

1 Model Derivations

2 Notation

3 The following table includes physical definitions of all parameters and variables used in the models presented:

Variable Name	Description
TOF	Time of flight
EXT	Optical extinction
$norm.EXT$	Normalized optical extinction (EXT/TOF)
L	Animal length along the long axis (μm)
W	Animal width (μm)
L_0	Animal length along the long axis (μm) at the start of a larval stage
W_0	Animal width (μm) at the start of a larval stage
V	Animal volume (μm^3)
a_1, a_2, b_1, b_2	Conversion parameters from COPAS BIOSORT measurements to length and width
$\varepsilon_L, \varepsilon_{Circ}$	Normalized Stretch along length and circumferential directions
E_L, E_C	Young's Modulus in the length and circumferential directions (psi)
ν_{cl}, ν_{lc}	Poisson's ratios (unitless)
σ_L, σ_{Circ}	Normalized force or "strain" in the length and circumferential directions (psi)
Δp	Internal pressure due to growth (psi)
d	Thickness of cuticle (μm)
S	Ratio of ΔW : ΔL (unitless)

5 Bootstrapping algorithm

6 To calculate a robust regression of the measured COPAS BIOSORT data we bootstrap the regression using
7 case-resampling (Davison and Hinkley 1997, pages 261-266). Each iteration of the algorithm (**Algorithm**
8 **S1**) involves resampling the data of interest with replacement and maintaining the size of the sample. At
9 each iteration the lokern regression is calculated for Red, Length, and Width data. Additionally any desired
10 function (for example, volume, pumping frequency times pharynx fraction, or derivatives) of these regressions

11 is calculated at each iteration. Regressions at each iteration are saved and the mean and variance of these
 12 regressions at each regression time point are used to determine the statistics of the regression.

Algorithm S1 Regression Bootstrapping with Case Resampling

```

Ndata = # animals in sample;
iterations = # resamplings;

for  $i = 0$  to iterations do
  Resample Ndata points with replacement. Collect Red, Length, Width data;

  Apply lokern regression to resampled Red, Length, Width;

  Calculate desired functions of Red, Length, Width regressions;

  Save Red, Length, Width, and combined regressions;
end for

Calculate Standard deviation at each regression time point of saved regressions;
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13 **Transformation of sorter measurements to volume units**

14 To utilize sorter measurements and convert them to meaningful units we define a linear transformation from
 15 the correlation plots (Figure S2)

$$L = a_1 TOF + b_1 \tag{1}$$

$$W = a_2 norm.EXT + b_2. \tag{2}$$

16 Using Equations (1) and (2) we can approximate the volume of any object that passes through the sorter
 17 by the expression

$$V = \frac{\pi}{4} (a_1 TOF + b_1) (a_2 norm.EXT + b_2)^2. \tag{3}$$

18 **Derivation of stretcher model**

19 We model the cuticle as a thin walled pressure vessel made of orthotropic, linear materials to capture the
 20 relationship between how much the cuticle stretches and how much force is applied to the cuticle. The
 21 relationship between the amount of stretch and the amount of applied pressure is described by the matrix:

$$\begin{bmatrix} \varepsilon_L \\ \varepsilon_{Circ} \end{bmatrix} = \begin{bmatrix} \frac{1}{E_L} & \frac{-v_{cl}}{E_c} \\ \frac{-v_{lc}}{E_L} & \frac{1}{E_c} \end{bmatrix} \begin{bmatrix} \sigma_L \\ \sigma_{Circ} \end{bmatrix} \quad (4)$$

Here E_L and E_C are the Young's modulus in the length and circumferential direction, and v_{cl} and v_{lc} are the appropriate Poisson's ratios. These material properties can be measured experimentally. Normalized stretch, ε , is defined as the change in size normalized by the initial size of a cuticle in length (L) and circumference (Circ) (Equations 5 - 6) . Here σ is the normalized force applied along the length and circumferential directions of the cuticle (Equations 8 - 9).

$$\varepsilon_L = \frac{\Delta L}{L_0} \quad (5)$$

$$\varepsilon_{Circ} = \frac{\Delta C_{irc}}{C_{irc0}} \quad (6)$$

Here L_0 and C_{irc0} are the length and circumference of the cuticle at the onset of stretch, or in other words at the start of a larval stage. Experimentally we measure width, not circumference, but the circumference of a circle is proportional to the width of the circle, so we can replace the circumference with the measured width:

$$\frac{\Delta C_{irc}}{C_{irc0}} = \frac{\pi \Delta W}{\pi W_0} = \frac{\Delta W}{W_0} \quad (7)$$

By approximating *C. elegans* as cylindrical, and assuming the only force working on the cuticle is isotropic internal pressure, we can determine the normalized force in terms of pressure and geometric properties:

$$\sigma_L = \frac{W}{4d} \Delta p \quad (8)$$

$$\sigma_{Circ} = \frac{W}{2d} \Delta p \quad (9)$$

Where W is the diameter or "width" of the cylinder and d is the thickness of the cuticle. We can now rewrite Equation (4) in terms of measurable quantities (length and width) by multiplying out the matrices making the appropriate substitutions.

$$\Delta L = \frac{L_0 W}{2d} \left(\frac{1}{2E_L} - \frac{v_{cl}}{E_c} \right) \Delta p = a_L \Delta p \quad (10)$$

$$\Delta W = \frac{W_0 W}{2d} \left(\frac{1}{E_C} - \frac{v_{lc}}{E_L} \right) \Delta p = a_W \Delta p \quad (11)$$

Here we compress the coefficients fixed by material and geometric properties into one constant

$$a_L = \frac{L_0 W}{2d} \left(\frac{1}{2E_L} - \frac{v_{cl}}{E_c} \right) \quad (12)$$

$$a_W = \frac{W_0 W}{2d} \left(\frac{1}{E_c} - \frac{v_{lc}}{E_L} \right) \quad (13)$$

When calculating the ratio stretch in the length and width directions, the common W term in the coefficients will cancel.

Stretcher slope calculations

The Stretcher model predicts a constant ratio between stretch in width, ΔW , and stretch in length, ΔL . This ratio will be defined as $S \equiv \frac{\Delta W}{\Delta L}$. These measures of stretch are changes in the length and width measurements over some period of time. To see how the ratio of $\frac{\Delta W}{\Delta L}$ changes throughout a larval stage, we need to measure the stretch instantaneously. To do this we take advantage of the derivative approximation

$$\Delta W \approx \frac{dW}{dt} \Delta t \quad (14)$$

$$\Delta L \approx \frac{dL}{dt} \Delta t \quad (15)$$

Equations (14, 15) are combined in the ratio found in Equation (3) to give:

$$S = \frac{\Delta W}{\Delta L} \approx \frac{W'(t)}{L'(t)} \quad (16)$$

Here $W' = \frac{dW}{dt}$ and $L' = \frac{dL}{dt}$. We differentiate the time series from the local regressions of length and width data. The ratio of derivatives gives the instantaneous stretch ratio as it changes over time. Due to the numerical difficulty of calculating both derivatives and ratios with accuracy, we expect large error bars for this ratio. To estimate the size of error bars we apply a first order error propagation formula to Equation (16).

$$\sigma_S^2 \approx \left| \frac{\partial S}{\partial(W')} \right|^2 \sigma_{(W')}^2 + \left| \frac{\partial S}{\partial(L')} \right|^2 \sigma_{(L')}^2 + 2 \frac{\partial S}{\partial(W')} \frac{\partial S}{\partial(L')} \sigma_{(W')(L')} \quad (17)$$

$$\sigma_S^2 \approx \left| \frac{1}{L'} \right|^2 \sigma_{(W')}^2 + \left| \frac{-(W')^2}{(L')^3} \right|^2 \sigma_{(L')}^2 - 2 \frac{W'}{(L')^3} \sigma_{(W')(L')} \quad (18)$$

49 The variance in Equation (18) is calculated for each time point in the regressions of length and width.
 50 We resample the data for each larval stage 2,000 times with replacement (see Methods for larval stage
 51 determination). For each of these resampled sets of data, we numerically differentiate the length and width
 52 regression to determine an estimate of the length and width derivatives over time. At each time point the
 53 mean regression length and width derivatives are used in place of W' and L' . The covariance matrix for the
 54 length and width derivatives is calculated at each time point using the 2,000 resampled regressions in the
 55 bootstrap analysis and used as the variance terms in Equation (18).