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Life Cycle Progression of Chlainomonas sp.: A Field Study

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ABSTRACT
Every year, there are blooms of algae in snowy alpine environments during the summer snow melts. One environment in particular, the snow-on-lake habitat on Bagley Lake in Mt Baker, has been the subject of study by the Kodner lab for many years. In this habitat, we find the genus Chlainomonas which has bloomed in late spring and early summer annually. Our lab has proposed a life cycle for the genus (Matsumoto et al 2024), and there are many morphologically distinct cell stages found in field collected samples. This study has expanded our understanding the life cycle dynamics by examining the cell morphology and abundance across the bloom season in 2022. This data helped us test if our life cycle hypothesis and suggests that our predictions about the life cycle progression are correct. Our data shows that there was a significant change in algae morphology distribution from week to week, which supports the predicted cycle of cell growth into cysts followed by development of sporangia and "pill" cells, or spores. We also used single cell PCR to amplify the 18S gene to confirm that pill cells were identical to large red cells, linking these two cell phases genetically.

INTRODUCTION
There have been alpine algae blooms studied across the globe for decades and over 40 described species of snow algae, but only a few species from four genera (Sanguina, Chlainomonas, Rosetta, and Chloromonas) create the large red and pink blooms seen in the late spring and early summer (Hoham and Remais 2020, Engstrom et al 2024). They grow as snowmelt begins, when water becomes available to them in the snowpack and sunlight is high (Hoham 2000). There has been recent interest in studying algal blooms because the blooms’ dark color decreases snow albedo, and the algae themselves are highly temperature sensitive, with Hoham (1975) observing Chlainomonas sp. losing their flagella in the laboratory at temperatures above 4 degrees C, and van Hees et al (2023) observed lysing of algae cells both in the field microscope and in lab microscopes when large cells are removed from cold temperatures. Being a temperature sensitive species makes them just as vulnerable to climate change as the snow they reside on. Continued study is crucial to characterize this bloom for our understanding of the ability of these microbial communities to weather these changes.

Within Washington, Matsumoto et al (2024) classified a new species of algae, Bagley Chlainomonas sp. which grows on Bagley Lake, on Mt. Baker, which is a part of the North Cascades Mountain range. Bagley Lake is an unusual environment in that it does not freeze over in the winter, but rather gathers a thick snowpack on top which floats atop the shallow lake and insulates it from icing over. This snowpack then melts in the spring and summer, returning the lake to its liquid status and creating a wet environment for algae to grow. Bagley Chlainomonas sp. was genetically analyzed via the rbcL gene and found to be sister to C. rubra and C. kolii, and closely related to C. Rubroleosa (Novis, 2023) but with a unique life cycle. They proposed a life
cycle in which at the beginning of spring, large, rounded cells with flagella swim up from lake sediment to the snow’s surface, where large quantities bloom in the sunlight. During this vegetative period, the algae lose their flagella and grow both larger and rounder, developing thick cell walls. By late summer, in a cyst stage, the cells then sink back down into the lake with their fresh resources. They were unable to observe the status of the cells in situ after this point, however, in dark and cold laboratory conditions (the refrigerator) the algae cysts divided into colonies of a morphology called “pills” for their oblong shape and half red-half green appearance. These pills are hypothesized to be the product of meiosis or mitosis and could either combine with each other as gametes to complete the life cycle and grow into the spring swimmer or could simply grow into the spring swimmer at the bottom of the lake bed over the course of the fall and winter. This species had also been studied in van Hees et al (2023), who characterized the bloom in 2021 as patches over the lake. The patches were found across the lake, and each behaved individually, which implies many individual blooms rather than a single synchronous bloom.

This informed the study of Bagley Lake in 2022, wherein sites were placed across the lake and monitored for the entirety of the bloom season. From these sites several measurements were taken by the Kodner lab, but for the purposes of this paper, we will examine the morphology seen by microscope photos and single cells pulled from these samples. In these patches across the lake, we categorized and quantified the morphological phases of the algal cells throughout the growing season, comparing them on both a patch to patch and week to week basis. We hypothesize that these cells follow Matsumoto’s proposed life cycle and that the pill morphology are of the same species.

METHODS
Study Site

Upper Bagley Lake is in the North Cascades mountains, located at the end of State Highway 542. It’s 1277 meters above sea level and is 11.8 kilometers northeast of Mt Baker, with the coordinates 48.8632° North, -121.6793° E. There is no ice cover during winter, instead being covered in snow through the summer, and is surrounded by snow covered slopes. The lake is shallow, only about 2-3 meters deep and the snowpack is about 0.5 meters deep. The algae blooms are observed to appear on the snow covering the lake between April and May and are present until snow is completely melted from the lake, which melts on the perimeter first but still has traversable snow rafts. The melt is generally complete by the middle of July or earlier, depending on weather conditions. In 2022, the bloom spanned from late May to early July, over the course of seven weeks with observations and samples taken on a weekly basis. Dates span from T0 (Time 0) which was May 26, 2022, to T6 (Time 6) which was July 7, 2022.

Sample Collection

In collecting samples for this, we created different sites across the lake (Figure1), labelling them by letter, within which we found individual patches, labelled by a number. We sampled three patches per site however, due to the appearance and disappearance of blooms in patches, they would change from week to week, as we sampled the algae which was present within the site. Entire sites would also disappear from week to week, however we always made sure to sample from 6 sites, with the exception of T0, which was a preliminary sampling week.
and only had two study sites. From the patches we took 50 mL of snowpack, which were then stored in snow in a thermos at >1 degree C until they could be imaged under the laboratory microscope within 36 hours of collection. These samples were then stored in the at 4 degrees C in their 50 mL tubes in a liquid state and monitored for pill formation. Following the same patches within sites, we collected 15 mL samples of snowpack which were preserved with 2-3 drops of Lugols while still frozen, to preserve them.

Other measurements taken from these sites include photosynthetic activity, temperature, snow wetness and density. We also collected samples for DNA extraction (which were used in Matsumoto et al).

![Figure 1: A map of Upper Bagley Lake, which has labelled sites across it lettered A, B, G, V, W, X, Y, Z.](image)

**Morphology Categorization**

Using a light microscope at 40x magnification, images were taken of fresh samples while still frozen. The images were taken to represent all morphology being seen within the sample representatively, taking more photos of the cells we saw more of. These photos were later used to create a key, judging each cell photographed on its morphology and counted into categories. We grouped them as seen in Figure 2, with cell type A being a small cell under stress from infection, cell type D being a swimmer-type of cell, with a pointed end on one side and large envelope, cell type E being a smaller cell with a close envelope, cell type F being similar to E except it has a folded edge on one side of the cell wall, resembling a collar or bow-tie. Cell type G is a perfectly round cell without a visible envelope, cell type H is displaying a reproductive behavior described in Matsumoto et al 2024, cell type J is a large and rounded cell which has filled the envelope, and cell type K which is similar to cell type J with the addition of a thickened outer cell wall, as a cyst.
Cell Size Methodology
Using the samples which had been preserved in Lugols, we measured the size of cells by calculating scale using the known distance of the grid of a hemocytometer on a light microscope to determine a universal scale at 40x magnification, which was entered into ImageJ. We measured in ImageJ with the oval tool and measurement function, taking the area of each cell we photographed by making the oval tool as large as the glowing outer cell envelope, with date site and cell size recorded. All cells had been photographed only within the central 1millimeter by 1 millimeter area, with three representative samples of each patch being taken and all cells measured from that sample of the patch sample.

PCR Methods
The fresh samples were stored in 4 degrees C for 14 months before being used for genetic analysis. The same microscopy samples were placed (12 microliters at a time) on a slide under a dissection microscope, where single cells were picked using a sterile glass pipette tip and placed into individual tubes. Single cells were taken of the bloom’s round morphology (due to deterioration of the sample over time, it is unclear what their morphology had been when the samples were fresh to categorize them as with the fresh samples) as well as the pill morphology, which were clear due to their oblong shape. Single cell amplification was performed, using an 18S primer (both forward and reverse, along with MasterMix). The thermocycler was set to 58
degrees C and 48 degrees C for our temperature for 35 cycles, after which gels were run using Invitrogen E-gel 2% agarose, with 5 microliters of PCR product and 15 microliters of E-gel loading buffer.

RESULTS
Size Distribution

After testing to ensure that the distributions of size were normal (T2 and T3 were not normal) and were log transformed (transformation not pictured in Figure 3, those are the direct measurements). With all data normal, ANOVA tests were performed on the size distributions, with a one-way ANOVA comparing the different patches, within each week. Inside of the week, the patches were significantly different from each other every week, with a p value of less than $2 \times 10^{-16}$. Furthermore, when a two-way ANOVA test comparing the patches and the weeks was performed, the weeks were also significantly different, with a p value of less than $2 \times 10^{-16}$. These size values indicate that the cells are larger at the end of the summer than at the beginning.
Morphology Distribution

After creating categories for each cell type (Figure 2), we counted each photo, noting the site and patch each sample was taken from. With this data, we were able to see that there is an increase in morphologies J and K (the larger cells and the cyst cells) during later weeks, with morphology D (the former swimmer cell) remaining consistent. This can be seen in Figure 4, which displays a week-by-week breakdown of the cell types. When breaking down into the patch-by-patch basis, we see fairly consistent results between sites. We performed ANOVA tests on these different groupings and there were no significant differences between the patches within a week, and there were no significant differences between weeks, although there is a visible trend, with the appearance of J and K.
Figure 4: Bar graph displaying the week in the X axis and the proportion of abundance in the Y axis, with a legend for the cell type which are coded by colors.
Figure 5: Seven bar graphs displaying different weeks morphological breakdown, with patch on the X axis and proportion of abundance on the Y axis. Week one, labelled T0 has only two patches, while the rest of the weeks (T1-T6) have 18 different patches, each with an individual composition of morphology, which are labelled by color in a legend to the right of the figure.

Single Cell DNA Analysis

Being 14 months after collection, the samples had degraded somewhat and it was unclear what their former morphology had been when picking single cells, however the cells which had remained intact were distinct from pill cells due to size and shape, which allowed us to pick single cells of both the typical, round, morphology, and pill morphology, along with pill colonies. With these single cells, using an 18S primer, they were amplified with a PCR reaction and run in an electrophoresis gel, to compare the length of the gene in both cell types. We ran a total of 24 single cells, and received bands from 4 of the products, which matched lengths between pill colonies, pills, and the control, which was DNA extracted from a pond on Mt Watson previously. The bands were at around 2,500 base pair (bp) lengths.
Figure 6: Photos of electrophoresis gels. In the gel, there are thin bands at around 2,500 bp in lanes M, 2, and 5, and wide bands at around 100 bp in lanes 1, 3, 5, 6, 8, and 9. From left to right, the products put in the gel are M – the ladder, 1 – the negative control, 2 – the positive control, 3 – single pill cell from T6 X6, 4 – single pill cell from T6 X6, 5 – single pill cell from T6 X6, 6 – single round cell from a bulk sample taken in 2023, 7 – single round cell from a bulk sample taken in 2023, 8 – single round cell from a bulk sample taken in 2023, 9 – single halosphaera cell, 10 – single halosphaera cell.

Figure 7: In the gel, there are thin bands at around 2,500 bp in lanes M, 2, and 6, and faint thin bands in lanes 9 and 10, and wide bands at around 100 bp in lanes 1, 3, 4, 5, 6, 7, 8, 9, and 10. From left to right, the products put in the gel are M – the ladder, 1 – the negative control, 2 – the positive control, 3 – single round cell from T4 G7, 4 – single round cell from T4 G7, 5 – single round cell from T4 G7, 6 – single pill cell from T4 G7, 7 – single pill cell from T4 G7, 8 – single pill cell from T4 G7, 9 – pill cells from T3 X3, 10 – pill cells from T3 X3.
DISCUSSION

Overall, the data gathered in this project supports the hypothesized life cycle posited by Matsumoto with quantification. The cell size increases across the lake over the course of the bloom, and so while the patches may be behaving individually (van Hees 2023), all the patches are following the same life cycle and grow larger with this pattern. By quantifying cell size, we are able to support the hypothesis of growth during the summer bloom season. The cells are growing in size together, which also supports the hypothesis that they are all following the same life cycle. With that, the morphology of the cells present are shifting with this size change. As the algae grow larger, the morphologies present reflect that, with an increase in the large and cyst cell types. While we were unable to find significant differences in the counts of cell types, this could be due to a need for increased sampling, or the limiting factors of the number of types categorized. Nonetheless, the visible increase of cyst morphology by the end of the season (which is best seen in Figure 4) clearly demonstrates the proposed life cycle.

Further supporting this life cycle, the PCR results had matching bands shows that the pill morphology has a long 18S sequence. This 18S sequence tells us that their sequence at that gene locus is the same for pills and pill colonies, and supports the idea that the pills are of the same species as the round morphology. If the pills truly are Bagley Chlainomonas sp. spores, then they would show the same sequence, which they do. We can therefore link the pill morphology with the rounder cell morphology and flagellated cell morphology, as a stage which the species develops. Developing in the dark refrigerator as they do, it would make sense for this to represent the phase of their life cycle that happens after they sink back into lake sediments, as it’s dark and cool in the lake, which we have not yet been able to observe in situ.

Some possible issues with this assessment include if the photos taken for morphology were not fully representative of real quantities. Though we worked to ensure that photography of samples was approximately accurate, human error can always affect studies. The cell size measurement was done entirely by hand, which again can create human error. Also, if the 18S sequence is simply conserved between species of algae, rather than the length supporting a single species. We were unable to get results from single round cells, likely due to their strong cell wall, which resists being broken open either with heat or cold. This weakens the argument for the pill genetics, which requires more study.

CONCLUSIONS

Future research could examine more genetic loci, to ensure that the pills are consistently containing the same genetic sequences as other morphologies. Additionally, breaking into cyst and flagellated cell types would be a rich area of future research. With more intensive studies of specific patches, visiting them more often than once a week could also elucidate more results on the exact life cycle of a single patch from its appearance to disappearance. By taking a closer eye to these algae, we can better understand all factors affecting these environments.

REFERENCES


