Balanced, Indigenous, Desirable Phytoplankton Populations in Virginia Tidal Waters

FINAL REPORT

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Prepared by
Claire Buchanan, PhD
Interstate Commission on the Potomac River Basin

for

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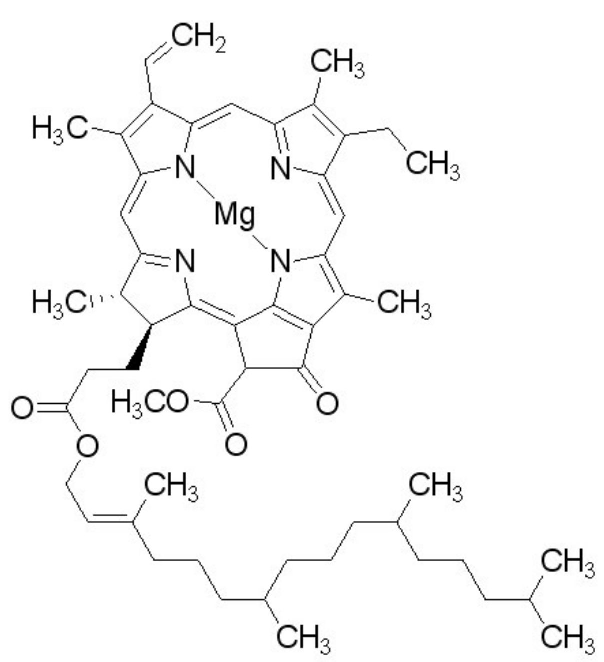

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Interstate Commission on the Potomac River Basin
30 West Gude Dr., Suite 450
Rockville, MD 20850
301-984-1908

Disclaimer

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Cover illustration: the molecular structure of the photopigment chlorophyll *a*

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Executive Summary

Results of this study further support the premise that estuarine phytoplankton in comparatively good water quality, herein called “Reference” conditions, are representative of the “balanced, indigenous, desirable” aquatic life invoked in Virginia’s water quality standards. Reference conditions have concentrations of dissolved inorganic nitrogen and phosphorus low enough to limit the formation of nuisance algal blooms and water clarity adequate for normal, unstressed photosynthesis in above-pycnocline waters. “Degraded” conditions are distinctly different, with excess concentrations of both nitrogen and phosphorus and inadequate, or poor, water clarity. The study examined the taxonomic composition of phytoplankton populations in Reference, Degraded, and two intermediate water quality conditions. The analysis was limited to spring and summer phytoplankton samples collected between 1984 and 2012 in high mesohaline and polyhaline salinities (>10‰) of Virginia’s Chesapeake Bay and tidal tributaries.

A diverse set of forty-five (45) taxa or taxonomic groups appear more frequently and/or in higher overall or maximum abundances in Degraded conditions. They include known or suspected toxin producers or nuisance bloom formers (HABs). Thirty-two (32) predominantly diatom taxa or taxonomic groups appear more frequently and/or in higher overall or maximum abundances in Reference conditions. Only one is a known toxin producer, but toxic strains have not been reported in the Mid-Atlantic. The 77 taxa and taxonomic groups were scored so as to accentuate differences found in their abundance distributions in Reference and Degraded conditions. On average, 4.9 and 4.8 of the 77 possible taxa were present and could be scored in the individual spring and summer samples, respectively.

A numeric Phytoplankton Taxonomic Index (PTI) was calculated for each sample by averaging the sample’s scored taxa. Like the PIBI index developed from community-level phytoplankton metrics, high values of the PTI index indicate the presence of traits characteristic of Reference water quality conditions. Populations in these well-lit, nutrient-limited conditions are distinctly different from populations in Degraded conditions. The PTI index classification efficiency, or ability to correctly identified both Reference and Degraded water quality conditions, was 89.1% in spring and 90.4% in summer. Phytoplankton populations in Reference conditions can be considered examples of the balanced, desirable populations called for in Virginia Water Quality Standards.

Phytoplankton growth in degraded water quality conditions frequently is suppressed by inadequate light levels, even in the presence of excessive amounts of nutrients. In these cases, the population’s chlorophyll *a* concentration will be low and may even meet chlorophyll *a* criteria, but it will not be associated with other desirable characteristics. Given the overriding importance of adequate light to photosynthetic organisms, information about water clarity could be useful in the chlorophyll *a* criteria assessment process and could increase confidence in final decisions on designated use attainment. SAV-based water clarity criteria have been promulgated in Virginia for tidal nearshore environments, and they are similar to the phytoplankton thresholds for adequate light in open waters. Attainment of these water clarity criteria in both nearshore and open water environments would reduce the risk of nuisance algal blooms and toxin-producing phytoplankton taxa. A straight-forward, linear relationship between attainment of the James River chlorophyll *a* criteria and balanced, desirable phytoplankton populations—expressed as the PIBI or PTI—develops as the confounding influence of poor water clarity recedes.

Balanced, Indigenous, Desirable Phytoplankton Populations in Virginia Tidal Waters

Introduction

A use designation in Virginia Water Quality Standards calls for water quality that supports a balanced, indigenous population of aquatic life (9 VAC 25-260-10) and requires conditions promoting undesirable or nuisance aquatic plant life to be controlled (9 VAC 25-260-20). The tidal James River experiences frequent blooms of undesirable and nuisance algae in its surface waters. Excessive nutrient concentrations are primarily responsible for the frequent algal blooms. Blooms can disrupt and harm other aquatic biota by impeding filter-feeding, producing toxins, blocking sunlight and forcing large diel swings in pH. Bacterial decomposition of the blooms when they die can deprive water of dissolved oxygen. In the tidal James River, the aquatic life designated use of most of the river's open water environments is currently listed as impaired on the basis of hypoxia and reduced water clarity. Algal blooms due to excessive nutrient concentrations, along with excessive sediments, are implicated as the causes of the impairment (VADEQ 2004).

Balanced indigenous populations of aquatic life in open waters are supported by similarly balanced populations of phytoplankton, the algae of those waters. As primary producers, phytoplankton are the base of the open water food web, and populations in good ("Reference") water quality conditions exhibit many desirable community-level features (Buchanan *et al.* 2005). Reference conditions have nutrient concentrations low enough to limit bloom formation and water transparency adequate for normal, unstressed photosynthesis in above-pycnocline waters. Several community-level features, or metrics, were used to develop and validate a bay-wide phytoplankton index of biotic integrity (PIBI) for Chesapeake Bay (Lacouture *et al.* 2006, Johnson and Buchanan 2013). The index is reference-based, meaning the statistical properties of phytoplankton populations in season- and salinity-specific Reference water quality conditions were used to create a numerical scale against which phytoplankton metrics in all conditions can be scored. Metrics are calculated from each sample's normalized taxonomic counts and from phytoplankton-related parameters in associated water quality samples (e.g., chlorophyll *a*, pheophytin). The metrics are scored and the scores averaged to obtain an index value for each sample. The index discriminates well between Reference and "Degraded" (i.e., excess nutrient and poor water transparency) conditions. The index's classification efficiency, or its ability to identify correctly both Reference and Degraded waters, ranges from 70% to 90%. This reiterates that light and nutrients are key environmental factors controlling phytoplankton populations.

Phytoplankton populations in Reference water quality conditions can be considered examples of the balanced populations called for in Virginia Water Quality Standards. The objective of this study is to characterize these balanced phytoplankton communities at a finer taxonomic level. If substantial taxonomic differences are found in Reference and Degraded conditions, this will support the premise that reducing nutrient loads to the James River and improving the estuary's water clarity will shift phytoplankton toward more desirable, balanced communities. An approach similar to the one used to develop the PIBI (above) is applied here to identify discriminating phytoplankton taxa, score them, and develop a numeric index capable of representing the taxonomic compositions of balanced phytoplankton populations.

Data

Differences in Maryland and Virginia laboratory enumeration protocols make merging the states' taxonomic cell counts somewhat problematic. Only phytoplankton counts performed by Old Dominion University for the Virginia Chesapeake Bay Program (CBP) monitoring program were analyzed in this study. Documentation describing the collection and enumeration protocols is available online at the CBP website. The 500 ml water samples preserved with Lugol's solution and buffered formaldehyde are put through several settling and siphoning stage and reduced to a final 20-40 ml concentrate. A known volume of the concentrate is placed in an Utermöhl settling chamber and counted with an inverted microscope. The microscopic examination is done with three procedures: 1) at 300X, taxa clearly identifiable at this magnification (usually >5 microns) are counted in 10 random fields or up to a minimum cell count of 200; 2) at 500X, all taxa not clearly discernable at the 300X magnification (usually <5 microns) are counted in 10 random fields; and 3) at 125X, the entire chamber is scanned for larger, rarer taxa (usually >60 microns) not previously enumerated. The counts are then normalized by the volumes examined under the three magnifications. Final units are number of cells of a given taxa per liter. Taxonomic identification is based on internationally accepted identification keys and counted species are paired with their corresponding taxon serial number (TSN) from the Integrated Taxonomic Information System (ITIS).

Taxonomic count data for spring (March – May) and summer (July – September) between 1985 and 2012 were downloaded from the CBP Data Center on October 28, 2014 with the assistance of M. Mallonee, the CBP Water Quality Data Manager. The data were merged with a previously developed data set containing the corresponding water quality data and the community-level metrics developed from the same taxonomic counts. Calculations of the community-level metrics are described in Lacouture *et al.* 2006 and online at the CBP website.

Data Analysis

This study used a straightforward, reference-based, binary scoring approach to detect taxonomic shifts associated with changing water quality conditions. Within each season and salinity zone, phytoplankton samples are assigned to one of four distinct water quality categories, which are based on the water column transparency (water clarity) and nutrient concentrations at the time the sample was collected. Categories range from Reference to Degraded. Phytoplankton taxa exhibiting distinctly different statistical properties across the four water quality categories in one or both seasons were identified as potential indicator taxa. Taxa abundances found predominantly in Reference conditions were scored 1; abundances most characteristic of Degraded were scored 0. The average score of all the scored taxa in each sample was calculated and used as an index of the sample's phytoplankton taxonomic composition. Samples with index scores approaching 1 represent Reference communities and those with index scores approaching 0 represent Degraded communities.

Water Quality Categories

Phytoplankton habitat conditions were evaluated in this study by two season (spring, summer) and five salinity zones (tidal fresh, 0 – 0.5‰; oligohaline, >0.5 – 5‰; low mesohaline, >5 - 10‰; high mesohaline, >10 – 18‰; and polyhaline, >18‰). Three parameters are used to categorize the water quality condition from which each phytoplankton sample was collected: Secchi depth, dissolved inorganic nitrogen (DIN) and ortho-phosphate (PO_4). Each of the nutrient parameters is classified into one of four classes, the boundaries of which are derived from the results of nutrient bioassay experiments (Fisher and Gustafson 2003) and two Relative Status Method analyses (see below). The four classes are, for ease of identification, called best, better, poor, and worst. Samples associated with Secchi depth, DIN and PO_4 that all classify as best or better are assigned to the Reference (REF) category. Samples associated with the three parameters all classifying as poor or worst are assigned to the Degraded (DEG) category. Samples with better or best Secchi depth but one or both nutrients classifying as poor or worst are assigned to the "Mixed Better Light" (MBL) category. Those with poor or worst Secchi depth but one or both nutrients classifying as better or best are assigned to the "Mixed Poor Light" (MPL) category. The two mixed categories have conditions intermediate between REF and DEG. The MBL category supports phytoplankton populations resembling REF and can be used as a surrogate for REF when the latter is not found in a particular season or salinity environment. The MPL category shares many traits with DEG and typically has the greatest range of metric values of any category, including the highest chlorophyll *a* concentrations. More details are provided in Buchanan *et al.* (2005) and Lacouture *et al.* (2006).

The original thresholds used to separate the poor and worst classes of DIN and PO_4 from the better and best classes were also used in this analysis: 0.07 mg DIN/liter and 0.007 mg PO_4/liter. Concentrations equal to or below these thresholds classified as better or best. The original thresholds used to separate all four Secchi classes were derived from a Relative Status Method (RSM) analysis of the Maryland and Virginia 1985 – 1990 water quality data (Buchanan *et al.* 2005, Olson 2009). It became apparent after this earlier RSM analysis that the mesohaline salinity zone in Chesapeake Bay encompasses too great a range of nutrient and light conditions. Subdividing the mesohaline into low (>5 - 10‰) and high (>10 – 18‰) zones produces two, more homogeneous habitats.

An RSM analysis was performed for this study, to identify Secchi depth classes for low and high mesohaline salinity zones. The analysis also examined the RSM thresholds of Secchi depth classes previously identified for tidal fresh, oligohaline, and polyhaline salinities, to verify these bay-wide thresholds. The analysis was performed on water quality data collected bay-wide between 1984 and 2013, in open water environments (i.e., surface waters at locations deeper than 2 m). Assembly and preparation of the dataset are described in Buchanan (2014). Sampling events with measurements for total nitrogen (TN), total phosphorus (TP), and total suspended solids (TSS) were extracted from the dataset (n = 52,631) and parsed into the five salinity zones (above), by season. The seasons were: spring (March – May), June, summer (July – September), autumn (October – November), and winter (December – February). The 33rd percentile of each parameter in each season-salinity habitat was identified, and samples with TN and TP and TSS concentrations below their respective 33rd percentiles were selected. The statistical properties of the Secchi depths for these selected sampling events were then determined (**Appendix A**). The 25th%iles, medians, and 75th%iles of their distributions are the thresholds used to separate the worst-poor, poor-better, and better-best classes of Secchi depth, respectively. Only the spring and summer thresholds are relevant to the purposes of this study. The original and revised Secchi thresholds are shown in **Appendix A Table A-1** and **Figure A-1**.

Phytoplankton Samples

The taxonomic count data for the phytoplankton samples were merged with their corresponding phytoplankton community-level metrics and water quality parameters, which include chlorophyll *a*. Each phytoplankton sample was assigned one of the four water quality categories (**Table 1**). In Virginia's tidal fresh, oligohaline and low mesohaline salinities, no REF phytoplankton samples were found in spring and only one was found in summer. Between 16 and 70 REF phytoplankton samples were found in spring and summer in Virginia's two higher salinity zones. The relatively low numbers of phytoplankton samples in REF, and even surrogate REF (MBL), water quality conditions in Virginia waters are due in part to the fact that water quality samples collected for various monitoring programs far outnumber phytoplankton taxonomic samples.

Table 1. Numbers of phytoplankton sampling events associated with each season- and salinity-specific water quality category in Virginia tidal waters. REF, Reference; MBL, Mixed Better Light; MPL, Mixed Poor Light; DEG, Degraded.

Salinity zone	REF	MBL	MPL	DEG	Total
Spring					
TF		8	32	154	194
OH			31	52	83
LoMH		3	32	26	61
HiMH	16	52	194	52	314
PH	70	27	166	10	273
Summer					
TF	1	10	49	76	136
OH		4	78	25	107
LoMH			54	21	75
HiMH	21	5	161	52	239
PH	59	84	338	68	549

The near absence of REF phytoplankton samples in Virginia's lower salinity waters precludes taxonomic characterizations of REF populations in tidal fresh, oligohaline, and low mesohaline salinity zones. Taxonomic characterizations of REF populations in the higher salinities are possible when the high mesohaline and polyhaline samples are combined. A preliminary examination of the taxonomic data, done for this study, indicated that the cell abundances and responses of many phytoplankton taxa to Virginia water quality conditions in these higher salinities differ little, if at all. The ranges of Secchi depth and of DIN and PO_4 concentrations in the resulting REF, MBL, MPL and DEG water quality categories (using the revised Secchi depth thresholds) are also similar in the higher salinities (**Appendix A Figures A2-A4)**. Phytoplankton samples from the high mesohaline and polyhaline salinity waters could be grouped, thereby increasing the total sample sizes (n) for the study's analysis. The remainder of this report focuses on phytoplankton taxa in the combined high mesohaline and polyhaline salinity waters of Chesapeake Bay and its tributaries. These taxa were evaluated for their potential use in characterizing the phytoplankton communities of REF conditions, and their ability to discriminate between REF and DEG conditions.

Approximately 530 distinct taxa have been identified in spring and summer in high salinity (>10 ‰) waters of Virginia's Chesapeake Bay over the course of the CBP monitoring program. A review of the data showed that between nine (9) and seventy-nine (79) taxa are identified in the approximately 200 cells counted from each phytoplankton sample. About two-thirds of the 530 taxa occur so infrequently in the sample counts (<5%) that they often are not statistically reliable indicators of water quality condition. The usefulness of the remaining taxa (~170) as indicators depends on their properties in the four water quality categories. For this study, taxa were considered potential candidates if they exhibited one or more of the following traits: 1) occur frequently in REF and/or DEG conditions; 2) occur primarily or only in REF *or* DEG conditions; 3) have relatively high abundances in REF and/or DEG conditions when they occur; 4) are a known toxin-producer; and 5) are known as high quality food for grazers. Identification of the known toxin-producers in Virginia waters was confirmed by Dr. Todd Egerton, head of Old Dominion University's Phytoplankton Taxonomy Laboratory. When rare but closely related species appeared to be responding similarly to water quality conditions, they were sometimes combined into genus-level taxonomic groupings.

Taxon Scoring

Indications of how a taxon is responding to nutrient- and light-related water quality conditions are often seen in the "tails" of the taxon's abundance distributions rather than the middles of the distributions. Phytoplankton community-level metrics show similar response patterns, where the central tendency (e.g., mean, median) of a metric's distribution is not as responsive to water quality conditions as its tails.

For example, the median chlorophyll *a* concentrations in DEG and REF conditions of high mesohaline waters are fairly close (9.9 and 7.5 µg/liter, respectively), whereas the maximum concentrations reach 194 µg/liter in DEG and only 24 µg/liter in REF. Some taxa can vary in the frequency of their appearances in different conditions. For example, *Anabaena*, *Cochlodinium*, and multiple diatom and green taxa occur predominantly or solely in DEG and MPL conditions; a smaller group of taxa, primarily diatoms, occur predominantly in REF and MBL conditions. These highly discriminating taxa are good metrics for an index.

Several scoring approaches were investigated for this analysis (e.g., 1 – 3 – 5 on a 1-5 scale; gradient from 0 – 1). The approach that produced the strongest numeric separation between REF and DEG conditions was one in which one or both tails of the taxa distributions in REF were scored (1, 0) and the middle or overlapping values of the distributions were not scored (Null). The following guidelines were established for scoring the phytoplankton metrics, in order to provide consistency across the variety of taxonomic responses to water quality. Some examples of the guidelines applied to data are shown in **Figure 1**.

1) Where possible, scoring criteria are based on percentiles of a taxon's abundance distribution in the REF conditions of the relevant season-salinity habitat (although a rare taxon found almost exclusively in certain water quality categories can be scored simply if it is present). The MBL category is sometimes used if the sample size of REF is very small.

2) Taxonomic metrics are scored 1, 0, or Null (no score). A score of 1 indicates the abundance of the taxon is most characteristic of the REF condition; a score of 0 indicates the abundance is most characteristic of the DEG condition; a score of Null indicates the abundance is not distinctly characteristic of either REF or DEG.

3) If taxon abundance distributions are sufficiently separated in REF and DEG conditions, both tails may be scored.

4) Taxa whose REF abundances correlate strongly with salinity, indicating they may be intolerant of broad fluctuations in salinity (stenohaline), are avoided.

5) Only seasons with clear response patterns are used. If a response pattern in one season differs from that of the same taxon in the other season, the response in the season with the highest overall abundances is assumed to be representative of the taxon, and the pattern in the other season is assumed to be masked by seasonal or other environmental factors.

All the numeric scores (1, 0) for a sample are averaged to obtain a measure, or index, of the status of the sample's phytoplankton population. Samples with index scores approaching 1 represent the best communities, with a preponderance of taxon characteristics typically found in REF conditions. Samples with index scores approaching 0 represent a preponderance of taxa and/or taxa abundances typically found in DEG conditions. By common use, index scores greater than or equal to 0.5 on an overall scale of 0 – 1, such as the one developed here, are considered indicative of REF conditions. Index scores less than 0.5 are considered indicative of a DEG conditions.

Using these guidelines, a classification efficiency can be determined for the resulting index. Classification efficiency (CE) is the average of a) the fraction of samples in REF that the index correctly

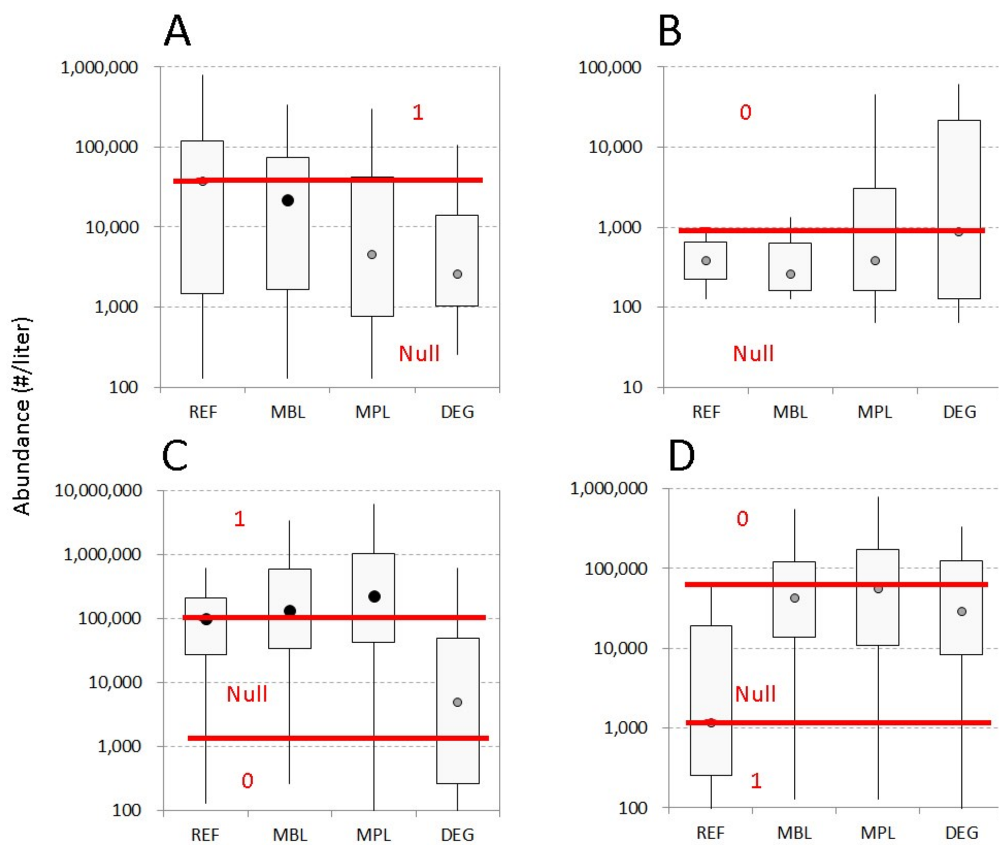

Figure 1 Examples of how taxonomic metrics are scored. Figure symbols (see text for further details): closed circle, median; larger closed circle, median abundance of taxon that occurs in more than 50% of samples of the given water quality category; box, 25th%ile to 75th%ile range; whiskers, minimum and 95%ile. A) Only high abundances in Reference (REF) clearly distinguish the REF and Degraded (DEG) distributions, so high abundances are scored 1. B) Only high abundances in DEG clearly distinguish the REF and DEG distributions, so high abundances are scored 0. In both A and B, low abundances, where REF and DEG distributions overlap strongly, are scored Null (no score). C) High abundances in REF and low abundances in DEG distinguish the distributions, so high abundances are scored 1 and low abundances are scored 0. D) High abundances in DEG and low abundances in REF distinguish the distributions, so high abundances are scored 0 and low abundances are scored 1. In both C and D, abundances between high and low scoring thresholds are scored Null.

identifies as REF (i.e., having an index value $\geq$0.5), and b) the fraction of samples in DEG conditions that the index correctly identifies as DEG (i.e., having an index value of <0.5). The equation is:

$$CE = \frac{\left(\frac{REF\ n_{\geq 0.5}}{REF\ n_{total}} + \frac{DEG\ n_{<0.5}}{DEG\ n_{total}}\right)}{2}$$

Results

Seventy-seven (77) taxa or groups of closely related taxa from the spring (Sp) and/or summer (Su) seasons were identified as responsive to differences in light and nutrient conditions in high salinity waters of Chesapeake Bay. Taxa abundance distributions in the four water quality categories and how they were scored are shown in **Appendix B Table B-1**.

The taxa or taxonomic groups that favor DEG conditions are diverse. Twenty-nine (29) of these appear more often in DEG conditions and typically have higher maximum abundances or higher overall abundances in DEG conditions as compared to REF. They are:

Blue-green: *Anabaena* sp (Sp, Su), *Chroococcus* sp (Su), *Phormidium* sp (Su)

Cryptomonad: *Cryptomonas erosa* (Su)

Desmid: *Staurastrum* spp (Sp, Su)

Diatom: *Amphiprora* spp (Sp, Su), *Amphora* spp (Sp, Su), *Asterionella formosa* (Sp), *Aulacoseira* spp (Sp, Su), *Chaetoceros subtilis* (Su), *Cyclotella striata* (Sp), *Cymbella* spp (Sp, Su), *Diploneis* sp (Su), *Eunotia* sp (Sp, Su), *Fragilaria* spp (Sp, Su), *Gyrosigma fasciola* (Su), *Navicula* spp (Sp, Su), *Nitzschia* spp (Su), *Pseudo-nitzschia seriata* (Sp, Su), *Surirella* spp (Sp, Su)

Dinoflagellate: *Akashiwo sanguinea* (Sp), *Cochlodinium* spp (Sp, Su), *Heterocapsa triquetra* (Sp, Su), *Heterocapsa rotundata* (Su), *Prorocentrum minimum* (Sp),

Euglenoid: *Euglena* sp (Sp, Su)

Green: *Ankistrodesmus falcatus* (Sp, Sum), *Dactylococcopsis rhaphidioides* (Sp, Su), *Scenedesmus quadricauda* (Sp, Su)

Another diverse set of sixteen (16) taxa or taxonomic groups have higher maximum abundances or higher overall abundances in DEG conditions, but appear in roughly the same frequencies in DEG and REF conditions. They are:

Cryptomonad: *Cryptomonas* sp <10µm (Sp, Su)

Diatom: *Cocconeis* sp (Su), *Coscinodiscus* spp (Sp, Su), *Cylindrotheca closterium* (Su), *Leptocylindrus minimus* (Su), *Melosira nummuloides* (Sp), *Pleurosigma angulatum* (Su), *Rhaphoneis amphiceros* (Sp, Su), *Skeletonema costatum* (Sp, Su), *Skeletonema potamos* (Sp, Su)

Dinoflagellate: *Cladopyxis claytonia* (Sp), *Diplopsalis lenticular* (Sp, Su), *Protoperidinuium* sp group (Su), *Scrippsiella trochoidea* (Sp, Su)

Euglenoid: *Eutreptia lanowii* (Sp, Su)

Green: *Chlorella* sp (Sp)

Taxa or taxonomic groups that favor REF conditions are dominated by diatoms. Four (4) taxa or taxonomic groups have higher maximum abundances or higher overall abundances in REF conditions, but appear in roughly similar frequencies in DEG and REF conditions. They are:

Diatom: *Chaetoceros compressus* (Su), *Coscinosira polychorda* (Sp, Su), unidentified pennales >60µm (Sp)

Green: *Pyramimonas* spp (Su)

Twenty-eight (28) diatom and dinoflagellate taxa or taxonomic groups appear more frequently in REF conditions and typically have higher maximum abundances or higher overall abundances in REF conditions. They are:

Diatom: *Asterionella glacialis* (Su), *Cerataulina pelagica* (Sp, Su), *Chaetoceros curvisetus* (Sp, Su), *Chaetoceros decipiens* (Su), *Chaetoceros neogracilis* (Su), *Cyclotella* sp >30µm (Su), *Cyclotella caspia* (Su), *Dactyliosolen fragilissimus* (Sp, Su), *Detonula pumila* (Su), *Ditylum brightwellii* (Su), *Guinardia delicatula* (Sp, Su), *Guinardia flaccida* (Su), *Hemiaulus* spp (Su), *Leptocylindrus danicus* (Su), *Proboscia alta* (Sp, Su), *Pseudo-nitzschia pungens* (Su), *Rhizosolenia imbricata* (Su), *Rhizosolenia setigera* (Sp, Su), *Rhizosolenia stolterfothii* (Su), *Rhizosolenia styliformis* (Sp, Su), *Thalassionema nitzschioides* (Su), *Thalassiothrix mediterranea* (Su)

Dinoflagellate: *Ceratium furca* (Su), *Ceratium lineatum* (Sp), *Eucampia zoodiacus* (Su), *Noctiluca scintillans* (Su), *Polykrikos kofoidi* (Su), *Prorocentrum micans* (Sp, Su)

Individual phytoplankton samples never contained all 77 taxa or taxonomic groups. As many as 12 taxa in some spring samples and 16 taxa in some summer samples were available to score in the analysis data set. On average, however, 4.9 and 4.8 taxa could be scored in the spring and summer samples, respectively. A phytoplankton taxonomic index (PTI) value is calculated for each sample by averaging the sample's available taxonomic scores. Samples with two or more scored taxa received an index value (95.4% of all spring samples, 89.7% of all summer samples). An index value was not calculated for samples with one or no scored taxa.

The PTI correctly classifies a high percentage of the samples with scored metrics coming from REF and DEG conditions. The CE for the spring index was 89.1%; the CE for the summer index was 90.4%. Distributions of the index scores in the four water quality categories in spring and summer are shown in **Figure 2,** and the percentiles are provided in **Appendix B Table B-2**. The high CEs are reflected in the very large differences seen in the PTI's REF and DEG distributions in **Figure 2**. The CEs are comparable to the CEs for the PIBI index which range from 70% to 90% in eight season- and salinity-specific habitats. The high CE percentages for both the PTI and PIBI indices attest to the critical importance of water transparency (light) and nutrients as environmental factors controlling phytoplankton.

Taxa richness also differs in the four water quality categories (**Figure 3**). Richness is highest in REF and MBL and lowest in DEG in both spring and summer. The degree of difference in taxa richness between REF and DEG is small compared to that in the PTI, suggesting richness is not as sensitive a measure of taxonomic change as the PTI index. As water quality conditions deteriorate, the declining abundances of taxa that do better in REF conditions would appear to be countered to a large extent by increasing abundances of other taxa that do better in DEG conditions.

High densities of individual taxa and taxonomic groups, identified here as "taxa blooms[1]," occur in all water quality categories (**Figure 4**). Some non-toxic taxa are more likely to bloom in REF conditions, e.g., *Cerataulina pelagica*, *Cyclotella* sp >30µm, *Cyclotella caspia*, *Dactyliosolen fragilissimus*, *Guinardia*

[1] A cell density of 250,000 cells per liter is used here as an arbitrary bloom threshold for individual taxa or taxonomic groups. Raising or lowering this threshold will add or subtract taxa from the list of bloom-formers and vertically shift the distributions shown in Figure 4 and the curves shown in Figure 7.

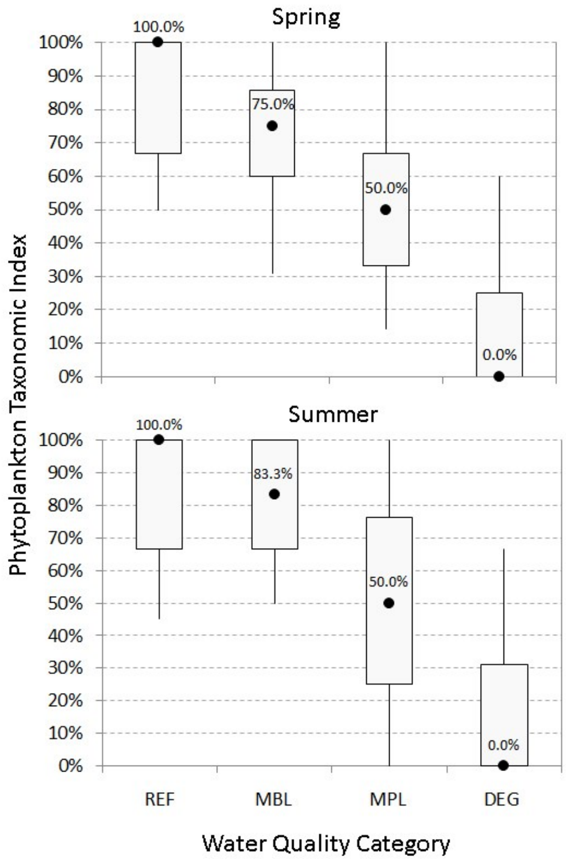

Figure 2. Distribution of phytoplankton taxonomic index scores in the four high salinity water quality categories. Shown are the 5th, 25th, 50th, 75th, and 95th percentiles. Water quality categories: REF, Reference; MBL, Mixed Better Light; MPL, Mixed Poor Light; and DEG, Degraded. See also Figure C-2 in Appendix C.

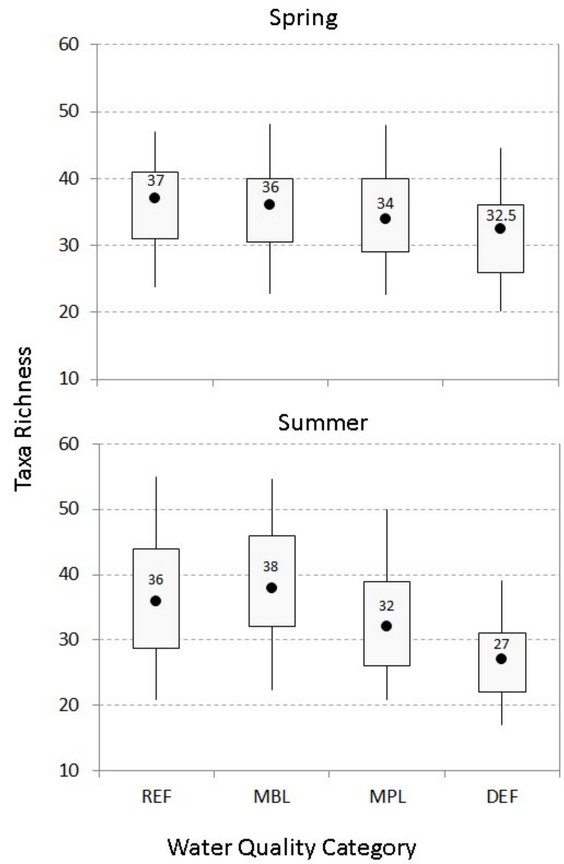

Figure 3. Distribution of taxa richness in four water quality categories in high salinity waters. Taxa richness is the number of taxa identified in an approximately 200-count sub-sample. See Figure 2 heading for more details. See also Figure C-3 in Appendix C.

delicatula, *Leptocylindrus danicus*, *Pyramimonas* spp., *Rhizosolenia stolterfothii*, *Thalassionema nitzschioides*, and unidentified pennales >60µm. Other non-toxic taxa are more likely to bloom in DEG conditions, e.g., *Ankistrodesmus falcatus, Chaetoceros subtilis*, *Chlorella* sp, *Chroococcus* sp, *Cryptomonas* sp <10µm, *Cyclotella striata*, *Cylindrotheca closterium*, *Heterocapsa triquetra*, *Heterocapsa rotundata*, *Leptocylindrus minimus*, *Phoridium* sp, *Skeletonema costatum*, and *Skeletonema potamos*.

Of the known toxin producers that can exceed 250,000 cells/liter, most exhibit higher frequencies of occurrence, higher maximum abundances, and often higher median abundances in DEG conditions. They include *Anabaena* sp., *Cochlodinium* spp., and *Pseudo-nitzschia seriata*. Other toxin producers also exhibit higher frequencies and/or densities in DEG but don't exceed 250,000cells/liter. They include *Amphora* spp. and *Scrippsiella trochoidea.* One potential toxin producer, *Pseudo-nitzschia pungens*, occurs more frequently, in higher maximal densities, in REF waters. The fact that toxin-producing taxa occur more often in particular water quality categories raises an interesting question: "can specific light and/or nutrient conditions trigger toxin production in cells genetically capable of producing toxins?"

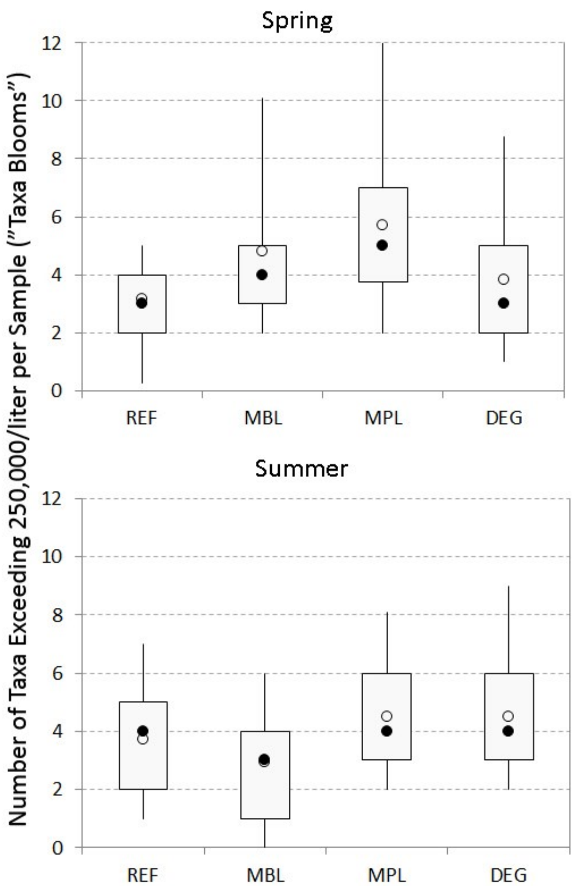

Figure 4. Number of taxa or taxonomic groups in four water quality categories in high salinity waters that exceed 250,000 cells/liter ("taxa blooms"). See Figure 2 heading for more details. See also Figure C-6 in Appendix C.

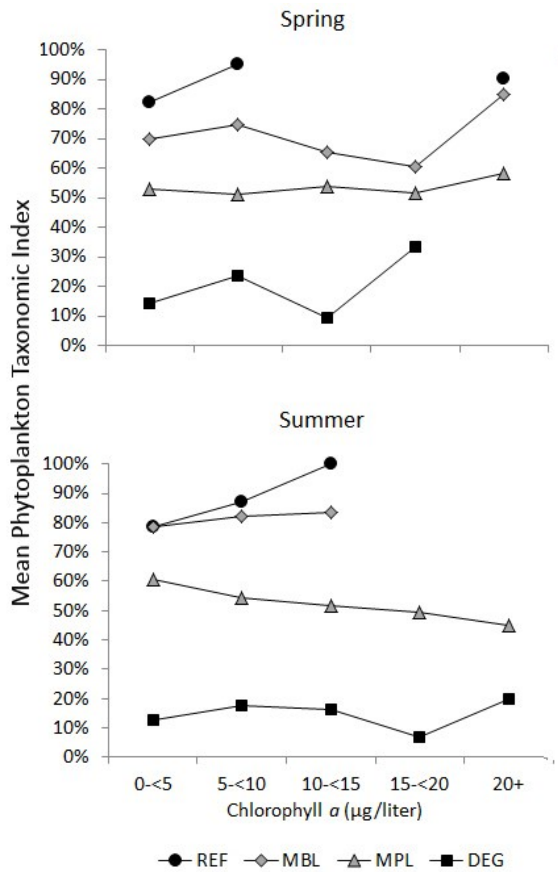

Figure 5. Mean phytoplankton taxonomic index (PTI) value versus chlorophyll *a* concentration in high salinity waters. See Figure 2 heading for water quality categories. See also Figure C-2 in Appendix C.

The PTI index is fairly unaffected by changes in total population biomass, as indicated by chlorophyll *a*. Within each water quality category, PTI scores remain approximately the same as chlorophyll *a* concentration increases (**Figure 5, Appendix C Figure C-2**). Scores of the PIBI, the index composed of community-level metrics, are also unaffected by chlorophyll *a* increases in each water quality category (**Appendix C Figure C-1**).

On the other hand, taxa richness and the number taxa blooms per sample appear to be affected by total phytoplankton biomass, again represented by chlorophyll *a* concentration. Taxa richness declines in three of the four water quality categories—REF, MBL, and MPL—as chlorophyll *a* increases (**Figure 6, Appendix C Figure C-3**) and the number of taxa blooms per sample tends to increase in all water quality categories as chlorophyll a increases (**Figure 7, Appendix C Figure C-4**). Interestingly, in most chlorophyll *a* bins, the mean number of taxa blooms in each of the four water quality categories is approximately the same. This suggests that, for a given chlorophyll *a* concentration, taxa blooms may be just as likely to occur in REF as in DEG. Differences in the frequency of taxa blooms seen in **Figure 4**, therefore, could be due to differences inherent in the chlorophyll *a* concentrations of the individual water quality categories (DEG and MPL have higher maximum chlorophyll *a* concentrations) rather than in the water quality categories *per se*.

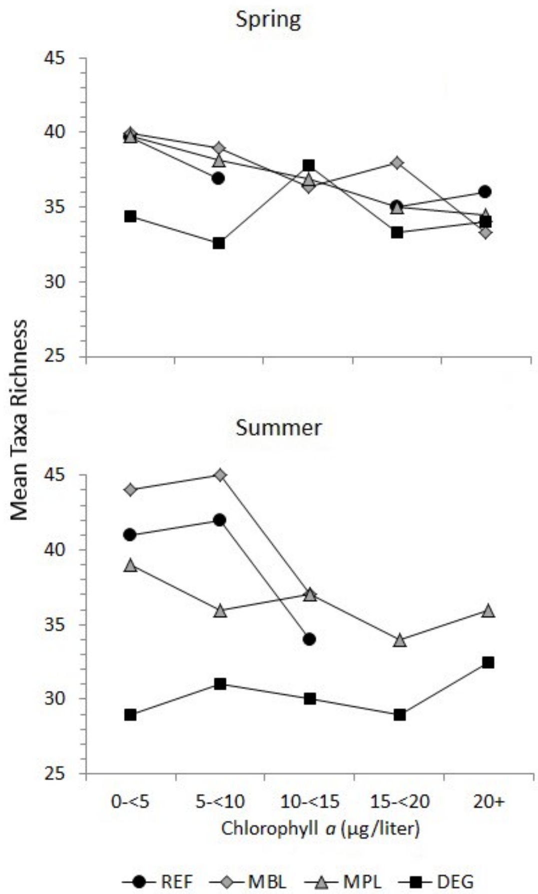

Figure 6. Mean taxa richness versus chlorophyll *a* concentration in high salinity waters. See Figure 2 heading for more details. See also Figure C-3 in Appendix C.

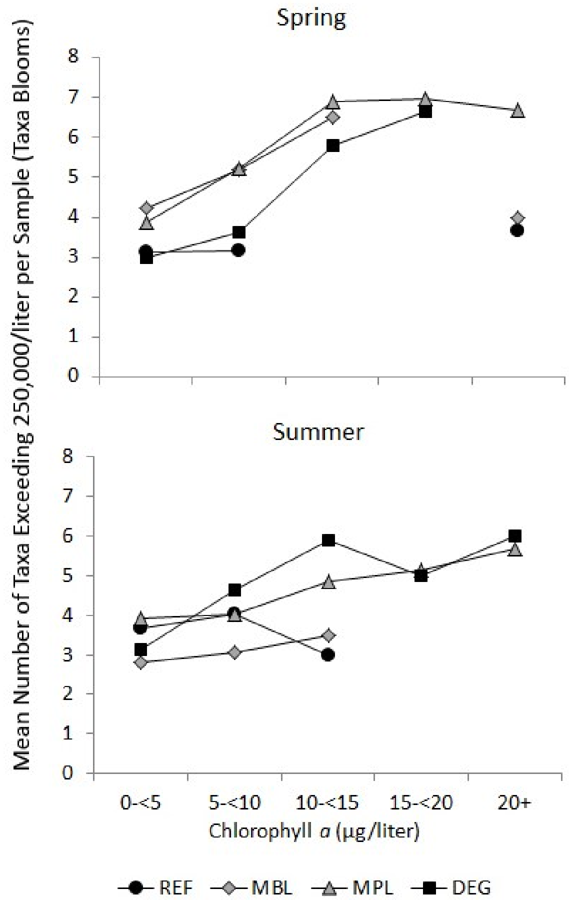

Figure 7. Mean number of taxa blooms versus chlorophyll *a* concentration in high salinity waters. See Figure 2 heading for more details. See also Figure C-6 in Appendix C.

The Shannon-Weiner Index and Pielou Evenness Index (also known as the Shannon Equitability Index) were also calculated. The results are inconclusive, but suggest there is little change in these measures of diversity across the four water quality categories and across the range of chlorophyll *a* concentrations in each category (**Appendix C Figures C-5** and **C-6**).

Discussion

A cast of over 500 phytoplankton taxa inhabit high salinity waters of Virginia' Chesapeake Bay in spring and summer. At any one time, their individual responses to light and nutrient conditions are superimposed on their responses to a myriad of other pressures, including temperature and salinity changes, episodic weather events, competition, and grazing. Samples collected once or twice monthly are snapshots of these organisms' drifting populations, and laboratory protocols count only a fraction of the individuals in each sample. A finite description of the taxonomic composition of a balanced, indigenous, desirable phytoplankton community for these waters is not possible. What is possible are semi-quantitative indications (indices) of the taxa that tend to occur more often, in distinctly higher or lower abundances, when water quality is "good" or "poor."

Society perceives good water quality for aquatic plants to be nutrient concentrations low enough to limit blooms of "undesirable or nuisance aquatic plant life" and water transparency deep enough to allow normal, unstressed photosynthesis. Good water quality is assumed to support balanced, indigenous, desirable populations of aquatic plants, and is deemed essential for meeting Virginia water quality standards (VADEQ 2004) and achieving key CBP restoration goals. Previous bioassay experiments and analyses of monitoring program data have identified numeric thresholds for nitrogen, phosphorus and Secchi depth that are useful in characterizing good water quality for phytoplankton. Called Reference (REF) in this report, these good conditions have DIN and PO_4 concentrations below 0.07 and 0.007 mg/liter, respectively, and Secchi depth above certain season- and salinity-specific thresholds (see **Appendix A**). They contrast with poor, or Degraded (DEG), conditions where all three water quality parameters fail to meet the thresholds.

Balance, Indigenous, Desirable Phytoplankton Populations

Wetzel (2001), Lampert and Sommer (1997), Wehr and Sheath (2003), and others have summarize a wealth of evidence linking phytoplankton community structure and function to nutrients and light. In previous Chesapeake studies, REF conditions were shown to have comparatively stable and predictable proportions of the major taxonomic groups; low concentrations of the toxin-producing species *Microcystis aeruginosa* and *Prorocentrum minimum*; low cellular levels of chlorophyll *a* (expressed as the chlorophyll-to-carbon, or Chla:C, ratio), indicating unstressed photosynthesis; relatively large average cell size in the population; amounts of the nano-micro phytoplankton size fractions (2–200 µm) eaten by fish and shellfish grazers that are the same or higher than those in DEG; rare occurrences of high (bloom) and low (bust) biomass events; and total chlorophyll *a* concentrations that do not reach the heights found in eutrophic systems (Buchanan *et al.* 2005). There is anecdotal evidence that the quality of phytoplankton as food for zooplankton may be higher in REF conditions. Carpenter *et al.* (2006) found that zooplankton in Chesapeake Bay polyhaline REF conditions were roughly four times more abundant than in DEG, more diverse, and heavily dominated by copepods and cladocerans.

Taxonomic differences between REF and DEG conditions in Chesapeake Bay, identified at the time as "Least-Impaired" and "Impaired," were analyzed in Marshall *et al.* (2006) and Lacouture *et al.* (2006). The results of these two studies are somewhat inconsistent due in part to the former focusing on mean and median values of taxa and taxonomic group distributions and the latter focusing on the upper and lower percentiles of the taxonomic group distributions. Many of the taxa identified in Marshall *et al.* (2006) are important members of Chesapeake phytoplankton communities, and most proved amenable to the scoring approach of this study.

The current study compared phytoplankton from REF and DEG conditions in Virginia's high salinity waters. Seventy-seven (77) taxa were identified that exhibited notable differences in their frequency of appearance and/or cell abundances. A diverse set of forty-five (45) taxa or taxonomic groups appear more frequently and/or in higher overall or maximum abundances ("taxon blooms") in Degraded conditions. They included the toxin producers *Anabaena* sp, *Prorocentrum minimum*, *Pseudo-nitzschia seriata*, and *Cochlodinium* spp. Thirty-two (32) predominantly diatom taxa or taxonomic groups appear more frequently and/or in higher overall or maximum abundances in Reference conditions. Most do not produce toxin, and the one potential toxin producer, *Pseudo-nitzschia pungens*, has not been reported to produce toxins in the Mid-Atlantic (World Register of Marine Species). The results show that phytoplankton taxonomic compositions in REF conditions can and do differ substantially from those in DEG conditions. The Phytoplankton Taxonomic Index, or PTI, calculated from scored taxonomic counts has very high classification efficiencies, indicating the taxonomic differences are strongly responsive to light and nutrient conditions in the open water environment. For a given range of chlorophyll *a* concentrations, the mean number of taxon blooms per sample changes little across the four water quality categories. Taxa blooms occur in REF conditions just as often as they do in DEG conditions, but are propagated by a different mix of taxa.

The results of the current study support the premise that phytoplankton populations in well-lit, nutrient-limited water quality conditions (REF) have desirable characteristics and are distinctly different from populations in DEG. Phytoplankton populations in REF water quality conditions can be considered examples of the balanced, desirable populations called for in Virginia Water Quality Standards. High values of the PTI index—as well as the earlier PIBI index developed from community-level metrics—will indicate the presence of balanced, desirable populations.

Community-level metrics such as the number of taxa blooms per sample have an inherent relationship with chlorophyll *a* concentration and will change as chlorophyll a concentration increases or decreases. Both the PTI and PIBI indices, however, are insensitive to changes in chlorophyll *a* and their values remain fairly consistent across a broad range of chlorophyll *a* concentrations. The two indices represent phytoplankton taxonomic and physiological responses to water quality condition that happen regardless of the total size of the population.

The overriding influence of estuarine water quality conditions is evident in the study's results. Both phytoplankton indices have high classification efficiencies in Chesapeake Bay's high salinity waters, correctly differentiating REF from DEG conditions most of the time. This demonstrates strong responses of multiple phytoplankton community and taxonomic features to water quality conditions. Nutrient concentrations and water clarity levels in the James River estuary can be expected to influence which taxa dominate the phytoplankton population and how abundant those taxa are likely to become.

Protectiveness of James River Chlorophyll a Criteria

Virginia currently uses chlorophyll *a* criteria to assess achievement of "balanced, desirable" aquatic life, namely phytoplankton, in the tidal James River. As described in USEPA (2007), "*a criterion threshold is a concentration that should rarely be exceeded by a 'population' of concentration data exhibiting healthy levels. The state-adopted concentration-based chlorophyll a criteria values are threshold concentrations that should only be exceeded infrequently since a low number of naturally occurring exceedances occur even in a healthy phytoplankton population*."

The current James River chlorophyll *a* criteria approximate the upper percentiles of chlorophyll *a* concentrations in REF conditions, so chlorophyll *a* concentrations that meet (i.e., do not exceeding) the James River criteria occur often in high salinity REF conditions. In high salinity waters bay-wide, 93% of all spring REF samples and 83% of all summer REF samples have chlorophyll *a* concentrations below the 12 and 10 μg/liter criteria, respectively, and concentrations very rarely exceed 25 μg/liter (**Figure 8**). However, low chlorophyll a concentrations are also prevalent in DEG conditions. In high salinity waters bay-wide, about 76% of all spring DEG samples and 68% of all summer DEG samples meet the 12 and 10 μg/liter criteria, respectively. A subset of exceptionally degraded sites meets the criteria more frequently. Phytoplankton growth in DEG conditions can be suppressed by inadequate light levels despite the presence of excessive amounts of nutrients, and populations will exhibit lower chlorophyll *a,* lower cell abundances, and lower total biomass in these conditions (Buchanan et al. 2005).

Chlorophyll *a* criteria by themselves can be counted on to indicate the presence of undesirable, potentially harmful populations when the frequency and/or extent of criteria exceedances are high in an

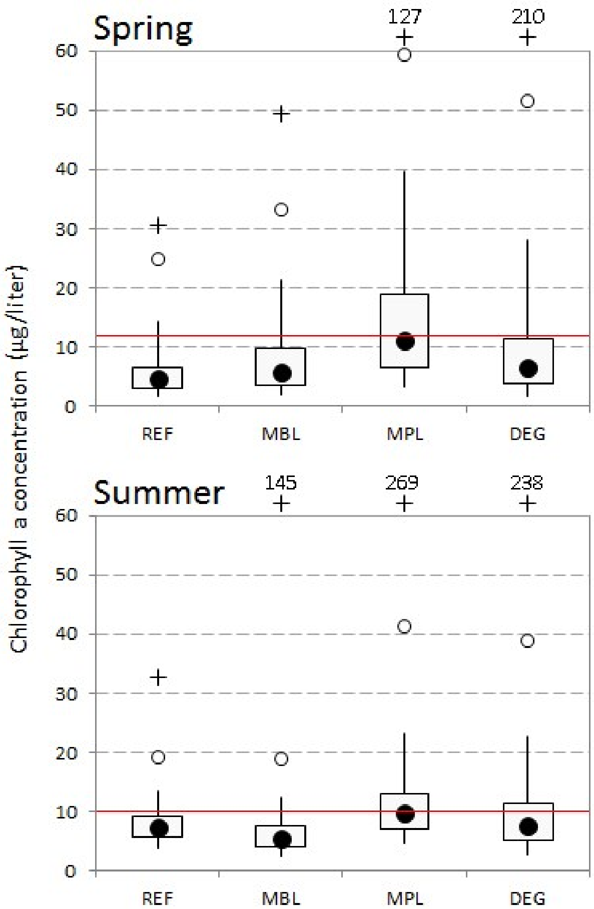

Figure 8. Chlorophyll *a* concentrations in four water quality categories in high salinity waters (>10 ‰) bay-wide. Box-and-whiskers - 5th, 25th, 50th, 75th and 95th percentiles; open circle – 97.5th percentile; cross – maximum concentration. Maximum concentrations greater than 60 µg/liter are indicated. Red lines indicate spring (12 µg/liter) and summer (10 µg/liter) James River chlorophyll a criteria. See Figure 2 heading for more details.

assessment period. When relatively few criteria exceedances occur in an assessment period, the results cannot be linked definitively to either undesirable, potentially harmful populations or balanced, desirable populations. Conclusions drawn from assessments that focus solely on chlorophyll *a* criteria attainment are prone to error if ambient water quality conditions are very degraded.

Given the overriding importance of adequate light to photosynthetic organisms, information about Chesapeake Bay water clarity could be useful in the chlorophyll *a* criteria assessment process and would increase confidence in final decisions on designated use attainment. Chesapeake Bay jurisdictions have water clarity criteria. They were originally developed for submerged aquatic vegetation (SAV) communities in nearshore habitats, and are somewhat lower than the Secchi depth thresholds identified for phytoplankton (**Table 2**). Attainment of the SAV-based water clarity criteria in the open waters environments would reduce the risk of nuisance algal blooms and toxin-producing phytoplankton taxa. As the confounding influence of poor water clarity recedes, a more straight-forward, linear relationship between attainment of the James River chlorophyll *a* criteria and balanced, desirable phytoplankton populations—expressed as the PIBI or PTI—develops. Attainment of the James River chlorophyll *a* criteria under these circumstances is protective of balanced, desirable phytoplankton populations. These relationships are discussed further in Buchanan *et al.* (in prep.).

Season/ Salinity Zone		Phytoplankton Thresholds *	Phytoplankton Thresholds (**Appendix A**)	SAV Restoration to 1 Meter **	PLW Secondary Requirement ***
Spring	TF	>0.9	>0.8	>0.725	>0.711 (PLW=13%)
	OH	>0.7	>0.8	>0.725	>0.711 (PLW=13%)
	MH	>1.8	>1.4 (LoMH) >1.8 (HiMH)	>0.967	>0.958 (PLW=22%)
	PH	>2.15	>2.1	>0.967	>0.958 (PLW=22%)
Summer	TF	>0.8	>0.8	>0.725	>0.711 (PLW=13%)
	OH	>0.6	>0.8	>0.725	>0.711 (PLW=13%)
	MH	>1.45	>1.2 (LoMH) >1.6 (HiMH)	>0.967	>0.958 (PLW=22%)
	PH	>1.85	>1.8	>0.967	>0.958 (PLW=22%)

Table 2. Thresholds of adequate water clarity for phytoplankton in open water habitats and submerged aquatic vegetation (SAV) in nearshore habitats, expressed as Secchi depth (meters). Sources: * Buchanan *et al.* (2005) for phytoplankton reference communities; ** Batiuk *et al.* (1992) from SAV Technical Synthesis I (Secchi depth = 1.45/kd); *** Batiuk *et al.* (2000) from SAV Technical Synthesis II (Secchi depth = 1.45/kd).

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Appendix A

Secchi depth thresholds and Virginia water quality conditions

Statistical distributions of the Secchi depths in selected, “good” samples
Water quality sampling events from open water environments, collected bay-wide between 1984 and 2013 and having measurements for total nitrogen, total phosphorus, and total suspended solids (n = 52,631), were extracted from a larger water quality dataset and parsed into five salinity zones and five seasons. The five salinity zones are: tidal fresh (TF, 0 – 0.5‰); oligohaline (OH, >0.5 – 5‰); low mesohaline (LoMH, >5 - 10‰); high mesohaline, (HiMH, 10 – 18‰); and polyhaline (PH, >18‰). The five seasons are: spring (March – May); June; summer (July – September); autumn (October – November); and winter (December – February). Per the Relative Status Method (RSM), the 33^{rd} percentile of each of the three water quality parameters was identified in each of the 25 season-salinity habitats. Samples with TN *and* TP *and* TSS concentrations below their respective 33rd percentiles, in the desirable or “good” end of the statistical distributions from the perspective of the Chesapeake Bay Program, were selected. The statistical properties of Secchi depths in these selected samples were then determined, and are shown in **Table A-1** and **Figure A-1** below. Thresholds separating the worst, poor, better, and best Secchi depth classes in each season-salinity habitat are the 25^{th}%ile, 50^{th}%ile, and 75^{th}%ile. For comparison, the original Secchi depth thresholds (50^{th}%iles) dividing the poor and better classes are provided.

DIN, PO_4, and Secchi depth in the four high salinity water quality categories
All available water quality data (1984 – 2013) collected at Virginia monitoring stations were classified as Reference (REF), Mixed Better Light (MBL), Mixed Poor Light (MPL), or Degraded (DEG) using the following thresholds (see **Appendix A** for season- and salinity-specific thresholds for Secchi depth):

REF	Reference	Secchi > threshold; DIN $\leq$0.07 mg/liter; PO_4 $\leq$0.007 mg/liter
MBL	Mixed Better Light	Secchi > threshold; no limits on DIN or PO_4
MPL	Mixed Poor Light	Secchi $\leq$ threshold; no limits on DIN or PO_4
DEG	Degraded	Secchi $\leq$ threshold; DIN >0.07 mg/liter; PO_4 >0.007 mg/liter

The resulting distributions of DIN, PO_4, and Secchi depth in the four water quality categories are shown in **Figures A-2, A-3,** and **A-4**, respectively.

Table A-1. Statistical properties (percentiles, sample size) of Secchi depths in "good" Chesapeake Bay water quality conditions, for five salinity zones. Distributions derived with the Relative status Method applied to all available bay-wide water quality data for open water environments, 1984 – 2013. See text for details. Original threshold: the threshold, corresponding to the 50th percentile, which separated the worst and poor Secchi depth classes from the better and best class in the original RSM analysis (Buchanan *et al.* 2005).

Season	Salinity Zone	5	25	50	75	90	95	n	Original Threshold
Spring	TF	0.4	0.6	0.8	1.1	1.31	1.6	320	0.9
	OH	0.41	0.7	0.8	1	1.3	1.5	143	0.7
	LoMH	0.8	1.2	1.4	1.7	2	2.3	284	1.8
	HiMH	1.2	1.5	1.8	2	2.56	2.9	355	1.8
	PH	1.22	1.7	2.1	2.6	3.3	3.8	224	2.15
June	TF	0.4	0.6	0.7	0.8	1	1.2	86	0.8
	OH	0.47	0.6	0.7	0.8	0.9	0.94	54	0.6
	LoMH	0.8	1	1.2	1.5	1.8	1.8	89	1.45
	HiMH	1	1.1	1.4	1.7	2	2.19	122	1.45
	PH	1	1.3	1.6	1.8	2.5	3	133	1.85
Summer	TF	0.4	0.6	0.8	1.1	1.3	1.5	275	0.8
	OH	0.5	0.6	0.8	1	1.1	1.2	167	0.6
	LoMH	0.7	0.9	1.2	1.4	1.7	1.8	253	1.45
	HiMH	1.1	1.4	1.6	1.8	2	2.2	645	1.45
	PH	1.2	1.5	1.8	2.1	2.6	3.2	534	1.85
Autumn	TF	0.5	0.7	0.9	1.23	1.5	1.99	124	0.9
	OH	0.46	0.6	0.8	1	1.14	1.44	73	0.5
	LoMH	0.9	1.1	1.2	1.55	1.88	2.28	43	2
	HiMH	1.27	1.6	2	2.3	2.7	3.03	235	2
	PH	1.2	1.7	2	2.5	3	3.44	194	2.1
Winter	TF	0.4	0.6	0.8	1.1	1.5	1.89	164	0.6
	OH	0.46	0.6	0.9	1	1.3	1.45	72	0.6
	LoMH	0.99	1.2	1.4	1.78	2	2.16	98	1.8
	HiMH	1.2	1.5	2	2.2	2.6	3	274	1.8
	PH	1.3	1.7	2.1	2.5	3	3.2	238	2.3

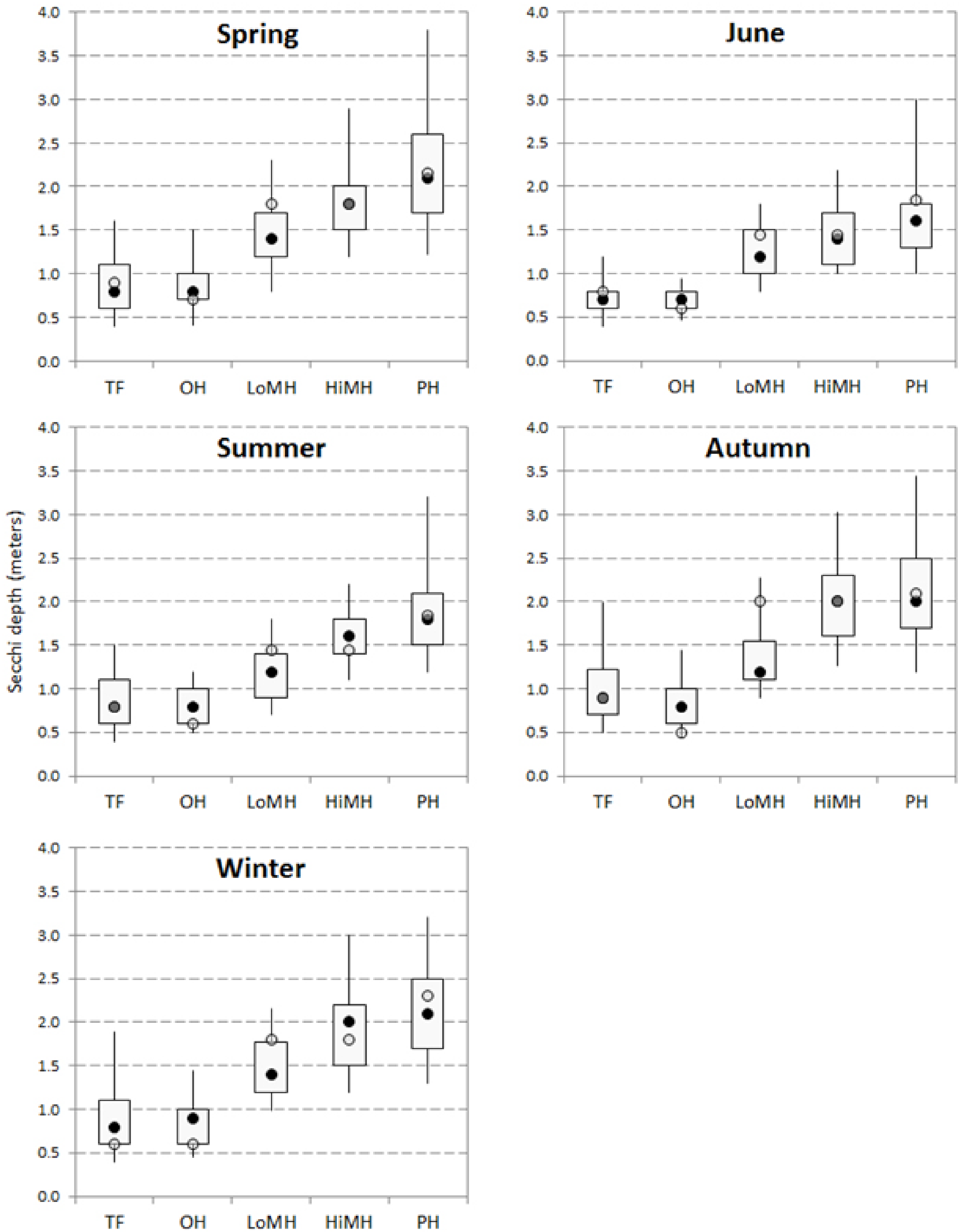


Figure A-1. Distributions of Secchi depth in seasonal “good” Chesapeake Bay water quality conditions, for five salinity zones. Distributions derived with the Relative Status Method applied to all available bay-wide water quality data for open water environments, 1984 – 2013. Closed circle, median; open circle, original threshold (low and high mesohaline salinities were not separated in the original analysis); box, 25^{th}%ile to 75^{th}%ile range; whiskers, 5^{th}%ile and 95%ile. See text for details.

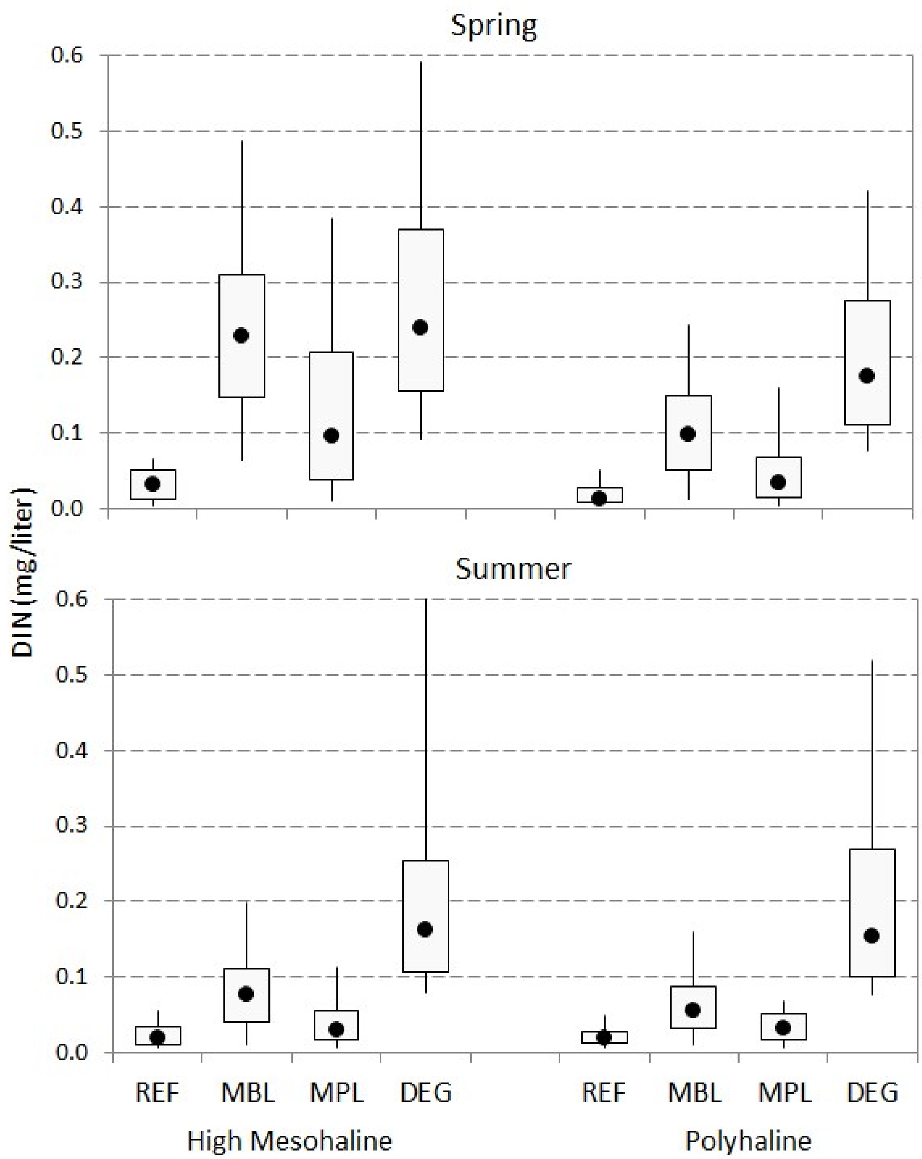


Figure A-2. High salinity, spring and summer distributions of dissolved inorganic nitrogen (DIN) in the four water quality categories: REF - Reference conditions; MBL – Mixed Better Light; MPL – Mixed Poor Light; DEG – Degraded conditions. High mesohaline - >10 – 18‰; polyhaline - >18‰. Closed circle, median; box, 25th%ile to 75th%ile range; whiskers, 5th%ile and 95%ile. Drawn from all available Virginia open water stations (>2 meter depth), 1984 – 2013. See text for details.

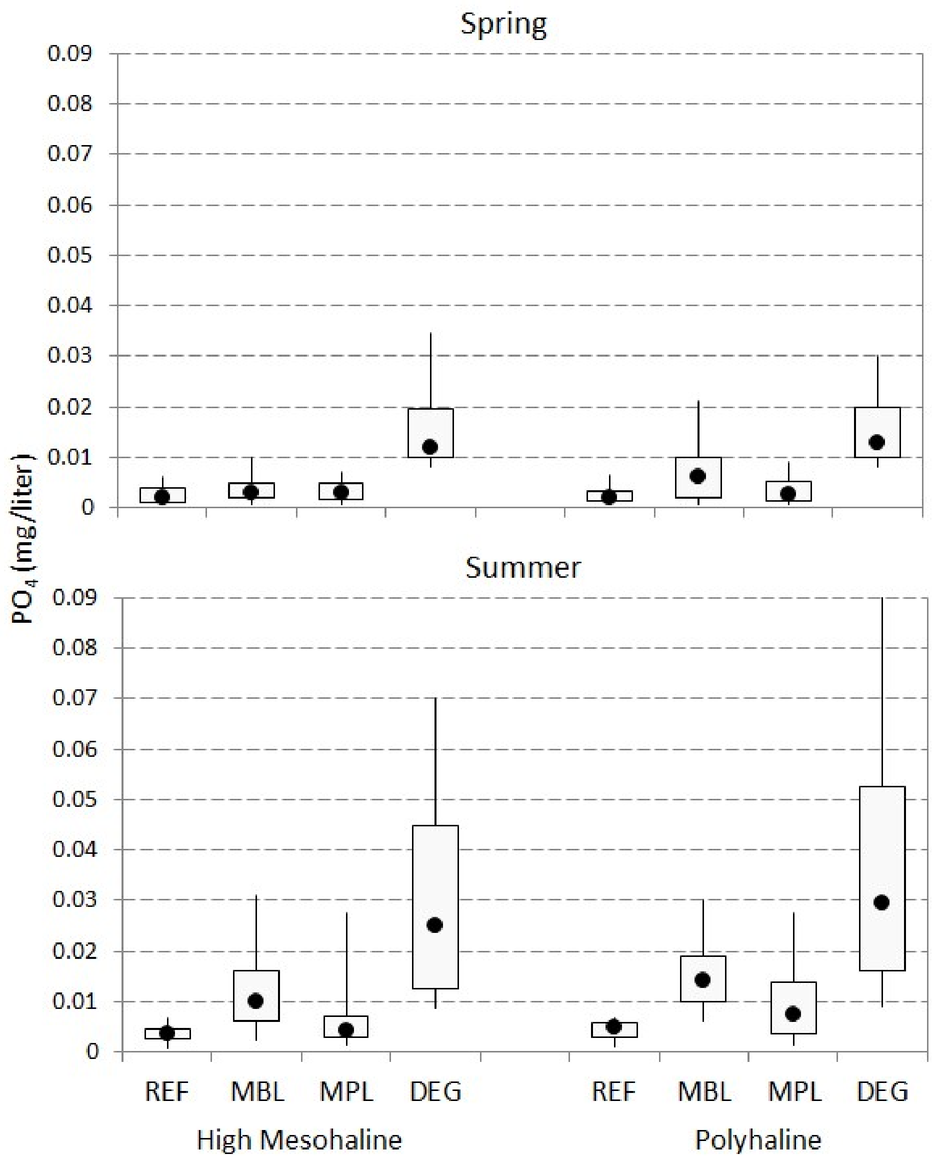


Figure A-3. High salinity, spring and summer distributions of ortho-phosphate (PO_4) in the four water quality categories: REF - Reference conditions; MBL – Mixed Better Light; MPL – Mixed Poor Light; DEG – Degraded conditions. High mesohaline - >10 – 18‰; polyhaline - >18‰. Closed circle, median; box, 25^{th}%ile to 75^{th}%ile range; whiskers, 5^{th}%ile and 95%ile. Drawn from all available Virginia open water stations (>2 meter depth), 1984 – 2013. See text for details.

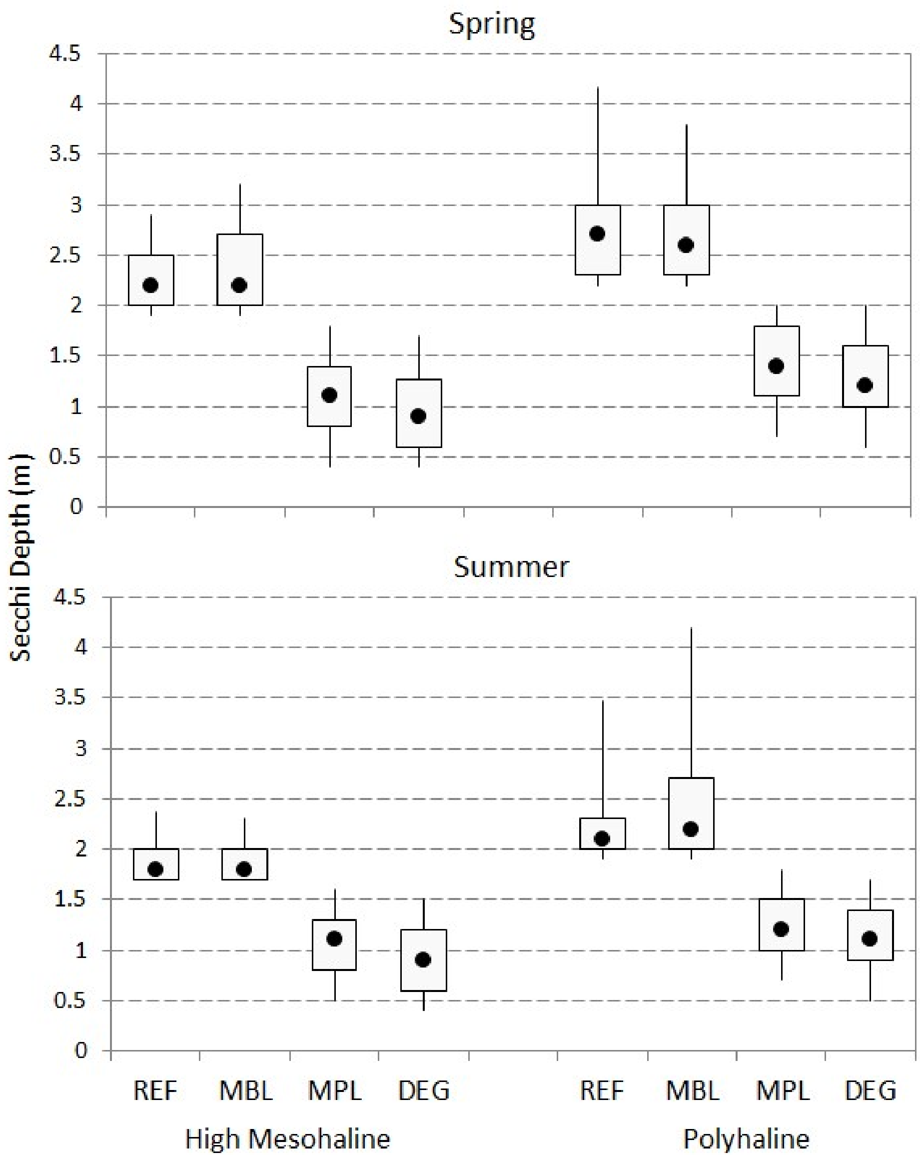


Figure A-4. High salinity, spring and summer distributions of Secchi depth in the four water quality categories: REF - Reference conditions; MBL – Mixed Better Light; MPL – Mixed Poor Light; DEG – Degraded conditions. High mesohaline - >10 – 18‰; polyhaline - >18‰. Closed circle, median; box, 25th%ile to 75th%ile range; whiskers, 5th%ile and 95%ile. Drawn from all available Virginia open water stations (>2 meter depth), 1984 – 2013. See text for details.

Appendix B

Taxon abundance distributions in four water quality categories

This appendix shows statistical distributions of the abundances (cells per liter) of 77 selected taxa in the four water quality categories, and possible spring and summer scoring thresholds for those taxa. The taxa were selected based several characteristics, including frequency of occurrence, toxin-producers, affinity for REF or DEG categories.

Symbols (see text for further details):

closed circle - median abundance of taxon in the given water quality category

larger closed circle - median abundance of taxon and it also occurs in more than 50% of samples in the given water quality category

box - 25^{th}%ile to 75^{th}%ile range

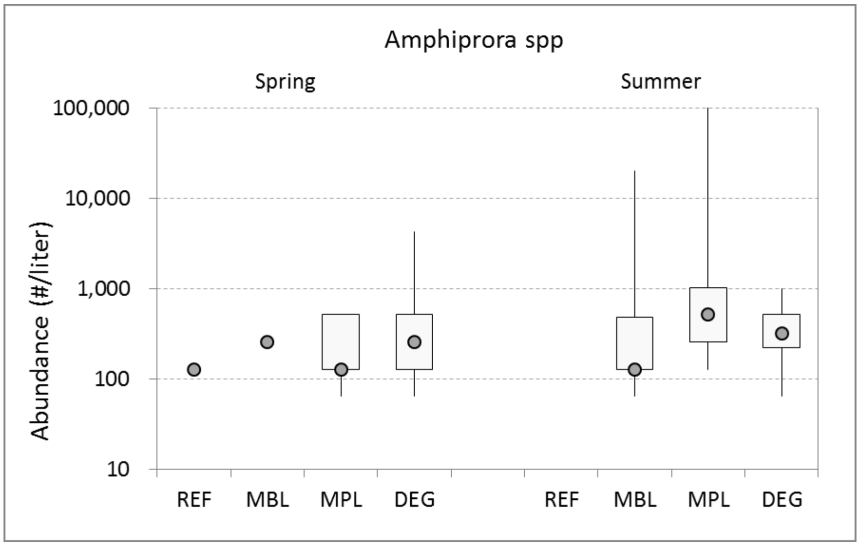
whiskers - minimum and 95%ile

If five or fewer records of a taxon are found in a water quality category, only the median value is shown.

Water quality categories (see **Appendix A** for season- and salinity-specific thresholds for Secchi depth):

REF	Reference	Secchi > threshold; DIN $\leq$0.07 mg/liter; PO_4 $\leq$0.007 mg/liter
MBL	Mixed Better Light	Secchi > threshold; no limits on DIN or PO_4
MPL	Mixed Poor Light	Secchi $\leq$ threshold; no limits on DIN or PO_4
DEG	Degraded	Secchi $\leq$ threshold; DIN >0.07 mg/liter; PO_4 >0.007 mg/liter

Table B-1. Scored taxa and taxonomic groups

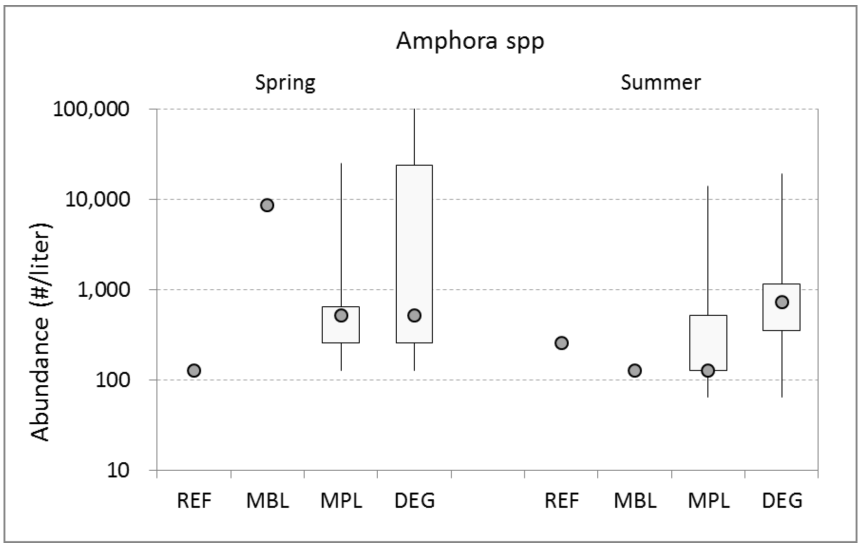


DIATOM

More frequent and higher maximum abundances in spring and summer DEG

Spring
Score 0: >512 (REF max)
Else: Null

Summer
Score 0: >512 (REF max)
Else: Null

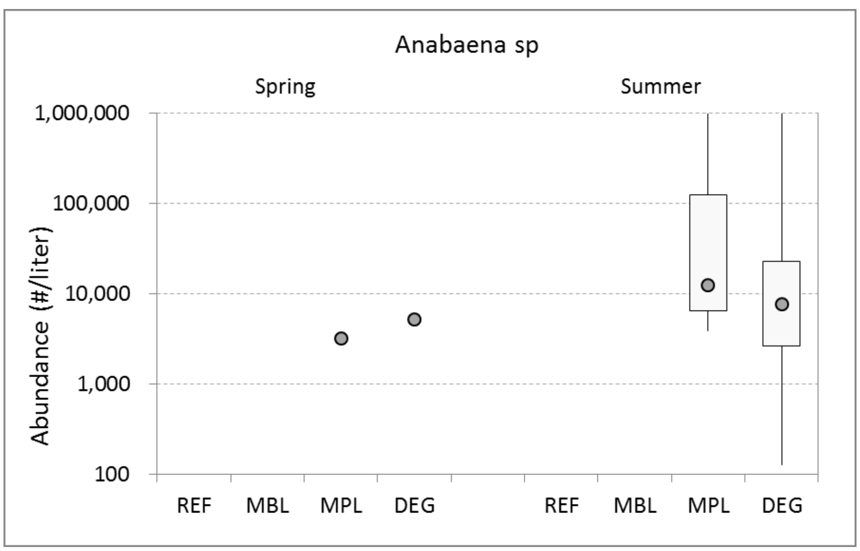


DIATOM
Toxin producer: *A. coffeaeformis*

More frequent and higher maximum abundances in spring and summer DEG

Spring
Score 0: >512 (Ref max)
Else: Null

Summer
Score 0: >512 (Ref max)
Else: Null

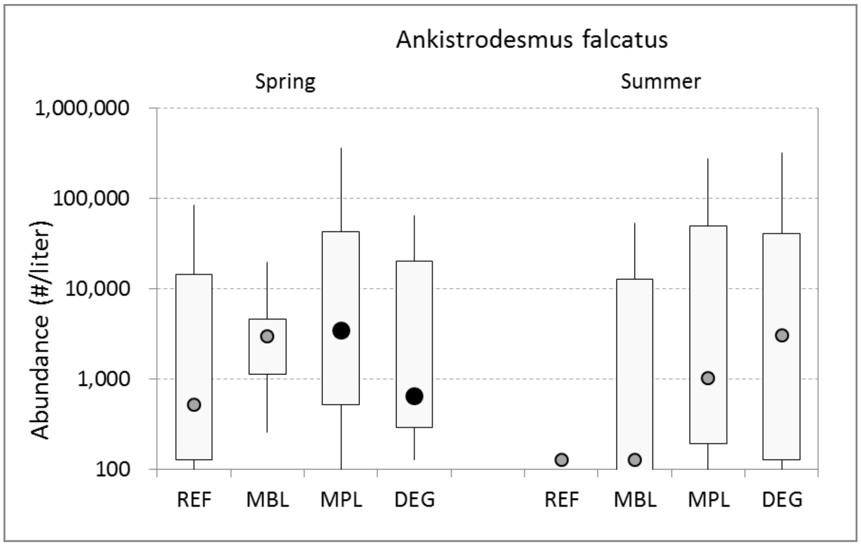


CYANOBACTERIA
Toxin producer

More frequent and higher maximum abundances in spring and summer DEG

Spring
Score 0: if present
Else: Null

Summer
Score 0: if present
Else: Null

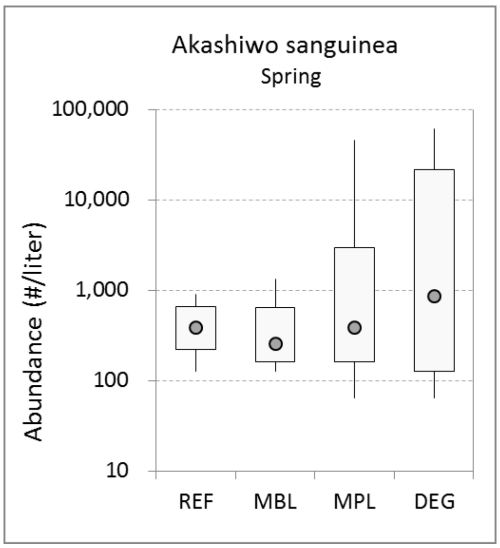


GREEN

More frequent and higher maximum abundances in spring and summer DEG

Spring
Score 0: >14,219 (REF 75%ile)

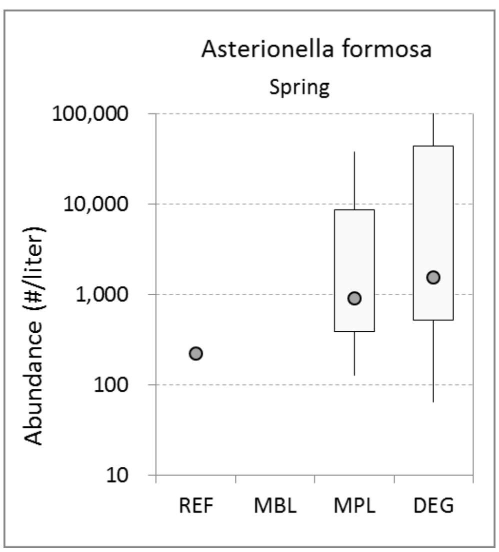
Summer
Score 0: >12,710 (MBL 75%ile)
Else: Null



DINOFLAGELLATE
Syn: *Gymnodinium splendens*

More frequent and higher maximum abundances in spring DEG

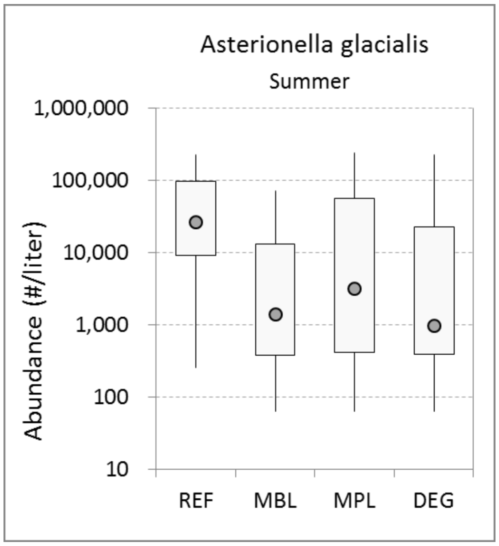
Spring
Score 0: >896 (REF max)
Else: Null



DIATOM

More frequent and higher maximum abundances in spring DEG

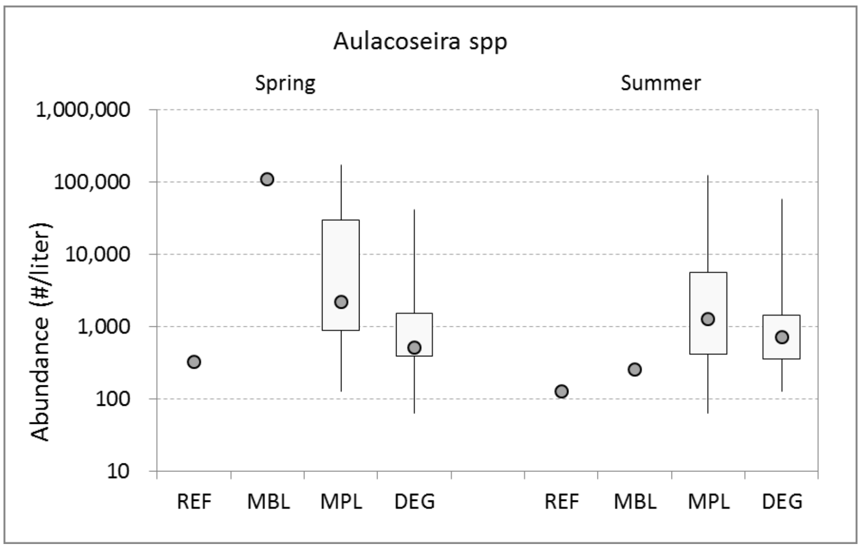
Spring
Score 0: >512 (REF max)
Else: Null



DIATOM

Higher overall abundances in summer REF

Summer
Score 1: ≥26,397 (REF 50%ile)
Else: Null

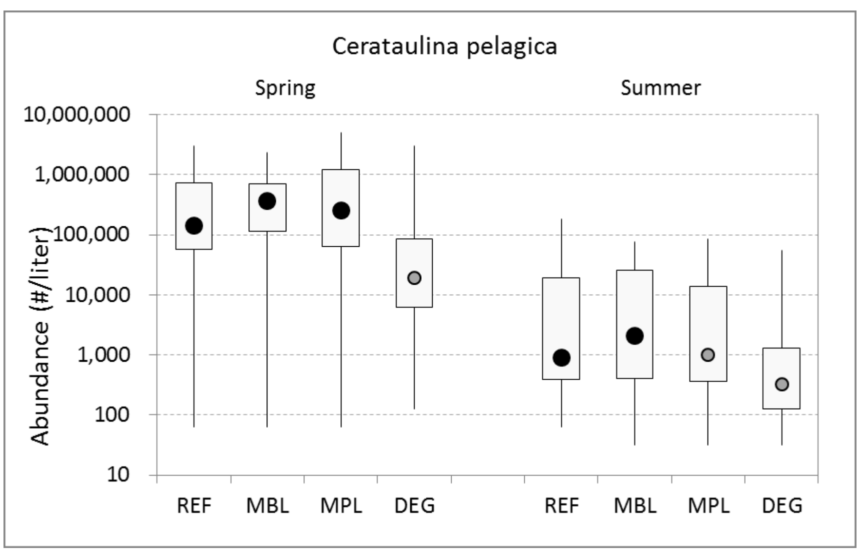


DIATOM

More frequent in spring DEG; more frequent and higher maximum abundances in summer DEG

Spring
Score 0: >384 (REF max)
Else: Null

Summer
Score 0: >384 (REF max)
Else: Null

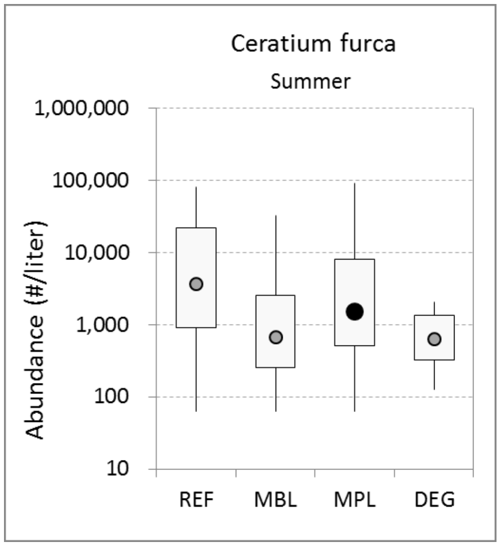


DIATOM

More frequent and higher overall abundances in spring and summer REF

Spring
Score 1: ≥229,868 (REF 60%ile)
Else: Null

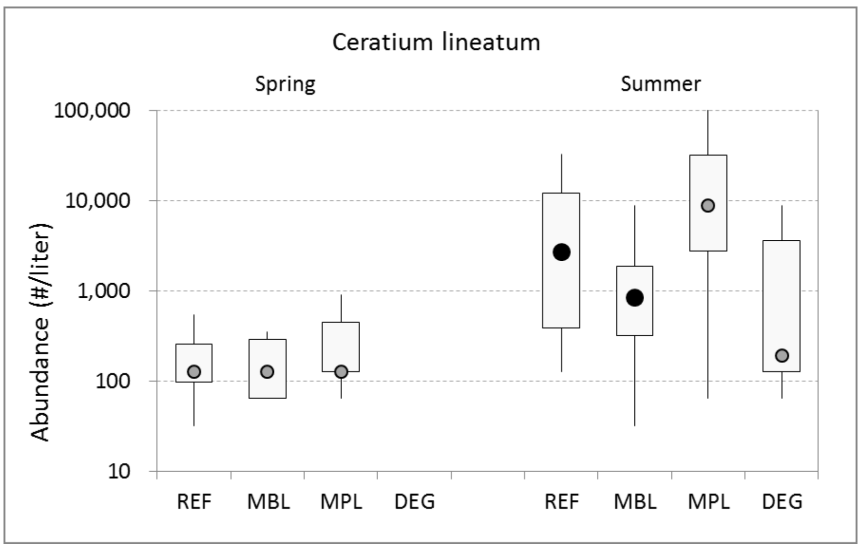
Summer
Score 1: ≥2,086 (REF 60%ile)
Score 0: <128 (REF10%ile)
Else: Null



DINOFLAGELLATE

More frequent and higher overall abundances in REF

Summer
Score 1: ≥2,886 (REF 40%ile)
Else: Null

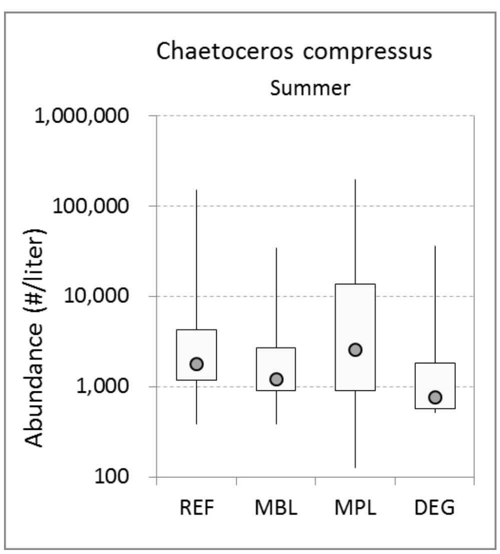


DINOFLAGELLATE

Different seasonal patterns. More frequent in spring REF. More frequent and higher maximum abundances in summer REF.

Spring
Score 1: if present
Else: Null

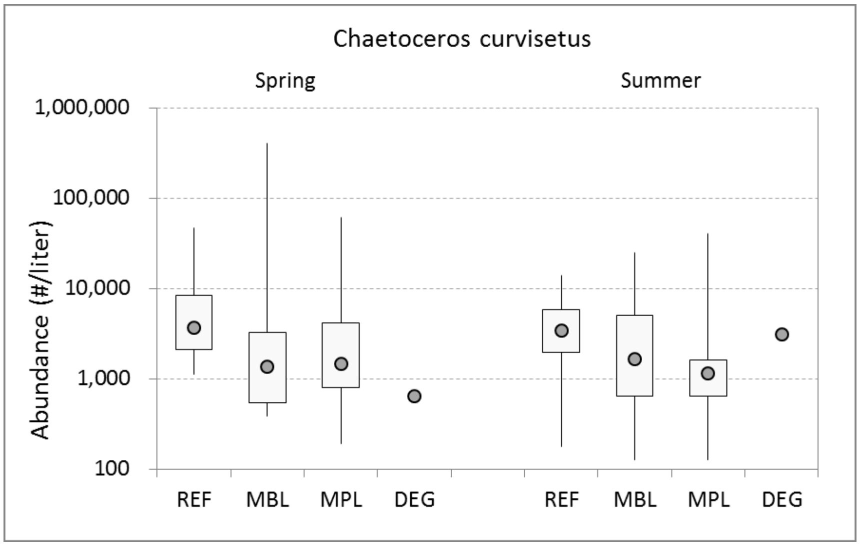
Summer
Score 1: ≥12,176 (REF 75%ile)
Score 0: <128 (REF 5%ile)
Else: Null



DIATOM

Higher overall abundances in summer REF

Summer
Score 1: ≥2,854 (REF 60%ile)
Else: Null

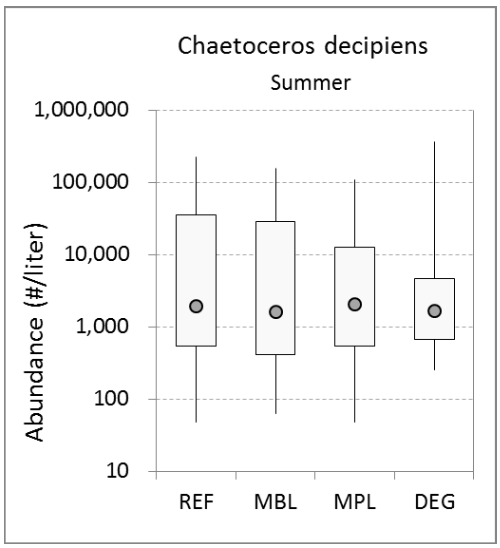


DIATOM

More frequent and generally higher abundances in REF

Spring
Score 1: >2,112 (REF 25%ile)
Else: Null

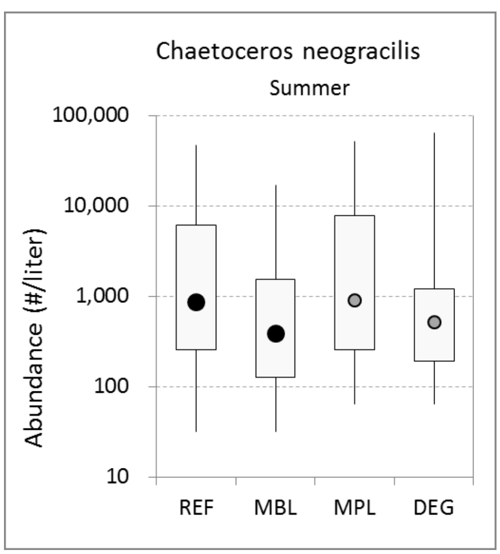
Summer
Score 1: >3,392 (REF 50%ile)
Else: Null



DIATOM

More frequent and often higher maximum abundances in REF

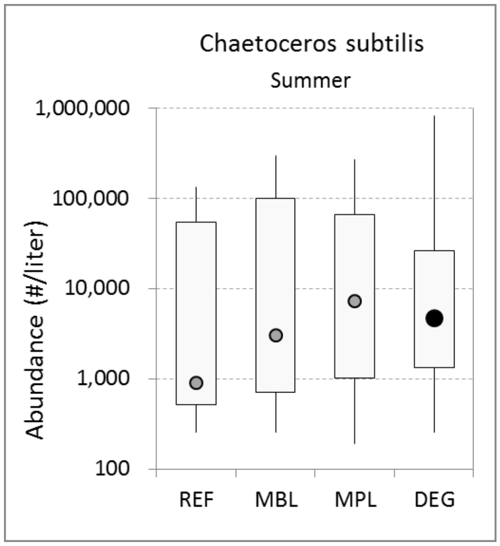
Summer
Score 1: >36,042 (REF75%)
Else: Null



DIATOM

More frequent and higher overall abundances in summer REF

Summer
Score 1: >6,117 (REF 75%ile)
Else: Null



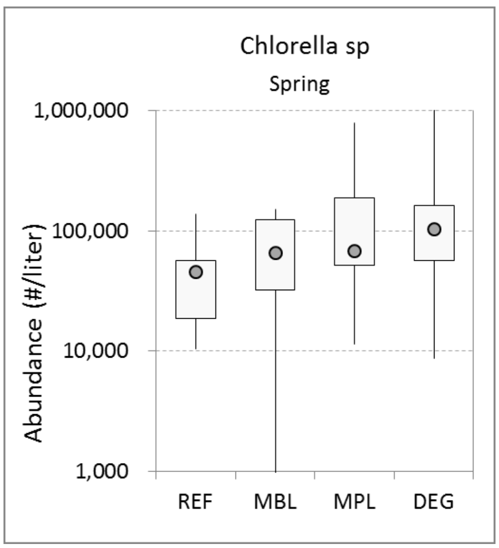
DIATOM

More frequent and higher maximum abundances in summer DEG

Summer

Score 1: ≤896 (REF 50%ile)

Else: Null



GREEN

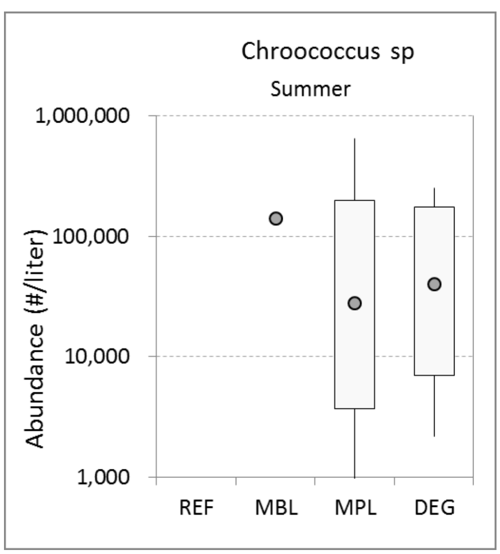
Higher maximum abundances in spring DEG

Spring

Score 0: >155,776 (REF max)

Score 1: ≤45,581 (REF 50%ile)

Else: Null



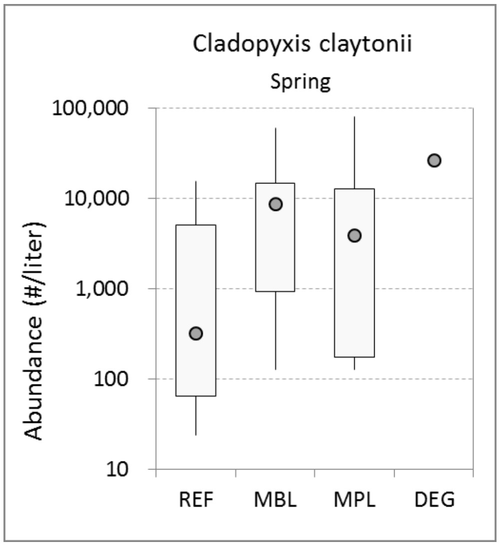
BLUE-GREEN

Most frequent in summer DEG

Summer

Score 0: if present

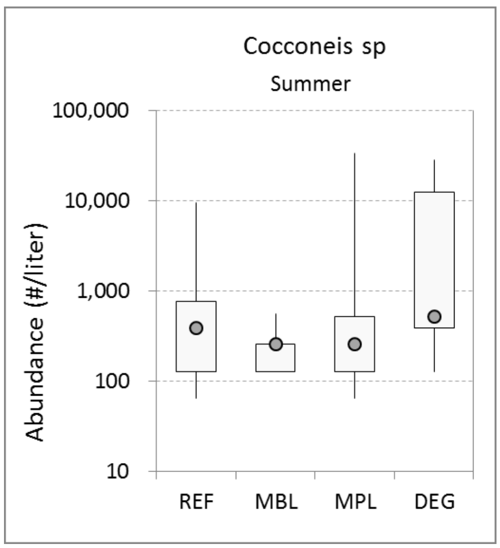
Else: Null



DINOFLAGELLATE

Higher maximum abundances but less frequent in spring DEG

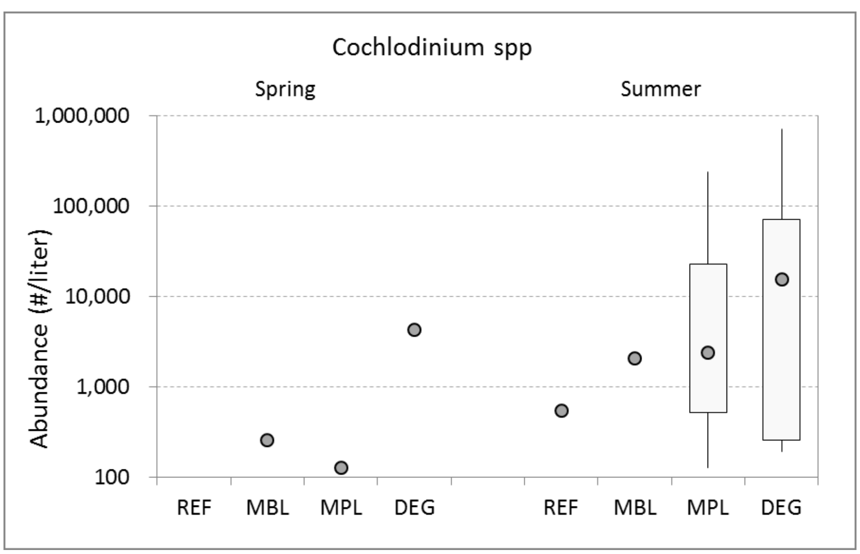
Spring
Score 1: <5,114 (REF 75%ile)
Score 0: >17,357 (REF max)
Else: Null



DIATOM

Higher abundances in summer DEG

Summer
Score 0: >762 (REF 85%ile)
Else: Null

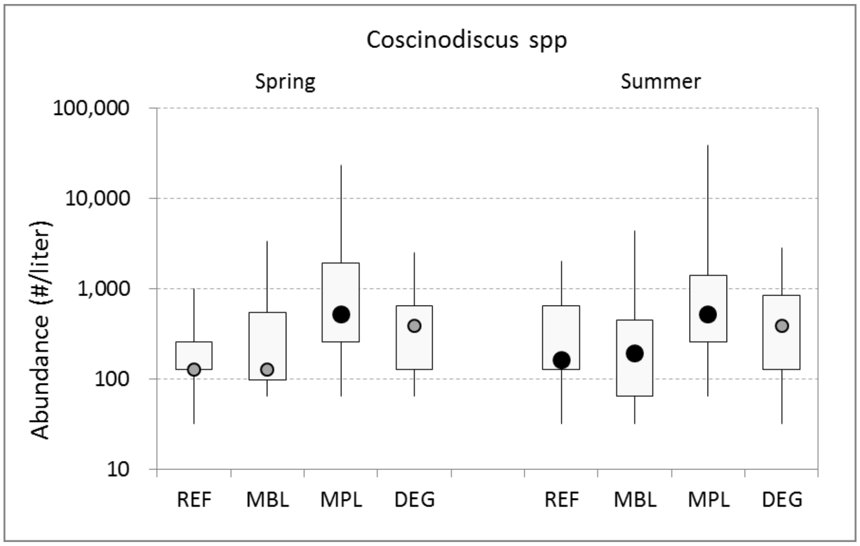


DINOFLAGELLATE
Toxin producer: *C. polykrikoides*

Higher abundances and more frequent in spring and summer DEG

Spring
Score 0: if present
Else: Null

Summer
Score 0: if present
Else: Null

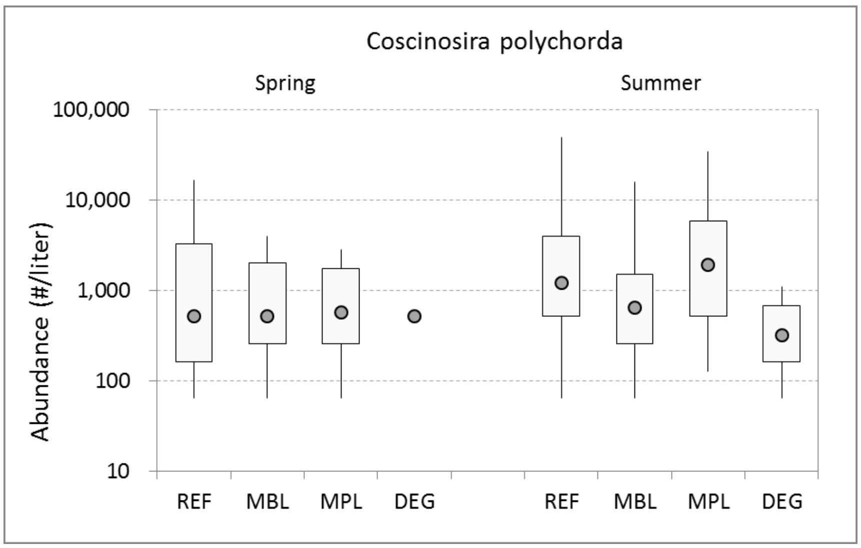


DIATOM

Higher maximum abundances in spring and summer DEG

Spring
Score 0: >256 (REF 75%ile)
Else: Null

Summer
Score 0: >2,032 (REF 95%ile)
Else: Null

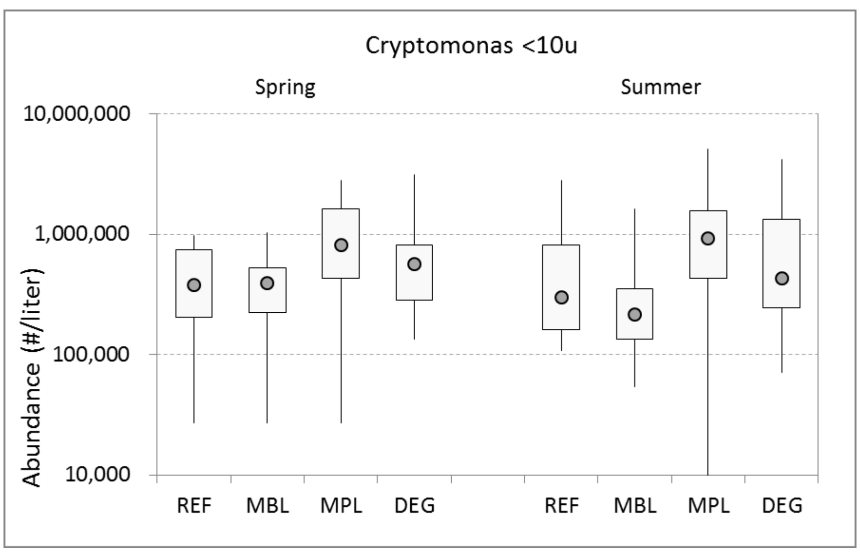


DIATOM

Higher maximum abundances in spring and summer REF

Spring
Score 1: ≥2,016 (MBL 75%ile)
Else: Null

Summer
Score 1: ≥1,216 (REF 50%ile)

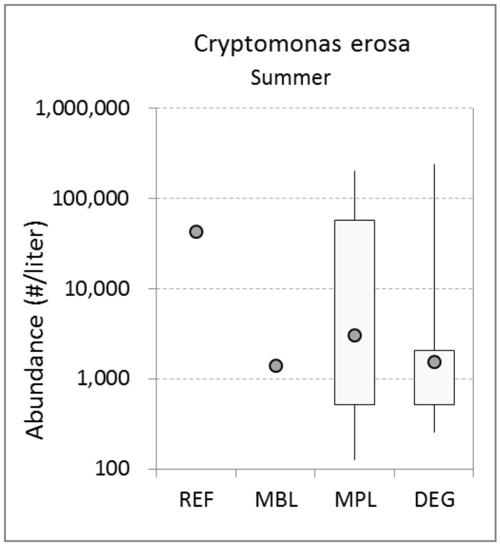


CRYPTOMONAD

Higher overall abundances in spring and summer DEG

Spring
Score 0: ≥967,138 (REF 95%ile)
Else: Null

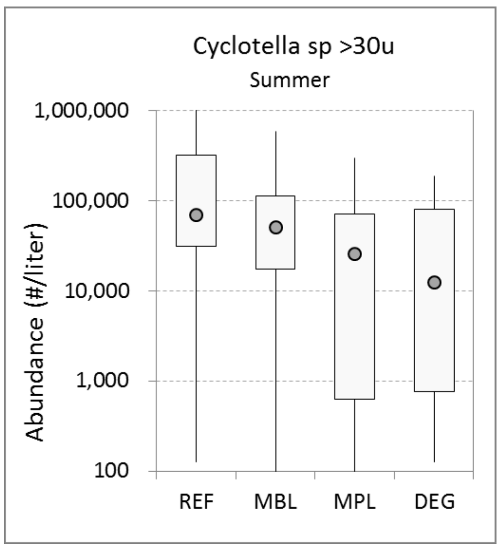
Summer
Score 0: ≥2,000,000 (~REF 90%ile)
Else: Null



CRYPTOMONAD

More frequent in summer DEG

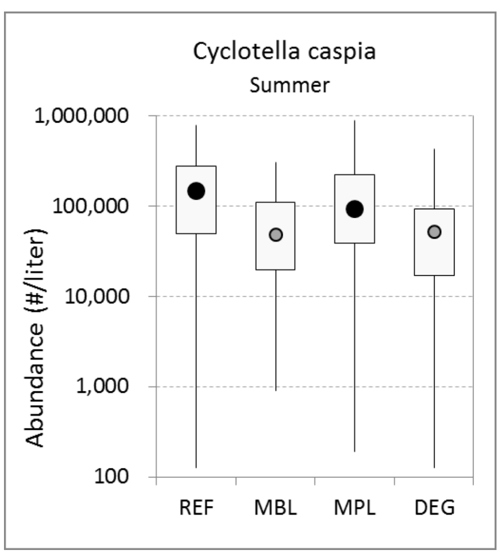
Summer
Score 0: if present
Else: Null



DIATOM

More frequent and higher maximum abundances in summer REF

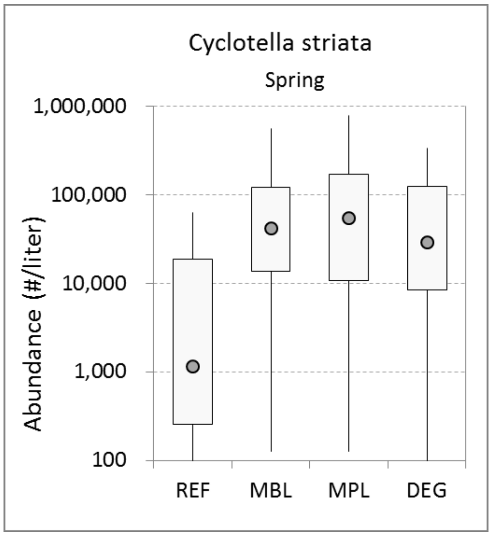
Summer
Score 1: ≥165,265 (REF 60%)
Else: Null



DIATOM

More frequent and higher maximum abundances in summer REF

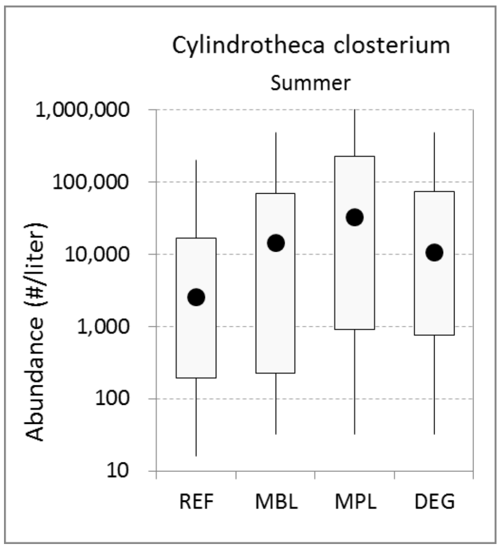
Summer
Score 1: ≥275,780 (REF 75%)
Score 0: <1,712 (REF 5%ile)
Else: Null



DIATOM

More frequent and higher overall abundances in spring DEG

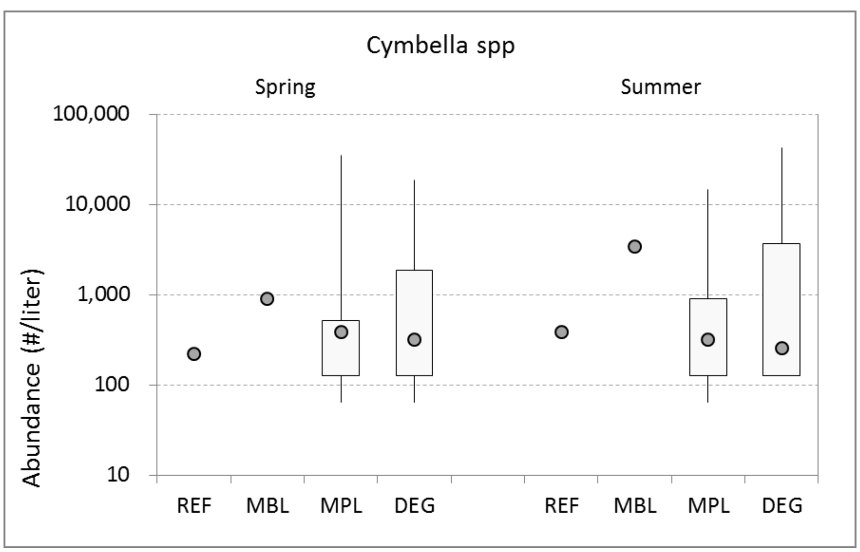
Spring
Score 0: >62,794 (REF 95%ile)
Score 1: < 1,152 (REF 50%ile)
Else: Null



DIATOM

Higher abundances in summer DEG

Summer
Score 1: <128 (REF20%ile)
Score 0: >198,912 (REF 95%ile)
Else: Null

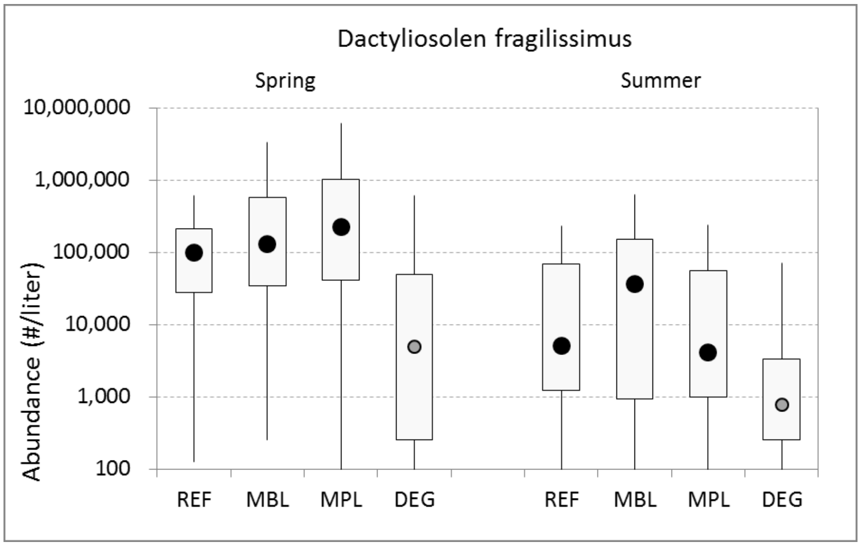


DIATOM

More frequent and higher maximum abundances in spring and summer DEG

Spring
Score 0: if present
Else: Null

Summer
Score 0: if present
Else: Null

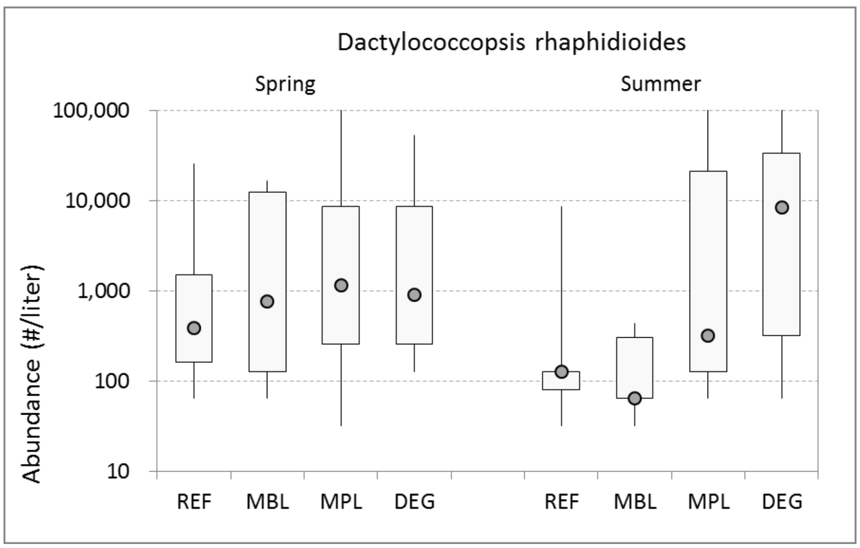


DIATOM

More frequent and higher overall abundances in spring and summer REF

Spring
Score 1: ≥100,147 (REF 50%ile)
Score 0: <1,312 (REF 15%ile)
Else: Null

Summer
Score 1: ≥69,184 (REF 75%ile)
Score 0: <442 (REF 10%ile)
Else: Null

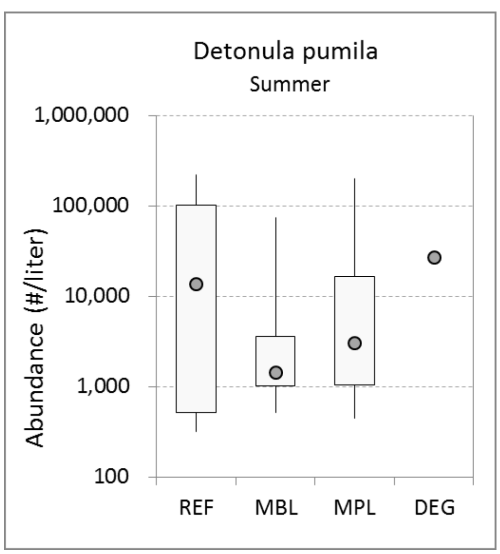


GREEN

More frequent and higher abundances in spring and summer DEG

Spring
Score 0: >4,948 (REF 85%ile)
Else: Null

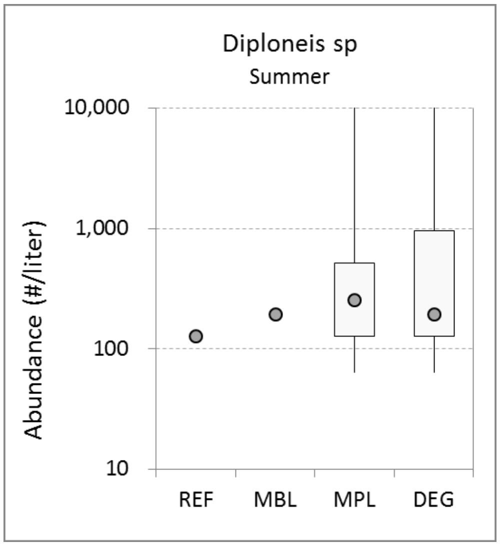
Summer
Score 0: >304 (MBL 75%ile)
Else: Null



DIATOM

More frequent and higher maximum abundances in summer REF

Summer
Score 1: >13,824 (REF 50%ile)
Else: Null

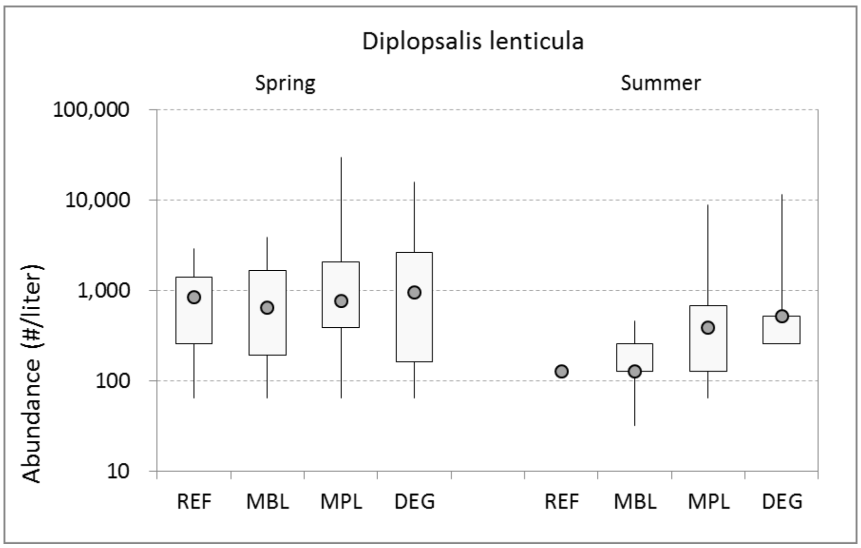


DIATOM

More frequent and higher maximum abundance in summer REF

Summer

Score 0: >128 (REF 50%ile)
Else: Null



DINOFLAGELLATE

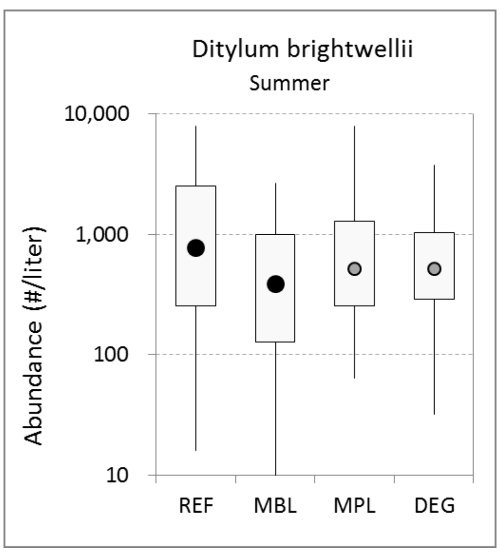
Higher maximum abundances in spring and summer DEG

Spring

Score 0: >2,925 (REF 95%ile)
Else: Null

Summer

Score 0: >512 (REF max)
Else: Null

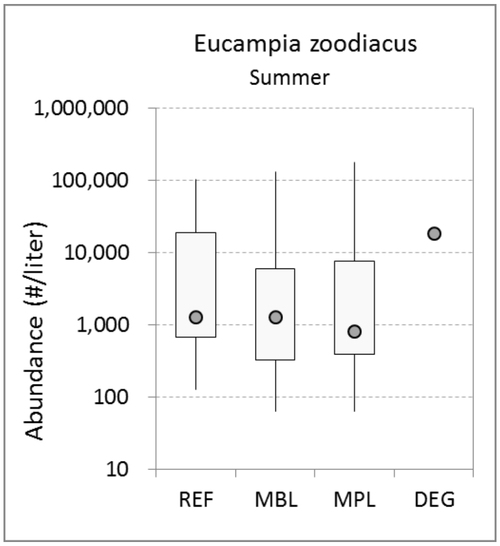


DIATOM

Slightly higher abundances and more frequent in summer REF

Summer

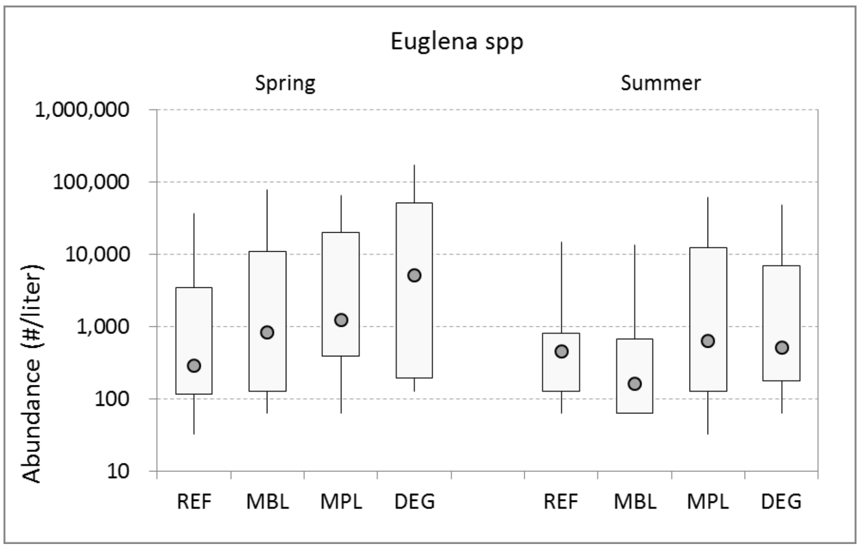
Score 1: ≥2,528 (REF 75%)
Else: Null



DIATOM

More frequent in summer REF

Summer
Score 1: ≥18,992 (REF 75%ile)
Else: Null

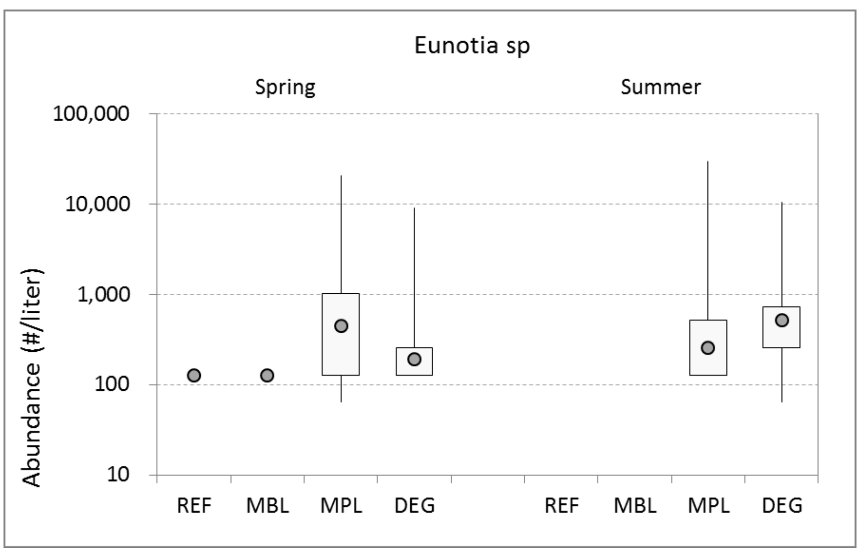


EUGLENOID

More frequent and higher maximum abundances in spring and summer DEG

Spring
Score 0: >20,092 (REF 90%ile)
Else: Null

Summer
Score 0: 9,955 (REF 90%ile)
Else: Null

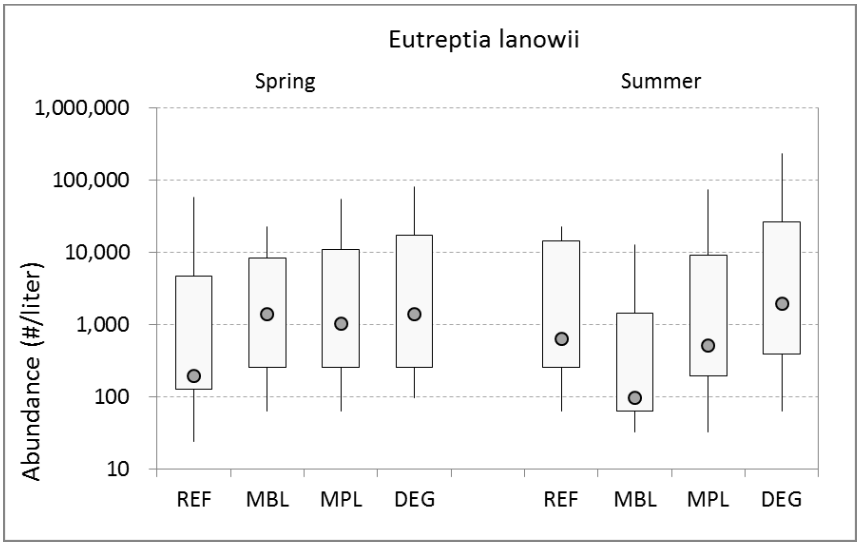


DIATOM

More frequent and higher abundances in spring and summer DEG

Spring
Score 0: >128 (REF max)
Else: Null

Summer
Score 0: if present
Else: Null

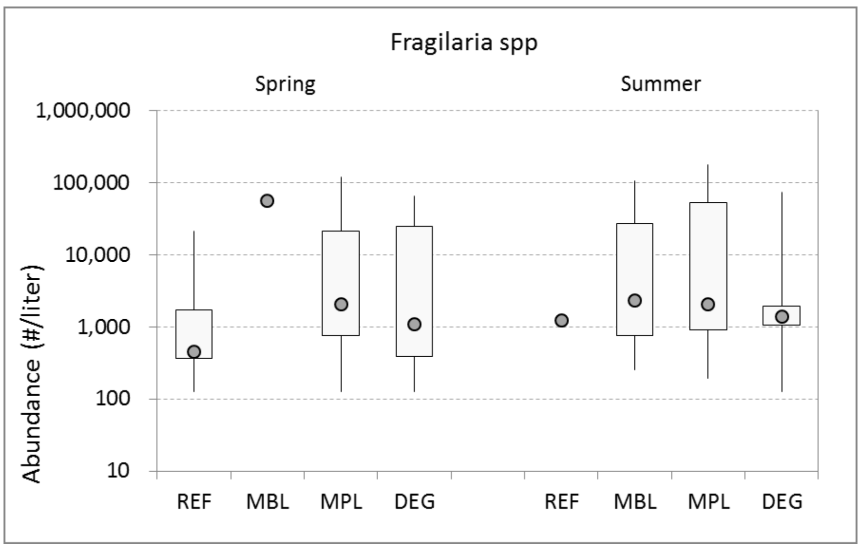


EUGLENOID

Higher maximum abundances in spring and summer DEG

Spring
Score 1: <192 (REF 50%ile)
Else: Null

Summer
Score 0: >22,702 (REF 95%ile)
Else: Null

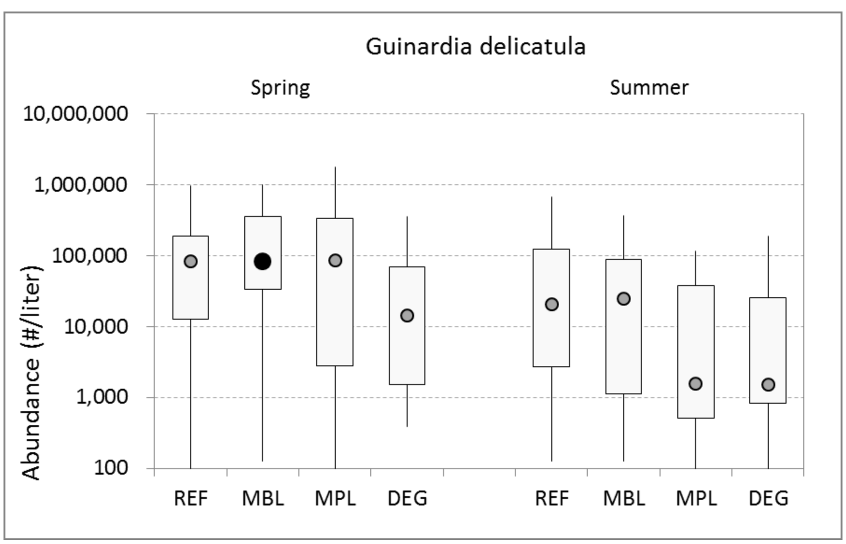


DIATOM

More frequent and higher maximum abundances in spring and summer DEG

Spring
Score 0: >30,515 (REF max)
Else: Null

Summer
Score 0: >56,832 (REF max)
Else: Null

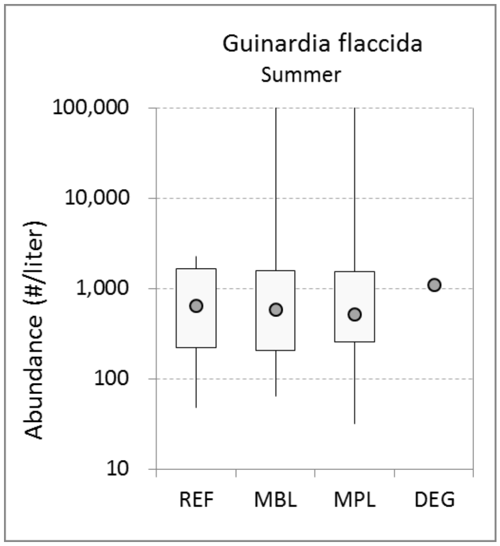


DIATOM

More frequent and higher maximum abundances in spring and summer REF

Spring
Score 1: $\geq$84,096 (REF 50%ile)
Else: Null

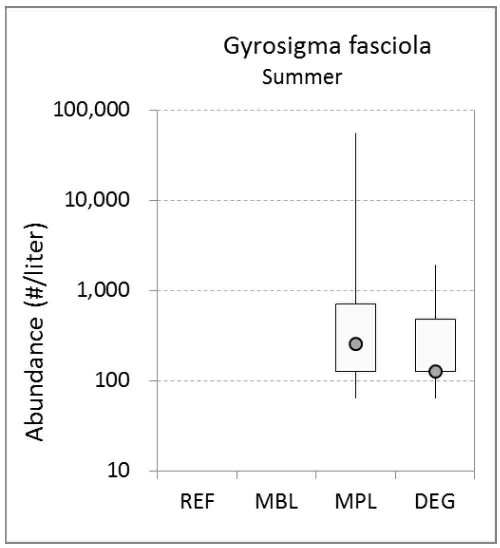
Summer
Score 1: $\geq$122,649 (REF 75%ile)
Else: Null



DIATOM

More frequent in summer REF

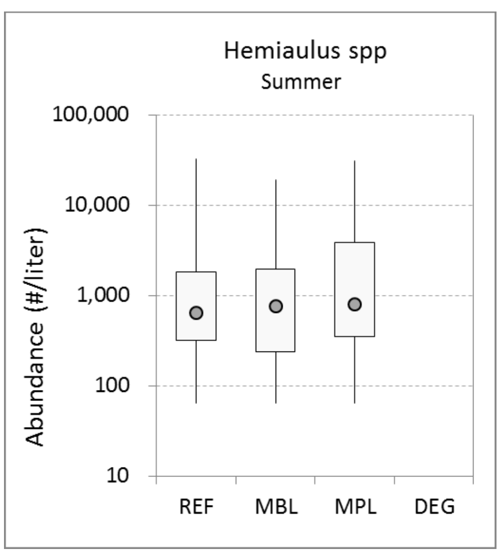
Summer
Score 0: >3,072 (REF max)
Else: Null



DIATOM

More frequent and higher abundances in summer DEG

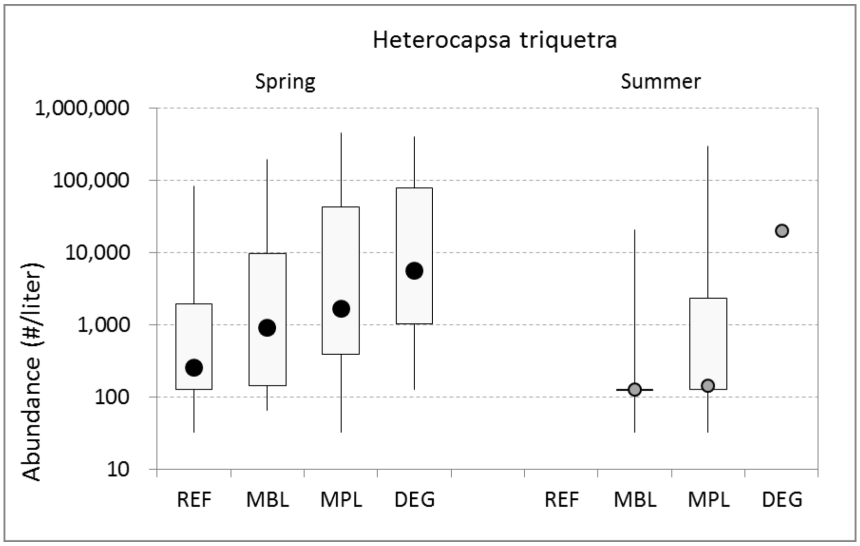
Summer
Score 0: present
Else: Null



DIATOM

More frequent in summer REF

Summer
Score 1: if present
Else: Null

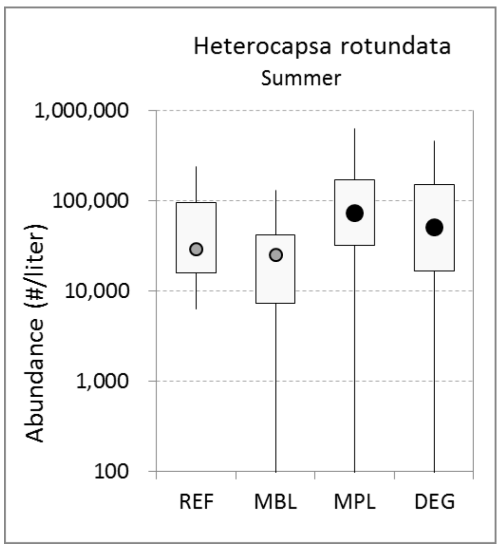


DINOFLAGELLATE
Syn: *Peridinium triquetra, Glenodinium triquetrum, Properidinium heterocapsa*

Higher overall abundances in spring and summer DEG

Spring
Score 1: ≤256 (REF 50%ile)
Score 0: >83,264 (REF 95%ile)
Else: Null

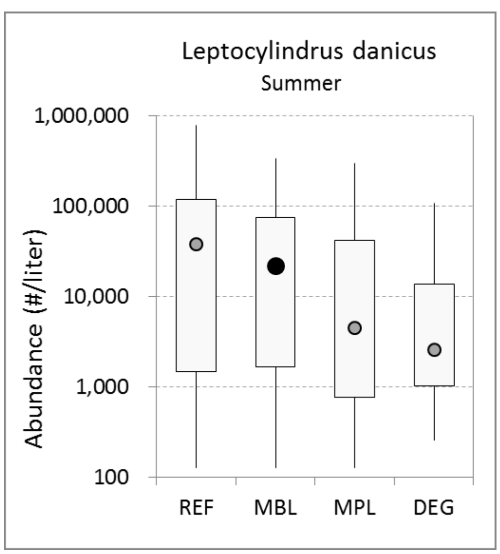
Summer
Score 0: if present
Else: Null



DINOFLAGELLATE
Syn: *Katodinium rotundum*

More frequent and higher maximum abundances in summer DEG

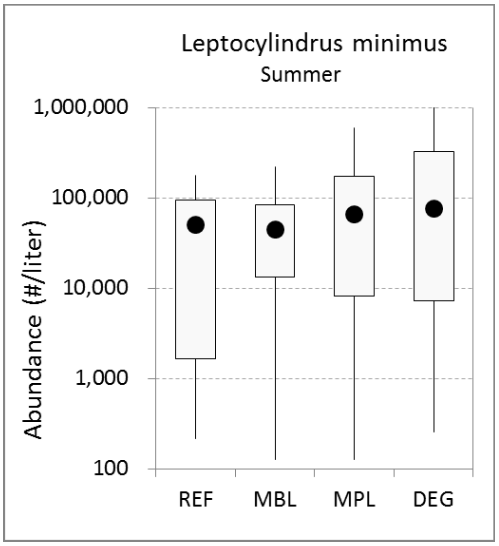
Summer
Score 0: >237,993 (REF 95%ile)
Else: Null



DIATOM

More frequent and higher maximum abundances in summer REF

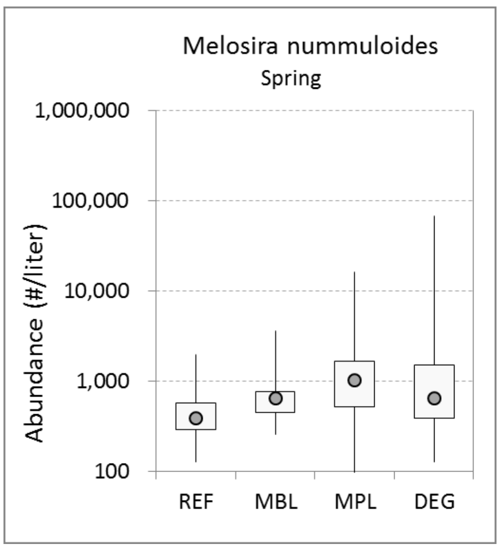
Summer
Score 1: ≥37,670 (REF 50%ile)
Else: Null



DIATOM

Higher maximum abundances in summer DEG

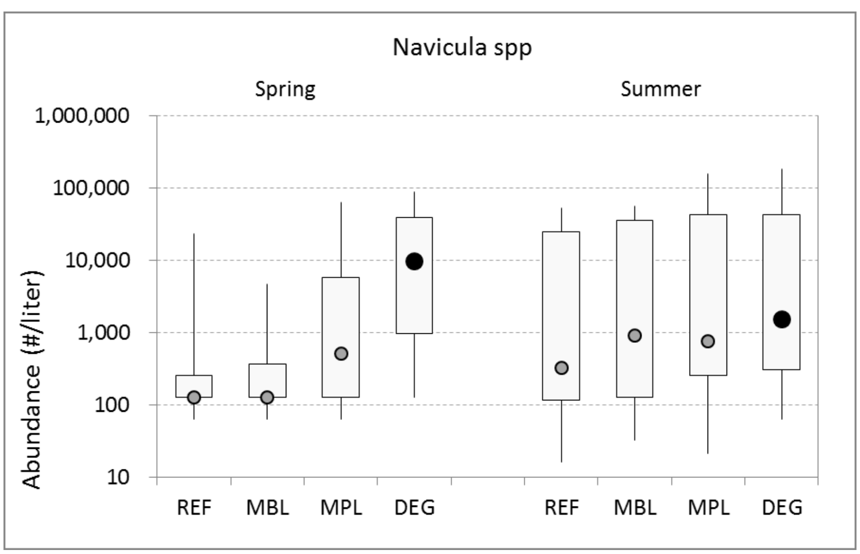
Summer
Score
Else: Null



DIATOM

Higher maximum abundances in spring DEG

Spring
Score 0: >4,966 (REF 90%ile)
Else: Null

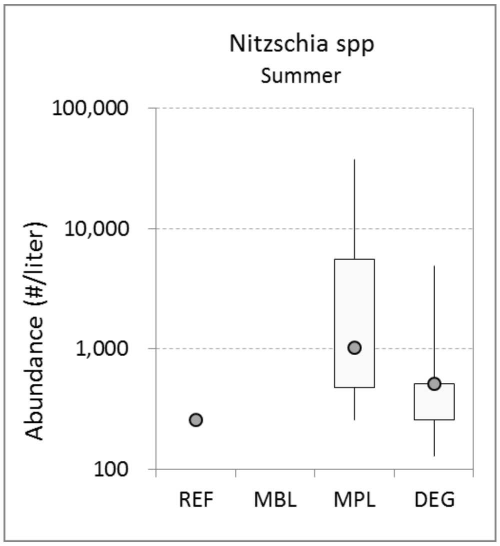


DIATOM

More frequent and higher maximum abundances in spring and summer DEG

Spring
Score 0: >4,966 (REF 85%ile)
Else: Null

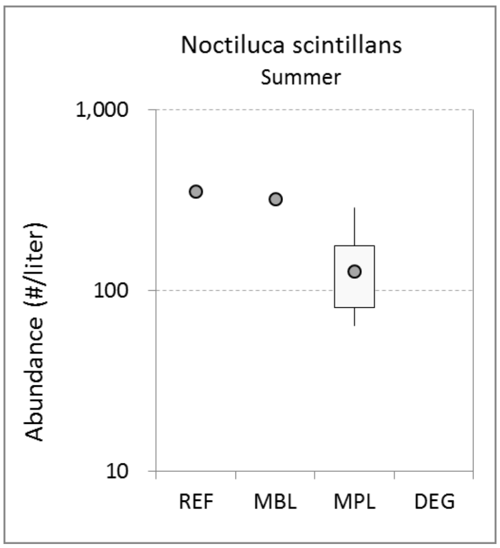
Summer
Score 0: >53,280 (REF 95%ile)
Else: Null



DIATOM

More frequent and higher overall abundance in summer DEG

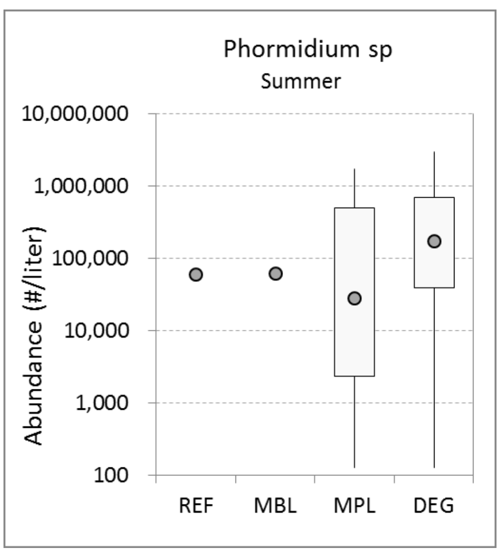
Summer
Score 0: >53,280 (REF max)
Else: Null



DINOFLAGELLATE

Rare, large-sized species not seen in summer DEG

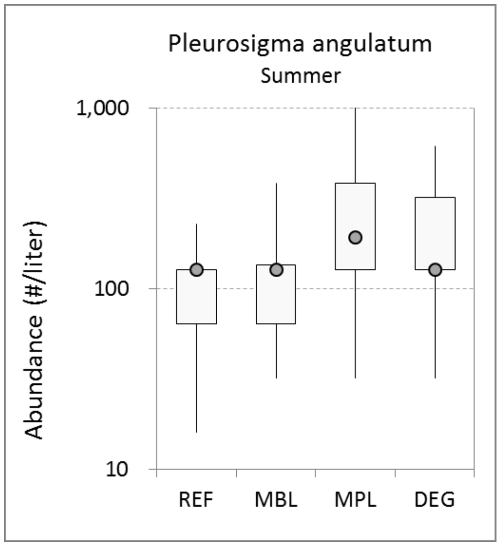
Summer
Score 1: if present
Else: Null



BLUE-GREEN

More frequent and higher maximum abundances in summer DEG

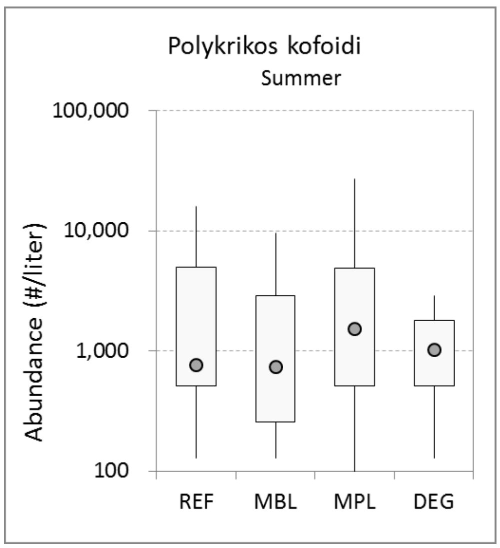
Summer
Score 0: >116,838 (REF max)
Else: Null



DIATOM

Higher maximum abundances in summer DEG

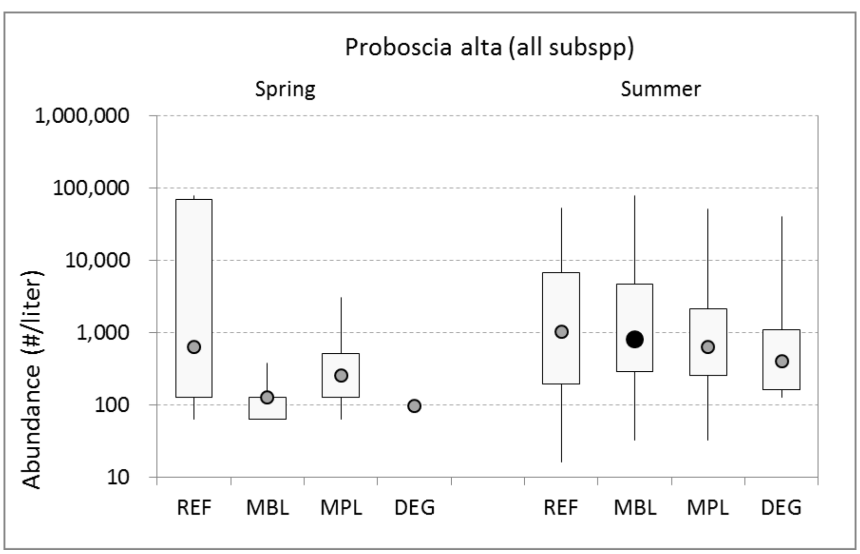
Summer
Score 0: >256 (REF max)
Else: Null



DINOFLAGELLATE

More frequent and higher maximum abundances in summer REF

Summer
Score 1: $\geq$ 4,992 (REF 75%ile)
Else: Null

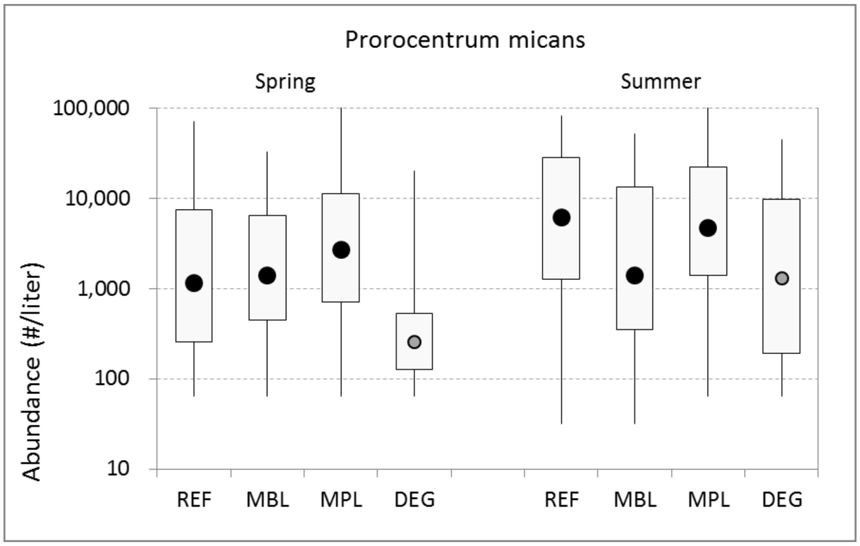


DIATOM

Most frequent and higher maximum abundances in REF

Spring
Score 1: $\geq$128 (REF 25%ile)
Else: Null

Summer
Score 1: $\geq$1,184 (REF 50%ile)
Else: Null



DINOFLAGELLATE

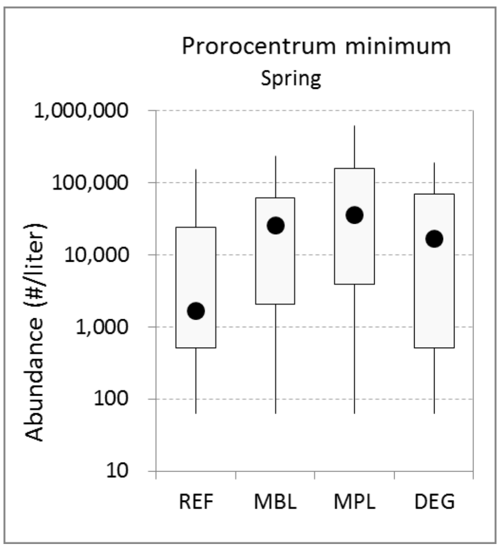
More frequent and higher maximum abundances in REF

Spring

Score 1: >=1,152 (REF 50%ile)
Score 0: <128 (REF 10%ile)
Else: Null

Summer

Score 1: 28,709 (REF 75%ile)
Else: Null

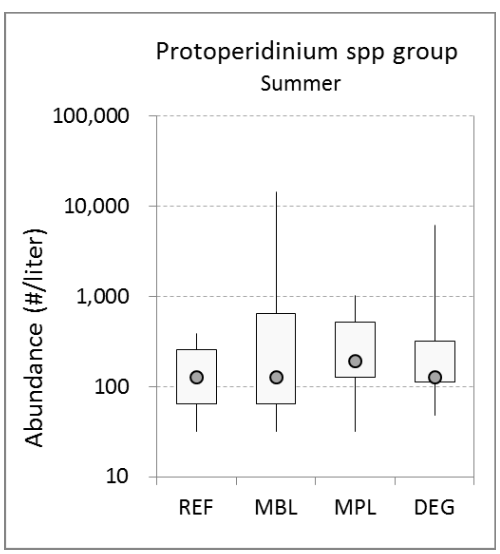


DINOFLAGELLATE
Toxin producer

Higher maximum abundances in spring DEG

Spring

Score 0: >51,017 (REF 90%ile)
Else: Null



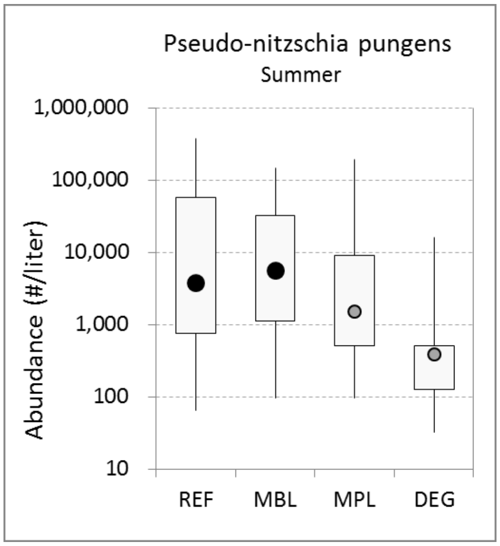
DINOFLAGELLATE

Species in group: *P. bipes, breve, brevipes, conicum, depressum, divergens, oblongum, oceanicum, ovatum,* and *pallidum*

Higher maximum abundances in summer DEG

Summer

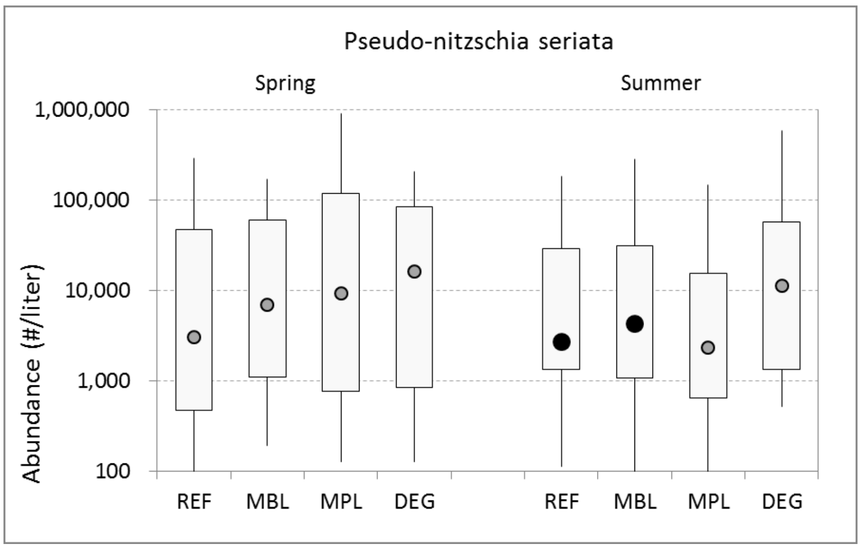
Score 0: >384 (REF max)
Else: Null



DIATOM
Toxin producer

More frequent and higher abundances in REF

Summer
Score 1: ≥3,776 (REF 50%ile)
Else: Null

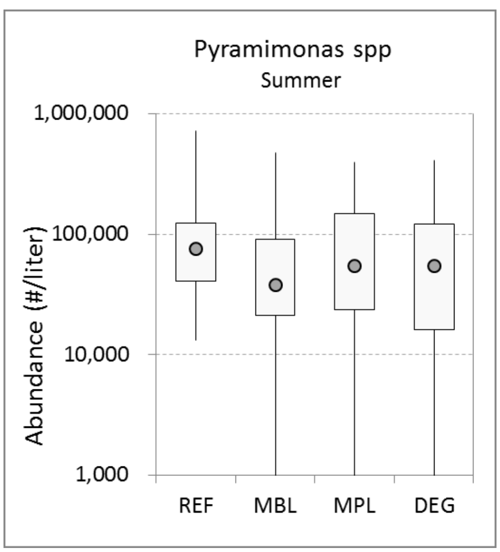


DIATOM
Toxin producer

More frequent in spring and summer REF, but higher median and maximum abundances in DEG

Spring
Score 1: ≤960 (REF 30%ile)
Else: Null

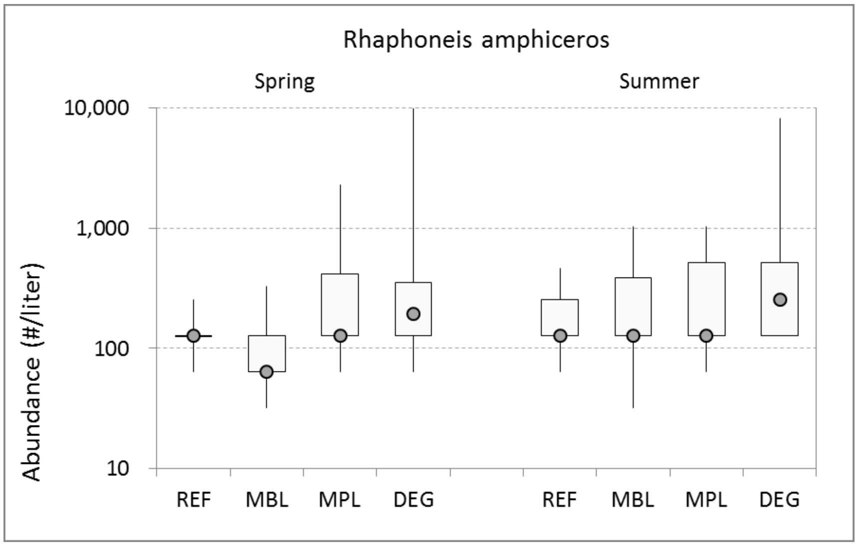
Summer
Score 1: ≤742 (REF 15%ile)
Else: Null



GREEN

Higher abundances in summer REF

Summer
Score 0: 13,248 (REF min)
Else: Null

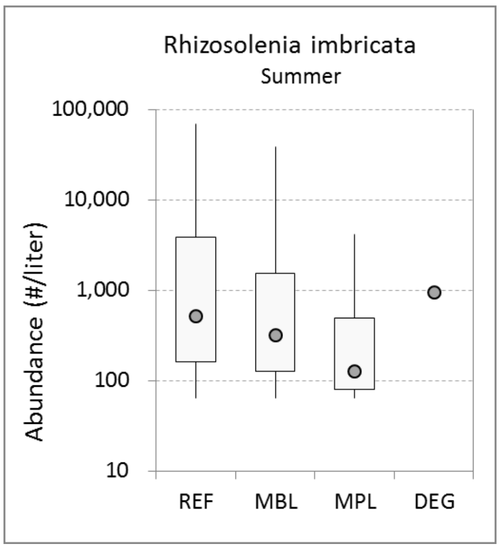


DIATOM

Higher abundances in spring and summer DEG

Spring
Score 0: >256 (REF 95%ile)
Else: Null

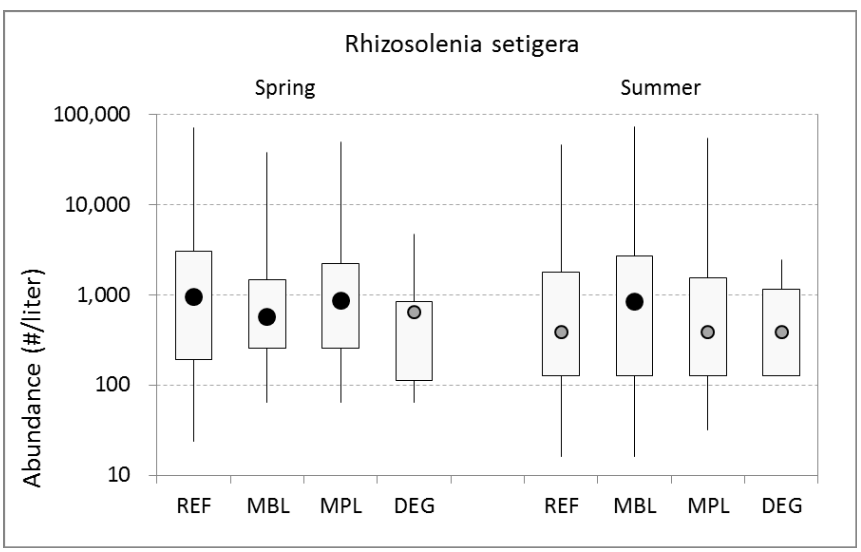
Summer
Score 0: >512 (REF max)
Else: Null



DIATOM

More frequent and higher maximum abundances in summer REF

Summer
Score 1: $\geq$1,546 (REF 65%ile)
Else: Null

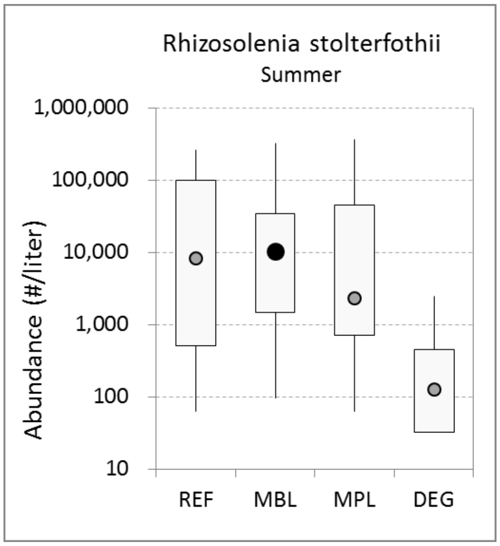


DIATOM

More frequent and higher maximum abundances in spring and summer REF

Spring
Score 1: $\geq$1,434 (REF 60%ile)
Else: Null

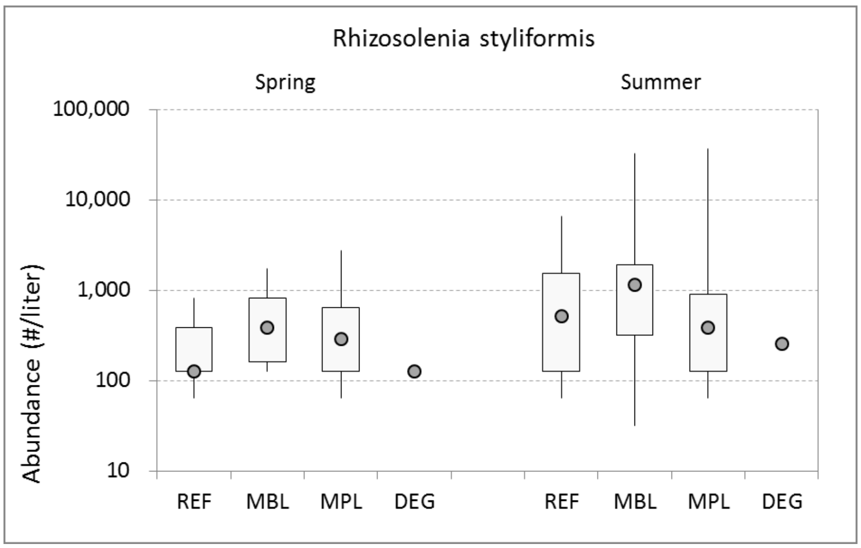
Summer
Score 1: $\geq$2,458 (REF 80%ile)
Else: Null



DIATOM

More frequent and higher maximum abundances in summer REF

Summer
Score 1: ≥3,520 (REF 45%ile)
Else: Null

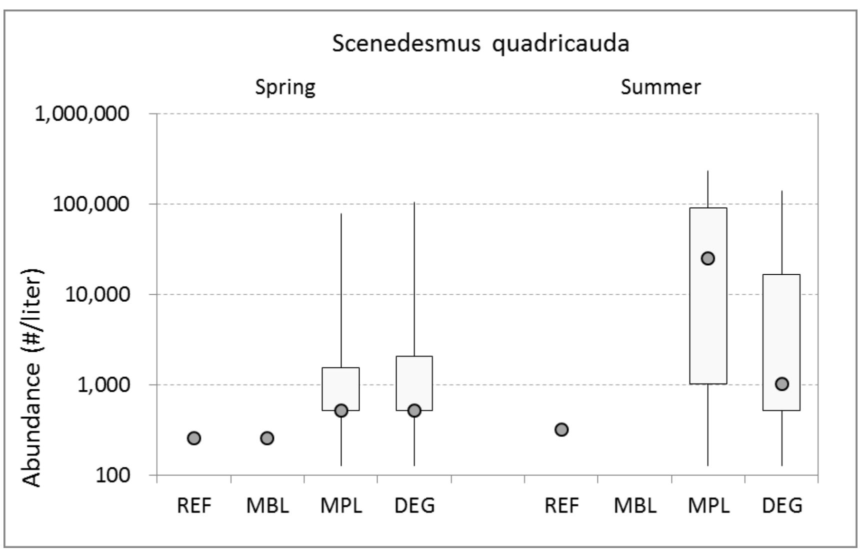


DIATOM

More frequent and higher maximum abundances in spring and summer REF

Spring
Score 1: ≥384 (REF 75%ile)
Else: Null

Summer
Score 1: >512 (REF 50%ile)
Else: Null

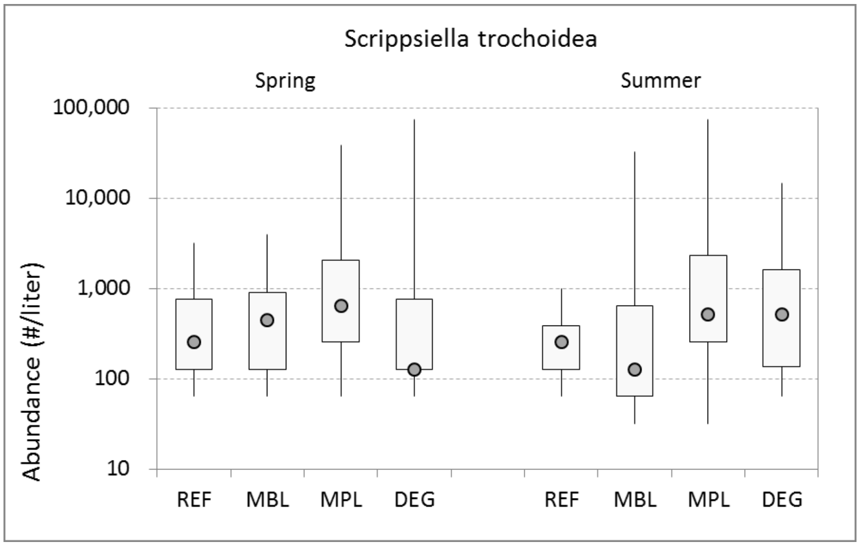


GREEN

More frequent and higher overall abundances in DEG

Spring
Score 0: >128 (REF max)
Else: Null

Summer
Score 0: >512 (REF max)
Else: Null

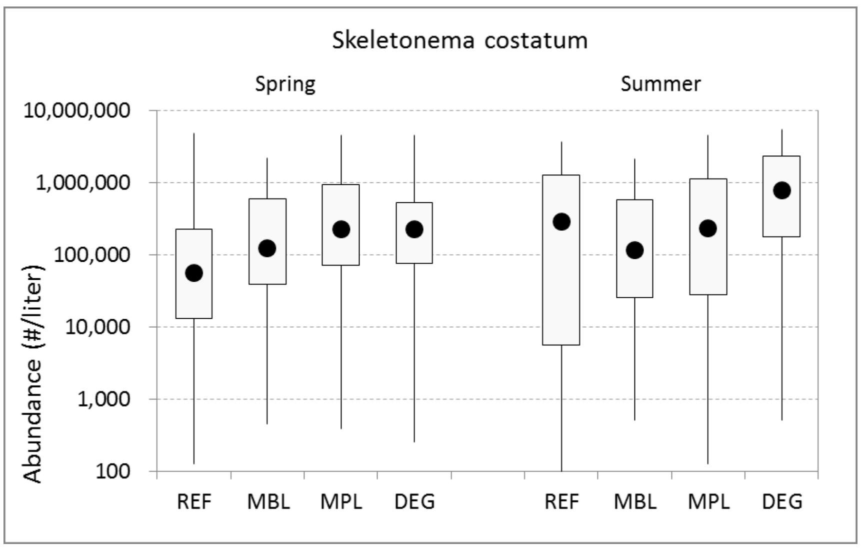


DINOFLAGELLATE
Toxin producer
Syn: *Glenodinium trochoideum*

Higher maximum abundances in spring and summer DEG

Spring
Score 0: >3,174 (REF 95%ile)
Else: Null

Summer
Score 0: >998 (REF 99%ile)
Else: Null

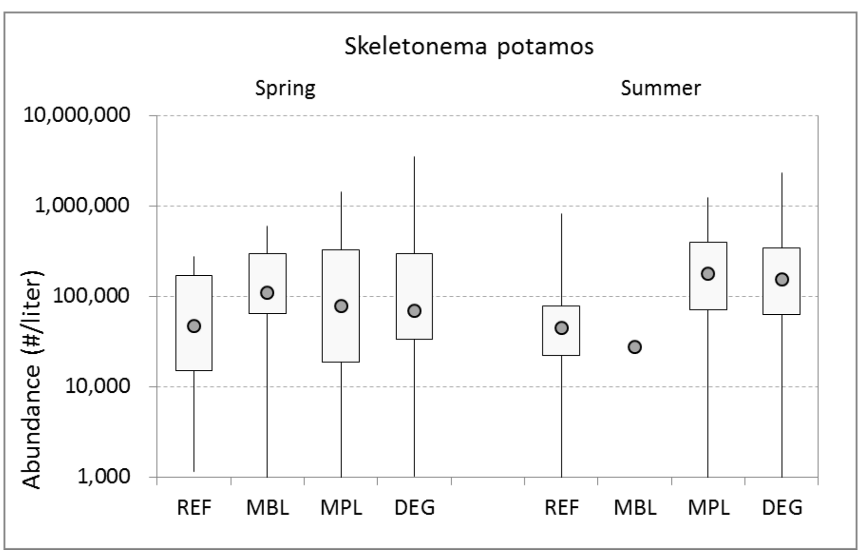


DIATOM

Higher overall abundances in spring and summer DEG

Spring
Score 1: <u><</u>13,061 (REF 25%ile)
Score 0: >2,118,300 (REF 95%ile)
Else: Null

Summer
Score 1: <u><</u>5,632 (REF 25%ile)
Score 0: >5,038,843 (REF 98%ile)
Else: Null

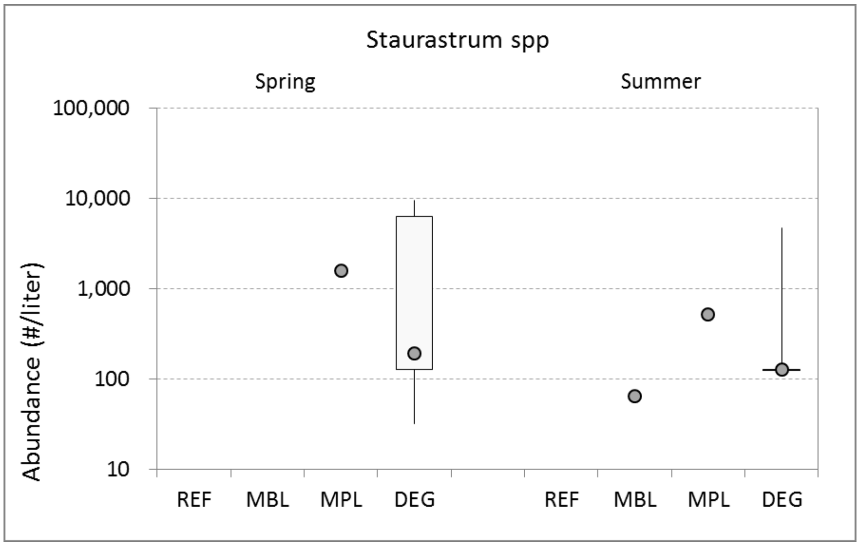


DIATOM

More frequent and higher maximum abundances in spring and summer DEG

Spring
Score 0: >284,160 (REF max)
Else: Null

Summer
Score 1: <u><</u>21,936 (REF 25%ile)
Score 0: >818,210 (REF 95%ile)
Else: Null

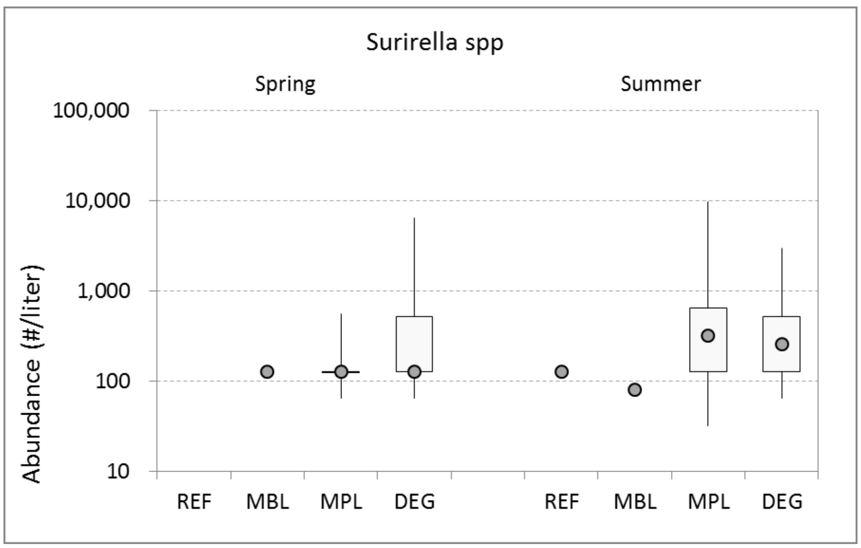


DESMID

More frequent in spring and summer DEG

Spring
Score 0: if present
Else: Null

Summer
Score 0: if present
Else: Null

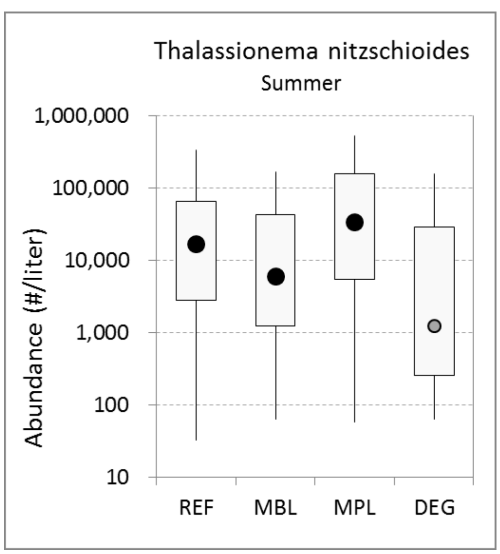


DIATOM

More frequent and higher maximum abundances in spring and summer DEG

Spring
Score 0: if present
Else: Null

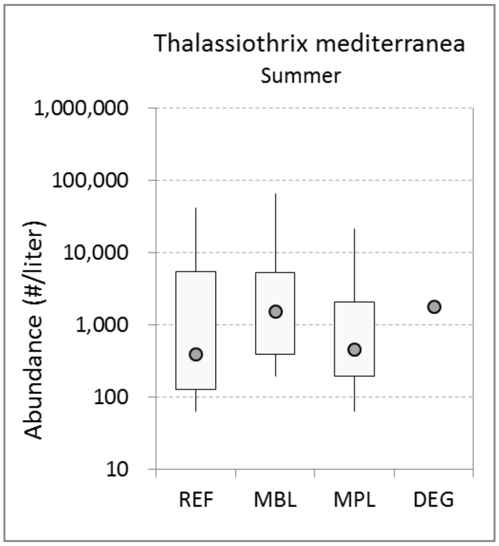
Summer
Score 0: if present
Else: Null



DIATOM

More frequent and higher abundances in summer REF

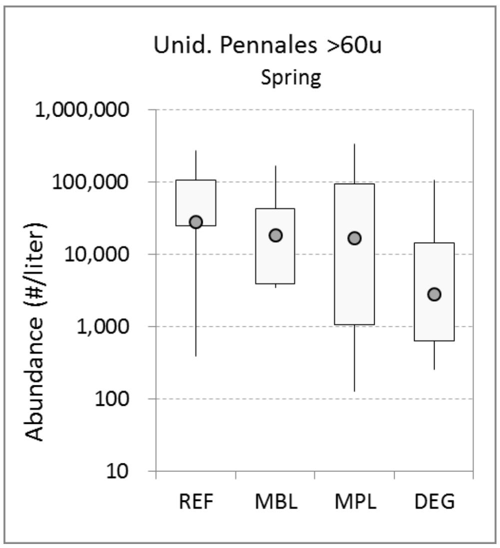
Summer
Score 1: >=252,822 (REF 92%ile)
Socre 0: <140 (REF 1.5%)
Else: Null



DIATOM

More frequent and higher maximum abundances in summer REF

Summer
Score 1: if present
Else: Null



DIATOM

Higher overall abundances in spring REF

Spring
Score 1: $\geq$28,416 (REF 50%ile)
Else:

Table B-2. Spring and summer distributions of the Phytoplankton Taxonomic Index in the four water quality categories. * Index values are calculated only for samples with two or more scored taxa.

SPRING

	REF	MBL	MPL	DEG
5%ile	50.0%	30.8%	14.3%	0.0%
25%ile	66.7%	60.0%	33.3%	0.0%
50%ile	100.0%	75.0%	50.0%	0.0%
75%ile	100.0%	85.7%	66.7%	25.0%
95%ile	100.0%	100.0%	100.0%	60.0%
% samples w/ index*	94.2%	94.9%	97.5%	86.4%

SUMMER

	REF	MBL	MPL	DEG
5%ile	45.0%	50.0%	0.0%	0.0%
25%ile	66.7%	66.7%	25.0%	0.0%
50%ile	100.0%	83.3%	50.0%	0.0%
75%ile	100.0%	100.0%	76.3%	31.3%
95%ile	100.0%	100.0%	100.0%	66.7%
% samples w/ index*	93.8%	92.1%	91.4%	78.3%

Appendix C

Statistical distributions of phytoplankton metric values in different water quality categories, across a range of chlorophyll *a* concentrations

Figure C-1. Phytoplankton Index of Biotic Integrity

Figure C-2. Phytoplankton Taxonomic Index

Figure C-3. Phytoplankton Taxa Richness

Figure C-4. Number of Taxa Blooms per Sample

Figure C-5. Phytoplankton Shannon-Wiener Diversity Index

Figure C-6. Phytoplankton Shannon's Equitability Index (Pielou's Evenness Index)

Phytoplankton Index of Biotic Integrity (PIBI)

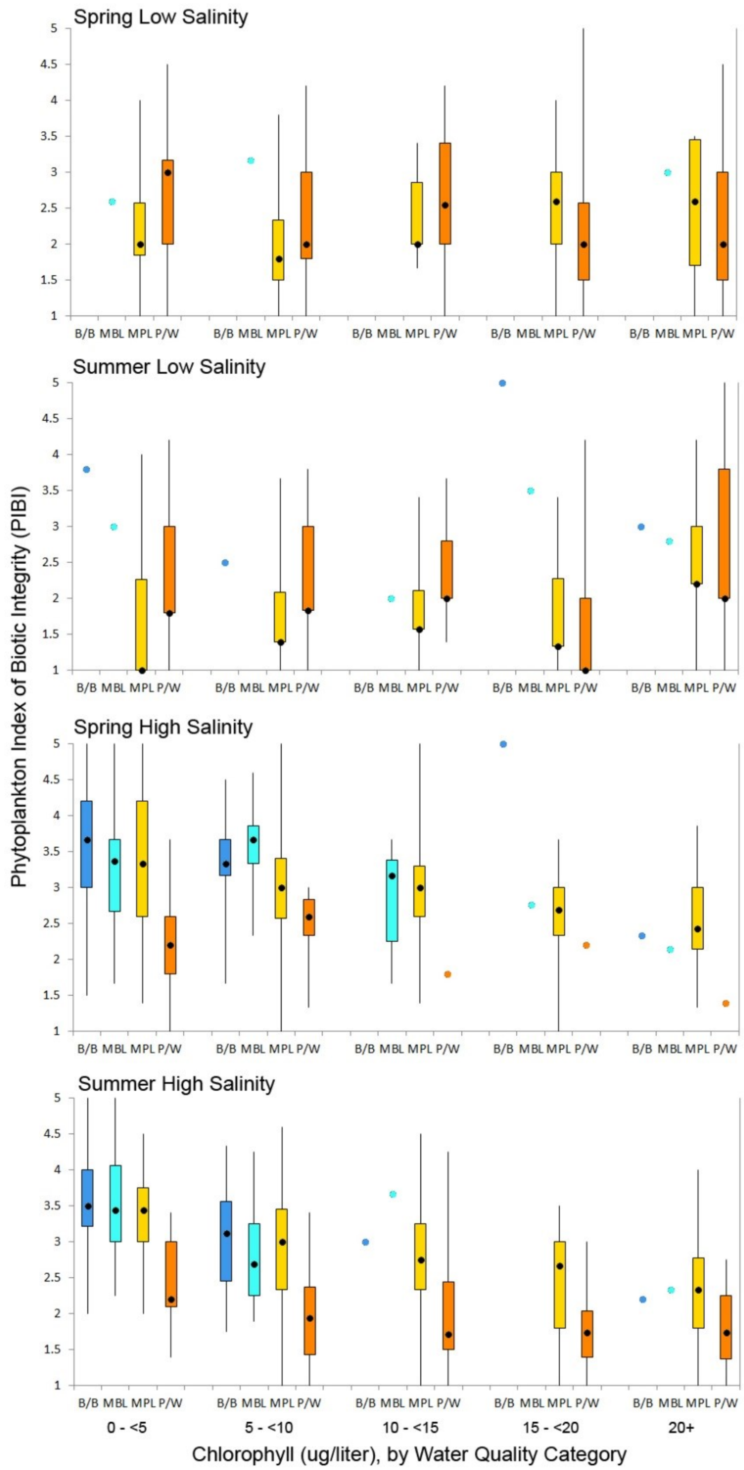


Figure C-1. Distributions of the Phytoplankton Index of Biotic Integrity (PIBI) values from Virginia waters, in the four water quality categories (REF, MBL, MPL, DEG) and five chlorophyll *a* concentration ranges. Low salinity, 0 - ≤10 ppt; high salinity, >10 ppt. Box-and-whiskers show 5th, 25th, 50th, 75th, and 95th percentiles. Only median is shown for water quality categories n≤5.

Phytoplankton Taxonomic Index (PTI)

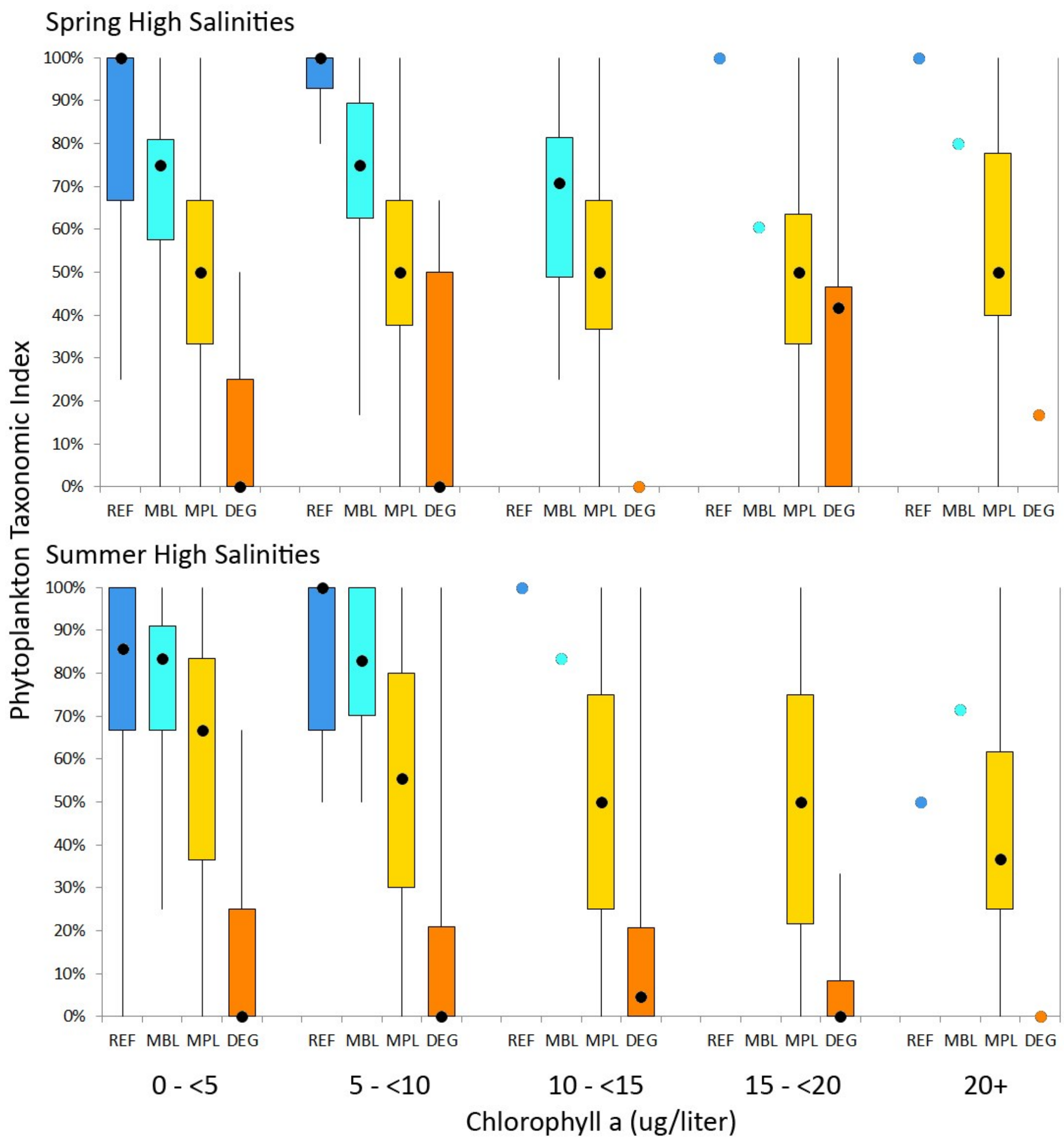


Figure C-2. Distributions of the Phytoplankton Taxonomic Index values from Virginia high salinity waters, in the four water quality categories (REF, MBL, MPL, DEG) and five chlorophyll *a* concentration ranges. High salinity, >10 ppt. Box-and-whiskers show 5th, 25th, 50th, 75th, and 95th percentiles. Only median is shown for water quality categories n$\leq$5.

Phytoplankton Taxa Richness

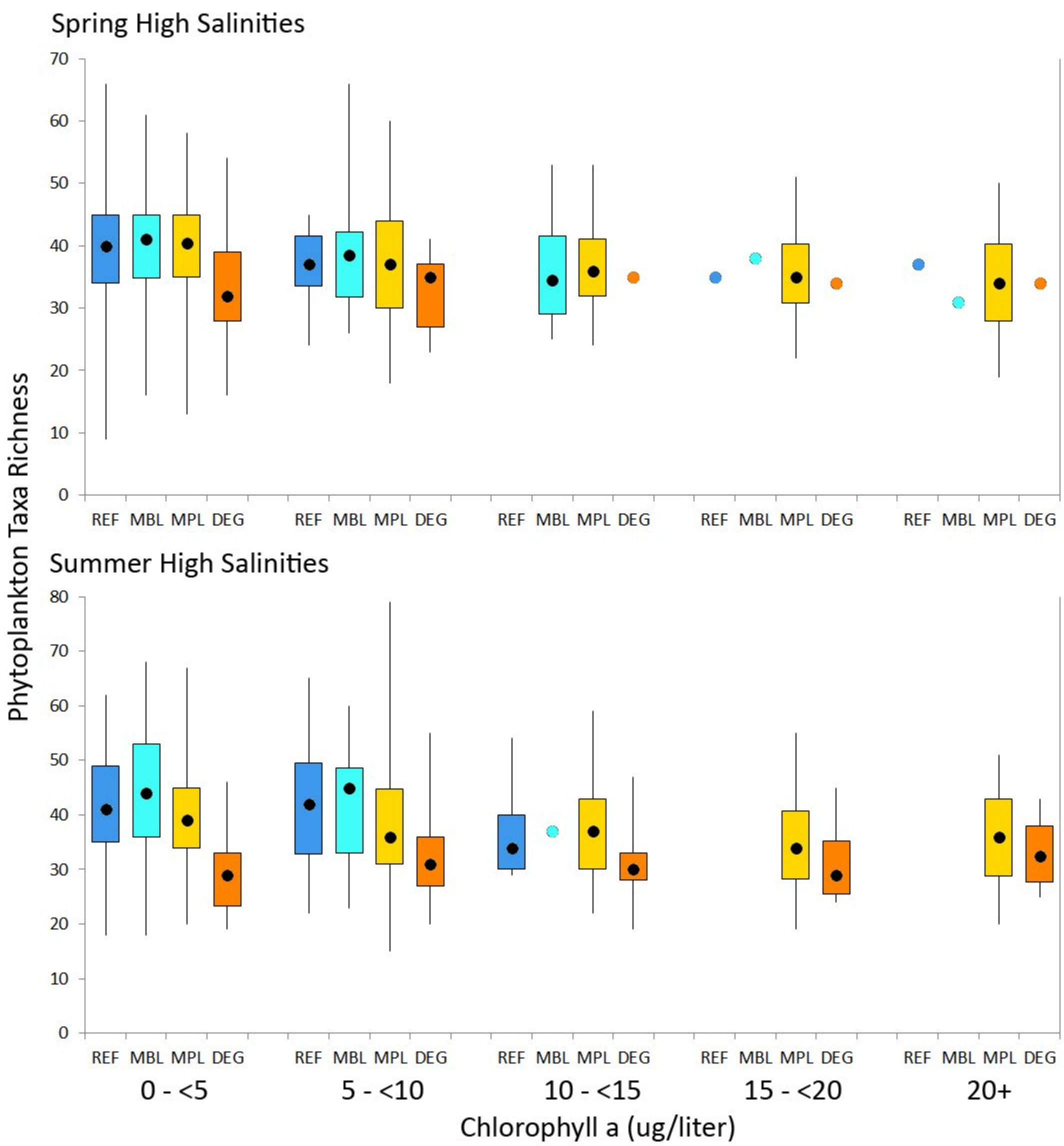


Figure C-3. Distributions of phytoplankton taxa richness values from Virginia high salinity waters, in the four water quality categories (REF, MBL, MPL, DEG) and five chlorophyll *a* concentration ranges. High salinity, >10 ppt. Box-and-whiskers show 5th, 25th, 50th, 75th, and 95th percentiles. Only median is shown for water quality categories n≤5.

Number of Taxa Blooms

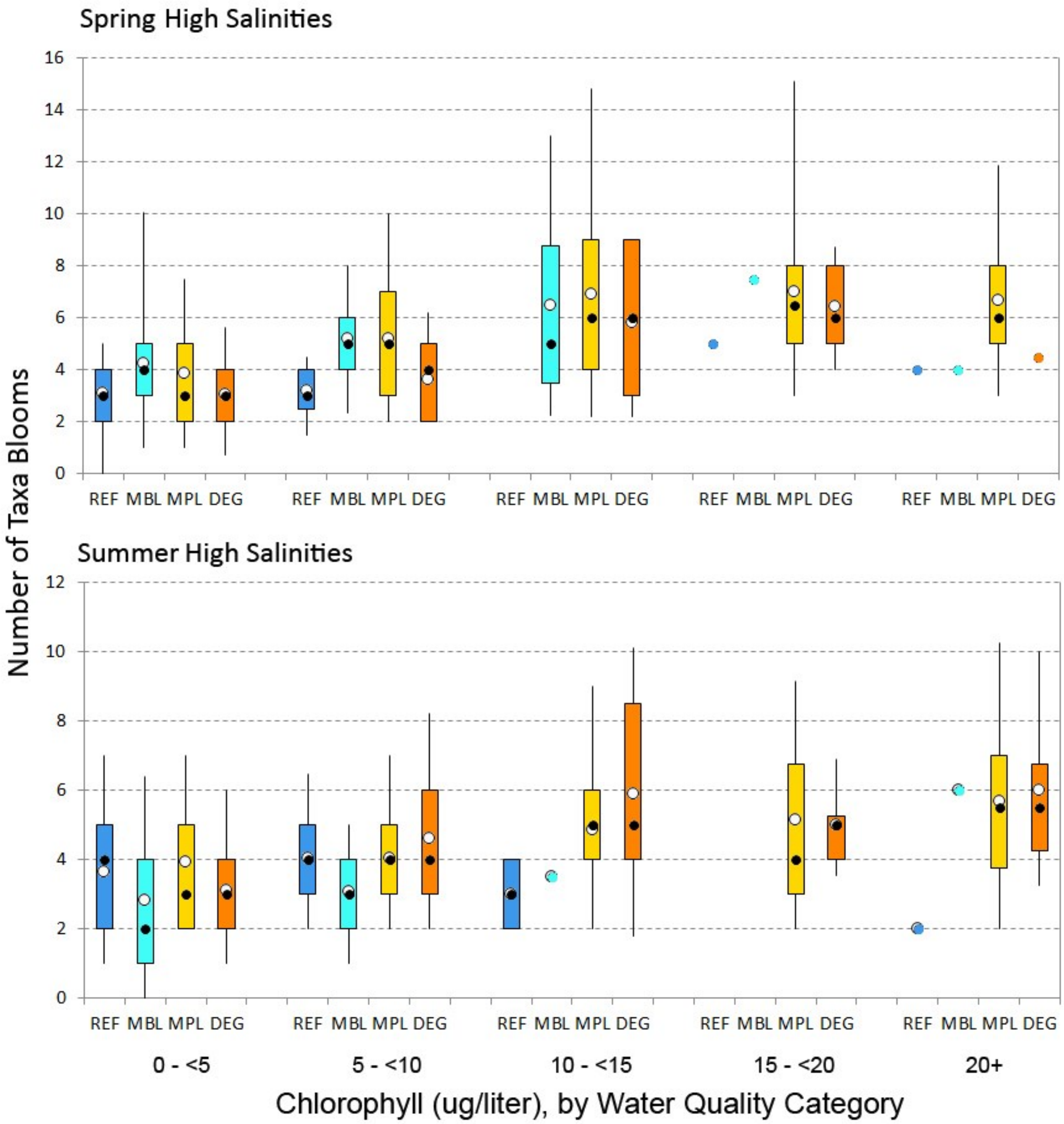


Figure C-4. Distributions of the number of taxa blooms per sample from Virginia high salinity waters, in the four water quality categories (REF, MBL, MPL, DEG) and five chlorophyll *a* concentration ranges. High salinity, >10 ppt. Box-and-whiskers show 5th, 25th, 50th, 75th, and 95th percentiles. Open circle is the mean. Only median and mean are shown for water quality categories with n≤5. Blooms are defined here as abundances of an individual taxon or taxonomic group that are greater than 250,000 cells per liter.

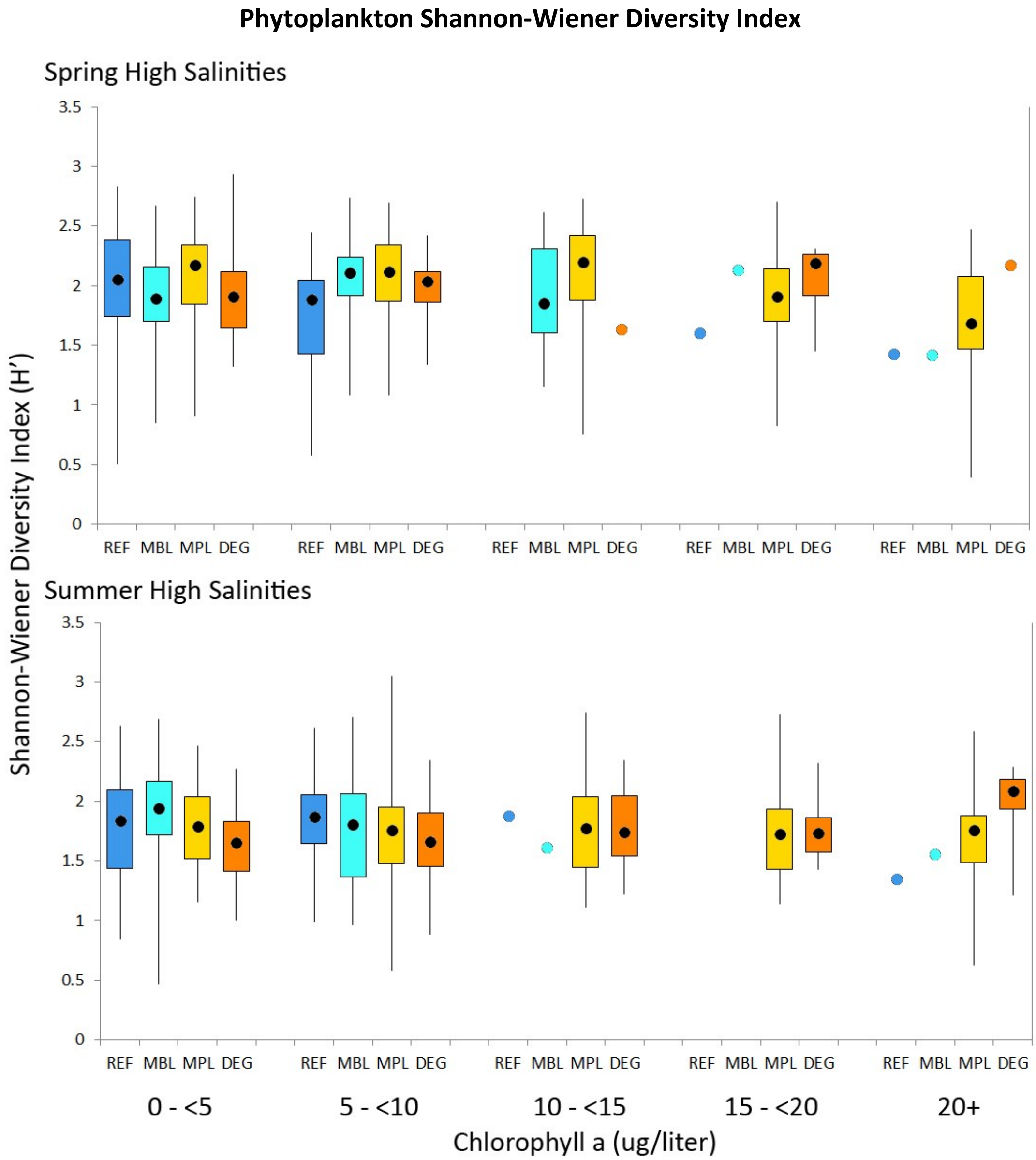


Figure C-5. Distributions of phytoplankton Shannon-Wiener Diversity Index (H') values from Virginia high salinity waters, in the four water quality categories (REF, MBL, MPL, DEG) and five chlorophyll *a* concentration ranges. High salinity, >10 ppt. Box-and-whiskers show 5th, 25th, 50th, 75th, and 95th percentiles. Only median is shown for water quality categories n$\leq$5. $H' = -\sum(p_i * \ln p_i)$, where p_i is the proportion of the ith taxa in the population.

Pielou's Evenness Index

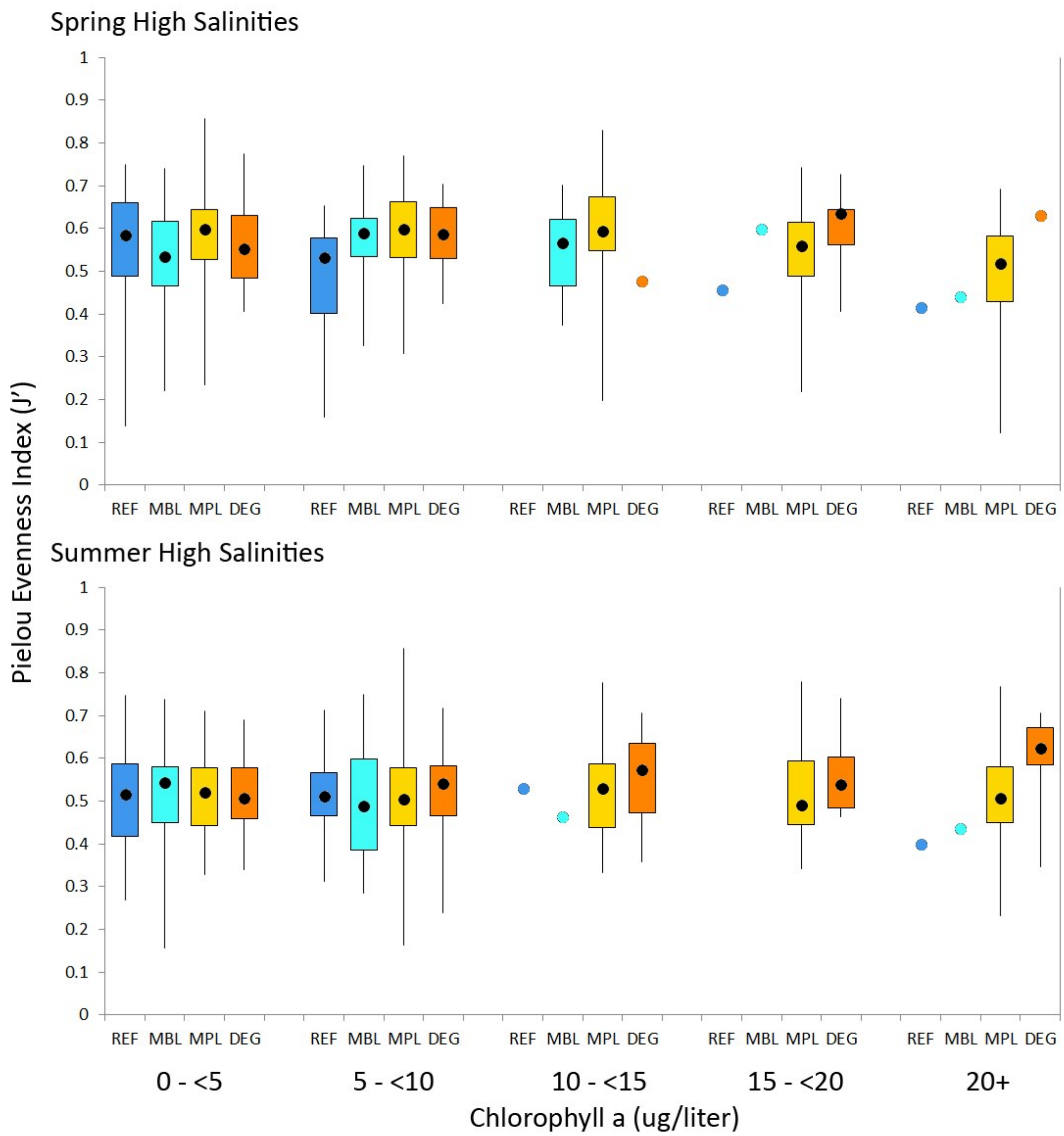


Figure C-6. Distributions of the phytoplankton Pielou Evenness Index (or Shannon Equitability Index) values from Virginia high salinity waters, in the four water quality categories (REF, MBL, MPL, DEG) and five chlorophyll *a* concentration ranges. High salinity, >10 ppt. Box-and-whiskers show 5th, 25th, 50th, 75th, and 95th percentiles. Only median is shown for water quality categories n≤5. J' is H'/H'max, where H'max is the natural log (ln) of taxa richness.