Model Derivations

Bootstrapping Algorithm

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

Transformation of sorter measurements to volume units

To utilize sorter measurements and convert them to meaningful units we define a linear transformation from the correlation plots (S1 Fig)

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

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

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

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

Derivation of Stretcher Model

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

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

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**).

$$\varepsilon_L = \frac{\Delta L}{L_0} \tag{5}$$

$$\varepsilon_{Circ} = \frac{\Delta Circ}{Circ_0} \tag{6}$$

Here L_0 and $Circ_0$ 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:

$$\frac{\Delta Circ}{Circ_0} = \frac{\pi \Delta W}{\pi W_0} = \frac{\Delta W}{W_0} \tag{7}$$

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:

$$\sigma_L = \frac{r}{2t}\Delta p \tag{8}$$

$$\sigma_{Circ} = \frac{r}{t}\Delta p \tag{9}$$

Where r is the radius of the cylinder and t 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.

$$\Delta L = \frac{L_0 r}{t}\left(\frac{1}{2E_L} - \frac{v_{cl}}{E_c}\right)\Delta p = a_L \Delta p \tag{10}$$

$$\Delta W = \frac{W_0 r}{t}\left(\frac{1}{E_C} - \frac{v_{lc}}{E_L}\right)\Delta p = a_W \Delta p \tag{11}$$

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

$$a_L = \frac{L_0 r}{t}\left(\frac{1}{2E_L} - \frac{v_{cl}}{E_c}\right) \tag{12}$$

$$a_W = \frac{W_0 r}{t}\left(\frac{1}{E_c} - \frac{v_{lc}}{E_L}\right) \tag{13}$$

Stretcher Slope Calculations

The Stretcher model predicts a constant ratio between stretch in width, ΔW, and stretch in length, ΔL. 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

$$\Delta W \approx \frac{dW}{dt}\Delta t \tag{14}$$

$$\Delta L \approx \frac{dL}{dt}\Delta t \tag{15}$$

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

$$\frac{\Delta W}{\Delta L} \approx \frac{W'(t)}{L'(t)} \tag{16}$$

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).

$$\sigma^2_{Ratio} \approx \left|\frac{\partial Ratio}{\partial(W')}\right|^2 \sigma^2_{(W')} + \left|\frac{\partial Ratio}{\partial(L')}\right|^2 \sigma^2_{(L')} + 2\frac{\partial Ratio}{\partial(W')}\frac{\partial Ratio}{\partial(L')}\sigma_{(W')(L')} \tag{17}$$

$$\sigma^2_{Ratio} \approx \left|\frac{1}{L'}\right|^2 \sigma^2_{(W')} + \left|\frac{-(W')^2}{(L')^3}\right|^2 \sigma^2_{(L')} - 2\frac{W'}{(L')^3}\sigma_{(W')(L')} \tag{18}$$

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