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Diet of the eastern oyster, *Crassostrea virginica*, growing in a eutrophic tributary of Chesapeake Bay, Maryland, USA

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ABSTRACT

A 2-year study was undertaken to understand feeding preferences of the eastern oyster *Crassostrea virginica* in the eutrophic Rhode River, a tributary of Chesapeake Bay, Maryland, USA. A subset of experimentally suspended oysters was collected monthly and environmental parameters were simultaneously measured. Oysters were measured in height to determine growth, and the phytoplankton in their gut were examined both microscopically and using indicator pigments and compared with phytoplankton abundance and composition in the water column. Growth was higher in the second year of the study when flow was lower and salinity higher. Food selectivity was calculated using a modified electivity index (E_i), which relates phytoplankton composition in the gut to that in the water. Oysters appeared to preferentially graze–or at least preferentially retain in the gut–various (unidentified) flagellates, Ochrophyta (diatoms) and Myzozoa (dinoflagellates), and appeared to generally reject cyanobacteria, especially picocyanobacteria, from their diet. The Myzozoa included several common harmful algal bloom taxa, including *Prorocentrum minimum* (=*P. cordatum*) and *Heterocapsa rotundatum*, that can detrimentally affect oyster growth. Reductions in eutrophication will likely be beneficial for oyster diets if such reductions result in fewer dinoflagellate blooms and in picocyanobacteria abundance during the critical feeding summer months.

1. Introduction

The eastern oyster, *Crassostrea virginica* (Gmelin, 1791), is ecologically and economically important in estuaries from Maritime Canada to the Gulf of Mexico, but declines in oyster populations have been observed in many estuaries over the past decades. Oysters have been negatively impacted by disease, declines in water quality and suitable habitat, as well as by over-harvesting (Wesson et al., 1999; Ocean Studies Board, 2004). For example, in Apalachicola Bay, Florida, recent and dramatic declines in oyster abundances have raised many questions about changes in ecology and habitat and therefore what actions might be appropriate to help reverse these trends (Oczkowski et al., 2011; Petes et al., 2012; Havens et al., 2013). Whereas disease and habitat loss are thought to be the major threats to oysters (e.g., Wesson et al., 1999; Garland and Kimbro, 2015), eutrophication has also been increasing over the past half century, leading to changes in nutrient loading, which in turn affect the phytoplankton community on which oysters feed (Hagy et al., 2004; Kemp et al., 2005; Glibert et al., 2005, 2007).

Characterizing the natural diet of oysters and how it may change under differing environmental conditions is a topic of considerable interest, as it has impacts for understanding the effects of habitat change or the suitability of habitat for aquaculture (e.g., Shumway et al., 1985; Kasim and Mukai, 2016; Hall et al., 2020). Stable isotope analyses indicate the bulk of assimilated carbon (C) comes from phytoplankton (Haines and Montague, 1979; Hughes and Sherr, 1983; Langdon and Newell, 1996). Although oysters mainly consume algae, they also consume bits of detrital matter, fragments of seagrasses and numerous other non-algae cells including small animals such as copepods and rotifers, as well as eggs and larvae of various species if they are taken in with the algae (e.g., Haines and Montague, 1979; Newell, 1988; Bayne, 2017). Oysters and other bivalves may discriminate and selectively feed on different foods based on particle size (e.g., Haven and Morales-Alamo, 1970; Riisgård, 1988; Stenton-Dozey and Brown, 1992, Defossez and Hawkins, 1997) and food quality (Arifin and Bendell-Young, 1997; Ward et al., 1997; Newell and Jordan, 1983) as well as quantity in terms of particle concentrations (Bayne et al., 1989,

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1993). Particle discrimination may also be mediated by factors related to the particles themselves, such as shape, surface properties, charge and other poorly understood characteristics (Rosa et al., 2013, 2018). In natural settings, factors such as temperature and salinity also affect feeding (e.g., Shumway, 1996). Material that is not digested may be released as pseudofeces or as feces, both of which are important sources of recycled C and nitrogen (N), and the proportion and composition of feces and pseudofeces may alter the subsequent biogeochemical cycling of that C and N, leading to either the favorable process of denitrification (e.g., Hoellein et al., 2015) or the regeneration of nutrients on which phytoplankton may thrive.

The natural diets of oysters–especially those growing in eutrophic waters–may include algae that are considered harmful or potentially toxic (e.g., Landsberg, 2002; Burkholder et al., 2018 and references therein). Harmful algae and harmful algal blooms (HABs) have several direct effects on oysters. They can render the oysters contaminated making them unsuitable for human consumption, but they can also affect growth and, in some cases, development and survivorship of larvae (e.g., Wikfors and Smolowitz, 1995; Kim-Brinson and Ramsdell, 2001, Jeong et al. 2004, Leverone et al., 2006; Padilla et al., 2006; Bricelj and MacQuarrie, 2007; Glibert et al., 2007; Stoecker et al., 2008). Harmful taxa within the gut may also cause damage to the oyster digestion system and reduced assimilation. Impacts of harmful algae differ by the growth state of the oysters at the time of their exposure, the particular HAB species or strain, as well as its stage of growth (Landsberg, 2002; Pate et al., 2003).

To better understand feeding preferences of *C. virginica*, seasonal changes in food supply and diet of oysters in the Rhode River, a tributary of Chesapeake Bay, USA, were examined. Chesapeake Bay, the largest estuary in the USA, has long been degraded by excess nutrient pollution and eutrophication and HAB proliferation (e.g., Kemp et al., 2005). In Chesapeake Bay, oyster abundances have declined nearly 100-fold over the last century (Rothschild et al., 1994; Jordan et al., 2002; Kimmel and Newell, 2007; Wilberg et al., 2011). Efforts to restore oysters in Chesapeake Bay have centered on rebuilding habitat and seeding with hatchery-spawned oysters with the hope of recovering their ecosystem services including water quality improvement that extends from their filtration activity (e.g., Kellogg et al., 2014). Oyster aquaculture is also expanding in Chesapeake Bay.

This study thus addressed the following questions: How does the natural diet of oysters vary seasonally in this eutrophic tributary in terms of phytoplankton taxa and their nutrient content; what phytoplankton taxa are preferentially ingested and/or digested; and, how is ingestion, digestion and growth affected by environmental conditions? Such information is important for understanding how changes in diet may affect growth over longer periods of time, a topic of considerable relevance for estuaries stressed from the multiple ecosystem changes. Oyster restoration is often motivated by the promise of improved water quality that extends from their filtration activity (e.g., Kellogg et al., 2014). However, if oyster feeding is inhibited because of detrimental environmental conditions or phytoplankton community, these ecosystem services will not be realized. Similarly, oyster aquaculture will not be sustainable if environmental conditions are unfavorable.

2. Materials and methods

2.1. Oyster feeding

The study lasted 26 months, and was comprised of two individual experiments, each lasting ~1 year. The first experiment was initiated on September 26, 2010, and lasted through September 19, 2011, and the second experiment was initiated on September 29, 2011 and continued until December, 2012. In each experiment, 100 adult *C. virginica* were obtained from a commercial oyster grower, Marinetics, Inc., Cambridge MD, and divided among three cages (2.54 cm mesh). Cages were suspended from the Smithsonian Environmental Research Center dock, approximately 30 cm above the bottom of the Rhode River (Fig. 1a).

Each month, 5 oysters were removed from the cages and returned to the laboratory for sampling (Fig. 1b). At the time of sampling, temperature and salinity were recorded in the surface and bottom waters using a YSI probe (Xylem, Inc.). In the laboratory, shell heights were first measured to the nearest 1 mm using a caliper ruler. Then, the oyster valves were separated and the samples of gut contents were collected with disposable Pasteur pipettes. Given the natural environment from which the oysters were collected, it was not possible to sample pseudofeces. Unpreserved material collected from the gut was placed in a Sedgewick-Rafter plankton counting cell and allowed to settle for 15 min. Identification and counting were done with either a Zeiss Axiovert 205 microscope at 40x or an Olympus phase-contrast compound microscope at 200X magnification. Cells were identified to the lowest taxon possible. Gut taxa were then grouped by phytoplankton functional group. Microscopic identifications did not include material or taxa other than phytoplankton. In all, 130 individual oysters were sampled, dissected, and gut contents identified.

After identifying cells from the gut of each of the 5 oysters individually, an equal aliquot from each oyster was then pooled into a combined gut sample (Fig. 1b). This combined material was placed on a GF/F filter. Filters were frozen at −80 °C and subsequently analyzed for pigment composition using a Hewlett Packard Series 1100 high-performance liquid chromatography (HPLC) system according to Van Heukelem and Thomas (2001). The rationale for pigment analysis in addition to that of individual microscopic enumerations is that pigment signatures may yield insight into food ingested and digested even when cells were no longer identifiable. Applying the methods used in Alexander et al. (2008), changes in the ratio of selected, diagnostic pigments relative to chlorophyll *a* (chl *a*), and relative to the initial ratio of these pigments in the algal diet, were used as indices of cell passage through the gut. Pigment content was normalized to chl *a* and the composition of fucoxanthin:chl *a* was taken as a measure of diatoms abundance, peridinin:chl *a* as a measure of peridinin-containing dinoflagellates, and zeaxanthin:chl *a* as a measure of cyanobacteria (Jeffrey and Wright, 1994; Jeffrey and Vesk, 1997). Phaeophytin *a* and phaeophorbide *a* were used as the diagnostic indicators of the degradation of chl *a*. In total, 26 monthly pooled samples were analyzed for gut pigment composition, although data on the degradation pigments are only available for 12 of those 26 sets of samples (primarily from experiment 1 in 2011).

2.2. Phytoplankton community composition in water

At the time of oyster sampling, phytoplankton samples were also collected from the river for comparison with that in the gut composition samples (Fig. 1b). Whole water samples were collected from near surface and near bottom at the site of the oyster cages and returned to the laboratory for near-immediate microscopic identification and enumeration of taxa present as above. In the laboratory, samples were inverted to mix and 1 ml was then transferred to a Sedgewick-Rafter chamber using a disposable glass Pasteur pipette. The chamber was examined on a Zeiss Axiovert 205 microscope at 40 ×. Cells were identified to the lowest taxon possible.

To compare the water column pigment composition with cell enumerations, the water samples were filtered, filters were frozen at−80 °C, and diagnostic pigments were subsequently analyzed by HPLC using the same techniques as described above (Section 2.1; Van Heukelem and Thomas, 2001; Fig. 1b).

2.3. Water quality and flow

As part of the broader Chesapeake Bay Monitoring Program (https://www.chesapeakebay.net/what/data), dissolved and particulate nutrient data (concentrations of NO_3^-, NH_4^+, PO_4^{3-}, particulate nitrogen (PN), particulate phosphorus (PP) and particulate carbon (PC))

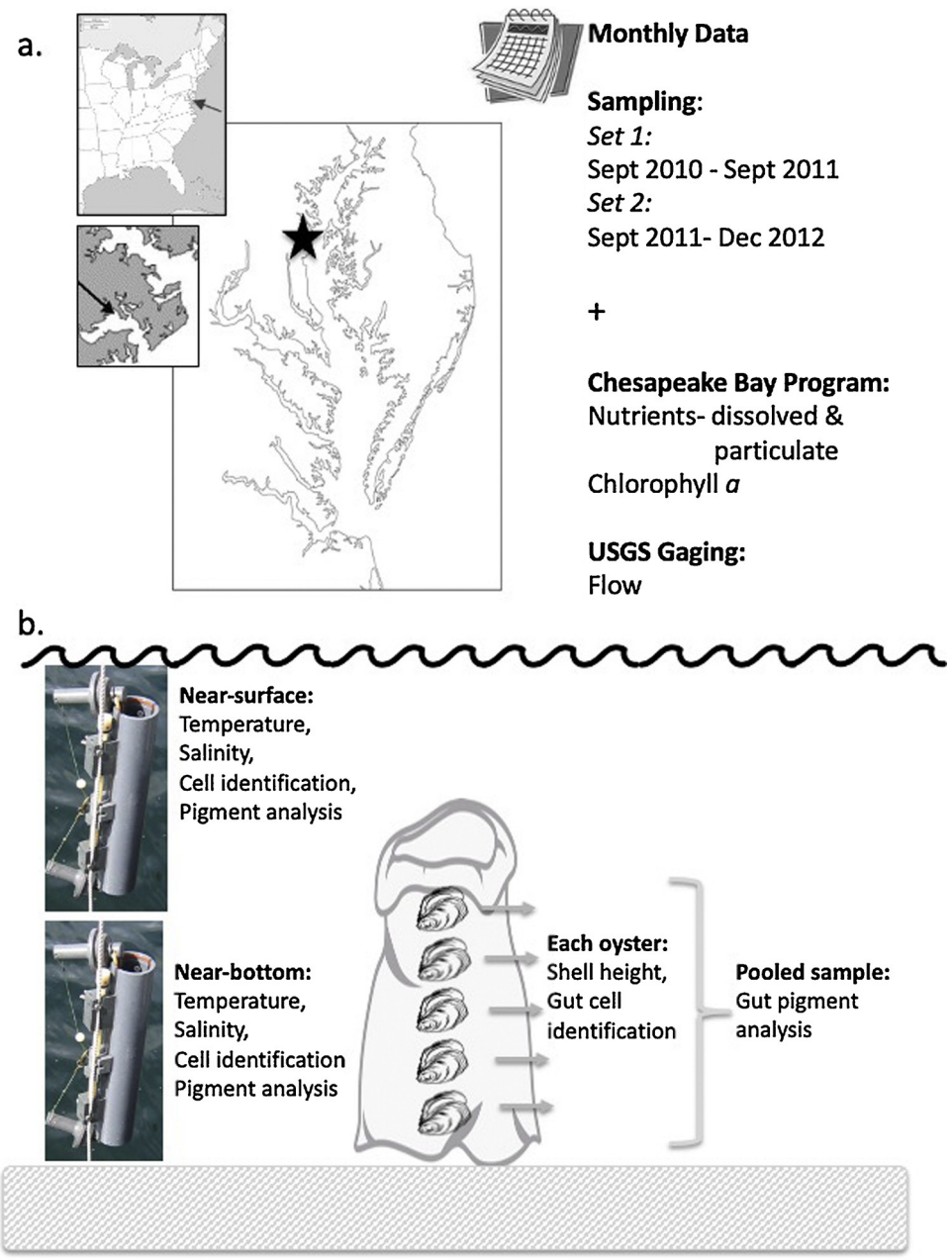

Fig. 1. (a) Chesapeake Bay with the location of the Rhode River (38° 53' 08'' N, 76 ° 32' 29'' W) identified; inset maps show the Chesapeake Bay on the US east coast and the study site on Rhode River; list of dates of sampling and other data accessed. (b) schematic diagram of the monthly sampling.

were collected on a monthly basis from a nearby site on the same river, although these samples were not taken on the same dates as the oyster collections. The Chesapeake Bay Program also reports concentrations of chl *a*, which were compared with data taken on the same day as oyster sampling.

As there are no flow gages located at the study site, monthly river flow data were downloaded from the USGS gaging site 01589795 at the adjacent South River. Units of $ft^3\ s^{-1}$ were converted to $m^3\ s^{-1}$ by multiplying by the constant 0.0283 (waterdata.usgs.gov). These data are indicative of long-term trends which Chesapeake Bay tributaries are experiencing and are not meant to represent immediate conditions for the oysters under study.

2.4. Data analysis and statistics

Phytoplankton taxonomic data for both oysters and the water column were summarized with respect to the major phytoplankton functional groups. Myzozoa (including dinoflagellates), Ochrophyta (including diatoms), Cyanobacteria, Chlorophyta, and various flagellates (which could not be further identified; hereafter referred to as unidentified flagellates) represented the dominant groups in both the water column and the gut flora, and thus emphasis is placed on these groups herein. While some samples included representatives of the Cryptophyta and the Euglenophyta, their abundances were typically small relative to the other functional groups. The complete oyster growth and phytoplankton and pigment data set is reported in Weissberger and Glibert (2021).

In order to determine differences in the phytoplankton composition and total chl *a* concentrations from samples from surface and bottom collections, correlation analysis was applied. Comparisons of surface and bottom-collected samples were made across all dates using ANOVA. Comparisons were also similarly made between the chl *a* concentrations

derived from the Bay Program data and those collected herein.

Variability in growth and ingestion within each monthly sample of 5 oysters was determined, as was standard deviation of shell height. To estimate the variability of ingestion of the different oysters by month, the relative standard error (RSE) of the gut content (based on cell enumeration by major phytoplankton group) was calculated by month, recognizing that this calculation is based on whole, identifiable cells. Growth of oysters was individually regressed against water quality parameters and phytoplankton community composition and results were also analyzed by principal components analysis. All statistical analyses were conducted using Excel or XLSTAT.

Food selectivity was calculated using a modified version of Ivlev's (1961) electivity index (E_i) (Rosa et al., 2013). Compared to the commonly used Ivlev's electivity index, this modified index is less sensitive to errors associated with rare species (Lechowicz, 1982). Values were calculated to relate phytoplankton composition in the gut to that in the water according to:

$$E_i = (r_i - p_i)/((r_i + p_i) - 2(r_i \times p_i))$$

where r_i is the relative abundance of a particular phytoplankton in the diet and p_i is the abundance of that taxa in the water column. Such calculations were made using both cell counts and indicator pigments. For the calculations using cell enumerations, the average abundance from each of the 5 oysters was used. Values range from +1 to −1, with positive values indicating selection for a particular food source, and negative values indicating rejection, and a value near 0 indicates consumption proportional to availability. As recommended by Lechowicz (1982) in applying such an index, due to the imperfect nature of electivity indices in capturing all associated errors and variabilities, the rank order of food preference is also reported by season. In interpreting rank order, the five major phytoplankton functional groups were compared, and thus values ranged from 1 (most preferred) to 5 (least preferred).

Digestion efficiency was calculated using the concentrations of chl *a* degradation pigments, according to a modification of a formula reported by Bayne (2017):

Digestion efficiency= 1- (phaeopigments in gut/ingested chlorophyll)

The Bayne formulation applied phaeopigments in the feces; here, the gut value was used.

3. Results

3.1. Flow and water quality parameters

During the course of the 2-year study, water quality and flow conditions varied widely. Water temperatures ranged from ~4–28.5 °C, and salinity ranged from 2.5 to 14.2 (Fig. 2a, b). Although temperatures were comparable during the different years of the study, 2011 was a

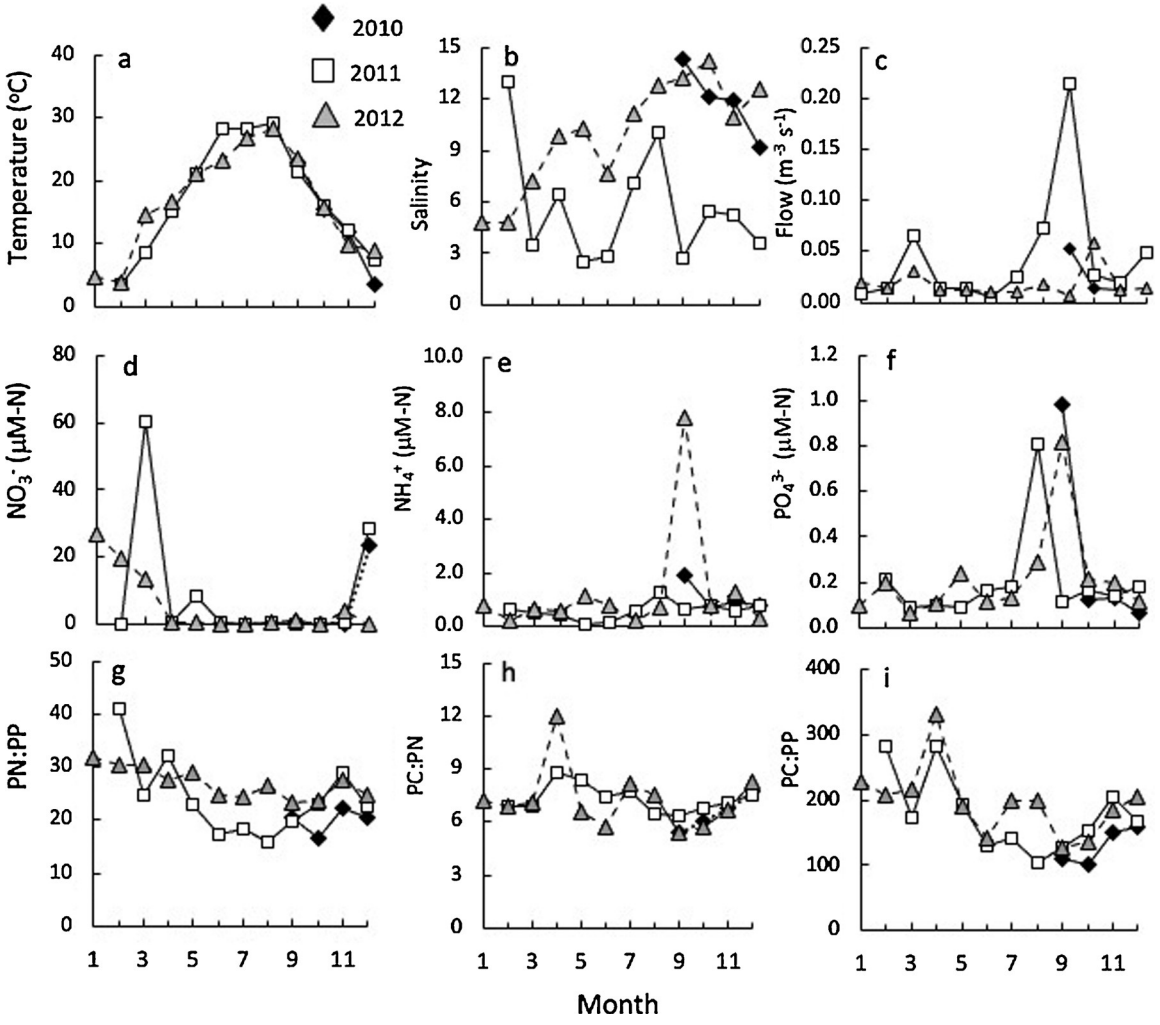

Fig. 2. Environmental and nutrient parameters of the Rhode River measured during the years of the study. (a) temperature; (b) salinity; (c) flow (data from the nearby South River); (d) NO_3-; (e) NH_4^+; (f) PO_4^{3-}; (g) ratio of particulate nitrogen: particulate phosphorus (PN:PP; molar basis); (h) particulate carbon: PN (molar); (i) PC:PP (molar).

much wetter year than either late 2010 or 2012 (Fig. 2c). Average flow in the South River in 2011 (4.22 $m^3 s^{-1}$) was nearly twice that of 2012 (2.57 $m^3 s^{-1}$), largely driven by a wetter summer, and this is further evidenced by the considerably lower salinities during 2011 (the month of February being an exception; Fig. 2b).

Nutrient concentrations were also highly variable between seasons and between years. Concentrations of NO_2^- ranged from a few tenths of a μM during the summer months to several tens of μM during the winter months (Fig. 2d). Concentrations of NO_3^- in 2011 reached higher concentrations than those in 2012, especially in late winter/spring, consistent with 2011 being a higher-flow year. Concentrations of NH_4^+ ranged between a few tenths of a μM and about 1 μM, although higher values were attained in late summer during the lower-flow year of 2012 (Fig. 2e). Concentrations of PO_4^{3-} remained in the range of 0.1–0.2 μM-P, except during mid to late summer in each year, when concentrations reached 0.8–1.0 μM-P (Fig. 2f). For particulate N:P (PN:PP), lowest values, approaching Redfieldian proportions (16 on a molar basis; Redfield, 1958), were seen during the summer months of 2011, while higher values, >40, were seen during the winter of 2011, when DIN concentrations were also very high and overall ratios were sustained at a higher level than in 2012 (Fig. 2g). Ratios of particulate C: PN (PC:PN) ranged from 5.4 to 12.0 on a molar basis (Fig. 2h). The highest PC:PN was seen during the spring months, and the highest overall value was observed during the lower-flow year, 2012. Ratios of PC:PP varied between summer lows nearing 100 during 2012, but values in excess of 200 during the winter and early spring months of both years (Fig. 2i).

3.2. Chlorophyll and cell abundances

Concentrations of total phytoplankton in surface-collected samples did not differ from those in bottom-collected samples across the time series (R^2 = 0.75, p < 0.01, n = 23). Furthermore, in spite of the different timing of sample collection, no significant differences were observed between chl *a* values derived from the Bay Program and those measured herein (R^2 = 0.42, n = 13, p < 0.05, not shown).

Temporal patterns in chl *a* and community composition varied between years. Concentrations of chl *a* ranged from 6.7 to 88.1 μg L^{-1} (Fig. 3a, b). Average annual total chl *a* in 2011 (35.50μg L^{-1}) was nearly 3-fold higher than that in 2012 (12.89 μg L^{-1}). Peaks in chl *a* (>75 μg L^{-1}) were seen in May and September 2011, while the highest chl *a* peak in 2012 was observed in July, with ~70 μg L^{-1}.

As with chl *a*, cell abundances were lower in 2012 than in 2011 (Fig. 3c, d). Throughout most of each year, the abundance of Myzozoa was low, generally representing <25 % of the phytoplankton assemblage on a cell basis (Figs. 3c, d, 4 a), but increased in abundance when both temperature and salinity were low (Fig. 4b). There were two distinct dinoflagellate blooms: during winter 2011 the bloom was dominated by *Heterocapsa rotundata* (Fig. 5a), while late in the year in 2012, *Prorocentrum minimum* (≒*P. cordatum*) was the dominant species (Fig. 5b).

Ochrophytes, including Bacillariophytes (diatoms), were approximately an order of magnitude more abundant in 2011 than 2012 especially during the fall months (Fig. 3c, d). They made up >15 % of the phytoplankton community throughout most of the study, and during periods of the spring and fall, they made up >50 to 75 % of the phytoplankton community, predominating when temperatures were >10 °C across all measured salinities (Fig. 4c, d). Of the Bacillariophyceae, *Cyclotella* sp. was the most common, especially during 2011, while *Skeletonema costatum* dominated the fall bloom in 2012 as well as late in 2010 (Fig. 5c, d).

Overall highest cell abundances were attained by Cyanobacteria (Fig. 3c, d). They dominated during summer months, making up >75 % of the assemblage at that time, based on cell numbers (Fig. 4e). Their abundances increased with temperature and salinity (Fig. 4f). In 2011, the Cyanobacteria increased in early summer, but the peak came later in 2012, in August and September. Cyanobacteria were dominated by *Cyanobium* sp. in 2011, and by *Synechococcus* sp. in 2012 (Fig. 5e, f).

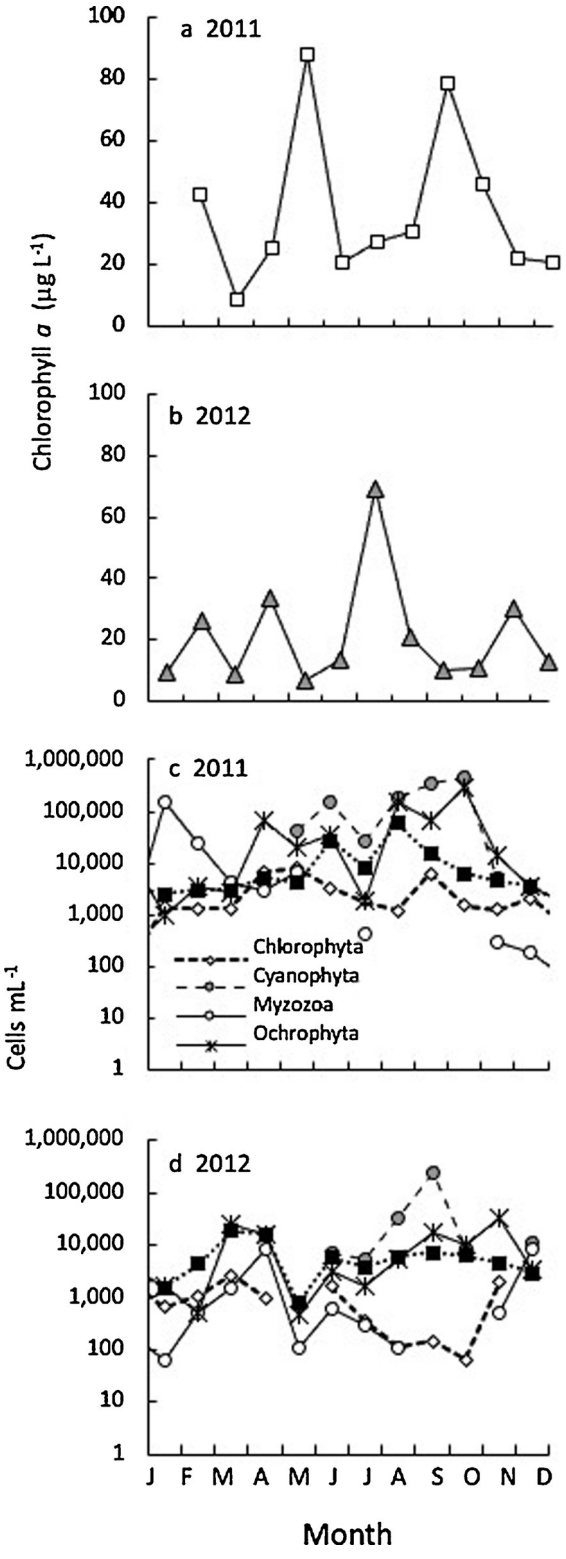

Fig. 3. (a, b) Concentration of chlorophyll *a* in the water column of the Rhode River for years 2011 and 2012; (c, d) abundance of dominant phytoplankton groups during years 2011 and 2012.

Various unidentified flagellates, which were found throughout the year, increased to highest levels in summer 2011 (Fig. 3b, d). They represented >25 % of the phytoplankton community throughout most of 2012, but a much smaller percentage in 2011 (Fig. 4g). They were found at all temperatures and salinities (Fig. 4h).

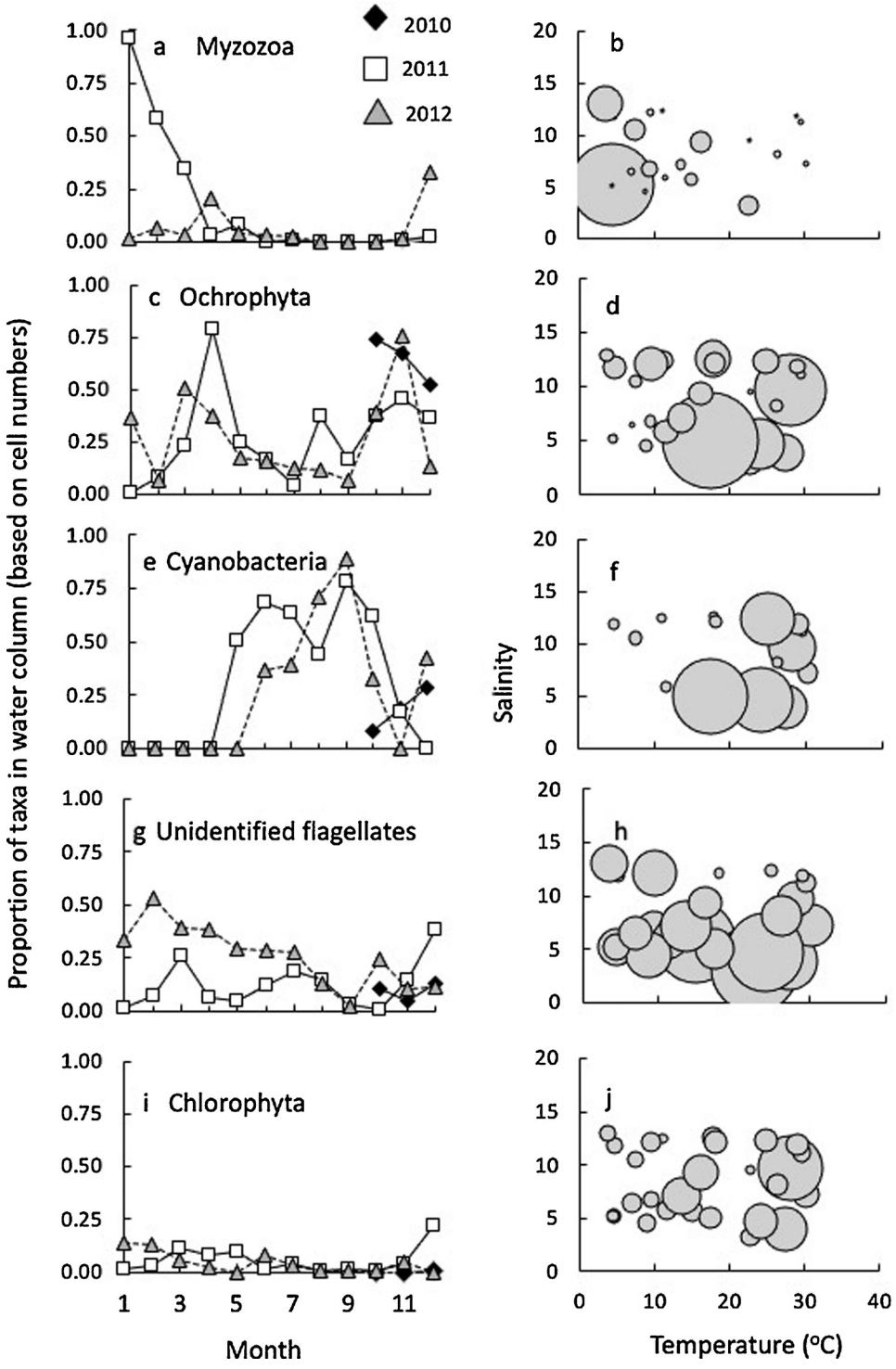

Fig. 4. (a, c, e, g, i) The proportion of individual phytoplankton groups in the water column for the years of the study based on cell number; (b, d, f, h, j) proportion of individual phytoplankton groups based on cell numbers in relation to water column temperature and salinity (size of bubble reflects relative abundance).

Chlorophytes consistently comprised <10 % of the phytoplankton community (except December 2011; Figs. 3c, d, 4 i). They were found at all temperatures and salinities, but did increase as temperatures rose (Fig. 4j). Chlorophytes were dominated by *Chlorococcum* sp. in the early months of both years and *Ankistrodesmus* sp. appeared late in 2011 (Fig. 5g, h).

3.3. Water column pigments

Concentrations of individual pigments relative to chl *a* also give insight into the relative proportion of different phytoplankton taxa. The proportion of peridinin in the water column relative to chl *a*, indicative of peridinin-containing dinoflagellates, varied throughout each year, typically in the range of 0.1 −0.3 (Fig. 6a) and abundances remained relatively constant with respect to temperature and salinity (Fig. 6b). Fucoxanthin relative to chl *a*, indicative of diatoms, in the water column

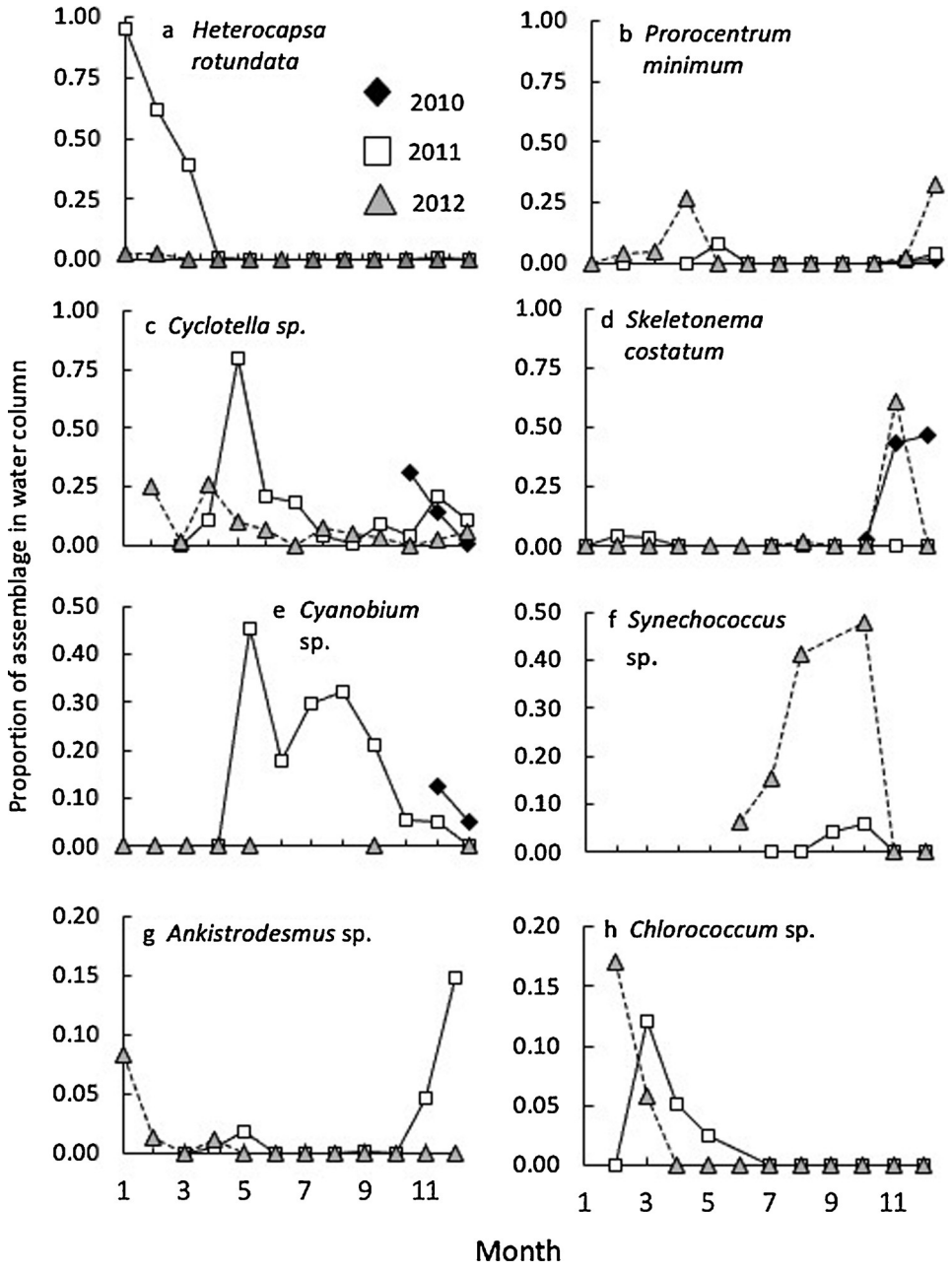

Fig. 5. Proportions in the water column of the most common species identified for each major phytoplankton group: (a, b) dinoflagellates; (c, d) diatoms, (e, f) cyanobacteria, and (g, h) chlorophytes.

was typically <0.20 for the first half of both years, but increased to >0.20 for the latter half of both years (Fig. 6c). Similar to peridinin, fucoxanthin was found in the water column at virtually all salinities and temperatures (Fig. 6d). Proportions of zeaxanthin/chl *a* in the water column reached 0.10 in both years in the summer or fall months (Fig. 6e) and increased with temperature, especially above 20 °C (Fig. 6f).

3.4. Oyster growth

The two sets of experimental oysters measured in different years had different rates of growth over their respective periods of sampling, as shown by changes in mean shell height and monthly change in size (Fig. 7). The first set of oysters averaged 83 ± 5 mm (mean ± SD) when deployed, had widely varying sizes in the first months of sampling, but from that point on, mean height steadily declined (Fig. 7a). The second set of oysters, averaged 76 ± 7 mm (mean ± SD) when deployed, and after initial variation in the first months, grew substantially, especially during the subsequent summer (Fig. 7b). From January through September, mean shell height increased 0.39 mm month^{-1} in 2011, while in 2012, it increased at a significantly faster rate, 1.47 mm month^{-1} ($p < 0.01$).

When compared with environmental variables, including abundances of the major phytoplankton groups, growth was most closely related to salinity (Fig. 8a), although the bivariable relationship was only significant at a level of $p = 0.052$ (Fig. 8b). Growth was weakly ($R^2 = 0.18$, $p < 0.05$) but significantly inversely related to Chlorophyta, as would be expected since this phytoplankton group tended to dominate in fresher waters (Fig. 8a, c).

3.5. Variability of oysters within sampling periods

Variability in gut flora of the 5 oysters sampled per month differed

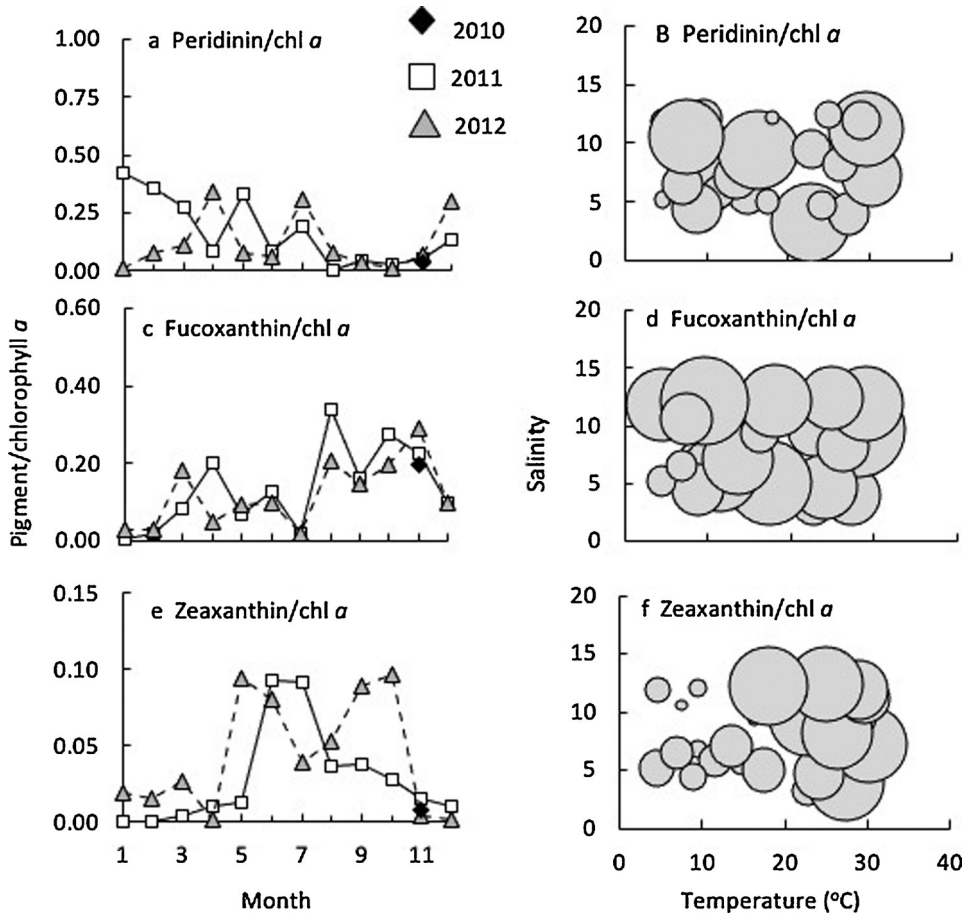

Fig. 6. (a, c, e) The proportion of individual phytoplankton groups in the water column for the years of the study based on signature pigments relative to chlorophyll *a* (note differing y-axis scales); (b, d, f) proportion of individual phytoplankton groups based on signature pigments in relation to water column temperature and salinity (size of bubble reflects relative abundance).

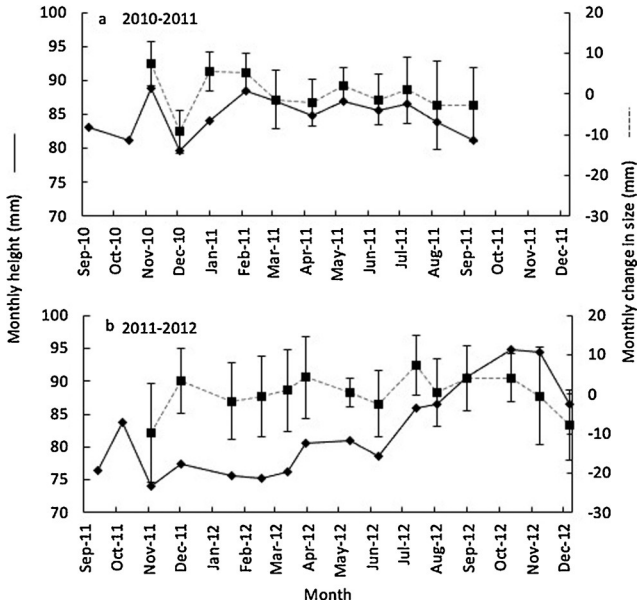

Fig. 7. Mean height (mm) of the 5 oysters sampled per month in the two individual experiments (diamonds) and the corresponding monthly mean change in size (squares). Error bars show standard deviation of the monthly change.

with season and taxa (Fig. 9). Lowest variability, based on RSE of cell abundances within the gut, was found for Myzozoa and unidentified flagellates, averaging 34.6 and 33.6 %, respectively (Fig. 9a, d). Variability in Ochrophyta varied from a low of 12.3 % in July when diatoms represented >25 % of the assemblage to 75.0 % when diatoms represented a much smaller percentage of the water column assemblage (Fig. 9b). On average, Cyanophyta had a RSE of 47.0 %, but they dominated only for a few months of the year (Fig. 9c).

Highest variability, averaging 49.5 %, was observed for Chlorophytes, which were consistently in low abundance (Fig. 9d; Fig. 3c, d).

3.6. Comparison of water and gut flora by cell enumeration

Differences were observed between the relative proportion of different taxa in the phytoplankton community in the water column and that found in the gut, and these proportions differed by phytoplankton taxon, year, and season. Myzozoans (dinoflagellates) during the winter *Heterocapsa* bloom of 2011 (Fig. 5a) were not similarly reflected in the gut (Fig. 10a). However, later in 2011, and in winter/spring of 2012 when *P. minimum* were more abundant in the water (Fig. 5b), more Myzozoans were recorded in the gut than in the water column (Figs. 10a, 4 a). Ochrophytes (diatoms) were present in substantial proportions in the gut throughout both years, although proportions were higher in 2011 than in 2012 (Figs. 10b). *Cyclotella* dominated in spring 2011 (Fig. 5c) and there was a corresponding peak in the gut (Fig. 10b). Similarly, the *Skeletonema* bloom of late 2012 (Fig. 5d) was reflected in the late 2012 peak in diatoms in the gut (Fig. 10b). Cyanobacteria, which comprised >75 % of the phytoplankton community in summer,

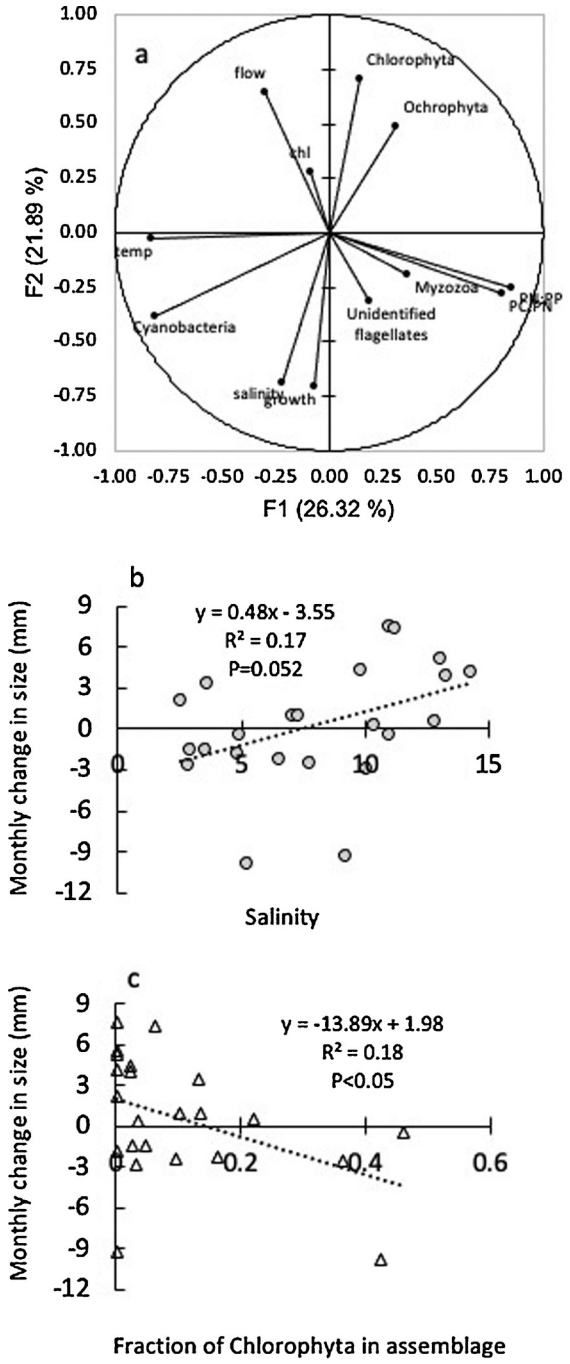

Fig. 8. (a) Principal components analysis of environmental factors and oyster growth; (b) correlation between oyster growth and salinity; (c) correlation between oyster growth and fraction of Chlorophyta in the water column assemblage.

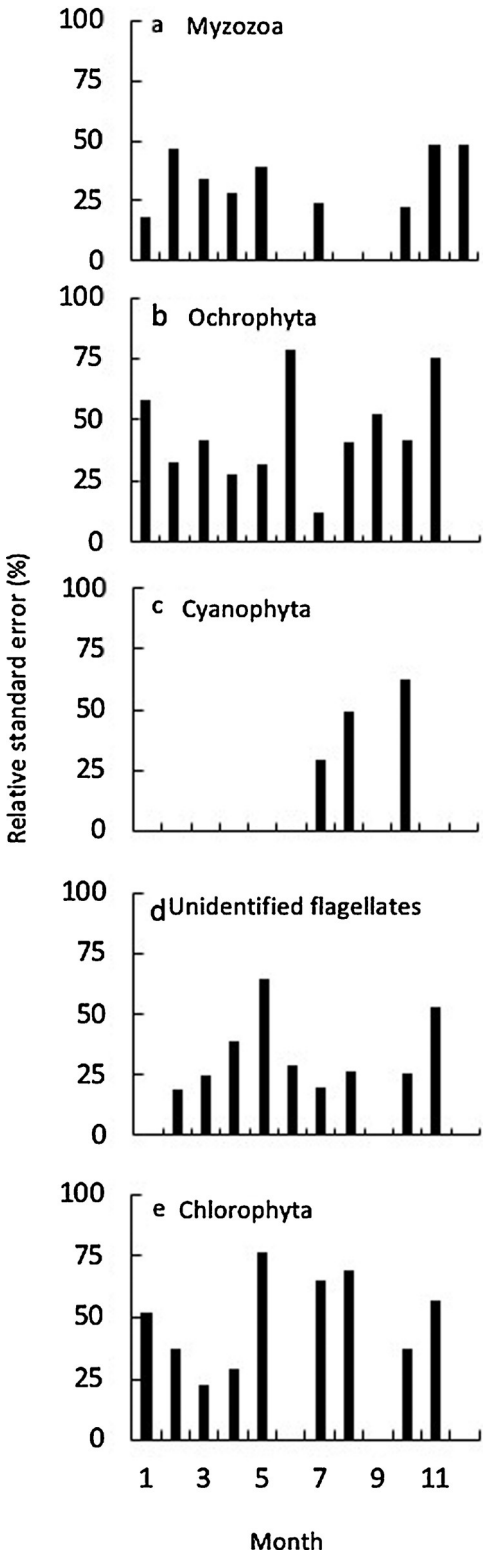

Fig. 9. Relative standard errors of the major phytoplankton groups quantified in the guts of oysters by month.

were also well represented in the gut during this same period of the year (Figs. 4e, 10 c). However, the *Cyanobium* bloom of 2011 (Fig. 5e) was maintained in the water column throughout the summer and fall was only seen in the gut in late summer (Fig. 10c). In 2012, when *Synechococcus* was the more dominant cyanobacterium (Fig. 5f), the timing of its presence in the gut was similar to that in the water column (Fig. 10c). Unidentified flagellates generally comprised 25–50 % of the phytoplankton in the gut, with the exception of the late summer months (Fig. 10d). Chlorophytes remained a low proportion of the phytoplankton in the gut, as was the case with chlorophytes in the water column (Figs. 4i, 10 e), and, as with the other taxa, the dominant species differed by year (Fig. 5g, h).

3.7. Comparison of water and gut flora by pigment composition

For comparisons of gut versus water column based on pigment composition, many trends were similar to those of cell abundance,

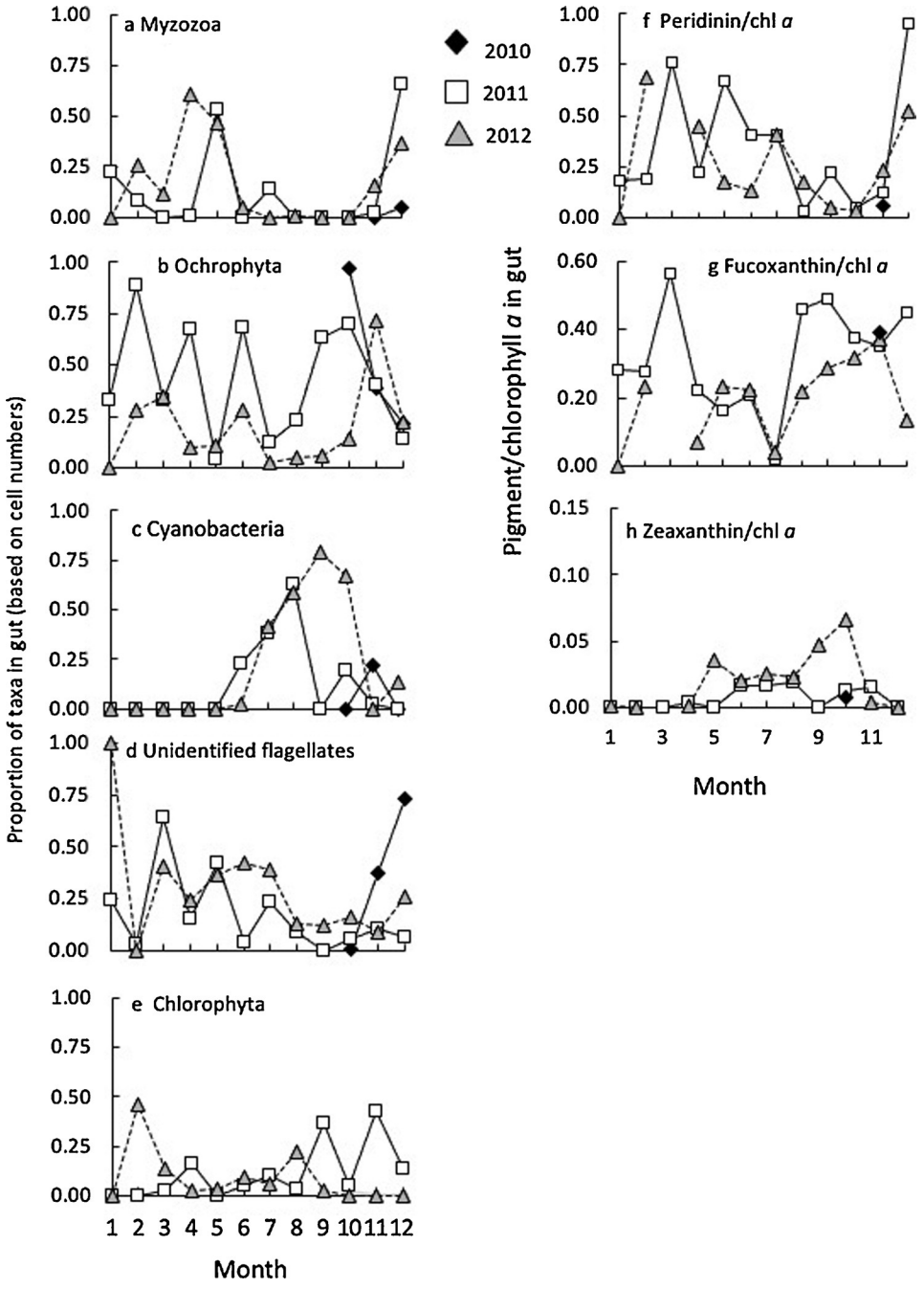

Fig. 10. (a-e) The proportion of individual phytoplankton groups in the gut for the years of the study based on cell number; (f-h) the proportion of individual phytoplankton groups in the gut for the years of the study based on signature pigments relative to chlorophyll *a*.

although there were some differences. The bloom of *P. minimum* seen in the water column in spring and again in winter of 2012 (Fig. 5b) contributed to a high abundance of peridinin:chl *a* in the gut at these same times (Fig. 10f). The winter 2011 bloom of *H. rotundata* (Fig. 5a) was not detected as increased peridinin in the gut in January, but a peridinin peak was seen by February (Fig. 10f). In contrast, in the gut, throughout 2011 (with the exception of September), levels of fucoxanthin/chl *a* exceeded 0.20, even reaching 0.56 in March, while in 2012, fucoxanthin/chl a proportions ranged from <0.05 to 0.25 through August, and only increased in the fall months (Fig. 10g; note missing data for March 2012), and late summer in both years. The spring 2011 peak of fucoxanthin/chl *a* in the gut corresponded with the timing of the *Cyclotella* bloom (Fig. 5c), while late summer of both years had high abundances of *Skeletonema* in the water column (Fig. 5d). Higher gut proportions of zeaxanthin were reached in 2012 relative to 2011 (Fig. 10h), corresponding to higher proportions of *Synechococcus* in that year (Fig. 5f).

3.8. Electivity indices

The modified E_i indices based on averaged cell abundances showed similarities to–as well as differences from–indices based on pigment composition. Whereas the E_i values for Myzozoa and Ochrophyta fluctuated between positive and negative values throughout both years of study based on cell abundance, when pigment values were used, both peridinin and fucoxanthin reflected preference across all seasons and years, with the exception of January 2012 (Fig. 11a–d). The Ɇ for Cyanobacteria based on both cell enumerations and zeaxanthin-based

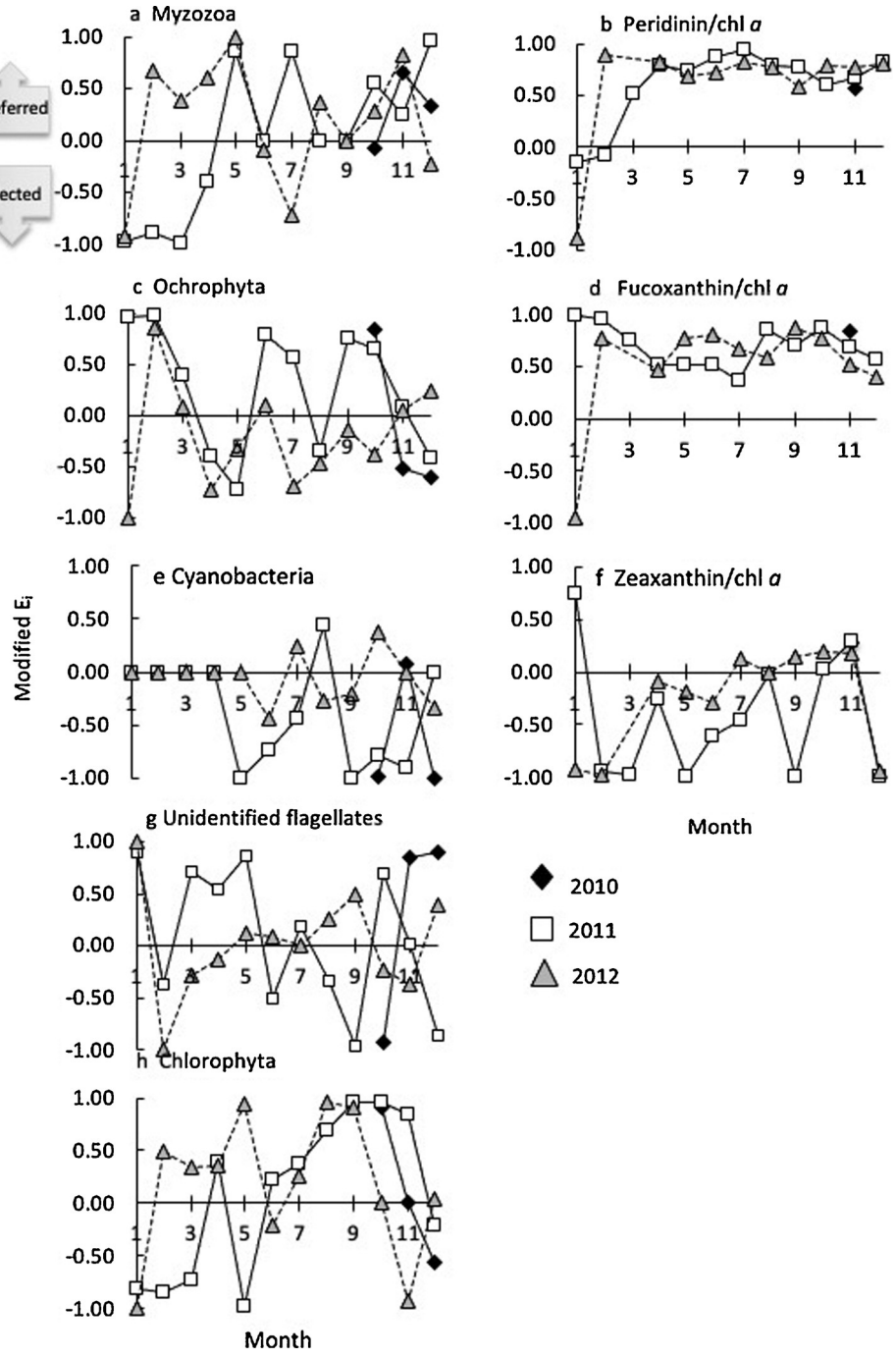

Fig. 11. Modified electivity index (E_i) by month for each year of the study. (a, c, e, g, h) E_i by taxa based on identified cells in oyster guts relative to the water column; (b, d, f) E_i by phytoplankton pigment based on concentrations in the pooled gut sample relative to the water column. Positive values reflect preference; negative values reflect rejection.

pigments were primarily negative throughout both years, indicative of rejection of this prey type. Higher Ei values were found for 2012 than for 2011 for both indices, especially later in the year when the *Synechococcus* bloom was observed (Fig. 11e, f).

For the unidentified flagellates, the E_i values were both highly variable, showing more frequent preference in spring (in 2011) or fall than in summer (Fig. 11g). For Chlorophyta, values were relatively higher in the late summer of both years (Fig. 11h). No comparable pigment data are available for these groups. Note that Chlorophyta were consistently a low proportion of the phytoplankton in the water and gut (Figs. 3b, c, 10e).

The rank preference varied by season (Fig. 12), with unidentified flagellates most preferred (rank $E_i = 1$) in the early seasons of the year, chlorophytes in summer and fall. Cyanobacteria were consistently ranked in the least preferred categories (rank Ei of 4–5). Myzozoans were the second most preferred from spring through fall, and Ochrophytes intermediate in rank preference at all seasons (rank Ei of 2–3).

3.9. Degradation pigments

Concentrations of both degradation pigments, phaeophytin and pheophorbide, varied with total chl *a* and the individual pigments in the gut (Fig. 13). Up to a value of ~2000 μg L^{-1} chl *a* in the gut, both degradation pigments increased, but as gut chl *a* concentrations

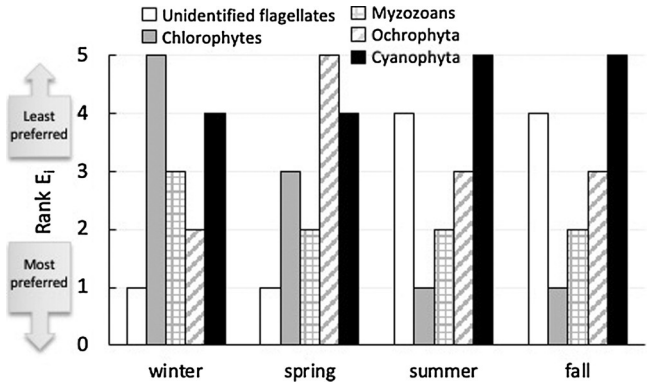

Fig. 12. Rank Ei by season for the dominant phytoplankton groups. Rank calculation is based on identified cells in oyster guts relative to the water column A rank of 1 indicates the most preferred taxon, a rank of 5 the least preferred.

increased even higher, concentrations of digestive pigments declined (Fig. 13a, b). For peridinin and for zeaxanthin, both degradation pigments also increased with concentration of the respective pigment in the gut up to a point, then declined (Fig. 13c, d, g, h). However, for fucoxanthin, while a pattern of saturation of phaeophytin with increasing pigment was found (with a decline at the highest levels), for phaeophorbide, a linear trend with increasing concentration was found, with no apparent saturation (Fig. 13e, f).

Both phaeophytin and pheophorbide in the gut increased significantly with salinity (excluding February data; $p = 0.005$ and 0.003 with or without excluded datum), suggestive of greater digestion as salinity rose (Fig. 14a, b). Relationships between phaeophytin and phaeophorbide in the gut and other environmental parameters (temperature, PC:PP, PN:PP and PC:PN) were not as strong (not shown); of these, the only relationship that was significant was phaeophorphide and temperature ($y = 6.60x-37.28$, $R^2 = 0.37$, $p = 0.04$; Fig. 14c–f). Digestion efficiency also was positively correlated with temperature ($p = 0.048$; Fig. 14h).

4. Discussion

This study aimed to understand the natural diet of oysters in conditions of eutrophication and variable flow in a tributary of Chesapeake Bay which historically had abundant oyster growth. With considerable interest and investment ongoing in oyster restoration not only in Chesapeake Bay but in waters worldwide, knowledge of how algal community composition affects oyster diets is important in understanding and predicting oyster recovery. Also, as oyster aquaculture develops in this region there is also interest in protecting such investments. Moreover, with increasing recognition of the diversity and increasing trends in HABs in Chesapeake Bay (e.g., Li et al., 2015), the potential exists for increased human health risks from oyster contamination (e.g., Oesterling and Luckenbach, 2008; Webster, 2009; Wolny et al., 2020).

Various methods have been used to establish selectivity or food preferences in natural populations, including flow cytometry, variable fluorescent signatures, direct counts, signature pigments, among other methods (e.g., Shumway et al., 1985; Pastoureaud et al., 1996; Soletchnik et al., 1997; Cognie et al., 2001). Herein, both microscopy and signature pigment analysis were applied to understand how electivity changed as seasonal abundance of different phytoplankton taxa changed.

4.1. Methodological considerations

There were several limitations to this study. First, given the natural setting and the suspension of oysters above the sediment, it was not possible to collect pseudofeces. This study also did not enumerate the myriad of other particles that can be taken in by oysters, such as detrital material. Detritus-bacteria complexes may contribute substantially to C requirements when bacterial concentrations are high (Crosby et al., 1989). To account for this, and their potential nutritive contribution, the particulate nutrient values of the seston in the water was examined.

Gut content collection and identification were challenging. Contents in the gut may have represented material that may have been recently taken in, that which was less easily digested, or both. For these reasons, complementary pigment analyses of the gut material were undertaken to compare with the cell enumeration data. Patterns in cell abundance and corresponding signature pigments did not consistently align. Small, unidentified dinoflagellates may not have been enumerated, or were characterized as unidentified flagellates, but their pigments were detected. Similarly, small diatoms may not have been adequately enumerated. Moreover, if the cells were consumed by small grazers, they would not have been enumerated as identifiable cells, but their pigments may have been extracted in the plankton sample.

Some analysts have employed isotopic ratios of the material in gut and the sources of C or N that oysters may have assimilated together with mixing models to characterize diets and all sources. Using such an approach, Decottingnies et al. (2007) concluded that benthic and planktonic microalgae dominated the diets of the 5 oysters (*Crassostrea gigas*) they collected on each of 3 dates. In another study, Riera and Richard (1996) used stable isotopes of C to assess *C. gigas* diets in a bay in France and also confirmed that when phytoplankton were abundant, they were fed upon preferentially relative to detritus. The isotopic approach did not allow those investigators to distinguish individual algal taxa, and the labor-intensive nature of isotopic approaches would make a two-year, monthly sampling approach as used herein, very difficult and costly.

4.2. Oysters in the Rhode River

The Rhode River has supported oysters for thousands of years, based on archeological sites from this river (Rick et al., 2016). These analyses suggest that human activities and climatic factors have influenced oyster productivity in this and other Chesapeake Bay tributaries over time (Rick et al., 2016). Using data from the St. Mary's and Patuxent Rivers, also tributaries of Chesapeake Bay, Kirby and Miller (2005) inferred faster oyster growth in the decades before 1850, and slower growth since, which they related to the detrimental effects of eutrophication, as well as disease and fishing pressure. A decrease in the growth in *C. virginica* growth observed by Kirby and Miller (2005) from the mid-1850s to 2000 was also related to an increase in dinoflagellates and cyanobacteria in the 20th century, inferred from classical studies (Loosanoff and Engle, 1947, Galtstoff 1964) and as evidenced from lipid biomarkers in sediment cores (Zimmerman and Canual, 2002).

4.3. Key trends and feeding preferences

This study encompassed two quite different years in terms of freshwater flow, salinity, nutrient availability, and relative phytoplankton composition. During the first year of this study, the slow growth of oysters was likely related to the low salinities which were often <6. In the second year of this study, salinities were higher but nevertheless rarely exceeded 12 (Fig. 2b). Optimal salinities for oysters are generally from 14 to 28 (Shumway, 1996), but there is variability between species and growth stage. Overall, growth was related to salinity, although the relationship just exceeded the 0.05 significance level (Fig. 8a, b). While oysters can tolerate such salinities, low salinities are physiologically stressful and may inhibit feeding activity, weaken digestion efficiency, and reduce scope for growth, resulting in loss of energy available for reproduction (e.g., Hutchinson and Hawkins, 1992; Shumway, 1996; Gray and Langdon, 2018; Sehlinger et al., 2019). As salinity fluctuates, oysters have to compensate for changes in ion concentrations and their

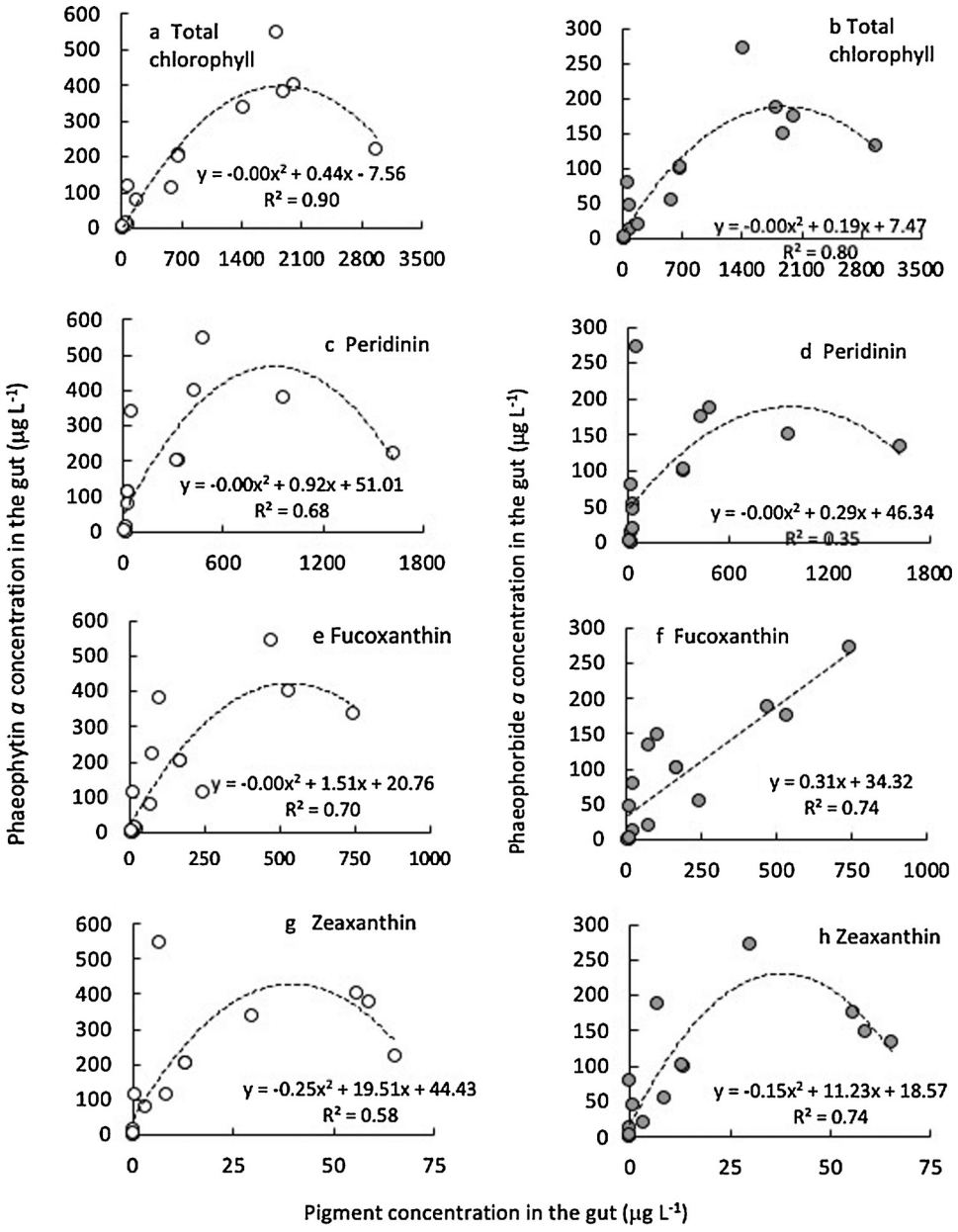

Fig. 13. Concentrations of degradation pigments phaeophytin (a, c, e, g) and phaeophorbide (b, d, f, h) in the guts of pooled oyster samples as a function of (a, b) total chlorophyll *a* and (c-h) other diagnostic pigments in the guts.

water intake across the plasma membrane (Hall et al., 2020). La Peyre et al. (2016) reported high mortality of oysters held in experimental cages at salinity <5, and also showed that mortality increased in low salinity conditions when high water temperatures (>30 °C) also occurred. For the oyster *Crassostrea nippona*, optimal salinity has been documented to be 25–30, and optimal temperatures 24 −28 °C (Wang and Li, 2018). The Asian oysters *Crassostrea sikamea* and *C. ariakensis* appear to prefer higher salinities (>30) during early stages of development, but somewhat lower salinities at later growth stages (Xu et al., 2011). These ranges imply that in spite of the fact that oysters have grown in the Rhode River for hundreds to thousands of years, none of the conditions experienced during this study fell in the optimal range.

Chlorophyll *a* averaged >30 µg L^{-1} during 2011, while values up to >70 µg L^{-1} were observed during 2012, concentrations which are consistent with the range previously observed for this river (e.g., Gallegos et al., 2005 and references therein). The high abundance of blooms of *P. minimum*, as seen in 2012, are also quite typical for this riverine system (Gallegos and Jordon, 2002; Gallegos et al., 2005). Large mid- to late-summer blooms of cyanobacteria are also commonly seen for many of the Chesapeake Bay tributaries (reviewed by Li et al., 2015). Thus, the Rhode River represented a site characterized by highly variable salinity, high nutrients, algal blooms, and other fluctuating natural conditions.

The E_i values and rankings indicated that Ochrophyta (diatoms) were either preferentially used or at least taken up in proportion to their availability. Langdon and Newell (1996) documented the preference for diatoms by *C. virginica* over many other taxa. Highest proportion of diatoms in the gut was seen in late 2010 - early 2011 based on cell abundance and pigment composition (Fig. 10b, g), and both the modified E^i value and rank E^i indicated preference (Figs. 11c and 12). Both *Cyclotella* sp. and *Skeletonema* sp. were present during this time (Fig. 5c, d). It is noteworthy that a large decrease in salinity and a correspondingly large increase in NO_3^- was seen from February to March in 2011 and this would be consistent with the increase in flow observed at that time. Diatoms are generally considered beneficial food for oysters and

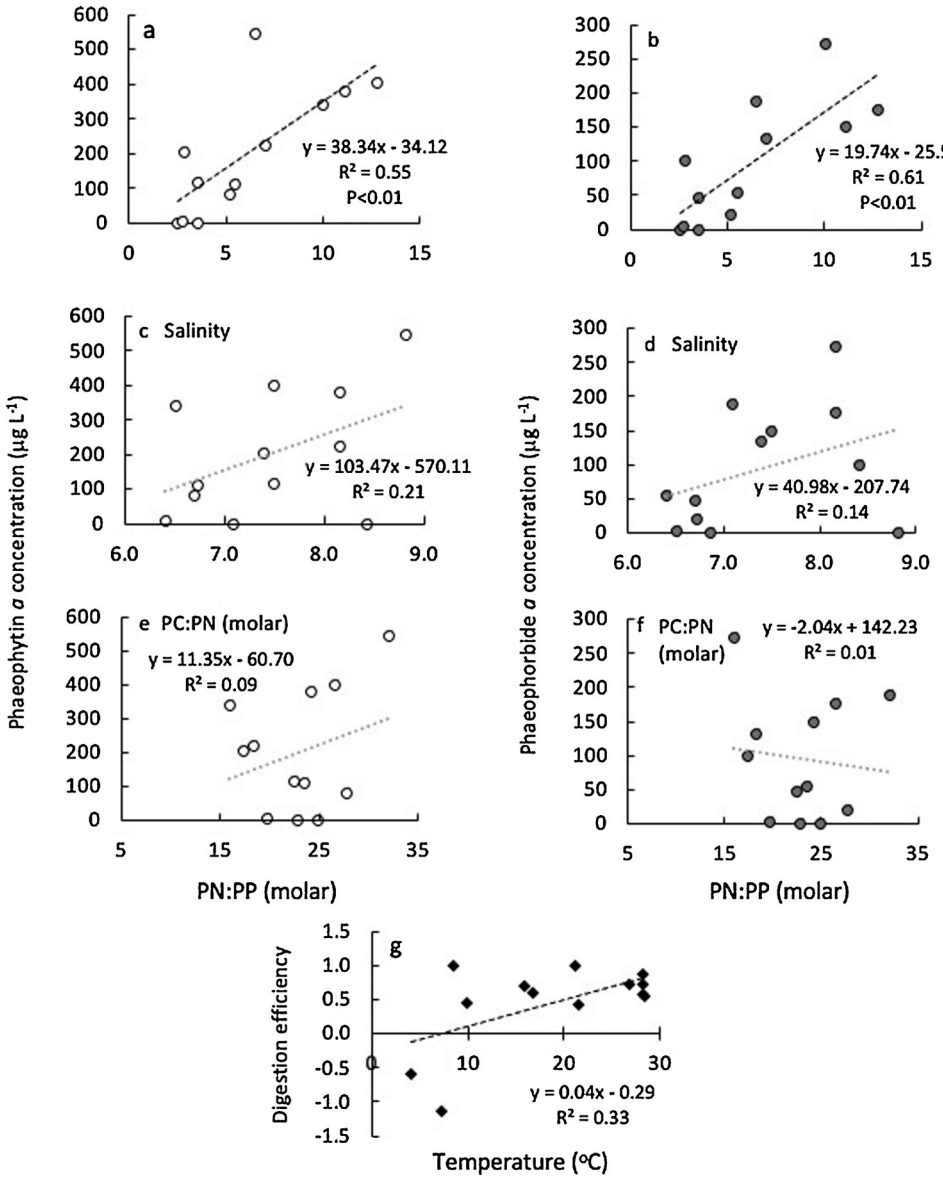

Fig. 14. Concentrations of degradation pigments phaeophytin (a) and phaeophorbide (b) in the gut as a function of water column salinity, as a function of PC:PN (c, d), and as a function of PN:PP (e, f). (g) digestion efficiency as a function of temperature. Relationships that are significant at $p < 0.05$ are drawn with darker lines.

have long been used in hatchery diets either as a sole diet or more often in combination with other species. Diatoms such as *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis*, *C. calcitrans*, *C. calcitrans* forma *pumilum*, *C. tenuissimus* have commonly been reported for this purpose (e.g., Ponis et al., 2006).

Myzozoa were often the second-most preferentially grazed and retained within the oysters' guts (Figs. 11 and 12). The dominant species of Myzozoa were the HAB taxa, *H. rotundata* in winter 2011, and *P. minimum* episodically in 2012 (Fig. 5a, b). In winter 2011, the *H. rotundata* was seemingly not grazed, as few such cells were observed in the gut at that time. The extent to which viable HAB cells pass through the gut of oysters, or are retained by the oysters, affects whether oyster detoxify when the HAB taxon contain toxins such as paralytic shellfish toxins (Bricelj et al., 1991). Although the consistency of feces was not documented herein, in previous experiments on the Asian oyster, *C. ariakensis*, feces changed considerably with diet. Thicker, more robust appearing feces and pseudofeces were observed when the oysters were given either a control diet of *Isochrysis* or a diet containing some *P. minimum*. Thinner, ropier material was produced when the oysters had the HAB dinoflagellate *Karlodinium veneficum* or raphidophytes in their diet (Alexander et al., 2008).

Blooms of *H. rotundata* often occur in winter months, and they are highly responsive to runoff events (e.g., Litaker et al., 2002). This bloom appeared during late 2010 but was sustained well into March of 2011. Although few studies have addressed effects of *H. rotundata* in the diet of oysters during winter, when their overall feeding rates were low, much is known about another member of this genus and its effects on oysters. *Heterocapsa circularisquama* is a bloom-former in many areas of oyster culture, particularly in Asia, and its detrimental effects have been well documented. It has been shown to cause cytoplasmic discharge, mass mucus production, irregular shape, delayed or inhibited mineralization of shell formation, as well as other effects on pearl oysters (Basti et al., 2011). Clearly, the effects of *H. rotundata* on *C. virginica* are worthy of additional study.

The other dinoflagellate, *P. minimum*, that bloomed sporadically throughout 2012 is a species about which much is known vis à vis its effects on oysters. *Prorocentrum minimum* has been shown to have significant, but variable, impacts on oysters. Early studies on impacts of *P. minimum* on *C. virginica* suggested no ill effects on feeding or growth (Connell and Cross, 1950). However, substantial effects have been

shown more recently. With *C. virginica* it has been observed experimentally, as well as anecdotally, that in the presence of *P. minimum* at 10^4 cells mL^{-1} spawning did not occur, and at an order of magnitude higher density, oysters reduced their filtration rates and died (Luckenbach et al., 1993). Within the Rhode River, concentrations of *P. minimum* fell within these ranges when it occurred. In other laboratory studies, *P. minimum* has also been shown to induce histological damage and to reduce growth of *C. virginica* larvae and juveniles (Luckenbach et al., 1993, Wikfors and Smolowitz, 1995). Observations on adult oysters exposed to *P. minimum* have shown a change in hemocyte profiles (H égaret and Wikfors, 2005), lysosomal destabilization (Keppler et al., 2005, 2006) and histopathological changes to gut tubules and sloughing of gut cells (Wikfors and Smolowitz, 1995; Pearce et al., 2005, Alexander et al., 2008). In the field, the impact of *P. minimum* on two populations of *C. virginica* has also been examined by exposing these animals to natural and simulated plankton blooms (H égaret and Wikfors, 2005). Oysters regularly experiencing such blooms had a higher rate of respiratory burst in hemocytes (Hégaret and Wikfors, 2005). Interestingly, Brownlee et al. (2005) found that *P. mimimum* blooms (10^4 cells mL^{-1}) from the Patuxent River, another tributary of Chesapeake Bay, had positive effects on growth of eastern oyster spat in 12-day laboratory experiments. In experiments with the Asian oyster, *C. ariakensis*, laboratory exposures to *P. minimum*, in mixtures with *Isochrysis* sp., yielded pigment signatures in the feces and pseudofeces that indicated release of *P. minimum* relative to the control food, especially in treatments for which *P. minimum* was provided at a higher relative abundance. Microscopic observations also indicated release of intact *P. minimum* cells in those experiments (Alexander et al., 2008). Wikfors (2005) speculated that *P. minimum* may be more toxic or less palatable to oysters when they are in a stage of growth decline compared with cells that are rapidly growing; this may help to resolve the discrepancy in the observations above.

Based on effects of *P. minimum* and spawning, an estimate can be made of the potential impact on recruitment in Chesapeake Bay. Natural spawning occurs over a 10 to 11-week period in spring and summer. Up to 5 spawning events can occur over this period (e.g., Mann et al., 2014). Blooms of *P. minimum* of 1–2 week duration are now common in Chesapeake Bay, especially in spring months (Li et al., 2015) and this was indeed the case in the Rhode River in 2012 (Fig. 5b). A 2-week delay in spawning from a single 2-week bloom alone may reduce the number of spawns, in turn reducing recruitment. When this estimate also considers the reduction in growth rates due to HABs, as is the case with *P. minimum* and its induction of histological damage impacting growth of oyster larvae and juveniles in laboratory studies (Luckenbach et al., 1993; Wikfors and Smolowitz, 1995), the total effect of HABs could be a substantial loss in oyster recruitment from a single HAB event of just a couple of weeks in duration (Glibert et al., 2007; Mann et al., 2014

The extent to which picocyanobacteria are taken up by oysters is a topic of considerable interest (reviewed by Rosa et al., 2018). Herein, cyanobacteria were largely discriminated against during feeding. This was seen in the modified E_i values throughout all seasons and in their rank preference (Figs. 11e and 12). The difference in E_i between 2011 and 2012 for cyanobacteria may be related to the different cyanobacterial composition during the two years. In 2011, *Cyanobium* was the dominant genus (Fig. 5e), while in 2012 it was *Synechococcus* (Fig. 5f). Both are small cells, generally <3 μm. Apparently *Cyanobium* was rejected to a greater extent than was *Synechococcus*. Motivated, at least in part, by the ability to quantify these particles robustly with instruments such as flow cytometers, there have been a number of recent studies on such picoplankton. Although these small cells may be filtered directly, taken in attached to other particles, or as particle aggregates, numerous studies have documented that oysters do not retain or digest as much phytoplankton when the phytoplankton are in the very small size ranges, <5 μm (e.g., Ryther, 1954, Riisgård, 1988). Methods used herein did not allow discrimination of these different pathways of ingestion.

Studies conducted in aquaculture settings have shown that picocyanobacteria can be taken up by bivalves but provide "just enough nutrition to support the basic maintenance needs, but not for the growth of the cultured organisms" (Avila-Poveda et al., 2014). Gallager et al. (1994) tested the picocyanobacterium *Synechococcus* as food for larvae of the bivalve *Mercenaria mercenaria* and found that, although the bivalves could derive some nutrition from these picoplankton, growth rates of larvae fed on this species as a sole diet were 2-fold lower than those of molluscs fed on a diet of a larger (5–6 μm length) algal species commonly used in aquaculture, the haptophyte *Isochrysis galbana.* Recently, a study conducted in Great Bay Estuary, New Hampshire, USA, also illustrated this same effect: at a site in which the phytoplankton was dominated by larger cells, in the 5–28 μm range, oyster absorption efficiency was higher than at a second site where the phytoplankton cells were mostly of the size range <5 μm (Hoellein et al., 2015). A recent study of the oyster *C. gigas* has shown that picoplankton are not necessarily digested, but may accumulate in multiple tissues, including the digestive gland, connective tissue, mantle and gonad (Avila-Poveda et al., 2014). Increasing cyanobacteria in the diet has the potential to change the availability of sterols and polyunsaturated fatty acids (PUFAs) that are considered essential for growth, especially during the larval stage. The absence of the required sterols and PUFAs has been shown to severely affect the growth of many higher trophic level organisms (e.g., Martin-Creuzburg and von Elert, 2009). *Synechococus*, the most common picocyanobacteria genus, lacks the sterols critical for growth (Martin Creuzburg et al., 2008). The PUFAs and sterols are critical because they are precursors for many bioactive molecules and are integral to cell membranes (Martin-Creuzburg and von Elert, 2009).

Although the E_i values suggested that Chlorophyta were consumed or retained preferentially in the gut, at least in winter and spring (Figs. 11 and 12), growth of the oysters was negatively correlated with their abundance (Fig. 8c). It should be noted that inefficient digestion may also be due to low temperatures of the season, unrelated to food.

These was a general upward trend of digestion pigments with increased C content of the ingested material (Fig. 14c, d), but these trends were not significant and there were no trends with PN:PP (Fig. 14e, f). Newell and Jordan (1983) previously reported mixed preferences for N (4 out of 4 trials) and for C (2 out of 4 trials) in *C. virginica*. Early work on digestion efficiency by Hawkins et al. (1986) in experiments with *Mytilus edulis* showed that digestion efficiency did not vary significantly with a high quality or low quality diet, based on particulate organic matter relative to total seston, but that high silt content strongly affected digestion efficiencies, leading to values that were low and/or negative. Bayne (2009) suggested that food electivity may change depending on the seasonal requirements for C, N or P depending on the metabolic need associated with growth, reproduction, or maintenance. Most researchers (reviewed by Ward and Shumway, 2004) now think that mechanical sorting is a more important process than selection based on nutritive value. Whether or not smaller particles are more digestible is a different question (reviewed by Ward and Shumway, 2004). On the one hand, if oysters are preferentially feeding on smaller particles, it would logically follow that these would be more digestible, but food value may scale with food size, resulting in larger cells having the more digestible components (Ward and Shumway, 2004). The clearest trends herein were those of increasing digestion pigments with increasing salinity (Fig. 14a, b).

As summarized by Bayne (2017), numerous studies have shown a response pattern in clearance rate of oysters and other filter feeders that first suggests a saturating response then declines at higher concentrations. High concentrations can clog the pallial ciliary tracks (e.g., Beninger et al., 1992). As examples, clearance rates in the scallop *Pecten maximus* and in the mussel *Atrina zelandica* have been shown to first increase then decrease as either total Chl *a* or total particulate matter increase (Strohmeier et al., 2009; Hewitt and Pilditch, 2004). In *C. gigas*, similar response relationships with respect to seston or particulate matter concentration have been reported (Barill é et al., 1993; Ren et al.,

2000). Here, a similar response was found for digestive pigments as a function of total Chl *a* and specific pigments in the gut (except phaeophorbide and fucoxanthin). Thus, not only is total intake a function of total available particulates, but so too is how fast they may be processed in the gut. The production of pseudofeces can also control the mass of particles taken in, but no measures were made of pseudofeces herein.

In addition to taxa differences with respect to feeding, these data give considerable insight into other aspects of nutrition. Both the dissolved and the particulate nutrient data show strong seasonal differences, with a tendency toward P limitation during the winter and spring months, and N limitation during the summer months. Of significance is the fact that cyanobacteria tended to dominate during the summer months, the months when P was most available. Discrimination against cyanobacteria due to its size (or other attributes) at a time when P is most available may limit the oyster's access to this critical element. Phytoplankton accumulation in the water was highest when nutrients in the water were in Redfield proportions (i.e, DIN:DIP ~16), but nutrient ratios well in excess of these proportions were common, throughout all but the mid-summer months, increasing the likelihood for P deficiency and associated N-rich biodeposits (e.g., Hoellein et al., 2015).

5. Conclusions

This study has shown the wide range of algal food both available for oysters in a eutrophic environment, and the relative preference or selectivity for certain taxa during certain times of the year. Oysters appeared to preferentially graze–or at least preferentially retain in the gut–unidentified flagellates, Ochrophyta and Myzozoa, including HAB dinoflagellates, and appeared to generally reject cyanobacteria from their diet. While electivity indices of Chlorophyta suggested preference, the overall abundance of this taxon was low compared to others. Both the retention of HAB dinoflagellates and the rejection of food during periods of cyanobacterial dominance are potentially of concern as small changes in diet can have large effects on absorption efficiency and effects of diet can be exacerbated by other environmental stressors; all of these may affect the scope for growth (e.g., Strohmeier et al. (2012).

While much work remains to be done to fully understand the extent to which phytoplankton species may or may not be affecting oyster growth and recruitment *in situ*, the trends presented here suggest that the phytoplankton assemblage of the eutrophic Rhode River creates challenges for oyster restoration and oyster aquaculture in this historically oyster-rich tributary of Chesapeake Bay. Continued efforts to reduce nutrient loads in this estuary will likely be beneficial for oysters if such nutrient reductions yield concomitant reductions in dinoflagellate blooms and picocyanobacteria during the summer months. In turn, the ecosystem services that oysters provide may be enhanced.

CRediT authorship contribution statement

Eric J. Weissberger: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing - original draft. **Patricia M. Glibert:** Investigation, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The views expressed in this article are the authors' own and do not necessarily represent the view of MD DNR. Any reference obtained to a specific product, process, or service does not constitute or imply an endorsement by the authors or their organizations.

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