Nutrient reduction by eastern oysters exhibits low variability associated with reproduction, ploidy, and farm location

Matthew Poach[1], Ryan Morse[2], Shannon L. Meseck[1], Annita Alvarado[3,7], Julie Reichert-Nguyen[4], Katherine McFarland[1], Hope Elliott[5], M. Lisa Kellogg[6], Mark W. Luckenbach[6], Julie M. Rose[1*]

[1]NOAA Fisheries NEFSC Milford Laboratory, 212 Rogers Ave, Milford CT 06460
[2]CASE Consultants International under contract to NOAA Fisheries NEFSC Narragansett Laboratory 28 Tarzwell Drive, Narragansett RI 02882
[3]Integrated Statistics under contract to NOAA Fisheries NEFSC Milford Laboratory, 212 Rogers Ave, Milford CT 06460
[4]NOAA Office of Habitat Conservation, Chesapeake Bay Office, 200 Harry S. Truman Parkway, Suite 460, Annapolis MD 21401
[5] NOAA Hollings Student Internship at NOAA Fisheries NEFSC Milford Laboratory, 212 Rogers Ave, Milford CT 06460
[6]Virginia Institute of Marine Science, Gloucester Point, VA 23062
[7]*present address*: University of Connecticut, 55 N. Eagleville Rd, Storrs, CT 06269

*corresponding author: julie.rose@noaa.gov

Keywords: eutrophication, *Crassostrea virginica*, nutrient management, nitrogen, phosphorus

Abstract

Enhancement of shellfish populations has long been discussed as a potential nutrient reduction tool, and eastern oyster aquaculture was recently approved as a nutrient reduction best management practice (BMP) in Chesapeake Bay, USA. This study addressed BMP-identified data gaps involving variation in nutrient concentration related to ploidy, effects of reproductive development, and a paucity of phosphorus concentration data. Diploid and triploid oysters were collected from farms in Maryland and Virginia across the typical local reproductive cycle. The nutrient concentration of tissue and shell was consistent with the currently implemented BMP. Minor variation observed in nitrogen and phosphorus concentration was within the previously reported range, for farm location, ploidy, and reproductive cycle timing. Ploidy-based differences in tissue dry weight were not observed at either farm, which contrasts with current nutrient reduction estimates. These results suggest separate crediting values for diploids and triploids may need further investigation and potential re-evaluation.

Acknowledgments

The authors would like to thank Scott Budden (Orchard Point Oyster Company), Anthony Marchetti (Rappahannock Oyster Company), and Patrick Oliver (Rappahannock Oyster Company) for their strong support of this project, including providing oysters, and sharing their

local knowledge and cultivation practices. Suzanne Bricker and Matt Parker coordinated oyster collection and shipping from Orchard Point. Funding for this work was provided by the NOAA Office of Aquaculture, Oyster Recovery Partnership, and NOAA Hollings Scholar Program.

Introduction

Excess nutrients from land and air sources have caused systemic problems in estuaries and coasts around the world (Breitburg et al., 2018; Bricker et al., 1999). Eutrophication, the accumulation of nutrients in an aquatic environment, leads to the overgrowth of plants such as microalgae and seaweeds (Rabalais et al., 2010). The symptoms of eutrophication include nuisance and harmful algal blooms, low dissolved oxygen, reductions to biodiversity, and loss of key habitats (Breitburg et al., 2009; Bricker et al., 2008; Deegan et al., 2012). Recognition of the linkage between excess nutrients and widespread environmental degradation drove the development of nutrient management programs in both freshwater and marine environments across the US and Europe during the 1970s and 80s (Boesch, 2019).

Nitrogen (N) is the primary focus of nutrient management programs in the marine environment, as this nutrient has been shown to most often limit the growth of phytoplankton and macroalgae in coastal and estuarine ecosystems (Howarth and Marino, 2006). Phosphorus (P) has also been identified as an important co-limiter of marine plant growth, and has been included in some coastal nutrient management programs as well (Howarth and Paerl, 2008; Wurtsbaugh et al., 2019). Initial nutrient management efforts were focused on point sources, including wastewater treatment and large animal feeding operations (Le Moal et al., 2019). In recent years, many programs have expanded to include nonpoint sources from land and air, such as fertilizers from crop agriculture and suburban lawns, car and industrial emissions, and septic systems (Lintern et al., 2020). As the targets of N and P management have broadened, the approaches and practices employed to reduce nutrient inputs have grown as well (Basu et al., 2022).

Shellfish have been identified as a potential tool for mitigation of the symptoms of eutrophication for over 40 years (Officer et al., 1982). Shellfish are filter feeders, and ingest N and P contained in planktonic organisms and organic detrital matter. A portion of these ingested nutrients are assimilated into tissue and shell as the animal grows (Clements and Comeau, 2019; Higgins et al., 2011). Through biodeposit production, nutrients can also be transported to the seafloor, where the N component of biodeposits may enhance naturally-occurring denitrification processes (Kellogg et al., 2013; Ray et al., 2021), and both N and P may be buried in sediments (Beseres Pollack et al., 2013; Kellogg et al., 2014).

Both large-scale shellfish restoration and shellfish aquaculture have been proposed as additional tools for N and P reduction in the coastal and marine environment (Lindahl et al., 2005; Newell, 1988; Rose et al., 2014). The Chesapeake Bay Program (CBP) has been on the forefront of efforts in the US to incorporate both shellfish restoration and shellfish aquaculture into approved nutrient reduction strategies to meet water quality goals through Clean Water Act programs. Shellfish aquaculture was approved by the CBP as a best management practice

(BMP) for N and P reduction in 2017 (Reichert-Nguyen et al., 2016). Under this BMP, the N and P contained in the tissue of hatchery-sourced, cultivated eastern oysters (*Crassostrea virginica*) can be counted towards subwatershed nutrient reduction goals after harvest.

The approval process for the oyster aquaculture BMP involved a literature review and synthesis by an expert panel, who established the mean N and P concentration (% of dry weight) of eastern oyster tissue, and generated robust regressions for the relationship between eastern oyster shell height and oyster tissue dry weight (Reichert-Nguyen et al., 2016). N and P reduction were calculated by multiplying the mean nutrient concentration (derived from values found in studies from the US Atlantic Coast) with animal biomass and number of oysters harvested by an individual farm (Carmichael et al., 2012; Grizzle and Ward, 2011; Higgins et al., 2011; Kellogg et al., 2013; Sebastiano et al., 2015) as cited in Reichert-Nguyen et al. (2016). The influence of seasonal temporal factors and of ploidy could not be assessed due to limited data availability, thus the same mean nutrient concentration was applied to both diploid and triploid oysters, regardless of time of harvest.

The ploidy of cultivated oysters varies across farms in Chesapeake Bay. Wild oysters, and some farmed oysters, are diploid, meaning that each individual contains two sets of chromosomes (one from each parent). Some farms instead grow triploid oysters, which have an extra set of chromosomes and are produced in hatcheries by cross-breeding a diploid oyster with a tetraploid oyster. Triploids are typically sterile, and can be faster-growing than diploids, attributed to energy savings from not reproducing. Since triploids do not undergo morphological changes associated with reproduction, triploid oyster tissue is less seasonally variable in morphology than that of diploid oysters. In addition to well-documented morphological variation of diploid oysters related to reproduction, it is possible that diploid oyster tissue nutrient concentration also varies across the oyster reproductive cycle.

Spatial and temporal factors such as location within Chesapeake Bay and season did not affect the tissue dry weight to shell height regression within the available literature reviewed by the panel (Reichert-Nguyen et al., 2016). Triploid oysters grown in aquaculture gear (e.g., bottom cages, floating bags, etc.) exhibited higher tissue dry weight to shell height relationship than wild or cultivated diploid oysters grown on the seafloor without gear, resulting in greater total N and P removal at harvest (Reichert-Nguyen et al., 2016). However, the effect of ploidy was confounded with farm practices because most farms that use aquaculture gear in Chesapeake Bay grow triploids while most farms that grow their oysters on bottom with no gear use diploids. Previous literature suggests that both ploidy and gear can affect oyster growth, e.g., (Dégremont et al., 2012; Walton et al., 2013). Subsequent analysis was conducted after approval of the oyster aquaculture BMP, with additional data on diploid and triploid oysters grown in gear, suggesting that gear and ploidy could both influence the relationship between oyster tissue dry weight and shell height (Cornwell et al., 2023).

The expert panel assigned a higher nutrient reduction to triploid oysters than diploids in the oyster aquaculture BMP, but recommended additional research into the potential effect of ploidy on oyster tissue nutrient concentration. The BMP identified other data gaps, including

insufficient data on the potential effect of reproductive development on diploid tissue N concentration, limited data available for triploid oyster N concentration, no data for triploid oyster P concentration, and limited data available for diploid oyster tissue and shell P concentration.

This study seeks to address all of these data gaps and limitations identified by the oyster BMP expert panel in the current eastern oyster literature. We partnered with oyster farmers in Maryland and Virginia to collect data reflecting real world conditions on shellfish farms. The data collected here can be used to inform future assessment of existing oyster aquaculture nutrient reduction programs in places like Chesapeake Bay and Massachusetts, USA. The analytical approach developed by the Chesapeake Bay Program, and applied here, is highly transferable to other geographic locations, and can be used to guide the development of new programs in other eutrophic estuaries.

Methods

Sites and sample collection

Eastern oysters, *Crassostrea virginica*, were collected from two commercial oyster farms within Chesapeake Bay, USA: Orchard Point Oyster Company (MD farm), located at 39.049461, -76.211852 in the Chester River, Graysonville, Maryland; and Rappahannock Oyster Company (VA farm), located at 37.597854, -76.429769 in the Rappahannock River, Topping, Virginia (Fig. 1). Both farms cultivate triploid and diploid oysters in subtidal bottom cages. For the MD farm, 10 mm diploids were planted in May 2015 at a stocking density of ~6000 oysters per cage, and 6-7 mm triploids were planted in May 2016 at a stocking density of ~31,000 oysters per cage. For the VA farm, diploids and triploid oyster seed were both planted in May 2015. The MD farm sourced their diploids from the Horn Point Hatchery in Maryland, and triploids were the LOLA line sourced from Oyster Seed Holdings (Grimstead, VA). The VA farm sourced their diploids from wild Rappahannock River oyster stock, and triploids were the LOLA line sourced from Oyster Seed Holdings.

For each farm, oyster collection occurred during five separate periods in 2017 to represent the typical reproductive cycle of eastern oysters in Chesapeake Bay, as described in (Guévélou et al., 2019; Mann et al., 2014). Samples were collected from farms in late February (inactive), early May (developing), early June (spawning), mid-August (post-spawn), and October (inactive). Thirty diploid and 30 triploid oysters were collected from each farm during each sampling period. Oysters were stored on ice and shipped overnight to the NOAA Fisheries NEFSC Milford Laboratory, Connecticut, USA for processing and nutrient analysis. Oysters were stored in the laboratory at -20^{o}C until analysis.

Although water quality data were not collected at each farm, water quality data were available for nearby monitoring stations from the CBP (https://data.chesapeakebay.net/WaterQuality). To assess differences in water quality between the two farms, we downloaded data for station LE3.4 in the lower Rappahannock River in Virginia and from station ET4.2 in the lower Chester River in Maryland (Fig. 1). Stations throughout Maryland and Virginia were sampled on a monthly basis, except in June and August when stations were sampled twice monthly (Chesapeake Bay Program, 2017).

Nutrient analysis

Individual oysters were measured with calipers to determine shell height, defined here as the longest distance between the hinge and the lip of the oyster, parallel to the long axis (Galtsoff, 1964). Oysters were shucked, tissue and shell were separated, and dried to constant weight at 60^{o}C in a drying oven before determination of tissue and shell dry weight, then samples were ground to a powder (Planetary Ball Mill PM 200, Retsch GmbH, Haan, Germany). Ground tissue and shell samples were stored in a desiccator until processing for elemental analysis of carbon, hydrogen, nitrogen (CHN), and P. Tissue samples from all collected oysters were ground for nutrient analysis. Only the shell from February samples (both farms) and ~50% of the May samples (MD only) were processed for nutrient analysis, due to funding constraints and damage to laboratory equipment from shell material. As nutrient concentration of shell material was not expected to vary seasonally, this limitation should not affect interpretation of results.

N analysis was performed on ground tissue and shell samples using a Costech ECS 4010 CHNS elemental analyzer (Valencia, CA). SRM1547; Peach Leaves (National Institute of Standards and Technology; U.S. Department of Commerce: Gaithersburg, MD 01 March 2027) was used to ensure accuracy of N analysis and was 29,665 ± 270 mg kg^{-1} (n=142), which was within 0.05% of the reported value.

P analysis was performed on ground tissue and shell using a modified combustion method from Boros and Mozsar (2015). Briefly, 100 mg of dried, ground oyster tissue or 50 mg of dried, ground oyster shell were placed into individual 50 mL glass test tubes and muffled overnight at 550°C. After muffling, 10 mL of 2.5M hydrochloric acid was added to each test tube and dried at 105°C for 1 hour. Samples were then transferred to 50 mL polypropylene conical centrifuge tubes using 10 mL of Milli-Q water and centrifuged at 3000 rpm for 10 minutes. P was determined on a neutralized aliquot (Lambda 35 UV/VIS spectrometer, Perkin Elmer, Waltham, MA, USA) using EPA Method 365.3. As for N analyses, SRM1547 was used to ensure accuracy of P analysis and resulting values (1419 ± 115 % n=88) were within 3.5% of the expected value.

Data analysis

All statistical analyses were conducted using the software program R version 4.2.0 (http://www.r-project.org). A two-way ANOVA with 20% trimmed means was used to compare main effects and interactions of site and ploidy on the N and P concentration of eastern oyster tissue and shell. Trimmed means were used to increase robustness to the potential for conditions of nonnormality, heteroscedasticity, and outliers, which can be common in biological and ecological datasets (Wilcox, 2022). To assess the effect of reproductive development on diploid tissue N and P concentration, triploids were used as a sterile control group, and diploids and triploids were compared for each month-site combination. A modified ANOVA, using 20% trimmed means and percentile bootstrap method, was used because it does not have the assumptions of normality or homoscedasticity and generally has higher power (Wilcox, 2022). The false discovery rate across multiple ANOVA tests was controlled using the Benjamini-Hochberg method.

The extreme curvature in the relationship between oyster dry weight and shell height presented a challenge to hypothesis testing. Shell height and oyster dry weight (tissue and shell) were natural log transformed to approximate linearity, an approach previously employed by Higgins et al. (2011). Robust regression analysis of the transformed data was conducted using the Theil-Sen regression estimator and percentile bootstrap method (Wilcox, 2022). Existing literature indicates that the relationship between oyster dry weight and shell height is best described by a power function, so nonlinear quantile regressions were generated using the R statistical package quantreg (Koenker, 2006, 2016). The 50th quantile was used as an estimate of the median of the dataset, as 50% of the tissue dry weight values lie above each value of shell height using this approach. The use of nonlinear quantile regression also facilitates direct comparison to the previous Chesapeake Bay oyster literature (Reichert-Nguyen et al., 2016).

The total N and P reduction effectiveness for 1 million harvested oysters was computed using the regressions reported herein using a shell height of 76.2mm, corresponding to a three-inch oyster, and compared to values derived from literature regressions (Cornwell et al., 2023; Reichert-Nguyen et al., 2016). Briefly, the total weights of tissue for oysters were converted to total weight of N and P using mean tissue N and P percent values of 8.2% and 0.9%, respectively, and mean shell N and P values of 0.2% and 0.04%, respectively (Reichert-Nguyen et al., 2016). The total tissue or shell weights were calculated using the power function $Y=ax^b$.

Results

Difference in water quality between farm sites

Assuming that data from nearby Chesapeake Bay water quality monitoring stations ET4.2 and LE3.4 are representative of water quality conditions at the Maryland (MD) and Virginia (VA) farms, respectively, the two oyster farm sites exhibit distinctly different hydrographic properties (Fig. 2). The MD farm had greater temperature extremes and lower salinity than the VA farm.

At the MD site, water temperature ranged from 0.5-27.9 □, with a mean annual temperature of 14.1□ at the bottom and 15.1□ at the surface (Fig. 2A). At the VA site, water temperature ranged from 5.47-29.11□ and mean annual temperature was 19.1□ at the bottom and 19.8□ at the surface. Salinity at the bottom at the MD site ranged from 8.12-19.31 with a mean annual salinity of 13.0, while the range at the surface was 4.0-15.1, with mean annual salinity of 10.5 (Fig. 2B). Nearly all values for surface salinity (85%) and the majority (60%) of bottom salinity values were below 14 PSU. Bottom salinity at the VA site ranged from 14.8-22.5 and salinity at the surface ranged from 13.2-19.2. Mean annual salinity was 18.1 at the bottom and 16.3 PSU at the surface. All values for bottom salinity were greater than 14, and only two values for surface salinity were less than 14.

The MD site also had much higher dissolved inorganic nitrogen (DIN) concentrations, generally higher chlorophyll *a* (Chl *a*) concentrations, and lower water clarity than the VA site. In spring and summer, DIN concentrations were much higher at the MD site than at the VA site (Fig. 2D). Both sites exhibited annual cycles in DIN concentrations with peaks coinciding with periods of lower salinity. Chl *a* concentrations were generally higher at the MD site than at the VA site, although both sites experienced episodic blooms with Chl *a* exceeding 15 µg/l in each year (Fig.

2C). The MD site experienced a major bloom with Chl *a* concentrations exceeding 90 µg/l in bottom waters in March and April of 2015. Water clarity, as measured by Secchi depth, was greater for the VA site with a range of 0.8-3.2 m and an overall mean of 1.72 m (Fig. 2F). The maximum value at the VA site increased in each successive year, suggesting improved water clarity. The MD site had a lower range of 0.5-2.6 m and an overall mean Secchi depth of 1.3 m.

Both sites experienced hypoxic conditions with oxygen concentrations below 5 mg/l in all three years (Fig. 2E). Hypoxic conditions were limited to bottom waters and occurred only in summer months.

Eastern oyster tissue and shell nutrient concentration

Eastern oyster tissue samples (n=622) had an overall mean N concentration of 7.7% (SD=1.2; range 4.8-11.3) and P concentration of 0.85% (SD=0.19; range 0.44-1.7), including both diploids and triploids across all farms and months (Table 1). Shell samples (n=129) had an overall mean N concentration of 0.21% (SD=0.07; range 0.05-0.43) and P concentration 0.045% (SD=0.004; range 0.03-0.05).

Farm location and ploidy effects on nutrient concentration

Tissue N concentration had small but significant main effects of both farm location and ploidy, but no interaction (Fig. 3A, Table 2; farm p=0.001, ploidy p=0.001, interaction p=0.987). The VA farm had slightly higher tissue N than the MD farm (Table 1; 8.1% vs. 7.2%), and the difference in means between diploids and triploids across farms was even smaller (7.8% vs. 7.5%).

Shell N concentration had a small but significant main effect of farm location, but the ploidy main effect and the interaction of the two factors were not significant (Table 2; farm p=0.001, ploidy p=0.107, interaction p=0.062). Similar to tissue N concentration, the VA farm shell N concentration was slightly higher when compared to the MD farm (Table 1; 0.24% vs. 0.17%).

Tissue P concentration had a small but significant main effect of ploidy, but the main effect of farm location and the interaction between the two factors were not significant (Fig. 3B; Table 2; farm p=0.062, ploidy p=0.001, interaction p=0.06). Diploid oysters had higher tissue P concentration than triploids across farms (0.89% vs. 0.80%).

Shell P concentration had small but significant main effects of both farm location and ploidy, but no interaction (Table 2; farm p=0.004, ploidy p=0.001, interaction p=0.268). The MD farm had slightly higher shell P concentration than the VA farm (Table 1; 0.046% vs. 0.044%), and triploids had slightly higher shell P concentration compared to diploids across farms (0.047% vs. 0.043%).

Effects of reproductive development on diploid nutrient concentration

Tissue N concentration at both farms was significantly different in May and June, although the direction of the difference varied between the two months (Fig. 4). In May, triploid N concentration was greater than diploids (MD 6.9 vs. 6.4% p=0.023, VA 8.1 vs. 7.3% p<0.001) while in June, diploid N concentration was greater (MD 7.1 vs. 6.3%, VA 9.6 vs. 8.1%, both

p<0.001). In February, at the Virginia farm only, diploid N concentration was significantly higher than triploids (6.8 vs. 6.2%, p=0.003). In August, at the Maryland farm only, diploid N concentration was significantly higher than triploids (8.3 vs. 7.5%, p<0.001). There were no significant differences between diploid and triploid N concentration at either farm in October (all p>0.05).

Tissue P concentration at both farms was significantly different in June, with diploids greater than triploids (Fig. 4; MD 1.02 vs. 0.8%, VA 1.15 vs. 0.85%, both p<0.001). In August, at the Maryland farm only, diploid P concentration was significantly higher than triploids (1.13 vs. 0.84, p<0.001). There were no significant differences between diploid and triploid P concentration at either farm in February, May, or October (all p>0.05).

Eastern oyster morphometrics and dry weight

Diploid oysters at the MD farm were consistently smaller in both shell height and tissue dry weight than diploid oysters at the VA farm, despite being planted at the same time (Fig. 5A). Triploid oysters at the MD farm were also consistently smaller than triploids at the VA farm (Fig. 5B), although the MD oysters were reported to have been planted a year later (2016 vs. 2015), so direct comparison of triploid oyster size across farms was not possible.

There was no significant difference between diploids and triploids in the relationship between natural log transformed oyster tissue dry weight and shell height, either in the combined dataset ($p = 0.76$) or when the farms were considered individually (MD $p = 0.85$; VA $p = 0.35$). There was a significant difference between diploids and triploids in the relationship between natural log transformed oyster shell dry weight and shell height (full data set $p < 0.001$), but this difference was driven solely by the Virginia farm ($p = 0.003$), with no difference observed at the Maryland farm ($p = 0.21$).

Quantile regressions were generated for the relationship between tissue dry weight and shell height for the combined MD and VA datasets, and compared to the 50th quantile regressions for diploid and triploid oysters from the Chesapeake Bay oyster aquaculture BMP (Fig. 6). The 50th quantile regression for diploid oysters from this study was virtually identical to the BMP diploid oyster regression for oysters grown without gear (Fig. 6; Table 3). In contrast, the 50th quantile regression for tissue dry weight to shell height of triploids from this study was less steep than that of diploid oysters, and was considerably less steep than that reported in the Chesapeake Bay oyster aquaculture BMP (Fig. 6, Table 3).

Discussion

Nutrient concentration of eastern oyster tissue and shell

Our findings validate the current management approach that assigns the same Bay-wide N and P tissue concentration values to both diploid and triploid eastern oysters, a management decision that was originally based on limited data and best professional judgment by an expert panel (Reichert-Nguyen et al., 2016). The two farms in this study reflect typical growing conditions for shellfish aquaculture across Chesapeake Bay (Fig. 2). There were statistically significant differences in tissue and shell nutrient concentration between the two farms, but the

magnitude of these differences was small when compared to the range of nutrient concentration reported in the literature for other parts of the Chesapeake Bay, and the Atlantic coast region more broadly. The difference in tissue N concentration between the MD and VA farm (7.2 and 8.1%, respectively) was within the previously reported range for tissue N concentration (7.28-9.27%), (Reichert-Nguyen et al., 2016). This was also the case for shell N (0.17 vs. 0.24% for MD vs. VA; literature range 0.08-0.32%), and shell P (0.046% vs. 0.044% for MD vs. VA; literature values only reported to one significant digit, 0.04%). There was no significant difference in tissue P concentration between farms. These findings are consistent with the low geographic variation in eastern oyster tissue and shell nutrient concentration reported in Reichert-Nguyen et al. (2016), Cornwell et al. (2023), and Clements and Comeau (2019).

Similarly, there were statistically significant differences in tissue and shell nutrient concentration between diploid and triploid oysters, but the magnitude of these differences was small and within the variation observed previously across the Chesapeake Bay and broader Atlantic coast region. The difference in tissue N concentration between diploids and triploids was 7.8% and 7.5%, respectively, within the reported range for tissue N concentration (7.28-9.27%), (Reichert-Nguyen et al., 2016). Shell N was not significantly different between diploids and triploids. Tissue P concentration was 0.89% for diploids and 0.80% for triploids, within the previously reported range (0.62-1.26%). Shell P concentration was 0.043% for diploids and 0.047% for triploids; as noted above, literature values were only reported to one significant digit, 0.04%. These results support the use of the same N and P concentration for both diploid and triploid oysters.

Effects of reproductive development on diploid nutrient concentration

In the Chesapeake Bay, oysters spawn multiple times within a season (Mann et al., 2014), but the reproductive cycle generally follows a trend of inactivity in the winter months, early gametogenesis by April, late stage gametogenesis by May, and spawning in June-July, with oysters largely being in the post spawn stage by early August (Guévélou et al., 2019; Mann et al., 2014).This reproductive cycle also marks significant changes in tissue weight due the building of reproductive material and plumping up in the late stages of gonad development and a rapid weight loss following spawning (Dridi et al., 2007). During this process, energy reserves are mobilized to fuel gametogenesis and spawning leading to a buildup and then a rapid decrease in glycogen and lipids associated with spawning events in the summer months (Dridi et al., 2007; Encomio et al., 2005). Glycogen and lipids are carbon-rich molecules, which may have influenced the overall nutrient composition of diploid tissues. Diploid tissue N concentration was significantly lower than triploid tissue N concentration in May at both farms, when diploids were undergoing gonad development. Both nutrients peaked in relative tissue concentration in the months during which peak gametogenesis is expected, which would be consistent with the loss of carbon-rich glycogen and lipids during spawning, and fell in the months following a spawning event (Fig. 4). The temporal variation observed therefore is likely a result of the natural variation in biochemical makeup of the oyster as it changes throughout the reproductive cycle.

The data from both farms reflected statistically significant but relatively small variability in tissue nutrient concentration, consistent in timing with the typical spawning cycle in Chesapeake Bay

(Fig. 4). During two of the five months sampled, significant differences in tissue N concentration were observed at both farms. Tissue N concentration was greater in triploids at both farms than diploids in May, but in June the reverse was observed, with diploid tissue N concentration greater than that of triploids. The magnitude of these differences was less than 1.5% between diploids and triploids for any month/farm combination. Tissue P concentration was significantly different between diploids and triploids at both farms during only one of the five months sampled, with diploids exhibiting higher tissue P concentration in June. The absolute difference between diploids and triploids was small, 0.22 in MD and 0.30 in VA, although since the mean P concentration of oyster tissue was also small, this represented a 24% and 30% elevation of P concentration in MD and VA, respectively (Fig. 4).

While triploid oysters are sterile, they do undergo some gonad development, though not typically to the ripe or spawning stage (Allen and Downing, 1990; Jouaux et al., 2010; Matt and Allen, 2021). In a recent study, Guévélou et al. (2019) followed the gonad development of diploid and triploid oysters across several sites within Virginia waters and found both ploidies to follow the expected seasonal cycle, but triploids were delayed in development compared to diploids and never reached a full ripe stage. The current study shows similar seasonal trends for both ploidies, but with larger variation in N and P concentration in diploids likely due to achieving more advanced gametogenesis. Diploid tissue N concentration was lower than that of triploids in May, as diploids were undergoing gonad development. For diploids, there is a clear peak in tissue nutrient concentration in June, corresponding to the expected peak in spawning activity (Guévélou et al., 2019; Mann et al., 2014). However, MD diploids had a prolonged peak going into August which could suggest a prolonged spawning period (Mann et al., 1994). It is plausible that MD oysters, being smaller (Fig. 5), were able to allocate more energy to reproduction allowing for increased frequency of spawning events within a season (Hofmann et al., 1994). However, interannual variation is to be expected within and among regions and can be caused by numerous factors. Environmental factors such as temperature, salinity, and food availability play a significant role in the timing of spawning (Cox and Mann, 1992) and may lead to some site specific differences and interannual variation on the exact timing. Even subtle shifts in temperature and/or food availability can cause measurable differences in timing and duration of the reproductive cycle among years and across geographic locations (Bernard et al., 2011; Hofmann et al., 1994; Hofmann et al., 1992), which could explain differences in the timing and magnitude of changes in N and P between MD and VA oysters. Further, low salinity events, as observed in MD during this study period, can cause delayed gametogenesis and variation in the timing among different genetic stocks can be expected (Encomio et al., 2005; Gregory et al., 2023). Although this study identified differences in the total N and P concentration among ploidies, geographic locations, and month sampled, the magnitude was small and likely to be negligible in the annual accounting of nutrient credits when inter and intra-annual variations are factored in.

The opposing direction of the differences in tissue N concentration between diploids and triploids in May and June suggests that any under-crediting of diploids that could occur on a farm in May would be essentially canceled out by over-crediting of diploids the following month, and vice-versa for triploid oysters. While tissue P concentration was significantly elevated in

diploids, and the relative magnitude of that difference was 24-30%, the limitation of this difference to a single month greatly reduces the overall impact to nutrient management in Chesapeake Bay. Harvest of cultivated oysters in Chesapeake Bay occurs year-round, and there is no reason to expect harvest numbers to consistently vary in one direction between May and June across farms Bay-wide. The logistical challenges and increased reporting and verification requirements involved in shifting to a monthly reporting structure for this nutrient best management practice would not appear to be justified by the size of the effects observed.

Using eastern oyster shell measurements to predict total nutrient concentration

While there were minimal differences in tissue and shell N concentration between farm locations, oyster sizes differed considerably (Fig. 5). Since diploid oysters were reported to have been planted on both farms at the same time, these morphological differences may have been due to faster growth at the VA farm relative to the MD farm. Data from nearby Chesapeake Bay Water Quality Monitoring Program stations indicate that salinity at the MD farm was consistently lower than salinity recorded at the VA farm (Fig. 2B). The MD farm regularly experienced prolonged periods of salinity levels falling below 10 psu, which can negatively affect eastern oysters (McFarland et al., 2022) and alter the timing of reproductive activity (Gregory et al., 2023). These data suggest that suboptimal environmental conditions may explain growth differences between the two farm locations.

Our findings validate the observed relationship between tissue dry weight and shell height for diploid oysters that is currently used in the Chesapeake Bay oyster aquaculture nutrient best management practice (Fig. 6A, regression “CB diploid”). The relationship reported here from the two farms studied is virtually identical to that generated from a much larger dataset of oysters collected Bay-wide (Reichert-Nguyen et al., 2016).

Interestingly, we were unable to replicate the difference between diploid and triploid oysters that was reported in both Reichert-Nguyen et al. (2016) and Cornwell et al. (2023). These previous studies observed a steeper relationship between tissue dry weight and shell height for triploids relative to diploids, where larger triploid oysters had higher predicted tissue dry weight than diploids. The oyster dataset evaluated in these studies was a synthesis of existing literature, did not allow for the direct comparison of triploids and diploids from the same location, and the 2016 triploid dataset was considerably smaller than the diploid dataset (5,750 diploids vs. 1,066 triploids). Additionally, all triploid data were from a single study, Kingsley-Smith et al. (2009), where oysters were from research plots that used near bottom cages and were not sampled directly from oyster farms. The 2016 analysis, on which the current BMP is based, identified a potential confounding factor related to cultivation practice in the data: triploid oysters in Chesapeake Bay are typically grown in aquaculture gear, while diploids are largely grown directly on bottom without the use of gear. The 2016 report recommended re-evaluation of this triploid/diploid difference when additional data were available to allow a better assessment of the gear factor.

The subsequent reanalysis in Cornwell et al. (2023) included a much larger number of triploids grown in gear through the addition of farm-collected data from Cubillo et al. (2018) (2,328 individuals; 3,394 individuals total from 2 studies), and expanded the dataset of diploids grown

in gear (additional 420 individuals for a total of 504 individuals vs. 84 individuals from Hinson et. al 2011 used in the 2016 report). This reanalysis had three conclusions: 1) validation of the previous regression for diploid oysters grown on bottom without gear, 2) observation that diploids grown in gear had a steeper relationship between tissue dry weight and shell height than diploids grown on bottom without gear, and 3) observation that triploids grown in gear had a steeper slope than diploids grown in gear, thus there was still a ploidy effect in the data after removal of the gear factor. The inclusion of additional triploid data in the reanalysis increased the calculation of N reduction effectiveness by triploids for individuals 3.5 inches and larger (Table H-2 in Cornwell et al. 2023; regressions recreated here in Fig. 6A “CB Diploid with Gear” and “CB Triploid with Gear”). The reanalysis recommended that the current oyster aquaculture BMP be evaluated for the oyster aquaculture practices that use gear and either diploid or triploid oysters.

In this study, diploid and triploid oysters were grown in the same location, using the same gear type (bottom cages), eliminating the potential confounding factors identified in the oyster aquaculture nutrient BMP. We were unable to verify the steeper slope for the triploid oysters that was observed in the 2016 oyster aquaculture BMP and the 2023 reanalysis, and the triploid oysters measured here at both farms exhibited tissue dry weight that was not statistically different from diploid oysters of a similar size (Fig. 6A). Additionally, the diploids grown in aquaculture gear in this study did not exhibit the enhanced relationship between dry tissue weight and shell height that was observed for diploids grown in gear in the 2023 BMP reanalysis, although our data fell within the overall observed range of diploids grown in gear in this reanalysis. Our findings are not consistent with the current oyster aquaculture BMP strategy of using separate regressions to calculate nutrient reductions for diploid and triploid oysters in Chesapeake Bay.

Possible explanations for the difference between the panel report and our findings are not obvious based on the information available for these data sets. The original 2016 analysis was based on a single study (Kingsley-Smith et al., 2009), but that dataset included samples from three tidal rivers across both Maryland and Virginia, from similar locations in the estuary that were sampled in this study (Severn, Patuxent, and York Rivers vs. Chester and Rappahannock Rivers sampled here). All oysters were grown subtidally across both studies, and sampling months in both studies included all four seasons. The reanalysis in 2023 added one additional dataset, tripled the total number of triploid samples evaluated, and supported and strengthened the original findings. One difference between the data analyzed here and the previous synthesis was that this study directly compared triploids and diploids grown under the same conditions at the same time and locations. Another difference was that the broodstock used in the Kingsley-Smith et al. study were from Aquaculture Genetics and Breeding Technology Center at the Virginia Institute of Marine Science, Gloucester Point, Virginia, which differed from the LOLA line from Oyster Seed Holdings that was used on both farms in this study.

Both farms sampled in this study employed bottom cages, which were linked to poor growth relative to other gear types by Walton et al. (2013). These same authors specifically noted a reduction in triploid tissue dry weight in bottom cages relative to other gear types, so gear type may have been a contributing factor in the results presented here. Kingsley-Smith et al. (2009)

used an experimental design where trays were also included in the cages for easy sampling and overcrowding was limited by removing spat from the shell substrate. It is unknown whether these experimental methods influenced the morphology of the triploid oysters. There is considerable literature on the growth advantage of triploid oysters in comparison to diploid oysters, both in terms of morphometry and biomass (e.g. as reviewed in Wadsworth et al. 2019). However, Wadsworth et al. (2019) did highlight several studies detecting no difference in growth rate between diploids and triploids (Callam et al., 2016; Ibarra et al., 2017; Shatkin, 1992; Stone et al., 2013; Walton et al., 2013), and identified contributing factors such as low salinity, gear type, cultivation practices, and limited spawning period in northern temperate locations. Low salinity may have affected oysters at the Maryland farm, but was unlikely to have been a problem at the Virginia farm (Fig. 2). The age difference of triploids vs. diploids at the MD farm may have influenced the ploidy comparison at this location, but triploids and diploids were the same age at the VA farm. It is important to note that we did not compare growth rate between diploids and triploids in this study, and oysters of the same shell height may have been different ages. Additional data at a broader geographic scale and across different cultivation practices are needed to help identify underlying mechanisms (environmental or farming strategy) that could explain our results.

The recent recommendation developed for eastern oyster restoration practices in Chesapeake Bay does include a regression for oyster shell height to shell dry weight, based on wild diploid oysters not associated with oyster farms (Cornwell et al., 2023). This previously-generated regression has a steeper slope than was observed in this study, for either the diploid, triploid, or combined datasets (Fig. 6B). A likely contributing factor to this difference was the use of aquaculture gear at both farms in this study. Previous studies have observed thicker and heavier shells on oysters that were grown on bottom without gear when compared to oysters that were grown in gear (summarized in Mizuta and Wikfors (2019)).

Eutrophication of the coastal environment is a global problem, and shellfish aquaculture can be a valuable additional tool to nutrient management programs across a broad geographic scale. Resource managers need robust science to have confidence in the predicted nutrient removal by shellfish aquaculture harvest. While this study was focused on eastern oyster aquaculture in Chesapeake Bay, USA, the approach for calculating oyster harvest nutrient removal, applied both here and by the CBP, is highly transferable to other eutrophic locations with nutrient management programs. Furthermore, this approach may be employed for calculating nutrient removal by other cultivated species with sufficient data availability.

Conclusions

Our findings highlight some of the positive attributes associated with using eastern oyster aquaculture for nutrient reductions in eutrophic estuaries. N and P concentration exhibited low variability in time and space, and regressions based on morphology continued to give high confidence in predicted total N and P reduction achieved at harvest. The data presented here fill specific gaps in the literature, including strengthening limited data available for triploid eastern oyster N concentration and diploid eastern oyster P concentration, and establishing baseline data for triploid eastern oyster P concentration. The limited difference in total nutrient removal

between diploid and triploid oysters at the two farms studied here suggests that nutrient reduction calculations for current and future oyster aquaculture nutrient best management practices may be able to be simplified, which could streamline reporting and verification requirements. Additional data at a broader geographic scale and across different cultivation practices would help determine if updated management practices are needed.

Tables

State	Ploidy	Tissue N (%)	Shell N (%)	Tissue P (%)	Shell P (%)
Maryland	Diploid	7.4 (1.0)	0.18 (0.06)	0.89 (0.22)	0.045 (0.002)
Maryland	Triploid	7.1 (0.9)	0.17 (0.05)	0.77 (0.12)	0.047 (0.004)
Virginia	Diploid	8.2 (1.3)	0.22 (0.06)	0.90 (0.23)	0.042 (0.004)
Virginia	Triploid	7.9 (1.1)	0.27 (0.08)	0.83 (0.16)	0.046 (0.004)
Maryland	All	7.2 (1.0)	0.17 (0.06)	0.82 (0.18)	0.046 (0.003)
Virginia	All	8.1 (1.2)	0.24 (0.07)	0.87 (0.20)	0.044 (0.005)
All	Diploid	7.8 (1.2)	0.20 (0.06)	0.89 (0.23)	0.043 (0.004)
All	Triploid	7.5 (1.1)	0.21 (0.08)	0.80 (0.14)	0.047 (0.004)
All	All	7.7 (1.2)	0.21 (0.07)	0.85 (0.19)	0.045 (0.004)

Table 1. Mean values of percent N and P concentration in oyster tissue and shells from farm sites in Maryland and Virginia by ploidy, overall mean values across both states by ploidy, and combined overall mean values across all samples. Standard deviations are shown in parentheses.

Factor	Tissue N	Shell N	Tissue P	Shell P
Farm	0.001	0.001	n.s.	0.004
Ploidy	0.001	n.s.	0.001	0.001
Interaction	n.s	n.s.	n.s.	n.s

Table 2. Results from two-way ANOVA tests showing p-values for main effects of farm and ploidy and interactions between the two terms. Non-significant values (p >0.05) are denoted by n.s.

Description	Ploidy	Cultivation	a	b	N removed (lbs per 1e6 oysters)	P removed (lbs per 1e6 oysters)	Combined N removal (lbs)	Combined P removal (lbs)	Source
Shell	All	Gear	0.00036	2.6020	125	25	-	-	*This study*
Tissue	Diploid	Gear	0.00051	1.7751	201	22	326	47	*This study*
Tissue	Triploid	Gear	0.00033	1.8144	154	17	279	42	*This study*
Shell	Diploid	No gear	0.00147	2.3964	210	42	-	-	CB BMP 2016
Tissue	Diploid	No gear	0.00037	1.8336	189	21	399	63	CB BMP 2023
Tissue	Diploid	Gear	0.00016	2.0771	235	26	444	68	CB BMP 2023
Tissue	Triploid	Gear	0.00002	2.6070	291	32	501	74	CB BMP 2023

Table 3. Effect of ploidy and farm cultivation practice on N and P removal per 1 million oysters with a shell size of three inches (76.2 mm). The total tissue or shell weights were calculated using the power function $Y=ax^b$. The combined nutrient removal columns indicate the sum of the shell and tissue nutrient weights for 1 million oysters.

Figures

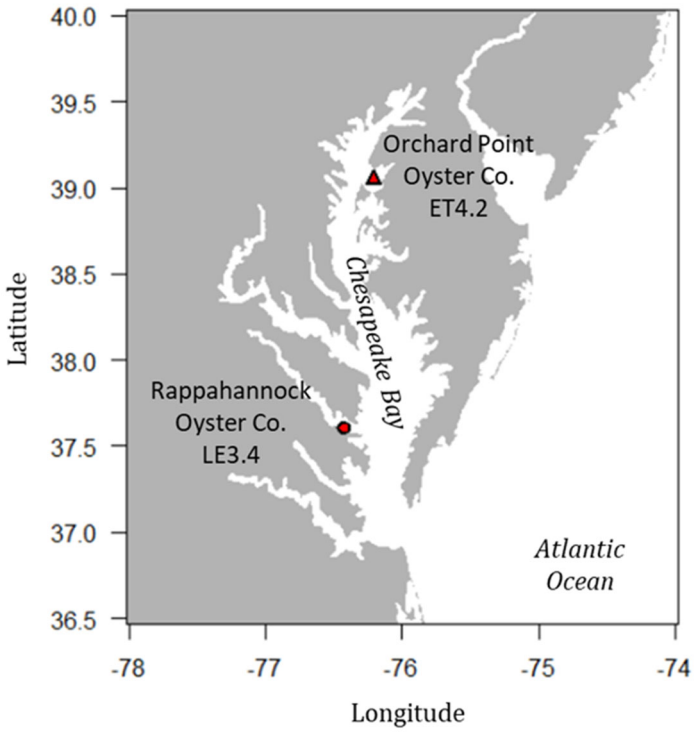
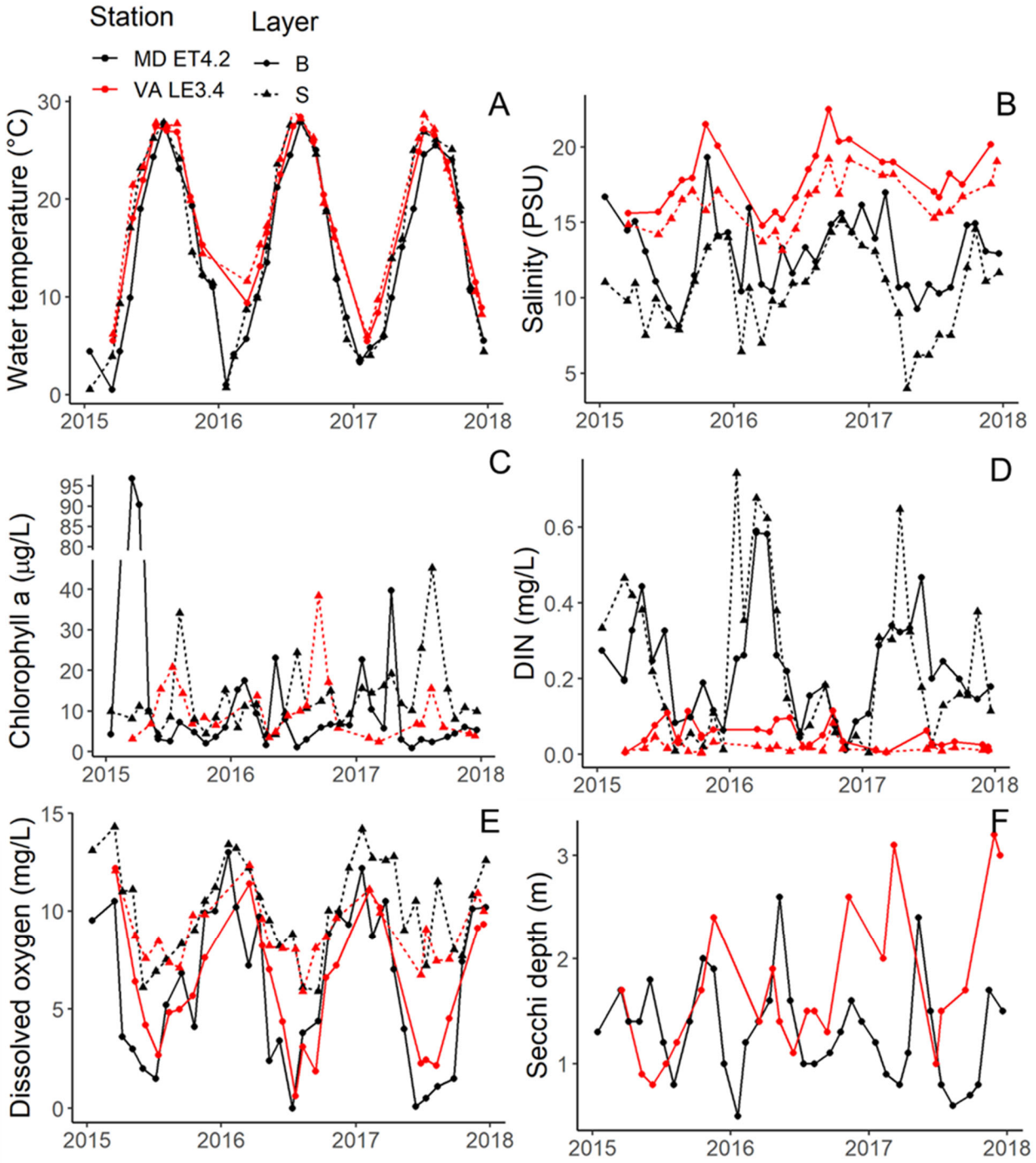
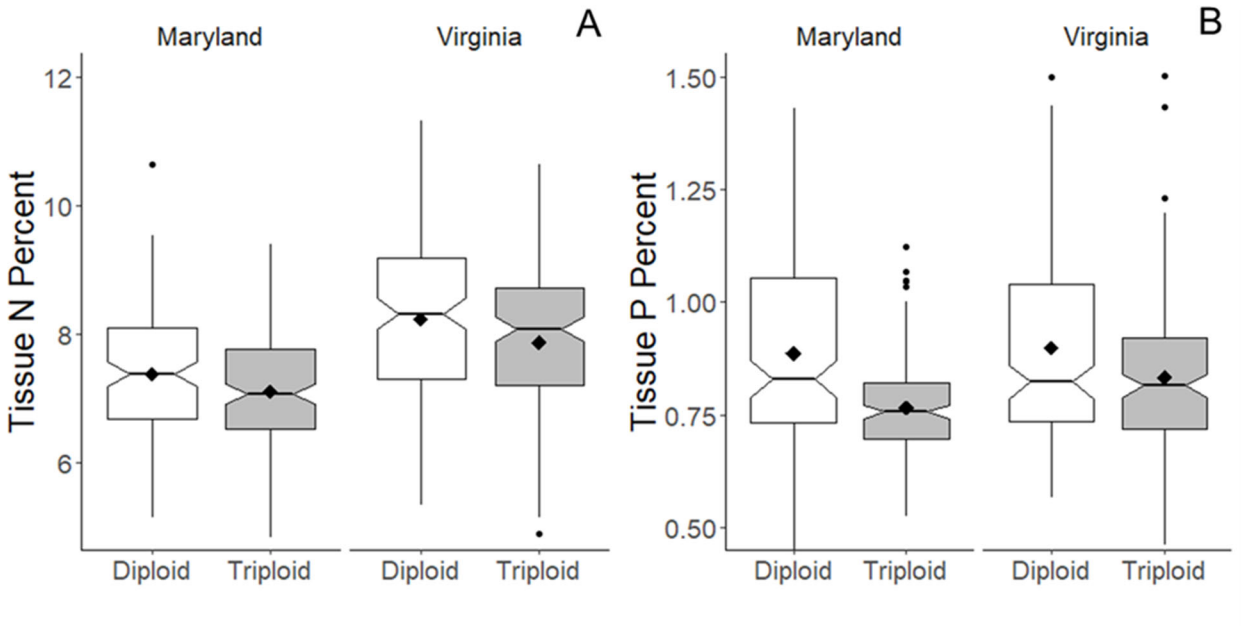


Figure 1. Location of Chesapeake Bay oyster farm sites for the Rappahannock Oyster Company in the Rappahannock River in Virginia (red circle), and the Orchard Point Oyster Company in the Chester River in Maryland (red triangle). The Chesapeake Bay Water Quality Monitoring Program stations LE3.4 and ET4.2 are located so close to the farm sites that they fall under the same symbol.



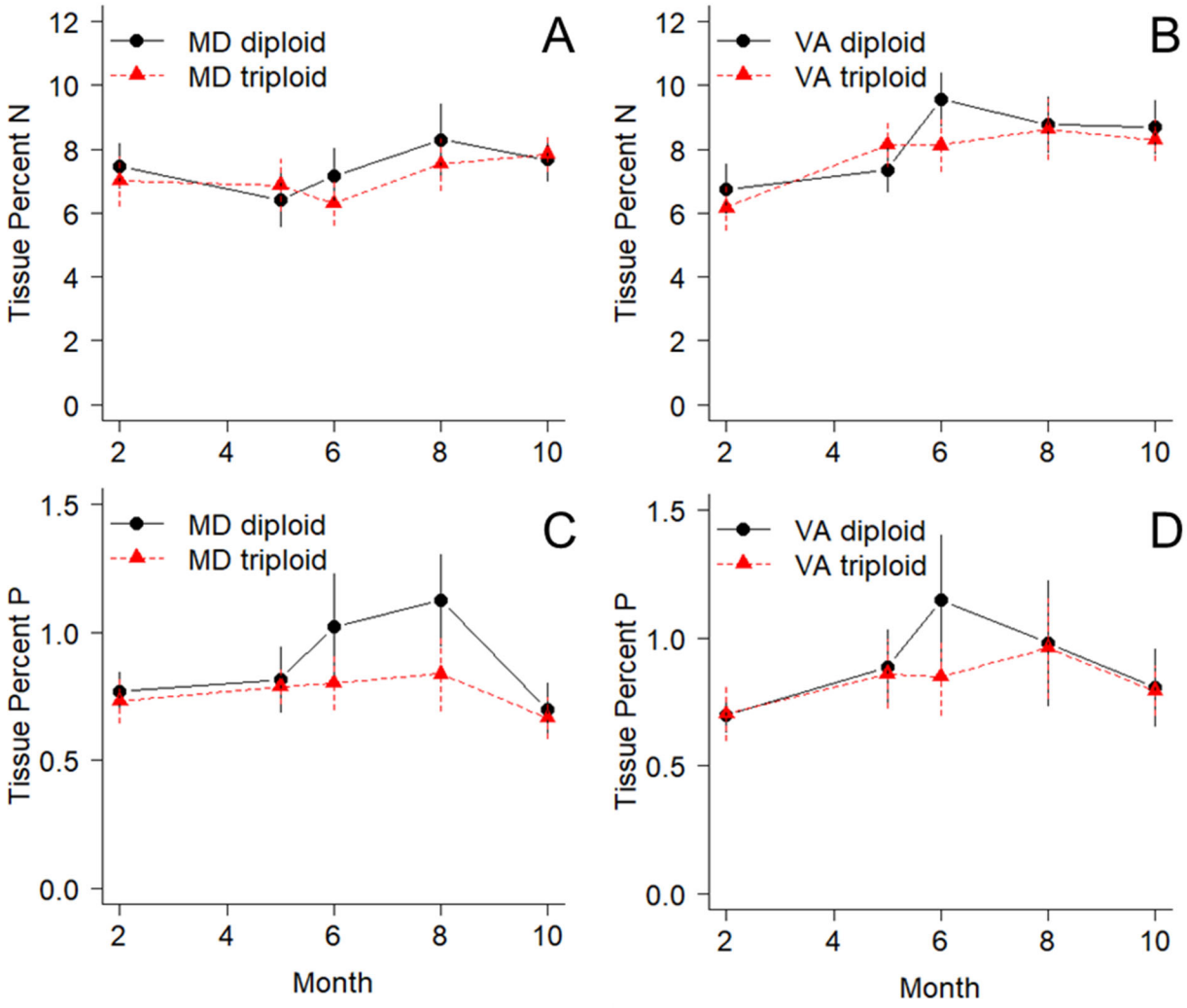
549

550 **Figure 2. Chesapeake Bay Program Water Quality Monitoring Program observations for**
551 **Station LE3.4 in the Rappahannock River (in red) and Station ET4.2 in the Chester River**
552 **(in black). Time series are presented for both bottom water (solid line with circles) and**
553 **surface water (dashed line with triangles) for water temperature (□), salinity, dissolved**
554 **oxygen concentration (mg/l), chlorophyll *a* concentration (µg/l), dissolved inorganic**
555 **nitrogen (DIN, mg/l), and Secchi depth (m).**



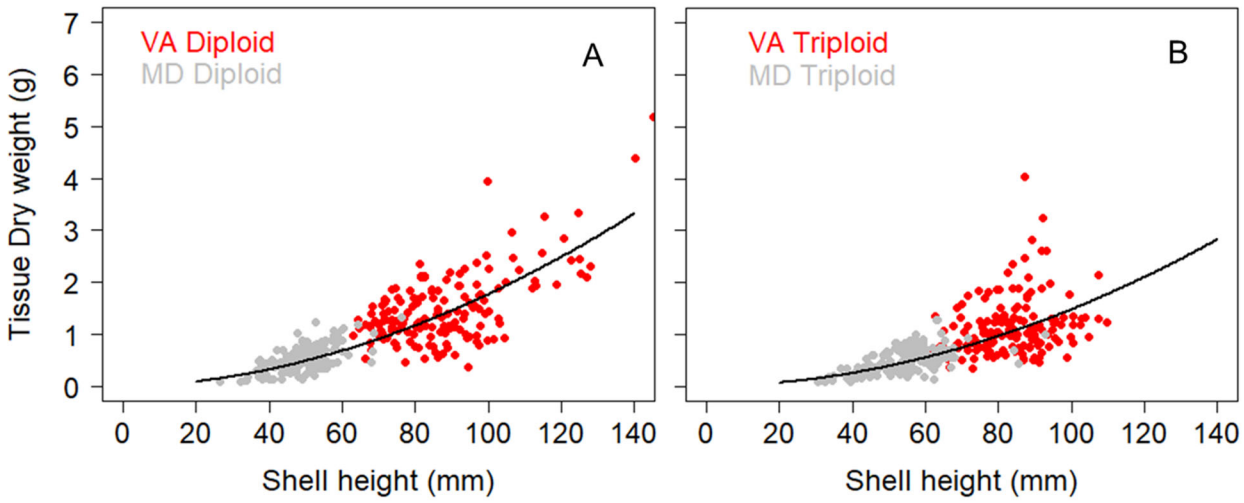
556

557 **Figure 3. Oyster tissue N (A) and P concentrations (B) between diploid (white) and**
558 **triploid oysters (gray) at each farm site. Differences are highlighted by both mean (black**
559 **diamonds) and median (horizontal lines) concentrations.**



560

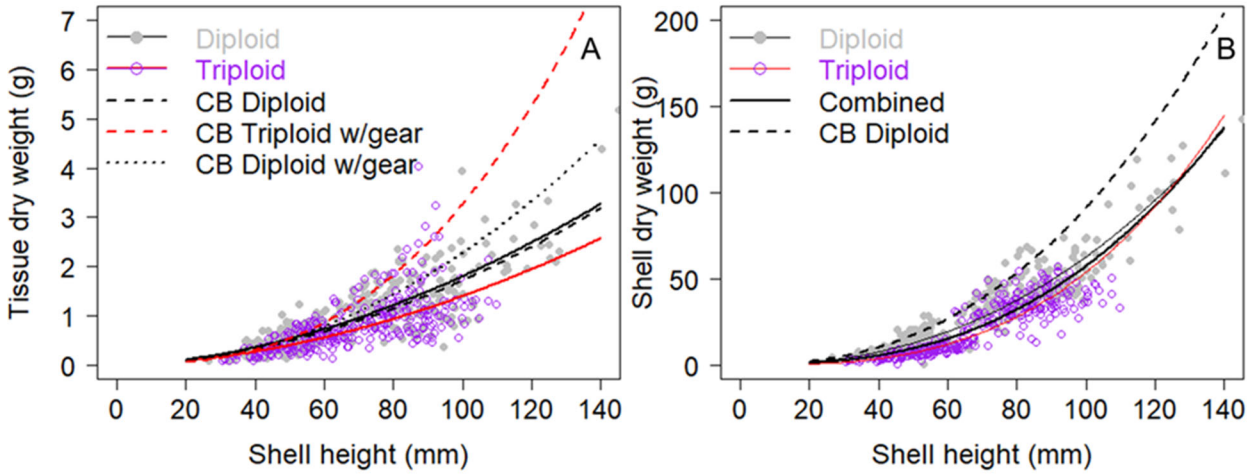
561 **Figure 4. Time series of oyster tissue N and P concentrations by month for the VA farm**
562 **(A, C) and the MD farm (B, D) showing differences in mean values between diploid (black**
563 **circles) and triploid oysters (red triangles). Error bars show +/- one standard deviation.**



564

565 **Figure 5. Differences in shell height to tissue dry weight relationships for diploid (A) and**
566 **triploid oysters (B) by farm location. Samples from the VA farm are shown in red, and**
567 **samples from the MD farm are shown in gray. Regressions are 50th quantile by ploidy.**

568



569

570 **Figure 6. Shell height to dry weight relationships for diploid oysters grown with gear**
571 **(gray points, solid black line) and triploid oysters grown with gear (purple points, solid**
572 **red line) from both farm sites. 50th quantile regressions are shown for tissue (A) and**
573 **shell (B; diploid and triploid combined). For comparison, model fits from the 2023 update**
574 **to the Chesapeake Bay Oyster Aquaculture BMP report are shown for diploid oysters**
575 **grown without gear (dashed black line), and with gear (dotted black line), and triploid**
576 **oysters grown with gear (dashed red line).**

577

References

Allen, S.K., Downing, S.L., 1990. Performance of Triploid Pacific Oysters, *Crassostrea gigas*: Gametogenesis. Canadian Journal of Fisheries and Aquatic Sciences 47, 1213-1222.

Basu, N.B., Van Meter, K.J., Byrnes, D.K., Van Cappellen, P., Brouwer, R., Jacobsen, B.H., Jarsjö, J., Rudolph, D.L., Cunha, M.C., Nelson, N., Bhattacharya, R., Destouni, G., Olsen, S.B., 2022. Managing nitrogen legacies to accelerate water quality improvement. Nature Geoscience 15, 97-105.

Bernard, I., de Kermoysan, G., Pouvreau, S., 2011. Effect of phytoplankton and temperature on the reproduction of the Pacific oyster Crassostrea gigas: Investigation through DEB theory. Journal of Sea Research 66, 349-360.

Beseres Pollack, J., Yoskowitz, D., Kim, H.-C., Montagna, P.A., 2013. Role and value of nitrogen regulation provided by oysters (Crassostrea virginica) in the Mission-Aransas estuary, Texas, USA. PLoS One 8, e65314.

Boesch, D.F., 2019. Barriers and Bridges in Abating Coastal Eutrophication. Frontiers in Marine Science 6.

Boros, G., Mozsar, A., 2015. Comparison of different methods used for phosphorus determination in aquatic organisms. Hydrobiologia 758, 235-242.

Breitburg, D., Levin, L.A., Oschlies, A., Grégoire, M., Chavez, F.P., Conley, D.J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G.S., Limburg, K.E., Montes, I., Naqvi, S.W.A., Pitcher, G.C., Rabalais, N.N., Roman, M.R., Rose, K.A., Seibel, B.A., Telszewski, M., Yasuhara, M., Zhang, J., 2018. Declining oxygen in the global ocean and coastal waters. Science 359, eaam7240.

Breitburg, D.L., Hondorp, D.W., Davias, L.A., Diaz, R.J., 2009. Hypoxia, nitrogen and fisheries: integrating effects across local and global landscapes. Annual Review of Marine Science 1, 329-349.

Bricker, S.B., Clement, C.G., Pirhalla, D.E., Orlando, S.P., Farrow, D.R.G., 1999. National Estuarine Eutrophication Assessment: Effects of Nutrient Enrichment in the Nation's Estuaries. NOAA, National Ocean Service, Special Projects Office and the National Centers for Coastal Ocean Science, Silver Spring, MD, p. 71.

Bricker, S.B., Longstaff, B., Dennison, W., Jones, A., Boicourt, K., Wicks, C., Woerner, J., 2008. Effects of nutrient enrichment in the nation's estuaries: A decade of change. Harmful Algae 8, 21-32.

Callam, B.R., Allen, S.K., Frank-Lawale, A., 2016. Genetic and environmental influence on triploid Crassostrea virginica grown in Chesapeake Bay: Growth. Aquaculture 452, 97-106.

Carmichael, Ruth H., Walton, W., Clark, H., 2012. Bivalve-enhanced nitrogen removal from coastal estuaries. Canadian Journal of Fisheries and Aquatic Sciences 69, 1131-1149.

627 Chesapeake Bay Program, 2017. Methods and quality assurance for Chesapeake Bay water
628 quality monitoring programs, CBP/TRS-319-17, May 2017, 231 p.
629
630 Clements, J.C., Comeau, L.A., 2019. Nitrogen removal potential of shellfish aquaculture
631 harvests in eastern Canada: A comparison of culture methods. Aquaculture Reports 13,
632 100183.
633
634 Cornwell, J., Bricker, S., Lacatell, A., Luckenbach, M., Marenghi, F., Moore, C., Parker, M.,
635 Paynter, K., Rose, J., Sanford, L., Wolinski, B., Caretti, O., Reichert-Nguyen, J., Slacum, W.,
636 2023. Oyster BMP Expert Panel Second Incremental Report, Report to the Chesapeake Bay
637 Program. Available online at
638 https://d18lev1ok5leia.cloudfront.net/chesapeakebay/documents/Oyster-BMP-Expert-Panel-
639 Second-Report.pdf.
640
641 Cox, C., Mann, R.L., 1992. Temporal and spatial changes in fecundity of eastern oysters,
642 Crassostrea virginica (Gmelin, 1791) in the James River (1992). Journal of Shellfish Research
643 11, 49-54.
644
645 Cubillo, A., Bricker, S.B., Parker, M., Ferreira, J.G., 2018. NCCOS Ecological Assessment to
646 support NOAA's Choptank Complex Habitat Focus Area: eutrophication and shellfish
647 aquaculture/restoration ecosystem services modeling. Final report submitted to NOAA NCCOS,
648 Cooperative Oxford Laboratory by Longline Environmental, Ltd., National Oceanic and
649 Atmospheric Administration, National Ocean Service, National Centers for Coastal Ocean
650 Science, Silver Spring, MD. Av.
651
652 Deegan, L.A., Johnson, D.S., Warren, R.S., Peterson, B.J., Fleeger, J.W., Fagherazzi, S.,
653 Wollheim, W.M., 2012. Coastal eutrophication as a driver of salt marsh loss. Nature 490, 388-
654 392.
655
656 Dégremont, L., Garcia, C., Frank-Lawale, A., Allen, S., K. , 2012. Triploid Oysters in the
657 Chesapeake Bay: Comparison of Diploid and Triploid *Crassostrea virginica*. Journal of Shellfish
658 Research 31, 21-31.
659
660 Dridi, S., Romdhane, M.S., Elcafsi, M.h., 2007. Seasonal variation in weight and biochemical
661 composition of the Pacific oyster, Crassostrea gigas in relation to the gametogenic cycle and
662 environmental conditions of the Bizert lagoon, Tunisia. Aquaculture 263, 238-248.
663
664 Encomio, V.G., Stickler, S.M., Allen, S.K., Chu, F.-L., 2005. PERFORMANCE OF “NATURAL
665 DERMO-RESISTANT” OYSTER STOCKS—SURVIVAL, DISEASE, GROWTH, CONDITION
666 AND ENERGY RESERVES. Journal of Shellfish Research 24, 143-155, 113.
667
668 Galtsoff, P.S., 1964. The American Oyster *Crassostrea virginica* Gmelin. Fishery Bulletin of the
669 Fish and Wildlife Service 64, 1-480.
670
671 Gregory, K.M., McFarland, K., Hare, M.P., 2023. Reproductive Phenology of the Eastern
672 Oyster, Crassostrea virginica (Gmelin, 1791), Along a Temperate Estuarine Salinity Gradient.
673 Estuaries and Coasts 46, 707-722.
674
675 Grizzle, R.E., Ward, K., 2011. Experimental quantification of nutrient bioextraction potential of
676 oysters in estuarine waters of New Hampshire. Report to the Piscata Region Estuaries

Partnership; available online at http://www.prep.unh.edu/resources/pdf/experimental_quantification_of-unh-11.pdf.

Guévélou, E., Carnegie, R.B., Small, J.M., Hudson, K., Reece, K.S., Rybovich, M.M., 2019. Tracking Triploid Mortalities of Eastern Oysters *Crassostrea virginica* in the Virginia Portion of the Chesapeake Bay. Journal of Shellfish Research 38, 101-113, 113.

Higgins, C.B., Stephenson, K., Brown, B.L., 2011. Nutrient bioassimilation capacity of aquacultured oysters: quantification of an ecosystem service. Journal of Environmental Quality 40, 271-277.

Hofmann, E.E., Klinck, J.M., Powell, E.N., Boyles, S., Ellis, M., 1994. Modeling oyster populations. II. Adult size and reproductive effort. Journal of Shellfish Research 13, 165-182.

Hofmann, E.E., Powell, E.N., Klinck, J.M., Wilson, E.A., 1992. Modeling oyster populations. 3. Critical feeding periods, growth, and reproduction. Journal of Shellfish Research 11, 399-416.

Howarth, R., Paerl, H.W., 2008. Coastal marine eutrophication: Control of both nitrogen and phosphorus is necessary. Proceedings of the National Academy of Sciences 105, E103.

Howarth, R.W., Marino, R., 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades. Limnology and Oceanography 51, 364-376.

Ibarra, A.M., Ascencio-Michel, R., Ramírez, J.L., Manzano-Sarabia, M., Rodríguez-Jaramillo, C., 2017. Performance of Diploid and Triploid *Crassostrea gigas* (Thunberg, 1793) Grown in Tropical Versus Temperate Natural Environmental Conditions. Journal of Shellfish Research 36, 119-139, 121.

Jouaux, A., Heude-Berthelin, C., Sourdaine, P., Mathieu, M., Kellner, K., 2010. Gametogenic stages in triploid oysters Crassostrea gigas: Irregular locking of gonial proliferation and subsequent reproductive effort. Journal of Experimental Marine Biology and Ecology 395, 162-170.

Kellogg, M.L., Cornwell, J.C., Owens, M.S., Paynter, K.T., 2013. Denitrification and nutrient assimilation on a restored oyster reef. Marine Ecology Progress Series 480, 1-19.

Kellogg, M.L., Smyth, A.R., Luckenbach, M.W., Carmichael, R.H., Brown, B.L., Cornwell, J.C., Piehler, M.F., Owens, M.S., Dalrymple, D.J., Higgins, C.B., 2014. Use of oysters to mitigate eutrophication in coastal waters. Estuarine, Coastal and Shelf Science 151, 156-168.

Kingsley-Smith, P.R., Harwell, H.D., Kellogg, M.L., Allen, S.M., Allen, S.K., Meritt, D.W., Paynter, K.T., Luckenbach, M.W., 2009. Survival and Growth of Triploid *Crassostrea virginica* (Gmelin, 1791) and *C. ariakensis* (Fujita, 1913) in Bottom Environments of Chesapeake Bay: Implications for an Introduction. Journal of Shellfish Research 28, 169-184, 116.

Koenker, R., 2006. Quantile regression in R: a vignette, Available online at http://www.econ.uiuc.edu/~roger/research/rq/vig.pdf.

Koenker, R., 2016. Package 'quantreg': Quantile Regression. Available online from the R Development Core Team: https://cran.r-project.org/web/packages/quantreg/quantreg.pdf.

Le Moal, M., Gascuel-Odoux, C., Ménesguen, A., Souchon, Y., Étrillard, C., Levain, A., Moatar, F., Pannard, A., Souchu, P., Lefebvre, A., Pinay, G., 2019. Eutrophication: A new wine in an old bottle? Science of The Total Environment 651, 1-11.

Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.-O., Olrog, L., Rehnstam-Holm, A.-S., Svensson, J., Svensson, S., Syversen, U., 2005. Improving marine water quality by mussel farming - a profitable solution for Swedish society. Ambio 34, 129-136.

Lintern, A., McPhillips, L., Winfrey, B., Duncan, J., Grady, C., 2020. Best Management Practices for Diffuse Nutrient Pollution: Wicked Problems Across Urban and Agricultural Watersheds. Environmental Science & Technology 54, 9159-9174.

Mann, R., Southworth, M., Carnegie, R.B., Crockett, R.K., 2014. Temporal Variation in Fecundity and Spawning in the Eastern Oyster, *Crassostrea virginica*, in the Piankatank River, Virginia. Journal of Shellfish Research 33, 167-176.

Mann, R.L., Rainer, J.S., Morales-Alamo, R., 1994. Reproductive activity of oysters, Crassostrea virginica (Gmelin, 1791) in the James River, Virginia, during 1987-1988. Journal of Shellfish Research 13, 157-164.

Matt, J.L., Allen, S.K., 2021. A classification system for gonad development in triploid Crassostrea virginica. Aquaculture 532, 735994.

McFarland, K., Vignier, J., Standen, E., Volety, A.K., 2022. Synergistic effects of salinity and temperature on the eastern oyster Crassostrea virginica throughout the lifespan. Marine Ecology Progress Series 700, 111-124.

Mizuta, D.D., Wikfors, G.H., 2019. Seeking the perfect oyster shell: a brief review of current knowledge. Reviews in Aquaculture 11, 586-602.

Newell, R.I.E., 1988. Ecological changes in Chesapeake Bay: are they the result of overharvesting the eastern oyster (*Crassostrea virginica*)?, in: Lynch, M.P., Krome, E.C. (Eds.), Understanding the estuary. Chesapeake Research Consortium Publication 129, Gloucester Point, VA, pp. 536-546.

Officer, C.B., Smayda, T.J., Mann, R., 1982. Benthic filter feeding: a natural eutrophication control. Marine Ecology Progress Series 9, 203-210.

Rabalais, N.N., Díaz, R.J., Levin, L.A., Turner, R.E., Gilbert, D., Zhang, J., 2010. Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 7, 585-619.

Ray, N.E., Hancock, B., Brush, M.J., Colden, A., Cornwell, J., Labrie, M.S., Maguire, T.J., Maxwell, T., Rogers, D., Stevick, R.J., Unruh, A., Kellogg, M.L., Smyth, A.R., Fulweiler, R.W., 2021. A review of how we assess denitrification in oyster habitats and proposed guidelines for future studies. Limnology and Oceanography: Methods n/a.

Reichert-Nguyen, J., Cornwell, J., Rose, J., Kellogg, L., Luckenbach, M., Bricker, S., Paynter, K., Moore, C., Parker, M., Sanford, L., Wolinski, B., Lacatell, A., Fegley, L., Hudson, K., French, E., Slacum, W., 2016. Panel recommendations on the oyster BMP nutrient and suspended sediment reduction effectiveness determination decision framework and nitrogen and

phosphorus assimilation in oyster tissue reduction effectiveness for oyster aquaculture practices, Report to the Chesapeake Bay Program. Available online at https://www.oysterrecovery.org/wp-content/uploads/2017/01/Oyster-BMP-1st-Report_Final_Approved_2016-12-19.pdf.

Rose, J.M., Bricker, S.B., Tedesco, M.A., Wikfors, G.H., 2014. A Role for Shellfish Aquaculture in Coastal Nitrogen Management. Environmental Science & Technology 48, 2519-2525.

Sebastiano, D., Levinton, J.S., Doall, M., Kamath, S., 2015. Using a Shellfish Harvest Strategy to Extract High Nitrogen Inputs in Urban and Suburban Coastal Bays: Practical and Economic Implications. Journal of Shellfish Research 34, 573-583, 511.

Shatkin, G., 1992. Production and growth of yearline triploid American oysters (*Crassostrea virginica*). University of Maine, Orono, ME, p. 95.

Stone, B.W., Hadley, N.H., Kingsley-Smith, P.R., 2013. Evaluating the Potential Growth Advantage of Triploid Eastern Oysters (*Crassostrea virginica*) in South Carolina Relative to Commercially Cultured Diploid Native Stocks. Journal of Shellfish Research 32, 647-655, 649.

Wadsworth, P., Wilson, A.E., Walton, W.C., 2019. A meta-analysis of growth rate in diploid and triploid oysters. Aquaculture 499, 9-16.

Walton, W.C., Rikard, F.S., Chaplin, G.I., Davis, J.E., Arias, C.R., Supan, J.E., 2013. Effects of ploidy and gear on the performance of cultured oysters, Crassostrea virginica: Survival, growth, shape, condition index and Vibrio abundances. Aquaculture 414-415, 260-266.

Wilcox, R.R., 2022. Introduction to robust estimation and hypothesis testing Elsevier, London.

Wurtsbaugh, W.A., Paerl, H.W., Dodds, W.K., 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. WIREs Water 6, e1373.