

Yolk Dynamics in Amphibian Embryos

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Abstract. In this paper we study the role of gravity in the development of freshly fertilized amphibian eggs in the hope that it will lead to a deeper understanding of the consequences of procreation in a microgravity environment. We formulate and solve mathematical models for the rotation of the egg and the settling of the yolk platelets. The model for rotation utilizes solid body mechanics and we find that there is an initial “up righting” phase consistent with experiments on the correct timescale. Our model for yolk-platelet motion involves the two-phase flow of yolk and albumin. We reduce this model to a one-dimensional hyperbolic nonlinear partial differential equation which we solve using the method of characteristics. The yolk settling times are consistent with experiments. Our work suggests that yolk motion is needed for the correct development of embryos.

Keywords. two-phase flow, embryo development, gravity, settling, rigid body rotation

1 Introduction

The impetus for this work is to attempt to understand the role that gravity plays in the development of embryos. There are three key processes observed in development of the egg prior to the first cell division:

- the up-righting phase (first minute),
- the delay until cortical rotation initiates (up to one hour),
- the cortical rotation (a further 30 minutes).

Our hope is that in understanding these simple processes, we will be able to understand some of the interesting behaviour of the egg described in Section 1.3 when it is rotated manually. This problem was proposed by the Manitoba Institute of Child Health and investigated at the First Fields-MITACS Industrial Problem-Solving Workshop in 2006.

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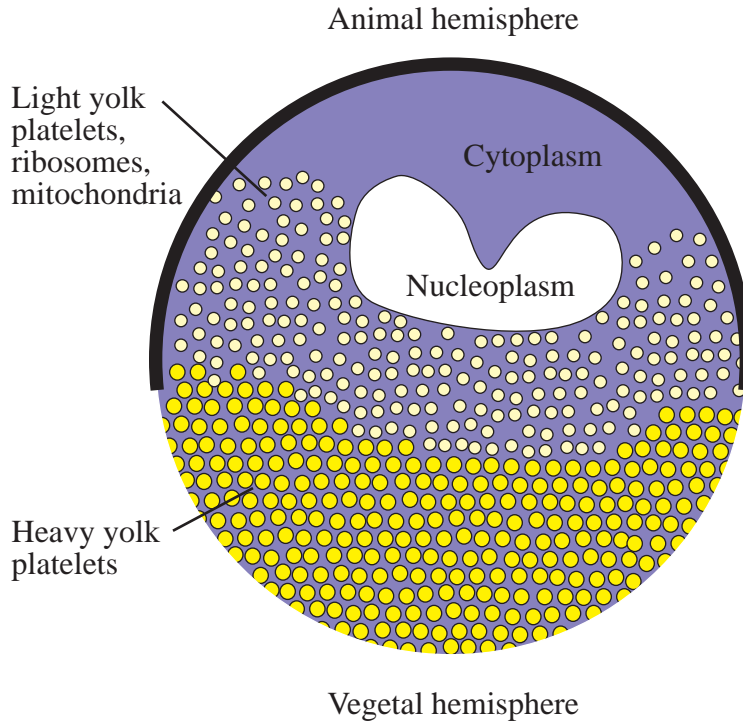


Figure 1: *Structure of a mature oocyte.*

A related problem was presented at the European Study Group with Industry in Bristol in 2003 which focussed on the artificial incubation of penguin eggs at Bristol zoo. During their development, eggs undergoing a natural incubation are rotated by the penguin at regular intervals. The report from the study group [4] concentrated on determining the reason for this behaviour in order to understand the requirements for artificial incubation. They found that the rotation did not significantly affect the temperature distribution of the egg but it was essential for the removal of waste products from near the embryo. They also discussed the settling of yolk and looked at the force balance on the embryo. The key difference between avian and amphibian eggs is that, in the former, the yolk is contained in a yolk sac on which the embryo develops whereas in the latter, the yolk platelets are free to move throughout the egg.

1.1 Structure of the egg

Figure 1 illustrates a mature amphibian oocyte (unfertilized egg) and its internal structure. Contained within the oocyte is a nucleus containing nucleoplasm, and suspended in viscous cytoplasm are yolk platelets, ribosomes, and mitochondria. The yolk platelets store lipoproteins that are packed into a regular array and provide energy for the developing cell. They vary in size and density with an average diameter of $12 \mu\text{m}$ [23] for those platelets located in the vegetal (lower) hemisphere.

The periphery of the cell is quite complicated and consists of a series of interconnected layers. Outermost is a monolayer of follicle cells which are bound to the follicular epithelium that encases the oocyte. Beneath this is a vitelline envelope which is roughly $1 - 2 \mu\text{m}$ in thickness and consists of a network of fibres that range in diameter from $40 - 70 \text{ nm}$. Within 200 nm of the surface of the plasma membrane are the cortical and pigment granules.¹

Two characteristics that distinguish between the hemispheres are that the vegetal hemisphere has significantly less pigment granules and has larger cortical granules [6] than the animal hemisphere.²

1.2 Egg activation

Fertilized eggs are released from the female in random orientations. In urodele amphibians such as the axolotl, many sperm enter the egg, but only one sperm nucleus fuses with the egg nucleus. When a fertilizing sperm penetrates the plasma membrane, the cortical granules break down and release their contents to the surface of the egg. Swelling on contact with water, the contents of the granules expand the space that lies between the plasma membrane and the vitelline envelope forming the perivitelline space [10]. At the same time, the vitelline envelope hardens and prevents any further penetration by sperm. Once the perivitelline space has formed, the animal hemisphere contracts, the egg is freed from its connections to its surrounding membranes, and reorientation of the eggs via gravity is permitted.

Average rates of rotation are typically 0.26 rpm [10] and the up-righting itself is hypothesized to be a result of the different yolk densities. Figure 2 shows axolotl eggs after up-righting. They appear to be axially symmetric, though inversion experiments [14, 21, 22] suggest that this may only apply to the cortex (cell membrane and underlying attached cytoplasm). It is possible that the embryo may be spherically symmetric at this stage.

Further asymmetry within the egg is introduced by a reorganization of the cytoplasm at about the halfway point between the moment of fertilization and time of first cell division. The reorganization occurs with a 30° rotation³ of the cortex relative to the rest of the cell body [10] and is depicted in Figure 3. The portion of the egg exposed by moving the animal hemisphere becomes covered with the clear vegetal hemisphere and, as a result, forms a grey crescent. This grey region of the egg becomes the dorsal (back) side of the embryo and the opposite side of the egg forms the ventral (belly) side of the embryo. The grey crescent is only visible in certain amphibian eggs but many different techniques have been developed to visualize the subcortical rotation in many species.

¹Stereo micrographs of these structures can be found in [9].

²A detailed view of the cell periphery can be found in [17] or in [6] and the references cited therein.

³Vincent [20] indicates rotations of $20^\circ - 38^\circ$ with an average of 28° . Maximum linear displacement is $300 \mu\text{m}$ at rates up to $10 \mu\text{m}/\text{minute}$.



Figure 2: *Typical axolotl eggs and their encapsulating gel after the up-righting phase (Courtesy of Susan Crawford-Young).*

1.3 The role of gravity

Gravity plays a role in the development of the embryo but its effect is paradoxical. This is demonstrated in a sequence of experiments where the egg was immobilized and rotated manually. Four situations are examined with an initially inverted egg.

1. An initial rotation of $15^\circ - 90^\circ$ held for 30 minutes followed by a manual up-righting of the egg causes the dorsal lip of the embryo to reposition to the side of the egg opposing gravity but otherwise produces normal offspring [3].
2. Eggs irradiated with ultraviolet light typically create offspring with morphological defects. These defects can be corrected provided that the egg is manually rotated within 30 minutes [3].
3. A sustained rotation of 165° (nearly inverted) produces offspring that have altered pigmentation. The probability of sterility is enhanced for those eggs with a lower viscosity [21].
4. A sustained inversion at 180° creates eggs that divide normally but fail to produce offspring [22].

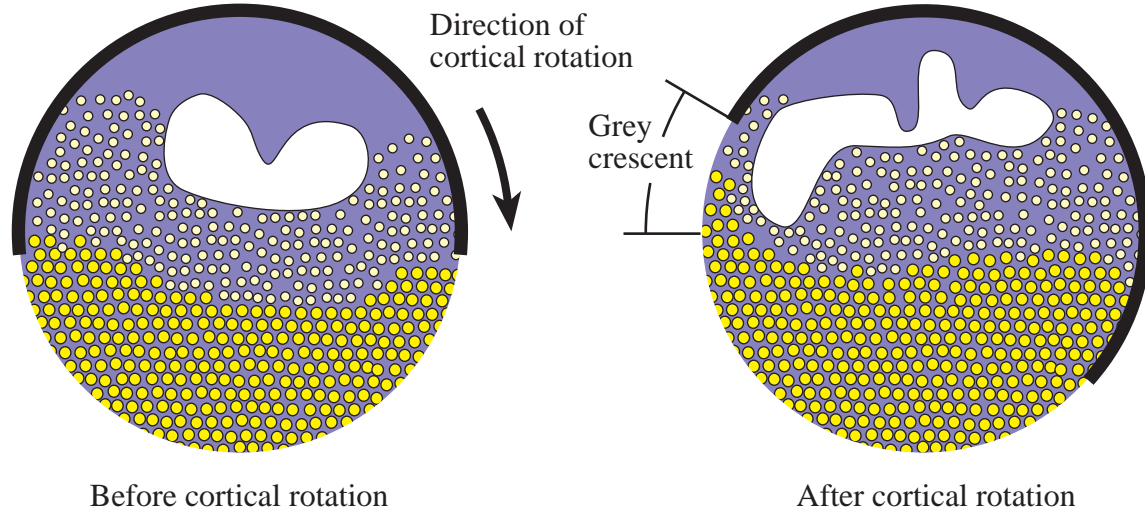


Figure 3: Eggs before and after cortical rotation.

A centrifuge has also been shown to be effective at orientating the cytoplasm, provided the procedure starts before the initiation of cortical rotation. For example, conjoined twin tadpoles can be formed by irradiating the egg with ultraviolet light, centrifuging at $30g$ for 4 minutes at a 90° inclination and then centrifuging at $10g$ for 4 minutes at a 0° inclination. These offspring were characterized as doubly rescued from the irradiation [1]. Despite the strength of the acceleration in this latter case the eggs produce viable offspring.

1.4 Overall timeline

Each of the processes discussed above occurs at a specific time within the first cell cycle. Figure 4 depicts the various events and is an accumulation of data from several sources [1, 10, 11, 12, 20, 24]. The time units are labelled in both normalized time (NT) and in minutes. A time of 0NT corresponds to the moment of fertilization and 1NT is the time when the egg divides for the first time. There is some ambiguity in the literature with respect to the absolute time scale since 1NT can take anywhere from 85 – 120 minutes depending on the temperature [1]. For the remainder of this report we have assumed the upper bound of 1 NT = 120 minutes.

1.5 Physical data

We illustrate the overall geometry, and regions of interest, of an amphibian egg in Figure 5. The pigmentation variation seen in the previous images is represented by the dark and light grey shaded regions.

The parameter values used in this article are presented in Table 1. Most of the data is taken from the African clawed frog (*Xenopus laevis*) species and inferred from the embryological literature.

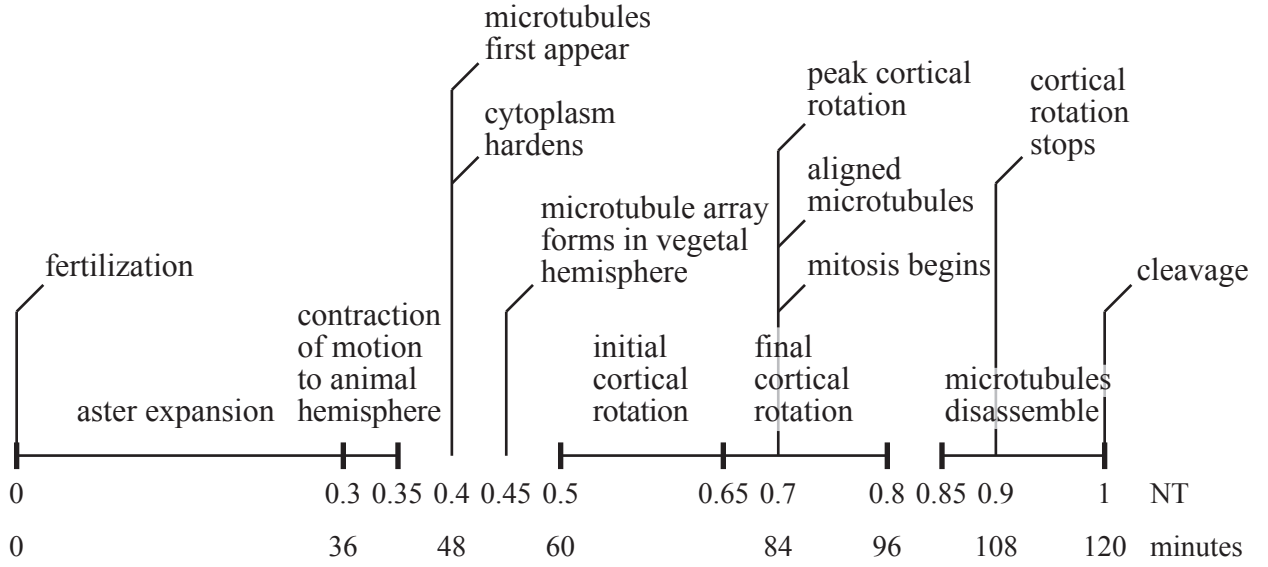


Figure 4: A timeline of significant events in the oocyte from fertilization to first cleavage.

Table 1: Physical properties of a typical amphibian egg.

Density (kg m^{-3})	
Nucleoplasm	$\rho_0 = 1000$
Cytoplasm	$\rho_1 = 1100$
Yolk platelets	$\rho_2 = 1200$ [15]
Dynamic Viscosity ($\text{kg m}^{-1} \text{s}^{-1}$)	
Nucleoplasm	$\mu_0 = 5 \times 10^{-3}$ [13]
Cytoplasm	$\mu_1 = 20 \times 10^{-3}$ [18]
Water	$\mu_2 = 1 \times 10^{-3}$
Lengths (m)	
Nucleus diameter	$2r_0 = 7 \times 10^{-4}$
Egg diameter	$2r_1 = 2 \times 10^{-3}$ [15]
Yolk platelet diameter	$a = 1.2 \times 10^{-5}$ [15]
Nucleus displacement	$s = 7 \times 10^{-4}$
Cortex width	$h_1 = 2 \times 10^{-5}$ [17]
Perivitelline space	$h_2 = 3 \times 10^{-6}$ [25]

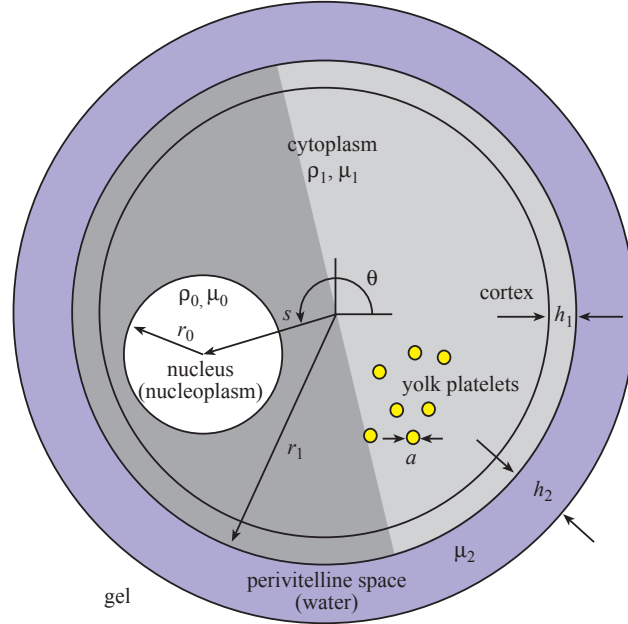


Figure 5: *An idealized amphibian egg and its physical parameters.*

We note however that reported sizes of yolk platelets range from $2 - 14 \mu\text{m}$ in diameter: Neff et al. [15] identified three categories (small, medium and large) of platelet with densities of 1208 , 1240 , and 1290 kg m^{-3} respectively. Wall et al. [23] specifies two sizes at different concentrations with a preponderance of the platelets about $12 \mu\text{m}$ in diameter. Light yolk platelets were found to have a density of 1210 kg m^{-3} in concentrations of 7 g ml^{-1} whereas heavy platelets are more dense and occur in higher concentrations: 1230 kg m^{-3} at 40 g ml^{-1} .

1.6 Plan

In the remainder of this paper we consider the up-righting phase in an attempt to determine whether a rigid body rotation is consistent with the observed timescale. We then propose a mathematical model that describes the distribution of platelets within the egg, reduce the model to its simplest limit and solve the resulting model for various initial conditions. Finally we draw conclusions about the about the settling time for the platelets and discuss possible extensions.

2 Up-righting phase

As previously described, once the eggs are fertilized, the contents of the cortical granules are released into the gap between the plasma membrane and the vitelline membrane. Upon hydration this gap expands to form the perivitelline space, freeing the egg and causing it to rotate with the dark

pigmentation area towards the top. Some of the characteristics of this up-righting motion are that it takes about a minute to complete [10] and appears to be overdamped [7].

If the interior of the cell is sufficiently viscous then the up-righting can be approximated with a rigid body rotation. As discussed earlier, two of the main components of the interior of the egg are yolk platelets and cytoplasm. The platelets are membrane-bound spheres containing high concentrations of yolk protein so that interior can be thought of as a colloidal suspension. To determine how long it would take a yolk platelet of diameter a to traverse the length of the egg we apply Stokes' law to find the terminal velocity

$$v_\infty = \frac{1}{18} \frac{(\rho_2 - \rho_1)ga^2}{\mu_1} = 3.92 \times 10^{-7} \text{ m s}^{-1}$$

using the data in Table 1. This implies that it would take 5.1×10^3 seconds for the platelet to fall the 2 mm length of the egg. Since the up-righting occurs on the time scale of a minute, during this phase the interior of the egg can be approximated as solid. The same approximation holds for avian eggs [4] and we note that this results in a speed of $1.4 \times 10^{-9} \text{ m s}^{-1}$, which is significantly slower than for amphibian eggs.

Referring back to Figure 5, the nucleus is set inside a cell body and has three torques acting on it. Gravity and buoyancy act through the centre of mass and centre of buoyancy (the centre of mass of the displaced fluid), respectively. The viscous drag opposes motion in the fluid bearing region of the perivitelline space. Denoting θ as the angular displacement of the nucleus, I_0 as the moment of inertia of the cell, and the various torques by τ_i , the angular force balance reads

$$I_0 \ddot{\theta} = \tau_{\text{buoy}} + \tau_{\text{gravity}} + \tau_{\text{drag}}, \quad \theta(0) = \theta_0, \quad \dot{\theta}(0) = 0. \quad (1)$$

Using the values in Table 1 we find that

$$I_0 \lesssim \frac{2}{5} m_1 r_1^2 = \frac{8}{15} \pi \rho_1 r_1^5 \simeq 1.8 \times 10^{-12} \text{ kg m}^2, \quad (2)$$

and

$$\tau_{\text{buoy}} + \tau_{\text{gravity}} = \frac{4}{3} \pi r_0^3 (\rho_1 - \rho_0) g s \cos \theta \simeq 3.7 \times 10^{-10} \cos \theta, \quad (3)$$

where I_0 neglects the effect of the lower density region occupied by the nucleus. For the drag we need to take into account the variation in speed over the surface of the sphere. At an azimuthal angle of φ , the infinitesimal torque is

$$d\tau_{\text{drag}} = -\mu_2 (r_1 \sin \varphi) \dot{\theta} \left(\frac{r_1 \sin \varphi}{h_2} \right) dA = -2\pi r_1^4 \frac{\mu_2 \dot{\theta}}{h_2} \sin^3 \varphi d\varphi.$$

Integration over $0 \leq \varphi \leq \pi$ gives the expression

$$\tau_{\text{drag}} = -\frac{4}{3} \pi r_1^4 \frac{\mu_2 \dot{\theta}}{h_2} \sim -1.4 \times 10^{-9} \dot{\theta}. \quad (4)$$

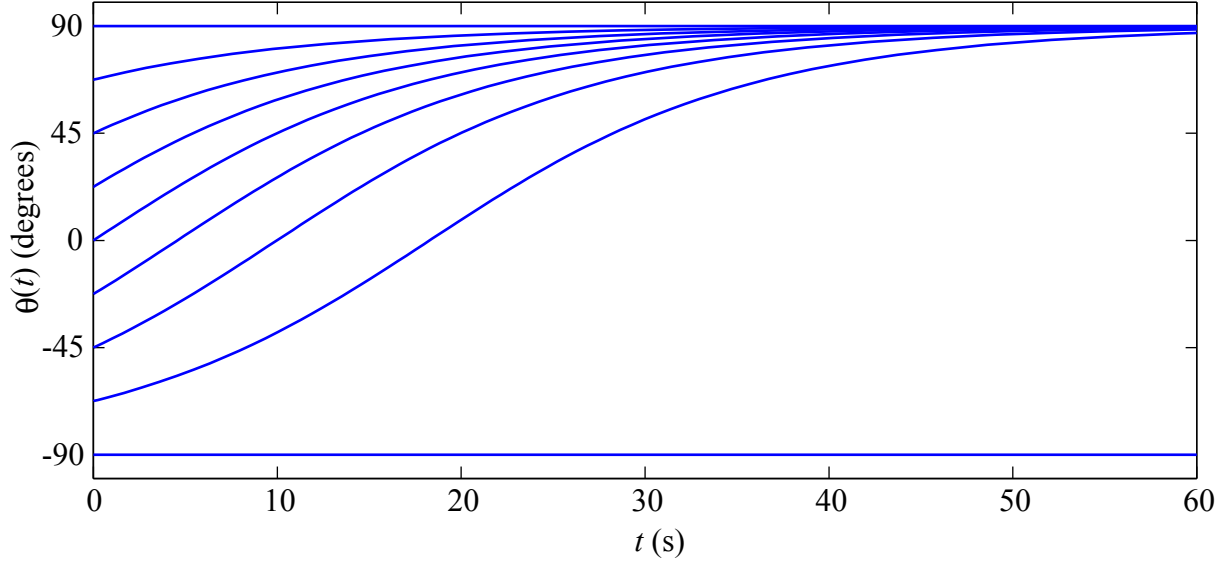


Figure 6: Solution curves to (5) with various initial values $-\pi/2 \leq \theta_0 \leq \pi/2$.

Due to their relative smallness, the inertial terms do not contribute significantly to the motion. Neglecting them reduces (1)-(4) to the simplified expression

$$\dot{\theta} \simeq \frac{sr_0^3}{r_1^4} \frac{h_2 g(\rho_1 - \rho_0)}{\mu_2} \cos \theta, \quad \theta(0) = \theta_0, \quad \dot{\theta}(0) = 0 \quad (5)$$

with solution curves illustrated in Figure 6. Notice that the up-righting time is typically less than a minute. Using reported rotational speeds of anywhere from 0.026 to 0.54 rpm [10] and the fact that the maximal rate of rotation occurs when $\theta = 0$ we can determine the size of the perivitelline space as

$$\frac{0.026(2\pi)}{60} \frac{r_1^4 \mu_2}{sr_0^3 g(\rho_1 - \rho_0)} \leq h_2 \leq \frac{0.54(2\pi)}{60} \frac{r_1^4 \mu_2}{sr_0^3 g(\rho_1 - \rho_0)}$$

or $0.092 \mu\text{m} \leq h_2 \leq 1.9 \mu\text{m}$. These values are quite possible since this space is created by the swelling contents of cortical granules that are themselves typically only $1.5 \mu\text{m}$ in diameter.

3 Delay until cortical rotation

Once the egg has up-righted, the yolk platelets in the cytoplasm will settle out until they reach an equilibrium distribution. To study this behaviour we model⁴ the interior of the egg using a one-dimensional two-phase-flow model (see [8]) for the yolk/cytoplasm colloid as illustrated in Figure 7. In what follows α and β are the volume fractions of the yolk and cytoplasm which move with speeds

⁴We neglect the presence of the nucleus in this section.

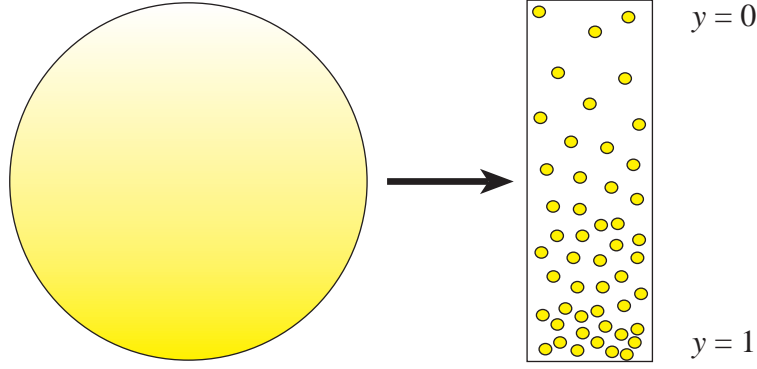


Figure 7: *One-dimensional approximation of the interior of the egg.*

u_α and u_β respectively. Conservation of mass for each phase can then be written as

$$\alpha_t + (\alpha u_\alpha)_y = 0, \quad \beta_t + (\beta u_\beta)_y = 0. \quad (6)$$

Assuming that space is completely filled by yolk and cytoplasm (i.e there are no voids), we write

$$\alpha + \beta = 1. \quad (7)$$

Neglecting inertia and introducing p_α and p_β for the averaged isotropic pressure in each phase, μ_α and μ_β for the viscosity of each phase, g as the acceleration due to gravity, and τ_α and τ_β for the deviatoric stresses, conservation of momentum for each phase leads us to

$$(-\alpha p_\alpha + \alpha \tau_\alpha)_y + (p_\alpha - \tau_\alpha^{\text{int}})\alpha_y - \frac{\mu_\alpha}{a^2} \alpha f(\alpha)(u_\alpha - u_\beta) + \rho_\alpha g \alpha = 0, \quad (8)$$

$$(-\beta p_\beta + \beta \tau_\beta)_y + (p_\beta - \tau_\beta^{\text{int}})\beta_y + \frac{\mu_\alpha}{a^2} \alpha f(\alpha)(u_\alpha - u_\beta) + \rho_\beta g \beta = 0, \quad (9)$$

where τ_α^{int} and τ_β^{int} are the stresses at the interfaces. The first two terms are the bulk-averaged and surface-averaged contributions to the stress from the phases themselves, the third term in these equations represents drag of one phase on the other, and the final term represents the gravitational force. Continuity of stress at the interface between yolk and cytoplasm gives

$$-p_\alpha + \tau_\alpha^{\text{int}} - (-p_\beta + \tau_\beta^{\text{int}}) = -\gamma \kappa, \quad (10)$$

where γ is the average interfacial tension and κ is the average interfacial curvature.

In order to close the model we have to pose four constitutive laws, describing the behaviour of the two fluids and what happens with the stress at the interface. Assuming both phases behave as viscous liquids, we write

$$\tau_\alpha = \mu_\alpha u_{\alpha y}, \quad \tau_\beta = \mu_\beta u_{\beta y}, \quad \tau_\alpha^{\text{int}} = -\mu_\alpha f_1(\alpha) u_{\alpha y}, \quad \tau_\beta^{\text{int}} = -\mu_\beta f_2(\beta) u_{\beta y},$$

where f_1 and f_2 are functions associated with the geometry of the system and the ease of motion of the liquids.

It is straightforward to reduce these ten expressions to three. We nondimensionalise the resulting equations using

$$y = L\hat{y}, \quad u_\alpha = U\hat{u} = \frac{\rho_\alpha g a^2}{\mu_\alpha} \hat{u}, \quad t = \frac{L\mu_\alpha}{\rho_\alpha g a^2} \hat{t}, \quad p = \frac{\mu_\alpha U L}{a^2} \hat{p}, \quad \epsilon = \frac{a}{L},$$

to yield, dropping hats,

$$\alpha_t + (\alpha u)_y = 0, \tag{11}$$

$$\alpha p_y = \alpha \left(1 - \frac{f u}{1 - \alpha} \right) + \epsilon^2 \left((\alpha u_y)_y + f_1 \alpha_y u_y \right), \tag{12}$$

$$p_y = \bar{\rho} + (1 - \bar{\rho})\alpha + \epsilon^2 \left((\alpha u_y)_y + f_1 \alpha_y u_y - (1 - \alpha)(f_1 u_y)_y + \Gamma \right) \tag{13}$$

where $\bar{\rho} = \rho_\beta / \rho_\alpha$, and

$$\Gamma = -\frac{\mu_\beta}{\mu_\alpha} \left[\left((1 - \alpha) \left(\frac{\alpha u}{1 - \alpha} \right)_y \right)_y - f_2 \alpha_y \left(\frac{\alpha u}{1 - \alpha} \right)_y + (1 - \alpha) \left(f_2 \left(\frac{\alpha u}{1 - \alpha} \right)_y \right)_y \right]. \tag{14}$$

To leading order in ϵ , we find that

$$\alpha_t + (\alpha u)_y = 0, \quad u = (1 - p_y) \frac{1 - \alpha}{f}, \quad p_y = \bar{\rho} + (1 - \bar{\rho})\alpha,$$

in otherwords,

$$\alpha_t + \left(\frac{(1 - \bar{\rho})\alpha(1 - \alpha)^2}{f} \right)_y = 0. \tag{15}$$

We suppose that we know the initial distribution of the yolk platelets, so that we write

$$\alpha(y, 0) = \alpha_0(y). \tag{16}$$

At the top and bottom of the egg, we suppose that there is no motion of either phase (that is, since the fluids are viscous we impose the no slip condition) and so we set $u = 0$. It is not possible to impose this condition on the leading-order outer solution at both the top and bottom surfaces, as close to the surfaces the viscous stresses that we have neglected to leading order become important. Scaling into the boundary layers at the top and bottom of the egg, we find that conservation of mass leads to the condition

$$u\alpha = 0$$

at $y = 0$ and $y = 1$. We pick $u = 0$ throughout the bottom boundary layer (with consequence that $\alpha = 1$ at $y = 1$). In the top boundary layer, we choose $\alpha = 0$. We now consider the evolution of three relevant initial conditions (a) a linear yolk gradient, (b) an inverted egg (with a linear gradient) and (c) a well-mixed egg. We set $f = 1$ for simplicity, since we are unsure of the exact

underlying microscopic processes, and we scale $t = \tau/(1 - \bar{\rho})$ to remove the explicit dependence on ρ . The timescale, T , for the drainage problem is therefore given by

$$T = \frac{L\mu_\alpha}{(\rho_\alpha - \rho_\beta)g\alpha^2} \sim 700 \text{ s}, \quad (17)$$

using the values given in Table 1.

Our problem is then to solve

$$\alpha_\tau + (\alpha(1 - \alpha)^2)_y = 0, \quad y \in (0, 1), \tau > 0, \quad (18)$$

with an initial condition appropriate to each case and keeping in mind the boundary conditions discussed in the previous paragraph.

3.1 Settling of yolk from a linear gradient

We suppose that

$$\alpha(y, 0) = y, \quad (19)$$

and we note that the initial linear gradient satisfies the boundary conditions discussed in the previous section. We solve (18) and (19) using the method of characteristics, which, for $\tau < 1/4$, yields

$$\alpha(y, \tau) = \begin{cases} 0, & 0 \leq y < \tau \\ \frac{1}{6} \left[4 - \frac{1}{\tau} + \sqrt{\left(4 - \frac{1}{\tau}\right)^2 - 12 \left(1 - \frac{y}{\tau}\right)} \right], & \tau < y \leq 1. \end{cases} \quad (20)$$

At $\tau = 1/4$ a shock forms and we use the Rankine-Hugoniot condition (see [16]) to find the position of the shock. Denoting the shock position by $y = s(\tau)$, we have

$$\frac{ds}{d\tau} = \frac{[\alpha(1 - \alpha)^2]}{[\alpha]}, \quad (21)$$

where the square brackets denote the jump in the quantity inside the brackets as the shock is traversed. In this case, since the volume fraction of yolk platelets is zero above the shock, (21) becomes

$$\frac{ds}{d\tau} = (1 - \alpha_+)^2, \quad (22)$$

where α_+ is the solution given in (20) and evaluated at $y = s$, i.e.

$$\alpha_+ = \frac{1}{6} \left[4 - \frac{1}{\tau} + \sqrt{\left(4 - \frac{1}{\tau}\right)^2 - 12 \left(1 - \frac{s}{\tau}\right)} \right]. \quad (23)$$

The initial condition for the shock position is $s = 1/4$ at $\tau = 1/4$. We solve (22) numerically and show the position of the shock in (y, τ) space in the upper figure of Figure 8. As $\tau \rightarrow \infty$, $s \rightarrow 1/2$, and the liquid separates into cytoplasm at the top of the egg, and yolk at the bottom. We show the volume fraction of the yolk platelets as it evolves in the lower figure of Figure 8. We note that the yolk takes an infinite amount of time to reach its steady state and that the drainage is fastest at the start. Indeed after 15 minutes, the yolk platelets reside below $y = 0.45$ (i.e. within 10% of their final position).

3.2 Settling of an inverted egg

In this case, we set

$$\alpha(y, 0) = 1 - y. \quad (24)$$

We find that a shock immediately develops at the bottom of the egg, across which the yolk volume fraction jumps to unity. A second shock propagates in from the top of the egg, initiating at $\tau = 1/2$, across which the volume fraction jumps from zero. Denoting these two shocks as $s_{\text{top}}(\tau)$ and $s_{\text{bot}}(\tau)$ respectively, we find that the solution is

$$\alpha(y, \tau) = \begin{cases} 0, & 0 \leq y \leq s_{\text{top}}(\tau) \\ \frac{1}{6} \left[4 - \frac{1}{\tau} + \sqrt{\left(4 - \frac{1}{\tau}\right)^2 - 12 \left(1 + \frac{1}{\tau} - \frac{y}{\tau}\right)} \right], & s_{\text{top}}(\tau) \leq y \leq s_{\text{bot}}(\tau) \\ 1, & s_{\text{bot}}(\tau) \leq y \leq 1 \end{cases} \quad (25)$$

where the position of the shocks can be found by solving

$$\frac{ds_{\text{top}}}{d\tau} = (1 - \alpha(s_{\text{top}}, \tau))^2, \quad s_{\text{top}}(1/4) = 0, \quad (26)$$

and

$$\frac{ds_{\text{bot}}}{d\tau} = -\frac{\alpha(s_{\text{bot}}, \tau) (1 - \alpha(s_{\text{bot}}, \tau))^2}{1 - \alpha(s_{\text{bot}}, \tau)}, \quad s_{\text{bot}}(0) = 0. \quad (27)$$

These two shocks collide at $y = 1/2$, $\tau = 2.6$ and the solution becomes

$$\alpha(y, \tau) = \begin{cases} 0, & 0 \leq y < 1/2 \\ 1, & 1/2 \leq y \leq 1, \end{cases} \quad (28)$$

for subsequent times, since the new shock created when these two collide satisfies $ds_{\text{end}}/d\tau = 0$. We show the characteristic projections and the evolution of the yolk volume fraction in Figure 9. We note that, in this case, the yolk reaches its steady state after a finite time, which is equivalent to 30 minutes.

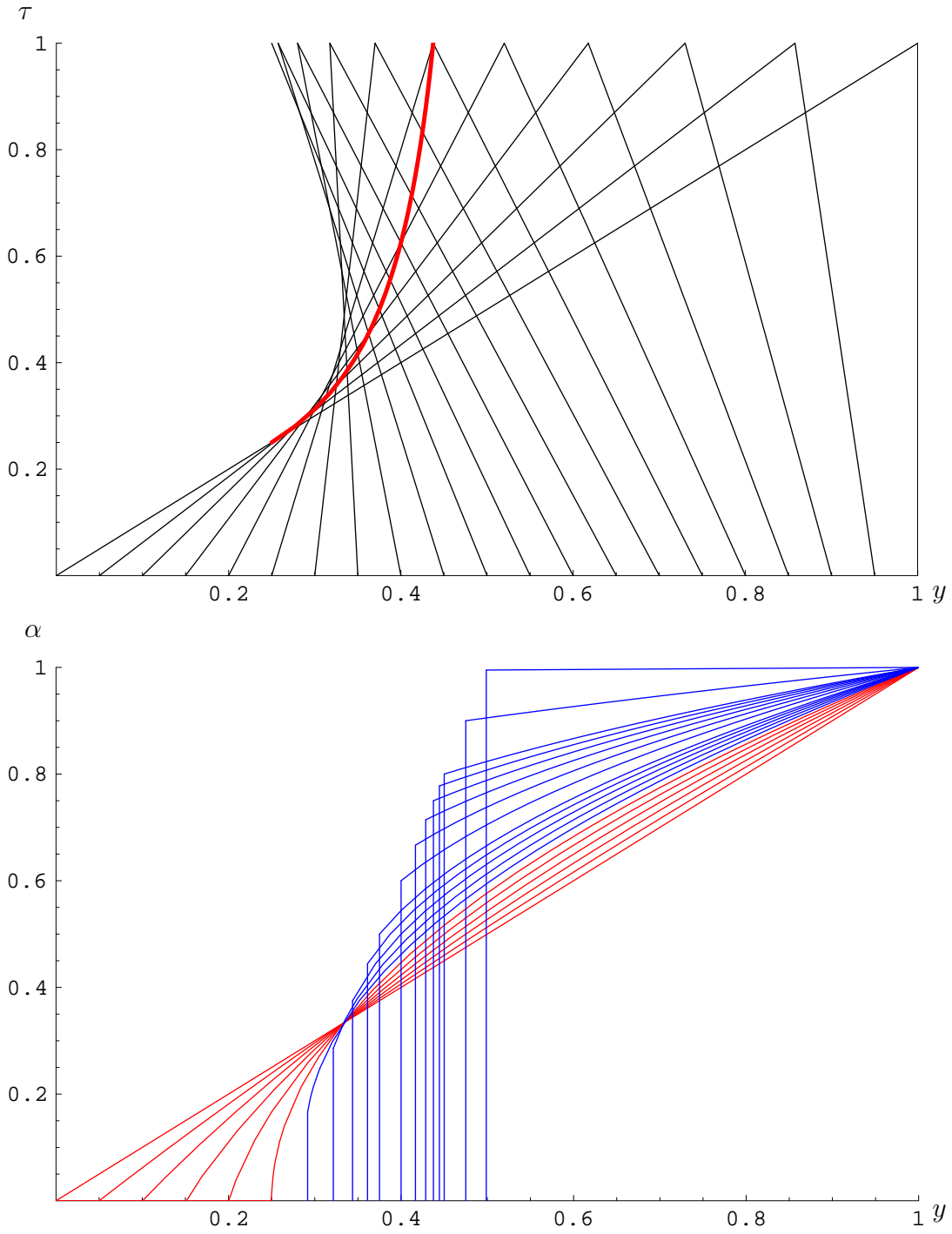


Figure 8: (upper) Characteristic projection in (y, τ) space. The red line shows the position of the shock. (lower) Graph showing the evolution of the yolk volume fraction. The red profiles are before the shock develops, the blue ones, afterwards.

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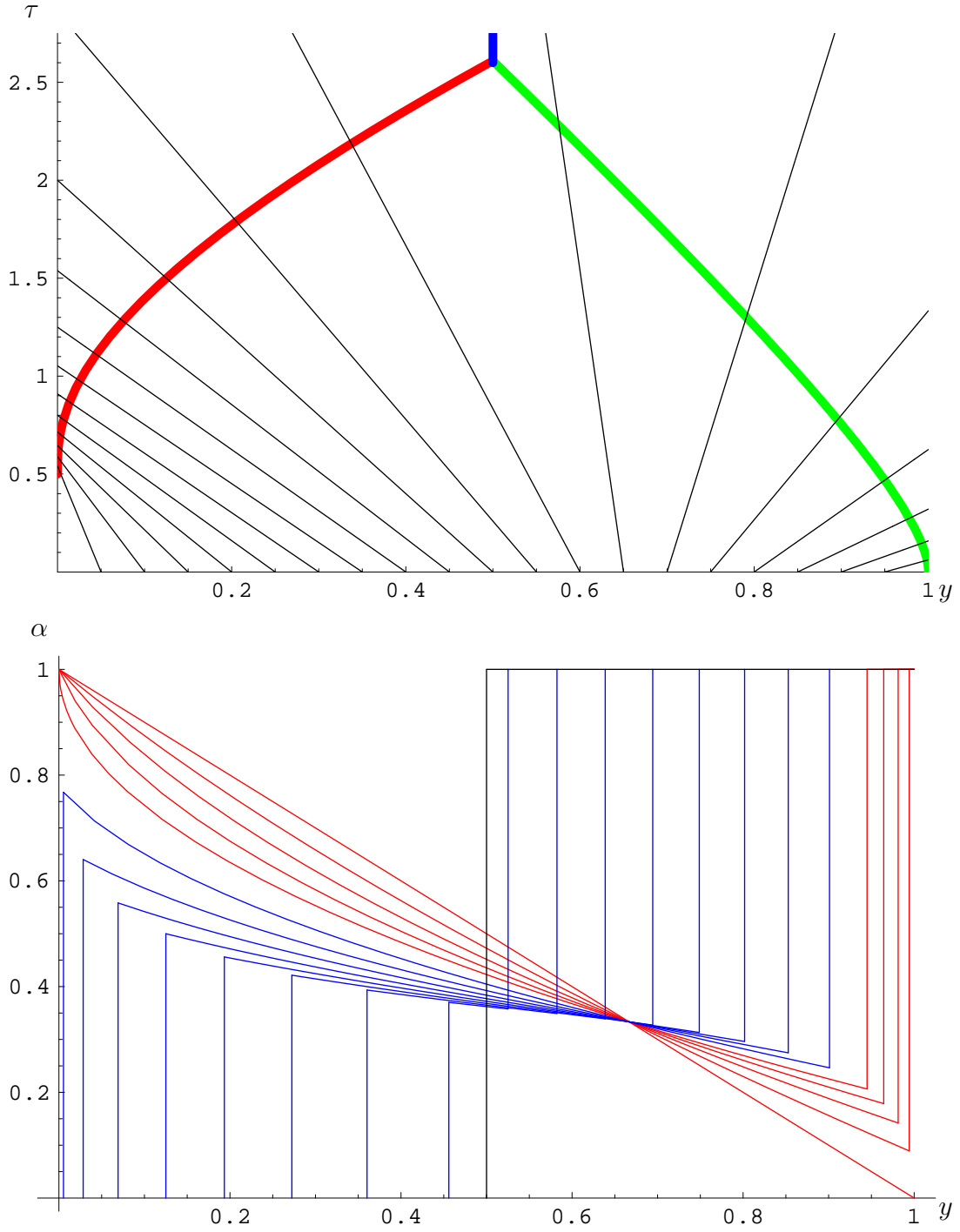


Figure 9: (upper) Characteristic projection in (y, τ) space. The red line shows the position of s_{top} , the green line shows the position of s_{bot} and the blue line shows the position of s_{end} . (lower) Graph showing the evolution of the yolk volume fraction. The red profiles are before the shock s_{top} develops, the blue ones, afterwards. The final state is shown in black.

3.3 Settling of a well-mixed egg

The final case we consider is the settling of an egg where the yolk is well mixed, so that the initial condition is

$$\alpha(y, 0) = 1/2. \quad (29)$$

In this case, the solution is $\alpha = 1/2$ along parallel lines $\tau = 4(y_0 - y)$. However, the initial data cannot propagate into the triangle $1 - \tau/4 \leq y \leq 1$, and we introduce an expansion fan there (with α ranging from $1/2$ to 1). Some of the characteristics in the fan intersect with those generated from the initial data so, as in the previous case, we have a shock propagating in from the base of the egg. The solution reads

$$\alpha(y, \tau) = \begin{cases} 0, & 0 \leq y \leq s_{\text{top}}(\tau) \\ 1/2, & s_{\text{top}}(\tau) \leq y \leq s_{\text{bot}}(\tau) \\ \frac{2 + \sqrt{1 - \frac{1-y}{\tau}}}{3}, & s_{\text{bot}}(\tau) \leq y \leq 1. \end{cases} \quad (30)$$

In this situation, it is easy to find $s_{\text{top}} = \tau/4$, and that s_{bot} satisfies

$$\frac{ds_{\text{bot}}}{dt} = \frac{\alpha^*(1 - \alpha^*)^2 - \frac{1}{8}}{\alpha^* - \frac{1}{2}}, \quad s_{\text{bot}}(0) = 1, \quad (31)$$

where

$$\alpha^* = \frac{2 + \sqrt{1 - \frac{1-s(\tau)}{\tau}}}{3}. \quad (32)$$

This solution holds until the two shocks collide, which occurs at τ^* , found by solving $s_{\text{top}}(\tau^*) = s_{\text{bot}}(\tau^*)$, and yielding $\tau = 1.788$, $y = 0.444$. Subsequently a final (slowly moving) shock propagates back down the egg allowing a relaxation to the steady state. The final shock initiates from $s_{\text{bot}}(\tau^*)$, and moves according to

$$\frac{ds_{\text{end}}}{dt} = (1 - \alpha^*)^2, \quad s_{\text{end}}(\tau^*) = s_{\text{bot}}(\tau^*). \quad (33)$$

We show the characteristic projection and the evolution of the yolk volume fraction in Figure 10, in which we can see that the yolk takes an infinite time to settle to the steady state. However, after 22 minutes, the yolk platelets are all below $y = 0.45$.

Yolk Dynamics in Amphibian Embryos

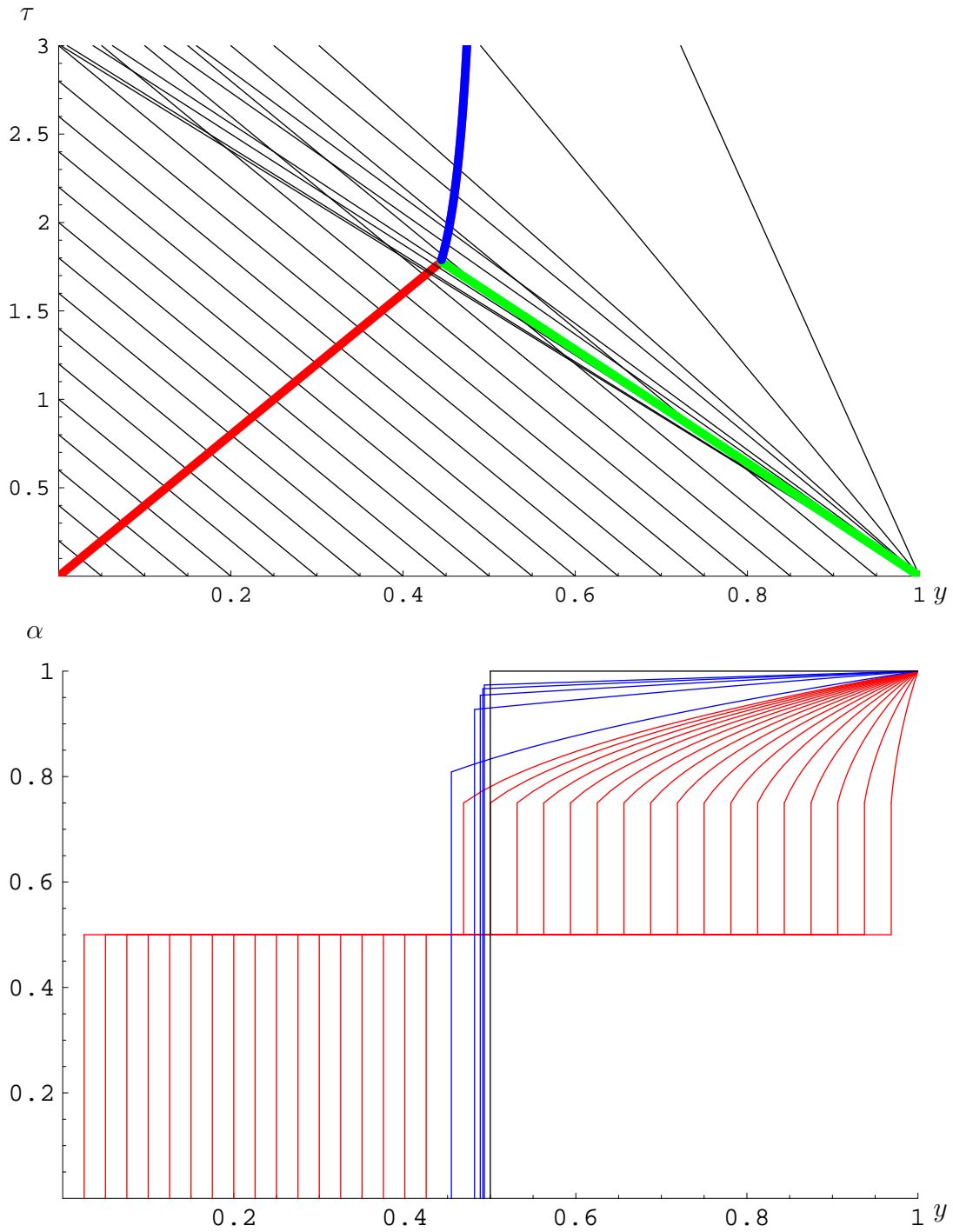


Figure 10: (upper) Characteristic projection in (y, τ) space. The red line shows the position of s_{top} , the green line shows the position of s_{bot} and the blue line shows the position of s_{end} . (lower) Graph showing the evolution of the yolk volume fraction. The red profiles are before the shock s_{end} develops, the blue ones, afterwards. The final state is shown in black.

4 Cortical rotation

We now turn our attention to the rotation of the cortex that takes place typically around $t = 0.5$ in normalized time units or at about 60 minutes. Some recent developments [2, 11, 12, 24] have begun to identify components involved in the cortical rotation but there is still a poor understanding of the details of the actual mechanism. What is known with some certainty is that the motor molecules dynein and kinesin both play a role in the process. In addition, recent measurements [5] have determined that one motor molecule is capable of producing about 6.2×10^{-12} N of force.

Even though the electrokinetics of microtubules is still in its infancy [19], it is possible to determine the number of motor molecules that would be required to perform the cortical rotation. We know that the rotation rate is typically $\dot{\theta} = 30^\circ$ in 30 minutes $= (\pi/6)/1800 \text{ rad s}^{-1}$. As a result the linear speed at the surface of the egg ($r_1 = 1 \times 10^{-3}$ m) is $v_1 = r_1 \dot{\theta}$ or $v_1 = 2.9 \times 10^{-7} \text{ m s}^{-1}$.

The drag force on the cortex is to a first approximation simply the surface area of the cortex multiplied by the viscous drag per unit area. Using the data in Table 1,

$$F_{\text{drag}} = 4\pi r_1^2 \mu_1 \left. \frac{\partial v}{\partial r} \right|_{r=r_1} = 4\pi r_1^2 \mu_1 \frac{v_1}{h_1} = 3.6 \times 10^{-9} \text{ N}$$

indicating that about 590 of the roughly 10^4 available motor molecules would be required to achieve the observed rotation rate. Irrespective of the actual mechanism chosen there seems to be enough energy available to allow the rotation to occur.

There are two main difficulties that need to be overcome before any further modelling can commence. First, the generation of microtubules inside the egg and the motion of motor molecules along these microtubules could be modelled but as mentioned earlier, it is not clear in the literature as to the actual mechanism. The second problem to overcome is an understanding of the initiation and cessation of the cortical rotation, for which there does not seem to be an appropriate biological model.

5 Conclusions and Future Work

In our analysis of the yolk dynamics of the egg prior to the first cell division, three sub-problems were investigated. The up-righting phase was modelled with a simple buoyancy argument for a low density nucleus trapped inside a viscous cytoplasm and predicts behaviour consistent with observations.

Once the egg is upright, the yolk platelets in the cytoplasm settle out until they reach an equilibrium distribution. We described the settling of yolk platelets through the cytoplasm using a two-phase model. We reduced the model to the simplest form possible and solved the resulting model analytically in three cases: a linear initial gradient of yolk platelets, an inverted egg, and a well-mixed egg. We found that the inverted egg settled in finite time, while the other cases settled in infinite time (with the evolution being 90% complete within 15 minutes and 22 minutes

respectively). In all three cases, a region devoid of yolk platelets formed near the top of the egg, and we were able to track the point at which the volume fraction became non-zero.

In the case where one inverts the egg, the yolk platelets are stationary after 30 minutes, in contrast with the upright case, where there is *always* motion of the fluid in the egg, albeit slow. This suggests that yolk *motion* is needed for correct development of embryos, consistent with the findings of [4] for avian eggs. However, further speculation about links between the yolk platelet evolution and the development of the embryo requires further experimentation.

With respect to cortical rotation, we verified that motor molecules within the egg will have sufficient energy to cause the cortical rotation. However, a clear mechanism for how the rotation takes place was not identified.

Future work along these lines could involve further development of the two-phase model to allow the inclusion of the nucleus and/or utilization of the actual egg geometry. An important step in the verification and refinement of the models used in this preliminary work is the direct observation of yolk platelets during the critical moments between fertilization and the first cell division.

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