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5 Title: “Vertical spatio-temporal patterns of phytoplankton due to migration
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8 behaviors in two shallow, microtidal estuaries: Influence on phytoplankton
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11 function and structure”
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45 Running header: vertical spatio-temporal patterns of phytoplankton due to migration
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Abstract: Vertical patterns of phytoplankton biomass driven by phytoflagellate migration constitute a natural, short-term process with potentially important impacts on estuarine primary production and community composition. Although often considered well-mixed, phytoflagellate vertical migration patterns have been observed in shallow estuaries. We investigated vertical migration patterns in two shallow estuaries to determine how often and under what environmental conditions vertical migration patterns occurred, and to understand the potential impacts of vertical migration on phytoplankton composition and productivity. Vertical migration patterns were determined from circumannual records of decimeter-scale, semihourly, vertical profiles of chlorophyll fluorescence in two shallow, microtidal estuaries, the New River Estuary and Neuse River Estuary, North Carolina, USA. Observed migration patterns were compared with coincident measures of temperature, salinity, light, turbidity, nutrients, vertical stratification, and wind speed. A simple light $\times$ biomass model was used to estimate the influence of vertical migration patterns on total water column primary production. Collectively, between two sites in each estuary, diel vertical migration (DVM) patterns were detected on about half of the days. A secondary migration pattern reflecting a midday descent was also observed and was attributed to avoidance of intense midday surface irradiance. The likelihood of detectable DVM patterns increased under warmer conditions and lower incident irradiance, and at two of the stations was lessened by elevated wind speeds or reduced stratification intensity. Even weak stratification may be sufficient to reduce vertical mixing to levels that allow effective depth regulation by directed swimming. Modeled depth-integrated primary production was increased modestly ($< 10\%$) by observed migration patterns compared to a hypothetical vertically-homogenous biomass distribution. Access to optimal light levels and elevated bottom water dissolved inorganic nitrogen

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4 concentrations provides selective advantages that may explain phytoflagellate dominance in
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6 these estuaries, particularly during the warmer months, when production in surface waters of
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8 both systems is strongly N-limited.
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14 **Key Words: phytoplankton, vertical migration, light, nutrients, primary production**
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19 **Regional Index Terms: New River Estuary and Neuse River Estuary, North Carolina,**
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1. Introduction

As the dominant primary producers in many estuaries, phytoplankton abundance and productivity play critical roles in determining water quality, fisheries yields, carbon and nutrient cycling (Paerl et al. 2014). Understanding the drivers of spatio-temporal patterns of phytoplankton is increasingly important for separating the effects of anthropogenic stressors (i.e. elevated nutrient and sediment loading) that are manageable at the watershed level from natural processes that are largely uncontrollable. It is becoming increasingly clear that natural externally-driven, short-term (hours to weeks) events and processes such as wind mixing events (Miller et al. 2006), floods and droughts (Paerl et al. 2014), heat waves and cold snaps (Cloern et al. 2005; Buskey et al. 1998) drive changes in estuarine phytoplankton communities by altering availability of nutrients and light, and changing the balance between population growth and losses due to grazing, settling, and advection from the system (Cloern and Jassby 2010; Peierls et al 2012). While the importance of these external short-term drivers on estuarine phytoplankton is well established, the importance of natural, internally-driven short-term processes has received comparatively less attention.

Vertical migration patterns represent a short-term process that is driven internally within the phytoplankton community. Synchronous diurnal vertical migration (DVM) is the most commonly documented migration pattern of phytoflagellates whereby cells swim to the well-lit surface waters during the day and migrate into deeper, often more nutrient rich, waters at night. Other vertical migration patterns include midday descents away from high near-surface irradiance (Passow 1991; Ault 2000; Hall and Paerl 2011), and asynchronous diel vertical migrations whereby only a portion of a flagellate community migrates to deeper depths on any particular night (Ralston et al. 2007).

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4 Changes in the depth distribution of phytoplankton coupled with the steep physical and
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6 chemical gradients in estuaries can result in dramatic increases in light and nutrient
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8 availability over short time (s to d) and spatial (cm to m) scales that can enhance intrinsic
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10 growth (Ault 2000; Hall and Paerl 2011; Peacock and Kudela 2014). At the population level,
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12 coupling between the depth distribution of phytoplankton and vertical gradients of horizontal
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14 current velocities may decrease advective losses (Anderson and Stolzenbach 1985; Crawford
15
16 and Purdie 1992). Additionally, mismatches in the vertical location of phytoplankton and
17
18 zooplankton patches can decrease grazing losses (Jones 1991; Salonen and Rosenberg 2000).
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20 Since phytoplankton taxa vary in their ability to regulate their depth, the vertical physical and
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22 chemical gradients that confer advantages on species more adept at depth regulation are
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24 important determinants of community structure (Margalef 1978; Ganf and Oliver 1982; Fogg
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26 1991) and may play an important role in determining the spatio-temporal patterning of
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28 estuarine phytoplankton assemblages.
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33 Water quality and ecosystem simulation models that are used to explore and predict
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35 impacts of human and natural drivers on estuaries rarely include vertical migration behaviors,
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37 even those that simultaneously model flagellated and non-flagellated phytoplankton
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39 functional groups (e.g. Cerco and Cole 1993; Heironymous and Bowen 2006). Is this effort
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41 to avoid overly complex models justified? Or, does it disregard an advantageous behavioral
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43 adaptation that is expressed with a high enough frequency to play an important role in
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45 shaping spatio-temporal patterns of estuarine phytoplankton assemblages and their impacts
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47 on production and biogeochemical cycling?
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55 We do not, therefore, always know until we have had a great deal of empirical
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57 experience, whether a given example of structure is very extraordinary, or a more trivial
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59 expression of something which we may learn to expect all the time (Hutchinson 1953).
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4 To our knowledge, there have been no studies that document how often the resultant
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6 temporal/ vertical structuring of phytoplankton biomass by vertical migrations occurs in
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8 estuarine systems. The simple presence of flagellate species with documented migration
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10 behaviors does not confirm that the behaviors are expressed. Under some physiological (e.g.
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12 strong nutrient deficiency) or environmental conditions (e.g. low temperatures/ steep salinity
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14 gradients), phytoflagellates may or may not undergo vertical migrations (Heaney and Eppley
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16 1981; Tyler and Seliger 1981; Doblin et al. 2006; Jephson and Carlsson 2009). The intensity
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18 and duration of intermittent turbulence also determines whether phytoflagellate swimming is
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20 effective in producing vertical movements of flagellate patches against the homogenizing
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22 effect of large turbulent eddies (Karp-Boss et al. 2000). Small-scale shear associated with
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24 turbulence can also impair migration at the cellular level through physical damage to flagella
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26 (Thomas and Gibson 1990). Shallow estuaries are highly susceptible to intermittent turbulent
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28 mixing from tidal and wind-driven shear, and the lack of persistent or seasonal stratification
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30 often leads to their characterization as being well-mixed (Mallin and Paerl 1992; Philips et al.
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32 2012). If median (i.e. most of the time) rates of vertical mixing in shallow estuaries are
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34 strong enough, vertical migration patterns may be a rather infrequent occurrence.
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43 This study examined four circumannual records of semi-hourly, decimeter-scale vertical
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45 profiles of chlorophyll in vivo fluorescence (IVF), salinity, temperature, and turbidity
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47 collected by autonomous vertical profiling (AVP) buoys at two sites in each of two shallow,
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49 microtidal estuaries, the Neuse River Estuary (NRE) and New River Estuary (NewRE),
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51 North Carolina, USA. Light and nutrient availability were monitored at each site to place
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53 observed phytoplankton depth distributions in the context of vertical gradients of growth
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55 limiting resources. The goals were to: 1) determine the frequency of occurrence of DVM
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populations, 2) determine the environmental conditions associated with DVM occurrence and the influence of DVM on access to light and nutrients, and 3) identify ecosystem-level ramifications of vertical migration on phytoplankton biomass, productivity, and composition.

2. Methods:

2.1. Study sites- Both the NewRE and NRE (Fig. 1) are shallow, microtidal estuaries with average depths and tidal amplitudes of ~1.5 m and 0.25 m in the NewRE (Ensign et al. 2004) and ~3.5 and < 0.1 m in the NRE (Luettich et al. 2000). Salinity ranges from oligohaline to polyhaline in the NewRE and oligohaline to mesohaline in the NRE (Hall et al. 2013; Luettich et al. 2000). Residence times for both estuaries are normally a few months but are longer during low river flows, and can be less than a week under very high flow conditions (Peierls et al. 2012). Both estuaries receive excessive anthropogenic nutrient loads and exhibit classic symptoms of eutrophication, including bottom water hypoxia, and harmful algal blooms comprised primarily of flagellated dinoflagellates and raphidophytes (Paerl et al. 1998; Hall et al. 2013; Mallin et al. 2005). Phytoplankton production of these estuaries is predominantly limited by nitrogen availability, but light availability is also an important factor (Mallin et al. 2005; Paerl et al. 2004). Suspended sediment concentrations in both estuaries are moderate but freshwater inputs contain high chromophoric dissolved organic matter (CDOM) levels (Anderson et al 2013; Woodruff et al. 1999) that create high background light attenuation even in the absence of phytoplankton absorption.

2.2. Autonomous Vertical Profilers- AVPs were deployed in the NewRE at Morgan Bay (34° 42.197' N, -77° 24.228' W) and Stones Bay (34° 35.758' N, -77° 24.690' W) (Fig. 1) on

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4 14 and 12 June 2008, respectively, and the data record analyzed ended on 31 September
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6 2012. Deployments in the NRE were conducted at NRE120 (34° 56.933' N, -76° 48.912' W)
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8 from 19 November 2001- 26 December 2002, and at NRE180 (35° 3.848' N, -76° 31.560'
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10 W) from 25 May 2003- 26 August 2004. Average depths at Morgan Bay, Stones Bay,
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12 NRE120, and NRE180 deployment sites were 3.0, 2.5, 4.6, and 6.9 m, respectively. The
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14 AVPs consisted of a moored floating platform housing a computer controlled winch
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16 mechanism (Reynolds-Fleming et al. 2002) that casted a YSI 6600 sonde (Yellow Springs
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18 Inc, Yellow Springs, Ohio). Profiles of temperature, salinity, turbidity, pH, dissolved oxygen
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20 and IVF of chlorophyll were produced every 30 minutes. The YSI 6025 IVF probe (model
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22 6025) utilizes a 470 nm light emitting diode excitation source and 650-700 nm band pass
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24 emission filter (YSI 6-Series User's Manual). Approximately ten percent of the water depth
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26 at the surface and bottom was not measured by the AVPs. Winch speed and sampling
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28 frequency of the YSI 6600 data sondes were set to achieve a nominal depth resolution of 10
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30 cm, except 25 cm at NRE120. During post-deployment data processing, data from the AVPs
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32 were gridded using 30 minute by 10 cm bins (except 25 cm bins at NRE120). Sondes were
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34 exchanged with cleaned and calibrated sondes on a weekly basis at sites NRE120 and
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36 NRE180, and a monthly basis in the NewRE following an improved anti-fouling design that
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38 kept the sonde above the water between casts. Turbidity and IVF probes were automatically
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40 wiped hourly to reduce fouling. All calibrations were performed according to the YSI user's
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42 manual, including a two-point calibration of IVF using deionized water and a 0.1 g L⁻¹
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44 rhodamine-*b* solution. Post calibration of the IVF data was not performed. Eastern Standard
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46 Time was maintained for all data.
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4 **2.3. Meteorological data-** In the NewRE, anemometers (RM Young Marine Wind Monitor
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6 0510) mounted to each AVP measured six minute average wind speed and direction every
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8 thirty minutes. Daily average wind speed for NRE120 were obtained from Marine Corp Air
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10 Station Cherry Point (Fig. 1; site KNKT 34°54.18' N, 76°52.85' W) through the State
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12 Climate Office of North Carolina. Hourly average wind speed for NRE180 was obtained
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14 from the National Data Buoy Center station CLKN7 located (34°37.30' N, 76°31.50 W) 45
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16 km south of station NRE180 (Fig. 1). Wind data from these sources accurately portray wind-
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18 stress at the two NRE sites (Reynolds-Fleming and Luetlich 2004; Whipple et al. 2006).
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21 Hourly average solar radiation (400-1100 nm) for the NRE sites came from the US EPA
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23 CASTNET site BFT142 (34° 53.088'N, 76°37.218'W) (Fig. 1) (www.epa.gov/castnet). For
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25 NewRE sites, hourly average solar radiation data came from the U.S. Forest Service Remote
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27 Automated Weather Station site CLJN7 (34°38.84' N, 77°29.11 W) (Fig. 1).
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36 **2.4. Light attenuation, nutrients, and phytoplankton-** Biweekly downstream transects of 11
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38 sites in the NRE and monthly transects of 8 sites in the NewRE were performed for sample
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40 collection and in situ measurements (Fig. 1). At NRE120 and NRE180, and Stones Bay
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42 (station 3 of the sampling transect) in the NewRE, samples were collected within 100 m of
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44 the AVP. Of the four proximal transect stations (5,6,7 & 8), transect station 5 was chosen to
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46 represent the Morgan Bay AVP location because it had the most similar salinity, dissolved
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48 oxygen, and IVF to measurements made by the AVP. Depth profiles of photosynthetically
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50 active radiation (PAR), temperature, salinity, dissolved oxygen, pH, and turbidity were made
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52 at each transect station at 0.5 m increments using a YSI 6600 multiparameter water quality
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54 sonde (Yellow Springs Inc, Yellow Springs, Ohio) linked to a LI-COR LI-192 quantum
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4 sensor (LI-COR Biosciences, Lincoln, Nebraska). Diffuse attenuation coefficients (K_d) were
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6 calculated according to Beer's law and euphotic zone depth was calculated as the depth of
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8 1% incident PAR. Water samples for determination of nutrient concentrations, phytoplankton
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10 biomass and community composition were collected from surface (0.2 m) and 0.5 m above
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12 the bottom and analyzed according to methods described in Peierls et al., (2012).
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19 **2.5. Data analyses-** Average daily patterns of relative IVF depth distribution from the AVP
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21 data were determined at each site for winter (Dec-Feb), spring (Mar-May), summer (Jun-
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23 Aug), and fall (Sep-Nov) seasons. Each IVF profile was converted to a relative IVF profile
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25 by dividing IVF at each depth by the mean profile IVF. For each season, relative IVF
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27 measurements at each depth interval and time of day (e.g. all measurements made at 1.1 m
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29 depth and 12:30 AM) were averaged.
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33 Density was calculated from salinity and temperature (Pond 1983) and stratification
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35 intensity ($\Delta\rho$ in kg m^{-3}) was calculated as the density difference between deepest and
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37 shallowest depths measured. The center of mass of the IVF depth distribution for each profile
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39 (Z_{COM}) and depth of the IVF maximum (Z_{MAX}) were used as metrics of changes in
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41 phytoplankton depth distribution that are independent of absolute IVF concentrations (Pearre
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43 1979). Even for a vertically homogenous IVF profile, changes in water depth due to tides
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45 and/or seiches could produce a daily or semi-diel cycle in Z_{COM} . For example, the Z_{COM} for
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47 homogenous IVF profiles in 3 and 3.5 m of water would be 1.5 m and 1.75 m, respectively.
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49 The effect of changes in water depth on variability in Z_{COM} were minimized by subtracting
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51 half of the depth of the water column from the Z_{COM} for each profile, effectively referencing
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53 Z_{COM} to the mid-depth of the water column rather than the surface. Data segments with depth
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profiles that were missing more than one third of their measurements and days when more than one third of profiles were missing were excluded from analyses.

The occurrence of appropriately-phased (i.e. up during day/ down at night) daily cycles in Z_{COM} and Z_{MAX} were used to objectively categorize whether or not DVM occurred during the AVP deployments. Wavelet spectral analysis (Torrence and Compo 1998) was used to visualize the timing of DVM cycles within the Z_{MAX} records. Prior to wavelet analyses, Z_{MAX} records were demeaned and data gaps were filled with zeros. Statistically significant diurnal cycles in Z_{MAX} were identified when the lower 95% confidence interval of power was greater than the 95% significance level (Torrence and Compo 1998). When significant diel power was observed, the phase of the wavelet transform ($\theta_{Wn(s)}$) was calculated as $\theta_{Wn(s)} = \tan^{-1}[\Im\{W_n(s)\}/\Re\{W_n(s)\}]$, where $\Im\{W_n(s)\}$ and $\Re\{W_n(s)\}$ are the imaginary and real parts of the wavelet transform (W) at wavelet scale (s) and time point (n) (Torrence and Compo 1998). Phase was calculated for the observed diel signal and the diurnal solar cycle represented by a cosine wave starting at 00:00 midnight and the phase shift was calculated by subtraction. A phase shift of 0 h indicates that the phase of the diel signal is perfectly synchronized with the solar cycle (i.e. nearest surface at 12:00 and nearest bottom at 00:00).

The Morlet wavelet (wavenumber = 6) smooths power of diel signals across several days which makes it inappropriate for assessing whether DVM occurred on any particular day. To determine whether DVM occurred on a day-by-day basis, least-squares harmonic analyses were conducted on Z_{COM} and Z_{MAX} for each day of data. A positive DVM detection was defined by a significant positive correlation ($p < 0.05$) between the observed diel cycle in both Z_{COM} and Z_{MAX} with a cosine wave nearest surface at noon (Hall and Paerl 2011).

For each day, binary scores of DVM occurrence (1= DVM detected; 0 = DVM not detected) were compared against daily mean values of environmental parameters to understand the dominant factors that lead to a vertically migrating phytoplankton community. Variables considered were daily means of depth-averaged salinity, depth-averaged temperature, depth-averaged turbidity, depth-averaged IVF, stratification intensity ($\Delta\rho$), wind speed, and incident solar radiation. Multiple logistic regressions were constructed for data collected from each AVP station with DVM occurrence as the response variable and the environmental parameters as independent variables. As a complement to the logistic regressions, classification trees were developed for the occurrence of DVM at each AVP site to visualize the impacts of the environmental predictors and to identify thresholds, which may lead to or allow DVM occurrence. Classification trees were grown using Matlab's "classregtree" routine which follows the CART algorithms of Breiman et al. (1984) including use of the Gini index as a splitting criteria, use of 10 fold cross validation for estimates of model error, and pruning by finding the smallest tree whose cost is within 1 standard error of the minimal cost tree. To prevent overly complex trees, minimum parent node and leaf size were set at 10 and 5 percent of the total number of observations (Adolf et al. 2006).

2.6. Productivity Model- A simple light $\times$ biomass model was used to estimate the influence of observed migration patterns on primary production. IVF profiles were converted to units of Chl *a* using the relationship $\text{Chl } a = \text{IVF} \times 0.72 + 3.29$ (Hall et al. 2008). This conversion has no effect on interpreting how migration patterns impact production. Total solar radiation (W m^{-2}) was multiplied by 2.04 to estimate incident PAR ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) (Fisher et al. 2003) and was linearly interpolated from hourly to semi-hourly time intervals.

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4 Turbidity, IVF, salinity, and light attenuation data collected during bi-weekly or monthly
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6 monitoring transects were used to construct linear regression models of light attenuation (K_d)
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8 in the water column for each AVP station. Salinity, IVF, and turbidity strongly relate to
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10 concentrations of CDOM (Bowers and Brett 2008; Anderson et al. 2013), phytoplankton, and
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12 other particulates that govern light attenuation (Anderson et al. 2013; Woodruff et al. 1999).
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14 The regressions explained 60-75% of the variation in K_d at three AVP sites where K_d varied
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16 from $\sim 1\text{-}3\text{ m}^{-1}$ but only explained 44% of the variability at NRE180 where K_d only ranged
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18 from $\sim 0.5\text{--}1.5\text{ m}^{-1}$ (see Appendix A). Using modeled light attenuation and Beer's Law, semi-
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20 hourly PAR profiles were constructed from incident PAR and salinity, turbidity, and IVF
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22 collected by the AVPs. Productivity at each depth within each profile was estimated from
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24 PAR as $P = B P_{\max}^B (\tanh(\alpha * \text{PAR} / P_{\max}^B))$, where P is primary productivity, $P_{\max}^B$ is the
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26 maximum production rate normalized to biomass (B) measured as Chl a , and α is the initial
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28 light limited slope (Jassby and Platt 1976). A model that included photoinhibition was
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30 considered but was rejected because as photosynthesis becomes photoinhibited, non-
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32 photochemical quenching (NPQ) of IVF should cause the estimate of B to be reduced by a
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34 similar proportion to the decrease in P^B (Vincent et al. 1984). Thus, inclusion of
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36 photoinhibition in the production model would have effectively double-counted the influence
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38 of photoinhibition. $P_{\max}^B$ and α were estimated as the mean values [$2.47\text{ g C g Chl } a^{-1}\text{ h}^{-1}$ and
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40 $0.0087\text{ g C g Chl } a^{-1} (\mu\text{mol photons m}^{-2}\text{ s}^{-1})^{-1}$] reported by Buzzelli et al. (2001) from natural
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42 phytoplankton communities in the lower NRE.
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53 To understand the influence of observed vertical variation in biomass on productivity,
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55 productivity based on observed Chl a profiles $B(z)$ was compared to a hypothetical case of a
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57 vertically homogenous Chl a distribution (B_{mean}) derived from the mean of each profile (Hall
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and Paerl 2011). Differences between daily production of observed and hypothetically-mixed profiles were calculated by computing the productivity of the profile of deviations of $B(z)$ from B_{mean} (i.e. $B(z) - B_{\text{mean}}$). The daily integral of the productivity of the deviation profiles were then normalized by the daily integral productivity of the hypothetically-mixed profiles to calculate the relative difference in productivity produced by vertical variation in B .

A sensitivity analysis was performed to determine how relative differences might be affected by choice of photosynthetic parameters and differences in photosynthetic parameters between the vertically migrating and non-migrating components of the phytoplankton community. Productivity calculations were rerun for both the deviation profiles and mean profiles with P_{max}^B set as before but with α adjusted to achieve the minimum ($81 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and maximum E_k ($393 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) values reported for the NRE (Buzzelli et al. 2001; Boyer et al. 1993). Calculations were rerun alternately using the highest and lowest α values for both the deviation profile and profile means to provide upper and lower bounds for the relative difference in production due to biomass heterogeneity.

3. Results

3.1. Environmental conditions during AVP deployments- At all AVP deployment sites, average water column temperature varied seasonally from $< 10^\circ\text{C}$ in January to $\sim 30^\circ\text{C}$ in August (Fig. 2A; Appendix B). Small-amplitude ($2\text{--}4^\circ\text{C}$), synoptic-scale temperature fluctuations were also observed. The importance of weather as a driver of these systems is exemplified by the solar radiation records (Fig. 2B and Appendix B) which exhibited daily to weekly variation that accounted for more than half of the total variability (Fig. 2B). Due to the ocean's high heat capacity, peak temperatures lagged solar radiation by about a month.

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4 Salinity at all sites tended to increase during the warmer months when river flow is low
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6 but this pattern was rapidly disrupted or amplified by weather events. For example, Tropical
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8 Storm Nicole led to a near freshening of the NewRE in late September 2010 when the estuary
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10 would have been near annual maximum salinity (Fig. 3 A and C). In contrast, multiple
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12 nor'easter storms in the winter of 2009-2010, amplified the seasonal cycle resulting in a
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14 multi-month period of depressed salinities (~10 at Morgan Bay and ~15 at Stones Bay). In
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16 the NRE, lower salinities at station NRE180 compared to the upstream station NRE120 (Fig.
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18 3 E and G), were driven by drought during the station NRE120 deployment, and unusually
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20 high precipitation in summer 2003 during the deployment at NRE180 (Paerl et al. 2010).
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25 In the NewRE, maximum stratification intensities were generally observed during low
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27 salinity periods when freshwater input was greatest (Fig. 3 A-D). However, even during these
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29 low salinity periods, stratification was frequently interrupted by synoptic-scale mixing
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31 events. The relationship between average water column salinity and stratification intensity
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33 was not obvious for the NRE (Fig. 3 E-H). In either estuary, continuous stratification with
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35 intensities greater than $\sim 2 \text{ kg m}^{-3}$ rarely lasted more than a few weeks.
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41 Light attenuation increased during high river flow, low salinity periods when CDOM
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43 concentrations are higher and riverine nutrient inputs stimulate higher phytoplankton biomass
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45 accumulation (Fig. 3 A,C,E, and G). With respective average depths of 3.0, 4.6, and 6.9 m,
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47 the lower portion of the water column was commonly below the euphotic zone at Morgan
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49 Bay, NRE120, and NRE180 (Fig. 3 A, E, and G). With lower light attenuation and shallower
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51 depth (2.5 m), the euphotic zone most often extended to the bottom at Stones Bay (Fig. 3 C).
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55 At the AVP stations, relatively high concentrations ($> 4 \mu\text{mol L}^{-1}$) of DIN in the surface
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57 waters occurred infrequently (Fig. 4 A-D). The majority of the time, surface water DIN was
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4 ~1 $\mu\text{mol L}^{-1}$ or less (Fig. 4 A-D). The relationship of surface water DIN with temperature
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6 was significantly negative at Morgan Bay (Fig. 4A) but exhibited no significant trends with
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8 temperature at the other stations. Bottom water DIN exhibited significant positive
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10 relationships with temperature at both NRE stations (Fig. 4 C, D). When water temperature
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12 was > 15 °C, bottom water DIN was generally greater than surface water DIN and during
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14 cooler periods surface water DIN was generally higher than in the bottom waters. This
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16 resulted in strong correlations between temperature and vertical DIN gradients at Stones Bay,
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18 NRE120, and NRE180, and a near significant correlation at Morgan Bay (Fig. 4 A-D).
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24 Additional records of environmental parameters can be found in Appendix B and
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26 information on downstream gradients and river flow for the two estuaries during the study
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28 period can be found in Paerl et al. (2010) and Hall et al. (2013).
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34 **3.2. Example observations from the AVP at NRE180-** From 4 - 13 November 2003, there
35
36 was a strong pattern of DVM interrupted by two wind mixing events that vertically
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38 homogenized the density and IVF distributions (Fig. 5). For the first 4 d, the water column
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40 was stratified (Fig. 5 D) with moderate ($<5 \text{ m s}^{-1}$) southeast to southwest winds (Fig. 5 E). A
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42 clear pattern of DVM was apparent with near surface accumulation of IVF (0.5 – 2 m) during
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44 the daytime and nocturnal aggregations near to or below the pycnocline at 5 – 6 m depth
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46 (Fig. 5 A, D). Both Z_{MAX} and Z_{COM} tracked the pattern of DVM well and resulted in positive
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48 assessments of DVM occurrence (Fig. 5 B). On 8 November, a mixing event driven by strong
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50 northeast winds ($\sim 12 \text{ m s}^{-1}$) homogenized both the phytoplankton and density distributions.
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52 Z_{MAX} was highly variable, Z_{COM} remained very close to the center of the water column, and a
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54 significant DVM pattern was not detected during the mixing event. A weak yet significant
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DVM pattern was detected on 9 November as the winds began to decrease. On 10 November, the wind relaxed, the water column restratified, and the strong DVM pattern in IVF, Z_{MAX} , and Z_{COM} resumed. On 13 November, a strong southwest wind mixed the water column again, and no DVM pattern was detected.

3.3. Seasonality of average daily fluorescence depth distributions- During summer and fall at all four AVP stations, the average daily IVF depth distributions showed obvious patterns indicative of DVM with elevated IVF near the surface during day and deeper in the water at night (Fig. 6). There was also an indication of avoidance of near surface waters at midday. The midday descent of the IVF maximum to 1.5 – 2 m depth was most pronounced at Stones Bay, particularly in summer (Fig. 6G) but the pattern was also apparent at NRE120 during winter and summer (Fig. 6 I,K). During the winter, highest IVF concentrations were often found just above the bottom and the only indication of DVM was a midday reduction of IVF along the bottom. In spring, IVF was highest along the bottom throughout the day at all stations except NRE180. During spring, there was an obvious DVM pattern at NRE180, and indications of weak DVM patterns at NRE120, Morgan Bay, and Stones Bay.

3.4. Frequency of diel vertical migration detection- Wavelet power spectra of Z_{MAX} revealed intermittent periods of diel oscillations for all four AVP stations (Fig. 7). Deeper AVP stations exhibited higher maximum magnitudes of diel power (note different scale bars for contour plots); an effect driven primarily by the higher maximum amplitudes of diel oscillations that are possible in deeper water.

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4 At Morgan Bay in the NewRE, the diel peak in power dominated the power spectra (Fig.
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7 7A). Peaks in diel power occurred throughout the year but extended spans of diel power
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9 lasting a month or more were most common during summer and fall. At Stones Bay (Fig.
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11 7B), peaks in diel power were obvious but were weaker and occurred less frequently than at
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13 Morgan Bay. Unfortunately, data gaps due to equipment malfunction occurred during many
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15 of the time spans with strong diel power. For example during late summer and fall of 2009,
16
17 three data gaps of a week or more make it impossible to determine whether the period of diel
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19 power was continuous. The Z_{MAX} record at NRE120 also had episodic peaks in diel power
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21 that were observed commonly during late spring through summer (Fig. 7C). Large data gaps
22
23 occurred during fall and winter, but peaks in diel power were still detected in late fall of 2001
24
25 and 2002. The diel peak in power at NRE180 dominated the power spectra (Fig. 7D). Data
26
27 gaps in the NRE180 record were concentrated in the summer and fall. However, when data
28
29 was collected during these seasons, a prominent peak of diel power was invariably observed.
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31 During winter, diel oscillations occurred occasionally at NRE180 but from mid-January to
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33 mid-March 2004 diel power was low and similar to higher and lower frequencies. This time
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35 frame coincided with a prolonged period of very low stratification intensity (Fig. 3 H).
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43 Power at longer and shorter periods than the diel period was also evident but the only
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45 other consistent pattern was a band of power with a period of 0.5 d. The 0.5 d oscillation in
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47 Z_{MAX} was apparent at all four AVP stations (Fig. 7 A-D), and commonly but not exclusively,
48
49 occurred during periods of elevated diel power.
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53 When power of the diel oscillation in Z_{MAX} was statistically significant, the phase at
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55 NRE120, NRE180, and Morgan Bay was highly consistent (74, 91, and 73 % of cases within
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57 +/- 3 h phase shift) with the up during midday, down at midnight pattern expected from
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DVM (Fig. 8A-C). At Stones Bay, Z_{MAX} exhibited a significant diel oscillation on approximately the same number of days as at Morgan Bay. However, the phase of the diel cycle (Fig. 8D) was split nearly evenly between the phase expected from DVM and a diel oscillation with a +/- 12 h phase shift (i.e. Z_{MAX} lowest in the water column at midday and highest in the water column at night). This bimodal phase distribution at the diel period at Stones Bay and the semi-diel (0.5 d period) period at all sites both likely reflect a downward midday migration in response to high surface irradiance. In combination with a strong DVM pattern, a midday descent can be detected as a semi-diel cycle by wavelet analysis, while the phase of the diurnal signal is still indicative of DVM (see Appendix C). This pattern occurred commonly at NRE120, NRE180, and Morgan Bay. An example from a single day can be seen in Fig. 5 on 6 November, when the depth of Z_{MAX} declined by ~ 1 m at midday, returned to nearer the surface later in the afternoon, and then migrated to ~4 m depth at night. However, if the amplitude of the midday descent is stronger than the DVM signal, peak power is at the diel period, but the phase of the diel signal is shifted by half a diel cycle (12 h) (Appendix C). This scenario that was apparently more common at Stones Bay.

Based on least-squares harmonic analyses, significant diel cycles with the appropriate up during day/ down at night phase of DVM were detected in both Z_{MAX} and Z_{COM} simultaneously on about half of days sampled at NRE180, NRE120, and Morgan Bay, but only ~20 percent of days at Stones Bay (Table 1).

3.5. Relationship of DVM detection with environmental conditions- Logistic regressions determined highly significant, positive relationships between temperature and DVM occurrence at all four stations (Table 2). Among the sites, regression trees identified a

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4 remarkably consistent threshold temperature of ~14-23 °C above which DVM was more
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6 likely (Fig. 9 A-D). A negative relationship of DVM occurrence with incident solar radiation
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8 was the only other significant environmental relationship common to all four sites. The
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10 regression tree for NR180 indicated that DVM was more frequent when incident solar
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12 radiation levels were lower than 251 W m⁻² (Fig. 9D), a value that would only be observed
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14 from spring through fall on clear-sky days (Fig. 2B). At the level of pruning used, regression
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16 trees for other sites did not form branches based on solar radiation but more complicated
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18 trees showed branching at similar solar radiation levels (not shown). DVM occurrence was
19
20 positively related to phytoplankton biomass measured as IVF at all stations except NRE120,
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22 and was negatively related to salinity at all stations except NRE180 (Table 2; Fig. 9). A
23
24 positive relationship between DVM occurrence and stratification intensity was only observed
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26 at Morgan Bay (Table 2) and, the regression tree identified a threshold stratification intensity
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28 of 1.5 kg m⁻³, above which DVM occurrence was more likely (Fig. 9A). DVM occurrence
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30 was negatively related to wind speed at Morgan Bay and NRE180 (Table 2), particularly for
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32 wind speeds greater than ~ 8 m s⁻¹ at NRE180 (Fig. 9D).
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41 Vertical nutrient gradients were not measured at a sufficient resolution for inclusion in
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43 the logistic regressions or regression trees. However, a comparison of DVM occurrence on
44
45 days when surface and bottom water DIN were measured indicated that DVM was about fifty
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47 percent more common at Morgan Bay, NRE120, and NRE180 when DIN increased with
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49 depth (Table 1). At Stones Bay, DVM occurred with a low and equal frequency regardless of
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51 the vertical DIN gradient (Table 1).
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4 **3.6. Influence of vertical migration on primary productivity-** On days when DVM was
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6 detected, the daily production estimate based on IVF derived Chla profiles was greater than
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8 under the hypothetical case of a vertically homogenous Chla distribution (Table 3; Fig. 5C).
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10 At times, the productivity enhancement was substantial. For example on 11 Nov. 2003 at
11
12 NRE180, DVM more than doubled the midday productivity maximum (Fig. 5C). However,
13
14 median values for the relative differences in production between observed and
15
16 hypothetically-mixed profiles were generally < 5%. On days when DVM was not detected,
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18 modeled productivity was slightly higher for a homogenous Chla profile.
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24 Altering the shape of the photosynthesis versus irradiance model for both the
25
26 hypothetically-homogenous Chla profile and for the deviations resulting from migration
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28 patterns resulted in minor changes to the increased production gained by observed migration
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30 patterns. As expected, the largest relative differences were calculated when the light
31
32 utilization efficiency (α) of the deviations was set to its maximum, and α for the mean
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34 profile was set to the minimum. However, even under this best-case scenario for maximizing
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36 productivity through migrations, the highest median relative difference between the observed
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38 and hypothetically-mixed profile was only around ten percent (Table 3).
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45 46 **4. Discussion**

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48 **4.1. Phytoplankton distributions and mixing regimes-** The observation of diel oscillations in
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50 phytoplankton depth distributions on half or more of days at all stations indicates that most of
51
52 the time vertical mixing in these shallow estuaries is weak enough that phytoflagellate
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54 swimming provides effective depth regulation. Rather than well-mixed, intermittently-mixed
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56 is a more accurate portrayal of the mixing regime of these shallow estuaries. Between mixing
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4 events, biomass heterogeneity is the norm. With swimming speeds of 1-2 m h⁻¹ (Sommer
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7 1988), phytoflagellates can traverse the whole water column of these shallow estuaries within
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9 a few hours to access steep gradients of light and nutrient resources.

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12 Half of the time is likely a conservative estimate of the percentage of days that flagellates
13
14 exhibited vertical migration behaviors. In order to detect a DVM signal from the IVF
15
16 profiles, the migratory component of the phytoplankton must be sufficiently large to stand
17
18 out amongst the background variability and patchiness of the non-migratory (i.e. diatoms,
19
20 picocyanobacteria) component. This effect likely drove the positive relationship between
21
22 average water column IVF and DVM occurrence at three of the four AVP sites (Table 3)
23
24 because high biomass blooms are most often formed by flagellates in these estuaries (Hall et
25
26 al. 2013; Pinckney et al. 1998). However, when the migrating community constituted a small
27
28 proportion of the total phytoplankton community, it may have gone undetected. DVM, as
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30 defined by the methods used here to detect it, corresponds to the symmetric up during the
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32 day, down at night migration of the IVF distribution. Deviations from synchronous DVM do
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34 occur, including ascents throughout the photoperiod to achieve near-surface accumulations in
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36 the afternoon or evening (Sullivan et al. 2010; Kiefer and Lasker 1973), and descents at mid-
37
38 day as was commonly observed in this study at Stones Bay. These migration behaviors add
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40 error to the DVM signal and likely led to some days being classified as “DVM negative”,
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42 when, in fact, both DVM and a midday descent occurred.
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51 Negative relationships between DVM occurrence and wind speeds at NRE180 and at
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53 Morgan Bay in the NewRE, and the positive relationship with stratification intensity at
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55 Morgan Bay were consistent with mixing playing an important role in determining
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57 occurrence of DVM. However, the relatively modest stratification threshold of 1.5 kg m⁻³
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4 that was identified at Morgan Bay suggests that even during weak stratification, rates of
5
6 vertical mixing may be insufficient to overcome directed swimming speeds of flagellates.
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8 Some flagellates are capable of reorienting their swimming direction faster than the rotation
9
10 rates of turbulent eddies (Karp-Boss et al. 2000). If this is common among phytoflagellates,
11
12 then turbulent mixing does not influence the oriented swimming behavior of phytoflagellates,
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14 and vertical movements of patches are possible under all but the most turbulent conditions
15
16 when large eddies destroy patches through deep, random advection (Karp-Boss et al. 2000).
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18 Mixing due to intermediate sized eddies would lead to more diffuse patch distributions but
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20 diel oscillations in Z_{MAX} and Z_{COM} may still be apparent (Yamazaki et al. 2014).
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26 The lack of significant relationships of DVM occurrence with wind speed or stratification
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28 at the other sites does not indicate that mixing was not an important driver of DVM
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30 occurrence at these sites. However, it may indicate that daily averages of stratification
31
32 intensity or wind speed are poor proxies for mixing. The combination and interaction of
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34 shear and the inhibiting effect of buoyancy forces from stratification collectively determine
35
36 the structure of turbulence in the water column. For example, a downstream wind (as
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38 opposed to upstream wind) can enhance shear flows but also strain the longitudinal density
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40 gradient and enhance stratification intensity with substantial reductions in eddy diffusivity
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42 throughout the water column (Scully et al. 2005). A more complex parameter that
43
44 incorporated both wind speed and direction and its interaction with stratification may have
45
46 provided greater explanatory power for occurrence of DVM. Additionally, wind and
47
48 stratification may vary significantly within a single day (Fig. 5 D, E). In combination with
49
50 continuous directed swimming, turbulence intermittency may create short periods of strong,
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52 deep mixing when swimming is ineffective at depth regulation followed by periods of weak
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4 mixing that allow effective population movements (Karp-Boss et al 2000). A daily mean
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6 would not capture the impacts of these short-term fluctuations in mixing on the efficacy of
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8 motility for vertical migration.
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11 Another possible explanation for the insignificant relationship between stratification
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13 intensity and DVM occurrence at most of the sites is that a relationship does exist but is non-
14
15 monotonic. Laboratory studies have shown that many phytoflagellate species do not cross
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17 very steep haloclines or thermoclines (Jephson and Carlsson 2009; Kamykowski 1981a), and
18
19 can be trapped within layers of enhanced shear (Durham et al. 2009). Thus, there may be an
20
21 intermediate level of stratification where mixing is slow enough to allow effective directed
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23 swimming, and the salinity/temperature/shear gradients along the pycnocline are weak
24
25 enough to allow crossing by phytoflagellates. Logistic regression (a monotonic function)
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27 would not capture such a complex, non-monotonic relationship.
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36 **4.2. Other potential sources of the diel vertical migration pattern-** The consistent up during
37
38 the day/ down at night phase and observation of the observed diel oscillation in both Z_{MAX}
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40 and Z_{COM} eliminates many other potential sources of the diel cycles attributed to DVM. If
41
42 tidal forcing produced the observed diel signals, the phase of the observed diel signal would
43
44 have shifted about fifty minutes each day, resulting in a more even distribution of phases
45
46 over the course of the circumannual deployments. The diel sea breeze could also drive a diel
47
48 oscillation in Z_{COM} by resuspending microphytobenthic cells during the day when wave-
49
50 stress reaches a maximum followed by settling during the night when wind speed relaxes
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52 (Blauw et al. 2012). However, under such a scenario where biomass is mixed upwards from
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54 the sediments, Fick's law dictates that maximum IVF should always be observed nearest the
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4 sediments, and there should have been no diel signal observed in Z_{MAX} . Additionally, a diel
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6 oscillation driven by the sea breeze should peak in the late afternoon when wind speeds are
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8 maximum (Litaker et al 1988), rather than near noon.
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11 A diel imbalance between phytoplankton growth and grazing losses could produce a diel
12
13 cycle in IVF depth distribution if the imbalance were more pronounced in the surface layer
14
15 (Sournia 1974; Litaker et al. 1988). Available evidence indicates that diel biomass maxima
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17 occur near the end of the photoperiod (Sullivan et al. 2010; Litaker et al. 1988). So, the
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19 expected phase of such a signal would also be shifted by ~ 6 h from the observed oscillations.
20
21 Additionally, such a cycle driven by growth and grazing would not explain the observed
22
23 nocturnal accumulation of IVF at depth (Fig. 5A & Fig. 6). Detection of gut fluorescence
24
25 within migratory mesozooplankton is also unlikely to produce the observed DVM patterns
26
27 because the phase would have shown the opposite pattern with increases near the surface at
28
29 night and near the bottom during the day (Fulton 1984; Mallin and Paerl 1994). A depression
30
31 of near surface IVF during the day due to depression of fluorescence yield by NPQ induced
32
33 by high near surface irradiance could generate a diel oscillation in Z_{COM} and Z_{MAX} (Kiefer
34
35 1973). Again, however, the phase generated by NPQ would be out of phase (180 degrees or
36
37 12 h) with the observed signal. Recovery of fluorescence yield from NPQ is complete in less
38
39 than an hour of darkness. So its impact on fluorescent yield is minimal at night (Falkowski
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41 and Kiefer 1985), and NPQ cannot explain the nocturnal reduction of IVF near the surface
42
43 and increase of IVF at depth. Elimination of other potential mechanisms that could have
44
45 produced a DVM like pattern in the IVF profiles coupled with previous observations of
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47 DVM in the NRE based on cell densities (Hall and Paerl 2011), provides strong evidence that
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49 the observed patterns were due to phytoflagellate DVM.
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7 **4.3. Temperature and vertical nutrient gradients-** The proximal cause for the strong
8
9 relationship between DVM occurrence and temperature may be related to reduced swimming
10 speeds due to enhanced kinematic viscosity (Larsen and Riisgard 2009) or inability to cross
11 haloclines at low temperature (Kamykowski and McCollum 1986; Tyler and Seliger 1981).
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13 Low abundance of phytoflagellates is an unlikely explanation because dinoflagellate blooms
14 are common during winter in these estuaries (Hall et al. 2013; Pinckney et al. 1998).
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21 The warm period from late spring through fall corresponds to annual maxima in
22
23 phytoplankton production and generally to minima of external riverine nutrients inputs
24 (Boyer et al. 1993; Hall et al. 2013). As a result, from late spring through early fall, surface
25
26 water nutrient availability, particularly of growth limiting dissolved inorganic nitrogen
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28 (Mallin et al. 2005; Pinckney et al. 1999), is generally very low ($\sim 1 \mu\text{mol L}^{-1}$) throughout the
29
30 middle and downstream regions of these estuaries (Fig. 3D,H; Fig. 4 D,H) (Hall et al. 2013;
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32 Peierls et al. 2012; Twomey et al. 2005). As temperature warms to 15-20 °C, increased
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34 respiration rates in the sediments drive the oxycline upwards (Anderson et al. 2013) and
35
36 fluxes of ammonium and phosphate from the sediments increase dramatically (Fisher et al.
37
38 1982; Anderson et al. 2013). Capacity for nocturnal nutrient retrieval by DVM
39
40 phytoflagellates is an important factor in determining their competitive success in systems
41
42 where surface waters are nutrient deplete but higher nutrient concentrations exist at depth
43
44 (Salononen et al. 1984; Amano et al. 1998; Fraga et al. 1992). Regardless of the proximal
45
46 cause for the elevated frequency of DVM during warmer months, the pattern is well attuned
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48 for maximizing growth within the seasonal pattern of vertical nutrient gradients.
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4 On a day-to-day basis, DVM also occurred when DIN was higher in the surface waters
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6 (35-50% of days between the sites; Table 1). Migrating into deeper layers when surface
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8 waters are nutrient replete appears contrary to a growth-optimization strategy at the cellular
9
10 level, but has been previously documented in both field and laboratory studies (Handy et al.
11
12 2005; Cullen and Horrigan 1981; Eppley et al. 1968). Such behaviors, however, may be
13
14 advantageous at the population level if nocturnal migration below the downstream-flowing
15
16 surface layer minimized advective losses (Anderson and Stolzenbach 1985) or reduced
17
18 encounter rates with migratory zooplankton predators (Salonen and Rosenberg 2000).
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26 **4.4. Light-** Although both of these estuaries are shallow, rapid light attenuation creates a
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28 situation, where on average, bottom waters are below the euphotic zone (Fig. 3) and strongly
29
30 limiting to microalgal growth (Mallin and Paerl 1992; Anderson et al. 2013). With such steep
31
32 light gradients, the ability to minimize settling into aphotic bottom waters is a significant
33
34 advantage gained by motility. This would be particularly the case during high flow
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36 conditions when long-lived stratification events are most common (Fig. 3) and high CDOM
37
38 concentrations lead to the most intense light limitation (Anderson et al 2013). The ability to
39
40 access light under nutrient-replete, high-flow conditions may allow flagellates to take
41
42 advantage of pulses of riverine nutrients, and may significantly contribute to the high
43
44 proportion of blooms dominated by phytoflagellates in these estuaries, including harmful
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46 dinoflagellate and raphidophytes (Carstensen et al. this issue; Hall et al. 2013).
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53 Beyond simply enhancing light availability, IVF patterns showed indications of responses
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55 to short-term, hourly to daily, changes in incident light that are consistent with migrations to
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57 optimize light levels for photosynthesis and minimize exposure to damaging near-surface
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4 irradiance. Previous studies have shown that phytoflagellates often halt their daytime ascent
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6 or descend away from the surface near midday to avoid damaging levels of irradiance on
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8 clear sky days. Conversely, on cloudy days phytoflagellates often form dense near-surface
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10 aggregations (Ault 2000; Passow 1991; Hall and Paerl 2011). The resultant relationship
11
12 between incident light and the amplitudes of vertical excursion during DVM would increase
13
14 the likelihood of detecting signals in both the Z_{COM} and Z_{MAX} records on days with low
15
16 incident light, and may explain the consistently observed negative relationship between
17
18 DVM occurrence and average daily solar radiation (Table 2).
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24 Particularly at Stones Bay, a pattern indicative of a midday descent was regularly
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26 observed as a 12 h phase shift of the diel oscillations (Fig. 8). Compared to other sites,
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28 clearer water and shallower depth likely led to a relative increase in the midday descent
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30 amplitude versus the amplitude of the DVM cycle, and thus a higher frequency of cases when
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32 the midday descent became the dominant diel cycle. From a physiological perspective, the 12
33
34 h phase shift of the diel vertical migrations at this optically shallow site are consistent with
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36 modeled growth optimization when near-surface layers receive photoinhibiting irradiance
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38 (Kamykowski 1981b). It is possible that NPQ could depress near surface IVF on bright
39
40 sunny days (Kiefer 1973; Vincent et al. 1984) and produce an artifact misinterpreted as
41
42 surface avoidance behaviors. However, given the accumulation of evidence for surface
43
44 avoidance that is based on cell densities rather than IVF (Ault 2000; Passow 1991; Hall and
45
46 Paerl 2011), it is likely that the observed tendencies toward higher frequency of DVM
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48 detection on low-light days and higher frequency of midday descents at Stones Bay are true
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50 indications of short-term optimization of light levels (Ault 2000; Clegg et al. 2003).
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4 **4.5. Influence of vertical migration patterns on primary production-** Increasing
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6 phytoplankton light absorption relative to absorption by CDOM and non-algal particulates
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8 has the potential to significantly increase system-wide primary production (Klausmeier and
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10 Litchman 2001). Modeled productivity estimates indicated that, on average, DVM behaviors
11
12 resulted in only modest increases (< 5%) in daily primary productivity of the estuaries (Table
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16 3). Admittedly, these calculations have weaknesses due to uncertainty in the relationship
17
18 between IVF and Chl a , error in estimates of light attenuation, and uncertainty in the relation
19
20 between light and biomass normalized photosynthesis (P_b). Of these, errors in estimates of
21
22 light attenuation are probably the least severe because attenuation estimates from multiple
23
24 regressions fit observed attenuation values well. The ability of IVF to accurately predict the
25
26 Chl a profile is of more concern. As mentioned previously, fluorescence yield can be strongly
27
28 reduced by NPQ caused by strong near-surface irradiance. The intentional neglect of
29
30 photoinhibition in the productivity model (see Methods) was meant to compensate for
31
32 potential decreases in near-surface Chl a estimates due to NPQ of IVF (Vincent et al. 1984).
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34 However, determining whether fluorescence yield decreased proportionately with
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36 photosynthesis due to high light was not possible with the study data.
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43 Photophysiological parameters within the productivity model are known to be highly
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45 variable due to dependencies on temperature, previous light history, nutrient limitation status,
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47 and phytoplankton community composition (Gallegos and Neale this issue). There is
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49 evidence that flagellates achieve maximum photosynthetic rates at lower irradiances than
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51 non-motile taxa such as diatoms (Richardson et al. 1983). However, maximum
52
53 photosynthetic rates of flagellates normalized to Chl a (P_b^m) are generally lower than for
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55 diatoms (Gallegos 1992; Chan 1980). By increasing alpha and leaving P_b^m unaltered, the
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sensitivity analysis used constituted a best-case scenario to observe differences between productivity of heterogeneous and homogenous biomass distributions. Even under this best-case scenario, average productivity of the vertically migrating fraction relative to the whole community only resulted in minor increases (up to 10%) in total water column productivity.

The weak increases in production may indicate that although DVM was common, on average the vertically migrating community comprised only a modest fraction of the total biomass. Neither of the depth indices used to determine DVM occurrence are specifically affected by the proportion of the migrating community relative to average water column Chla. Even though DVM patterns only led to minor enhancements of total community productivity, the productivity enhancement of the taxa performing observed DVM behaviors might still be significant and form a strong selective pressure favoring phytoflagellates over non-motile taxa (Hall and Paerl 2011; Amano et al. 1998).

Vertically migrating phytoplankton can also stimulate system-wide productivity by redistributing nutrients from aphotic bottom waters into the euphotic zone (Fraga et al. 1992; Villareal et al. 1993; Olli et al. 1998). A rough calculation demonstrates the potential significance of nocturnal nutrient retrieval from the bottom waters of the two estuaries. Based on observations by Hall and Paerl (2011) in the NRE, assume a *Scrippsiella trochoidea* cell concentration of 8.0×10^8 cells m^{-3} in the bottom 1 m of the water column for 10 h at night. *S. trochoidea* has an N quota of 0.2×10^{-5} $\mu\text{mol N cell}^{-1}$ (Lirdwitayaprasit et al 1990) and a maximum dark NH_4^+ uptake rate of 0.02 hr^{-1} (Paasche et al., 1984), giving a maximum cellular uptake of $\sim 4.3 \times 10^{-6}$ $\mu\text{mol cell}^{-1}$ or $\sim 3,400 \mu\text{mol NH}_4^+ \text{m}^{-3}$ over the 10 h period. This is $\sim 140 \%$ of the $\sim 2,400 \mu\text{mol m}^{-2} \text{d}^{-1}$ NH_4^+ flux (Rizzo and Christian 1996) from the sediments into the bottom 1 m. Given the high frequency of DVM occurrence in these

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4 estuaries, nocturnal nutrient retrieval may form a significant compensatory mechanism for
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6 upward nutrient flux into the euphotic zone when vertical mixing is weak.
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11 **4.6. Conclusions-** Diel vertical migration patterns were a regular occurrence in these
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13 shallow, microtidal estuaries, with coherent diel oscillations detected on half or more days at
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15 all four sites. Shallow depth should not be used as a sole criterion for characterizing estuaries
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17 as well-mixed, at least with respect to phytoflagellate vertical distributions. The seasonal
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19 synchrony of DVM and nutrient release from the sediments during the warm months
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21 exemplifies the strong benthic-pelagic coupling in shallow estuaries. The flux of NH_4^+ from
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23 the sediments exceeds external inputs of N and is predicted to become increasingly important
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25 in shallow estuaries as waters warm due to climate change (Anderson et al. 2013; Luettich et
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27 al. 2000). As models are developed to understand how estuarine food webs will respond to
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29 external pressures driven by anthropogenic (i.e. changing nutrient loads) and climatic
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31 changes, integrating internal processes driven by phytoplankton behavior will provide more
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33 accurate depictions of responses of the phytoplankton community and nutrient flows within
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35 shallow, intermittently-mixed estuaries. Demonstration of the frequent occurrence of these
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37 behaviors and their potential impacts provides impetus for further research to address
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39 whether these advantages are worth the additional model complexity.
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53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4 **Literature Cited:**
5

6 Adolf, J. E., C. L. Yeager, W. D. Miller, M. E. Mallonee, and L. W. Harding Jr. 2006.
7

8
9 Environmental forcing of phytoplankton floral composition, biomass, and primary
10
11 productivity in Chesapeake Bay, USA. *Estuarine, Coastal and Shelf Science* 67:108-122.
12

13
14 Amano, K., M. Watanabe, K. Kohata, and S. Harada. 1998. Conditions necessary for
15

16 *Chatonella antiqua* red tide outbreaks. *Limnology and Oceanography* 43: 117-128.
17

18
19 Anderson, D. M. and K. D. Stolzenbach. 1985. Selective retention of two dinoflagellates in a
20

21 well-mixed estuarine embayment: the importance of diel vertical migration and surface
22
23 avoidance. *Marine Ecology Progress Series* 25: 39-50.
24

25
26 Anderson, I. C., M. J. Brush, M. F. Piehler, C. A. Currin, J. W. Stanhope, A. R. Smyth, J. D.
27

28 Maxey, and M. L. Whitehead. 2013. Impacts of climate-related drivers on the benthic
29
30 nutrient filter in a shallow photic estuary. *Estuaries and Coasts*. DOI: 10.1007/S12237-
31
32 013-9665-5.
33
34

35
36 Ault, T. R. 2000. Vertical migration by the marine dinoflagellate *Prorocentrum triestinum*
37

38 maximizes photosynthetic yield. *Oecologia* 125: 466-475.
39

40
41 Blauw, A. N., E. Beninca, R. W. P. M. Laane, N. Greenwood, and J. Huisman. 2012.
42

43 Dancing with the tides: Fluctuations of coastal phytoplankton orchestrated by different
44
45 oscillatory modes of the tidal cycle. *PlosOne* 7(11): e49319. doi:10.1371
46

47
48 Bowers, D. G. and H. L. Brett. 2008. The relationship between CDOM and salinity in
49

50 estuaries: An analytical and graphical solution. *Journal of Marine Systems* 73:1-7.
51

52
53 Boyer, J. N., R. R. Christian, and D. W. Stanley. 1993. Patterns of phytoplankton primary
54

55 productivity in the Neuse River estuary, North Carolina, USA. *Marine Ecology Progress*
56
57 *Series* 97: 287-297.
58
59
60
61
62
63
64
65

- 1
2
3
4 Breiman, L., J. Friedman, R. Olshen, and C. Stone. Classification and Regression Trees.
5
6 Boca Raton, FL: CRC Press, 1984.
7
8
9 Buskey, E.J., B. Wysor, and C. Hyatt. 1998. The role of hypersalinity in the persistence of
10
11 the Texas 'brown tide' in the Laguna Madre. *Journal of Plankton Research* 20: 1553-
12
13 1565.
14
15
16 Buzzelli, C. P., R. A. Luetlich, Jr., H. W. Paerl, J. Fear, J. Fleming, L. Twomey, B. Peierls,
17
18 E. Clesceri, M. J. Alperin, and C. S. Martens. Neuse River. 2001. Neuse River modeling
19
20 and monitoring project phase 2: Monitoring of hydrography and water quality,
21
22 circulation, phytoplankton physiology, and sediment-water coupling. University of North
23
24 Carolina Water Resources Research Institute. Report No. 426.
25
26
27 Cerco, C., and T. M. Cole. 1993. Three-dimensional eutrophication model of Chesapeake
28
29 Bay. *Journal of Environmental Engineering*, 119:1006- 1025.
30
31
32 Chan, A. T. 1980. Comparative physiological study of marine diatoms and dinoflagellates in
33
34 relation to irradiance and cell size. II Relationship between photosynthesis, growth, and
35
36 carbon/ chlorophyll a ratio. *Journal of Phycology* 16:428-432.
37
38
39 Clegg, M. R., S. C. Maberly, and R. I Jones. 2003. The effect of photon irradiance on the
40
41 behavioral ecology and potential niche separation of freshwater phytoplankton
42
43 flagellates. *Journal of Phycology* 39: 650-662.
44
45
46 Cloern, J.E., Jassby, A.D., 2010. Patterns and scales of phytoplankton variability in
47
48 estuarine-coastal ecosystems. *Estuaries and Coasts* 33, 230-241.
49
50
51 Cloern, J. E., T. S. Schraga, C. B. Lopez, N. Knowles, R. G. Labiosa, and R. Dugdale. 2005.
52
53 Climate anomalies generate an exceptional dinoflagellate bloom in San Francisco Bay.
54
55
56
57
58 *Geophysical Research Letters* 32: L14608. , doi:10.1029/2005GL023321.
59
60
61
62
63
64
65

- 1
2
3
4 Crawford, D. W. and D. A. Purdie. 1992. Evidence for avoidance of flushing from an estuary
5
6 by a planktonic, phototrophic ciliate. *Marine Ecology Progress Series* 79: 259- 265.
7
8
- 9 Cullen, J. J. and S. G. Horrigan. 1981. Effects of nitrate on the diurnal vertical migration,
10
11 carbon to nitrogen ratio, and the photosynthetic capacity of the dinoflagellate
12
13 *Gymnodinium splendens*. *Marine Biology* 62:81-89.
14
15
- 16 Doblin, M. A., P.A. Thompson, A.T. Revill, E.C.V. Butler, S.I. Blackburn, and G.M.
17
18 Hallegraeff. 2006. Vertical migration of the toxic dinoflagellate *Gymnodinium catenatum*
19
20 under different concentrations of nutrients and humic substances in culture. *Harmful*
21
22 *Algae* 5:665-677.
23
24
- 25 Durham, W. M., J. O. Kessler, and R. Stocker. 2009. Disruption of vertical motility by shear
26
27 triggers formation of thin phytoplankton layers. *Science* 323:1067-1070.
28
29
- 30 Ensign, S. H., J. N. Halls, and M. A. Mallin. 2004. Application of digital bathymetry data in
31
32 an analysis of flushing times of two North Carolina Estuaries. *Computers and*
33
34 *Geosciences* 30: 501–511.
35
36
- 37 Eppley, R. W., O. Holm-Hansen, and J. D. H. Strickland. 1968. Some observations on the
38
39 vertical migration of dinoflagellates. *Journal of Phycology* 4: 333–340.
40
41
- 42 Falkowski, P. and D. A. Kiefer. 1985. Chlorophyll *a* fluorescence in phytoplankton:
43
44 relationship to photosynthesis and biomass. *Journal of Plankton Research* 7: 715-731.
45
46
- 47 Finlayson-Pitts, B. J., and J. N. Pitts Jr. 2000. *Chemistry of the Upper and Lower Atmosphere*
48
49 *Theory, Experiments, and Applications*. San Diego, California: Academic Press. 969 p.
50
51
- 52 Fisher, T. R., P. R. Carlson, and R. T. Barber. 1982. Sediment nutrient regeneration in three
53
54 North Carolina estuaries. *Estuarine, Coastal and Shelf Science* 14: 101-116.
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3
4 Fisher, T. R., A. B. Gustafson, G. M. Radcliffe, K. L. Sundberg, and J. C. Stevenson. 2003.
5
6 A long-term record of photosynthetically active radiation (PAR) and total solar energy at
7
8 38.6 N, 78.2 W. *Estuaries* 26:1450-1460.
9
10
11 Fogg, G. E. 1991. Tansley review No 30: The phytoplankton ways of life. *New Phytologist*
12
13 118: 191–232.
14
15
16 Fraga, F., F. F. Perez, F. G. Figueiras, and A. F. Rios. 1992. Stoichiometric variations of N,
17
18 P, C and O₂ during a *Gymnodinium catenatum* red tide and their interpretation. *Marine*
19
20 *Ecology Progress Series* 87: 123-134.
21
22
23
24 Fulton, R. S. III. 1984. Distribution and community structure of estuarine copepods.
25
26 *Estuaries* 7: 38-50.
27
28
29 Gallegos, C. L. 1992. Phytoplankton photosynthesis, productivity, and species composition
30
31 in a eutrophic estuary- comparison of bloom and non-bloom assemblages. *Marine*
32
33 *Ecology Progress Series* 81: 257-267.
34
35
36 Ganf, G. G., and R. L. Oliver. 1982. Vertical separation of light and available nutrients as a
37
38 factor causing replacement of green algae by blue-green algae in the plankton of a
39
40 stratified lake. *Journal of Ecology* 70: 829-844.
41
42
43 Hall, N. S., R. W. Litaker, E. Fensin, J. E. Adolf, H. A. Bowers, A. R. Place, and H. W.
44
45 Paerl. 2008. Environmental factors contributing to the development and demise of a toxic
46
47 dinoflagellate (*Karlodinium veneficum*) bloom in a shallow, eutrophic, lagoonal estuary.
48
49 *Estuaries and Coasts* 31:402-418.
50
51
52
53 Hall, N. S., and H. W. Paerl. 2011. Vertical migration patterns of phytoflagellates in relation
54
55 to light and nutrient availability in a shallow, microtidal estuary. *Marine Ecology*
56
57 *Progress Series* 425:1–19.
58
59
60
61
62
63
64
65

- 1
2
3
4 Hall, N. S., H. W. Paerl, B. L. Peierls, A. C. Whipple and K. L. Rossignol. 2013. Effects of
5
6 climatic variability on phytoplankton biomass and community structure in the eutrophic,
7
8 microtidal, New River Estuary, North Carolina, USA. *Estuarine and Coastal Shelf*
9
10 *Science* 117:80-92.
11
12
13
14 Handy, S. M., K. J. Coyne, K. J. Portune, E. Demir, M. A. Doblin, C. E. Hare, S. C. Cary,
15
16 and D. A. Hutchins. 2005. Evaluating vertical migration behavior of harmful
17
18 raphidophytes in the Delaware Inland Bays utilizing quantitative real-time PCR. *Aquatic*
19
20 *Microbial Ecology* 40: 121-132.
21
22
23
24 Heaney, S. I. and R. W. Eppley. 1981. Light, temperature and nitrogen as interacting factors
25
26 affecting diel vertical migrations of dinoflagellates in culture. *Journal of Plankton*
27
28 *Research* 3: 331-344.
29
30
31 Hieronymus J. and J. D. Bowen. 2006. Calibration and verification of a two-dimensional,
32
33 laterally averaged mechanistic model of the Neuse River Estuary. University of North
34
35 Carolina North Carolina Water Resources Research Institute report 343- C.
36
37
38 Hutchinson, G. E. 1953. The concept of pattern in ecology. *Proceedings of the Academy of*
39
40 *Natural Science of Philadelphia* 105:1-12.
41
42
43 Jassby, A. D., and T. Platt. 1976. Mathematical formulation of the relationship between
44
45 photosynthesis and light for phytoplankton. *Limnology Oceanography* 21:540-547.
46
47
48 Jephson, T., and P. Carlsson. 2009. Species- and stratification-dependent diel vertical
49
50 migration behavior of three dinoflagellate species in a laboratory study. *Journal of*
51
52 *Plankton Research* 31: 1353-1362.
53
54
55 Jones, R. I. 1991. Advantages of diurnal vertical migrations to phytoplankton in sharply
56
57 stratified, humic forest lakes. *Archiv Fur Hydrobiologie* 120: 257-266.
58
59
60
61
62
63
64
65

- 1
2
3
4 Kamykowski, D. 1981a. Laboratory experiments on the diurnal vertical migration of marine
5
6 dinoflagellates through temperature gradients. *Marine Biology* 62: 57-64.
7
8
9 Kamykowski, D. 1981b. Dinoflagellate growth rate in water columns of varying turbidity as a
10
11 function of migration phase with daylight. *Journal of Plankton Research* 3:357-367.
12
13
14 Kamykowski, D. and S. A. McCollum. 1986. The temperature acclimatized swimming speed
15
16 of selected marine dinoflagellates. *Journal of Plankton Research* 8: 275-287.
17
18
19 Karp-Boss, L, E. Boss, and P. A. Jumars. 2000. Motion of dinoflagellates in a simple shear
20
21 flow. *Limnology and Oceanography* 45:1594-1602.
22
23
24 Kiefer, D. A. 1973. Fluorescence properties of natural phytoplankton populations. *Marine*
25
26 *Biology* 22: 263-269.
27
28
29 Klausmeier, C. A. and E. Litchman. 2001. Algal games: The vertical distribution of
30
31 phytoplankton in poorly mixed water columns. *Limnology and Oceanography* 46: 1998-
32
33 2007.
34
35
36 Larsen, P. S., and H. U. Riisgard. 2009. Viscosity and not biological mechanisms often
37
38 controls the effects of temperature on ciliary activity and swimming velocity of small
39
40 aquatic organisms. *Journal of Experimental Marine Biology and Ecology* 381: 67-73.
41
42
43 Lirdwitayaprasit, T., T. Okaichi, S. Montani, T. Ochi, and D. M. Anderson. 1990. Changes in
44
45 cell chemical composition during the life cycle of *Scrippsiella trochoidea* (Dinophyceae).
46
47 *Journal of Phycology* 26: 299-306.
48
49
50 Litaker, W., C. S. Duke, B. E. Kenney, and J. Ramus. 1987. Short-term variability and
51
52 phytoplankton abundances in a shallow tidal estuary. I. Winter and summer. *Marine*
53
54 *Biology* 96: 115-121.
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3
4 Litaker, W., C. S. Duke, B. E. Kenney, and J. Ramus. 1988. Diel chl *a* and phaeopigment
5
6 cycles in a shallow tidal estuary: potential role of microzooplankton grazing. *Marine*
7
8 *Ecology Progress Series* 47: 259-270.
9
- 10
11 Luettich, R. A., J. E. McNinch, H. W. Paerl, C. H. Peterson, J. T. Wells, M. A. Alperin, C. S.
12
13 Martens, and J. L. Pinckney. 2000. Neuse River Estuary modeling and monitoring project
14
15 stage 1: Hydrography and circulation, water column nutrients and productivity,
16
17 sedimentary processes and benthic-pelagic coupling, and benthic ecology. University of
18
19 North Carolina Water Resources Research Institute No. 325B.
20
21
22
- 23
24 Mallin, M. A., M. R. McIver, H. A. Wells, D. C. Parsons, and V. L. Johnson. 2005. Reversal
25
26 of eutrophication following sewage treatment upgrades in the New River Estuary, North
27
28 Carolina. *Estuaries* 28: 750-760.
29
30
- 31
32 Mallin, M. A. and H. W. Paerl. 1992. Effects of variable irradiance on phytoplankton
33
34 productivity in shallow estuaries. *Limnology and Oceanography* 37: 54-62.
35
- 36
37 Mallin, M. A., and H. W. Paerl. 1994. Planktonic trophic transfer in an estuary- Seasonal,
38
39 diel, and community structure effects. *Ecology* 75:2168-2184.
40
- 41
42 Margalef, R. 1978. Life-forms of phytoplankton as survival alternatives in an unstable
43
44 environment. *Oceanologica Acta* 1: 493-509.
45
- 46
47 Miller, W. D., L. W. Harding Jr., and J. E. Adolf. 2006. Hurricane Isabel generated an
48
49 unusual fall bloom in Chesapeake Bay. *Geophysical Research Letters* 33: L06612,
50
51 doi:10.1029/2005GL025658
52
- 53
54 Olli, K., A. S. Heiskanen, and K. Lohikari. 1998. Vertical migration of autotrophic micro-
55
56 organisms during a vernal bloom at the coastal Baltic Sea - coexistence through niche
57
58 separation. *Hydrobiologia* 363: 179-189.
59
60
61
62
63
64
65

- 1
2
3
4 Paasche, E., I. Bryceson, and K. Tangen. 1984. Interspecific variation in dark nitrogen uptake
5
6 by dinoflagellates. *Journal of Phycology* 20: 394-401.
7
8
9 Paerl, H. W., N. S. Hall, B. L. Peierls, and K. L. Rossignol. 2014. Evolving paradigms and
10
11 challenges in estuarine and coastal eutrophication dynamics in a culturally and
12
13 climatically stressed world. *Estuaries and Coasts* 37: 243–258.
14
15
16 Paerl, H. W., J. L. Pinckney, J. M. Fear, and B. L. Peierls. 1998. Ecosystem responses to
17
18 internal and watershed organic matter loading: consequences for hypoxia in the
19
20 eutrophying Neuse River Estuary, North Carolina, USA. *Marine Ecology Progress Series*
21
22 166: 17-25.
23
24
25
26 Paerl, H. W., K. L. Rossignol, N. S. Hall, B. L. Peierls, and M. S. Wetz. 2010. Phytoplankton
27
28 community indicators of short- and longterm ecological change in the anthropogenically
29
30 and climatically impacted Neuse River Estuary, North Carolina, USA. *Estuaries and*
31
32 *Coasts* 33: 485-497.
33
34
35
36 Paerl, H. W., L. M. Valdes, M. F. Piehler, and M. E. Lebo. 2004. Solving problems resulting
37
38 from solutions: The evolution of a dual nutrient management strategy for the eutrophying
39
40 Neuse River Estuary, North Carolina, USA. *Environmental Science & Technology*
41
42 38:3068-3073.
43
44
45
46 Passow, U. 1991. Vertical migration of *Gonyaulax catenata* and *Mesodinium rubrum*.
47
48 *Marine Biology* 110:455–463.
49
50
51 Peacock, M.B., and R.M. Kudela. 2014. Evidence for active vertical migration by two
52
53 dinoflagellates experiencing iron, nitrogen, and phosphorus limitation. *Limnology and*
54
55 *Oceanography* 59: 660-673.
56
57
58
59
60
61
62
63
64
65

- Pearre, S. Jr. 1979. Problems of detection and interpretation of vertical migration. *Journal of Plankton Research* 1: 29-44.
- Peierls, B. L., N.S. Hall and H. W. Paerl. 2012. Non-monotonic responses of phytoplankton biomass accumulation to hydrologic variability: a comparison of two coastal plain North Carolina estuaries. *Estuaries and Coasts* 35:1376-1392.
- Phlips, E. J., S. Badylak, J. Hart, D. Haunert, J. Lockwood, K. O'Donnell, D. Sun, P. Viveros, and M. Yilmaz. 2012. Climatic Influences on Autochthonous and Allochthonous Phytoplankton Blooms in a Subtropical Estuary, St. Lucie Estuary, Florida, USA. *Estuaries and Coasts* 35: 335-352.
- Pinckney, J.L., H.W. Paerl, M.B. Harrington, and K.E. Howe. 1998. Annual cycles of phytoplankton community structure and bloom dynamics in the Neuse River Estuary, NC (USA). *Marine Biology* 131: 371–382.
- Pinckney, J.L., H.W. Paerl, and M.B. Harrington. 1999. Responses of the phytoplankton community growth rate to nutrient pulses in variable estuarine environments. *Journal of Phycology* 35: 1455–1463.
- Pond, S. 1983. *Introductory Dynamical Oceanography*. 1st ed. Oxford, New York: Pergamon Press. 310 p.
- Reynolds-Fleming, J. V., J. G. Fleming, and R. A. Luetlich Jr. 2002. Portable autonomous vertical profiler for estuarine applications. *Estuaries* 25: 142-147.
- Reynolds-Fleming, J. V., and R. A. Luetlich Jr. 2004. Wind-driven lateral variability in a partially mixed estuary. *Estuarine, Coastal and Shelf Science* 60: 395-407.
- Richardson, K. J. Beardall, and J. A. Raven. 1983. Adaptation of unicellular algae to irradiance: an analysis of strategies. *New Phytologist* 93: 157-191.

- 1
2
3
4 Rizzo, W. M., and R. R. Christian. 1996. Significance of subtidal sediments to
5
6 heterotrophically-mediated oxygen and nutrient dynamics in a temperate estuary.
7
8 *Estuaries* 19: 475-487.
9
- 10
11 Salonen, K. and M. Rosenberg. 2000. Advantages from diel vertical migration can explain
12
13 the dominance of *Gonyostomum semen* (Raphidophyceae) in a small, steeply-stratified
14
15 lake. *Journal of Plankton Research* 22: 1841-1853.
16
17
- 18
19 Scully, M. E., C. Friedrichs, and J. Brubaker. 2005. Control of estuarine stratification and
20
21 mixing by wind-induced straining of the estuarine density field. *Estuaries* 28:321-326.
22
23
- 24 Sommer, U. 1988. Some size relationships in phytoflagellate motility. *Hydrobiologia*
25
26 161:125–131.
27
- 28
29 Sournia, A. 1974. Circadian periodicities in natural populations of marine phytoplankton.
30
31 *Advances in Marine Biology* 12: 325-289.
32
- 33
34 Sullivan, J. M., P. Donaghay, and J. B. Rines. 2010. Coastal thin layer dynamics:
35
36 Consequences to biology and optics. *Continental Shelf Research* 30: 50-66.
37
- 38
39 Thomas, W. H. and C. H. Gibson. 1990. Effects of small-scale turbulence on microalgae.
40
41 *Journal of Applied Phycology* 2: 71-77.
42
- 43
44 Torrence, C. and G. P. Compo. 1998. A practical guide to wavelet analysis. *Bulletin of the*
45
46 *American Meteorological Society* 79: 61–78.
47
- 48
49 Twomey, L. J., M. F. Piehler, and H. W. Paerl. 2005. Phytoplankton uptake of ammonium,
50
51 nitrate and urea in the Neuse River Estuary, NC, USA. *Hydrobiologia* 533: 123–134.
52
- 53
54 Tyler, M. A. and H. H. Seliger. 1981. Selection for a red tide organism- physiological
55
56 responses to the physical environment. *Limnology and Oceanography* 26:310-324.
57
58
59
60
61
62
63
64
65

Villareal, T. A., M. A. Altabet, and K. Culverrymsza. 1993. Nitrogen transport by vertically migrating diatom mats in the North Pacific-Ocean. *Nature* 363: 709-712.

Vincent, W. F., P. J. Neale, and P. J. Richerson. 1984. Photoinhibition: Algal responses to bright light during diel stratification and mixing in a tropical alpine lake. *Journal of Phycology* 20: 201-211.

Whipple, A. C., Luettich, R. A. Jr., and H. E. Seim. 2006. Measurements of Reynolds stress in a wind-driven lagoonal estuary. *Ocean Dynamics* 56:169-185.

Woodruff, D. L., R. P. Stumpf, J. A. Scope, and H. W. Paerl. 1999. Remote Estimation of Water Clarity in Optically Complex Estuarine Waters. *Remote Sensing of the Environment* 68: 41-52.

Yamazaki, H., C. Locke, L. Umlauf, H. Burchard, T. Ishimaru, and D. Kamykowski. 2014. A Lagrangian model for phototaxis-induced thin layer formation. *Deep Sea Research II* 101: 193-206.

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4 **Figure Captions:**
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7 Figure 1. Map showing geographic locations and bathymetry of the Neuse and New River
8 Estuaries, bi-weekly to monthly sampling stations (circles), autonomous vertical profiler
9 deployments at stations 120 and 180 in the Neuse River Estuary and Morgan Bay (MB) and
10 Stones Bay (SB) in the New River Estuary (squares), and meteorological stations (triangles).
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19 Figure 2. Water temperature and solar radiation for the New River AVP sites during 2009.
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21 (A) Daily average water column temperature at Morgan Bay (MB) and Stones Bay (SB). (B)
22 Daily averages of observed (Obs.) incident solar radiation and top of atmosphere (T.O.A.)
23 solar radiation calculated according to Finlayson-Pitts and Pitts (2000).
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31 Figure 3. Time series of daily mean stratification intensity measured as bottom minus surface
32 density, and daily means of water column average salinity at the four AVP sites. Euphotic
33 zone depths are plotted with salinity.
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41 Figure 4. Relationship between temperature and surface (open circles) and bottom water
42 (closed circles) dissolved inorganic nitrogen (DIN) concentration. Blue (red) vertical lines
43 connect sampling times when surface values were greater (less) than bottom water values. R_S ,
44 R_B , and R_{S-B} values are Spearman's rank correlation coefficients of temperature versus
45 surface, bottom, and surface minus bottom water DIN values. * and *** indicate p-values
46 less than 0.05 and 0.001.
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Figure 5. Example data from station 180 in the Neuse River Estuary from 4-13 November 2003. A) Depth/time contour plot of vertical profiles of in vivo fluorescence of chlorophyll, B) Time series of the depth of the center of mass of the fluorescence profiles (Z_{COM}) and depth of the fluorescence maxima (Z_{MAX}). + or - symbols centered at noon of each day indicate that a significant diel vertical migration pattern was or was not detected on that day (See methods for details). C) Modeled primary productivity based on photosynthetically active radiation (PAR), and both observed chlorophyll *a* profiles (Obs) and a hypothetical, vertically-homogenous distribution of the profile mean (Mix). D) Depth/ time contour plots of density (ρ), E) Wind speed with arrows pointing in the downwind direction. North is up. Solid black lines in panels A and D represent bottom depth, and blank spaces represent periods when data was not collected.

Figure 6. Average daily pattern of in vivo fluorescence depth distribution by season for the four deployment sites of autonomous vertical profilers.

Figure 7. Time localized wavelet power spectra of the depth of the fluorescence maximum (Z_{MAX}) records at the four AVP sites. Black patches along top of each panel indicate data gaps.

Figure 8. Wavelet phase estimates from significant diurnal signals in the depth of the fluorescence maximum (Z_{MAX}) at the four stations. A phase shift of zero indicates a Z_{MAX} cycle closest to the surface at noon and closest to the bottom at midnight.

Figure 9. Classification trees for the occurrence of a significant DVM cycle based on daily averages of water column average temperature (T , °C), water column average salinity (S), stratification intensity ($\Delta\rho$, kg m^{-3}), water column average in vivo fluorescence (IVF, arbitrary units), incident solar radiation (SR , W m^{-2}), turbidity ($Turb$) and daily average wind speed ($Wind$). Leaf purity is indicated by the fraction of properly categorized days over the total days in the leaf.

Table 1. Fraction of days classified as having a detectable diel vertical migration pattern based on both the depth of the fluorescence maximum, and the depth of center of mass having a significant and appropriately phased diel cycle (DVM+). The fraction of days exhibiting a significant diel vertical migration pattern when coincident nutrient measurements indicated that dissolved inorganic nitrogen in the bottom waters was greater ($DIN_B > DIN_S$) and less than ($DIN_B < DIN_S$) in the surface waters. N_{total} is the total number of days classified. N_{DIN} is the number of days with coincident assessments of DVM occurrence and nutrient concentrations.

Station	N_{total}	DVM+	N_{DIN}	$DIN_B > DIN_S$	$DIN_B < DIN_S$
Morgan Bay	1244	0.43	35	0.26	0.17
Stones Bay	1306	0.21	42	0.12	0.12
NRE120	183	0.50	11	0.27	0.18
NRE180	338	0.63	24	0.38	0.21

Table 2. Sign of coefficients from multiple logistic regressions of environmental predictors on the detection of DVM. Predictors were daily averages of stratification intensity ($\Delta\rho$), incident solar radiation (SR), and wind speed (Wind), and daily averages of mean water column temperature (T), salinity (S), in vivo fluorescence (IVF), and turbidity (Turb).

Station	$\Delta\rho$	T	S	IVF	SR	Turb	Wind
Morgan Bay	*** +	*** +	*** -	*** +	*** -	NS	** -
Stones Bay	NS	*** +	*** -	*** +	*** -	NS	NS
NRE120	NS	*** +	* -	NS	* -	NS	NS
NRE180	NS	*** +	NS	** +	** -	NS	*** -

Symbols + or – indicate the sign of statistically significant logistic regression coefficients. *,

, * indicate $p < 0.05$, 0.01 , 0.001 , respectively. NS indicates not significant.

Table 3. Modeled primary productivity based on observed (Obs) vertical profiles of chlorophyll *a* and a hypothetical vertically homogenous (Mix) chlorophyll *a* profile for days when diel vertical migration was (DVM +) and was not (DVM -) detected. Values are medians of daily productivity values (g C m⁻² d⁻¹) and the medians of the relative differences between “Obs” and “Mix” (% Diff). % Diff values in parentheses indicate scenarios (1,2) when the productivity model was altered so that α for the biomass deviations from the mean profile was set to the maximum (scenario 1) or minimum (scenario 2) observed value and E_k for the mean biomass profile was set to the minimum (scenario 1) or maximum (scenario 2) (see Methods). All % Diff values were significantly different from zero ($p < 0.05$) based on a Wilcoxon rank sign test.

Station	DVM +/-	Obs Mix	% Diff
Morgan Bay	DVM +	1.10 > 0.99	5.4 (2.5, 7.3)
	DVM -	1.11 < 1.12	-0.6 (-0.4, -0.7)
Stones Bay	DVM +	0.68 > 0.65	3.6 (1.6, 4.8)
	DVM -	0.74 < 0.75	-0.2 (-0.2, -0.7)
NRE120	DVM +	2.71 > 2.64	1.8 (0.9, 2.3)
	DVM -	1.81 < 1.91	-2.2 (-0.8, -2.4)
NRE180	DVM +	1.99 > 1.76	4.5 (1.9, 11.6)
	DVM -	1.59 < 1.70	-3.6 (-1.7, -6.2)

Figure 1

Figure 1.

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