Journal of
Plankton Research

plankt.oxfordjournals.org

J. Plankton Res. (2013) 35(4): 877–893. First published online April 5, 2013 doi:10.1093/plankt/fbt028

Seasonal dynamics of *Mesodinium rubrum* in Chesapeake Bay

MATTHEW D. JOHNSON[1]*, DIANE K. STOECKER[2] AND HAROLD G. MARSHALL[3]

[1]BIOLOGY DEPARTMENT, WOODS HOLE OCEANOGRAPHIC INSTITUTION, WOODS HOLE, MA 02543, USA, [2]UNIVERSITY OF MARYLAND CENTER FOR ENVIRONMENTAL SCIENCES, HORN POINT LABORATORY, CAMBRIDGE, MD 21613, USA AND [3]DEPARTMENT OF BIOLOGICAL SCIENCES, OLD DOMINION UNIVERSITY, NORFOLK, VA 23529, USA

*CORRESPONDING AUTHOR: mattjohnson@whoi.edu

Received October 26, 2012; accepted March 10, 2013

Corresponding editor: John Dolan

The photosynthetic ciliate *Mesodinium rubrum* is a common member of coastal phytoplankton communities that is well adapted to low-light, turbid ecosystems. It supports the growth of, or competes with, harmful dinoflagellate species for cryptophyte prey, as well as being a trophic link to copepods and larval fish. We have compiled data from various sources ($n = 1063$), on the abundance and distribution of *M. rubrum* in Chesapeake Bay and its tributaries. Because *M. rubrum* relies on obtaining organelles from cryptophyte algae to maintain rapid growth, we also enumerated cryptophyte algae in the portion of these samples that we collected ($n = 386$). *Mesodinium rubrum* occurred in oligohaline to polyhaline regions of Chesapeake Bay and throughout the year. Blooms (>100 cells mL^{-1}) primarily occurred during spring, followed by autumn. When compared across all seasons, *M. rubrum* abundance was positively correlated to temperature and cryptophytes, and negatively correlated with salinity. However, more focused analyses revealed that *M. rubrum* abundance during spring was associated with surface layer warming and decreased salinity, while early autumn assemblages were associated with surface cooling. These results imply that there are distinct seasonal niches for *M. rubrum* blooms. Blooms were more common in tributaries than in the main stem Bay and tended to be restricted to salinities under 10 PSU. Despite the rarity of "red water" events, *M. rubrum* is a ubiquitous mixotroph in Chesapeake Bay and at times likely exerts a strong influence on cryptophyte algal abundance and hence planktonic food web structure.

available online at www.plankt.oxfordjournals.org

KEYWORDS: *Mesodinium rubrum*; cryptophytes; Chesapeake Bay; phytoplankton; red-tides

INTRODUCTION

Mesodinium rubrum (=*Myrionecta rubra*) is a mixotrophic estuarine and neritic Litostome ciliate that occurs nearly year round in plankton assemblages and is capable of forming red tides (Taylor *et al.*, 1971; Crawford, 1989; Stoecker *et al.*, 2009). *Mesodinium rubrum* has been a subject of interest due to its phycoerythrin-rich cryptophyte plastids and its ability to form spectacular reddish-pink blooms (Powers, 1932; Ryther, 1967; Taylor *et al.*, 1969; Crawford, 1989). However, in recent years, it has received greater attention due to the discovery of its reliance on ingestion of cryptophytes and establishment of stable cultures (Gustafson *et al.*, 2000), and the initial discovery of its trophic link to the harmful dinoflagellate, *Dinophysis acuminata* (Park *et al.*, 2006). Herein, we have compiled data on *M. rubrum* spanning 22 years, in order to evaluate seasonal population dynamics in the Chesapeake Bay ecosystem. Our use of data from a variety of sources, including monitoring programs, maximizes the scope of our investigation of *M. rubrum*'s ecology in a large, spatially diverse, temperate estuary. We use these data to test the hypotheses that (i) *M. rubrum* is more abundant during "wet" (higher rainfall) years, (ii) it can exploit distinct hydrodynamic seasonal niches and (iii) its abundance is positively related to cryptophyte algae.

Blooms of *M. rubrum* are common in temperate estuaries, on continental shelves and in upwelling regions, and are usually ephemeral and highly productive events (Crawford, 1989). These blooms often occur in thin layers and may have diel cycles of vertical migration within the water column (Dale, 1987; Crawford and Purdie, 1992; Crawford and Lindholm, 1997; Sjöqvist and Lindholm, 2011). The vertical distribution of *M. rubrum* within the water column is highly variable, and may be governed by factors such as light, nutrients and tidal cycles (Crawford and Purdie, 1992; Crawford and Lindholm, 1997). Red tides of *M. rubrum* are typified by high primary productivity, with reports as high as 2187 mg C m^{-3} h^{-1}, or 16 pg C (pg chl *a*)$^{-1}$ h^{-1} recorded in the Peruvian upwelling zone, which is one of the highest productivity measurements recorded for phytoplankton (Smith and Barber, 1979). However, productivity of *M. rubrum* in a temperate estuarine habitat (salt pond) are more modest, measuring between 1.8 and 8.6 pg C (pg chl a)$^{-1}$ h^{-1} (Stoecker *et al.*, 1991). *Mesodinium rubrum* blooms have a profound effect on the action and absorption spectra of phytoplankton communities due to their phycobillin-containing plastids, and coincide with dramatic increases in community maximum quantum yields of photosynthesis (Kyewalyanga *et al.*, 2002). Field populations of *M. rubrum* also have high nitrate reductase activity (Packard *et al.*, 1978). Nitrogen uptake rates within *M. rubrum* blooms have measured between 2 and 5 μg-at N L^{-1} h^{-1}, with estimates of integrated nitrate uptake in vertically migrating populations of 24 mg-at m^{-2} day^{-1} (Packard *et al.*, 1978; Wilkerson and Grunseich, 1990). Blooms of *M. rubrum* off Peru can be massive, with patches measuring >250 km^2 (Ryther, 1967). Such blooms generally occur during periods of calm, warm weather following upwelling events (Ryther, 1967; Dugdale *et al.*, 1987). Most blooms of *M. rubrum* are associated either with a sudden increase in water column stability (Kyewalyanga *et al.*, 2002), fronts in upwelling zones (Packard *et al.* 1978) or estuarine plumes (Crawford *et al.*, 1997).

Research on cultures of *M. rubrum* has shown that they require the ingestion of cryptophyte algal prey in order to survive (Gustafson *et al.*, 2000). The role of feeding on cryptophytes by *M. rubrum* is complex; while the ciliate sequesters foreign organelles from cryptophyte algae, it differs profoundly from kleptoplastidic ciliates. *Mesodinium rubrum* can only utilize certain cryptophyte species as a source of organelles (e.g. plastids, mitochondria, nucleus) and it maintains the plastids and mitochondria in a quasi-symbiotic state, having the ability to divide these organelles (Johnson, 2011). Studies on a strain of *M. rubrum* from Antarctica suggest that feeding on cryptophyte prey is most important to replace the cryptophyte nucleus, which remains transcriptionally active, but is incapable of division (Johnson *et al.*, 2007). The presence of this foreign nucleus coincides with maximum plastid activity and division, and allows the ciliate to function as a phototroph (Johnson *et al.*, 2007). Studies of an Antarctic culture of *M. rubrum* have also demonstrated the resilience of the photosystem in the ciliate and its ability to harvest light under exceedingly low irradiance levels (Moeller *et al.*, 2011).

While *M. rubrum* will ingest a variety of cryptophyte species, all cultured and field populations only possess plastids from the *Geminigera/Teleaulax* clade (Park *et al.*, 2007; Myung *et al.*, 2011; Hansen *et al.*, 2012). Laboratory studies on multiple *M. rubrum* strains indicate that its reliance upon mixotrophic ingestion of carbon for growth is minor (Yih *et al.*, 2004; Johnson

and Stoecker, 2005; Smith and Hansen, 2007). The recently described *M. chamaeleon*, however, has much higher ingestion requirements for cryptophyte algae to maintain growth, and their sequestered organelles are less stable and organized differently compared with *M. rubrum* (Moestrup *et al.*, 2012).

Estuarine blooms of *M. rubrum* have been widely reported and, like all red tides, their causes appear to vary. Recent studies of *M. rubrum* blooms in the Columbia River Estuary, a salt wedge system, have provided intriguing insights into the dynamics of *M. rubrum* bloom initiation and its genetic diversity within a population (Herfort *et al.*, 2011a, b). Blooms of *M. rubrum* in the Columbia River first develop near the mouth of the estuary, coinciding with maximum *in situ* growth rates, while later the bloom becomes more apparent within the open channel of the estuary (Herfort *et al.*, 2011a). This shift in population distribution may have been due to flanking *M. rubrum* populations becoming entrained within the main estuary channel and coincided with neap tides and increased salt wedge intrusion into the river (Herfort *et al.*, 2011a). Interestingly, of five identified *M. rubrum* variants (A–E), based on partial18S–28S rDNA sequences, only one (variant B) was associated with red-water events (Herfort *et al.*, 2011b).

Chesapeake Bay is a partially mixed estuary formed from a drowned river valley (Pritchard, 1967) (Fig. 1). It is the largest estuary in the USA, at 320 km long and 40 km at its widest point, but is relatively shallow (<18 m) (Hack, 1957). Chesapeake Bay has numerous tributaries that empty along both shores, with the Susquehanna River at its northern boundary being the largest. These tributaries form sub-estuarine systems that frequently host independent phytoplankton bloom events (Glibert *et al.*, 2001). Circulation within Chesapeake Bay is mostly two-layer and partially mixed, and is influenced most strongly by river flow (Pritchard, 1952). While *M. rubrum* is common within Chesapeake Bay, it has rarely been reported as a red-tide forming species. Perhaps this explains why previous studies of ciliates within the system have focused on heterotrophic species (Dolan and Coats, 1990).

METHOD

Acquisition of historical and monitoring program data

The Rhode River *M. rubrum* abundance data set (including temperature and salinity) was collected by Dr. D.W. Coats between 1992 and 1994. Cell counts for the Rhode River were conducted using quantitative Protargol staining from surface samples (<1 m), as described previously (Montagnes and Lynn, 1993).

The Chesapeake Bay *M. rubrum* counts were acquired from R.V. Lacouture and S.G. Sellner and were generated through the Chesapeake Bay Water Quality Monitoring Program. These counts represent a composite sample of the surface-mixed layer by the combination of two independent samplings from five depths above the pycnocline. Subsamples from these composites were preserved with 1.5% acid Lugol's solution (final concentration by volume, BV) and with 2% (BV) buffered formalin. Corresponding salinity, temperature, dissolved inorganic nitrogen (DIN) and Susquehanna River flow data were acquired from the Chesapeake Bay Program Data Hub (www.chesapeakebay.net).

Data from the southern Chesapeake Bay come from a broad phytoplankton monitoring program of Virginia tidal rivers and streams from April 1998 through December 2009 that was sponsored by the Virginia Department of Health (VDH) and the Center for Disease Control and Prevention. The emphasis in this program was on the identification and distribution of potentially harmful species and the presence of harmful algal blooms in Virginia waters (Marshall *et al.*, 2009). Over 400 water samples were collected annually by the VDH Division of Shellfish Sanitation. Although the presence of *M. rubrum* was not specifically monitored in the Virginia Study, their occurrence in bloom concentrations (>100 cells mL^{-1}) was recorded using light microscopy.

Choptank, Patuxent and Pocomoke river samples

Cell counts for *M. rubrum* and cryptophyte abundance for the Choptank (2002–2004), Patuxent (2002–2004) and Pocomoke (1999–2001) Rivers were generated from archived preserved samples at Horn Point Laboratory, and represent surface (<1 m) samples. Sampling methods for water collection and salinity and temperature data in these tributaries have been described previously (Stoecker *et al.*, 2000, 2008; Reaugh *et al.*, 2007). Briefly, the samples from the Choptank, Patuxent and Pocomoke Rivers were fixed in 1% (BV) gluteraldehyde and refrigerated until used for making slides. Slides were made by gently (<10 PSI) filtering 3–5 mL of sample onto a 2.0 μm polycarbonate filter and mounting the filter on a glass microscope slide with emersion oil and a coverslip. All slides were frozen until enumeration, which was conducted on a Nikon Eclipse inverted microscope using fluorescence filter sets B-2A (band pass, BP, excitation: 450–490 nm; long-pass, LP, dichromatic beam splitter, DM, 500 nm; LP

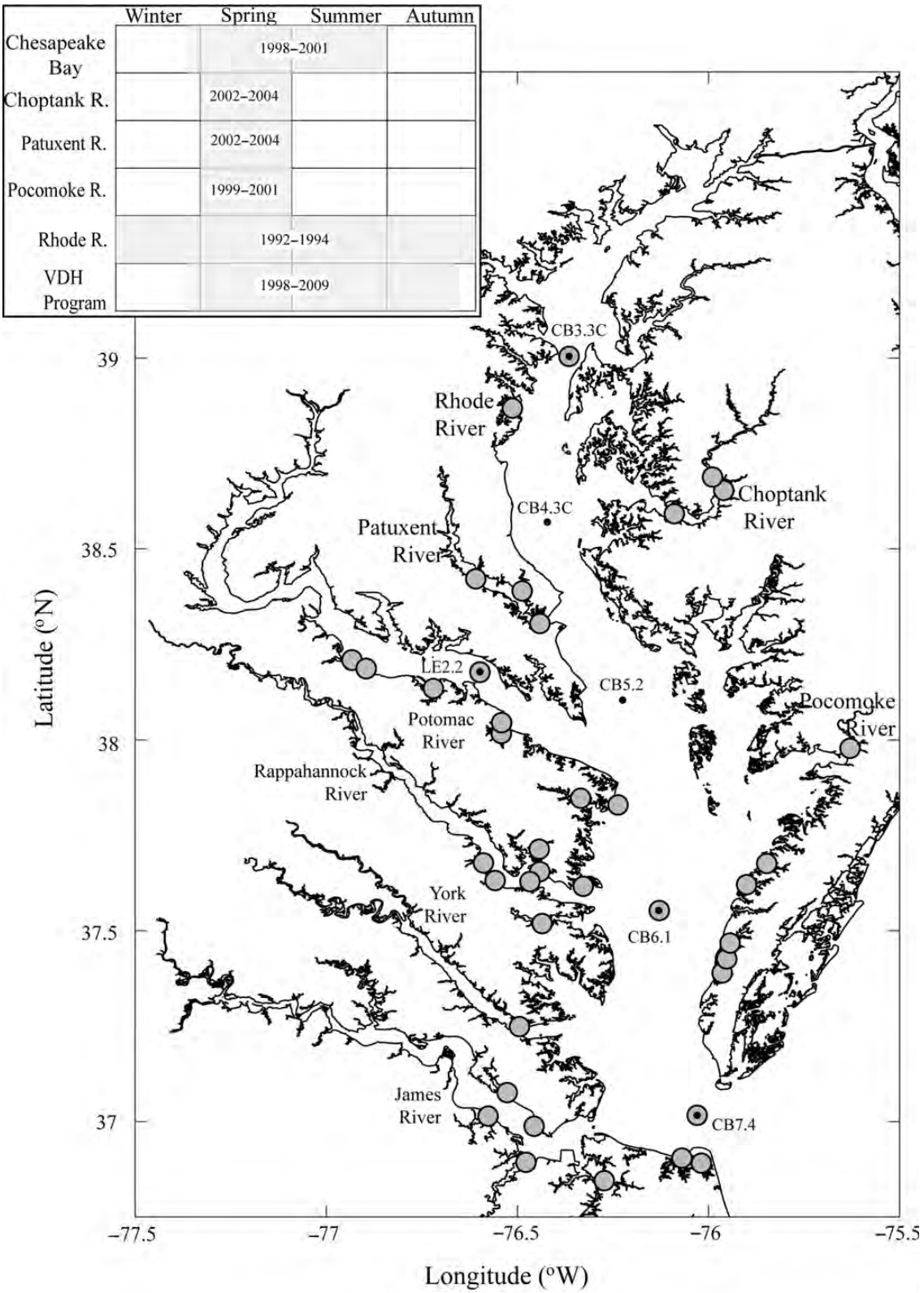

Fig. 1. Occurrence of *M. rubrum* blooms (>100 cells mL^{-1}) within the Chesapeake Bay system (gray circles); stations where data were obtained from the Chesapeake Bay Water Quality Monitoring Program are labeled with a black dot; *inset*: table showing the years and seasonal resolution of sampling from various data sources; VDH, Virginia Department of Health.

barrier filter, BA, 515 nm) and G-1A (BP excitation: 541–551 nm; LP DM, 575 nm; LP BA, 580 nm). Cell counts were conducted by making four or two transects on archived slides at 400–1000× magnification for *M. rubrum* and cryptophyte algae, respectively. Cells were identified based on their morphology and phycoerythrin fluorescence.

Statistical analysis

Normality of all data was tested using the Shapiro–Wilk test. Data with non-parametric distributions were log-transformed prior to statistical analysis in order to stabilize the mean/variance relationship and to create a more uniform distribution. *Mesodinium rubrum* cell count data were $\log(x+1)$ transformed, where x = cells mL^{-1}, in order to retain counts with a value of zero within the data set. In cases where normality was achieved, data were analyzed using analysis of variance (ANOVA) and the Tukey–Karmer minimum significant difference procedure to determine significance between annual cell abundance and river flow data for various tributaries. However, in most cases, normality was not achieved and a non-parametric analysis, Kruskal–Wallis ANOVA on ranks with Dunn's method for pairwise comparisons of groups, was used to determine significance. ANOVA was used to test the hypothesis that *M. rubrum* is more abundant during wet years. In order to test the hypothesis that *M. rubrum* can exploit distinct hydrodynamic regimes and that it is positively related to cryptophyte abundance, we used Spearman's rank correlation analysis to test the statistical dependence between cell abundance and environmental variables. All data were analyzed using Sigma Plot and Sigma Stat software (Systat Software, Inc.).

RESULTS

Overall data set

We compiled 1063 observations of *M. rubrum* and 386 observations of cryptophyte algal abundance from four Chesapeake Bay tributaries and portions of the main bay (Tables I and II; Fig. 1). Most of the samples were collected during spring or summer, 54 and 28%, respectively, while autumn and winter comprised ~10 and 8%, respectively (Table I). The Rhode River is the only data set that includes observations from all seasons. The majority of samples (76%) were from mesohaline regions of Chesapeake Bay, which is the dominant salinity class within the system (Table I). Very few samples (2.3%) were from polyhaline regions of Chesapeake Bay, and thus our analysis of *M. rubrum* bloom conditions are most representative of oligo- and mesohaline regions. *Mesodinium rubrum* occurred within a broad temperature and salinity range, with a central tendency of 18.8 ± 6.7°C and 10.6 ± 4.3 PSU ($n = 1063$; Table II).

"Blooms" of *M. rubrum*, defined here as a concentration >100 cells mL^{-1}, occurred on average at 19.4 ± 4.4°C and 6.9 ± 3.3 PSU ($n = 128$), while the highest concentrations (>1000 cells mL^{-1}) of the ciliate occurred on average at 18.1 ± 2.2 °C and 6.1 ± 2.5 PSU ($n = 16$). Most blooms of *M. rubrum* were associated with salinity levels that fell below the central tendency of their distribution (Fig. 2). Overall *M. rubrum* abundance was positively correlated with temperature, $r_{(900)} = 0.285$ ($P < 0.0001$), and negatively correlated to salinity, $r_{(929)} = -0.400$ ($P < 0.0001$). Spring and summer *M. rubrum* abundance was associated with declines in surface salinity and surface water warming, while autumn production was related to declines in surface temperature (Table III).

Rhode River

The Rhode River data set is the most comprehensive ($n = 540$), spanning 3 years and a portion of all seasons. While each year was unique, a general pattern included a large spring bloom of *M. rubrum* between May and early June when the temperature averaged 18.3 ± 2.4°C and salinity averaged 6.0 ± 3.1 PSU. Spring blooms resulted in >100 cells mL^{-1} throughout the River sampling area during all 3 years (Fig. 3). A second smaller peak around October appeared when surface temperatures cooled below 18°C in all 3 years (Fig. 3A–C). Lesser sporadic peaks also occurred in summer, usually July, when temperature averaged 27.7 ± 1.7°C and salinity averaged 9.2 ± 2.4 PSU. The first of the 3 years (1992) was a dry year compared with the 20 year (1990–2010) annual mean (40 834 $ft^3\ s^{-1}$) for Susquehanna River discharge, while the next 2 years were the third and second wettest, respectively (Table IV, Fig. 3D–F). During the first year (1992), the spring bloom was the smallest and shortest of the 3 years and occurred later (Fig. 3), while the annual mean level of *M. rubrum* in the river was the lowest (Table IV). The annual mean concentrations of *M. rubrum* during 1994 were the highest of the 3 years, with the ciliate rarely <10 cells mL^{-1} throughout the Rhode River sub-estuary (Fig. 3C).

Choptank and Patuxent Rivers

Sampling of the Choptank and Patuxent Rivers was between April and June during three consecutive years,

Table I: Description of data used in this study by season and salinity with the occurrence of M. rubrum *blooms*

System	Total (*n*)	Blooms[a] *n* (%)	Samples by season (*n*)				Samples by salinity (*n*)				
			Winter	Spring	Summer	Fall	<0.5	0.5–5	5–18	18–30	ND
Rhode River	540	66 (12)	30	168	234	108	2	59	435	0	44
Choptank River	285	21 (7.4)	27	153	5	0	0	8	140	1	36
Chesapeake Bay	68	3 (2.2)	12	66	58	1	0	0	97	40	0
Patuxent River	127	15 (12)	14	113	0	0	2	4	99	0	22
Pocomoke River	74	24 (32)	0	74	0	0	7	10	33	1	23
Sum (*n*)	1063	129 (12)	83	574	297	109	11	81	804	42	125
Blooms [*n* (%)]	0 (0)	104 (18)	15 (5.1)	10 (9.2)	0 (0)	35 (43)	78 (9.7)	0 (0)	16 (13)		

[a]Bloom: >100 cells mL^{-1}; ND, not determined.

Table II: Descriptive statistics for M. rubrum, *cryptophyte algae, salinity and temperature for all Chesapeake Bay and tributary stations used in this study*

	n	Mean	Median	SD	CI of mean	Range
Mesodinium rubrum[a]	1063	76.7	8	254	15.5	3300
Cryptophytes[a]	386	1432	908	1880	191	15,720
Temperature (°C)	956	18.8	19	6.7	0.43	31.3
Salinity (PSU)	951	10.6	10.7	4.3	0.28	22.7

[a]Cells mL^{-1}.

and includes counts for total cryptophyte abundance in addition to *M. rubrum*. River flow was greater in both systems during 2003 and 2004; however, no pattern was discernible between river flow and *M. rubrum* abundance over the 3 years (Table IV). Cryptophyte abundance was similar in both sub-estuaries, with surface concentrations typically near 1000 cells mL^{-1} throughout much of the sampling area (Table IV; Figs 4 and 5). The Patuxent River had the greatest mean abundance of cryptophyte algae during the wettest of the 3 years (2003), while no difference was observed for cryptophyte levels during the 3 years within the Choptank River (Table IV).

Blooms of *M. rubrum* occurred during two of the three years within the upper portion of the Patuxent River, and were generally at temperatures above 10°C (Fig. 4B, E and H) and salinity below 15 PSU (Fig. 4C, F and I). *Mesodinium rubrum* abundance in the upper Patuxent River appeared to coincide with high levels of cryptophytes (Fig. 4A, D and G) and inputs of freshwater to the system (Fig. 4C, F and I). During spring 2002, *M. rubrum* abundance increased at the upper Patuxent River sampling stations along a steeply declining salinity gradient, which remained a consistent feature throughout most of the sampling period (Fig. 4C). A 2004 bloom of *M. rubrum* in the upper Patuxent River occurred during a period of pronounced surface water warming and slight salinity decline (Fig. 4G–I).

Mesodinium rubrum distribution within the Choptank River differed over the 3 years, with short-lived blooms restricted mostly to the upper Choptank Stations (Fig. 5). In 2002, a small bloom of *M. rubrum* coincided with an increase in cryptophyte abundance throughout the sampling region, water column warming and a slight decline in surface salinity (Fig. 5A–C). In 2003, a bloom of *M. rubrum* occurred amid relatively low cryptophyte concentrations, when water temperature exceeded 16°C and within a strong salinity gradient (Fig. 5D–F). During spring 2004, an intense bloom occurred in the upper Choptank, with elevated cell numbers throughout the sampling region. This bloom peaked at 3200 cells mL^{-1}, and was associated with a reduction in cryptophyte abundance within the entire river, water column warming and with freshwater input (Fig. 5G–I). Overall *M. rubrum* abundance in both rivers was positively correlated with cryptophyte abundance, $r_{(df=383)} = 0.141$ ($P = 0.0056$), while cryptophyte abundance was positively correlated to temperature, $r_{(303)} = 0.289$ ($P < 0.0001$), but did not reveal a relationship with salinity.

Open bay stations

Mesodinium rubrum abundance was about one order of magnitude lower at open Chesapeake Bay stations than in the tributaries, averaging 7.2 cells mL^{-1}. Bloom-like concentrations of *M. rubrum* were in only a few samples from Chesapeake Bay (Table I). However, because open bay station counts were integrated composites of the entire surface layer (see the Method section), and all other cell counts represented discrete samples from the upper 1 m of surface water, direct comparisons are misleading. Furthermore, sampling resolution in the open Chesapeake Bay was much lower ($n = 68$) than within

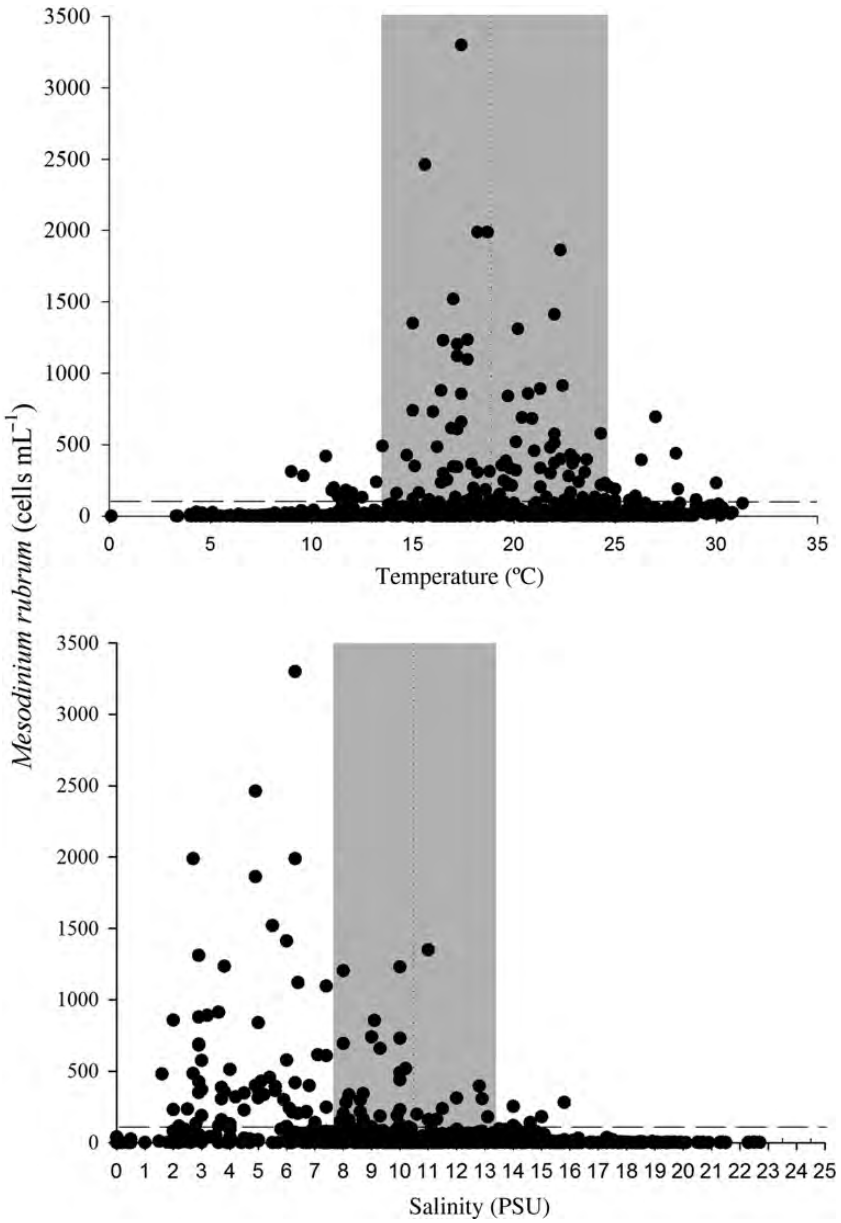

Fig. 2. Abundance of *M. rubrum* versus (**A**) temperature and (**B**) salinity. The horizontal dashed line indicates "bloom" levels of *M. rubrum* (100 cells mL^{-1}). The vertical dashed line represents the median and the gray box indicates the inter-quartile range (25th−75th percentiles) for temperature and salinity values, respectively, corresponding to *M. rubrum* abundance.

the various tributaries. Water column profiles compared before and during spring blooms of *M. rubrum* in the Potomac River and upper Chesapeake Bay revealed increases in surface layer stratification and a decrease in DIN levels during bloom events (Fig. 6). Observations of water column profiles before and during autumn blooms in southern Chesapeake Bay, however, show a decline in temperature and little change in salinity, or DIN preceding a bloom event (Fig. 7).

Southern bay tributaries

No quantitative or systematic sampling for *M. rubrum* in the southern Chesapeake Bay was available for this study. However, a qualitative phytoplankton monitoring program in the Virginia (southern) portion of Chesapeake Bay noted where samples had bloom-like concentrations (>100 cells mL^{-1}) of *M. rubrum* (Fig. 1, Table V). In contrast to other data sources, blooms were reported most often during summer in southern

Table III: Spearman's rank correlation results for seasonal M. rubrum *abundance with temperature and salinity*

Season	Samples (*n*)	Mean (cells mL^{-1})	Median (cells mL^{-1})	Max (cells mL^{-1})	Blooms[a] (*n*)	Temperature (*r*)	Salinity (*r*)
Spring	574	111	7.6	3300	104	0.402***	−0.388***
Summer	297	29	11.3	694	15	0.268*	−0.382***
Autumn (all dates)	109	69	21.7	1351	10	−0.096	−0.280
Autumn (through October)	75	95	31.1	1351	9	−0.455**	−0.172

***$P < 10^{-6}$, **$P < 10^{-5}$, *$P < 10^{-4}$.
[a]Days where bloom (>100 cells mL^{-1}) conditions were encountered.

Bay tributaries and were associated with higher salinity and temperature values (Table V).

DISCUSSION

Due to our use of archived samples that were either taken from the upper 1 m or which were integrated water column samples, we may have missed thin, subsurface accumulations of *M. rubrum* if they were present. This may have resulted in an underestimation of maximum abundances, particularly under highly stratified conditions and, in the case of surface samples, may have also resulted in a biased estimate of average water column abundance. However, by using archived samples and historical data, we were able to assess the occurrence of *M. rubrum* over a wide spatial area (from the Bay mouth to the upper Bay) and in major tributaries as well as in the main stem Bay. It also allowed us to use samples and data from many years, so that both wet and dry years were included. However, this wide coverage of necessity results in a lack of detailed information on vertical distribution, such as has been addressed in more spatially and temporarily restricted studies (Crawford and Purdie, 1992; Crawford and Lindholm, 1997; Herfort *et al.*, 2011a, b).

Physical factors that influence M. rubrum abundance in Chesapeake Bay

We have shown that *M. rubrum* abundance in Chesapeake Bay is related to temperature and salinity, but that the strength and direction of this correlation varies with season. As in previous studies of *M. rubrum* blooms (Crawford *et al.*, 1997), we also found a relationship between increased water column stability and *M. rubrum* abundance during spring. This was manifested by increased surface water temperature and lowered salinity (Table III). This water column pattern was associated with several May "blooms" (>100 cells mL^{-1}) in Chesapeake Bay (CB3.3C) and the Potomac River (LE2.2), where, from April to May, the surface layer increased to above 15°C and became more stratified, while DIN declined (Fig. 6). In the Newport River estuary in North Carolina, spring blooms of *M. rubrum* follow *Heterocapsa triquetra* blooms within the mesohaline frontal region of the estuary, and also coincide with increases in water temperature above 15°C (Litaker *et al.*, 2002). In the Columbia River estuary, *M. rubrum* abundance during summer coincided with neap tides, increases in salt wedge intrusion and decreases in river flow, suggesting that declines in turbulence during otherwise favorable growth conditions allow *M. rubrum* to grow and accumulate in the surface layer (Herfort *et al.*, 2011a). During summer in the Chesapeake Bay, *M. rubrum* abundance was associated with increased temperature and lower salinity, indicating that periodic rain events may stimulate production (Table III). During autumn, *M. rubrum* abundance was related to surface water cooling (Table III), with blooms generally occurring between 16 and 20°C. This pattern occurred during a large *M. rubrum* red tide in autumn 1995 in southern Chesapeake Bay, where the water column cooled to around 20°C from September to October and became increasingly mixed (Fig. 7). Seasonal blooms in Chesapeake Bay are mainly restricted to tributaries, and appear to be driven by different hydrodynamic regimes in spring and fall, suggesting that the ciliate is either highly opportunistic or that cryptic species or strains may have distinct seasonal niches.

We observed lower concentrations of *M. rubrum* at the open Chesapeake Bay stations relative to tributaries, which is consistent with its absence from previous studies of ciliates in surface waters of the main estuary (Dolan and Coats, 1990). The cause of lower *M. rubrum* abundance within the open Bay is uncertain, and most tributary-associated blooms appear to remain within these sub-estuaries. Within tributaries, stronger riverine influence on water column stratification and decreased light penetration probably help to structure the distribution of *M. rubrum* within the upper surface layer. *Mesodinium rubrum* may also become more easily entrained within tributary circulation systems by responding to tidal flow and riverine nutrient inputs.

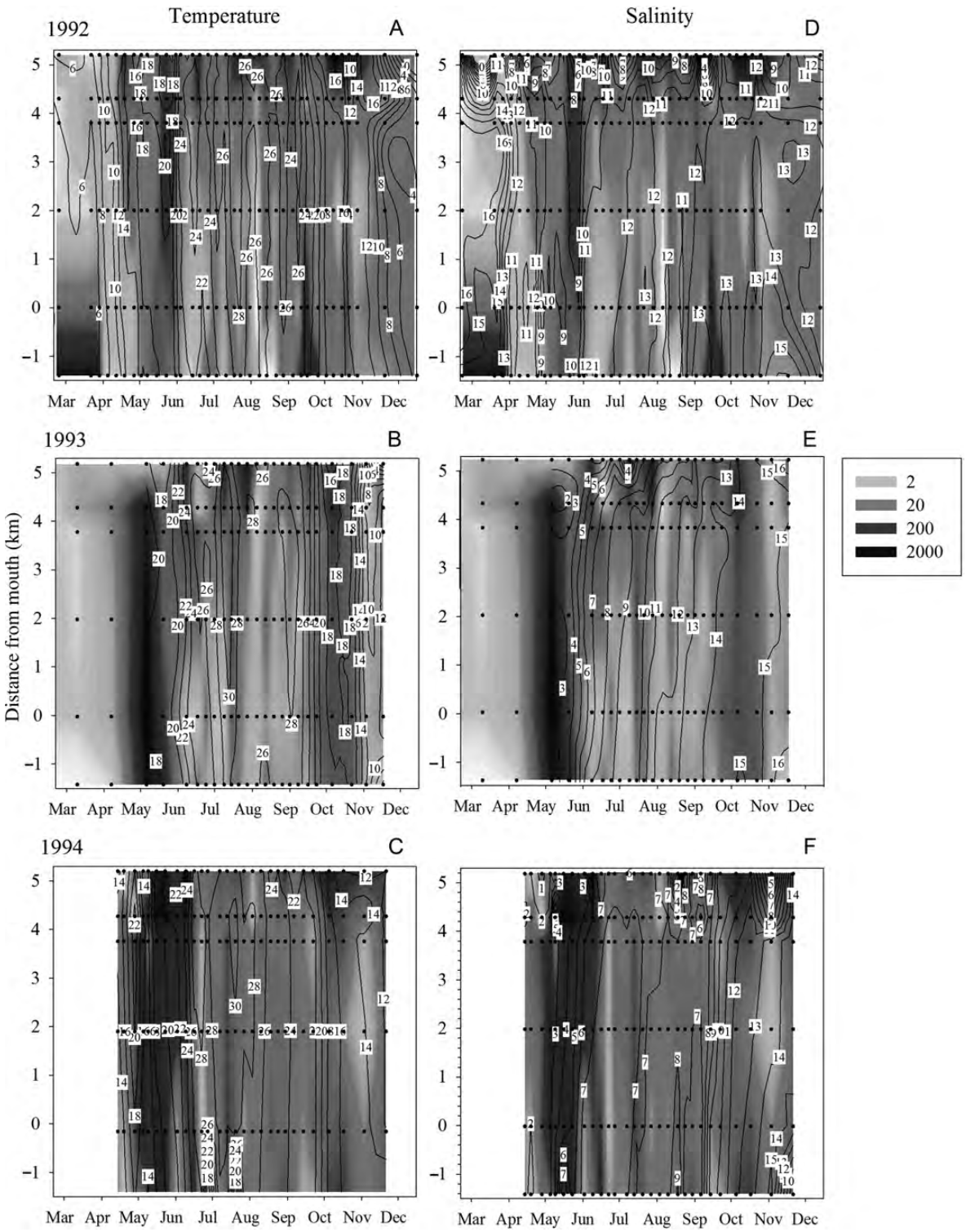

Fig. 3. Abundance of *M. rubrum* (gray scale contours) in the Rhode River by year with temperature (**A**–**C**) and salinity (**D**–**F**) line contours overlaid. Numbers on plots refer to levels salinity or temperature along line contours. Legend indicates *M. rubrum* abundance in cells mL^{-1}. *Y*-axis indicates distance from mouth of river (km) and black dots indicate sampling stations.

Table IV: A comparison of daily mean river flow data, M. rubrum *and cryptophyte algal abundance for three Chesapeake Bay tributaries across 3-year sampling periods*

Tributary	Year	River flow[1] ($ft^3\ s^{-1}$)	Cryptophyte (cells mL^{-1})	*M. rubrum* (cells mL^{-1})
Rhode River[2] (annual means)	1992	35 497 (1256)[a*]	NA	48 (10)
	1993	52 476 (4008)[b*]	NA	68 (18)
	1994	51 700 (3171)[c*]	NA	98 (15)[*]
	Statistical test	KW: $H = 101.7$, 2 d.f, $P < 0.001$	NA	KW: H = 17.9, 2 d.f, $P < 0.001$
Choptank River (spring means)[3]	2002	89 (3)[a*]	1401 (182)	56 (21)[a]
	2003	374 (15)[b*]	985 (118)	68 (58)
	2004	186 (16)[c*]	1178 (128)	164 (64)[a*]
	Statistical test	KW: $H = 71.3$, 2 d.f, $P < 0.001$	ANOVA: NS	KW: $H = 7.787$, 2 d.f, $P = 0.020$
Patuxent River (spring means)	2002	245 (11)	879 (329)	108 (55)[a]
	2003	867 (35)[a*]	1624 (216)[*]	47 (29)
	2004	515 (16)[a*]	913 (135)	118 (63)[a*]
	Statistical test	KW: $H = 90.0$, 2 d.f, $P < 0.001$	KW: $H = 22.9$, 2 d.f, $P < 0.001$	KW: $H = 16.2$, 2 d.f, $P < 0.001$

All values are mean (± standard error of the mean).
KW, Kruskal–Wallis one-way ANOVA on ranks; superscript letters indicate data groupings from Dunn's test and an asterisk indicates a significant difference ($P < 0.05$); no letter or the same letter indicates no significant difference; NA, not available; NS, ANOVA not significant.
[1]USGS Chesapeake Bay River Input Monitoring Program.
[2]River flow data are for the Susquehanna River (data not available for Rhode).
[3]Spring river flow data from 1 March to 20 June.

Such behavior has been demonstrated in Southampton Water, where during flood tide, the ciliate aggregated near the surface, while being dispersed away from the surface during ebb tide (Crawford and Purdie, 1992). In the open Chesapeake Bay, the most favorable physical conditions for *M. rubrum* blooms to develop may occur during periods of strong neap tides when turbulence declines and strong stratification can occur (Li and Zhong, 2009). However, the availability of nutrients, light and optimal cryptophyte prey are also important factors. In most regions of the main stem of Chesapeake Bay, bloom levels of *M. rubrum* would likely become dispersed due to tidal mixing and a general lack of a pronounced near surface physical structure to retain populations within a given area.

Possibility of a species complex

Previous studies on *M. rubrum* have noted distinct cell size classes (Lindholm, 1978; Montagnes *et al.*, 2008) raising the possibility that there may be a complex of cryptic species. Evidence supporting this hypothesis comes from the Columbia River Estuary and Oregon coastal margin, where at least five variants (A–E) of *M. rubrum* were identified during spring and summer (Herfort *et al.*, 2011b). Interestingly, only one of these variants (B) was associated with red-water events in the Columbia River (Herfort *et al.*, 2011b). A detailed investigation of the *Mesodinium* genus using cultures and isolated cells from coastal Denmark identified at least one novel species within this complex (variant D), *Mesodinium major* and a new variant (F) of the species complex (Garcia-Cuetos *et al.*, 2012). We observed a wide size range for *M. rubrum* in this study, but cell measurements were not made. Cryptic strains, or species in Chesapeake Bay and other coastal ecosystems, may explain why blooms of the ciliate have been reported under such a wide gradient of temperature and salinity (Figs 1 and 2) and at different times.

Trophic factors that may influence M. rubrum abundance in Chesapeake Bay

Cryptophyte algae are abundant in estuaries (Mallin *et al.*, 1991; Marshall *et al.*, 2005; Adolf *et al.*, 2006) and thrive in turbid, low light conditions (Marin *et al.*, 2011). Their exploitation of this niche is likely due both to their ability to absorb light in the blue–green portion of the spectrum (Marin *et al.*, 2011), which generally penetrates deeper than blue light in turbid or brackish estuarine waters, and to their ability to utilize dissolved organic carbon for mixotrophic growth (Lewitus *et al.*, 1991). In North Carolina estuaries, abundance of cryptophyte algae has been linked to rainfall events and they are one of the dominant phytoplankton classes in cool-weather blooms (Mallin *et al.*, 1991). In the Neuse River Estuary (North Carolina) stratified, turbid and low nitrate conditions favor cryptophyte biomass (Pinckney *et al.*, 1999). Likewise, *M. rubrum* frequently occur in low light habitats, such as in deep layers in the Baltic Sea (Setälä *et al.*, 2005) and in turbid estuaries (Crawford *et al.*, 1997; Herfort *et al.*, 2011a). In this and past studies of Chesapeake Bay (Li *et al.*, 2000; Adolf *et al.*, 2008), cryptophyte abundance was high, periodically exceeding 1000 cells mL^{-1}. In the main stem of Chesapeake Bay, cryptophyte-associated alloxanthin pigments had a

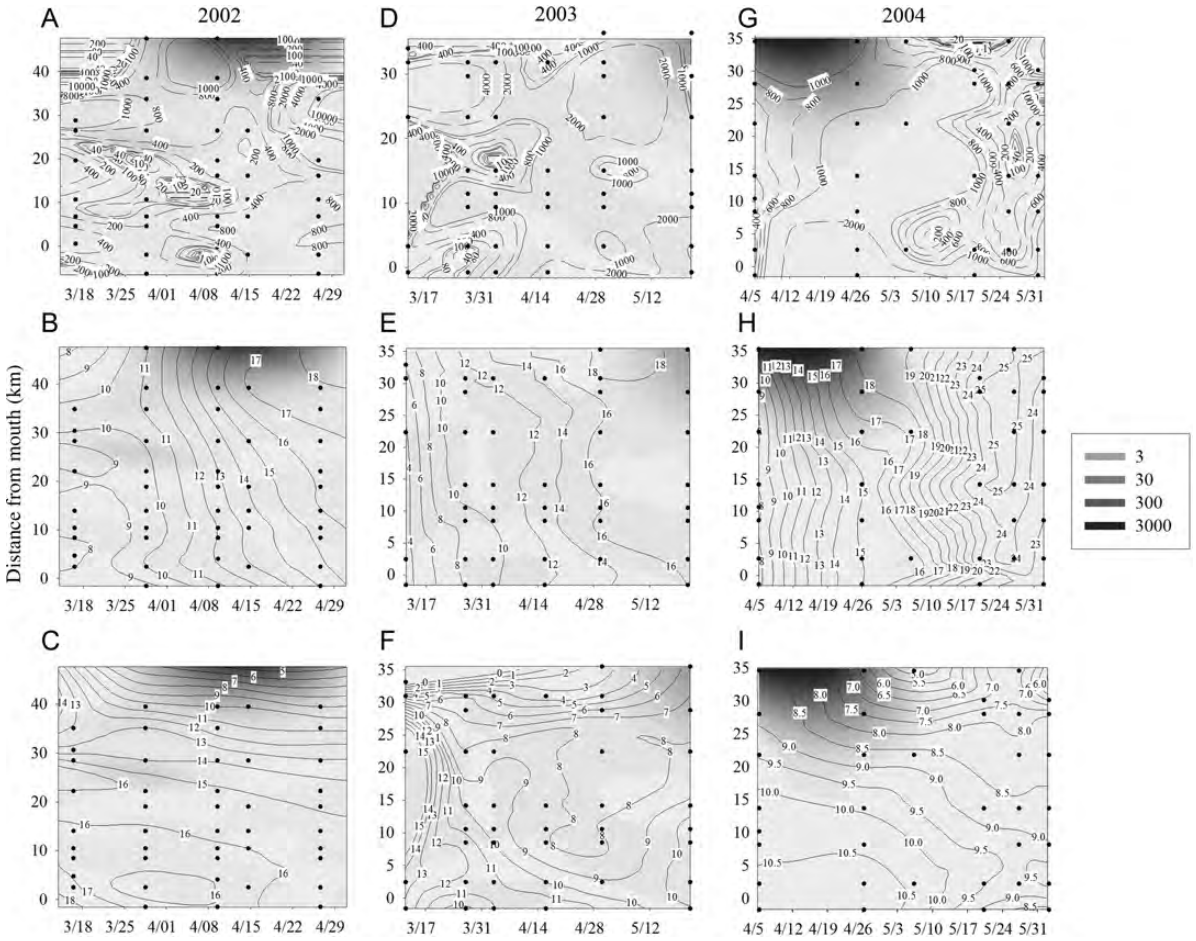

Fig. 4. Abundance of *M. rubrum* (gray scale contours, all plots) with cryptophyte algae (cells mL^{-1}; **A**, **D** and **G**), temperature (°C; **B**, **E** and **H**) and salinity (PSU; **C**, **F** and **I**) overlaid as lined contours, during spring in the Patuxent River (2002–2004). Numbers on plots refer to levels of cryptophytes, salinity or temperature along line contours. Gray-scale legend indicates *M. rubrum* abundance in cells mL^{-1}. *Y*-axis indicates distance from mouth of river (km) and black dots indicate sampling stations.

strong seasonal and regional signature, with populations peaking during autumn in the upper and lower Bay (Adolf *et al.*, 2006). While cryptophyte abundance was positively correlated to temperature but not salinity, our data were limited only to spring sampling within the Choptank, Patuxent and Pocomoke Rivers.

The precise nature of the relationship between the abundance and diversity of free-living cryptophyte algae and *M. rubrum* in nature remains obscure. However, high levels of cryptophytes have been observed prior to and during *M. rubrum* blooms in the Columbia River Estuary during three successive years (Peterson *et al.*, 2013). While the weak positive correlation between *M. rubrum* and cryptophyte algae observed here underscores their co-occurrence (see above), we found indirect evidence to support a grazing impact on spring assemblages of cryptophyte algae by the ciliate. During May 2004 in the Choptank River, high abundances of *M. rubrum* were found throughout the sampling region and coincided with a dramatic decline in cryptophyte abundance (Fig. 5G). However, these declines in cryptophyte populations could be due to other predators or environmental parameters.

Mixotrophic dinoflagellates are abundant in Chesapeake Bay from early spring through summer (Stoecker *et al.*, 1997; Li *et al.*, 2000; Adolf *et al.*, 2008), and are likely one of the main competitors of *M. rubrum* for cryptophyte prey. Formation of blooms in Chesapeake Bay of the toxic dinoflagellate *Karlodinium veneficum* are thought to be driven in large part by mixotrophic grazing on cryptophytes and perhaps other protist species (Adolf *et al.*, 2008). In culture, *K. veneficum* can ingest up to 8 cryptophyte cells^{-1} day^{-1} (Li *et al.*, 1999), while *M. rubrum* has been shown to ingest a maximum of ~9 cryptophyte cell^{-1} day^{-1} (Yih *et al.*, 2004), despite having a low ingestion requirement for sustaining maximum growth (Yih *et al.*, 2004; Johnson and Stoecker, 2005; Smith and Hansen, 2007). Thus, the ingestion rates

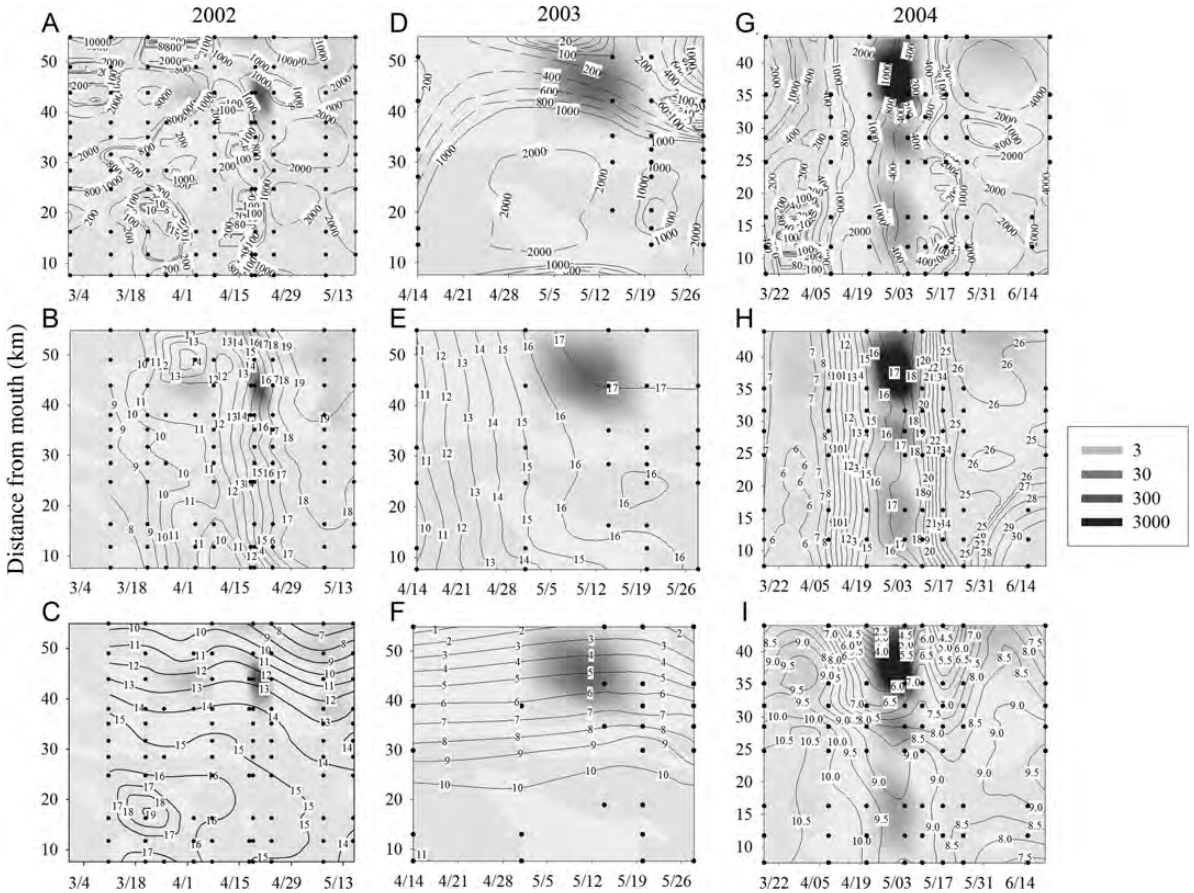

Fig. 5. Abundance of *M. rubrum* (gray scale contours, all plots) with cryptophyte algae (cells mL^{-1}; **A**, **D** and **G**), temperature (°C; **B**, **E** and **H**) and salinity (PSU; **C**, **F** and **I**) overlaid as lined contours, during spring in the Choptank River (2002–2004). Legend indicates *M. rubrum* abundance in cells mL^{-1}. *Y*-axis indicates distance from mouth of river (km) and black dots indicate sampling stations.

of the dinoflagellate and the ciliate appear to be roughly similar. However, some populations or strains of the ciliate may have mechanisms to rapidly exploit high levels of cryptophyte algae in order to maximize their growth potential (Peterson *et al.*, 2013). While *M. rubrum* appears to have relatively specific requirements for *Teleaulax/Geminigera* cryptophyte species for acquiring organelles, it will ingest a wider range of genera (Park *et al.*, 2007; Myung *et al.*, 2011; Hansen *et al.*, 2012). However, unlike mixotrophic dinoflagellates, it is unknown whether *M. rubrum* benefits from enhanced growth by ingesting cryptophyte species from which they cannot sequester organelles. Thus, while populations of *M. rubrum* likely exert a profound impact on overall cryptophyte algal abundance within tributaries, the complete role of cryptophyte ingestion and diversity in structuring *M. rubrum* populations remains to be determined. Likewise, the effect of *M. rubrum*'s competition for cryptophytes on mixotrophic dinoflagellate populations is unexplored.

With such high levels of cryptophyte abundance in Chesapeake Bay (Table II), it is perhaps surprising that greater levels of *M. rubrum* are not encountered more frequently. Factors that may constrain the production of *M. rubrum*, such as cryptophyte diversity, or physical structure within the water column, and grazing pressure by micro- or mesozooplankton need to be investigated further. Dilution experiments in the Rhode River Estuary have shown that *M. rubrum* growth rate increases with dilutions (Dolan *et al.*, 2000), which is consistent with *in situ* microzooplankton grazing pressure constraining the net growth of *M. rubrum*. Among the mixotrophic dinoflagellates, both toxic *Dinophysis* spp. (Park *et al.*, 2006) and *Neoceratium furca* (Stoecker, personal obs.) are known to feed on *M. rubrum*. Estuarine and marine copepods are important predators of ciliates (Stoecker and Capuzzo, 1990), including *M. rubrum* (Merrell and Stoecker, 1998; Fileman *et al.*, 2007), while studies of copepod nauplii have revealed minimal grazing on the ciliate (Turner *et al.*, 2001).

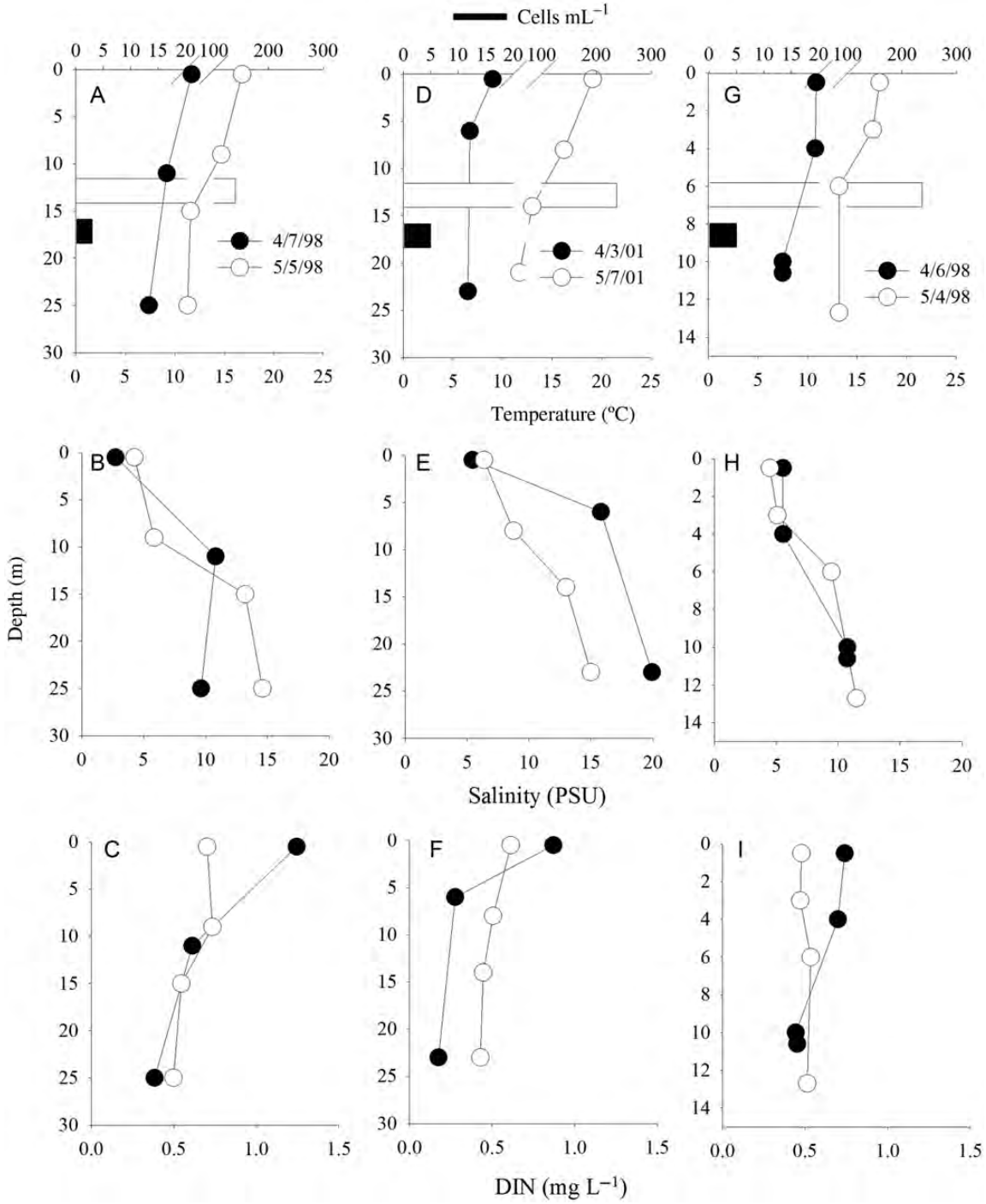

Fig. 6. Temperature, salinity and DIN levels during three *M. rubrum* blooms in the upper Chesapeake Bay and Potomac River. **A**–**C** are Station CB3.3C (upper bay) in spring 1998, stations **D**–**F** are CB3.3C during spring 2001 and stations **G**–**I** are LE2.2 (Potomac River) during spring 1998. Figures A, D and G show integrated surface layer abundance of *M. rubrum* (cells mL^{-1}; bars; upper *x*-axis) and temperature (°C; circles; lower *x*-axis), B, E and H show salinity (PSU) and figures C, F and I show DIN (mg L^{-1}).

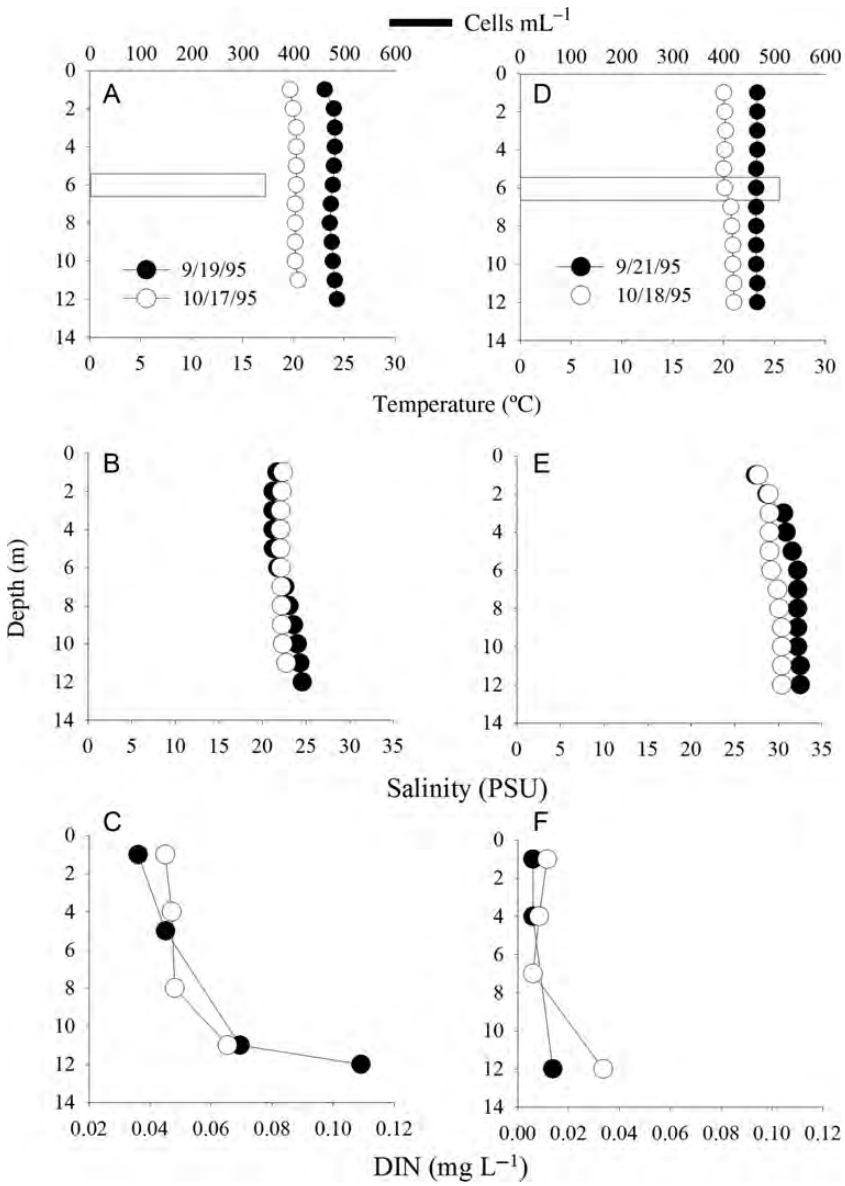

Fig. 7. Temperature, salinity and DIN levels during two *M. rubrum* blooms in lower Chesapeake Bay. **A**–**C** are Station CB6.1 in fall 1995, and stations **D**–**F** are CB7.4 during autumn 1995. A and D show integrated surface layer abundance of *M. rubrum* (cells mL^{-1}; bars; upper *x*-axis) and temperature (°C; circles; lower *x*-axis), B and E show salinity (PSU) and C and F show DIN (mg L^{-1}).

The impact of M. rubrum on Chesapeake Bay

The contribution *of M. rubrum* to phytoplankton community chlorophyll and primary production is high in many coastal and estuarine ecosystems (Smith and Barber, 1979; Stoecker *et al.*, 1991). In Chesapeake Bay, *M. rubrum* biomass can be on par with small blooms of red tide forming dinoflagellates, and capable of exceeding 100 μg L^{-1} chlorophyll *a* (calculated from published cellular chlorophyll levels and observed abundance). However, *M. rubrum* blooms in Chesapeake Bay are usually restricted to relatively small regions within tributaries. While blooms of *M. rubrum* may be conspicuous in other ecosystems and may exceed densities of 10^4 cells mL^{-1} (Taylor *et al.*, 1971), such events have not been reported in Chesapeake Bay. In a eutrophic ecosystem such as Chesapeake Bay (Kemp *et al.*, 2005), blooms of *M. rubrum* may largely go unnoticed due to high levels of phytoplankton community chlorophyll and colored dissolved organic matter (CDOM). Another possibility is that varieties of *M. rubrum* in Chesapeake Bay grow less prolifically than those elsewhere. One bloom recorded near the

Table V: Summary of environmental data for M. rubrum *blooms*[a] *observed in the Southern Chesapeake Bay (1998–2009)*

Season	*n*	Salinity PSU	Temperature (°C)
Spring	10	7.9 (4.5)[b]	20.7 (5.2)
Summer	31	16.7 (4.1)	27.1 (1.4)
Fall	7	14.6 (5.8)	18.5 (2.3)

[a]Blooms were estimated to be between 100 and 300 cells mL^{-1}.
[b]Mean (SD).

mouth (36° 59′ 36″, −76° 00′ 38″) of the bay in October 1995 exceeded 500 cells mL^{-1} (Marshall, 1996) and was noted for producing visible red water (L. Harding, personal communication), perhaps due to lower community chlorophyll and CDOM levels in this region. This is the only documented polyhaline (22.7–28.5 PSU) bloom of *M. rubrum* in the main stem of Chesapeake Bay. Monitoring blooms of *M. rubrum* in meso- and polyhaline regions of Chesapeake Bay may be useful as an early indicator of potentially toxic *Dinophysis* spp. (Campbell *et al.*, 2010), which have been reported at high levels in the Potomac River (Tango *et al.*, 2004). This is particularly relevant to the shellfish industry in meso- and polyhaline regions of Chesapeake Bay, due to potential accumulation of *Dinophysis* toxins in bivalves. The low number of observed *M. rubrum* red tides within the main body of the bay, despite high nutrients and an abundance of cryptophyte algae, is enigmatic and could point to generally unfavorable hydrodynamic conditions for this species or high losses to grazers. Despite their lack of numerical dominance, *M. rubrum* remains a nearly ever-present part of the plankton community throughout the year in most regions of Chesapeake Bay.

ACKNOWLEDGEMENTS

We kindly thank Dr. D. Wayne Coats for donating his Rhode River Data Set and Stella Sellner for providing cells counts of *M. rubrum* from the Chesapeake Bay Water Monitoring program.

FUNDING

Support for the production of this manuscript was provided by grants from the National Science Foundation (OCE-1031718 and OCE-1031344).

REFERENCES

Adolf, J., Yeager, C. L., Miller, W. D. *et al.* (2006) Environmental forcing of phytoplankton floral composition, biomass, and primary productivity in Chesapeake Bay, USA. *Estuar. Coast. Shelf Sci.*, **67**, 108–122.

Adolf, J. E., Bachvaroff, T. and Place, A. R. (2008) Can cryptophyte abundance trigger toxic *Karlodinium veneficum* blooms in eutrophic estuaries? *Harmful Algae*, **8**, 119–128.

Campbell, L., Olsen, R., Sosik, H. *et al.* (2010) First harmful *Dinophysis* (Cinophyceae, Dinophyciales) bloom in the U.S. is revealed by automated imaging flow cytometry. *J. Phycol.*, **46**, 66–75.

Crawford, D. W. (1989) *Mesodinium rubrum*: the phytoplankter that wasn't. *Mar. Ecol. Prog. Ser. Oldendorf*, **58**, 161–174.

Crawford, D. W. and Lindholm, T. (1997) Some observations on vertical distribution and migration of the phototrophic ciliate *Mesodinium rubrum* (=*Myrionecta rubra*) in a stratified brackish inlet. *Aquat. Microb. Ecol.*, **13**, 267–274.

Crawford, D. W. and Purdie, D. A. (1992) Evidence for avoidance of flushing from an estuary by a plankton, phototrophic ciliate. *Mar. Ecol. Prog. Ser.*, **79**, 259–265.

Crawford, D. W., Purdie, D. A., Lockwood, A. P. M. *et al.* (1997) Recurrent red-tides in the Southampton Water estuary caused by the phototrophic ciliate *Mesodinium rubrum*. *Estuar. Coast. Shelf Sci.*, **45**, 799–812.

Dale, T. (1987) Diel vertical distribution of planktonic ciliates in Lindaspollne, Western Norway. *Mar. Microbial Food Webs*, **2**, 15–28.

Dolan, J., Gallegos, C. L. and Moigis, A. (2000) Dilution effects on microzooplankton in dilution grazing experiments. *Mar. Ecol. Prog. Ser.*, **200**, 127–139.

Dolan, J. R. and Coats, D. W. (1990) Seasonal abundances of planktonic ciliates and microflagellates in mesohaline Chesapeake Bay waters. *Estuar. Coast. Shelf Sci.*, **31**, 157–175.

Dugdale, R. C., Wilkerson, F. P., Barber, R. T. *et al.* (1987) Changes in nutrients, pH, light penetration and heat budget by migrating photosynthetic organisms. *Oceanol. Acta*, SP, **6**, 103–107.

Fileman, E., Smith, T. and Harris, R. (2007) Grazing by *Calanus helgolandicus* and *Para-Pseudocalanus* spp. on phytoplankton and protozooplankton during the spring bloom in the Celtic Sea. *J. Exp. Mar. Biol. Ecol.*, **348**, 70–84.

Garcia-Cuetos, L., Moestrup, O. and Hansen, P. J. (2012) Studies on the genus *Mesodinium* II. Ultrastructural and molecular investigations of five marine species help clarifying the taxonomy. *J. Eukaryot. Microbiol.*, **59**, 374–400.

Glibert, P., Magnien, R., Lomas, M. *et al.* (2001) Harmful algal blooms in the Chesapeake and Coastal Bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events. *Estuar. Coasts*, **24**, 875–883.

Gustafson, D. E., Stoecker, D. K., Johnson, M. D. *et al.* (2000) Cryptophyte algae are robbed of their organelles by the marine ciliate *Mesodinium rubrum*. *Nature*, **405**, 1049–1052.

Hack, J. T. (1957) Submerged river system of Chesapeake Bay. *Geol. Soc. Am. Bull.*, **67**, 817–830.

Hansen, P. J., Moldrup, M., Tarangkoon, W. *et al.* (2012) Direct evidence for symbiont sequestration in the marine red tide ciliate *Mesodinium rubrum*. *Aquat. Microb. Ecol.*, **66**, 63–75.

Herfort, L., Peterson, T. D., Campbell, V. *et al.* (2011a) Myrionecta rubra (*Mesodinium rubrum*) bloom initiation in the Columbia River estuary. *Estuar. Coast. Shelf Sci.*, **95**, 440–446.

Herfort, L., Peterson, T. D., McCue, L. A. *et al.* (2011b) Myrionecta rubra population genetic diversity and its cryptophyte chloroplast specificity in recurrent red tides in the Columbia River estuary. *Aquat. Microb. Ecol.*, **62**, 85–97.

Johnson, M. D. (2011) Acquired phototrophy in ciliates: a review of cellular interactions and structural adaptations. *J. Eukaryot. Microbiol.*, **58**, 185–195.

Johnson, M. D., Oldach, D., Delwiche, C. F. *et al.* (2007) Retention of transcriptionally active cryptophyte nuclei by the ciliate *Myrionecta rubra*. *Nature*, **445**, 426–428.

Johnson, M. D. and Stoecker, D. K. (2005) Role of feeding in growth and photophysiology of *Myrionecta rubra*. *Aquat. Microb. Ecol.*, **39**, 303–312.

Kemp, W. M., Boynton, W. R., Adolf, J. E. *et al.* (2005) Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Mar. Ecol. Prog. Ser.*, **303**, 1–29.

Kyewalyanga, M., Sathyendranath, S. and Platt, T. (2002) Effect of *Mesodinium rubra* (=*Myrionecta rubra*) on the action and absorption spectra of phytoplankton in a coastal marine inlet. *J. Plankton Res.*, **24**, 687–702.

Lewitus, A. J., Caron, D. A. and Miller, K. R. (1991) Effects of light and glycerol on the organization of the photsynthetic apparatus in the faculatative heterotroph *Pyrenomonas salina* (cryptophyceae). *J. Phycol.*, **27**, 578–587.

Li, A., Stoecker, D. K. and Adolf, J. E. (1999) Feeding, pigmentation, photosynthesis and growth of the mixotrophic dinoflagellate *Gyrodinium galatheanum*. *Aquat. Microb. Ecol.*, **19**, 163–176.

Li, A., Stoecker, D. K. and Coats, D. W. (2000) Spatial and temporal aspects of *Gyrodinium galatheanum* in Chesapeake Bay: distribution and mixotrophy. *J. Plankton Res.*, **22**, 2105–2124.

Li, M. and Zhong, L. (2009) Flood-ebb and spring-neap variations of mixing, stratification and circulation in Chesapeake Bay. *Cont. Shelf Res.*, **29**, 4–14.

Lindholm, T. (1978) Autumnal mass development of the "red water" ciliate *Mesodinium rubrum* in the Aland archipelago. *Memo. Soc. Fauna Flora Fenn.*, **54**, 1–5.

Litaker, R. W., Tester, P. A., Duke, C. S. *et al.* (2002) Seasonal niche strategy of the bloom-forming dinoflagellate *Heterocapsa triquestra*. *Mar. Ecol. Prog. Ser.*, **232**, 45–62.

Mallin, M. A., Paerl, H. W. and Rudek, J. (1991) Seasonal phytoplankton composition, productivity and biomass in the Neuse River estuary, North Carolina. *Estuar. Coast. Shelf Sci.*, **32**, 609–623.

Marin, A., Doust, A. B., Scholes, G. D. *et al.* (2011) Flow of excitation energy in the cryptophyte light-harvesting antenna phycocyanin 645. *Biophys. J.*, **101**, 1004–1013.

Marshall, H. (1996) Toxin producing phytoplankton in Chesapeake Bay. *Va. J. Sci.*, **47**, 29–37.

Marshall, H. G., Burchardt, L. and Lacouture, R. (2005) A review of phytoplankton composition within Chesapeake Bay and its tidal estuaries. *J. Plankton Res.*, **27**, 1083–1102.

Marshall, H., Lane, M., Nesius, K. *et al.* (2009) Assessment and significance of phytoplankton species composition within Chesapeake Bay and Virginia tributaries through a long-term monitoring program. *Environ. Monit. Assess.* **150**, 143–155.

Merrell, J. and Stoecker, D. (1998) Differential grazing on protozoan microplankton by developmental stages of the calanoid copepod *Eurytemora affinis* Poppe. *J. Plankton Res.*, **20**, 289–304.

Moeller, H. V., Johnson, M. D. and Falkowski, P. G. (2011) Photoacclimation in the phototrophic marine ciliate *Mesodinium rubrum* (Ciliophora). *J. Phycol.*, doi:10.1111/j.1529–8817.2010.00954.x.

Moestrup, Ø., Garcia-Cuetos, L., Hansen, P. J. *et al.* (2012) Studies on the genus *Mesodinium* I: ultrastructure and description of *Mesodinium chamaeleon* n. sp., a benthic marine species with green or red chloroplasts. *J. Eukaryot. Microbiol.*, **59**, 20–39.

Montagnes, D. J. S., Allen, J., Brown, L. *et al.* (2008) Factors controlling the abundance and size distribution of the phototrophic ciliate *Myrionecta rubra* in open waters of the North Atlantic. *J. Eukaryot. Microbiol.*, **55**, 457–465.

Montagnes, D. J. S. and Lynn, D. H. (1993) A quantitative protargol stain (QPS) for ciliates and other protists. In Kemp, P. F., Sherr, B. F., Sherr, E. B. *et al.* (eds), *Handbook of Methods in Aquatic Microbial Ecology.* CRC Press, Boca Raton, pp 229–240.

Myung, G., Kim, H. S., Park, J. S. *et al.* (2011) Population growth and plastid type of *Myrionecta rubra* depend on the kinds of available cryptomonad prey. *Harmful Algae*, **10**, 536–541.

Packard, T., Blasco, D. and Barber, R. (1978) *Mesodinium rubrum in the Baja California upwelling system*. Springer-Verlag, Berlin.

Park, J. S., Myung, G., Kim, H. S. *et al.* (2007) Growth responses of the marine photosynthetic ciliate *Myrionecta rubra* to different cryptomonad strains. *Aquat. Microb. Ecol.*, **48**, 83–90.

Park, M. G., Kim, S., Kim, H. S. *et al.* (2006) First successful culture of the marine dinoflagellate *Dinophysis acuminata*. *Aquat. Microb. Ecol.*, **45**, 101–106.

Peterson, T. D., Golda, R. L., Garcia, M. L. *et al.* (2013) Associations between *Mesodinium rubrum* and cryptophyte algae in the Columbia River estuary. *Aquat. Microb. Ecol.*, doi:10.3354/ame01598.

Pinckney, J. L., Paerl, H. W. and Harrington, M. B. (1999) Responses of the phytoplankton community growth rate to nutrient pulses in variable estuarine environments. *J. Phycol.*, **35**, 1455–1463.

Powers, P. B. A. (1932) *Cyclotrichium meunieri* Sp. Nov. (Protozoa, Ciliata); cause of red water in the Gulf of Maine. *Biol. Bull.*, **63**, 74–80.

Pritchard, D. W. (1952) Estuarine hydrography. In Landsberg, H. E. (ed), *Advances in Geophysics 1*. Academic Press, New York, pp. 243–280.

Pritchard, D. W. (1967) What is an estuary, physical viewpoint. In Lauf, G. H. (ed), *Estuaries*. American Association for the Advancement of Science, Washington, DC.

Reaugh, M., Roman, M. and Stoecker, D. K. (2007) Changes in plankton community structure and function in response to variable freshwater flow in two tributaries of the Chesapeake Bay. *Estuar. Coasts*, **30**, 403–417.

Ryther, J. H. (1967) Occurrence of red water off Peru. *Nature*, **214**, 1318–1319.

Setälä, O., Autio, R., Kuosa, H. *et al.* (2005) Survival and photosynthetic activity of different *Dinophysis acuminata* populations in the northern Baltic Sea. *Harmful Algae*, **4**, 337–350.

Sjöqvist, C. O. and Lindholm, T. J. (2011) Natural co-occurrence of *Dinophysis acuminata* (Dinoflagellata) and *Mesodinium rubrum* (Ciliophora) in thin layers in a coastal inlet. *J. Eukaryot. Microbiol.*, **58**, 365–372.

Smith, M. and Hansen, P. J. (2007) Interaction between *Mesodinium rubrum* and its prey: importance of prey concentration, irradiance and pH. *Mar. Ecol. Prog. Ser.*, **338**, 61–70.

Smith, W. O. and Barber, R. T. (1979) Carbon budget for the autotrophic ciliate *Mesodinium rubrum*. *J. Phycol.*, **15**, 27–33.

Stoecker, D. K. and Capuzzo, J. M. (1990) Predation on Protozoa: its importance to zooplankton. *J. Plankton Res.*, **12**, 891–908.

Stoecker, D. K., Johnson, M. D., de Vargas, C. *et al.* (2009) Acquired phototrophy in aquatic protists. *Aquat. Microb. Ecol.*, **57**, 279–310.

Stoecker, D. K., Li, A., Coats, D. *et al.* (1997) Mixotrophy in the dinoflagellate *Prorocentrum minimum*. *Mar. Ecol. Prog. Ser.*, **152**, 1–12.

Stoecker, D. K., Putt, M., Davis, L. H. *et al.* (1991) Photosynthesis in *Mesodinium rubrum*: species-specific measurements and comparison to community rates. *Mar. Ecol. Prog. Ser.*, **73**, 245–252.

Stoecker, D. K., Stevens, K. and Gustafson, D. E. (2000) Grazing on *Pfiesteria piscicida* (Dinamoebiales, Pyrrhophyta) by Microzooplankton. *Aquat. Microb. Ecol.*, **22**, 261–270.

Stoecker, D. K., Thessen, A. E. and Gustafson, D. E. (2008) "Windows of opportunity" for dinoflagellate blooms: reduced microzooplankton net growth coupled to eutrophication. *Harmful Algae*, **8**, 158–166.

Tango, P. J., Butler, W., Lacouture, R. *et al.* (2004) An unprecedented bloom of *Dinophysis acuminata* in Chesapeake Bay. In Steidinger, K. A., Landsberg, J. H., Tomas, C. R. *et al.* (eds), *Harmful Algae 2002*. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, St. Pete Beach, FL, USA, pp 358–361.

Taylor, F. J. R., Blackbourn, D. J. and Blackbourn, J. (1969) Ultrastructure of the chloroplasts and associated structures within the marine ciliate *Mesodinium rubrum* (Lohmann). *Nature*, **224**, 819–821.

Taylor, F. J. R., Blackbourn, D. J. and Blackboun, J. (1971) Red-water ciliate *Mesodinium rubrum* and its incomplete symbionts—review including new ultrastructural observations. *J. Fish. Res. Board Canada*, **28**, 391–407.

Turner, J., Levinsen, H., Nielsen, T. *et al.* (2001) Zooplankton feeding ecology: grazing on phytoplankton and predation on protozoans by copepod and barnacle nauplii in Disko Bay, West Greenland. *Mar. Ecol. Prog. Ser.*, **221**, 209–219.

Wilkerson, F. P. and Grunseich, G. (1990) Formation of blooms by the symbiotic ciliate *Mesodinium rubrum*: the significance of nitrogen uptake. *J. Plankton Res.*, **12**, 973–989.

Yih, W., Kim, H. S., Jeong, H. A. *et al.* (2004) Ingestion of cryptophyte cells by the marine photosynthetic ciliate *Mesodinium rubrum*. *Aquat. Microb. Ecol.*, **36**, 165–170.