Diel variation in motility of prymnesiophyte *Isochrysis galbana* under different irradiance

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**Abstract:** We investigated the diel variation in motility under different irradiance of the prymnesiophyte *Isochrysis galbana* Parke. The effectiveness of nutrient uptake by motility relative to molecular diffusion in seawater is quantified by the Sherwood number $Sh$, which is a function of cell size ($\mu m$) and motility rate ($\mu m s^{-1}$). We calculated $Sh$ for the prymnesiophyte *I. galbana* by determining its cellular equivalent spherical diameter ($ESD, \mu m$) and motility rate (numbers of $ESD s^{-1}$). Nutrient-enriched batch cultures were grown at 25°C in 12:12h light:dark cycles at light-limiting or light-saturated irradiances for 48 h. Observed motilities and $Sh$ values were within the range of previously reported values and indicated a significant relationship between motility and $Sh$ with $ESD$. Diel variations in motility and $Sh$ showed maxima at the middle of the light period and minima at the middle of the dark period under both light conditions. Diel patterns of motility and $Sh$ might be related to those of photosynthesis due to the supply of energy of motility.

**Key words:** diffusion, effectiveness of uptake, resource acquisition, Sherwood number

**Introduction**

Marine nanophytoplankton (2–20 $\mu m$) are autotrophic organisms that mainly inhabit coastal areas characterized by nutrient-replete conditions (Brun et al. 2015, Reynolds 2006). The biomass of nanophytoplankton accounts for approximately half of the global phytoplankton carbon biomass (Uitz et al. 2006). These organisms seldom sink because of their small size and instead remain suspended in the water column (Karp-Boss et al. 1996, Kiorboe 1993, Mann & Lazier 2006). As the surrounding fluid is not exchanged, cells would exhaust their supply of nutrients unless they can move (Munk & Riley 1952). Most nanophytoplankton are motile, possess flagella, and are thus called nanoflagellates. To take up nutrients effectively, nanoflagellates must exploit fresh fluid.

The nutrient uptake rate $\rho$ (amount of substrate time$^{-1}$) of a spherical cell with radius $r$ (length) can be expressed as follows (Pasciak & Gavis 1974):

$$r = 4\pi C'Dr,$$

(1)

where, $C'$ is the difference between the nutrient concentrations in the surrounding fluid and at the cell surface (amount of substrate length$^{-3}$) and $D$ is the diffusion coefficient (length$^2$ time$^{-1}$). Assuming the surrounding fluid is stagnant, nutrient uptake by motility of nanoflagellates ($\rho'$) can be expressed as a function of cell size (length) and motility rate $u$ (cell length time$^{-1}$) (Armstrong 2008, Karp-Boss et al. 1996):

$$\frac{\rho'}{\rho} = f\left(\frac{ru}{D}\right)$$

(2)

This equation assumes that $\rho$ is constant even while the cell is moving. The value of $\rho' / \rho$ is known as the Sherwood number, $Sh$, which indicates the effectiveness of nu-
nutrient uptake by motility, relative to the molecular diffusion in seawater and the nutrient flux can be increased to cells. This value is larger than the purely diffusional flux through the boundary layer around the cell. Thus, it is always greater than one. Equations have been considered for a better estimation of Sh that would take into account size and motility or sinking (Karp-Boss et al. 1996, Karp-Boss & Jumars 1998, Knorrboe et al. 2001, Logan & Aldredge 1989, Munk & Riley 1952, Pahlow et al. 1997, Pasciak & Gavis 1974). Sommer (1988) reported that the Sh in nanoflagellates was approximately 1.3-fold based on empirical data. Increasing their uptake 1.3-fold via motility might not be enough for nanoflagellates to move away from a nutrient-depleted fluid surrounding the cell. Thus, the motility of nanoflagellates has been thought to be linked to finding high nutrient patches, and not to enhance the nutrient uptake (Mann & Lazier 2006).

Nanoflagellates require energy to move. The energy for motility is stored in organic compounds produced through photosynthesis and respiration (Goldstein 1992). Because the energy for motility is related to photosynthesis, photosynthesis can affect motility. When the photosynthesis-produced energy is compared, the amount of energy available for motility is higher under light-saturated (LS) conditions than under light-limited (LL) conditions. Measurement of motility should also be conducted under LL conditions because nanoflagellates also exist under these conditions in the water column of the surface mixed layer. Since nanoflagellates are known to exhibit diel variation in photosynthesis (Joint & Pomroy 1986), motility might oscillate during a day. However, diel variations in motility and Sh in nanoflagellates have not been reported. Is there relationship between diel patterns of motility, Sh, and irradiance which affects photosynthesis? Investigating diel variations in motility and Sh under different irradiance could lead to clarify the nutrient uptake strategy of nanoflagellates. We studied diel variations in motility and Sh related to resource acquisition under different irradiance in the prymnesiophyte Isochrysis galbana because prymnesiophytes are ubiquitous in coastal areas (e.g., Goela et al. 2014).

**Materials and Methods**

**Culture conditions**

*Isochrysis galbana* (NEPCC633) was obtained from the North East Pacific Culture Collection at the University of British Columbia. Cultures were kept at 25°C. Light was provided on a 12 : 12h light : dark cycle by cool-white fluorescent tubes (FL30SEX-N, National, Tokyo, Japan). Aged seawater collected from coastal waters off Manazuru, Japan, was pre-filtered through membrane filters with a pore size of 0.2 μm. Nutrients, trace metals, and vitamins were added at f/2 concentrations (880 μM nitrate, 36 μM phosphate) (Guillard & Ryther 1962).

Batch experiments were conducted under LL and LS irradiance of 70 and 550 μmol m⁻² s⁻¹, respectively. Cells increased in size logarithmically from day 0 to day 7 and from day 0 to day 4 in the LL and LS experiments, respectively. Duplicate subsamples were harvested on day 7 and day 4 in the LL and LS experiments, respectively. Samples were harvested every 6 h for 48 h to include the light–dark cell division cycle (e.g., Prézelin 1992). Photosynthetically active radiation was determined using a scalar quantum sensor (Model QSL 100, Biospherical Instruments, San Diego, CA, USA) at the center of the culture vessel. Careful aseptic techniques were implemented to minimize bacterial contamination throughout incubation.

**Motility measurements**

The motility of 100 cells was measured using a stop-watch with a hemocytometer chamber (0.1-mm deep; 35103, Erma Inc., Tokyo, Japan) at a magnification of 400×. The chamber was kept at the same temperature as the cultures. All measurements of motility were conducted during the first minute after the start of the photoperiod. We confirmed that the temperature of the chamber did not increase significantly within 1 min. The motility patterns of the nanoflagellates seemed to be consistent with rotation and gyration of the cell as previously reported (cf. Thronsdén 1973). We recorded the speed based on the time required to move within one grid in the chamber (50-μm distance) as the motility rate (μm s⁻¹).

The motility (s⁻¹) is a relative velocity that represents how far cells are able to move per second. The motility was estimated as follows:

$$\text{Motility} = \frac{u}{ESD} \quad (3)$$

where $u$ is the motility rate (μm s⁻¹), and ESD is the equivalent spherical diameter of a cell (μm). The ESD values were estimated every 6 h from published data for *Isochrysis galbana* (Ishiwata et al. 2013) to calculate the motility. Time courses of Sh under the 2 light regimes in each 6-h time interval were calculated as follows by using a value of $10^3 \mu m^2 s^{-1}$ for the diffusion coefficient, $D$ (Karp-Boss et al. 1996):

$$Sh = \frac{1}{2} \left\{ 1 + \left[ 1 + 2 \left( \frac{ru}{D} \right)^{\frac{1}{3}} \right] \right\} \quad (4)$$

The motility and Sh of nanoflagellates were calculated from the results of published sources (Bauerfeind et al. 1986, Berdalet et al. 2007, Kamynowski et al. 1992, Rich-ter et al. 2007, Solari et al. 2011) as well as the present study. When microflagellates were included in the published sources, only cells with ESD<20 μm were used.

**Mathematical and statistical analysis**

The magnitudes of diel variation (MDV) in motility and
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Sh were calculated for the light and dark periods as follows:

$$\text{MDV} = \frac{\text{Max} - \text{Min}}{\text{Min}} \times 100\% \quad (5)$$

where Max is the maximum value and Min is the minimum value of each variable, respectively.

Statistical differences in the maxima and minima of motility and Sh between the LL and LS were analyzed using a t-test. Statistical differences in MDVs in motility and Sh between the LL and LS were also analyzed using a t-test.

**Results**

**Motility**

Distinct diel variations in motility were observed, with maxima in the middle of the light periods and minima in the middle of the dark periods under both LL and LS conditions (Fig. 1). The maximum motility was significantly faster (1.5 times) under LS than LL conditions (t-test; $p<0.001$), and the minimum motility was significantly faster (1.2 times) under LS conditions than under LL conditions (t-test; $p<0.05$). The MDV in motility was significantly higher (1.8 times) under LS than LL conditions (t-test; $p<0.05$, Fig. 2).
The Sh

Distinct diel variations of Sh were observed, with maxima in the middle of the light periods and minima in the middle of the dark periods under LS conditions (Fig. 3). The maximum and minimum Sh under LS conditions were 1.08 and 1.04, respectively (Fig. 3). The maximum and minimum Sh under LL conditions were 1.06 and 1.03, respectively (Fig. 3). The MDV in Sh was significantly higher (2.4 times) under LS than LL conditions (t-test; *p*<0.01, Fig. 4).

Relationship between ESD, motility, and Sh

To study the relationship between ESD and motility, as well as between ESD and Sh in nanoflagellates, we used published data (Bauerfeind et al. 1986, Berdalet et al. 2007, Kamykowski et al. 1992, Richter et al. 2007, Solari et al. 2011) and the results of the present study. Motility was significantly correlated to ESD (Fig. 5; *p*<0.001) and Sh was also significantly correlated to ESD (Fig. 6; *p*<0.001). Motility and Sh decreased as ESD increased (Figs. 5 and 6).
**Discussion**

The motility rate ($\mu$m s$^{-1}$) measurement is critical for estimating the motility (numbers of ESD s$^{-1}$). The present estimates of motility were within the range of previous studies, despite the variety of methodologies and experimental conditions (Table 1). The cell-size dependency of motility and $Sh$ was also consistent with previous results (Figs. 5 and 6, respectively). The energy which is required for movement is supplied from photosynthesis and respiration. Photosynthesis occurs during the light period and respiration occurs throughout the light-dark cycle. Diel patterns of motility and $Sh$, with maxima at the middle of the light period and minima at the middle of the dark period, were similar to those of photosynthesis (Prézelin 1992). It is suggested that the energy required for motility could be supplied mainly from photosynthesis during the light period. In the dark period, it could be supplied via respiration. Nanoflagellates can uptake nutrients more effectively with higher motility during the light period. It enables them to enhance the nutrient flux into the cell during this period. By enhancing the nutrient flux into the cell, nanoflagellates can grow their cells and divide during this dark period. This tendency may be true with the relationships between $MDV$'s of motility and $Sh$ and irradiance due to higher photosynthetic rate under LS conditions. Diel variations in motility and $Sh$ of nanoflagellates are suggested as a reasonable response in terms of growth of cells.

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