BT5051: **Transport Phenomena in Biological Systems**

Choose-Focus-Analyse Exercise

Tardigrade Survival under Desiccation Stress

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BE21B004

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1. Background

Tardigrades, also known as 'water bears' or 'moss piglets', are microscopic eight-legged organisms that thrive in a wide range of habitats on Earth. They are highly resilient to multiple abiotic stresses such as extreme temperature, extreme pressure, dehydration, radiation and starvation. Experiments have shown that these organisms can even survive in outer space [1]!

Figure 1: A tardigrade perched on moss
(Source: National Geographic)

Tardigrades employ a wide array of mechanisms in order to tolerate and overcome harsh environmental conditions. These strategies have spurred biological and biomedical engineers to propose novel tardigrade-inspired biomaterials, especially for vaccine and cell preservation [2].

In order to reliably replicate the extremotolerant mechanisms of tardigrades, it is essential to first create a rigorous theoretical framework to understand them. However, there are no existing mathematical models that address this issue. This exercise is aimed at creating a model for one of the mechanisms used by the tardigrade to survive dehydration. This model will be created by analysing the tardigrade under the lens of one of the fundamental fluxes involved in transport phenomena in biological systems – mass flux [3].

When subjected to dehydrating / desiccating conditions, tardigrades rapidly lose water through the **chitin**-rich **cuticle** that covers their body, retaining only a fraction of their original water content, and reducing their body volumes by 85-90%. This allows them to enter a latent state, called the **tun state** [4].

In the tun state, all metabolic activity is ceased and the tun is observed to be capable of tolerating exposure to other abiotic stresses [5]. This phenomenon is generally known as **cryptobiosis** (literally, hidden life), while this situation in particular is known as **anhydrobiosis**. Once the dehydration stress is removed, the tun gets rehydrated and the tardigrade resumes its normal activity.

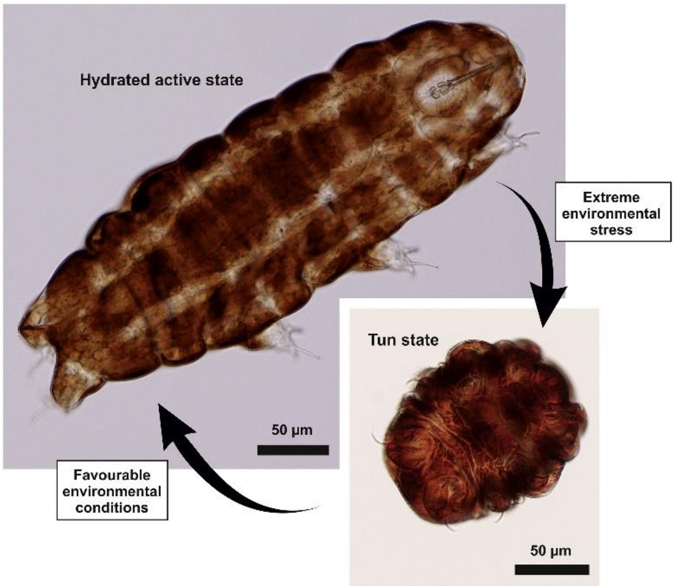

Figure 2: The tardigrade tun state
(Source: Møbjerg and Neves 2021)

We wish to create a mathematical model for the formation of the tun state under desiccation stress and perform some analyses using this model.

2. Principles

2.1 Mass balance

Consider a system through which there is input and output of a chemical component. The component can also be consumed or generated within the system. The rate at which this component accumulates in the system is given by

$$\boldsymbol{r_i - r_o + r_g - r_c = \frac{dm}{dt}}$$

r_i is the input rate.

r_o is the output rate.

r_g is the generation rate.

r_c is the consumption rate.

$\frac{dm}{dt}$ is the rate of accumulation.

We assume that the system is well-mixed and there is no position variation of the component within the system.

2.2 Mass flux

Mass flux is the amount of mass crossing a unit cross-sectional area per unit time. The mass flux of a component i is denoted by $\vec{n}_i$.

The mass flux of a component i with respect to the average mass flux of the bulk fluid is denoted by $\vec{j}_i$ (with a small j). It is related to the mass flux of the component $\vec{n}_i$ and the total mass flux due to bulk flow $\vec{n}_T$ by

$$\boldsymbol{\vec{j}_i = \vec{n}_i - w_i\vec{n}_T}$$

Here, w_i is the mass fraction of the component in the bulk fluid.

2.3 Fick's first law

Fick's first law is a constitutive relation between the mass flux of a component and its concentration gradient. In terms of mass fraction, it is given by

$$\boldsymbol{\vec{j}_i = -\rho D_i \vec{\nabla} w_i}$$

Here, ρ is the density of the bulk fluid and D_i is the diffusivity of the component.

2.4 Equation of continuity

For a component i in a multicomponent mixture that is flowing, the equation of continuity is given by

$$\boldsymbol{\frac{\partial \rho_i}{\partial t} + \vec{\nabla} \cdot \vec{n}_i = r_i}$$

Here, ρ_i is the density of the component and r_i is the net rate of generation of the component per unit molar mass.

3. Analysis

3.1 Kinetics of tun formation

Owing to its characteristic barrel shape, we can crudely approximate the hydrated tardigrade to be a cylinder of length L, radius R, and initial volume $V_0 = \pi R^2 L$. We assume that as it loses water, the tardigrade shrinks into a smaller cylinder with a reduced length but with the same radius R. Both these entities are covered by a thin cuticle. The hydrated tardigrade's cuticle has an initial thickness δ_0.

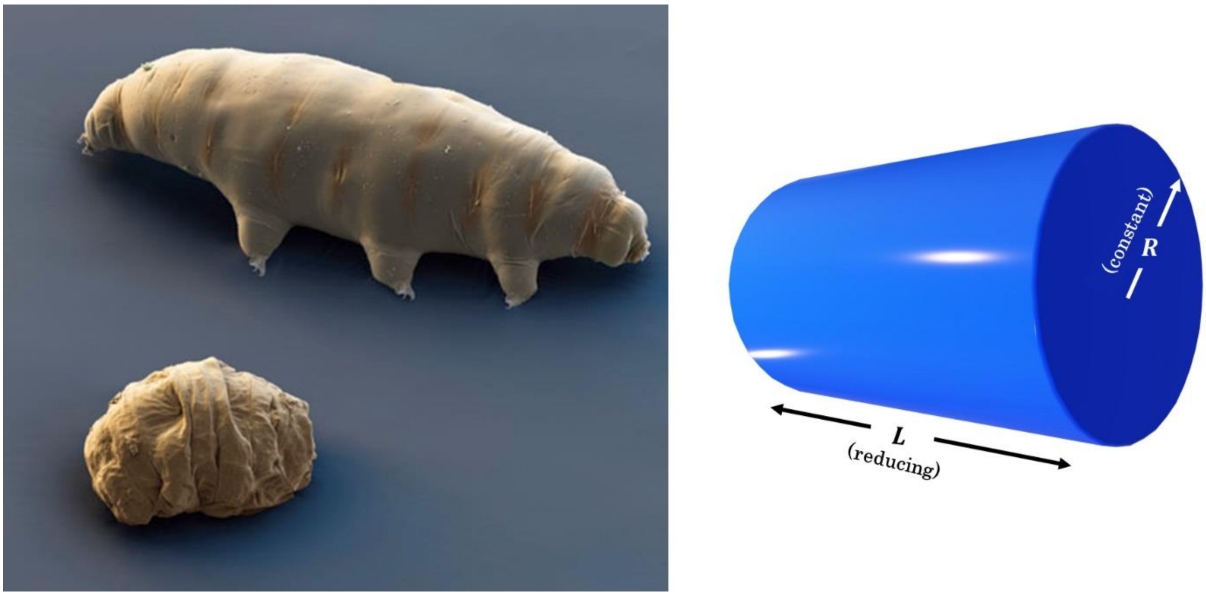

Figure 3: Approximating the shape of a tardigrade and its tun as a cylinder
(Source for the image on the left: American Scientist)

The volume of the tardigrade as it is shrinking is given by $V(t) = \pi R^2 l(t)$. Thus,

$$\frac{V_0}{V(t)} = \frac{L}{l(t)} \qquad \text{Eq. 1}$$

Also, the volume V and curved surface area S of the tardigrade are related by

$$\frac{V(t)}{S(t)} = \frac{\pi R^2 l(t)}{2\pi R l(t)} = \frac{R}{2} \qquad \text{Eq. 2}$$

This implies that the surface area to volume ratio remains a constant.

Assuming that there is no loss of any material that constitutes the cuticle during the transition to the tun state, the total volume of the cuticle must remain constant. Let the thickness at time t be $\delta(t)$. The cuticle is essentially an annular cylinder, and thus its volume can be found by

$$\pi(R + \delta_0)^2 L - \pi R^2 L = \pi\big(R + \delta(t)\big)^2 l(t) - \pi R^2 l(t)$$

$$\Rightarrow \pi\big(R^2 + {\delta_0}^2 + 2R\delta_0 - R^2\big)L = \pi(R^2 + \delta(t)^2 + 2R\delta(t) - R^2)l(t)$$

$$\Rightarrow \big({\delta_0}^2 + 2R\delta_0\big)L = \big(\delta(t)^2 + 2R\delta(t)\big)l(t)$$

As the thickness of the cuticle is likely to be small in comparison to the radius of the tardigrade, we can ignore its quadratic terms. This gives

$$2R\delta_0 L = 2R\delta(t)l(t)$$

Cancelling the common terms and then using Eq. 1 gives

$$\delta(t) = \delta_0 \frac{L}{l(t)} = \delta_0 \frac{V_0}{V(t)} \qquad \text{Eq. 3}$$

We have a relation between the tardigrade's instantaneous volume and the instantaneous thickness of its cuticle.

Now, consider the cuticular membrane through which the tardigrade loses water. The diffusion of water through this will be much faster than the rate of change of the tardigrade's volume or water content. Thus, the diffusion through the cuticle can be approximated to be at **pseudo steady state**.

We will also assume that water only diffuses across the curved surface of the cylinder and not the flat surfaces (which would correspond to the tardigrade's mouth and cloaca).

Let the mass fraction of water inside the tardigrade at a given time be $w_i(t)$ and the mass fraction of water outside be constant at w_o ($w_o < w_i$ during desiccation). The initial mass fraction of water inside the tardigrade is w_{i_0}. The mass fraction of water in the fluid crossing the membrane is w_m.

From the equation of continuity,

$$\cancelto{0 \text{ (steady state)}}{\frac{\partial \rho}{\partial t}} + \vec{\nabla} \cdot \vec{n}_m = \cancelto{0 \text{ (no reaction)}}{r_m}$$

After cancelling the irrelevant terms, we get

$$\vec{\nabla} \cdot \vec{n}_m = 0$$

Using $\vec{n}_m = -\rho D_m \vec{\nabla} w_m + w_m \vec{n}_T$, we get

$$\vec{\nabla} \cdot \left(-\rho D_m \vec{\nabla} w_m + w_m \vec{n}_T\right) = 0$$

Neglecting the bulk flow term as there are no velocities and assuming that the total density ρ of fluid in the membrane is constant and the same as that of the fluid inside the tardigrade, we get

$$\nabla^2 w_m = 0$$

In cylindrical coordinates, this can be written as

$$\frac{1}{r}\frac{\partial}{\partial r}\left(r \frac{\partial w_m}{\partial r}\right) + \frac{1}{r^2}\frac{\partial^2 w_m}{\partial \theta^2} + \frac{\partial^2 w_m}{\partial z^2} = 0$$

As the diffusion is only occurring in the radial direction, this simplifies to

$$\frac{d}{dr}\left(r \frac{d w_m}{dr}\right) = 0$$

As the derivative of $r\frac{dw_m}{dr}$ with respect to r is 0, it implies that $r\frac{dw_m}{dr}$ must be a constant. Thus,

$$r\frac{dw_m}{dr} = A$$

$$dw_m = A\frac{dr}{r}$$

Integrating both sides gives

$$w_m(r) = A\ln r + B$$

The boundary conditions, assuming a partition coefficient of unity, are:

At $r = R$ $\quad w_m(r) = w_i(t)$

At $r = R + \delta(t)$ $\quad w_m(r) = w_o$

Thus, $w_i(t) = A\ln R + B$ and $w_o = A\ln\big(R + \delta(t)\big) + B$

Subtracting the two gives

$$w_o - w_i(t) = A\ln\left(1 + \frac{\delta(t)}{R}\right)$$

$$\Rightarrow A = \frac{w_o - w_i(t)}{\ln\left(1 + \frac{\delta(t)}{R}\right)}$$

This allows us to write

$$\frac{dw_m}{dr} = \frac{A}{r} = \frac{1}{r}\frac{w_o - w_i(t)}{\ln\left(1 + \frac{\delta(t)}{R}\right)}$$

Using $j = -\rho D_m \frac{dw_m}{dr}$, we get

$$j = \frac{\rho D_m}{r}\frac{w_i(t) - w_o}{\ln\left(1 + \frac{\delta(t)}{R}\right)}$$

At the inner surface of the cuticle, i.e., at $r = R$, the mass flux is

$$j = \frac{\rho D_m}{R}\frac{w_i(t) - w_o}{\ln\left(1 + \frac{\delta(t)}{R}\right)} \qquad \text{Eq. 4}$$

Now, we apply a mass balance on the tardigrade's water content,

$$r_i - r_o + r_g - r_c = \frac{dm}{dt}$$

There is no input of water. We will assume that there is no generation or consumption of water within the tardigrade during the formation of the tun state. This is in line with the fact that the tun state is an ametabolic state.

$$-r_o = \frac{dm}{dt}$$

The rate of output of water is the mass flux of water times the surface area of the tardigrade. Using Eq. 4,

$$r_o = jS(t) = \frac{\rho D_m}{R} \frac{w_i(t) - w_o}{\ln\left(1 + \frac{\delta(t)}{R}\right)} S(t)$$

The rate of accumulation of water in the tardigrade is rate of change of mass fraction of water times the total mass ρV.

$$\frac{dm}{dt} = \frac{d}{dt}\left(w_i(t)\rho V(t)\right)$$

Substituting these in the water mass balance, we get

$$-\frac{\rho D_m}{R} \frac{w_i(t) - w_o}{\ln\left(1 + \frac{\delta(t)}{R}\right)} S(t) = \frac{d}{dt}\left(w_i(t)\rho V(t)\right)$$

Let us assume that during the formation of the tun state, the tardigrade somehow manages to keep the total density ρ constant.

Using Eq. 2 to substitute $S(t)$ in terms of $V(t)$ and Eq. 3 to replace $\delta(t)$, we get

$$-\frac{\rho D_m}{R} \frac{w_i(t) - w_o}{\ln\left(1 + \frac{\delta_0 V_0}{R} \frac{1}{V(t)}\right)} \frac{2}{R} V(t) = \rho \frac{d}{dt}\left(w_i(t)V(t)\right)$$

We can cancel ρ on both sides.

$$-\frac{2D_m}{R^2} \frac{V(t)(w_i(t) - w_o)}{\ln\left(1 + \frac{\delta_0 V_0}{R} \frac{1}{V(t)}\right)} = \frac{d}{dt}\left(w_i(t)V(t)\right)$$

Also, by definition of mass fraction,

$$w_i(t) = \frac{\text{Mass of water in tardigrade}}{\text{Total mass}} = \frac{\text{Total mass} - \text{Mass of other components}}{\text{Total mass}}$$

$$w_i(t) = 1 - \frac{m_{\text{other}}}{\rho V(t)}$$

m_{other} is a constant as the tardigrade does not lose these components.

Substituting for $w_i(t)$ in the previous equation,

$$-\frac{2D_m}{R^2}\frac{V(t)\left(1-\frac{m_{\text{other}}}{\rho V(t)}-w_o\right)}{\ln\left(1+\frac{\delta_0 V_0}{R}\frac{1}{V(t)}\right)}=\frac{d}{dt}\left(\left(1-\frac{m_{\text{other}}}{\rho V(t)}\right)V(t)\right)$$

Taking $V(t)$ inside the parentheses on the RHS, multiplying, and then evaluating the derivative,

$$-\frac{2D_m}{R^2}\frac{V(t)\left(1-\frac{m_{\text{other}}}{\rho V(t)}-w_o\right)}{\ln\left(1+\frac{\delta_0 V_0}{R}\frac{1}{V(t)}\right)}=\frac{dV(t)}{dt}$$

We can use the fact that $R^2=\frac{V_0}{\pi L}$ by definition of cylinder volume, $\frac{m_{\text{other}}}{\rho}=w_{\text{other}_0}V_0$ by definition of mass fraction and $\frac{V_0}{R}=\frac{S_0}{2}$ from Eq. 2. This gives

$$-\frac{2D_m\pi L}{V_0}\frac{V(t)\left(1-\frac{w_{\text{other}_0}V_0}{V(t)}-w_o\right)}{\ln\left(1+\frac{\delta_0 S_0}{2}\frac{1}{V(t)}\right)}=\frac{dV(t)}{dt} \qquad \text{Eq. 5}$$

We finally have a differential equation exclusively in terms of $V(t)$.

Now, we have to identify the values of the parameters in the differential equation. w_o is independent of the tardigrade as it describes the amount of water in the tardigrade's surroundings.

Parameter	Description	Value	Reference
D_m	Diffusivity of water through the cuticular membrane	$0.15\times10^{-10}\ \text{m}^2\text{s}^{-1}$	[6]
L	Initial length of the tardigrade	687 μm	[7]
R	Radius of the tardigrade	89 μm	[7]
V_0	Initial volume of the tardigrade	$\pi R^2 L$	
S_0	Initial curved surface area of the tardigrade	$2\pi RL$	
δ_0	Initial thickness of the cuticle	1.915 μm	[8]
w_{other_0}	Initial mass fraction of non-water components in the tardigrade	0.1927	[9]

The value of D_m, taken from [6], is the diffusivity of water through a chitin-chitosan membrane when the external water content is low, as obtained by molecular dynamics simulations. We use this value as the tardigrade cuticle is rich in chitin. There are no experimental measurements for the diffusivity of water through the tardigrade cuticle.

The values of L, R, δ_0, and w_{other_0} are those of the marine / coastal tardigrade *Milnesium tardigradum*. Marine tardigrades are capable of achieving the tun state in the order of seconds [4].

We will track the volume of the tardigrade over the course of 1 minute. To do so, we will solve the differential equation obtained in Eq. 5 numerically using Python's SciPy library. All codes used can be found in the Appendix.

For the sake of plotting a graph, let us assume that the tardigrade is placed in an environment with no water. This means that the external water content w_o is 0.

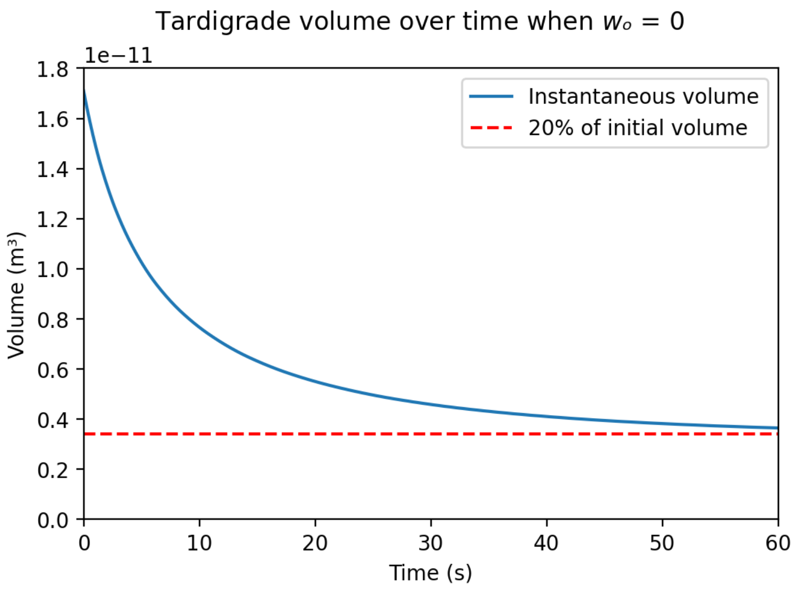

Figure 4: Plot of tardigrade volume $V(t)$ when $w_o = 0$

From the plot, we can see that the volume indeed decreases with time. By the end of 1 minute, the volume has shrunk to 20% of the initial volume and appears to have reached a steady value.

We can also track the water content in the tardigrade by plotting $w_i(t)$. This is related to the volume $V(t)$ by

$$\begin{aligned} w_i(t) &= 1 - \frac{m_{\text{other}}}{\rho V(t)} \\ &= 1 - \frac{w_{\text{other}_0} V_0}{V(t)} \end{aligned}$$

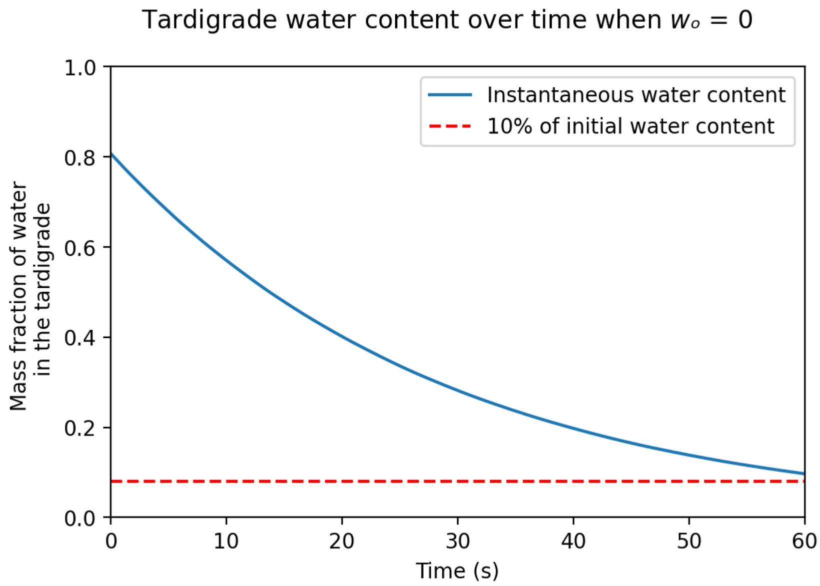

Figure 5: Plot of tardigrade water content $w_i(t)$ when $w_o = 0$

In the same duration of time, the water content of the tardigrade has dropped to 10% of its original value. Thus, the tardigrade is able to enter the tun state very rapidly once subjected to desiccating conditions.

3.2 Permeability slump

From the plot in Figure 4, it is clearly visible that the rate at which the tardigrade shrinks decreases over time. We can plot $\frac{dV(t)}{dt}$ against t to observe this.

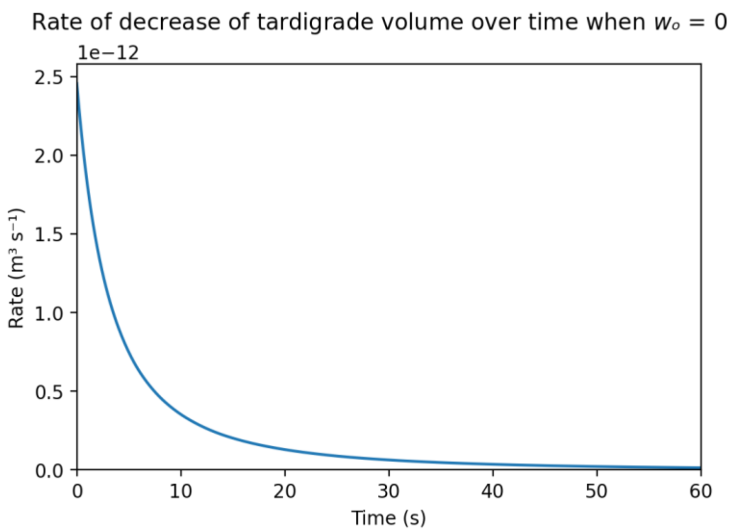

Figure 6: Plot of $\frac{dV(t)}{dt}$ when $w_o = 0$

We can see that the rate of decrease quickly drops to 0 as time proceeds, indicating that a steady volume has been reached.

This is also an indication that the diffusion of water is slowing down, presumably due to the increased thickness of the cuticle and the decreased difference in water content between the interior of the tardigrade and its environment.

This phenomenon, first observed in [10], is known as the **permeability slump**.

We define the cuticle's permeability P based on Eq. 4.

$$j = \frac{\rho D_m}{R} \frac{w_i(t) - w_o}{\ln\left(1 + \frac{\delta(t)}{R}\right)} = P(w_i(t) - w_o)$$

$$P(t) = \frac{\rho D_m}{R \ln\left(1 + \frac{\delta(t)}{R}\right)} = \frac{\left(\frac{\text{Total mass}}{V_0}\right) D_m}{R \ln\left(1 + \frac{\delta(t)}{R}\right)}$$

Using Eq. 3,

$$P(t) = \frac{\left(\frac{\text{Total mass}}{V_0}\right) D_m}{R \ln\left(1 + \frac{\delta_0 V_0}{R} \frac{1}{V(t)}\right)} \qquad \text{Eq. 6}$$

From [9], we can find that the total mass of *M. tardigradum* is 6.54 μg.

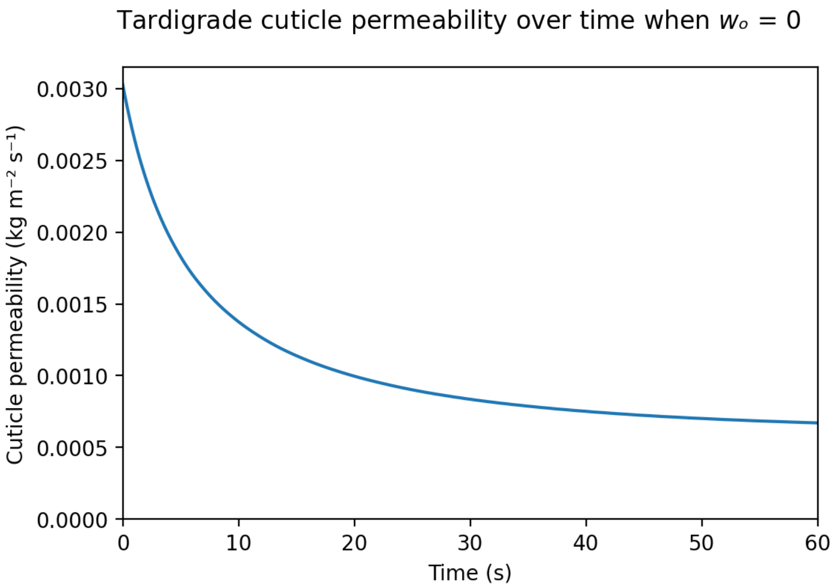

Figure 7: Plot of $P(t)$ when $w_o = 0$

We can see that the permeability of the cuticle gradually decreases over time and reaches a relatively steady value.

3.3 Effect of external water content

The volume lost by the tardigrade while entering the tun state will vary depending on how severe the dehydration stress is. To study this, we can modify the external water content w_o and track the tardigrade's volume over time.

3.3.1 Constant external water content

We can compare the tardigrade volume over time at different values of w_o, i.e., at different intensities of desiccation stress. These are kept constant over the duration of the study.

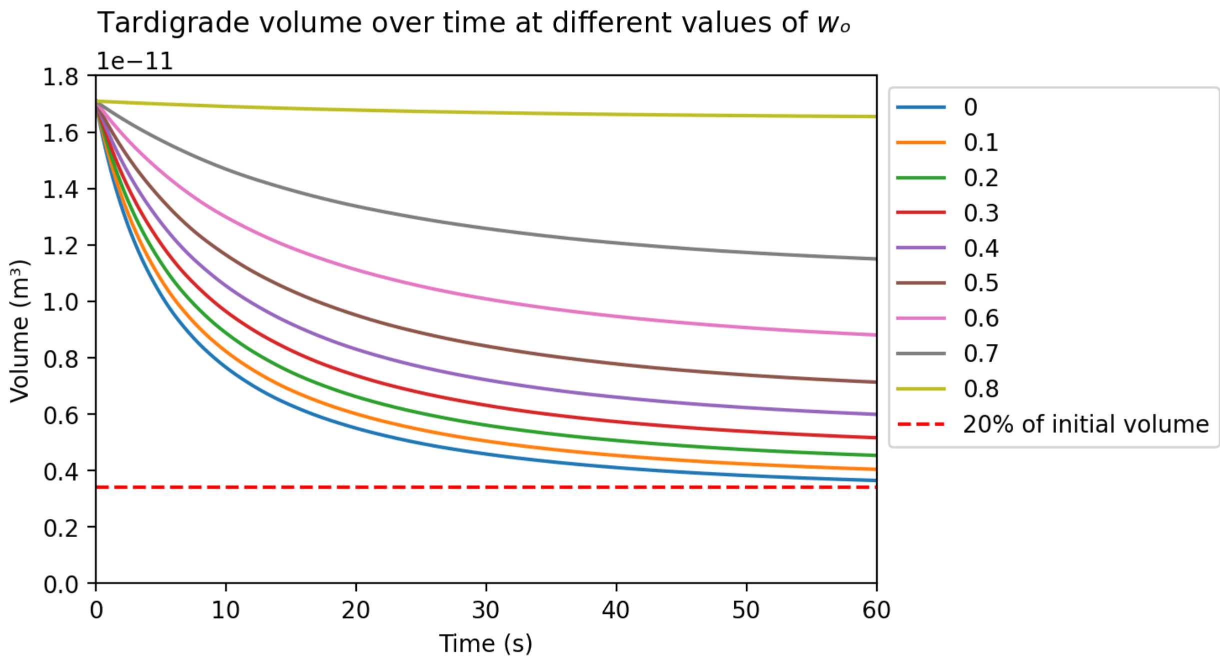

Figure 8: Plot of tardigrade volume $V(t)$ at different values of w_o

We can see that the volume decrease is much greater when the external water content is low. In fact, when the external water content is close to that of the tardigrade's internal water content, the volume barely changes.

Thus, the tardigrade changes its volume strictly in accordance with the ambient conditions.

3.3.2 Oscillating external water content

In the natural environment of the tardigrade, the external water content is unlikely to remain constant and will vary with time. In the coastal environment that *M. tardigradum* is present in, the tides and the ambient temperature will highly influence the local moisture content. Inspired by the experimental data in [11], we can crudely model the amount of water in the external environment as

$$w_o(t) = 0.125 \sin\left(\frac{\pi}{12} \times \frac{t}{3600}\right) + 0.325$$

Here, t is in seconds. This shows that the moisture content varies between 0.2 and 0.45 with a period of 24 hours, i.e., there is a **diurnal variation**.

The initial moisture content is $w_o = 0.325$. At this value of w_o, the steady volume of the tardigrade can be found to be $0.2855V_0$ by solving Eq. 5 numerically.

Starting from this volume, the tardigrade is subjected to the diurnally oscillating external water content. Its volume is tracked over a period of 48 hours.

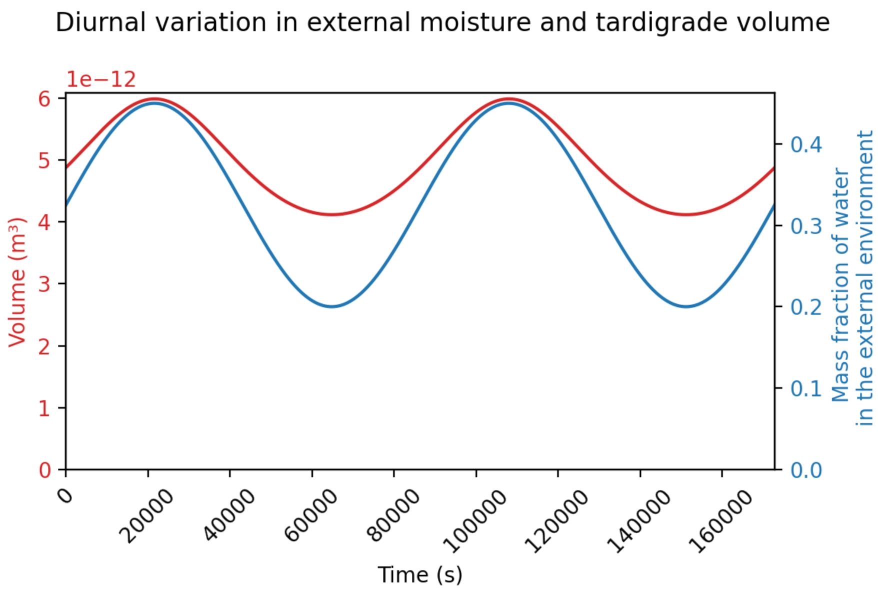

Figure 9: Diurnal variation in external moisture and tardigrade volume

We can see that the tardigrade volume also oscillates in phase with the moisture oscillation. However, the intensity of the volume oscillation is not as much compared to the moisture oscillation.

This oscillation in the tardigrade's volume is likely a mathematical consequence of the differential equation developed earlier and the oscillatory nature of the external moisture being considered. There are no experimental documentations of this behaviour. It would be interesting if such an observation was actually made.

3.3.3 Decaying external water content

While studying tun formation in tardigrades in a lab setting, scientists gradually decrease the external water content over the course of a day. If water is removed rapidly, the tardigrades may die. This has been highlighted by Dr. Sandeep Eswarappa from the Indian Institute of Science (IISc) while discussing tardigrades on the Joyful Microbe podcast [12]. To model this gradual decrease in external water content, we can describe w_o by

$$w_o(t) = 0.7 \exp\left(-\frac{t}{8 \times 3600}\right)$$

Here, t is in seconds. At $t = 0$, $w_o = 0.7$. w_o then gradually decreases to a value close to 0 after 24 hours.

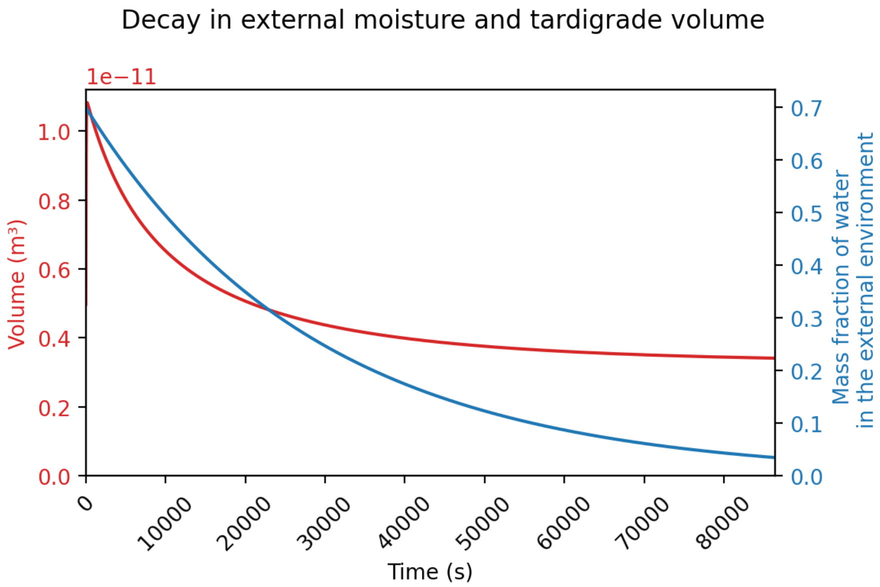

Figure 10: Decay in external moisture and tardigrade volume over 24 hours

We can see that the decay in volume is not as drastic as the decay in the mass fraction of water in the external environment. This enables the tardigrade to gradually enter the tun state without dying.

3.4 Effect of tardigrade dimensions

The dimensions of the tardigrade itself could impact the kinetics of tun formation. To visualise this, we can plot the fractional reduction in volume $\frac{V(t)}{V_0}$ for tardigrades of different dimensions when placed in an environment with $w_o = 0$ for 1 minute.

We can use the range of L and R of different tardigrade species given in [7] to create our plot.

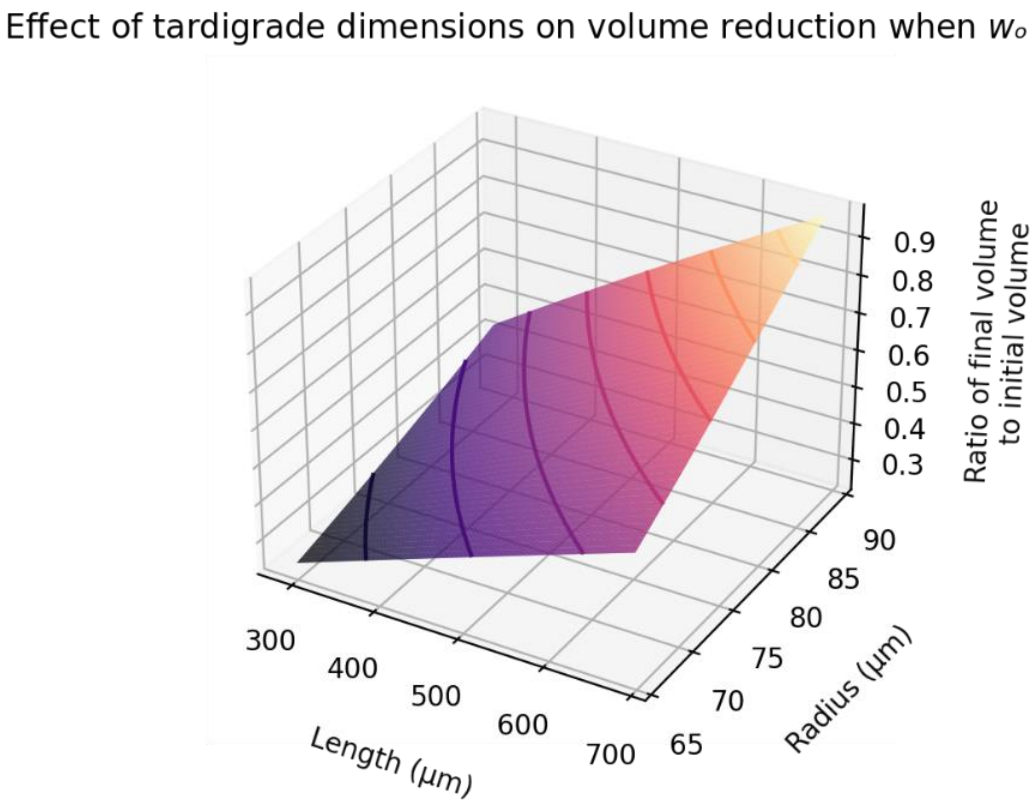

Figure 11: Effect of tardigrade dimensions on volume reduction when $w_o = 0$

This plot shows that tardigrades with larger dimensions do not reduce their volumes as much as those with smaller dimensions. Smaller tardigrades have a higher surface area to volume ratio and can thus lose water more rapidly than larger tardigrades that have a smaller ratio. This has also been noted in [13].

In this, we have assumed that the initial cuticle thickness and water content is the same for tardigrades of all dimensions. In reality, this may not be true.

3.5 Survival in the tun state

As mentioned earlier, the tardigrade can survive other abiotic stresses while in the tun state. Along with internal molecular mechanisms, the cuticle itself protects the tardigrade by acting as a barrier [13].

We can visualise this by using Eq. 3 to plot the variation of the cuticle thickness δ with time.

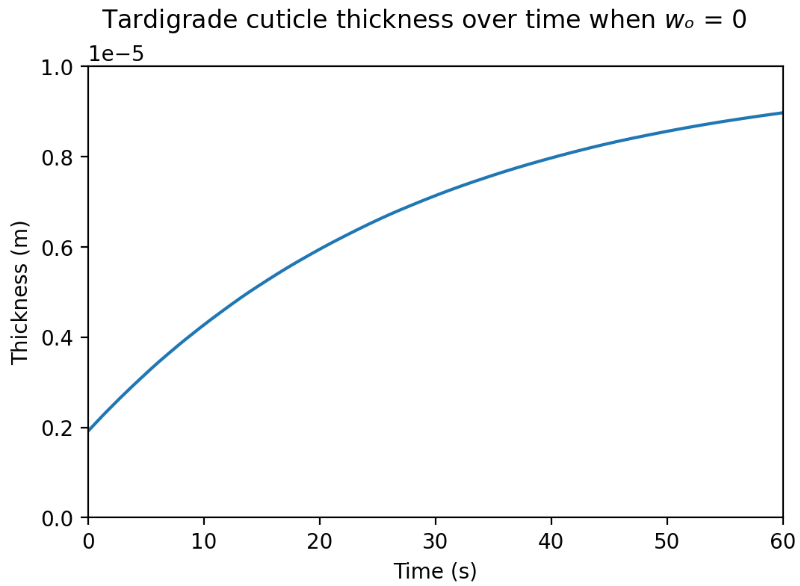

Figure 12: Plot of cuticle thickness $\delta(t)$ when $w_o = 0$

As the tardigrade shrinks, the cuticle's thickness gradually increases and reaches a relatively steady value. This increased thickness contributes to the tardigrade's survival while in the tun state. It could also prevent further loss of water from the tardigrade.

4. Conclusions

We find that the tardigrade can rapidly enter the tun state by shrinking its volume when exposed to desiccating conditions. It also loses most of its water content in the process.

The rate at which the volume reduction happens gradually decreases with time. This is because the cuticle becomes thicker and less permeable to water. This phenomenon is referred to as the permeability slump.

Tardigrades lose water more rapidly when the desiccation stress is more severe. The change in volume is negligible when the external water content is similar to the water content within the tardigrade.

When the external water content varies with time, the tardigrade suitable adapts its volume in sync with the ambient conditions.

Tardigrades with a smaller surface area to volume ratio can lose water and shrink faster than those with a larger ratio. Thus, they have a better chance of survival.

The increased thickness of the cuticle in the tun state could potentially help the tardigrade tide over other abiotic stresses it may experience by acting as a barrier.

5. Future Work

We have modelled the tardigrade as a homogenous cylinder. This is however a very coarse approximation. We will have to consider a more sophisticated geometry to get better results. The volume contributed by the tardigrade's eight legs is also significant and will have to be accounted for.

We have also assumed that the radius of the tardigrade remains constant during tun formation, but this might not actually be the case. A time-varying length and radius would make for a more complicated analysis.

The density of the fluid inside the tardigrade is unlikely to remain constant during tun formation. The volume reduction is not just due to the loss of water but also due to the contraction of the tardigrade's muscles [4]. This contraction could potentially alter the density.

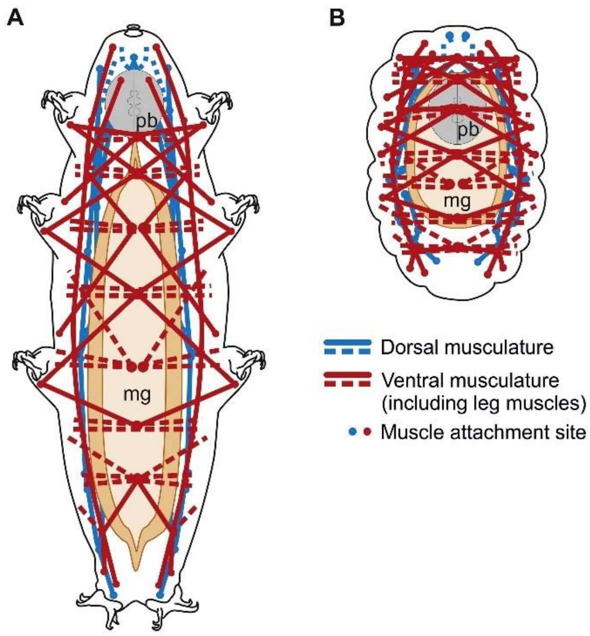

Figure 13: Contraction of the tardigrade's muscles during tun formation
(Source: Møbjerg and Neves 2021)

An experimentally determined value for the diffusivity of water through the tardigrade cuticle would improve the accuracy of the model developed. Accounting for the role of osmotic pressure on tun formation is also needed.

It is known that during the formation of the tun, numerous tardigrade-specific **intrinsically disordered proteins** (IDPs) form a vitrified "glass" as water is being lost. This prevents other proteins from being denatured permanently and offers structural integrity to the tardigrade's cells [14]. Including the role of these proteins, as well as others like the **DNA damage suppressor protein** (Dsup), in our model for tun formation would be an interesting extension.

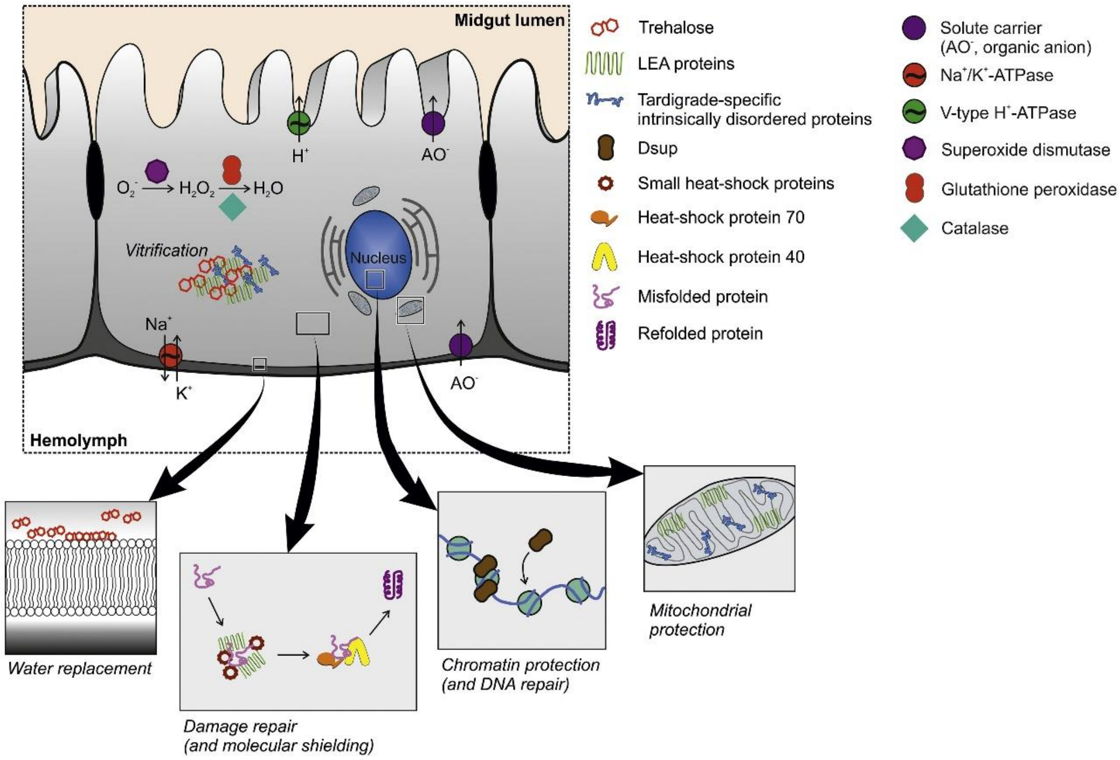

Figure 14: Molecular mechanisms employed by the tardigrade for survival under abiotic stresses (Source: Møbjerg and Neves 2021)

Acknowledgement

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Appendix

Code to plot Figure 4

```
import numpy as np
import scipy.integrate as sp
import matplotlib.pyplot as plt


def volume(V,t,w_o):

    pi = np.pi

    D_m = 0.15e-10
    L = 687e-6
    R = (178e-6)/2
    V_0 = pi*R*R*L
    S_0 = 2*pi*R*L
    delta_0 = 1.915e-6
    w_other_0 = 0.1927

    c1 = 2*D_m*pi*L/V_0
    c2 = w_other_0*V_0
    c3 = (delta_0*S_0)/2

    dVdt = -(c1*V*(1-(c2/V)-w_o))/(np.log(1+(c3/V)))

    return dVdt


V_0 = np.pi*(178e-6/2)*(178e-6/2)*(687e-6)
t = np.linspace(0, 60, 600)
w_o = 0
vol = sp.odeint(volume, V_0, t, args = (w_o,))

plt.figure(dpi=200)
plt.plot(t,vol,label="Instantaneous volume")
plt.axhline(V_0*0.2,c="r",ls="--",label="20% of initial volume")
plt.xlim(0,60)
plt.ylim(0,1.8e-11)
plt.xlabel("Time (s)")
plt.ylabel("Volume (m\u00b3)")
plt.suptitle("Tardigrade volume over time when $\it{w\u2092}$ = 0")
plt.legend()
plt.show()
```

Code to plot Figure 5

```
import numpy as np
import scipy.integrate as sp
import matplotlib.pyplot as plt


def volume(V,t,w_o):

    pi = np.pi

    D_m = 0.15e-10
    L = 687e-6
    R = (178e-6)/2
```

```
    V_0 = pi*R*R*L
    S_0 = 2*pi*R*L
    delta_0 = 1.915e-6
    w_other_0 = 0.1927

    c1 = 2*D_m*pi*L/V_0
    c2 = w_other_0*V_0
    c3 = (delta_0*S_0)/2

    dVdt = -(c1*V*(1-(c2/V)-w_o))/(np.log(1+(c3/V)))

    return dVdt


V_0 = np.pi*(178e-6/2)*(178e-6/2)*(687e-6)
t = np.linspace(0, 60, 600)
w_o = 0
vol = sp.odeint(volume, V_0, t, args = (w_o,))

water = [1-((0.1927*V_0)/V) for V in vol]

plt.figure(dpi=200)
plt.plot(t,water,label="Instantaneous water content")
plt.axhline(water[0]*0.1,c="r",ls="--",label="10% of initial water content")
plt.xlim(0,60)
plt.ylim(0,1)
plt.xlabel("Time (s)")
plt.ylabel("Mass fraction of water \n in the tardigrade")
plt.suptitle("Tardigrade water content over time when $\it{w\u2092}$ = 0")
plt.legend()
plt.show()
```

Code to plot Figure 6

```
import numpy as np
import scipy.integrate as sp
import matplotlib.pyplot as plt


def volume(V,t,w_o):

    pi = np.pi

    D_m = 0.15e-10
    L = 687e-6
    R = (178e-6)/2
    V_0 = pi*R*R*L
    S_0 = 2*pi*R*L
    delta_0 = 1.915e-6
    w_other_0 = 0.1927

    c1 = 2*D_m*pi*L/V_0
    c2 = w_other_0*V_0
    c3 = (delta_0*S_0)/2

    dVdt = -(c1*V*(1-(c2/V)-w_o))/(np.log(1+(c3/V)))

    return dVdt


V_0 = np.pi*(178e-6/2)*(178e-6/2)*(687e-6)
t = np.linspace(0, 60, 600)
```

```
w_o = 0
vol = sp.odeint(volume, V_0, t, args = (w_o,))

rate = [abs(volume(V,0,0)) for V in vol]

plt.figure(dpi=200)
plt.plot(t,rate)
plt.xlim(0,60)
plt.ylim(0)
plt.xlabel("Time (s)")
plt.ylabel("Rate (m\u00b3 s\u207b\u00b9)")
plt.suptitle("Rate of decrease of tardigrade volume over time when $\it{w\u2092}$ = 
0")
plt.show()
```

Code to plot Figure 7

```
import numpy as np
import scipy.integrate as sp
import matplotlib.pyplot as plt


def volume(V,t,w_o):

    pi = np.pi

    D_m = 0.15e-10
    L = 687e-6
    R = (178e-6)/2
    V_0 = pi*R*R*L
    S_0 = 2*pi*R*L
    delta_0 = 1.915e-6
    w_other_0 = 0.1927

    c1 = 2*D_m*pi*L/V_0
    c2 = w_other_0*V_0
    c3 = (delta_0*S_0)/2

    dVdt = -(c1*V*(1-(c2/V)-w_o))/(np.log(1+(c3/V)))

    return dVdt


def permeability(V):

    tot_mass = 6.54e-9
    V_0 = np.pi*(178e-6/2)*(178e-6/2)*(687e-6)
    D_m = 0.15e-10
    R = (178e-6)/2
    delta_0 = 1.915e-6
    rho = tot_mass/V_0

    c = (delta_0*V_0)/R

    return (rho*D_m)/(R*np.log(1+c/V))



V_0 = np.pi*(178e-6/2)*(178e-6/2)*(687e-6)
t = np.linspace(0, 60, 600)
w_o = 0
vol = sp.odeint(volume, V_0, t, args = (w_o,))
```

```python

P = [permeability(V) for V in vol]

plt.figure(dpi=200)
plt.plot(t,P)
plt.xlim(0,60)
plt.ylim(0)
plt.xlabel("Time (s)")
plt.ylabel("Cuticle permeability (kg m\u207b\u00b2 s\u207b\u00b9)")
plt.suptitle("Tardigrade cuticle permeability over time when $\it{w\u2092}$ = 0")
plt.show()
```

Code to plot Figure 8

```python
import numpy as np
import scipy.integrate as sp
import matplotlib.pyplot as plt


def volume(V,t,w_o):

    pi = np.pi

    D_m = 0.15e-10
    L = 687e-6
    R = (178e-6)/2
    V_0 = pi*R*R*L
    S_0 = 2*pi*R*L
    delta_0 = 1.915e-6
    w_other_0 = 0.1927

    c1 = 2*D_m*pi*L/V_0
    c2 = w_other_0*V_0
    c3 = (delta_0*S_0)/2

    dVdt = -(c1*V*(1-(c2/V)-w_o))/(np.log(1+(c3/V)))

    return dVdt

plt.figure(dpi=200)
for w_o in [0,0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8]:
    V_0 = np.pi*(178e-6/2)*(178e-6/2)*(687e-6)
    t = np.linspace(0, 60, 600)
    vol = sp.odeint(volume, V_0, t, args = (w_o,))
    plt.plot(t,vol,label=w_o)

plt.axhline(V_0*0.2,c="r",ls="--",label="20% of initial volume")
plt.xlim(0,60)
plt.ylim(0,1.8e-11)
plt.xlabel("Time (s)")
plt.ylabel("Volume (m\u00b3)")
plt.suptitle("Tardigrade volume over time at different values of $\it{w\u2092}$")
plt.legend(bbox_to_anchor=(1, 1))
plt.show()
```

Code to plot Figure 9

```python
import numpy as np
import scipy.integrate as sp
```

```
import matplotlib.pyplot as plt


def volume(V,t,w_o):

    pi = np.pi

    D_m = 0.15e-10
    L = 687e-6
    R = (178e-6)/2
    V_0 = pi*R*R*L
    S_0 = 2*pi*R*L
    delta_0 = 1.915e-6
    w_other_0 = 0.1927

    c1 = 2*D_m*pi*L/V_0
    c2 = w_other_0*V_0
    c3 = (delta_0*S_0)/2

    dVdt = -(c1*V*(1-(c2/V)-w_o))/(np.log(1+(c3/V)))

    return dVdt


def soil_moisture(t):

    T = t/3600

    return 0.125*np.sin(np.pi*T/12)+0.325


V_0 = 0.2855*np.pi*(178e-6/2)*(178e-6/2)*(687e-6)
moisture = [soil_moisture(t) for t in range(3600*48)]
volumes = []

for time in range(3600*48):

    w_o = moisture[time]
    t = np.linspace(time,time+1,2)
    vol = sp.odeint(volume, V_0, t, args = (w_o,))
    volumes.append(vol[1][0])
    V_0 = vol[1][0]

fig, ax1 = plt.subplots(dpi=200)

colour = "tab:red"
ax1.set_xlabel("Time (s)")
ax1.set_ylabel("Volume (m\u00b3)", color=colour)
ax1.plot(range(3600*48), volumes, color=colour)
ax1.tick_params(axis="y", labelcolor=colour)
plt.xlim(0,3600*48)
plt.ylim(0)
plt.xticks(rotation=45)

ax2 = ax1.twinx()
colour = "tab:blue"
ax2.set_ylabel("Mass fraction of water \n in the external environment", color=colour)
ax2.plot(range(3600*48), moisture, color=colour)
ax2.tick_params(axis="y", labelcolor=colour)
plt.xlim(0,3600*48)
plt.ylim(0)

plt.suptitle("Diurnal variation in external moisture and tardigrade volume")
fig.tight_layout()
plt.show()
```

Code to plot Figure 10

```
import numpy as np
import scipy.integrate as sp
import matplotlib.pyplot as plt


def volume(V,t,w_o):

    pi = np.pi

    D_m = 0.15e-10
    L = 687e-6
    R = (178e-6)/2
    V_0 = pi*R*R*L
    S_0 = 2*pi*R*L
    delta_0 = 1.915e-6
    w_other_0 = 0.1927

    c1 = 2*D_m*pi*L/V_0
    c2 = w_other_0*V_0
    c3 = (delta_0*S_0)/2

    dVdt = -(c1*V*(1-(c2/V)-w_o))/(np.log(1+(c3/V)))

    return dVdt


def external_moisture(t):

    T = t/3600

    return 0.7*np.exp(-T/8)


V_0 = 0.2855*np.pi*(178e-6/2)*(178e-6/2)*(687e-6)
moisture = [external_moisture(t) for t in range(3600*24)]
volumes = []

for time in range(3600*24):

    w_o = moisture[time]
    t = np.linspace(time,time+1,2)
    vol = sp.odeint(volume, V_0, t, args = (w_o,))
    volumes.append(vol[1][0])
    V_0 = vol[1][0]

fig, ax1 = plt.subplots(dpi=200)

colour = "tab:red"
ax1.set_xlabel("Time (s)")
ax1.set_ylabel("Volume (m\u00b3)", color=colour)
ax1.plot(range(3600*24), volumes, color=colour)
ax1.tick_params(axis="y", labelcolor=colour)
plt.xlim(0,3600*24)
plt.ylim(0)
plt.xticks(rotation=45)

ax2 = ax1.twinx()
colour = "tab:blue"
ax2.set_ylabel("Mass fraction of water \n in the external environment", color=colour)
ax2.plot(range(3600*24), moisture, color=colour)
ax2.tick_params(axis="y", labelcolor=colour)
plt.xlim(0,3600*24)
plt.ylim(0)
```

```
plt.suptitle("Decay in external moisture and tardigrade volume")

fig.tight_layout()
plt.show()
```

Code to plot Figure 11

```
import numpy as np
import scipy.integrate as sp
import matplotlib.pyplot as plt


def volume(V,t,w_o,L,R):

    pi = np.pi

    D_m = 0.15e-10
    V_0 = pi*R*R*L
    S_0 = 2*pi*R*L
    delta_0 = 1.915e-6
    w_other_0 = 0.1927

    c1 = 2*D_m*pi*L/V_0
    c2 = w_other_0*V_0
    c3 = (delta_0*S_0)/2

    dVdt = -(c1*V*(1-(c2/V)-w_o))/(np.log(1+(c3/V)))

    return dVdt


volume = np.vectorize(volume)


def final_volume(L,R):

    w_o = 0
    t = np.linspace(0, 60, 600)
    vol = sp.odeint(volume, V_0, t, args = (w_o,L,R))

    return vol[-1][0]/vol[0][0]


final_volume = np.vectorize(final_volume)

x = np.linspace(287,687,687-287+1)*1e-6
y = (np.linspace(132,178,178-132+1)/2)*1e-6
X, Y = np.meshgrid(x, y)
Z = final_volume(X,Y)

fig = plt.figure(dpi=200)
ax = plt.axes(projection="3d")
ax.plot_surface(X*1e6, Y*1e6, Z, rstride=1, cstride=1,cmap="magma",edgecolor="none")
ax.contour3D(X*1e6, Y*1e6, Z,cmap="magma")
ax.set_xlabel("\n\nLength (µm)")
ax.set_ylabel("\n\nRadius (µm)")
ax.set_zlabel("\n\nRatio of final volume \n to initial volume")
plt.title("Effect of tardigrade dimensions on volume reduction when $\it{w\u2092}$
= 0")
fig.tight_layout()
plt.show()
```

Code to plot Figure 12

```python
import numpy as np
import scipy.integrate as sp
import matplotlib.pyplot as plt


def volume(V,t,w_o):

    pi = np.pi

    D_m = 0.15e-10
    L = 687e-6
    R = (178e-6)/2
    V_0 = pi*R*R*L
    S_0 = 2*pi*R*L
    delta_0 = 1.915e-6
    w_other_0 = 0.1927

    c1 = 2*D_m*pi*L/V_0
    c2 = w_other_0*V_0
    c3 = (delta_0*S_0)/2

    dVdt = -(c1*V*(1-(c2/V)-w_o))/(np.log(1+(c3/V)))

    return dVdt


V_0 = np.pi*(178e-6/2)*(178e-6/2)*(687e-6)
t = np.linspace(0, 60, 600)
w_o = 0
vol = sp.odeint(volume, V_0, t, args = (w_o,))

delta = [(1.915e-6*V_0)/V[0] for V in vol]

plt.figure(dpi=200)
plt.plot(t,delta)
plt.xlim(0,60)
plt.ylim(0,10e-6)
plt.xlabel("Time (s)")
plt.ylabel("Thickness (m)")
plt.suptitle("Tardigrade cuticle thickness over time when $\it{w\u2092}$ = 0")
plt.show()
```