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PRIMARY RESEARCH PAPER

Tracking of algal cells: case study of swimming speed of cold-adapted dinoflagellates

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Abstract Swimming is a fundamental trait of many protists and optimizes growth and survival. We present an easy to use set-up for filming algal cells using *Apocalathium aciculiferum* and *Borghiella dodgei* as case studies; both dinoflagellates are model organisms of psychrophilic dinoflagellates. We filmed at low temperature (4.5°C) using a digital camera directly connected to a microscope and used open-source software to track their movement. We encountered several technical issues that were solved by using specific software settings (scaling, grey scale of images, restrictive particle recognition), and thus we obtained unbiased speed estimates. *Borghiella dodgei* showed a faster (minimum = 80, maximum = 255, median = 141 μm s^{-1}) swimming speed than *A. aciculiferum* (minimum = 29, maximum = 134, median = 85 μm s^{-1}). We linked differences in swimming speed to the dinoflagellates' environmental niche, and suggested that *B. dodgei* is more adapted to turbulent spring conditions than *A. aciculiferum*, occurring under ice. The use of a generic digital camera and open-source software makes filming and tracking of plankton movement very affordable. We provided code and detailed instructions to disseminate this type of movement analysis of plankton.

Keywords Movement ecology · Trackdem · Psychrophilic algae · Lake Tovel · Filming

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Introduction

Since Margalef (1978) conceptualized the importance of turbulence for species succession, researchers recognize the importance of motility for community dynamics. Recently, researchers have gained a better understanding of the interaction between cells and fluid motion: the motility of phytoplankton influences avoidance of deleterious turbulence patches during vertical migration, favours cell-to-cell encounters for sexual reproduction, determines vertical migratory patterns, and leads to cell accumulation in patches or layers, making cells more vulnerable to predation (Wheeler et al., 2019). Phytoplankton movement can be active or passive, and characteristics of active swimming are either directly assessed through filming

(e.g. Baek et al., 2009; Persson et al., 2013; Nielsen & Kiørboe, 2015) or indirectly through counts of algal abundance from different depths at different time periods (e.g. Ault, 2000; Regel et al., 2004). While filming generally requires specialized equipment and/or commercial software, here we present an easy to use set-up for filming algal cells using *Apocalathium aciculiferum* (Lemmermann) Craveiro, Daugbjerg, Moestrup et Calado; formerly known as *Peridinium aciculiferum*) and *Borghiella dodgei* Moestrup, Hansen et Daugbjerg as case studies; these species are model systems for psychrophilic dinoflagellates (Flaim et al., 2010, 2012, 2014; Obertegger et al., 2011). Both algae are found in Lake Tovel, a cold water lake whose surface water temperature seldom reaches 20°C. In Lake Tovel, *B. dodgei* disappears from the main basin in summer while it can be found in the smaller basin with water temperatures < 7°C and photosynthetic active radiation $> 50\%$ (Flaim et al., 2010), and *A. aciculiferum* occurs under ice and from January to April before spring mixing sets in (Calliari et al., 2004; Hansen & Flaim, 2007). Specifically, *A. aciculiferum* is a key species in winter phytoplankton and often develops blooms under ice (Rengefors, 1998; Rengefors & Legrand, 2001; Calliari et al., 2004; Hansen & Flaim, 2007) while *B. dodgei* forms occasional blooms in early spring (Flaim et al., 2006).

In dinoflagellates, two flagella provide the impulse for movement, and the different action of these organelles allows the cell to orientate itself according to chemical gradients (Fenchel, 2001). Dinoflagellates move at low Reynolds numbers because of their small size (Fenchel, 2001), implying that viscous forces dominate over inertial ones. Thus, dinoflagellates swimming pattern is helical (Fenchel, 2001), and the acceleration and stopping distances are much smaller than the cell length (Levandowsky & Kaneta, 1987). Most information on dinoflagellate movement comes from marine environments (Levandowsky & Kaneta, 1987; Smayda, 2010) and concerns dinoflagellate bloom formation (Smayda, 2010) and vertical migration (e.g. Ault, 2000; Baek et al., 2009). Most dinoflagellates have lower growth rates than diatoms, and their faster swimming speed is regarded as an adaptation to outcompete diatoms under turbulent conditions (Smayda, 2010). Sengupta et al. (2017) highlight the advanced level of control that dinoflagellates can exert on their migratory behaviour, especially in turbulent conditions. While chain-forming dinoflagellates swim faster than solitary cells, their superior swimming speed does not, however, directly translate into success in upwelling systems (Smayda, 2010). Environmental parameters affect swimming patterns of dinoflagellates differently. Small-scale turbulence decreases swimming speed of certain marine dinoflagellates and decreases growth with respect to still conditions (Berdalet et al., 2007). The marine dinoflagellate *Alexandrium tamarense* (Lebour) Balech decreases chain length and swimming speed in the presence of the kairomone of copepod predators (Selander et al., 2011). Swimming speed of the marine dinoflagellate *Cochlodinium polykrikoides* Margalef decreases with increasing abundance of diatoms (Lim et al., 2014). In diel vertical migration, the swimming speed of the dinoflagellate *Ceratium furca* (Ehrenberg) Vanhoeffen is higher during the day than at night (Baek et al., 2009). Despite low Reynolds number hindering active feeding, mixotrophic dinoflagellates produce small-scale currents to increase prey encounter rates (Nielsen & Kiørboe, 2015). Thus, as for other flagellates, swimming plays an important role in dinoflagellate ecology.

While species at low temperatures face a harsh environment, growth in cold waters is favoured by reduced competition and grazing due to low temperature (Rose & Caron, 2007). In fact, several dinoflagellates actively grow during winter. For example, the brackish cold-stenothermal dinoflagellate *Heterocapsa triquetra* (Ehrenberg) F.Stein grows under ice, but shows a decrease in cell diameter as an adaptation to low temperature (Baek et al., 2011). When temperatures are too low to support growth, different marine dinoflagellates swim with decreasing speed (Kamykowski & McCollum, 1986). Reduced swimming speed at low temperature might be expected because the viscosity of water increases with decreasing temperature, and thus Reynolds numbers are lowest at low temperature. Nothing is known about swimming characteristics of truly stenothermal taxa growing within narrow ranges of temperature < 10°C (i.e. psychrophilic species sensu Butterwick et al., 2005).

While traditionally dinoflagellates have been considered a predominantly marine group with freshwater taxa receiving less attention (Flaim et al., 2010), this is the first study for analysing and comparing swimming characteristics of two psychrophilic freshwater dinoflagellates. Here, we linked their swimming characteristics to their environmental niche, and by

filming these two species within their temperature optimum, their swimming characteristics were not impaired by physiological constraints arising outside of their optimal environmental requirements. We provided code and instructions for the different analytical steps with open-source software to disseminate this type of movement analysis.

Methods

Culture conditions

Apocalathium aciculiferum (hereafter referred to as *Apocalathium*) was cultivated in 250-mL flasks with 150 mL of WMC + Se medium (http://www.sccap.dk/media/freshwater/3.asp). *Borghiella dodgei* (hereafter referred to as *Borghiella*) cells were maintained in DY IV medium (Andersen et al., 1997). Both cultures were kept at a mean temperature of 4.5°C (mean temperature during day: 5°C, during night: 4°C) with a light regime of 14:10 light:dark cycle and a photon irradiance rate of approximately 100-125 µmol m^{-2} s^{-1}. The dinoflagellates were filmed in logarithmic phase. Both dinoflagellates were isolated from Lake Tovel (Italy) and are available from the Scandinavian Culture Collection of Algae and Protozoa (Scandinavian Culture Collection of Algae and Protozoa (*Apocalathium* SCCAP K-0998 and *Borghiella* SCCAP K-0998). Like most cold-adapted dinoflagellates growth rates are low: for *Borghiella* 0.2 cells day^{-1} at a temperature between 4 and 5°C (Flaim et al., 2010) and for *Apocalathium* 0.25 cells day^{-1} at 3.9°C (Flaim et al., 2014).

Filming

Dinoflagellates were filmed in the same walk-in cold room where the cultures were kept. Cell abundance of the filmed cultures was 116×10^6 cells L^{-1} for *Apocalathium* and 16×10^6 cells L^{-1} for *Borghiella*. For filming, a Canon EOS 60D camera was directly connected (i.e. without additional optical elements) to a Leica DMLB microscope with a C-EOS ring adapter. With a glass pipette, a small volume of culture was pipetted into the drop area (diameter = 9 mm, height = 1 mm; 0.064 ml) on a glass slide, created with Press-to-Seal™ silicone isolators; a cover slip was also used to prevent optical distortions during filming. The filming area was 3% of the drop area. All videos were filmed at 100 × magnification. The camera was controlled remotely with the Canon EOS Utility software. Dinoflagellates were filmed for 29 to 32 s (s) at 25 frames s^{-1}.

Data analysis

To analyse the recordings of dinoflagellates, we performed several steps utilizing open-source software (Table 1). The video format ".mov" was converted to ".avi" format using the open-source software FFmpeg (http://ffmpeg.org/). The avi. video was converted to an image sequence in ImageJ (Schneider et al., 2012) and then analysed with the open-source R software (R Core Team 2019) using the package trackdem (Bruijning et al., 2018; http://github.com/marjoleinbruijning/trackdem). The analysis comprised image sequence generation, particles identification, particles tracking, and statistical analysis. We used package trajr (McLean & Volponi, 2018) to extract speed from the swimming trajectories. The longer a movement trajectory, the more information can be extracted. Here, we assessed the influence of considering different trajectory lengths on the estimate of mean swimming speed by subsampling a trajectory to 100, 250, 400, 550, and 700 frames (i.e. five categories of different frame length in steps of 150 frames) and compared the mean swimming speed by a non-parametric one-way ANOVA (Kruskal–Wallis test). Considering our unbalanced design (i.e. the number of trajectories differed between categories of frame lengths), we randomly downsampled to the lowest number of trajectories per frame length category (function downSample of library caret; Kuhn, 2019). When significant differences between the five frame length categories were found, we applied post hoc testing corrected for multiple testing (pairwise Wilcoxon rank sum tests with Benjamini–Hochberg correction) to test for differences between categories. Only when differences in swimming speed were greater than 10%, did we assess speed differences between categories.

We compared the mean swimming speed of dinoflagellate species by a *t*-test; considering that it is very difficult to get the same number of trajectories for both species in each recording, we randomly selected n trajectories (n = number of trajectories of the species with the least ones) from the species with the most trajectories, repeated the *t*-test 1000 times,

Table 1 Workflow of different analytical steps with the major tasks, file type analysed, and the equipment (software or digital camera) used

Task			File type	Software-tool
1	a	Film	.mov	Digital camera
2	a	Convert.mov to.avi	.avi	FFmpeg
3	a	Create sequence of images in grey scale	.png	ImageJ
	b	Scale images to 50%		
4	a	Load images	.png	trackdem
	b	Identify background and subtract it from images		
	c	Identify particles (set threshold to minimum value to avoid tracking out of focus cells)		
	d	Create trajectories of moving cells with ID (high penalty for merging tracks of two cells)		
5	a	Convert pixel to μm (scaling to 50% implies that one pixel corresponds to 2x lengt)	.png	ImageJ
6	a	Filtering step: only trajectories > 100 frames	.csv	base, trajr
	b	Calculate speed of cells from frame to frame		
	c	Analyse mean speed dependent on trajectory length		
	d	Compare speeds with a t-test		
7	a	Overlap the original film with the paths of tracked cells		ImageJ

The detailed commands are given as Supplementary Material

and reported how often a significant difference was found.

We performed all analyses on an Intel©Core™ i5 CPU 2.5 GHz, 16 GB RAM, × 64-based computer.

Results

Technical issues and solutions

During recording, we noticed that too many trajectories were split into two and many cells collided and/or were out of focus when cell abundance per filming area was too high. Thus, we preferred recording two low abundance sets of cells to get meaningful numbers and to avoid having too many cells that hinder each other in their swimming. We, furthermore, set very restrictive settings for the identification of moving cells (function identifyParticles): we set (i) a small range for the area of moving particles, and (ii) the lowest possible threshold for the recognition of dark particles on a white background; these two settings guaranteed that cells that were particularly out of focus were not tracked. This aspect was important considering that filming captures a two-dimensional space (length, width) while cells move in three dimensions (length, width, height). Thus, only when cells were parallel to the observational plane (i.e. camera sensor), was the inference of speed unbiased. To determine cell area in pixels, we measured it with ImageJ on a single-scaled image. Furthermore, we set a very high penalty on merging trajectories of different cells (function trackParticles), and thus only few trajectories were interrupted. Generally, the identification and tracking of particles required more computational power than our computer possessed (i.e. 16 GB RAM), and this led to the interruption of computational steps. Thus, we scaled the images of single frames to 50%; this step decreased the size of each image and thus decreased the computational burden. In addition, scaling increased the relative size of a pixel. To convert pixel size to μm, we adopted the following strategy: we photographed a micrometre glass slide at the same settings used for filming (here × 100 magnification), scaled the image to 50%, and then counted the number of pixels for 100 μm length, thus obtaining a pixel to μm factor. This step was very important for a correct estimate of speed because the software (package trajr) calculates speed as pixel s^{-1} requiring conversion of speed estimates into μm s^{-1}.

Once cells were tracked, we further restricted the rendering of tracked cells to those that had a trajectory longer than 100 frames (i.e. 4 s of tracked cell movement). We adapted this strategy to base our

speed calculations on sufficiently long trajectories. Once the swimming trajectories (function plot(records)) were rendered, we checked the resulting recordings for interrupted trajectories. This happened when cells were too close to each other and the software did not know which cell to follow. Interruptions of trajectories were not frequent (*Apocalathium*: four and three times; *Borghiella*: three and two times), and we excluded the trajectory with the shorter length to avoid pseudoreplication. The last validation step (i.e. inspecting all tracked trajectories) and merging the original recording with the animated trajectory of tracked cells did not indicate any major issues (Supplementary material: video of *Apocalathium* and *Borghiella* tracked cells). As a consequence of the filtering steps and settings used, some cells that were visible in the original recording, where not tracked because of changes in their swimming plane, out of focus, had a too short trajectory, and/or were the second trajectory of the same cell. A detailed description of all steps can be found in the Supplementary Material (complete R code and description of ImageJ and FFmpeg steps).

Swimming speed of dinoflagellates

By merging the results of the two recordings, we obtained similar numbers of recorded trajectories for *Apocalathium* and *Borghiella* (Table 2). Regarding the influence of trajectory length on the estimate of mean swimming speed, few significant differences were found that were mainly $< 10\%$, and were thus regarded as not biologically meaningful. Only few trajectories gave a higher speed estimate with shorter than with longer trajectories (Table 3).

Based on these results, we compared the mean swimming speed of *Apocalathium* and *Borghiella* considering all trajectories of different length. The mean swimming speed of *Apocalathium* was 85 μm s^{-1} (minimum = 29, maximum = 134, median = 85, standard deviation (sd) = 22 μm s^{-1}) and of *Borghiella* 147 μm s^{-1} (minimum = 80, maximum = 255, median = 141, sd = 32 μm s^{-1}) (Fig. 1). A *t*-test indicated a statistically significant difference ($P < 0.001$ in 1000 tests; see "Methods" section) between the mean swimming speeds of the two species.

Discussion

This study used an easy to use experimental set-up and open-source software to film algal cells and to determine their swimming speed. We encountered several technical issues (e.g. insufficient computational power, merging of trajectories, tracking of cells that were out of focus, etc.) that were solved by preparations of images (i.e. grey scale and scaling) and using specific software settings. We provided code (Supplementary Material) to repeat our analysis in the framework of open, transparent, and reproducible science (Powers & Hampton, 2019). A complete comparison of our results with former studies was hampered by incomplete descriptions on filming details: Kamykowski et al. (1992) do not state for how long they filmed and how many trajectories were analysed; Jang et al. (2015) do not report frame rate and video analysis software used. The use of open-source software and readily available equipment will increase the use of filming in scientific research (e.g. Colangeli et al., 2016; Obertegger et al., 2018; Colangeli et al., 2019) and allow easier comparison between studies. Even though statistical differences in speed estimates were found, the differences were small and even short trajectories of 4 s (i.e. trajectory length of 100 frames) gave accurate results.

Table 2 Summary statistics of swimming trajectory length (length) as assessed by two recordings for each psychrophilic dinoflagellate; length values are given in frames; number of recorded trajectories (number), standard deviation (sd)

	Apocalathium	*Borghiella*
Number	26; 25	34; 36
Length		
Range	102–797; 102–598	102–725; 110–487
Median ± 1 sd.	312 ± 211; 236 ± 118	293 ± 184; 234 ± 107

Table 3 Number of trajectories (nr.), as assessed by non-parametric Kruskal–Wallis test, where the estimate of mean swimming speed differs between trajectory length categories (i.e. 100, 250, 400, 550, and 700 frames per trajectory)

	Apocalathium	*Borghiella*
nr. (speed differences between frame length categories > 10%)	4 (1); 5 (1)	13 (5); 6 (1)
nr. of trajectories with the highest speed in the highest frame length category	1; 1	3; 1

In parentheses, we report the number of trajectories where significant differences in estimated speed were > 10%. For these trajectories, we used pairwise Wilcoxon rank sum tests to test for differences between frame length categories; values are given for both recordings for each psychrophilic dinoflagellate

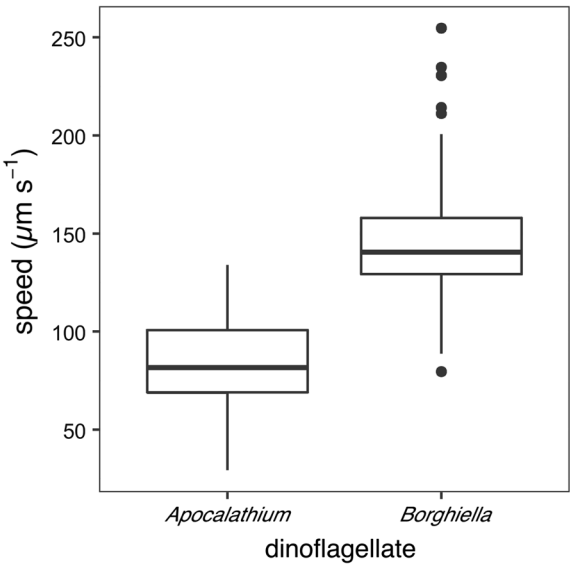

Fig. 1 Boxplots of mean swimming speed of the psychrophilic dinoflagellates as estimated from the different trajectories of different lengths; dots are outliers (i.e. speed is outside 1.5 times the interquartile range below the lower quartile)

Nevertheless, considering technical issues linked to tracking, we advocate for filming longer, and we suggest that filming at 25 frames s^{-1} for 20 to 30 s would be sufficient.

Dinoflagellate swimming has long attracted interest (Levandowsky & Kaneta, 1987), mainly linked to bloom formation (e.g. red tides). A detailed overview on swimming speed of mostly marine dinoflagellates is given in Levandowsky and Kaneta (1987), Kamykowski et al. (1992), and Smayda (2010). The temperate marine dinoflagellate *Prorocentrum minimum* (Pavillard) J.Schiller growing at a constant temperature (20°C) shows decreasing mean swimming speed with increasing viscosity (Sohn et al., 2013). Similarly, different marine dinoflagellates swim with decreasing speed when temperatures are too low to support growth (Kamykowski & McCollum, 1986). However both psychrophilic freshwater dinoflagellates, *Apocalathium* and *Borghiella* are adapted to low temperature, and thus despite high viscosity combined with low temperature, these dinoflagellates showed swimming speeds similar to other warm-water species of dinoflagellates (Levandowsky & Kaneta, 1987).

Even though the two dinoflagellates are morphologically quite different (Hanson & Flaim, 2009), it was also possible to discern them based on swimming speed. We suggest that these differences are related to their niche differentiation as psychrophilic species. In fact, several studies (Baek et al., 2009; Hall & Pearl, 2011) indicate that niche partitioning in marine dinoflagellates is mediated by their different swimming characteristics, and swimming speed is regarded as an adaptation under turbulent conditions (Smayda, 2010). *Borghiella* blooms are tied to turbulent conditions associated with snow-melt (Cellamare et al., 2016), and this observation is in line with the hypothesis that some dinoflagellates are adapted to turbulent conditions (Margalef, 1978; Smayda, 2010). In contrast, *Apocalathium* is restricted to a short temporal window (under ice, ice-out to spring overturn; Flaim et al., 2014) with reduced turbulence. We suggest that differences in swimming speed can be linked to adaptation to turbulence; *Borghiella* with its faster swimming speed may be better adapted to turbulence than *Apocalathium*.

This study provided a basic understanding on the variability and mean swimming speed of cold-adapted dinoflagellates that can be linked to their environmental niche. The use of commonly available digital cameras, access to open-source software, and the code we provided for all analytical steps will help other researchers to include filming in their toolbox. Discerning swimming patterns in protists can shed

important insights into the dynamics of plankton, but also for all inhabitants of a fluid environment (Sengupta et al., 2017; Wheeler et al., 2019). We tried to follow Reynolds encouragement (1998) that freshwater ecologists should see their work in the broader context—i.e. the development of ecological theory and understanding of ecosystem function and behaviour. In this sense, filming of live algal cells can open new challenges in research, for example the impact of predators or different diets on swimming behaviour, to mention just a few.

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