appendix F

Phytoplankton Reference Community Data Analyses

This appendix describes various analyses performed with the 1984-2001 Chesapeake Bay Program water quality and plankton monitoring data that supported determination of the phytoplankton reference community chlorophyll *a* concentrations reported in Chapter V.

REFERENCE PHYTOPLANKTON COMMUNITIES AND WATER QUALITY CONDITION CLASSIFICATIONS

Biological populations found in pristine or minimally impaired habitats provide essential information about how restoration efforts might improve ecosystem structure and function. Called 'reference communities,' these populations serve as benchmarks for measuring ecosystem impairment. Ecosystem impairment is assessed with a suite of physical, chemical and biological performance indicators which are measurable attributes of the ecosystem linked directly to restoration objectives. The properties of the performance indicators in biological reference communities furnish the evaluation (scoring) criteria needed to quantify ecosystem impairment at other sites (National Research Council 1992). Chlorophyll *a* has long been used as a surrogate measure of phytoplankton biomass and as a performance indicator of nutrient enrichment across a wide spectrum of aquatic systems (see Chapter V). Chlorophyll *a* as an indicator is directly linked to a restoration objective of the Chesapeake Bay Program, namely the reduction of excess, uneaten phytoplankton that accumulates in the water column and contributes to reduced water clarity and summer oxygen depletion in bottom waters, ultimately stressing the food webs the phytoplankton support.

Chlorophyll *a* concentrations for season- and salinity-specific phytoplankton reference communities for Chesapeake Bay tidal waters are described in this appendix and elsewhere (Buchanan et al., in review). The reference communities are based on phytoplankton populations currently found in waters least impaired by poor water clarity and nutrients in excess of phytoplankton growth requirements. Water quality

condition classifications were determined with three parameters crucial to phytoplankton growth: light penetration (measured as Secchi depth), dissolved inorganic nitrogen (DIN) and ortho-phosphate (PO_4).

ANALYSIS APPROACH

Chesapeake Bay water quality and phytoplankton data collected at Chesapeake Bay Program biomonitoring stations between 1984 and 2001 were first analyzed to identify samples that were least impaired by poor water clarity and excess nutrients. Seasonal and salinity-specific phytoplankton 'reference' communities for the Chesapeake Bay were then derived from the populations in those samples. The reference communities are used in this analysis to quantify chlorophyll *a* concentrations in the least-impaired water quality conditions currently found in the Chesapeake Bay and its tidal tributaries.

The Chesapeake Bay Monitoring Program has coordinated the year-round collection of plankton and water quality data at more than 26 stations for all salinity zones in the Chesapeake Bay mainstem and its major tidal tributaries since August 1984. Data for some parameters were collected over shorter periods or only by one state. The primary data and data documentation are available at http://www.chesapeakebay.net/data. Phytoplankton parameters that are measured (primary data) or derived from measured data include chlorophyll *a*, pheophytin, species abundances, biomasses of individual species in the nano (2–20 micron) and micro (20–200 micron) size fractions, phytoplankton biomass in pico (<2 micron) size fractions, average cell size of the nano-micro phytoplankton and the ratio of phytoplankton biomass (as carbon) to chlorophyll *a*. Productivity cannot be used for baywide analyses because Maryland and Virginia methodologies are different. In this study, water quality and phytoplankton data from the mixed upper layer of the water column (usually identified as 'above-pycnocline,' or AP) were analyzed, with the exception of a few tidal-fresh stations where samples were from the whole water column (WC). Data from each sampling event at an individual station were sorted into two seasons and four salinity zones for examination: spring (March, April and May) and summer (July, August and September); and tidal-fresh (0.0 to 0.5 ppt), oligohaline (>0.5 to 5.0 ppt), mesohaline (>5.0 to18.0 ppt) and polyhaline (>18.0 ppt). This minimizes the influence of season and salinity regime on the analysis.

Phytoplankton and water quality data within each season-salinity group were binned (further grouped) into six categories using Secchi depth, DIN and PO_4 thresholds shown in tables F-1 and F-2. The thresholds classify the Secchi depth, DIN, and PO_4 values of each data record as 'worst,' 'poor,' 'better,' or 'best'. The DIN and PO_4 thresholds separating 'better' and 'poor' values in tables F-1 and F-2 have been experimentally shown to be resource limitation thresholds for natural Chesapeake Bay phytoplankton populations (Fisher et al. 1988, 1999; Thomas Fisher personal communication). The Secchi depth thresholds separating 'better' and 'poor' values were empirically determined from the monitoring data using the Relative Status, or benchmark, method (Olson 2002). The 'better' water clarity levels are those

Table F-1. Spring (March through May) classification criteria for determining 'worst', 'poor', 'better' and 'best' water quality parameter conditions. Key: Secchi-Secchi depth (meters); DIN-average dissolved organic nitrogen in surface mixed layer (mg liter^{1}); PO_4—average orthophosphate (SRP) in surface mixed layer (mg liter^{1}); TF—tidal fresh salinities (0 to 0.5 ppt); OH—oligohaline salinities (>0.5 to 5 ppt); MH—mesohaline salinties (>5 to 18 ppt); PH—polyhaline (>18 ppt). The 25th percentile, median and 75th percentile of the parameter's values at stations identified as 'good' by the Relative Status Method are given for comparison purposes. See Buchanan et al. (in review) for details.

Parameter		Selected Spring Classification Criteria				Relative Status Method
		Worst	Poor	Better	Best	*25th%/median/75th%*
Secchi	TF	<0.7	=<0.9	>0.9	>1.1	*0.7 | 0.9 | 1.10*
Secchi	OH	<0.5	=<0.7	>0.7	>1.1	*0.5 | 0.7 | 1.10*
Secchi	MH	<1.35	=<1.8	>1.8	>2.25	*1.35 | 1.80 | 2.25*
Secchi	PH	<1.6	=<2.15	>2.15	>2.55	*1.6 | 2.15 | 2.55*
		Worst	Poor	Better	Best	*75th%/median/25th%*
DIN	TF	>.585	>0.070	=<0.070	<0.030	*.585 | .434 | .290*
DIN	OH	>.885	>0.070	=<0.070	<0.030	*.885 | .680 | .464*
DIN	MH	>.265	>0.070	=<0.070	<0.030	*.265 | .150 | .070*
DIN	PH	>.070	>0.070	=<0.070	<0.030	*.063 | .020 | .011*
		Worst	Poor	Better	Best	*75th%/median/25th%*
PO_4 (SRP)	TF	>0.020	>0.003	=<0.003	=<0.003	*.020 | .136 | .010*
PO_4 (SRP)	OH	>0.010	>0.003	=<0.003	=<0.003	*.010 | .005 | .004*
PO_4 (SRP)	MH	>0.003	>0.002	=<0.002	=<0.002	*.003 | .002 | .0006*
PO_4 (SRP)	PH	>0.005	>0.003	=<0.003	=<0.003	*.005 | .004 | .0007*

associated with the least impaired stations currently monitored in the Chesapeake Bay. They also approximate the light levels required for growth of underwater bay grasses (Batiuk et al. 2000). For the purpose of establishing phytoplankton reference communities, a water quality parameter classification of 'worst or 'poor' is considered impaired while a water quality parameter classification of 'better' or 'best' is considered unimpaired.

When all three parameters were classified as 'worst,' the data record was placed in the 'worst' water quality category. When all three parameters classified as 'poor' or 'worst' (includes all 'worst'), the data record was placed in the 'poor' water quality category. 'Poor' and 'worst' water quality conditions are characterized by low levels of light, and concentrations of DIN and PO_4 that exceed phytoplankton nutrient requirements. 'Worst' is an extreme subset of 'poor.' Similarly, when all three parameters classified as 'best,' the data record was placed in the 'best' water quality category. When all three classified as 'best' or 'better' (includes all 'best'), the data record was placed in the 'better' water quality category. 'better' and 'best' water quality conditions had high levels of light and limiting (low) concentrations of DIN and PO_4. 'Best' is an extreme subset of 'better'. Data records were placed in a

Table F-2. Summer (July through September) classification criteria for determining 'worst', 'poor,' 'better,' and 'best' water quality parameter conditions. Key: Secchi-Secchi depth (meters); DIN-average dissolved organic nitrogen in surface mixed layer (mg liter^{1}); PO_4—average orthophosphate (SRP) in surface mixed layer (mg liter^{1}); TF—tidal fresh salinities (0 to 0.5 ppt); OH—oligohaline salinities (>0.5 to 5 ppt); MH—mesohaline salinties (>5 to 18 ppt); PH—polyhaline (>18 ppt). The 25th percentile, median and 75th percentile of the parameter's values at stations identified as 'good' by the Relative Status Method are given for comparison purposes. See Buchanan et al. (in review) for details.

Parameter		Selected Summer Classification Criteria				Relative Status Method
		Worst	Poor	Better	Best	*25th%/median/75th%*
Secchi	TF	<0.6	=<0.8	>0.8	>1.0	*0.6 | 0.8 | 1.0*
Secchi	OH	<0.55	=<0.6	>0.6	>0.7	*0.55 | 0.6 | 0.7*
Secchi	MH	<1.2	=<1.45	>1.45	>1.7	*1.2 | 1.45 | 1.7*
Secchi	PH	<1.55	=<1.85	>1.85	>2.35	*1.55 | 1.85 | 2.35*
		Worst	Poor	Better	Best	*75th%/median/25th%*
DIN	TF	>.390	>0.070	=<0.070	<0.030	*.390 | .240 | .125*
DIN	OH	>.090	>0.070	=<0.070	<0.030	*.090 | .050 | .028*
DIN	MH	>.074	>0.070	=<0.070	<0.030	*.074 | .035 | .014*
DIN	PH	>.070	>0.070	=<0.070	<0.030	*.028 | .011 | .008*
		Worst	Poor	Better	Best	*75th%/median/25th%*
PO_4 (SRP)	TF	>0.025	>0.003	=<0.003	=<0.003	*.025 | .020 | .010*
PO_4 (SRP)	OH	>0.010	>0.003	=<0.003	=<0.003	*.010 | .009 | .004*
PO_4 (SRP)	MH	>0.008	>0.002	=<0.002	=<0.002	*.008 | .005 | .0035*
PO_4 (SRP)	PH	>0.010	>0.003	=<0.003	=<0.003	*.010 | .008 | .005*

'mixed poor light' category if Secchi depth classified as 'poor' or 'worst' and one or both of the nutrient parameters classified as 'better' or 'best'. Data records were placed in a 'mixed better light' category if Secchi depth classified as 'better' or 'best' and one or both of the nutrient parameters classified as 'poor' or 'worst'.

SUMMARY OF CHLOROPHYLL *A* RESULTS

The 'better' water quality conditions (includes 'best') occurred in 1.6 percent (spring) and 5.8 percent (summer) of the mesohaline biomonitoring records, and 21.1 percent (spring) and 10.4 percent (summer) of the polyhaline biomonitoring records collected between 1984 and 2001. Therefore, reference communities could be characterized directly from the phytoplankton associated with these least-impaired water quality data. Because values of most phytoplankton parameters in the mesohaline and polyhaline 'mixed better light' categories, including chlorophyll *a*, closely resembled those in 'better' categories, 'mixed better light' data were used to augment the small number of spring mesohaline 'better' data records. Median chlorophyll *a* concentrations were 5.6 (spring) and 7.1 (summer) μg liter^{-1} in the

mesohaline reference communities, and 2.9 (spring) and 4.4 (summer) μg liter^{-1} in the polyhaline reference communities. Reference community chlorophyll *a* values are within the 2-7 μg liter^{-1} range identified by Molvaer et al. (1997) for mesotrophic marine waters, but are slightly higher than the 1-3 μg liter^{-1} chlorophyll *a* range identified as mesotrophic by Smith et al. (1990). They can be considered high mesotrophic. The reference community medians are 50 percent (spring) and 58 percent (summer) of the Poor category median concentrations in mesohaline waters and 32 percent (spring) and 72 percent (summer) of the 'poor' category median concentrations in polyhaline waters. These differences are significant (Wilcoxon test, $p<0.01$). Chlorophyll *a* concentrations in the 'poor' categories classify as eutrophic in mesohaline waters and borderline eutrophic in polyhaline waters.

Tidal-fresh and oligohaline reference community chlorophyll *a* concentrations are based primarily on phytoplankton in the 'mixed better light' water quality category, which is the least impaired category commonly found in low salinity waters of the Chesapeake Bay. 'Better' water quality conditions occurred in less than 1 percent of all samples. The combined 'mixed better light' and 'better' categories occurred in 4.7 percent (spring) and 21.5 percent (summer) of the tidal fresh biomonitoring records and in 18.7 percent (spring) and 29.9 percent (summer) of the oligohaline biomonitoring records collected between 1984 and 2001. Median chlorophyll *a* concentrations were 4.3 (spring) and 8.6 (summer) μg liter^{-1} in the tidal fresh reference communities, and 9.6 (spring) and 6.0 (summer) μg liter^{-1} in the oligohaline reference communities. Reference community chlorophyll *a* values are within the ranges identified by Wetzel (2001) and Novotny and Olem (1994) for mesotrophic fresh waters, but sometimes exceed the ranges identified by Smith et al. (1998) and Ryding and Rast (1989). These values can be considered high mesotrophic. Median chlorophyll *a* concentrations of the reference community are 64 percent (spring) and 34 percent (summer) of those in tidal fresh 'poor' category waters, and 52 percent (spring) and 35 percent (summer) of those in oligohaline 'poor' category waters. These differences are significant (Wilcoxon test, $p<0.01$). Chlorophyll *a* concentrations in the tidal-fresh and oligohaline 'poor' categories classify as eutrophic to highly eutrophic.

Reference communities were also distinguishable from 'poor' category phytoplankton populations by their smaller chlorophyll *a* ranges (Figure F-1). Typically, ranges of chlorophyll *a* concentrations in the reference communities were $^1/_5$ to ½ the span of those in 'poor' water quality conditions. The large ranges of chlorophyll *a* concentrations found in the 'worst,' 'poor,' and 'mixed poor light' water quality categories of all salinity zones demonstrate the occurrence of frequent algal blooms in these categories. Marshall et. al. (in draft) show that the species compositions of phytoplankton associated with the lowest quartile (minimum—25th percentile) of chlorophyll *a* values in 'worst', 'poor' and 'mixed poor light' water quality conditions are generally mixed, while species compositions in the highest quartile of chlorophyll values (75th percentile–maximum) are dominated by 'bloom-forming' species. Mesohaline and polyhaline bloom-forming species include the diatoms

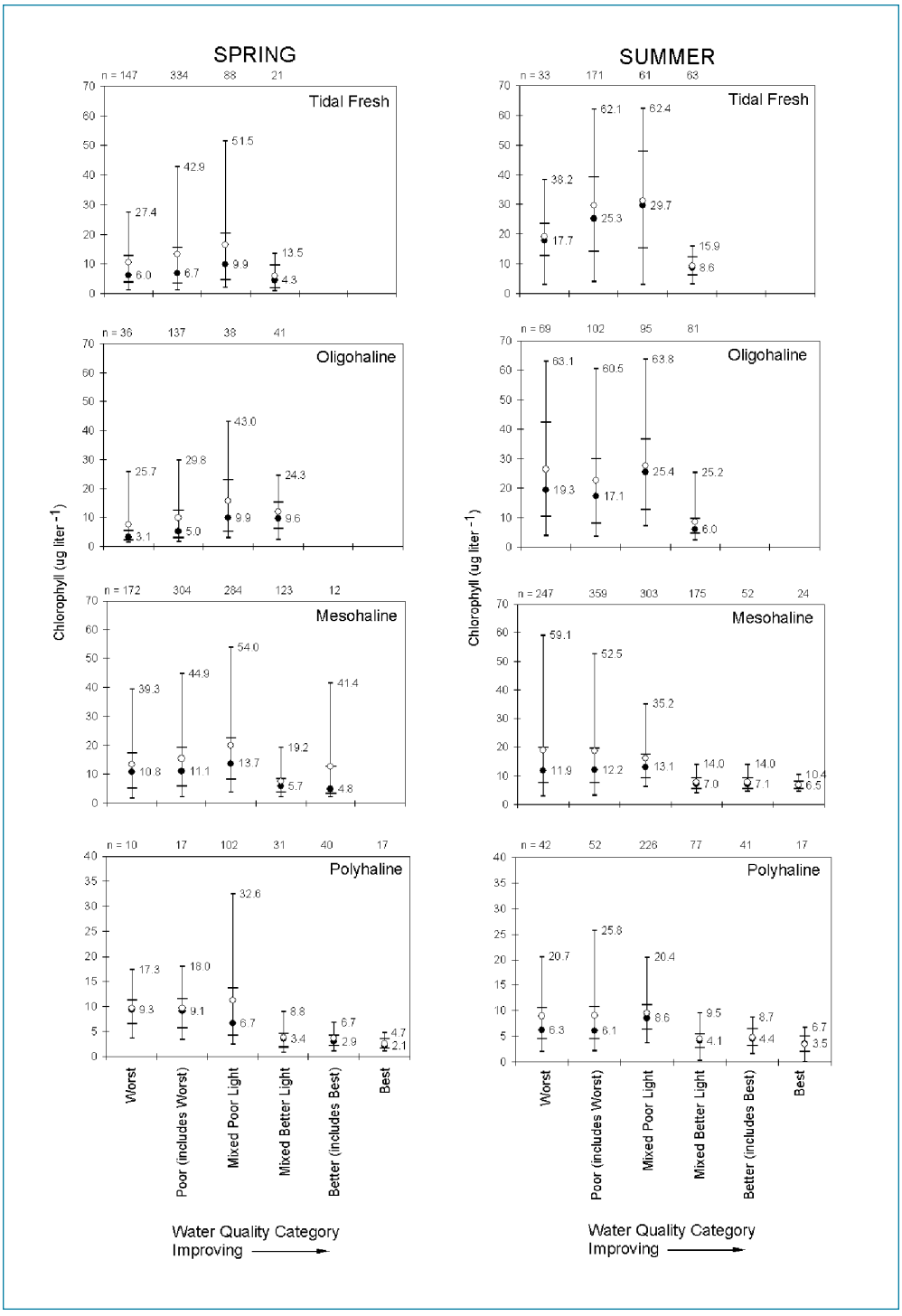

Figure F-1: Chlorophyll *a* concentrations (μg liter^{-1}) for six water quality conditions in eight season-salinity groups (see text for details). Symbols: median (•), average (°), and 5th, 25th, 75th and 95th percentiles (—). Median and 95th percentile values are shown. A blank indicates <10 data points were available in the water quality category.

Chaetoceros spp., *Cyclotella* spp. and (at times) the small, unidentified centric diatom, and the dinoflagellates *Gymnodinium* spp., *Katodinium rotundatum* and *Prorocentrum minimum*. Tidal-fresh and oligohaline bloom-forming species include colonial bluegreens such as *Microcystis aeruginosa*, filamentous bluegreen genera such as *Oscillatoria* and *Raphidiopsis*, diatoms such as *Coscinodiscus* spp., *Leptocylindrus minimus*, small unidentified centrics and *Melosira varians*, greens such as *Coelastrum* spp., and the dinoflagellate *Gymnodinium* spp. Coincident water quality data suggests the high chlorophyll *a* groups in 'worst,' 'poor,' and 'mixed poor light' conditions may represent blooms at their peak, while the low chlorophyll *a* groups may represent populations unable to use the available nutrients and blooming due to low light levels. Specifically, DIN concentrations in the high chlorophyll *a* groups are sometimes as little as half of those in the low chlorophyll *a* groups, indicating increased nitrogen utilization in the high chlorophyll *a* groups.

The ranges of chlorophyll *a* concentrations (5^{th} percentile–95^{th} percentile) observed in the phytoplankton reference communities indicate the peak concentrations that should be expected in populations currently inhabiting unimpaired Chesapeake waters. Chlorophyll *a* concentrations above these peak values constitute excess phytoplankton production fueled by high nutrient concentrations and are potentially harmful to the Chesapeake ecosystem. Peak chlorophyll *a* concentrations of the reference communities, expressed as μg liter^{-1}, are 13.5 (tidal-fresh), 24.3 (oligohaline), 24.6 (mesohaline) and 6.7 (polyhaline) in spring, and 15.9 (tidal-fresh), 25.2 (oligohaline), 14.0 (mesohaline) and 8.7 (polyhaline) in summer.

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