Article

A 21-year record of vertically migrating subepilimnetic populations of *Cryptomonas* spp.

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Abstract

The vertical distribution and diel migration of *Cryptomonas* spp. were monitored continuously for 21 years in mesotrophic Cross Reservoir, northeast Kansas, USA. The movements of these motile algae were tracked on multiple dates during July–October of each year using *in situ* fluorometry and optical microscopy of Lugol's iodine-preserved samples. Episodes of subepilimnetic diel vertical migration by *Cryptomonas* were detected and recorded on 221 different days between 1994 and 2014, with just 2 of these years (1998 and 2013) lacking any sampling events with deep peaks sufficiently large enough to track. Whenever a subepilimnetic layer of *Cryptomonas* was detectable, it was generally observed to ascend toward the bottom of the epilimnion beginning approximately at sunrise; to descend toward the lake bottom during the late afternoon and evening; and to remain as a deep-dwelling population until dawn of the following day. Moreover, there was high day-to-day consistency in the absolute water column depths at which the migrating algal cells would cease their ascending or descending movement. We believe this unique and remarkable dataset comprises the most detailed record of diel migratory behavior for any planktonic freshwater alga reported for a single freshwater lake.

Key words: *Cryptomonas,* DCM, deep chlorophyll maxima, motile phytoplankton, vertical migration**s**

Introduction

For more than a century of limnological research, freshwater plankton ecologists have focused their attention primarily on events and processes that occur in the epilimnion of lakes. As a result, we have a rich understanding of both the physical drivers and the biological interactions that regulate plankton communities in the mixed layer. Although the exact balance between these controlling factors may differ from lake to lake, succession in epilimnetic plankton seems to be triggered by physical events and then subsequently develops according to a set of predictable empirical rules. These rules have been well elaborated in the Plankton Ecology Group (PEG) model (Sommer et al. 1986, 2012) for temperate zone lakes, and de Senerpoint Domis et al. (2013) have extended this general model to other climatic regions, particularly polar and tropical latitudes.

By contrast, much less is known about the rules that govern the structure and function of the plankton

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communities that can develop below the mixed layer. It has been known since the early studies of Yoshimura (1939) that deep chlorophyll maxima (DCM) tend to occur in lakes of low to moderate fertility when water column transparency allows light to penetrate into or below the thermocline. DCM have been reported in natural lakes worldwide, including Africa (Descy et al. 2010), Asia (Ichimura et al. 1968, Lohman et al. 1988, Kim and Takamura 2002), Europe (Ilmavirta 1988, Jones 1991), all 5 of the Laurentian Great Lakes (Barbiero and Tuchman 2001), diverse natural lakes throughout North America (Pick et al. 1984, deNoyelles and Likens 1985, Konopka 1989, Gross et al. 1997), South America (de Matos Bicudo et al. 2009), and Tasmania (Croome and Tyler 1984). DCM also have been reported in artificial freshwater impoundments where sufficient light is available in the upper metalimnion to allow *in situ* phytoplankton growth (e.g., Knowlton and Jones 1989).

Mechanisms that contribute to DCM formation and maintenance include a local maximum in phytoplankton growth rate near the nutricline; photoacclimation of cellular pigment content that results in elevated chlorophyll relative to phytoplankton biomass at depth; and a range of physiologically influenced behaviors that can lead to the aggregation of phytoplankton in discrete subthermocline layers, subject to grazing and physical control (Cullen 2015). The phytoplankton assemblages that form deep layers in freshwater lakes are dominated by a wide variety of taxa, including cyanobacteria, chlorophytes, cryptophytes, chrysophytes, dinophytes, euglenophytes, prasinophytes, and bacillariophytes (cf. table 1 in Lindholm 1992). DCM are estimated to contain a majority of the total phytoplankton biomass and can account for as much as 23–90% of total annual primary production in the lakes in which DCM are present (Moll et al. 1984, Konopka 1989, Gasol et al. 1993). Although they typically are not sampled in most monitoring efforts and are often ignored by many plankton ecologists, it is increasingly evident that these deep phytoplankton communities can have great ecological importance (Lindholm 1992).

For example, although the epilimnetic plankton of Lake Cisó, Spain, has been found to conform well to the PEG model (Gasol et al. 1992a), a prominent metalimnetic community develops in Lake Cisó following thermal stratification that subsequently remains stable throughout the summer. Nonetheless, the presence and dynamics of these deep phytoplankton communities are not predicted by the PEG model as it is currently formulated, and Gasol et al. (1992a, 1992b, 1993) have suggested that additional model statements may need to be added to the PEG model to account for lakes that develop stable and persistent metalimnetic communities. To do so, however, requires expanding our understanding of factors that regulate the structure, function, and behavior of these deep-dwelling species.

Phytoplanktonic flagellates are a diverse group of unicellular or colonial eukaryotic microorganisms that combine finely controlled motility with oxygenic photosynthesis; they are common in both freshwater and marine habitats and form an important functional component of the phytoplankton community (Clegg et al. 2007). During periods of thermal stratification in freshwater lakes, when flagellates are often most abundant and when the water column typically exhibits strong vertical resource gradients, phytoflagellates can select and then maintain favorable positions within the water column using behaviors that include depth regulation, layer formation, and diel vertical migrations (Clegg et al. 2007). These movements can help maximize population growth and survival (Raven and Richardson 1984), but the mechanisms underlying them are not yet fully understood (Clegg et al. 2007).

This paper reports what we believe is an unparalleled long-term study of vertical migration behavior by subepilimnetic populations of *Cryptomonas*, a small freshwater phytoflagellate. Deep-dwelling *Cryptomonas* have been observed to undergo pronounced diel vertical migrations with amplitudes as large as 5 m (Arvola et al. 1991). We report here detailed measurements of fine-scale variation in the spatial distribution of the biflagellated alga *Cryptomonas*, including short time-scale diurnal vertical migration events recorded from 1994 to 2014 in Cross Reservoir, Kansas, USA. The data obtained from these efforts provide important new insights into the complex movements exhibited by natural *Cryptomonas* populations and also reveal remarkable consistency in their day-to-day ascending and descending swimming behavior.

Methods

Study site

This research was performed at Cross Reservoir, a 3 ha surface area, 12 m deep artificial impoundment constructed in 1991 at the University of Kansas Field Station in northeast Kansas (39.0524°N, 95.1846°W) in the Midwestern United States. It has a small protected 50 ha watershed consisting entirely of grasslands and forests. Water inflow is derived from direct precipitation and from 2 ephemeral streams. A drainage standpipe regulates reservoir water level and serves as the main outflow, and an auxiliary emergency overflow spillway is situated on the right overbank side of the dam. Because Cross Reservoir has no permanent surface inflows, it is not susceptible to strong hydraulic flushing events, evidenced by the fact that the

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auxiliary spillway overtopped just once (4–5 October 1998) during the 21 year study. Its water column exhibits low levels of suspended soils and dissolved organic color and therefore maintains consistently high water clarity because of the limited fluvial inputs. Hills and trees are located on 3 sides of the reservoir, shielding the lake surface from the wind, and the east end toward the dam is relatively open. As a result, thermal stratification begins each year in April, and complete mixing occurs by mid-November. The bottom of the epilimnion is typically located at 3 m in July and usually deepens to ~9 m by early November. The bottom of the epilimnion is defined here as the bottom of the first 1 m depth interval that exhibited a >1 °C temperature decrease per meter. The water column also consistently exhibits strong chemical stratification during the summer months (Fig. 1). Sporadic ice cover can occur during December–February.

Phytoplankton monitoring

Phytoplankton vertical distribution in Cross Reservoir was monitored during >6000 separate sampling occasions for 21 years on 531 days, with 445 days during July–October, using a combination of fluorometry and direct optical microscopy. Sampling occurred on 221 days during July–October when there was a DCM, with profiles recorded from before or soon after sunrise through midday and often later. On 117 days during July–October no DCM was present, and on 107 days during July–October a DCM was present but sampling was insufficient to follow DCM movement. For the 86 days during other months, DCM were sometimes present in June and November but were not followed. Sampling was performed from a boat anchored at a fixed station located at the deepest part of the lake (12 m). *In vivo* algal fluorescence was recorded multiple times on most sampling dates using 1 of 2 fluorometers, either a Chelsea MiniTracka *in situ* fluorometer (Chelsea Instruments LTD, West Molesey, Surrey, UK) or a Turner Model 10-005 R field flow-through fluorometer (Turner Designs, Inc., Mt. View, CA, USA), by lowering the unit or the hose, respectively, and timing 25 cm each 15 s. Both fluorometers were calibrated using known chlorophyll standards. Because chlorophyll fluorescence is an imprecise measure of chlorophyll (Cullen 2015), we used both instruments primarily as a tool to locate and monitor the movements of subsurface phytoplankton communities and to identify the water layers to be sampled for microscopical analysis of phytoplankton species composition and abundance. The subepilimnion layers that could be most accurately followed typically exhibited fluorescence values >3 times the values observed within the epilimnion. The Chelsea fluorometer was employed for the most detailed recordings from 1999 to 2012 analyzed here because the filter set for its detector only reports signals for *Cryptomonas* and other eukaryotic algae; fluorescence signatures from cyanobacteria and photosynthetic bacteria that could obscure the algal signal are not registered by this instrument. Identification of precise descent depths with Turner fluorescence profiles from 1994 to 1997 was confounded by comingled prokaryotes occurring at descent depths but not at ascent depths.

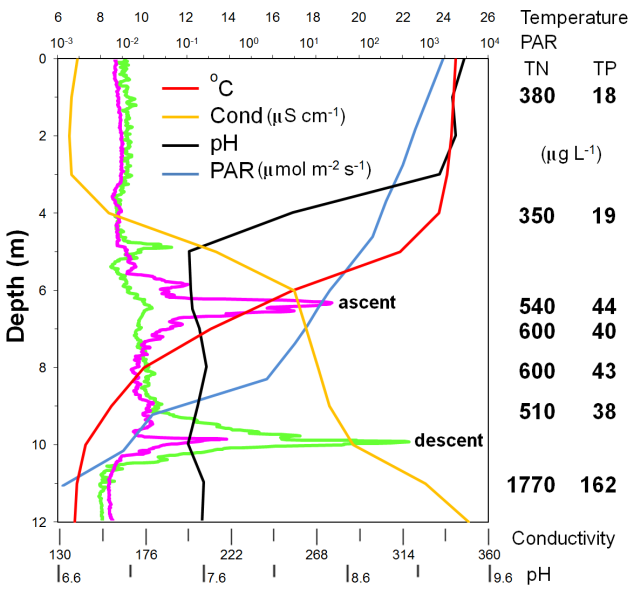

Fig. 1. Example of strong vertical gradients of light, temperature, and water chemistry in Cross Reservoir, KS, superimposed on 2 observations from a single vertical migration event by *Cryptomonas* spp., 9 Sep 2003. The pH values were unusually high on this date.

All fluorescence data were recorded on a deck-mounted Servogor 102 chart recorder, and this information was used to select the discrete water column depths sampled. Individual 100 mL water samples were pumped up to the boat from the selected depths through the fluorometer hose of the Turner fluorometer, or through a hose attached to the Chelsea fluorometer, and immediately preserved with Lugol's iodine (APHA 1995); all of these preserved samples have been archived. Algal identification and enumeration were performed on settled samples from each of the 221 pairs of ascent and descent depths and from other depths from these days and other days using a Wild M-1 inverted microscope at 560× magnification (detection limit, 23 cells mL^{-1}). Hardcopy fluorescence profile charts were scanned and digitized using ByteScout Graph Digitizer Scout software.

For the 221 sampling days when one or more DCM were followed, microscopical analysis indicated that one or more species of *Cryptomonas* always formed the primary migratory DCM. Of these 221 observed migration events, 158 vertical migrations examined during 1999–2012 using the Chelsea fluorometer were selected for detailed study,

and this best-characterized subset of the entire database is the focus of most of the present analysis. Space limitations do not permit detailed discussions of each of these events, but the main highlights are summarized.

Other limnological measurements

Vertical profiles of temperature, conductivity, pH, turbidity, and dissolved oxygen were taken on most of the 531 sampling dates and on all of the 221 observed DCM migration dates with a Water Quality Checker U-10 (Horiba Instruments, Kyoto, Japan). Light intensity was measured at multiple times and depths on each sampling date with a LI-COR LI-250 light meter (Lincoln, NE, USA) equipped with a spherical quantum sensor. The average July–October Secchi disk transparency during 1994–2014 was 3.11 m, and the average depth of the bottom of the epilimnion was 4.61 m.

Incident solar irradiance as photosynthetically active radiation (PAR, measured as µmol m^{-2} s^{-1}) was obtained from continuous measurements recorded at the University of Kansas Field Station weather station 600 m west of the lake. Measurements of water column nutrients were collected during July through October on 20 sampling dates for the 8-year period from 2002 to 2009. Specific conductivity levels increased with water depth during the period of thermal stratification for all years sampled, reflecting a trend of increasing inorganic nutrient availability with depth. The Turner fluorometer was used to obtain pumped samples from discrete depths of 1, 4, 6, 7, 8, 9, and 11 m on each of these 20 dates. Total nitrogen (TN) and total phosphorus (TP) concentrations were determined using a Lachat Model 4200 analyzer after appropriate chemical digestion, and soluble reactive phosphorus (SRP) and acid-corrected concentrations of chlorophyll *a* were determined from 0.45 µm Gelman Acrodisc-filtered samples using an Optical Technologies fluorometer (APHA 1995; Table 1).

Results

During the July–October stratified period from 1999 to 2012 providing the 158 migration events studied in detail, *Cryptomonas* abundances in the epilimnion averaged 427 cells mL^{-1} when DCM were absent versus an average of only 88 cells mL^{-1} when DCM were present. During the entire 21-year study period and 221 observed DCM migration events (Fig. 2), twenty-nine 1-day *Cryptomonas* migration events and 192 consecutive-day migrations (fifty-nine 2-day, fourteen 3-day, four 4-day, two 5-day, and one 6-day) for subepilimnion populations were recorded. During 1999 to 2012 and 158 migration events

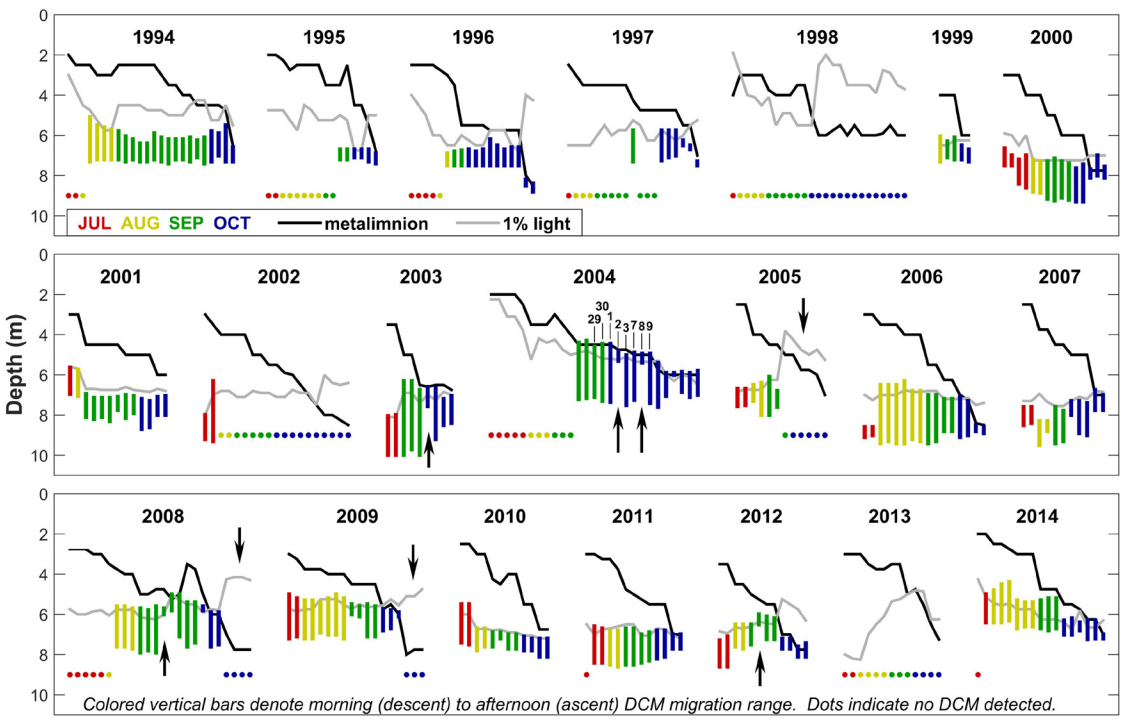

Fig. 2. A total of 221 diel *Cryptomonas* migration events (along with 117 days lacking DCM) were recorded in Cross Reservoir, KS, from 1994 to 2014. Seasonal variations in the bottom of the epilimnion (black line) and the 1% light level depth (gray line) are shown for each year. The top of each colored vertical bar represents the uppermost daytime location (the ascent depth) to which the *Cryptomonas* swam from the previous night's deepest location (the descent depth, represented by the bottom of each bar). Date details are provided only for the dense Sep–Oct 2004 sampling period. Upper and lower arrows are described in the text.

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Table 1. Summary of the major physicochemical features of Cross Reservoir, KS (USA) from 2002 to 2009 for the 8-year samples and 1999 to 2012 for the 14-year samples.

Parameter	Average	Range	# of sampling dates (# of years sampled)
Chlorophyll *a*, µg L^{-1} (epilimnion)	3.7	1.1–15.8	20 (8)
Chlorophyll *a*, µg L^{-1} (8 m)	59.6	4.1–119.5	20 (8)
TN, µg L^{-1} (epilimnion)	374	300–470	20 (8)
TN, µg L^{-1} (8 m)	726	380–1438	20 (8)
TP, µg L^{-1} (epilimnion)	17.2	8.9–26.3	20 (8)
TP, µg L^{-1} (8 m)	55.1	15.0–125.0	20 (8)
SRP, µg L^{-1} (epilimnion)	6.11	1.02–15.4	20 (8)
SRP, µg L^{-1} (8 m)	10.7	2.94–78.0	20 (8)
TN:TP (by mass: epilimnion)	23.2	13.0–38.0	20 (8)
TN:TP (by mass: 8 m)	14.8	8.0–25.0	20 (8)
Conductivity, µS cm^{-1} (epi)	169	129–252	158 (14)
Conductivity, µS cm^{-1} (8 m)	299	228-407	158 (14)
pH (epilimnion)	8.51	7.56–9.98	158 (14)
pH (8 m)	7.24	6.66–8.15	158 (14)
Depth of zero O_2 (m)	6.99	5.25–10.00	158 (14)
Depth of 1% incident PAR (m)	6.48	4.80–8.10	158 (14)

studied in detail, seven 1-day events and 151 consecutive-day migrations (fifty-two 2-day, eight 3-day, three 4-day, one 5-day, and one 6-day) were recorded. For the purpose of describing the *Cryptomonas* movement, the bottom of the epilimnion (as defined in the Methods section) is the bottom of the first meter with a >1 °C decrease.

For 2 of the 21 years studied (1998 and 2013, which account for 38 of the 338 July–October sampling days shown in Fig. 2), no DCM were detected at any time during the growing season, and cell densities throughout the water column at that time were typically <2000 cells mL^{-1}. By contrast, phytoplankton abundances in subepilimnetic populations observed on 158 days during 1999–2012 at their ascent depth averaged >13 000 cells mL^{-1} and achieved maximum values as high as 110 000 cells mL^{-1}. In 1998, the lack of a DCM was attributed to reduced transparency caused by heavier than normal periods of rainfall and runoff. Note the inversion between the metalimnion and the 1% light depth in early October 1998 (Fig. 2), a result of mixing due to the extreme runoff event that caused the overtopping of the auxiliary spillway mentioned earlier. In 2002, the lost DCM coincided with the presence of vertically migrating populations of *zoochlorella*-containing *Paramecium bursaria*, which were never recorded in other years. A *Paramecium bursaria* layer containing up to 87 000 cells L^{-1} was tracked on each of 25 days through the zone where *Cryptomonas* remained in greatly reduced numbers. The reasons for a lack of DCM formation in 2013 continue to be investigated, but the formation of deep algal layers exhibiting migratory behavior resumed in 2014 and continued with 15 recorded July–October events in 2015 (results not shown). During the other 19 study years, DCM containing mostly *Cryptomonas* spp. displayed diel vertical migration each time they were detected and followed on 221 days sampled during July–October (Fig. 2). Departures from typical migratory behavior (documented below) are attributed to extreme changes in underwater light conditions.

From 1999 to present, only the more taxon-discriminating Chelsea fluorometer was used to track algal migrations. The vertically migrating algal species most closely tracked for 158 days during 1999–2012 were identified microscopically as *Cryptomonas marssonii* Skuja 1948 emend Hoef-Emden et Melkonian; *Cryptomonas pyrenoidifera* Geitler 1992 emend Hoef-Emden et Melkonian; and *Cryptomonas ovata* Ehrenberg 1832 emend Hoef-Emden et Melkonian. Because significant morphological variation exists within this genus, taxonomic revisions of *Cryptomonas* reducing the total number of species have been made using molecular phylogenetic analyses (Hoef-Emden and Melkonian 2003); some *Cryptomonas* species previously reported in the literature thus are no longer accepted as unique taxa.

Consistent with Knapp et al. (2003) and Chapin et al. (2004) for Cross Reservoir, much of the water column below the epilimnion was anaerobic during the periods of observed *Cryptomonas* spp. movement, with a mean anoxic depth of 7 m (Table 1). The mean dissolved sulfide

levels immediately below the zone of migration in 1997–1999 averaged 307 µg L^{-1} S. Two consistent indicators of DCM formation were the formation of a sulfide–oxygen interface and anaerobic conditions located close to the depth of 1% incident PAR. For 122 of the 158 sampled migration days occurring during 1999–2012, *Cryptomonas* was observed to move upward out the anoxic zone during the day and then return to the anoxic layer at night. In 1994, the strong influence of light was confirmed experimentally using submersed lighting in a 3 m diameter, 5 m high limnocorral placed at night to enclose the water column, including the *Cryptomonas* DCM (Bergin 1997, Knapp et al. 2003). During the night, *Cryptomonas* rose 1 m well before sunrise; cell densities at the ascent depth located within the tube increased more than 4-fold, from 5917 to 28 420 cells mL^{-1}.

The details of 2 consecutive, pronounced vertical migration events monitored during 29–30 September 2008 were recorded (Fig. 3). Data from the 91 separate *in vivo* fluorescence profiles recorded during the 38 h sampling period reveal a clear vertical movement of *Cryptomonas* cells from their nighttime location at a depth of 7.6 m (the descent depth on this particular date) to a location just below the epilimnion during the day (an ascent depth of 5.4 m). This pattern was repeated during the next 24 h (upward migration from a 7.4 m descent depth to a 5.4 m ascent depth). A relatively small, somewhat evenly dispersed residual population of *Cryptomonas* appears to remain behind in the migration zone following each descent (Fig. 3), then rise along with the subsequent ascent (evidenced in Fig. 3b by the darker shading in the space above each down cycle compared to the lighter shading in the space below each up cycle). Thus, not every algal cell participated in downward movement phases of this particular sequence of migration events.

The vertical movements of *Cryptomonas* broadly tended to reflect changes in incident solar irradiance on these 2 sampling dates (Fig. 3b); note, however, that

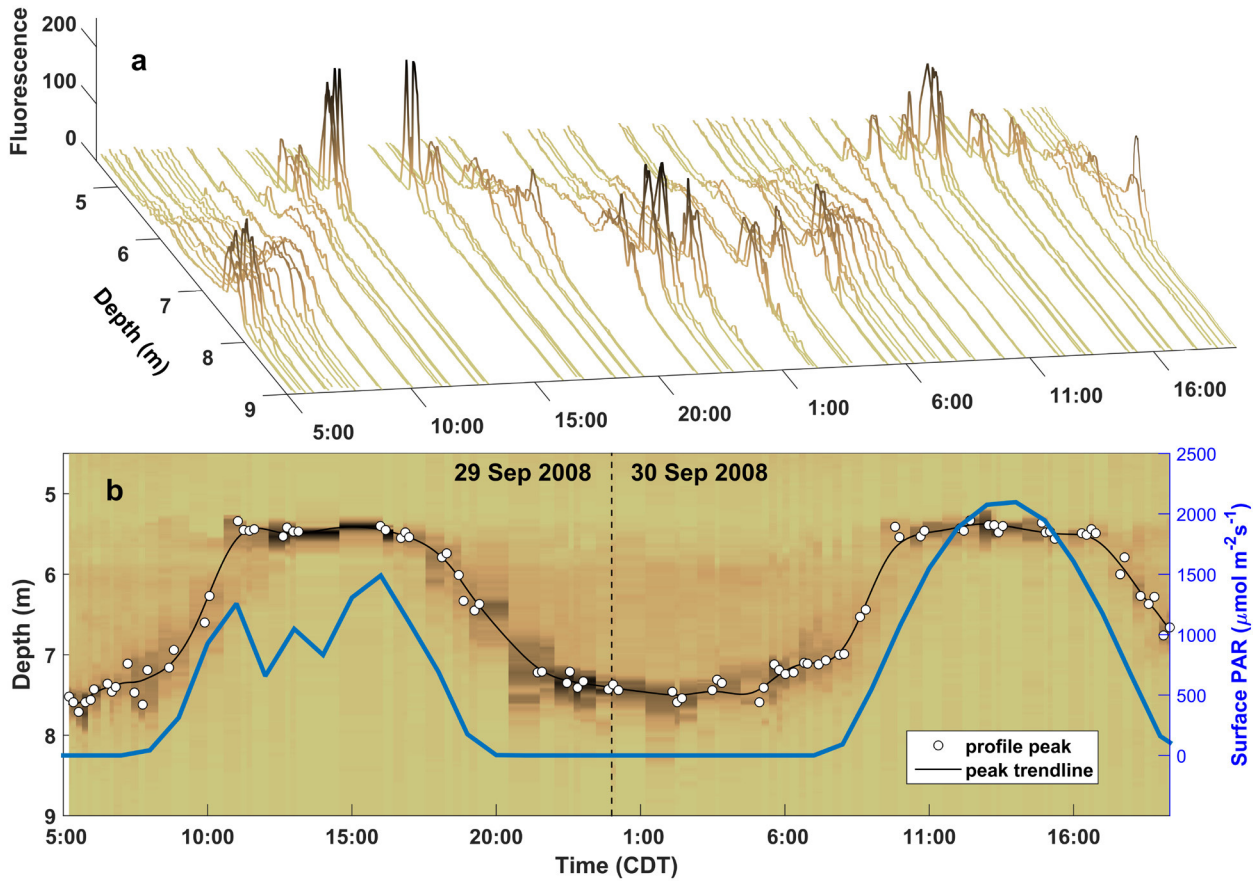

Fig. 3. (a) Example of a 2-day migration event monitored between 05:12 h on 29 Sep and 19:20 h on 30 Sep 2008, obtained using 91 separate *in vivo* fluorescence profiles taken during the 38 h period. For clarity, only the subepilimnion profile portions from 4.5 to 9 m, which encompass the entire zone of vertical migration by *Cryptomonas* on these dates, are displayed here. (b) To create this gapless flat-map version of panel (a), profiles were temporally drawn out in both directions to the midpoints of the gaps between the previous and subsequent profiles. Dots representing profile peaks are placed at the actual profile times. The vertical migration of *Cryptomonas* strongly tracked the pattern of incident irradiance on the lake surface, as monitored at a nearby weather station. The black line represents the smoothed trendline for the depth of peak algal fluorescence, computed using singular spectrum analysis (Ghil et al. 2002). All times are recorded as local Central Daylight Time.

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Table 2. Summary of algal migration data for the 1999–2012 period most analyzed in this study. The ascent span and the descent span data reflect the total distance travelled upward or downward, respectively, during a given vertical migration event by the *Cryptomonas* cells.

Parameter	Average	Range	# of sampling dates
Ascent depth (m)	6.53	4.3–8.6	158
Ascent span (m)	1.43	0.1–3.7	158
Ascent swimming rate (cm h^{-1})	41.3	20.4–66.7	94
Descent depth (m)	7.96	5.3–10.0	158
Descent span (m)	1.44	0.0–3.4	88
Descent swimming rate (cm h^{-1})	41.8	21.3–61.7	24
Depth of 1% incident PAR (m)	6.48	4.80–8.10	158

ascending swimming movements by these motile cells began before sunrise and before first light at depth from *in situ* measurements. This behavior was commonly seen on 29 additional sampling dates in which detailed predawn measurements of the DCM were performed, possibly suggesting the presence of innate circadian rhythms that warrant further study.

The details of all algal migration events recorded on 158 different dates between 1999 and 2012 (Table 2) are from an average of 32 fluorescence profiles measured per day on each of these sampling dates. The *Cryptomonas* cells moved as much as 3.7 m during their diel migration events, with similar ascent and descent spans and similar average swimming speeds; however, descent speed seems fairly uniform whereas ascent speed seems to have 2 phases, a slow phase prior to sunrise, perhaps attributable to circadian rhythm, followed by a fast phase after sunrise, perhaps a response to light inception (Fig. 3b). In addition, high consistency was observed in ascent and descent depths to which they moved on successive sampling dates. Strong correlations ($r^2 > 0.9$) were found both for the paired ascent depths (the uppermost daily position in the water column) and for the paired descent depths (the subsequent lowermost daily position) attained by *Cryptomonas* during all consecutive 2-day, regular vertical migration events (Fig. 4; regular meaning typical descending behavior was observed, with some exceptions described later). Almost equally strong ascent and descent depth relationships were observed across multiple 3-day sampling periods; plots of paired (day 1–day 3) ascent depths revealed a correlation of $r^2 = 0.93$, and paired (day 1–day 3) descent depths exhibited a similarly strong correlation of $r^2 = 0.87$ (data not shown).

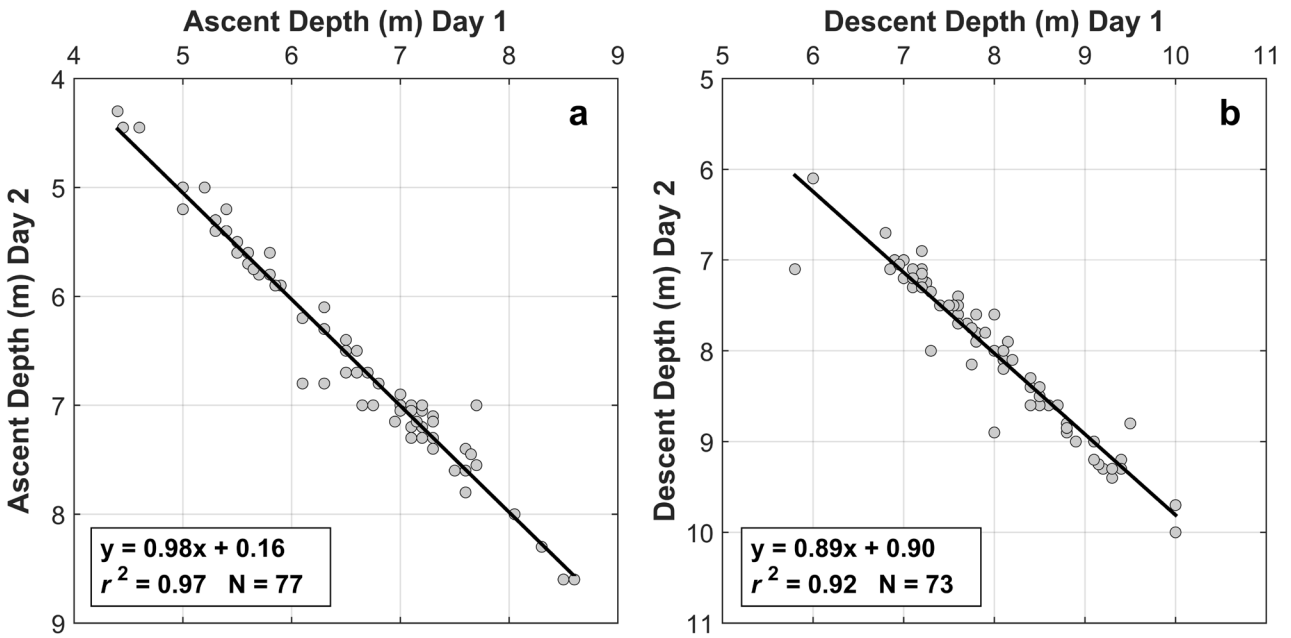

Fig. 4. Strong correlations are seen between 2-day consecutive ascent and descent depths from 158 *Cryptomonas* vertical migration events recorded from 1999 to 2012. (a) Comparison between the ascent depth on day 1 to the ascent depth on the following day. (b) Comparison between the descent depth on day 1 to the descent depth on the following day.

Further statistical analyses of the 1999–2012 data revealed a strong positive dependence of the *Cryptomonas* ascent depth on the transparency of the water column above the DCM, as reflected in the depth of 1% incident PAR (r^2 = 0.72; Fig. 5a). When light during the 21 years penetrated more deeply into the subepilimnion zone (Fig. 2 and 5), the *Cryptomonas* DCM generally were deeper in the water column; under these more transparent conditions, the motile cells exhibited both deeper ascent depths and deeper descent depths. During the 158 total days monitored during 1999–2012, the average 1% light depth was 6.48 m and the mean ascent depth was 6.53 m. By contrast, the cells' ascent depth was poorly correlated (r^2 = 0.16) with the absolute light levels experienced by the algal cells when they achieved their uppermost vertical location. The mean difference between midday light intensities experienced by *Cryptomonas* at their ascent depth and the corresponding midday light intensities measured at their previous descent depth was 13 µmol m^{-2} s^{-1} (range 0.1–72.5 µmol m^{-2} s^{-1}). This value provides a rough estimate of the light availability increase obtained by the migrating cells; however, this difference likely is slightly overestimated due to shading effects of the ascended DCM on light readings measured at the descent depth.

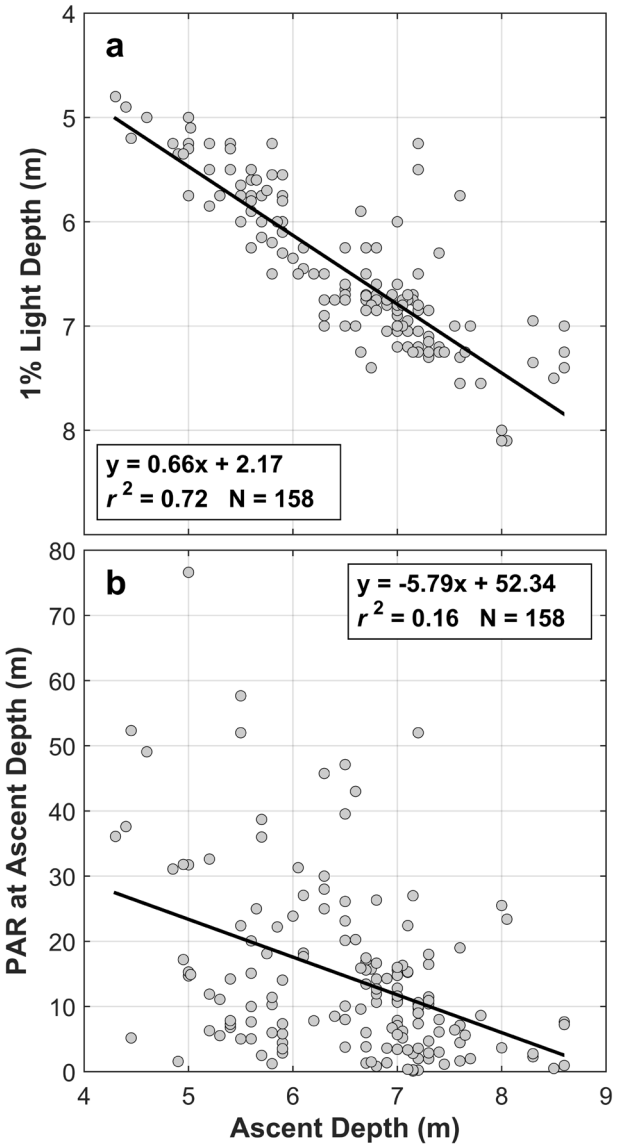

Fig. 5. Ascent depth and accompanying light data from 158 vertical migration events recorded from 1999 to 2012. (a) *Cryptomonas* ascent depth was fairly strongly correlated with water column transparency above the DCM, as indicated by the depth of 1% incident PAR. (b) By contrast, *Cryptomonas* ascent depth was weakly correlated with the absolute light level experienced by the cells when they reached that depth.

Significant departures from these general relationships were sometimes observed. First, we documented 5 events (lower arrows in Fig. 2) during the 21 years in which algal descent was delayed until after the next daytime period. Two of these shallower nighttime descents occurred in 2004, with this behavior captured during the monitoring of 8 of 11 consecutive days (Fig. 2). All 5 events were accompanied by periods of extremely heavy daytime cloud cover, with incident solar radiation being reduced to ≤20% of that of recent clear days. By contrast, all DCM days that exhibited regular migratory behavior and for which recent nearby-day fluorescence data were available for comparison were characterized by incident solar radiation >20% of that of recent clear days. On these 5 highlighted short migration dates, the range of subsurface PAR measured at the cells' ascent depth was low (1.5–5.2 µmol m^{-2} s^{-1}) relative to clear-day conditions, when light intensities measured at the ascent depth were usually 10-fold higher (24–43 µmol m^{-2} s^{-1}). Our provisional explanation for this abrupt change in swimming behavior is that although *Cryptomonas* ascended toward the bottom of the epilimnion during the day on each of these 5 events, insufficient photosynthetic energy was obtained during the day to allow a subsequent, energy-intensive descent during the night.

Other short migration days were apparent (Fig. 2), such as the third short migration in 2004 and the second and third short migrations in 2008. These samples and others lacked the necessary nearby-day fluorescence data to be examined in the same manner as the 5 described earlier. Specifically, the first 2008 short migration event (highlighted in Fig. 2) was preceded by 3 consecutive days of regular migration behavior, but the apparent second short migration event that immediately follows was not from the next day and lacked the necessary data to be similarly highlighted. Thus, the apparent back-to-back short migrations in September 2008 should not be interpreted as consecutive short migration days.

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Departures from the cells' typical migration behavior also were observed to result from periods of extended light reduction that accompanied episodes of reduced epilimnetic transparency. On 3 occasions (upper arrows in Fig. 2) following runoff from heavy rainfall, *Cryptomonas* moved into the epilimnion and remained in the mixed layer throughout the rest of the growing season. For example, late in the 2005 growing season, the 1% light level moved upward from 6.3 to 3.8 m, and the subsurface PAR value measured at the cells' previously recorded ascent depth was reduced from 27.0 to 1.2 µmol m^{-2} s^{-1}. Following this change in light availability, *Cryptomonas* abundance in the DCM at its ascent depth decreased sharply from 13 924 to 519 cells mL^{-1} at that same depth 2 weeks later while increasing 20-fold in the epilimnion. A similar phenomenon was observed in 2008, when the 1% depth moved from 5.8 to 4.3 m over 6 successive days, and the subsurface PAR value measured at the cells' previously observed ascent depth was reduced from 7.4 to 0.2 µmol m^{-2} s^{-1}. For this event, *Cryptomonas* abundance in the DCM at its ascent depth decreased from 15 010 to 779 cells mL^{-1} at that same depth 6 days later while increasing 5-fold in the epilimnion as a significant fraction of the total population moved upward into the mixed layer.

During the entire 21-year span of this study, *Cryptomonas* DCM were almost always absent when the depth of 1% light penetration occurred entirely within the epilimnion. For example, during the growing seasons of both 2005 and 2008 discussed earlier, the 1% light depth was observed to rise into the epilimnion while the DCM disappeared from the fluorescence profiles. The only exceptions to this general pattern occurred when the epilimnion was quickly deepening; as the growing season was ending each year in late October, *Cryptomonas* typically remained below the deepening epilimnion, following it down with progressively decreasing levels of incident solar radiation. Although diel subepilimnetic cell movements continued for several weeks, these migrations ended with the November mixing. The largest *Cryptomonas* populations subsequently persisted within the uppermost 3 m of the lake's water column until subepilimnetic DCM could form during the following summer.

Discussion

The factors that determine the abundance and spatial distribution of organisms are a primary focus in ecology (Brown 1984, Mellard et al. 2012). Phytoplankton are ideal organisms for examining these interacting processes because they require both inorganic nutrients and light to grow, and these essential resources are often present in strongly opposing vertical supply gradients in poorly mixed aquatic environments (Mellard et al. 2012). Fine-scale vertical heterogeneity in phytoplankton distributions is therefore common in the world's surface waters and may be a critical feature influencing trophic coupling in planktonic systems (Prairie et al. 2011). The formation of subsurface DCM in stratified systems is not a unique ecological response to environmental conditions; instead, it results from a broad range of interacting processes that contribute to the formation of nearly ubiquitous, persistent layers of elevated chlorophyll *a* (Cullen 2015).

In this study, we described an unprecedented 21-year record of DCM formation and behavior by phytoplankton. Other authors have reported longer records of phytoplankton vertical distribution, such as the 45 consecutive years of study in Lake Baikal (Hampton et al. 2014); however, the large (10–50 m) depth intervals sampled in Lake Baikal are exceptionally coarse relative to the centimeter-scale depth intervals analyzed here. We also note that Navarro and Ruiz (2013) have studied the formation of >9000 seasonal DCM throughout the world's oceans; however, their global-scale analysis does not capture the fine time-scale of DCM movements reported here (our measurements were made at intervals ranging from minutes to hours, frequently across multiple consecutive days). Smolander and Arvola (1998) studied phytoplankton vertical migrations in 10 successive diurnal experiments between May and September in a small, steeply stratified humic lake; however, these authors used a Blakar-type sampler that could not capture the detailed, 1 cm-scale cell movements recorded here using *in situ* fluorometry. In the study by Gervais (1997), fluorescence measurements were taken every 4 s, resulting in a spatial resolution of 1 cm; however, this analysis was restricted to multiple dates within a single growing season (30 Jun–31 Aug 1995). None of these studies match the level of spatial and temporal detail reported here.

The potential mechanisms that can result in the formation and persistence of DCM in lakes and oceans have been reviewed elsewhere (Camacho 2006, Prairie et al. 2011, Cullen 2015) and are not re-reviewed here. We emphasize, however, that motile algae in poorly mixed water columns can be thought of as playing a competitive game in opposing vertical gradients of nutrients and light. Using a spatially explicit dynamical model, Klausmeier and Litchman (2001) showed that highly motile phytoplankton species like *Cryptomonas* can form stable, thin layers in poorly mixed water columns (Mellard et al. 2012).

We conclude that the evidence presented here and by Knapp et al. (2003) suggests a key role for light availability in regulating the formation and diel vertical movements of subepilimnetic *Cryptomonas* in Cross Reservoir, Kansas (USA). As reported by numerous other investigators (e.g., Smolander and Arvola 1998, Camacho et al. 2001, Camacho 2006), the phytoflagellate populations in

Cross Reservoir were phototactic, moving upward to a predictable ascent depth within the oxygenated region of the water column during the daytime; during the nighttime, the strongest fluorescence signals and highest cell densities were recorded at a descent depth often located within the sulfide-rich anoxic layer (Fig. 1–3). Active swimming movements were used for these changes in the vertical location of *Cryptomonas*, and the observed swimming speeds of the algal cells (Table 2) are consistent with previously published values for vertical migration rates (up to ~70 cm h^{-1} in 4 m laboratory columns; Arvola et al. 1991).

We also stress that the absolute depths to which the *Cryptomonas* cells actively migrated were highly consistent across both 2- and 3-day intervals of continuous fluorometric measurements (Fig. 4). Moreover, a strong correlation was observed between the *Cryptomonas* cells' ascent depth and the transparency of the mixed layer, as reflected in the depth of 1% incident solar radiation ($r^2 = 0.72$; Fig. 5a). Currently, we are unsure why these migrating cells keyed in more closely on transparency than on the absolute light level experienced at their ascent depth (Fig. 5b). Light arriving to the deepest waters of lakes, however, primarily corresponds to the central part of the visible light spectrum from 550 to 630 nm (see figure 2 in Camacho 2006); these wavelengths of light can selectively be detected and harvested by phytoplankton species that contain high intracellular concentrations of phycobiliprotein accessory pigments, such as *Cryptomonas*. Consequently, we provisionally speculate that these phytoflagellates were responding to variations in both light intensity and light quality. Further work is needed to explore this hypothesis using underwater spectroradiometry.

Empirical evidence reported here suggests possible circadian rhythmicity in the movement of the *Cryptomonas* populations inhabiting Cross Reservoir (Fig. 3b). Circadian rhythms are well documented across the Tree of Life (Wijnen and Young 2006, Rosbash 2009), and algae clearly can tell the time of day (Suzuki and Johnson 2001). Ascending swimming movements by these phytoflagellates often began before sunrise and before first light at depth (Fig. 3 and data from 29 sampling dates, not shown). To our knowledge innate circadian rhythms have not been previously reported for either laboratory or field populations of *Cryptomonas*, and we strongly urge further study of this exciting possibility.

Cullen (2015) recently posed the intriguing question, "Subsurface chlorophyll maximum layers: enduring enigma or mystery solved?" We suggest that DCM can provide many interesting new mysteries to solve, and we hope the results reported here will stimulate new research on the spatial and temporal dynamics of subepilimnetic phytoplankton. In large freshwater and marine ecosystems such as the Laurentian Great Lakes and the oceans, new tools such as the FIDO-Φ instrument package (Prairie et al. 2011) can potentially be used to generate important new data and insights and to stimulate exciting new hypotheses.

We agree with Navarro and Ruiz (2013) that instead of passively reacting to instantaneous external forcings, DCM may modify their physical and chemical environments to become self-preserving biological structures. Once formed, the DCM itself thus controls the vertical distribution of nutrients and light through the competition mechanism identified by Klausmeier and Litchman (2001) to the extent that they persist in a highly cohesive and predictably responsive population over ecological time. These ideas should be tested further, and we join Cullen (2015) in hoping that existing theory, tracing back to Riley, Yentsch, and Steele, and buttressed with careful new observational, experimental, and theoretical work, will be part of these efforts.

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