

# Methodology for Testing Instantaneous Grinding Protocol

## Assumptions that must be met to use this methodology:

- Larvae are separated from frass and substrate prior to grinding.
- Larvae are roughly normally distributed in body size.
- Mean larval body mass is between  $0.0063 \pm 0.0029$  and  $0.2346 \pm 0.0307$  g.

## Replication recommendation:

You should create a new copy of spreadsheet ‘Supplemental File 2’ for each trial (e.g., independent time you set up and run your machine) and repeat Steps 1 - 3. We recommend three trials be conducted to account for variation in the way your machine processes larvae or variation in larval body size at time of slaughter in your facility.

## Step 1: Determining Larval Weight

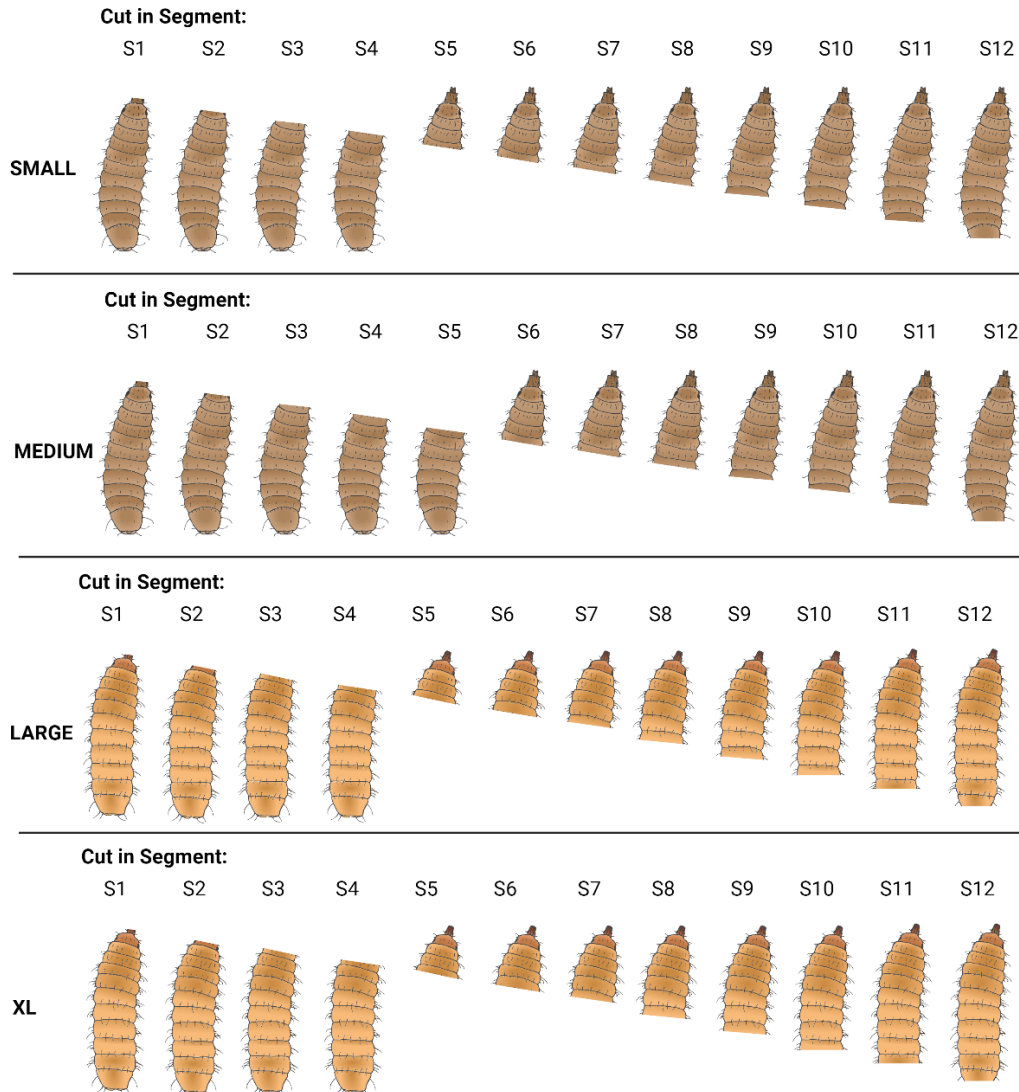
1. Sift larvae from substrate and frass.
2. Weigh five groups, each with ten, randomly-selected larvae from the batch. Input these numbers, in grams, into rows A2-A6 of the tab ‘Step 1’ in Supplemental File 2.
3. The mean larval body mass of a single larvae will automatically appear in cell C2. To confirm that the mean mass is within the range where this experimental methodology can be employed, check cells E2 & E3. **If either cell reads ‘No’ you cannot continue.**
4. **If cells E2 AND E3 read ‘Yes’, then** look at cell H2, which will direct you to the tab to use in Steps 2 & 4, and the part of Figure A to reference in Step 3.

## Step 2: Collecting Your Samples

1. Weigh all the larvae, in grams, prior to inputting into the machine. Put this number in cell A2 of the tab you were directed to use in Step 1.
2. Run the larvae through the machine, collecting the entire sample output in a container of known weight. Stir the sample gently, to homogenize.
  - a. You may have two or more populations of larvae (e.g., those stuck at the blade and those that made it past the particle plate in Barrett et al. 2023). If so, collect and stir both populations separately.
  - b. We have set up the example spreadsheet as if there were two subpopulations, named ‘Blade’ and ‘Plate’. If you have only one subpopulation, delete the extra rows. If you have more than two, make a copy of rows 5-7 for each additional subpopulation.
3. Weigh each subpopulation of larvae. Input these numbers into the first cell of each subpopulation (e.g., C2 for ‘Blade’ and C5 for ‘Plate’).
4. Take three, ~10 g samples from within each subpopulation of larvae. Weigh each sample and input the number, in grams, into the appropriate cell in column F (e.g., F2-F4 for ‘Blade’ subsamples and F5-F7 for ‘Plate’ subsamples).

**Step 3: Counting Your Samples** - Complete this step separately for each sample you collected in Step 2.

1. Count all whole larvae with no injuries and input the number of individuals into [cell H](#).
2. For all larvae in your sample that have been cut in half:
  - a. Use the part of Figure A that matches the larval body size you are using to count *only the halves of the larvae that are considered 'alive' per the diagram*. This prevents double-counting of individuals and ensures you are only counting the responsive half.



**Figure A. The half of the larva that is considered 'alive' when receiving a cut in a particular body segment, organized by larval body size.**

- b. For example, a larva complete from S1-S3 would not be considered 'alive' for any larval body size; however, a larva complete from S3-S12 would be considered 'alive' for all larval body sizes.
3. Input these data into [cells I-T](#), for each sample.
4. Once you have completed all samples for all subpopulations, [Cell Z2](#) will automatically calculate the % instantaneous death for the overall trial.
  - a. The percent instantaneous death for each subpopulation can be found in column X
  - b. The proportion of larvae within each subpopulation can be found in column D