Fluid Mechanics of Flagellated Microorganisms and Possible Pathways for the Evolutionary Transition to Multicellularity

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Midterm Report

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1 Introduction

Evolutionary transitions in organisms from unicellularity to multicellularity are thought to arise from trade-offs between benefits, costs and requirements. The volvocalean green algae offer a model biological system for the study of metabolite exchange. The algae are photosynthetic eukaryotes ranging in organization from individual cells to small groups of identical cells to large colonies of cells that exhibit germ-soma differentiation. The somatic cells that comprise these organisms have attached to them two flagella which yield propulsion important to chemotaxis and phototaxis. It has not been well researched, however, how this collective flagellar beating stirs the fluid around the organism thereby creating a higher rate of nutrient turnover not attainable by diffusive transport alone. The study of this phenomenon is the primary goal of this research project.

There are multiple reasons for choosing this particular group of organisms to study. For one, they have all been well researched and studied. Much is already known about Volvox in particular. Some examples of well researched facets of the organism include its life cycle, its reproductive cycle, its mobility, the type of environments in which it exists and where it can be found in nature. Also, as physicists and mathematicians, we prefer to work with bodies and problems that exhibit as much symmetry as possible in order to make the calculations and models easier to work with. Almost everyone has heard the expression "spherical cow". The volvacalean green algae, and more importantly the species Volvox, brandishes a spherical symmetry and thus it is a prime candidate with which to study. The figure on the following page shows a few examples of the algae.

2 Goals

There were three primary goals to this semester long research project:

1. Understand the model that has been developed to determine the fluid flow around a Volvox, a specific colony of the volvocalean green algae, and how this model applies to the collective flagellar hydrodynamics that remove the diffusive bottleneck thereby allowing for transitions to multicellularity. (theory)

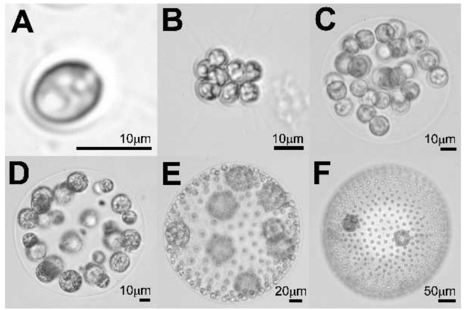

Figure 1: Species of Volvocalean green algae spanning a large range in size. (A) the single-cell *Chlamydomonas reinhardtii*, undifferentiated colonies (B) *Gonium pectorale* (8 cells) and (C) *Eudorina elegans* (32), and those with germ-soma differentiation (D) *Pandorina californica* (64), (E) *Volvox carteri* (∼1000) and (F) *V. Rousseletii* (∼2000).

2. Verify a relationship that has been found between the translational and rotational velocity components such that

$$\frac{U}{R\Omega} = -\cot\alpha \ , \tag{1}$$

   where U is the translational velocity, Ω is the rotational velocity, R is the radius of the Volvox and α is the angle that determines how stress is directed with respect to lines of longitude on the Volvox. The apparatus used to gather this data will include a darkfield illumination setup where the central light rays that ordinarily pass through or around the specimen are blocked only allowing oblique rays to illuminate the specimen. (experiment)

3. Apply this knowledge to a system of two Volvox organisms and determine their interaction. (theory)

   (a) Study the nearby swimming of pairs of Volvox. (experiment)

Fortunately, I joined a research group that had already developed a model for the fluid flow around a single Volvox. Therefore, my first task was to understand the problem at hand and the methodology by which the model was created by working through Martin Short's derivation for the fluid flow around the organism. The following arguments follow closely the method proposed by Martin Short. Rather than re-deriving the results here, though, I will only provide the main methods by which the solution was obtained.

3 Analysis

It has been found that a Volvox resides in the realm of low Reynolds number. The Reynolds number can be thought of as a ratio of the inertial forces to the viscous forces. This indicates that in the low Reynolds number regime the viscous forces dominate and the inertial forces are of no serious consequence. Moving through a low Reynolds fluid as a human would be comparable to swimming in a pool of molasses. Since the Volvox resides in the low Reynolds number regime, it is a well established fact that the fluid dynamical equations that govern the fluid flow can be approximated with confidence to be that of normal Stokes flow

$$\eta \nabla^2 \mathbf{u} = \nabla P \ . \tag{2}$$

Here, η is the viscosity, $\mathbf{u}$ is the fluid velocity and P is the pressure. The fluid that the Volvox prefers to live in (the ideal conditions being that of water mixed with some additional minerals and vitamins) is an incompressible fluid, and thus another equation that governs the fluid flow is the equation of incompressibility

$$\frac{\partial}{\partial r}(r^2 u_r) + \frac{r}{\sin\theta}\frac{\partial}{\partial\theta}(\sin\theta u_\theta) + \frac{r}{\sin\theta}\frac{\partial u_\phi}{\partial\phi} = 0 \ . \tag{3}$$

These are the fundamental equations that define the velocity of the fluid around a swimming volvox that resides in the low Reynolds number regime.

Since the Volvox is approximately a spherical organism (remember the "spherical cow") the geometry of the problem suggests that spherical coordinates should be used to solve the problem, and hence, the choice of spherical coordinates to represent fluid incompressibility. Here, the coordinates are labeled using the convention typically followed by physicists. That is, r is still measured from the origin, θ is the polar angle measured from the z-axis, and ϕ is the azimuthal angle measured from the x-axis. Noting that the general solutions to Laplace's equation in spherical coordinates yields spherical harmonics (associated Legendre functions multiplied by an exponential term) multiplied by a function of the radius alone, one might expect that the overall fluid velocities may take on a similar form. Therefore, the velocity component in the radial direction was proposed to be

$$u_r = \sum_{l=1}^{\infty}\sum_{m=0}^{l} F_l(r) P_l^m(w) e^{im\phi} \ . \tag{4}$$

To determine what kind of form the angular velocity components are to take, consider the fact that when all three components of velocity are placed into the incompressibility equation, the spherical harmonics must still satisfy Legendre's equation, that is, the same spherical harmonics must be returned. Legendre's equation is

$$\frac{\partial^2 y}{\partial\theta^2} + \frac{\cos\theta}{\sin\theta}\frac{\partial y}{\partial\theta} + \Big[l(l+1) - \frac{m^2}{\sin^2\theta}\Big] y = 0 \ . \tag{5}$$

After careful thought and some algebraic manipulation it was found that the following choices allow this to occur

$$u_\theta = \sum_{l=1}^{\infty}\sum_{m=0}^{l} G_l(r)\frac{\partial}{\partial\theta}P_l^m(w)e^{im\phi} \ , \tag{6}$$

$$u_\phi = \sum_{l=1}^{\infty}\sum_{m=1}^{l} G_l(r)\frac{im}{\sin\theta}P_l^m(w)e^{im\phi} \ . \tag{7}$$

When these components are substituted into the incompressibility equation the following relationship is found between the radial functions of the above expansions

$$G_l(r) = \frac{2F_l(r) + r\frac{\partial F_l(r)}{\partial r}}{l(l+1)} \tag{8}$$

The next step in the process requires a bit of imagination. Recalling the vector identity for two curls of a vector field

$$\nabla \times (\nabla \times \mathbf{A}) = \nabla(\nabla \cdot \mathbf{A}) - \nabla^2 A \ , \tag{9}$$

we find that for a vector field without a divergence (incompressible fluid flow)

$$\nabla^2 A = -\nabla \times \nabla \times A \ . \tag{10}$$

Also, note that the curl of a gradient is always zero. If we then take the curl of both sides of Stokes' equation we find

$$\nabla \times \nabla \times \nabla \times \mathbf{u} = 0 \ . \tag{11}$$

At first glance this may not seem beneficial. We have reduced our problem of solving a partial differential equation to that of solving a triple curl. However, todays computing software is more than capable of reducing this problem even further. Therefore, Mathematica was used to reduce this problem significantly. After many Legendre polynomial identities were used and a power series solution of the resulting differential equation was proposed the following resulted

$$F_l(r) = A_l r^{l+1} + B_l r^{l-1} + C_l r^{-l} + D_l r^{-l-2} \ . \tag{12}$$

If one looks closely, it can be noted that a ϕ component of the velocity field that is independent of ϕ still maintains compressibility if it is included in the velocity profile. This is done to account for the absence of the $m = 0$ term in the ϕ component of the velocity field. Upon substitution of this into the governing equation and again proposing a power series solution of the resulting differential equation one finds the following

$$H_l(r) = E_l r^l + I_l r^{-l-1} \ . \tag{13}$$

We are now equipped with the solutions to the flow field in general. All that is left is to apply the boundary conditions.

To simplify the problem even further assume an azimuthal symmetry to the geometry of the fluid flow. This approximation is valid because the Volvox organism actually exhibits this characteristic. Also, assume that the Volvox is swimming in the positive z direction with a speed U. If we place ourself in a reference frame that is traveling with the Volvox at the origin it appears as if the velocity of the fluid is $-U\hat{z}$ as r goes to infinity. If we express this velocity in spherical coordinates we can apply this as one boundary condition to the velocity profile. The second boundary condition arises because the fluid velocity in the radial direction at the surface of the organism must go to zero. That is, fluid is not entering or exiting the organism at a high enough rate as to disturb the velocity profile (the uptake rate of nutrients entering the organism is insignificant when compared to the fluid flow). The final boundary condition requires a little more ingenuity. One way to model the fluid motion around the organism is to suppose that the self-propelled motion of the Volvox can be described by the stress that the organism imposes on the fluid at its surface. We can model this as an average force per unit area that is uniform over the surface of the Volvox. Somatic cells, which the flagella are attached to, are distributed uniformly around the exterior of organism. Each of these cells helps to propel the organism by their collective flagellar beating. While this model hides the details of the motion of the fluid due to the propulsion by the organism's flagella (and their associated beating frequencies, length and stroke characteristics) it still provides an accurate description of the overall motion of the fluid. Therefore, the final boundary conditions that must be imposed are those of the stress tensor with the normal direction perpendicular to the surface of the Volvox acting in the direction of ϕ and θ with the stress components being modeled by an average force per unit area. These are the shear stresses at the surface of the organism.

$$\sigma_{-r\theta} = -\frac{\eta}{R}\Big[r\frac{\partial}{\partial r}\Big(\frac{u_\theta}{r}\Big) + \frac{1}{r}\frac{\partial u_r}{\partial \theta}\Big]_{r=1} \tag{14}$$

$$\sigma_{-r\phi} = -\frac{\eta}{R}\Big[r\frac{\partial}{\partial r}\Big(\frac{u_\phi}{r}\Big) + \frac{1}{r\sin\theta}\frac{\partial u_r}{\partial \theta}\Big]_{r=1} \tag{15}$$

In order to find the translational and the rotational velocities of the Volvox we turn to the appropriate reciprocal theorems which transfer us from the reference frame moving with the Volvox to one that is not. Rather than working through all of the mathematics, which are not trivial and are quite involved, I will simply quote the results.

$$\frac{u_r}{U} = -\Big(1 - \frac{1}{r^3}\Big)P_1(w) - \sum_{l=2}^{\infty}(r^{-l} - r^{-l-2})\frac{g_l P_l(w)}{g_1}\ , \tag{16}$$

$$\frac{u_\theta}{U} = -\Big(1 + \frac{1}{2r^3}\Big)P_1^1(w) + \sum_{l=2}^{\infty}\big((l-2)r^{-l} - lr^{-l-2}\big)\frac{g_l P_l^1(w)}{g_1 l(l+1)}\ , \tag{17}$$

$$\frac{u_\phi}{U} = -2\tan\alpha\sum_{l=1}^{\infty} r^{-l-1}\frac{(2l+1)g_l P_l^1(w)}{l(l+1)(l+2)g_1}\ , \tag{18}$$

where

$$g_l = \int_{-1}^{1} P_l^1(w)dw \ , \tag{19}$$

$$U = \frac{\tau \cos \alpha R}{\eta} \frac{\pi}{8} \ , \tag{20}$$

$$-\frac{1}{g_1} = \frac{2}{\pi} \ . \tag{21}$$

This brings us to the conclusion of the first goal of the research project: understanding the existing model in hopes that similar tactics may be employed when attempting to solve a multiple body problem. I extend my gratitude to Martin Short for all of his help in working through the model he developed. As one can see, though, a result of this model is that there is a relationship between the swimming speed and rotational speed of the organism. This ultimately brings us to the second goal.

We find that the model predicts a relationship between the translational and rotational swimming speeds of the Volvox as described below

$$\frac{U}{\Omega} = -R \cot \alpha \ . \tag{22}$$

It would be beneficial then to try and verify this relationship experimentally. Therefore an apparatus was constructed to take movies of individual and groups of Volvox organisms swimming (see figure below). From these movies, the translational and rotational swimming speeds can be calculated and the prediction made by the model can hopefully be verified. The apparatus includes an analog ccd camera with an adjustable lens. This camera lies on an adjustable track. The lens is directed at a platform that has an adjustable x, y and z base upon which a cuvet that contains the specimen is placed. A ring light is used to obtain a darkfield image of the swimming organism. Thus far, two preliminary movies have been taken in order to obtain the best possible images. Data collection will hopefully begin within the next few weeks. By the end of the semester, sufficient data should be collected and reduced so that an accurate plot reveals a cogent relationship between the translational and rotational velocities.

The final and significantly more difficult goal of this research project was to model the swimming behavior of pairs of Volvox organisms. There were two approaches to solving this problem that were considered. The first method was to borrow approximation results from similar problems that have been solved in the realm of sedimenting particles. The second method was to attempt to solve the problem in its entirety borrowing from the methods stated above for the single body problem. I chose the latter method to begin the study of the two body problem.

If you recall, this method relied upon finding a coordinate system which exhibited the appropriate symmetries of the problem. A solution was then proposed by expanding the velocity components of the fluid in terms of the

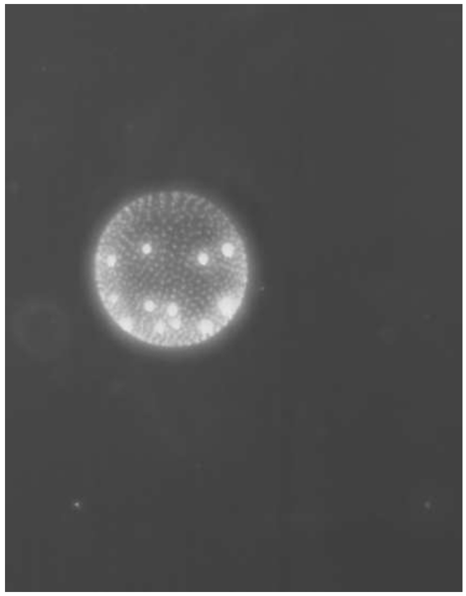

Figure 2: Image of a swimming Volvox taken from a preliminary movie.

coordinate system's natural harmonic functions (in spherical coordinates these were the spherical harmonics). The governing equations were reduced using vector identities and boundary conditions were applied to obtain a final solution. I attempted the same approach with the two body problem. Thus, an appropriate coordinate system needed to be used that exhibited the symmetries of the two body problem. It turns out that bispherical coordinates produce the required symmetries. Bispherical coordinates uses the variables η, θ and ϕ to label the coordinates. Surfaces of constant η are described by spheres that lie on the z axis with centers at $a \cot \eta$ and have radius $\frac{a}{\sinh \eta}$. This coordinate system is rather difficult to visualize. However, pictures of the coordinate system signify that the required symmetries existed. Therefore, the next step in the process was to discover what the natural harmonic functions of the coordinate system were. To do this, the technique of separation of variables was used to solve Laplace's equation in the new coordinate system. Laplace's equation in bispherical coordinates where f is the function is given by

$$\frac{\sin\theta}{(\cosh\eta-\cos\theta)^3}\Big[\frac{\partial}{\partial\theta}\Big(\frac{\sin\theta}{\cosh\eta-\cos\theta}\frac{\partial f}{\partial\theta}\Big)+\frac{\partial}{\partial\eta}\Big(\frac{\sin\theta}{\cosh\eta-\cos\theta}\frac{\partial f}{\partial\eta}\Big)+\frac{\partial}{\partial\phi}\Big(\frac{\csc\theta}{\cosh\eta-\cos\theta}\frac{\partial f}{\partial\phi}\Big)\Big]=0 \tag{23}$$

To separate variables, propose the function

$$f(\theta,\eta,\phi)=(\cosh\eta-\cos\theta)^{\frac{1}{2}}X(\theta)Y(\eta)Z(\phi)\ . \tag{24}$$

Substituting this function into Laplace's equation and then reducing using the standard techniques (no trivial calculation) one finds that, like spherical coordinates, the solution is an expansion of spherical harmonics multiplied by a function of η. But this is exactly what was obtained for the one body problem! Analogously to the one body problem, the next step in the process was to propose a solution of the fluid velocity profile as an expansion of the harmonic functions and plug these solutions into the governing equations. Thus, an expression for the equation for incompressibility in bispherical coordinates was needed and is as follows

$$\frac{(\cosh\eta-\cos\theta)^2}{a\sin\theta}\Big[\sin\theta\frac{\partial}{\partial\eta}\Big(\frac{u_\eta}{(\cosh\eta-\cos\theta)^2}\Big)+\frac{\partial}{\partial\theta}\Big(\frac{\sin\theta u_\theta}{(\cosh\eta-\cos\theta)^2}\Big)\Big]+\frac{\cosh\eta-\cos\theta}{a\sin\theta}\frac{\partial u_\phi}{\partial\phi}=0\,. \tag{25}$$

Similar to the one body problem, in order for incompressibility to be maintained when derivatives are taken we must get back the same spherical harmonics. We can therefore use Legendre polynomial identities to reduce the equations. After many different trial functions were proposed it was found that the following functions satisfied the conditions regarding their spherical harmonics

$$u_\eta=(\cosh\eta-\cos\theta)^2\sum_{l=1}^{\infty}\sum_{m=0}^{l}F(\eta)P_l^m(w)e^{im\phi}\ , \tag{26}$$

$$u_\theta=(\cosh\eta-\cos\theta)^2\sum_{l=1}^{\infty}\sum_{m=0}^{l}G(\eta)\frac{\partial}{\partial\theta}P_l^m(w)e^{im\phi}\ , \tag{27}$$

$$u_\phi=\sum_{l=1}^{\infty}\sum_{m=0}^{l}G(\eta)P_l^m(w)\Big(\frac{im(\cosh\eta-\cos\theta)}{\sin\theta}\Big)e^{im\phi}\ . \tag{28}$$

We seem to be close to obtaining a solution to the two body problem. Everything we have done thus far has followed directly from the one body problem. This may seem rather strange because problems typically get exponentially more difficult when more degrees of freedom are inserted. If intuition served the reader correctly, an uneasy feeling would have built up by this point in preparation for mathematical hardship. This feeling, in fact, would be well justified. If we follow the same prescription as for the one body problem of reducing Stokes' equation to that of a triple curl the resulting equations are ghastly. The curl of a vector in bispherical coordinates is unforgiving, let alone three curls, and is unreasonable to reduce. Let us suppose, however, that by some stroke of luck we reduce the equations to something manageable. The problem of applying boundary conditions still exists. It was not entirely difficult finding expressions for the fluid dynamical stress tensor in spherical coordinates. I have had some difficulty of finding the expressions in bispherical coordinates, however. I could, in theory, learn how to transform from one coordinate space into another by reading through a differential geometry text and applying these rules to the stress tensor. This task seems rather pointless, though, until we find a way to reduce the equations that are obtained after three curls of the vector field are taken in bispherical coordinates. If this cannot be accomplished the boundary conditions will never have reason to be applied.

4 Conclusion

We seem to be at a standstill then. Solving the problem exactly seems to be unreasonable provided the methods outlined above. While some insight to the problem was gained, finding an exact solution to the two body problem has so far proved unsuccessful. I move, therefore, to study the approximation methods that have been outlined for sedimenting particles. The main method that has traditionally been used is the method of reflections. This method will hopefully provide an accurate description of the two body problem and extend itself to the many body problem.