



Extant mat microbes synchronize vertical migration to a diel tempo

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ARTICLE INFO

Article history:

Received 12 July 2022

Accepted 14 October 2022

Available online 3 November 2022

Communicated by Jenny Fisher

Keywords:

Microbial mats

Diel vertical migration

Cyanobacteria

Photosynthesis

Sulfur oxidizing bacteria

Chemosynthesis

ABSTRACT

Animal migrations mark the largest daily movement of biomass on Earth today, but who performed the first diel migration and what are the implications? Modern-day benthic microbial mats resembling those of early Earth inhabit Lake Huron's low-oxygen, high-sulfur, submerged sinkholes. Over several days during multiple years, we gathered time-lapse images of motile, phototactic, purple-pigmented cyanobacteria capable of oxygenic and anoxygenic photosynthesis, and chemotactic, pigment-free, chemosynthetic sulfur-oxidizing bacteria indulging in diel vertical migration of just 1–2 mm across the mat-sediment interface. As the world turns, alternating waves of vertically migrating photosynthetic and chemosynthetic filaments responded rapidly and non-linearly to daily fluctuating sunlight and chemical gradients. The photosynthesizing cyanobacteria and sulfur oxidizing bacteria exhibited their fastest motility following dawn and dusk, which turned the mat surface visibly purple during day and white at night, respectively. Our high-frequency, time-lapse images visually capture a modern day synchronized diel migration similar to what might have been the first and largest daily mass movement of life during the long Precambrian. During that time, benthic microbial mat motility would have played a critical role in directly optimizing photosynthesis and chemosynthesis, as well as indirectly burying carbon and oxygenating the biosphere. Further studies of these mat ecosystems will add to the expanding knowledge of Earth's extant biodiversity and physiologies.

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Introduction

"Beginnings are apt to be shadowy, and so it is with that great mother of life, the sea. ..."

In its mysterious past it encompasses all the dim origins of life. . ."
(Carson, 1950).

Evidence of widespread microbial mat distribution during the Archean and Proterozoic is found today in the geological record of microbialites such as stromatolites (Grotzinger and Knoll, 1999; Stal, 1995). Fortunately, even today, microbial mats similar to life in the shallow, anoxic, sometimes euxinic (high hydrogen sulfide, low dissolved oxygen) seas of the early biosphere thrive in globally distributed refugia (Biddanda et al., 2009; Cohen et al., 1975; Prieto-Barajas et al., 2018; Yang et al., 2011). Several of these modern-day mat ecosystems potentially resemble life in

the Precambrian when the biosphere was mostly microbial and benthic (Brocks et al., 2017; Dick et al., 2018; Perez-Ortega, 2020; Sánchez-Baracaldo, 2015; Schopf, 1970).

Survival in such extreme environments required microorganisms to evolve numerous structural and physiological adaptations (Falkowski et al., 2009). Microbes within a mat operate in a 3-dimensional world, using motility to adjust to changing environmental gradients such as light, oxygen, and sulfide on a diel basis (Kruschel and Castenholz, 1998; Lichtenberg et al., 2020; van Gemerden, 1993). Past and recent studies support the idea that cyanobacteria adjust their vertical position within the mat ecosystem to optimize photosynthesis depending on ambient light (Lichtenberg et al., 2020; Tamulonis et al., 2011; Whale and Walsby, 1984). Along with photosynthetic cyanobacteria, motile chemosynthetic sulfur-oxidizing bacteria (SOB) take advantage of varying H₂S and O₂ levels within the upper μm to mm thickness of mat-sediment complexes (Castenholz et al., 1991; Jørgensen et al., 1986; Kinsman-Costello et al., 2017; Klatt et al., 2016a; Nold et al., 2010). Given inorganic carbon assimilation related to anoxygenic photosynthesis (AP), oxygenic photosynthesis (OP), and chemosynthesis occurs at the mat-water interface where light, sulfur, and oxygen variably interact, it requires active diel

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migration of cyanobacteria and SOB in response to diel fluctuating redox (Dick et al., 2018; Klatt et al., 2016b). It also requires variable metabolic activity, such that while most cyanobacteria are inhibited by sulfide, some are tolerant and others can use sulfide as the preferred electron donor over water, switching between AP and OP (Bühning et al., 2011; Dick et al., 2018; Jørgensen et al., 1986; Klatt et al., 2016b).

Benthic microbial mats, perhaps similar to those of shallow pools and coastal margins of Precambrian Earth, are currently found in the modern-day coastal waters of northwestern Lake Huron (Electronic Supplementary Material (ESM) Figs. S1 and S2) (Biddanda et al., 2009; Biddanda et al., 2012; Reinhard et al., 2013). Here, dissolution of Silurian-Devonian bedrock has produced karstic sinkhole formations, through which anoxic, sulfurous groundwater emerges onto the lake floor, fueling growth of microbial mats. The microbial mats that cover the floor and walls of Lake Huron's shallow submerged sinkholes are primarily composed of communities of purple-pigmented photosynthetic cyanobacteria and white-appearing chemosynthetic SOB (Biddanda et al., 2009; Voorhies et al., 2012). Hereafter, these filamentous purple cyanobacteria and white SOB will be referred to as simply cyanobacteria and SOB, respectively. Cyanobacteria and SOB together with the layers of sulfate-reducing bacteria and methanogenic bacteria in the organic matter-rich sediments beneath these mats, establish a vertical redox tower (Biddanda et al., 2012; Nold et al., 2010). Organisms inhabiting these mat ecosystems are closely related to those at the bottom of hot springs and Antarctic lakes (Andersen et al., 2011; Castenholz et al., 1991), and are analogous to the most ancient forms of life on Earth (Allwood et al., 2006; Falkowski et al., 2008) – making them model systems for exploring the biosphere's evolution, physiology, biodiversity, and biogeochemistry (Biddanda et al., 2012; Dick et al., 2018; Voorhies et al., 2012). Furthermore, based on the extensive Karst geology underlying the lower Great Lakes and the recent discovery of additional sinkholes in Lake Erie and Lake Huron, we predict that submerged sinkholes are a more common feature than expected in all of the lower Great Lakes (Michigan, Huron, Erie and Ontario; Biddanda et al., 2012).

In previous studies of Lake Huron's submerged sinkholes, we made several in-field and onboard/laboratory observations of freshly collected purple mats that subsequently turned white within hours when stored in darkness. Similarly, day-long dive/ROV observations in-field revealed evidence of the mat surface turning increasingly purple as the day progressed. The recent development of a field-deployable underwater camera system capable of high-frequency time-lapse photography provided the opportunity to directly observe this day-night shift in situ. Here, we chronicle the in situ shift in mat surface color over the course of several days. We corroborated field observations with a laboratory study of intact mats under simulated day-night conditions.

Materials and methods

Study site

The Middle Island Sinkhole is 17.3 km northeast of Alpena, Michigan in Thunder Bay National Marine Sanctuary, Lake Huron (ESM Figs. S1, S2 and S3). Due to karst geology, a portion of the Lake Huron bottom collapsed, creating a submerged sinkhole. Groundwater containing dissolved marine evaporites is pushed via hydrostatic pressure, causing oxygen-poor, sulfur-rich water to seep into the sinkhole (Biddanda et al., 2012; Ruberg et al., 2008). While 23 m in maximum depth, groundwater fills only the bottom 1 m of the sinkhole with 22 m of overlying Lake Huron

water. Only 5–10 % of ambient surface light reaches the surficial microbial mat covering the sinkhole bottom (Snider et al., 2017).

The main groundwater source into Middle Island Sinkhole comes from the “Alcove” at 15 m deep, and averages 9°C, 7.1 pH, 2,300 $\mu\text{S}/\text{cm}$, 0 μM dissolved oxygen, 13 mM sulfate (SO_4^{2-}). The groundwater then flows over a ledge down to the “Arena” study area. The Arena groundwater averages a somewhat steady 11 °C, 2,000 $\mu\text{S}/\text{cm}$, 53 μM dissolved oxygen with a density (1,001.03 kg/m^3 at 4 °C) greater than the overlying Lake Huron water (1000.10 kg/m^3 at 4 °C). Colorful microbial mats line the Alcove as well as in patches attached to rocks on the wall where the Alcove flows into the Arena. While the upper mm of mat can contain small amounts of dissolved oxygen, the concentration decreases to near 0 after the first 1–2 mm of the surficial sediment, with a portion of sulfate also being converted to H_2S by that point (~ 2 mM H_2S at 5 mm depth) (Kinsman-Costello et al., 2017; Nold et al., 2010).

The microbial mat mainly consists of purple-pigmented cyanobacteria of *Planktothrix* (formerly *Oscillatoria*) and *Phormidium* genera, which are capable of OP and AP, and whitish filaments of chemosynthesizing SOB (Biddanda et al., 2012; Kinsman-Costello et al., 2017). The SOB are morphologically similar to known sulfur-oxidizer, *Beggiatoa* (Nold et al., 2010), and coarse genetic analyses have confirmed the presence of *Beggiatoa* and *Epsilonproteobacteria* in the mats (Kinsman-Costello et al., 2017).

Time lapse imagery

Images were captured by a GoPro Hero 4 camera with a CamDo Blink attachment to enable time-lapse image capture. The camera setup was deployed within a GP4c Underwater Housing (Marine Imaging Technologies). Four LED lights (Blue Robotics) attached and synced to the camera system provided flash photography during the night (~ 5 s before each image). Complete Camera setup integrated by engineers at Marine Imaging Technologies (<https://www.marineimagingtech.com>).

The camera housing was attached to a PVC mount which angled the camera slightly downward. This system was deployed on the sinkhole bottom by NOAA divers from the Thunder Bay National Marine Sanctuary (Fig. 1). Images were taken every 30 min from July 10, 2018 at 12:00 noon until July 12, 2018 at 10:00 A.M. EDT. Concurrent light measurements (lux) were gathered by a HOBO Pendant Light/Temperature logger.

On June 10–11, 2019, we repeated the time-lapse study with images taken every 15 min over a 24-hour day-night cycle to confirm the 2018 results (ESM Video S1). This observation cycle occurred during somewhat cloudy conditions compared to the sunny-fair conditions of the 2018 study. Unfortunately, severe marine weather prevented us from gathering photos over the course of a complete day and night cycle. Further imaging was taken every 15 min during July 19–21, 2021, with partially cloudy and sunny conditions occurring on July 20 and 21, respectively (ESM Video S2).

Time-lapse image analysis

We estimated the percent coverage by cyanobacteria and SOB in each 2018 image. Images were overlaid with a 10×10 perspective-corrected grid enclosing a $1 \text{ m} \times 1 \text{ m}$ area (ESM Fig. S4). We estimate each individual square of the grid (100 total) to be covered mostly by cyanobacteria or SOB by the binary method. This method allows us to broadly estimate mat surface coverage, not taking into account thin veils of purple or white across a majority white or purple square. All squares were totaled to calculate percent coverage of the mat surface by cyanobacteria or SOB at each 30-minute time point.

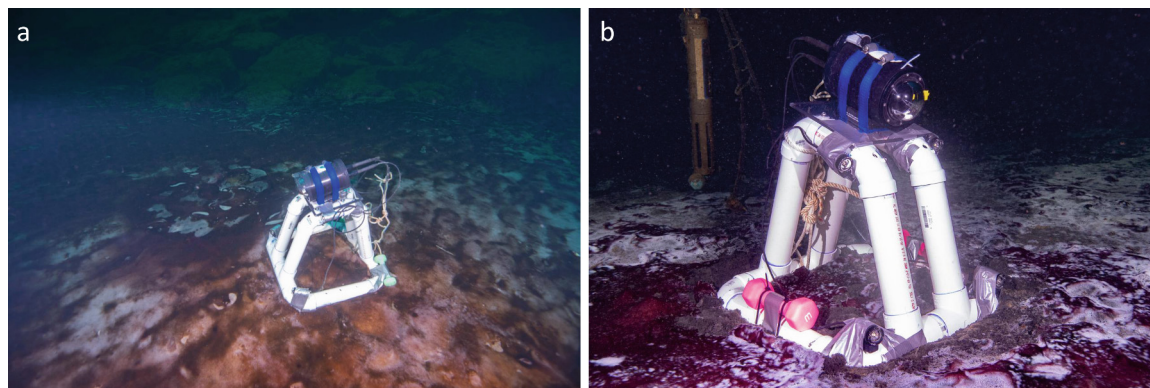


Fig. 1. Diver images of the deployment position of the time-lapse submersible camera system mounted on top of a PVC frame on microbial mat at the bottom of Middle Island Sinkhole, Lake Huron at ~23 m depth in 2018 (a) and 2021 (b). In the background of image (b) is a YSI multi-parameter water quality sonde monitoring the composition of the groundwater flowing over the mat ecosystem.

In-lab validation and analyses

Divers hand-collected 7-cm diameter, 20-cm long cores with clear PVC tubes from Middle Island Sinkhole in the same area the camera system was taking time-lapse imagery. Cores contained intact 1–2 mm thick discs of mat atop several centimeters of undisturbed sediment. They were sealed on board the research vessel and kept vertically stable on ice in a dark cooler until return to the lab at Annis Water Resources Institute in Muskegon, Michigan. Cores were put into an incubator, kept at 10 °C, on a 16-hour light/8-hour dark cycle. Light was applied from directly overhead and shaded with a neutral density filter to mimic the intensity and color of light they would experience in situ. Unlike the natural sinkhole environment, the side of the cores below the mat line were not shielded from receiving light exposure and light came on instantly during “daytime” as opposed to the gradual increase and decrease of light exposure in situ.

In addition to verifying the mat surface color change in a lab setting, we also tested the mat's specific response to light by changing the light/dark cycle timing. Instead of applying light from 6 am to 10 pm and darkness from 10 pm to 6 am as they experienced in situ, we applied light from 4 pm to 8 am and darkness from 8 am to 4 pm. This allowed us to manually photograph the mats at the end and beginning of the light period. Cores were allowed 4 light/dark cycles to adjust to the new diel period before we began to take photos. The groundwater over each core was gently replaced with fresh groundwater every day. Pictures were taken 30 min before each light and dark cycle would end.

Results

Time-lapse color changes

Time-lapse, in situ photography of the bottom of Lake Huron's Middle Island Sinkhole revealed nearly complete changes in mat surface coverage by cyanobacteria and SOB throughout the diel cycle in July 2018, June 2019, and July 2021 (Figs. 2 and 3). During daylight, cyanobacteria were the dominant surface dwellers, covering nearly the entire surface area of the mat ecosystem in June 2018. During night, SOB emerged upwards, covering much of the mat surface. After dawn when sunlight became available, cyanobacteria covered the mat surface 100 % in 5–6 h. Following nightfall, SOB covered the mat surface 90–93 % within 8–11 h. These striking changes in mat surface color between day and night were tracked in 2018 via time-lapse, spanning 2 days spread over 2 sunsets and 2 sunrises (93 images at 30 min intervals; ESM Video

S1). A shorter (24 hrs), higher frequency time-lapse (15 min image intervals) deployment in June 2019 confirmed these field observations, as did a longer (41 hrs) high frequency time-lapse in July 2021: during the day cyanobacteria surfaced, and at night SOB surfaced (Fig. 3; ESM Video S2).

Macroscopically, mats composed primarily of cyanobacteria or SOB at the surface appear purple or white, respectively (Figs. 2, 3 and 4a) (Biddanda et al., 2015; Voorhies et al., 2012). However, mat coloration was never evenly distributed – with surface patches of SOB filaments visible no matter the time of day. During night, only the darkest purple mat patches of the day remained covered by cyanobacteria remaining lightly purple.

Migration rates

Estimates of predominant mat surface color within a 1 m × 1 m grid overlay in successive time-lapse images through day-night cycles indicated remarkable changes in mat surface coloration from day to night (Table 1; Fig. 5; ESM Fig S5; ESM Videos S1 and S2). Under low light conditions 0.5 to 1.0 h before sunset, SOB started to cover the mat surface, gaining 25 % coverage within 1 h after dark (Table 1; Fig. 5; ESM Fig. S5). This was a much faster change in coverage compared to cyanobacteria, which only attained 25 % mat surface coverage 3 h after sunrise. As far as time to maximum coverage, cyanobacteria only took 5.5 h to reach 100 % coverage; however, SOB more slowly reached maximum coverage, taking over 8 h to do so and never reaching 100 %. Between the times of minimum and maximum coverage for cyanobacteria and SOB, their individual observed rates of change in coverage also appear to be different. The maximum rate of increase in coverage for cyanobacteria was 12.5 % per hour at 4 h after sunrise, while SOB was 8.5 % per hour at 1.5–3.5 h after sunset.

Microbes involved in diel vertical migration

Predominant composition of the mat community (as revealed by microscopy) consisted of cyanobacterial filaments (*Planktothrix* and *Phormidium*), and colorless SOB filaments (*Beggiatoa*) with dark inclusions inside, presumably elemental sulfur granules stored within their cells (Fig. 4b). Cyanobacterial filaments are typically 100–10,000 µm in length (Biddanda et al., 2015) and SOB filaments appeared to be of similar length under the microscope. A high magnification image shows details of cyanobacterial and SOB filaments (Fig. 4c). Both functional guilds of microbes (photo-synthetic cyanobacteria and chemosynthetic SOB) play an active role in diel vertical migration (Fig. 4d).

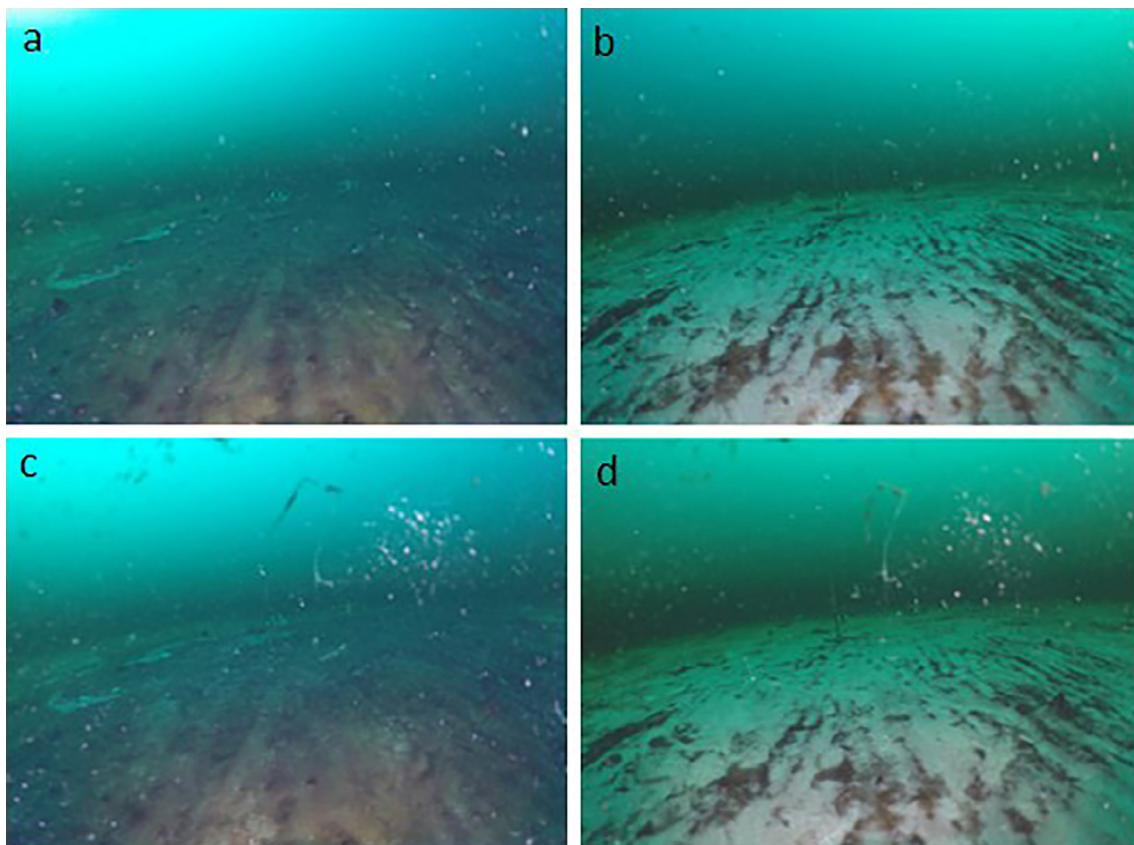


Fig. 2. Day and night shifts in mat surface color. Images from the time-lapse camera (Fig. 1) at dusk (20:00 hrs) and dawn (7:00 hrs) of microbial mats on the sinkhole bottom during July 10 to 12, 2018. Day 1 dusk (a), day 2 dawn (b), day 2 dusk (c), and day 3 dawn (d). Note: Planktonic debris has settled on the top of the camera dome on days 2 and 3.

In-lab validation of microbial diel vertical migration

In-lab simulations of day and night conditions with intact sediment cores collected from the sinkhole in 2018 produced similar results as in the field (ESM Fig. S6). Photographs taken just before simulated nightfall and daybreak showed mat surfaces to be mostly covered by cyanobacteria during day and by SOB at night. We followed two separate cores over several diel cycles (~14 days) and consistently observed day-night shifts in mat appearance confirming active diel vertical migration and corroborating field observations. Indeed, the study identified the central role of daylight in diel vertical migration control, not just due to the color change in response to light, but also due to the alteration of the time of day the mats would have experienced day light and night dark. The mats would have normally experienced dark from 21:30 to 06:30; however, in the lab we exposed the mats to darkness from 08:00 to 16:00. Thus, exposed to darkness in a time period shifted from their normal cycle, the mat microbes still responded with SOB surfacing during darkness and cyanobacteria surfacing during light.

Discussion

Diel journey: chasing sunlight, reduced sulfur, and dissolved oxygen

Observed overall changes in mat color in situ in the present study (~5–10 h) are slower than the <1 h to 2 h reported in some laboratory settings (Garcia-Pichel et al., 1994; Richardson and Castenholz, 1987). Faster changes were likely due to the instant application or removal of light in the laboratory at full intensity,

compared to gradual natural light cycles in situ. We did not measure rates of color change in the lab, but we also applied or removed light instantaneously. So, we may have seen quicker rates of change than in situ, similar to other laboratory experiments. In a previous study, Middle Island Sinkhole cyanobacteria (same as in the current study) in a laboratory setting had comparable movement speeds ($50\text{--}200\ \mu\text{m min}^{-1}$) as seen in other cyanobacterial mat studies which would allow time for the diel vertical migration we see in situ to occur (Biddanda et al., 2015). While we are uncertain about the precise mechanisms of motility employed by the cyanobacterial and SOB filaments to engage in diel vertical migration in the present study, earlier studies have revealed physiological and structural features such as slime secretion and surface proteins that facilitate movement (Hoiczky, 2000), and our live microscopic observations suggest gliding motility that is either phototactic, chemotactic or both (Biddanda et al. 2015; present study).

Despite having ample time to migrate, mat surface color was never completely uniform across the entire observable area. This is an indication that the horizontal redox environment below the mats may be quite patchy in both space and time. Such patchiness could be driven by variable ground water flow above the mat contours, and variable chemical and microbial composition of the sediment below (Biddanda et al., 2015; Kinsman-Costello et al., 2017). Indeed, random patches of mat are often lifted into finger-like projections 10–30 cm high – buoyed up by CH_4 gas produced by methanogens in the sediments below (Fig. 4 a,d) (Bižić et al., 2020; Nold et al., 2013; Teske et al., 1998).

The reasons for and mechanisms of migration in microbial mats have been established in a laboratory setting by decades of work;

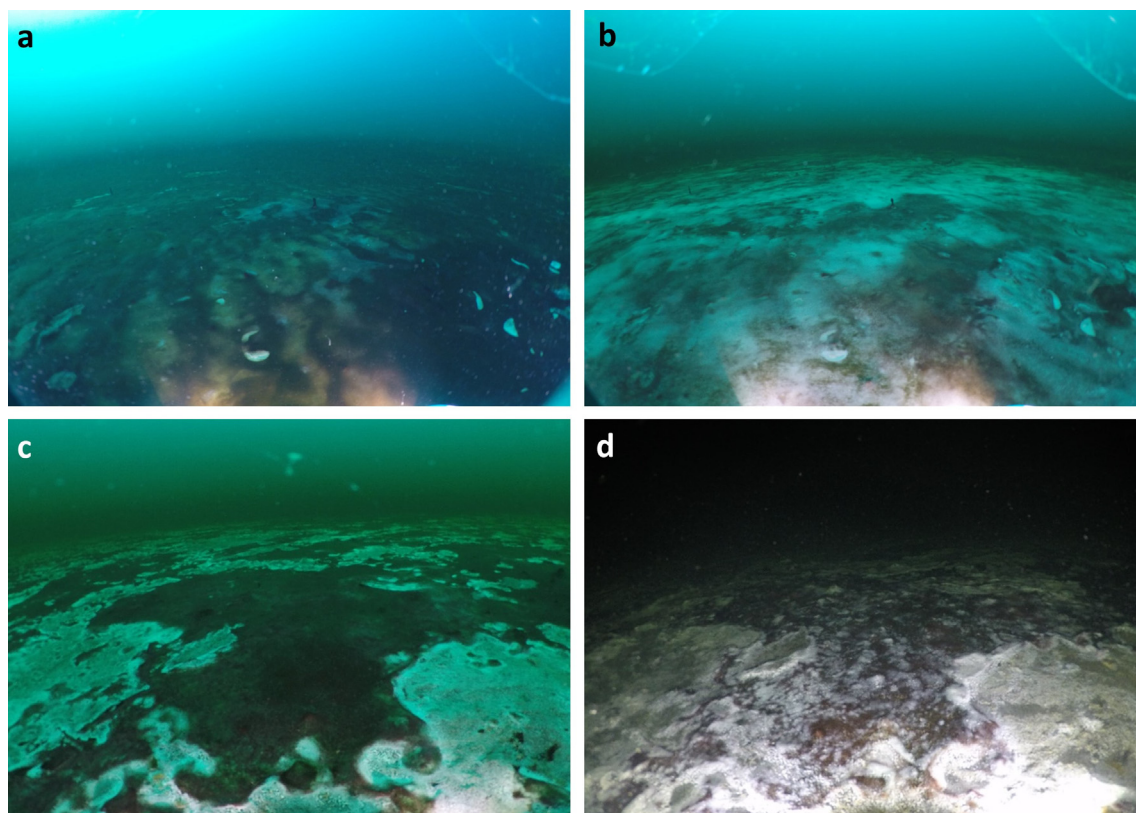


Fig. 3. In-field re-validation of diel vertical migration in 2019 and 2021: (a) dusk – June 11 20:00 and (b) dawn – June 12 7:00 and 2021: (c) midday – July 20 12:30 (d) midnight – July 20 0:30 time-lapse photos from June 2019 and July 2021 in-field camera deployment in Middle Island Sinkhole show the same diel changes in mat color as seen in July 2018 (Fig. 1, and Extended Data ESM Videos S1 and S2).

however, verification in situ has only recently become available. Similarities in environmental characteristics and microbes from other laboratory research allow us to infer how the Middle Island Sinkhole system works and the chemical changes occurring within the mat complex. Starting with SOB, we know that they follow the O_2/H_2S boundary to minimize exposure to too high or low concentrations of either (Dillon et al., 2009; Gray and Head, 1999; van Gernerden, 1993). The nearly complete surface coverage by SOB at the end of the night suggests that this boundary moves upwards vertically within the mat throughout the night to the point that the SOB straddle this boundary at the very top of the mat (Jørgensen et al., 1986; Klatt et al., 2016b). Above them they have O_2 (and other electron acceptors such as SO_4^{2-}), as indicated by direct sonde measurements from the overlying water, and that below the SOB should be relatively enriched with H_2S from sulfate reducers present in the sediment below the mat (van Gernerden, 1993; Klatt et al., 2021). As for the rates of change we see, the quick takeover of the surface by white SOB as the sun sets is likely in response to higher respiration rates without OP occurring (Pages et al., 2014). It is still unclear why SOB take over the surface so slowly later in the night, and why some mat patches continue to be covered by cyanobacteria throughout the night.

Cyanobacteria on the other hand respond to H_2S and light availability during the day, which can create two periods for them. In the early morning, the cyanobacteria start out in a H_2S -rich zone below the SOB, who only limit light penetration by $\sim 10\%$ (Lichtenberg et al., 2020). The presence of light and H_2S gives cyanobacteria the ingredients to perform the more energetically preferred AP as they migrate to the surface of the mat above SOB (Jørgensen et al., 1986; Klatt et al., 2020). The high light penetration through SOB could be the reason for such slow surface take-

over by the cyanobacteria in the morning. However, as H_2S is used by both SOB and cyanobacteria, cyanobacteria rise to the mat surface, maximize light availability, and perform OP. That is not to say that they can't perform both simultaneously in the intermittent period (Jørgensen et al., 1986; Klatt et al., 2016). The observed quick surface takeover by cyanobacteria later in the day could be the result of the combined surface movement by cyanobacteria seeking light maximization as well as downward movement by SOB following the O_2/H_2S boundary downwards after H_2S is used up and O_2 is produced near the mat surface. Over the diel cycle, cyanobacteria and SOB deal with competing signals of light, O_2 , and reduced sulfur to optimize their locations within the mat ecosystem, and such patches may be a reflection of the prevailing variable sediment redox conditions underneath the mats (Fig. 6) (Dillon et al., 2019; van Gernerden, 1993).

Optimization of mat ecosystem function by coordinated diel vertical migration

Modern microbial mats that are found in globally distributed sulfidic low- O_2 refugia exhibit a range of carbon and sulfur physiologies such as OP, AP, chemosynthesis, sulfate reduction, and methanogenesis, and provide a window into paleomicrobial lifestyles in our biosphere's past (Bižić et al., 2020; Bühring et al., 2011; Dick et al., 2018; Falkowski et al., 2008; Klatt et al., 2016; Souza et al., 2012; Yang et al., 2011). Mats like these are known to efficiently use and recycle carbon and sulfur between users and producers (Canfield and Des Marais, 1993; Jørgensen et al., 1979). Evaluating AP, OP, and chemosynthesis in the light of motility illustrates the advantage motility provides for optimizing resource use in these ecosystems where the oxic/anoxic boundary

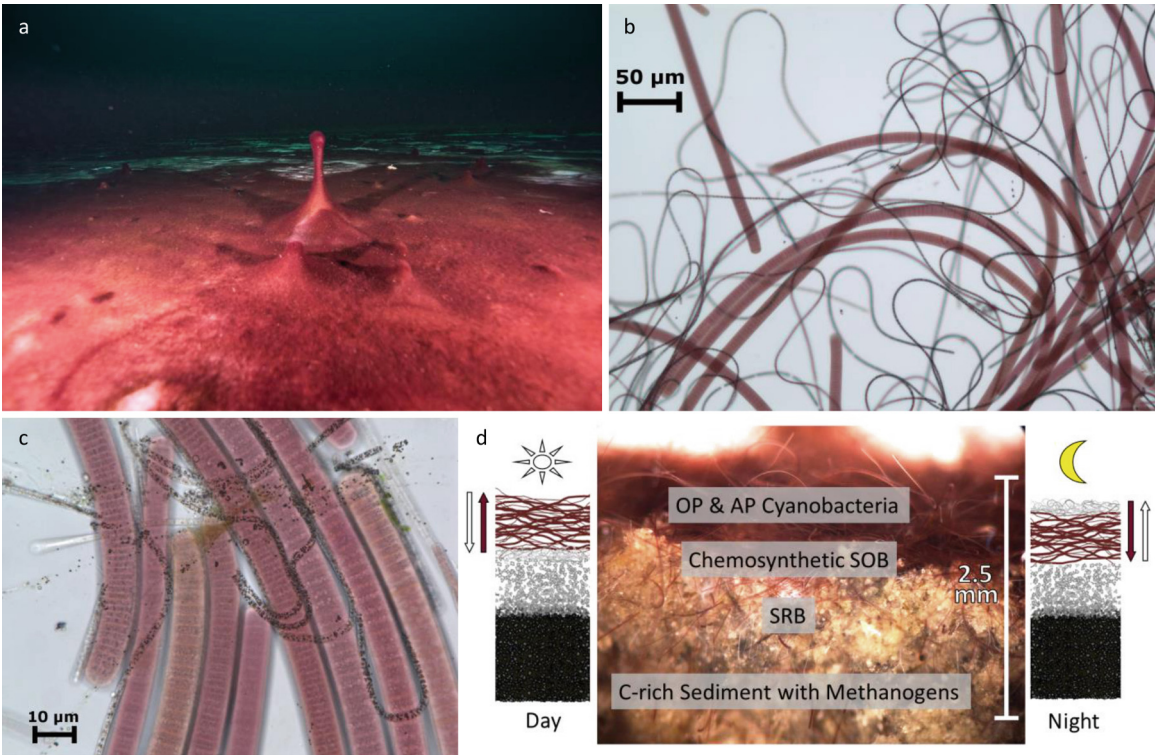


Fig. 4. Microbes of mat diel vertical migration: (a) Underwater diver photo during daytime showing the predominantly cyanobacteria-dominated purple coloration at the surface along with a minority of white patches where sulfur oxidizing bacteria continue to prevail (Photo credit: Phil Hartmeyer, NOAA Thunder Bay National Marine Sanctuary). Finger-like mat projections in the center of the image (10–30 cm tall) are buoyed up by excess CH₄ gas trapped beneath them. (b) 10x and (c) 40x Bright-field microscopy of microbes in the upper 1–2 mm of mat reveals the presence of purple cyanobacteria and chemosynthetic bacteria containing dark sulfur granules. (d) A cross-sectional stereo microscopic image of mat with intact sediment below reveals successive layers of motile, filamentous purple cyanobacterial and white sulfur oxidizing bacteria, a zone of semi-permanent white carbonate crystals, and a larger zone of black carbon-rich sediment at the bottom colonized by sulfur reducing bacteria and methanogens. Schematic diagrams beside the mat cross section depict scenarios of day and night-time status at the mat surface following vertical migration of alternating waves of photosynthetic and chemosynthetic filaments. Fig. (d) is composed from source information in [Nold et al. \(2010\)](#), [Biddanda et al. \(2012\)](#), [Voorhies et al. \(2012\)](#), and the present study.

Table 1

Diel color coverage over the mat surface: Time required for white and purple filaments to cover a given % of the camera viewing area on the mat surface during night and day periods, based on counts from the image analysis of a grid overlay (1 m × 1 m square cells) on each of the time-series images.

Time to Cover Mat Surface (hrs)						
Mat Coverage (%)	White Night #1		Purple Day #1		White Night #2	
Daily Minimum	0.0	0%	0.0	10%	0.0	0%
25	1.8		3.0		2.0	
50	3.0		3.5		3.5	
75	5.0		4.0		5.0	
Daily Maximum	8.5	90%	5.5	100%	10.5	93%

fluctuates on a diel basis ([Biddanda et al., 2012](#); [Bühning et al., 2011](#); [Snider et al., 2017](#); [Voorhies et al., 2012](#)). Motility enables microbes in the mat ecosystem to pursue ideal conditions rather

than waiting for diffusion. Future studies should investigate how the interplay of varying levels and duration of sunlight and sulfide may have exerted critical controls on O₂ production over evolutionary time.

Close metabolic coupling between phototrophs and heterotrophs is common in cyanobacterial mat communities, such as in hot springs, and Middle Island Sinkhole is no exception ([Stal, 1995](#); [Voorhies et al., 2012](#)). The big questions here are: how have meters of organic carbon gotten buried underneath the mats given the near balance between production and respiration within the mat system above, and where are heterotrophs getting their carbon from? One option is direct carbon supply by phototrophs and chemosynthesizers who are leaky or get lysed by viruses ([Voorhies et al., 2016](#)). The other option is an outside source of indirect carbon supply, like settling planktic material. As a consequence of microbial diel vertical migration, filaments seeking sunlight, O₂, or H₂S, climb over settled plankton from above, which subducts organic matter into anaerobic sediments below ([Biddanda et al., 2015](#); [Nold et al., 2010](#)). In these sinkholes, mat phototrophs likely indirectly transfer carbon and energy to their heterotrophic partners by burying sinking planktic production, leading to meters of organic carbon accumulation over mere thousands of years. This would explain how prior measurements of mat production and benthic respiration rates using benthic chambers revealed tight metabolic balance ([Voorhies et al., 2012](#)), even as considerable sedimentary carbon accumulates ([Nold et al., 2013](#)). Thus, carbon is buried as a by-product of metabolic optimization by microbial mats.

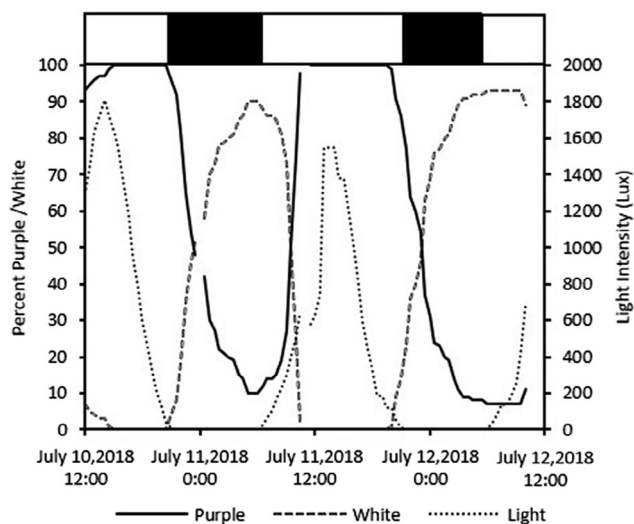


Fig. 5. Following the sun by diel vertical migration. Percent coverage of purple photosynthetic cyanobacteria and white chemosynthetic bacteria on the surface of the mat in relation to ambient light (Lux, dotted line) from July 10 to July 12, 2018. Missing values for 0:00 on July 11, 2018 due to technical error in time-series camera scheduling, and 11:00 on July 11, 2018 represents a cloudy image caused by sediment kicked up by divers during mid-day operations nearby. Note: The bars at the top represent times when daylight is available (white) or unavailable (black).

The 1 mm journey: speculations on diel vertical migration in a early Earth mat world

Diel vertical migration of zooplankton, fish, and marine mammals in lakes and oceans constitute the largest daily mass movement of life on the planet today (Behrenfeld et al., 2019; Hays, 2003). But what was the largest daily migration in the early biosphere before free-floating plankton, when prokaryotic microbes formed benthic mats on the Precambrian seafloor (Brocks et al., 2017; Sánchez-Baracaldo, 2015)? Could Lake Huron's low-oxygen, high-sulfur mat ecosystems, and other modern-day "extreme" microbial ecosystems such as these, provide an opportunity to peer into our distant evolutionary past?

We know that throughout Earth's history, massive colonial mats composed of microorganisms, like stromatolites, have left their fossilized reefs in the geological record and in the composition of the air and water (Falkowski et al., 2008; Schopf, 1970; Lyons et al., 2014). Despite the physical record, little is known directly about the physiology of cyanobacteria that thrived during that time, most of which did not leave fossil records, but did leave paleo-biomarkers (Hamilton et al., 2016). However, we can make some inferences based on physiological processes in modern-day analogs (Cohen et al., 1975). If motile diel vertical migration traits such as those observed in the present study prevailed among photosynthetic and chemosynthetic benthic microbes during the Precambrian into the Proterozoic, they would have represented the largest daily mass movement of life even if they were only moving 1–2 mm per day. Earth's modern-day extreme ecosystems like these may not only have helped shape the early biosphere by their daily activities of producing organic carbon and releasing oxygen in the past (Hamilton et al. 2016) as they do now (Biddanda et al., 2015; Klatt et al., 2021), but they also contribute critically to Earth's extant biodiversity and physiologic potential. Life's distant marine origins are apt to be shadowy under the fog of time (Carson, 1950), but careful observations of modern-day microbial mat ecosystems fueled by ancient marine evaporites may reveal clues to answering these enduring questions.

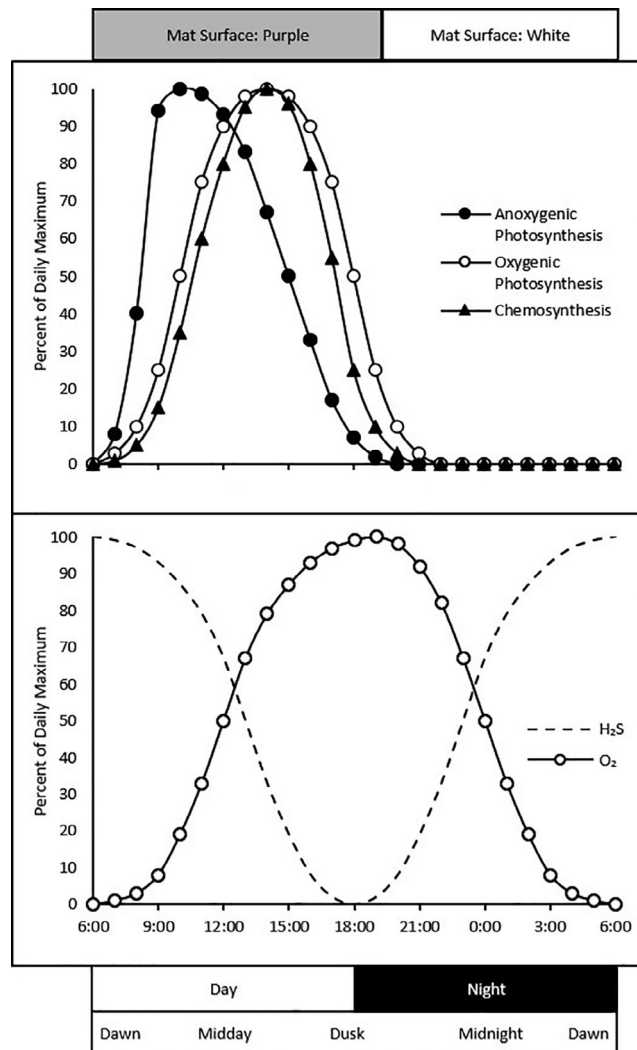


Fig. 6. A day in the life of a mat. Schematic model of the scenarios of the diel varying production processes (top) and substrate-products (bottom) in modern-day microbial mats in the Middle Island Sinkhole. Rates and concentrations are expressed relative to daily minimum and maximum. Sequential and coordinated vertical motility of anoxygenic/oxygenic photosynthetic cyanobacteria and chemosynthetic microbes across rapidly fluctuating redox may have favored the survival and success of microbial mats through time. Approximate rates and concentrations on daily mat dynamics are based on data in Jørgensen et al. (1986), Castenholz et al. (1991), Klatt et al. (2016), and the present study.

Conclusions

Today, benthic microbial mats covering submerged sinkhole ecosystems of Lake Huron exhibit rapid motility of photosynthetic and chemosynthetic filaments in response to daily environmental variations. Motility in sync with changing light and redox may play a critical role in mat formation and dynamics, photosynthesis and chemosynthesis, sedimentary carbon accrual, and net oxygen evolution in mat ecosystems. Thus, our motility-related findings have important implications for advancing our understanding of not only the ecophysiology and biogeochemistry of modern-day microbial mats, but also the evolution of early Earth's ocean and atmosphere as influenced by analogous microbial mat communities in the Precambrian seas. Our diel study also suggests promising leads for future hypothesis-driven observational and experimental investigations into the everyday life of modern-day mats to assess their emergent ecosystem level roles. These include new insights into how dynamic the early biosphere might have

been leading up to the oxygenation of the planet, the significance of modern-day mat ecosystems to Earth's current biodiversity and physiologic potential, and ideas about what a return to a future mat world scenario may be like, a scenario not implausible since many geobiological phenomena cycle and recycle again (Schlesinger and Bernhardt, 2013; Lyons et al., 2014). Additionally, Earthly mats such as those in the submerged sinkholes of Lake Huron that are and suitable for deploying human divers and remotely operated/autonomous observing vehicles may serve as useful working models in our search for life in extraterrestrial planets with active hydrospheres (Biddanda et al., 2021).

Microbes obtain and use information from their microenvironment to meet the challenges of everyday life (Dusenbery, 1996; Hoiczy, 2000), and microbes responding to microenvironmental gradients in their habitats are the engines that drive the cycling of major bioactive elements on Earth (National Academy of Sciences, 2007; Falkowski et al., 2008; Schlesinger and Bernhardt, 2013; Stocker, 2012). Over their long geobiologic history, microbes have evolved ecologically advantageous features such as motility that allow them to survive and succeed in sharply varying light and redox environments such as mat, soil and sediment ecosystems in which they often occur in abundance. In this context, the challenge will be to comprehend the daily “biogeochemical tango” taking place in Earth's extant mat microbiomes, and determine its significance to the biosphere's past, present and future. Such know-how will be increasingly critical in an Earth undergoing rapid environmental change.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Vessel and dive support were provided by NOAA's Thunder Bay National Marine Sanctuary during 2018 and 2019. NOAA's research vessel R5002 *Storm* skippered by Andrew Yagiela in 2018 and Travis Smith in 2019 and 2021 served as the platform for underwater deployments and dive operations. Thunder Bay National Marine Sanctuary's scientific dive team consisted of Wayne Lusardi, John Bright, Stephanie Gandulla and Phil Hartmeyer, and credit for underwater dive images goes to Phil Hartmeyer. Evan Kovacs and David Ullman at Marine Imaging Technologies, Boston, MA, contributed to submersible camera/light development and integration. This project was supported by National Science Foundation grants (EAR1637093 and OCE2046958) and National Aeronautical and Space Administration grant (Michigan Space Grants Consortium 80NSSC20M0124).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jglr.2022.10.006>.

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