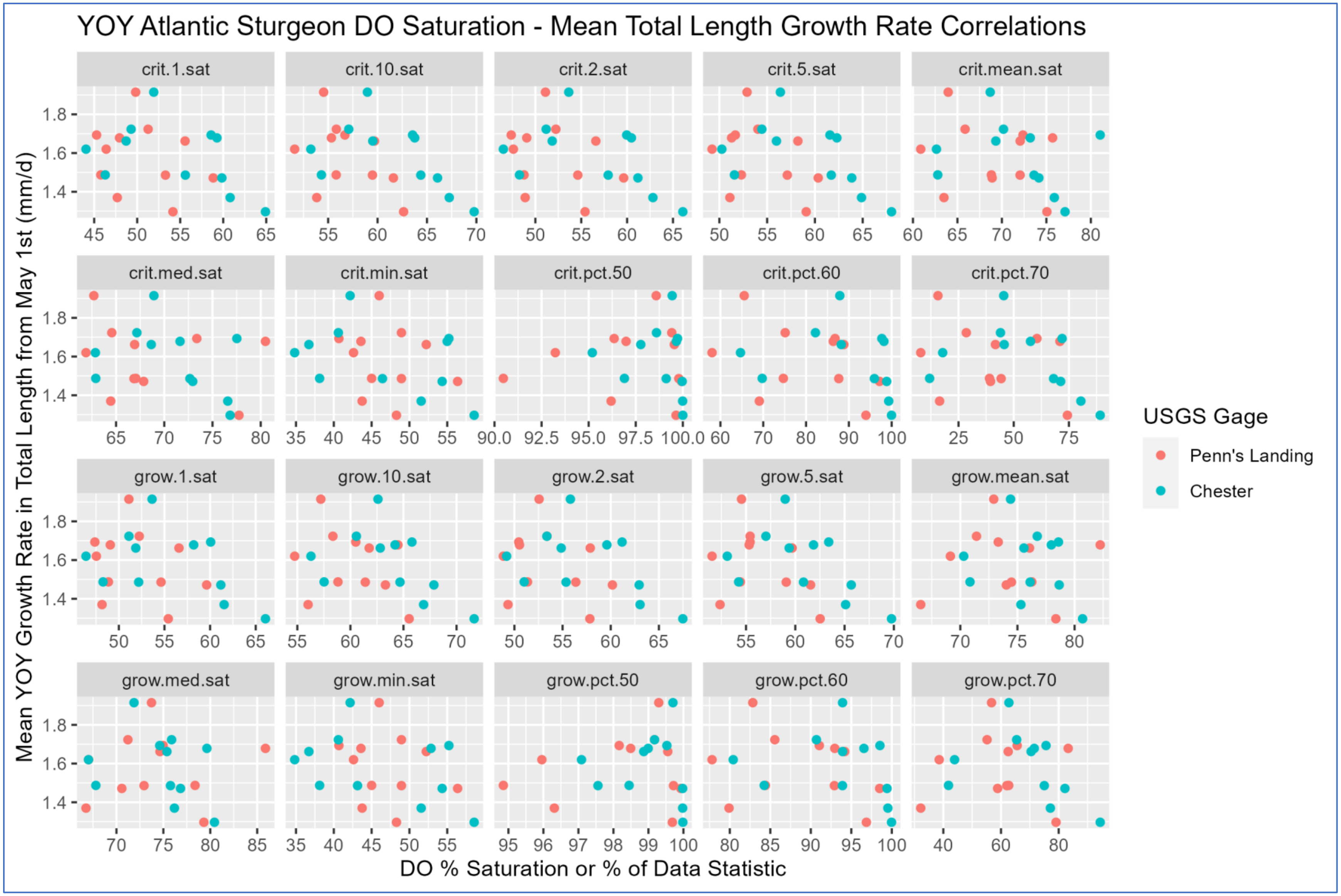

Figure 5.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon mean growth in length from May 1st, 2009-2022

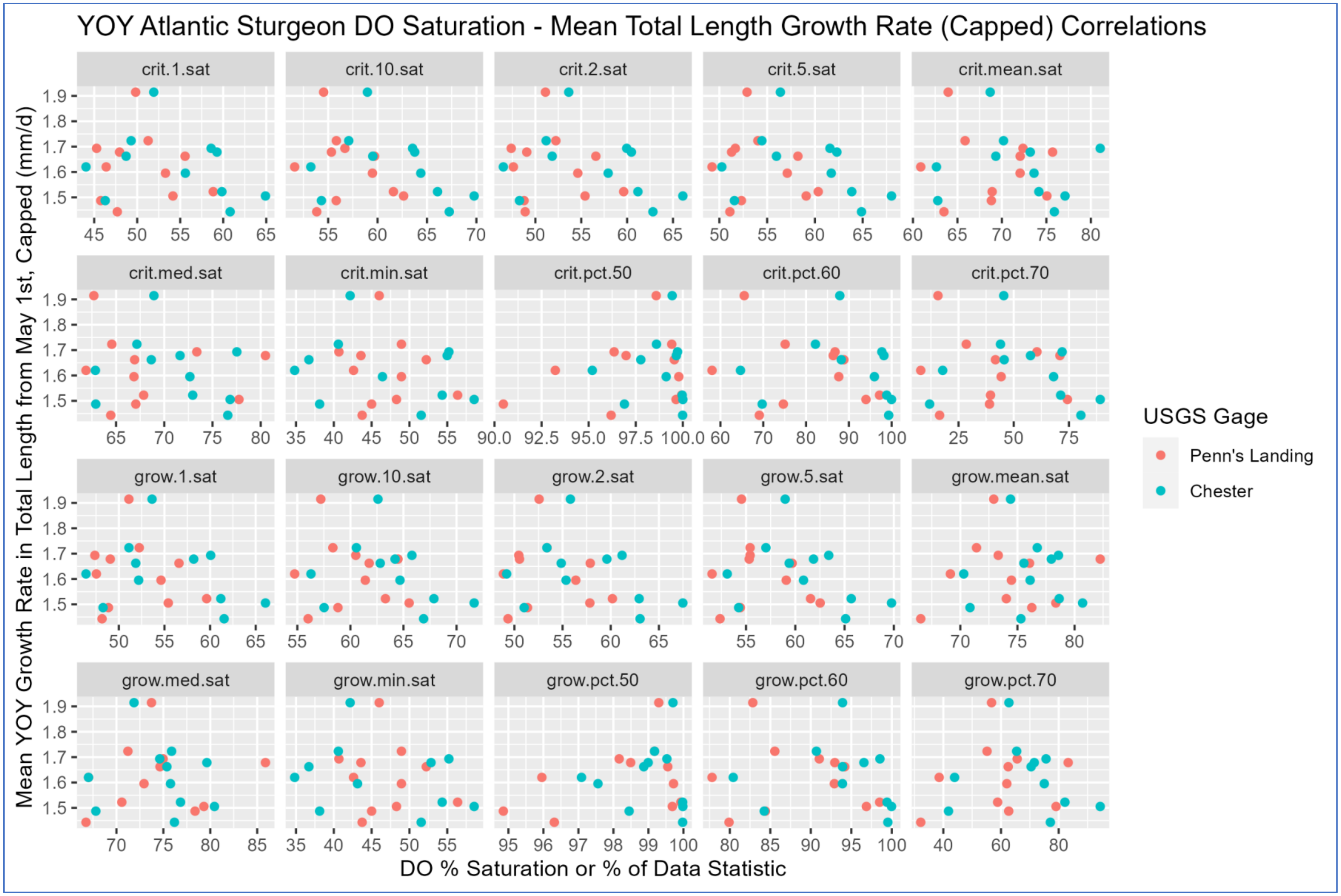

Figure 6.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon mean growth in length from May 1st, "capped" assuming winter growth was complete by Jan 1st, 2009-2022

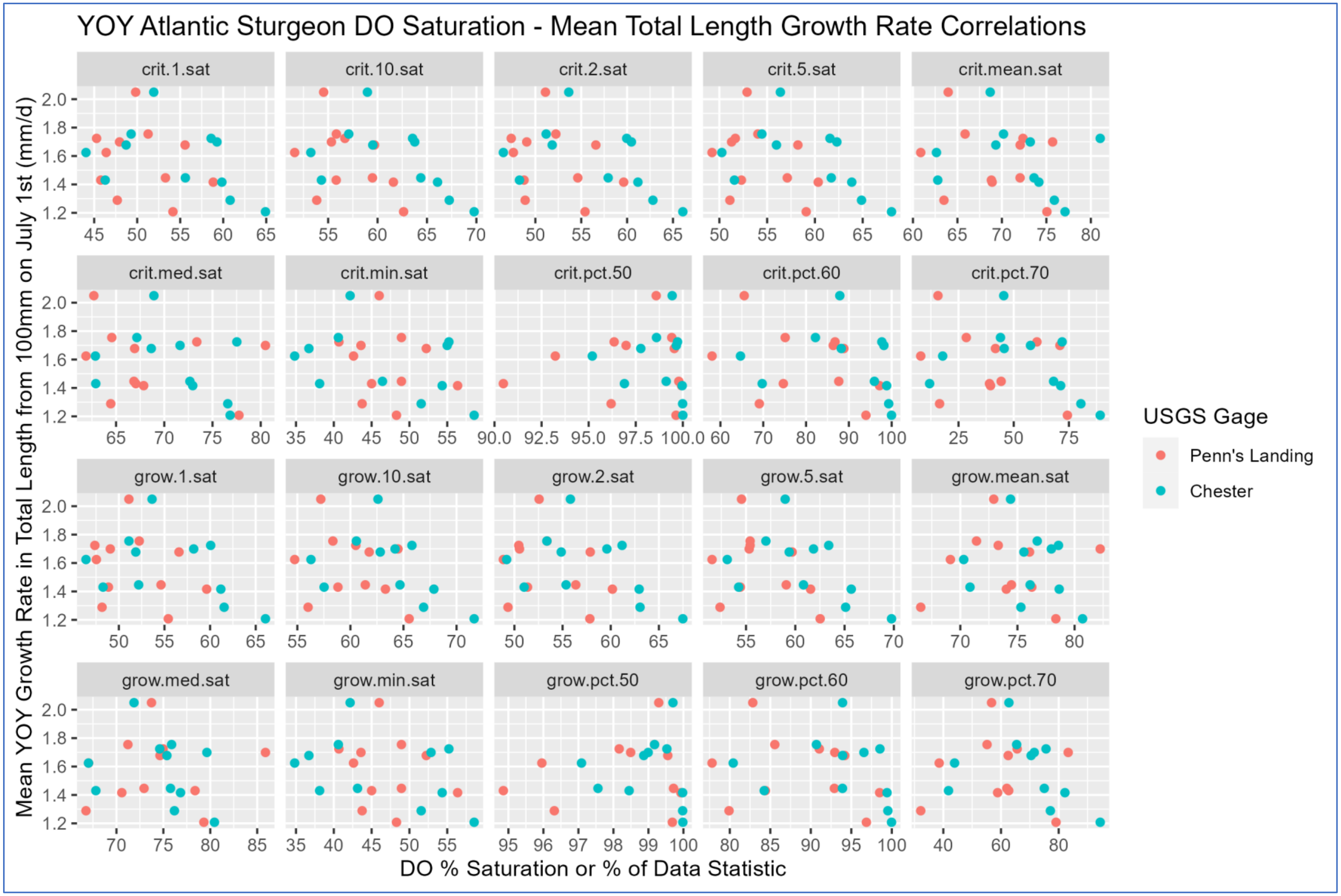

Figure 7.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon mean growth in length assuming fish were 100mm on July 1st, 2009-2022

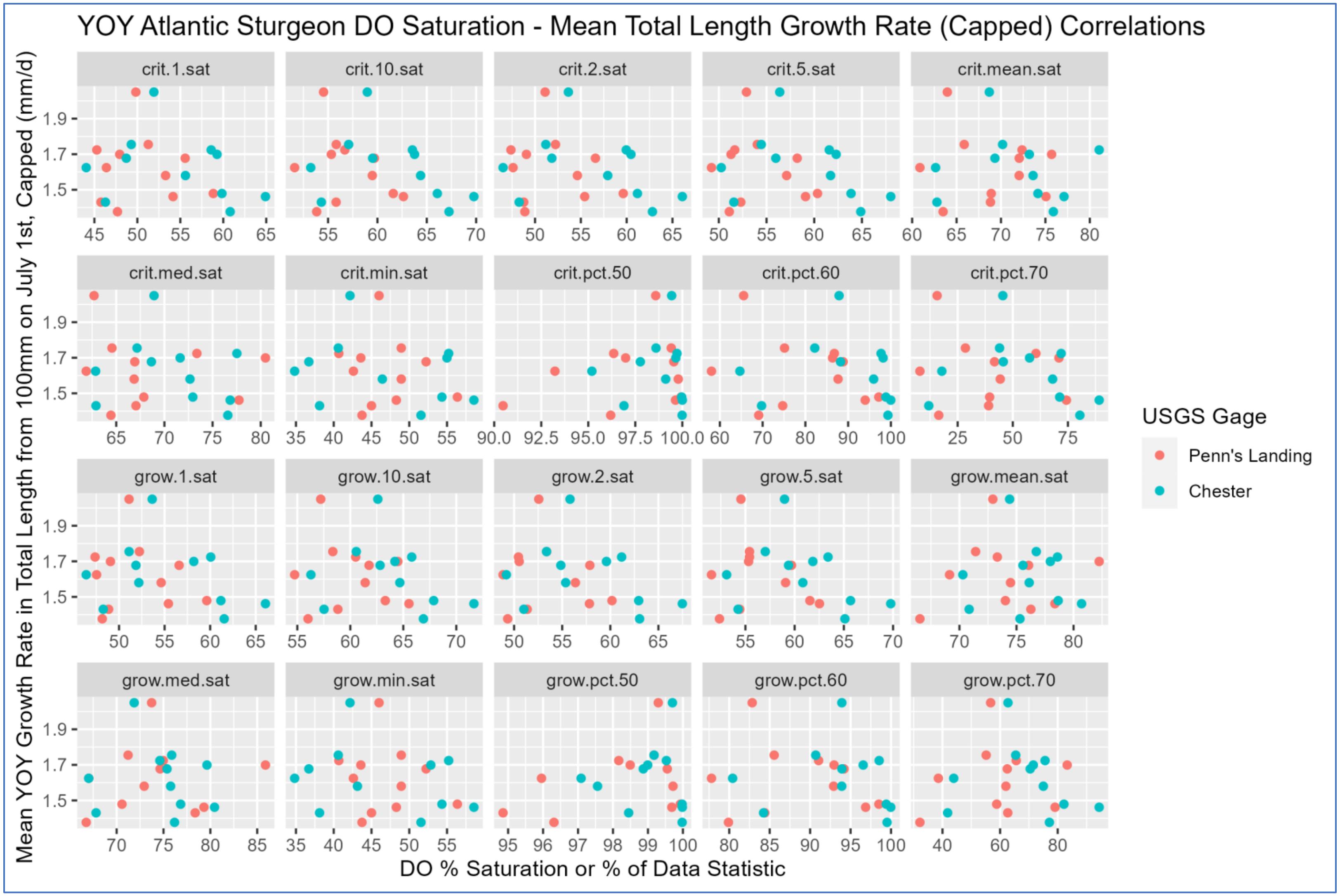

Figure 8.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon mean growth in length assuming fish were 100mm on July 1st, "capped" assuming winter growth was complete by Jan 1st, 2009-2022

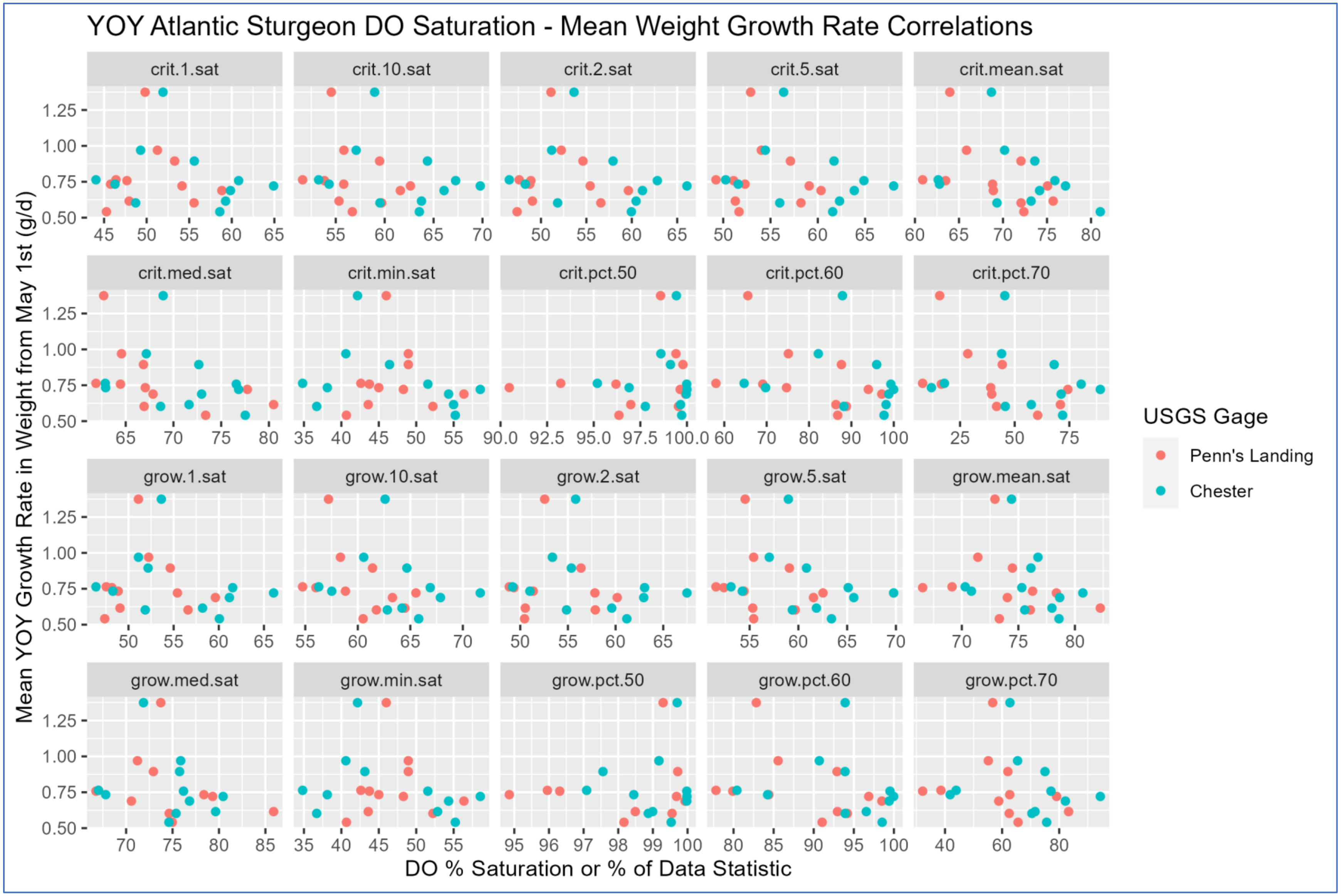

Figure 9.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon mean growth in weight from May 1st, 2009-2022

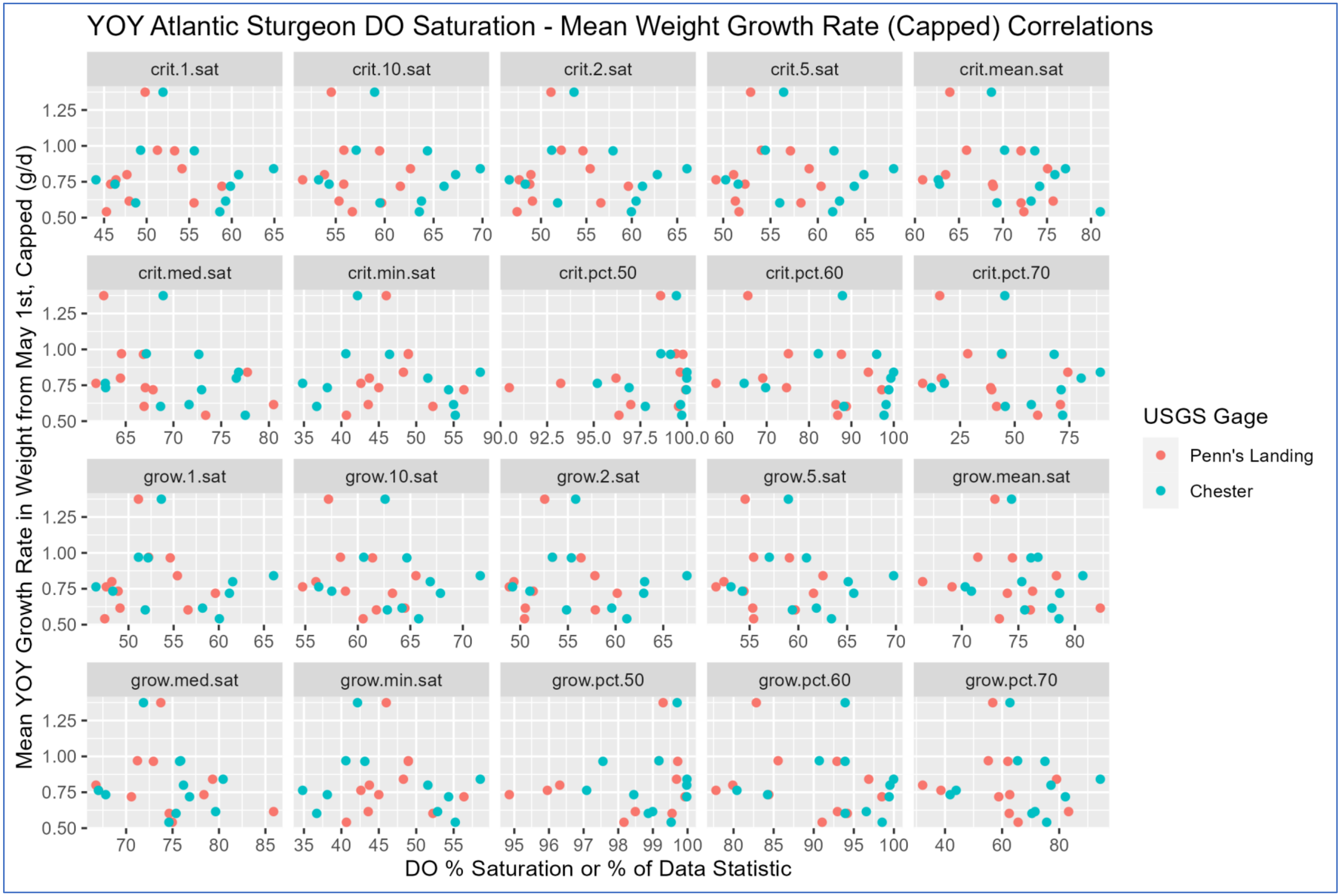

Figure 10.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon mean growth in weight from May 1st, "capped" assuming winter growth was complete by Jan 1st, 2009-2022

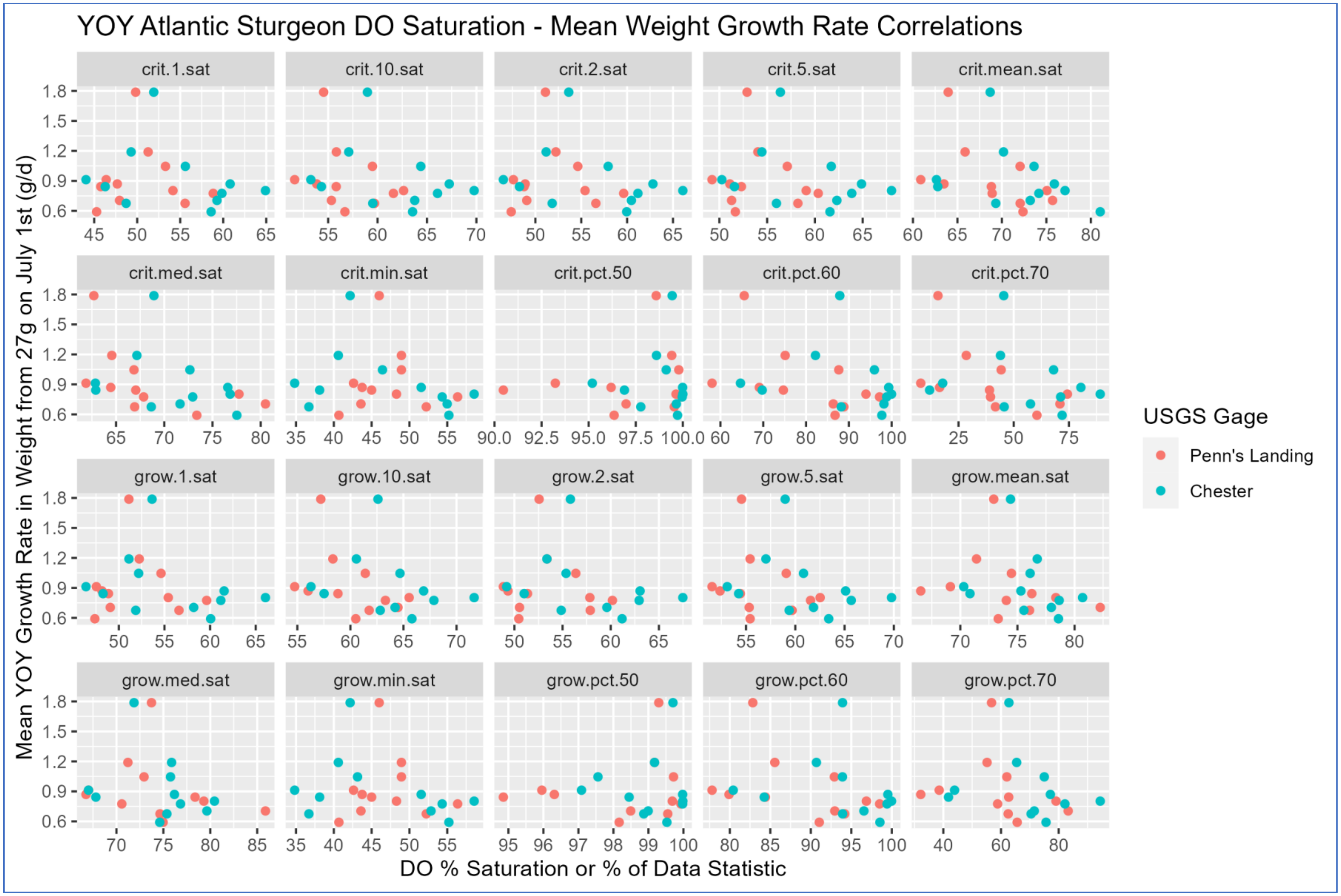

Figure 11.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon mean growth in weight assuming fish were 27g on July 1st, 2009-2022

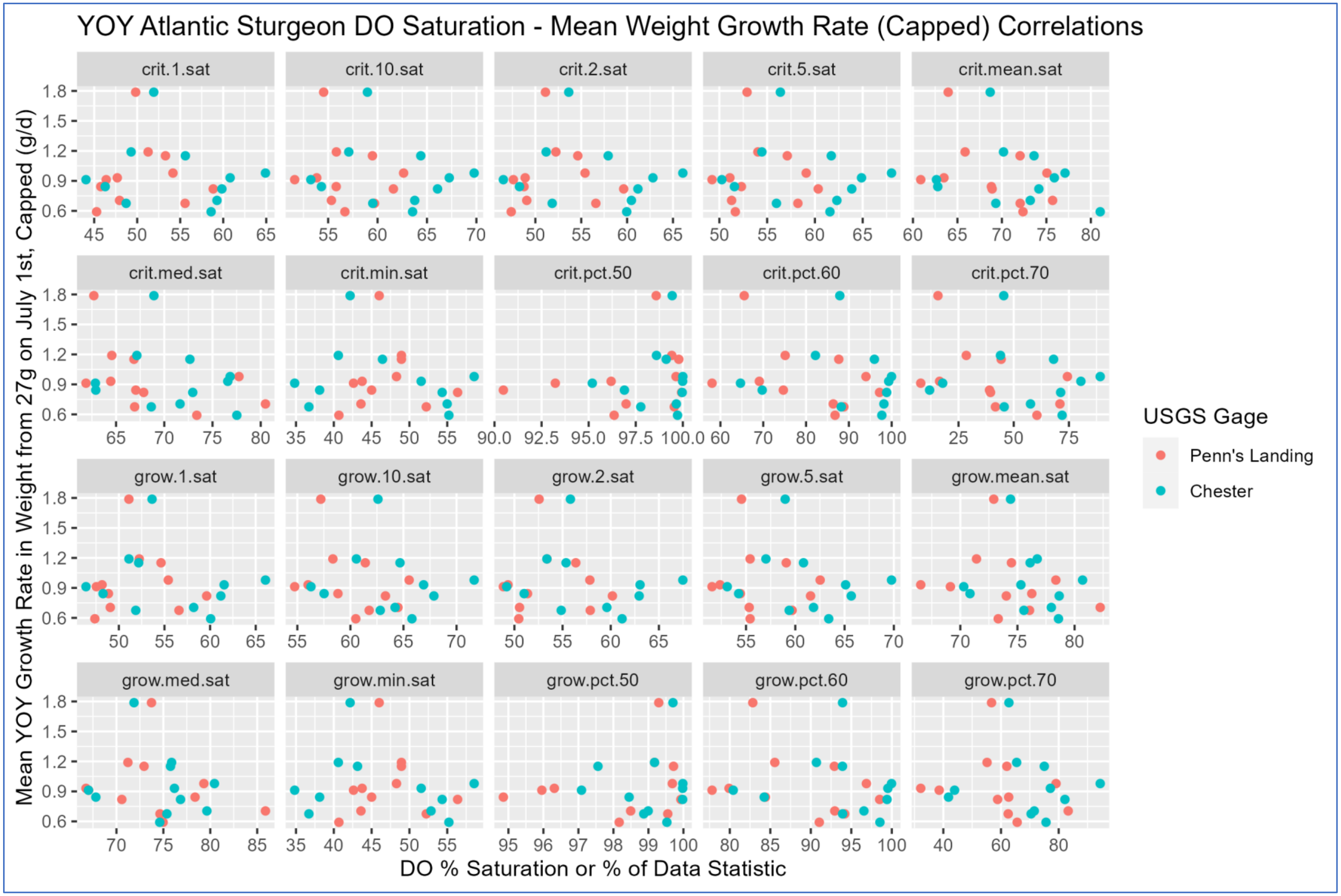

Figure 12.) Correlation for Delaware River seasonal DO statistics and YOY Atlantic sturgeon mean growth in weight assuming fish were 27g on July 1st, "capped" assuming winter growth was complete by Jan 1st, 2009-2022