The Egg-Counter: A novel microfluidic platform for characterization of *Caenorhabditis elegans* egg-laying

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Electronic Supplementary Information

Extensive supplemental materials are available online in three associated collections [1–3] to assist those readers wishing to implement the Egg-Counter platform. In addition to those collections, we provide the following supplemental figures for the general readership.

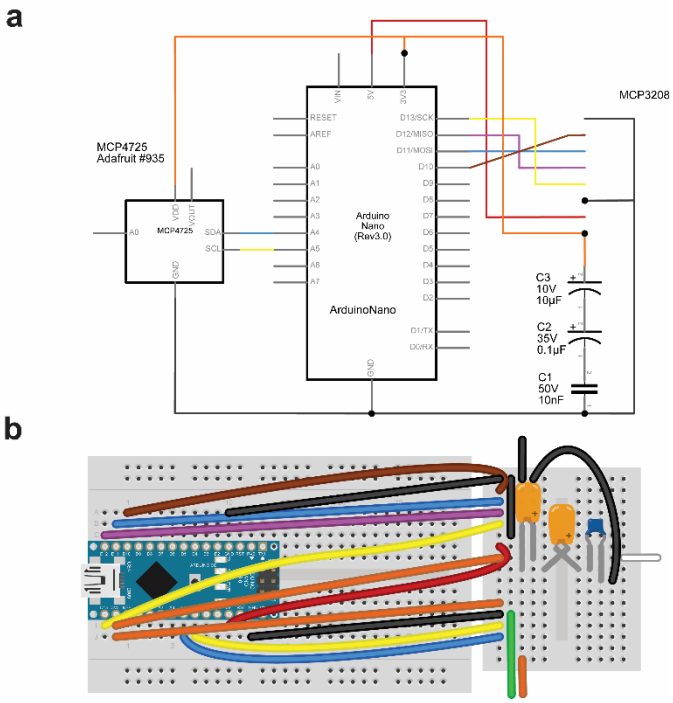

Fig. S1 Egg-Counter temperature monitor schematics. (a) Electrical schematic and (b) breadboard diagram for the Egg-Counter Temperature Monitor. Parts list and necessary software are available in online supplements [52] and [53] respectively.

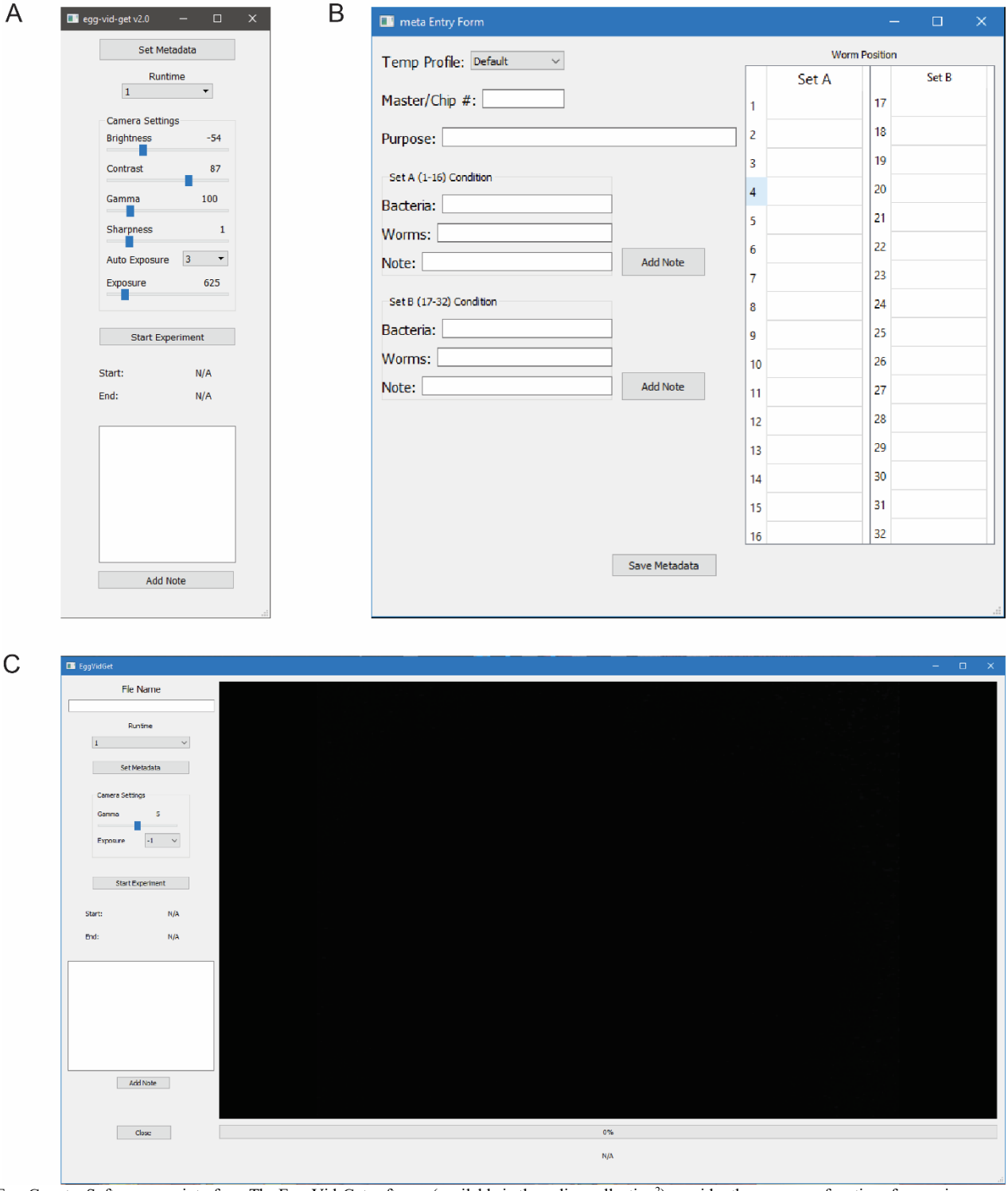

Fig. S2 Egg-Counter Software user interface. The Egg-Vid-Get software (available in the online collection[2]) provides the necessary functions for running an experiment on the Egg-Counter. Upon launching the software, (A) The provided GUI enables the experimenter to set the image acquisition parameters, enter experimental metadata, and initiate the experiment. When selecting the "Set Metadata" option, a (B) GUI is launched that allows the user to enter experimental metadata for the egg laying experiment. When selecting the "Start Experiment" button, a (C) window is provided with a real-time presentation of the experiment in progress.

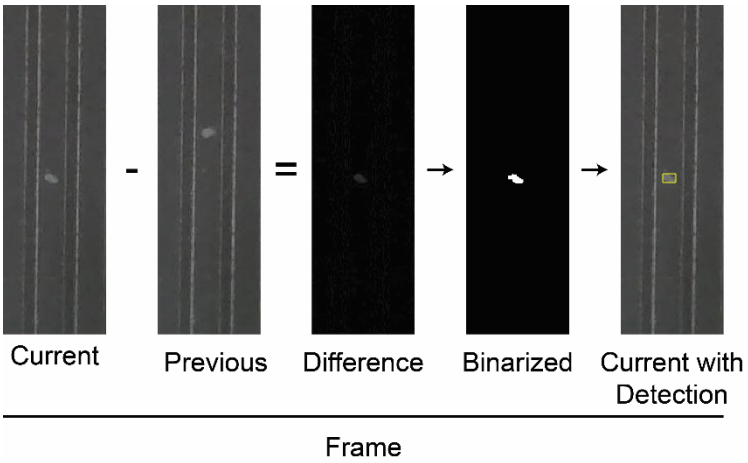

Fig. S3 The Egg-lane-proc software (available in the online collection[2]) and the egg-vid-get software determine which video frames contain objects of interest. In general, the process uses sequential frames to identify areas of change by simple difference determination. For assembling the list of egg-like objects for use in the Egg-Anno software, several parameters to minimize false positives and streamline annotation (see online collection[2] for software details).

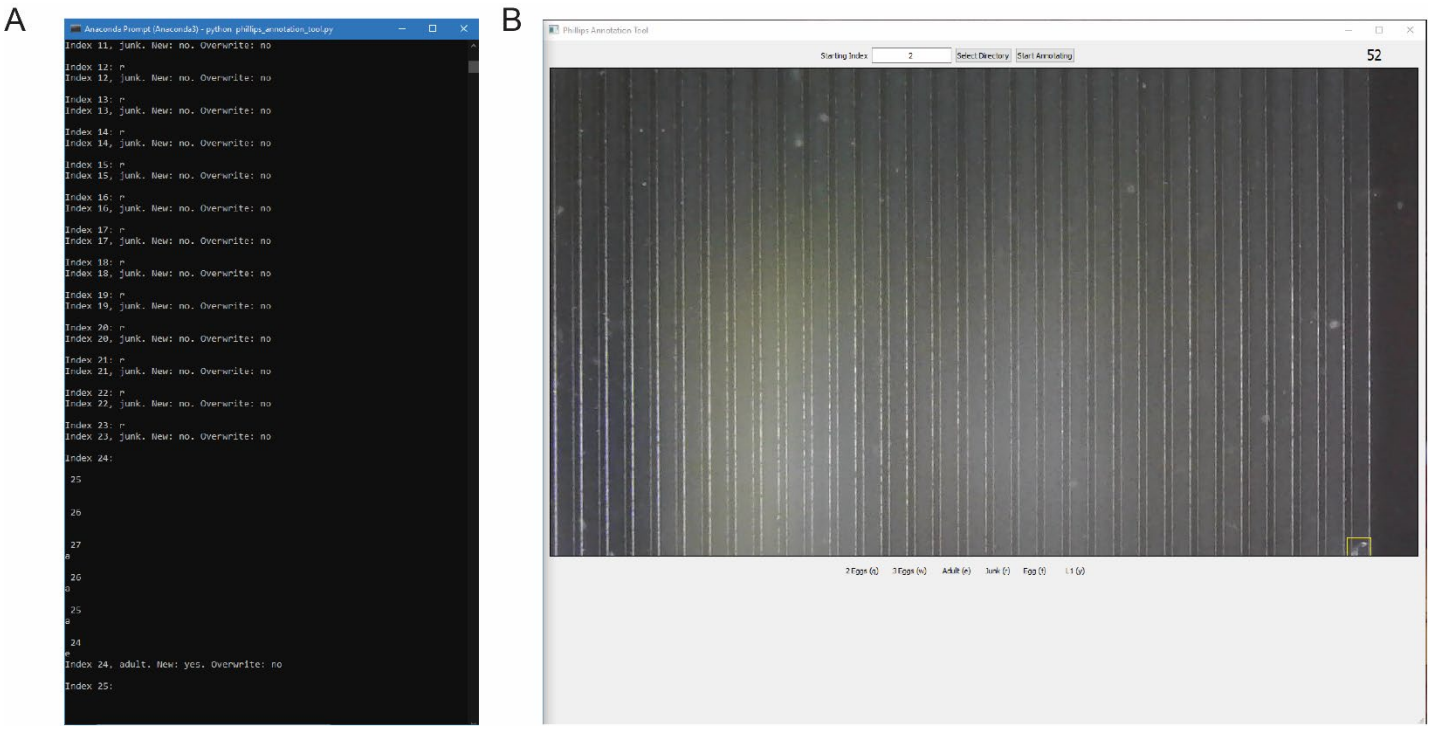

Fig. S4 The Egg-Counter computer aided annotation. The Egg-Vid-Anno software (available in the online collection[2]) provides the necessary functions for processing the movie files after an experiment on the Egg-Counter is completed. Upon launching the software, (A) a command window enables the annotation of each egg-like object that was detected by the Egg-Ana software. To make determinations, the user is presented with each frame in which a detected object is captured (B). With each entry, the annotations are saved to a post-analysis dataset file.

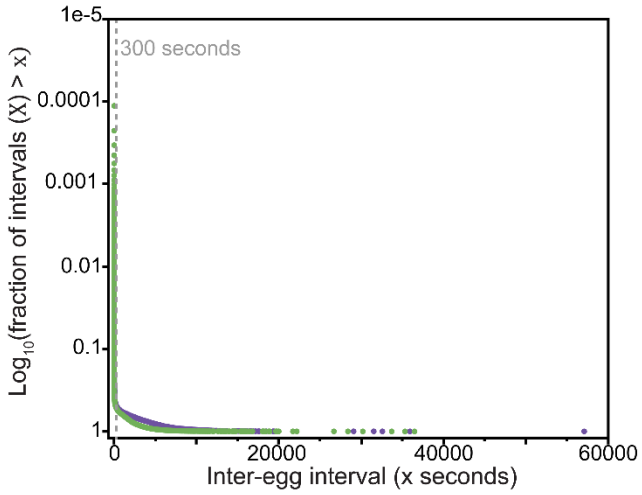

Fig. S5 Cutoff for within versus between bout intervals. The log-tail interval distribution plots for the inter-egg-intervals at 15°C (purple) and 20°C (green) provide a visualizable transition between the two classes of intervals, as previously published[4,5]. A transition is observable at ~300 seconds, well between the previously published mean inter-bout-interval of ~20 minutes and the published mean within-bout-interval of ~20 seconds[4,5]. We therefore selected 300 seconds as a cutoff for separating inter-bout and within-bout intervals.

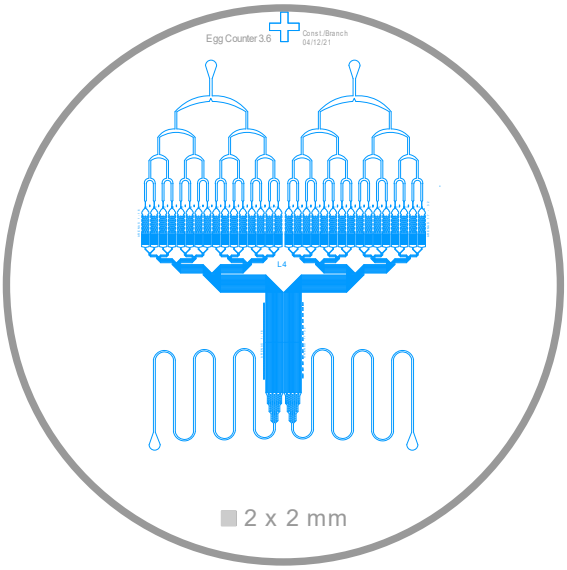

Fig. S6 A novel microfluidic Egg-Counter.

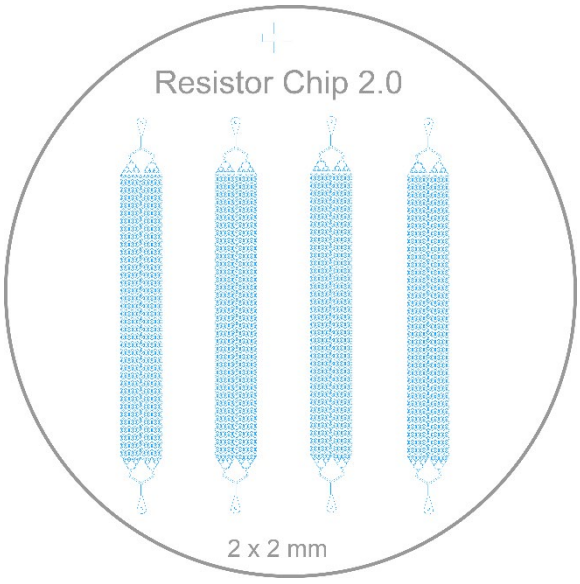

Fig. S7 Egg-Counter Resistance Chip.

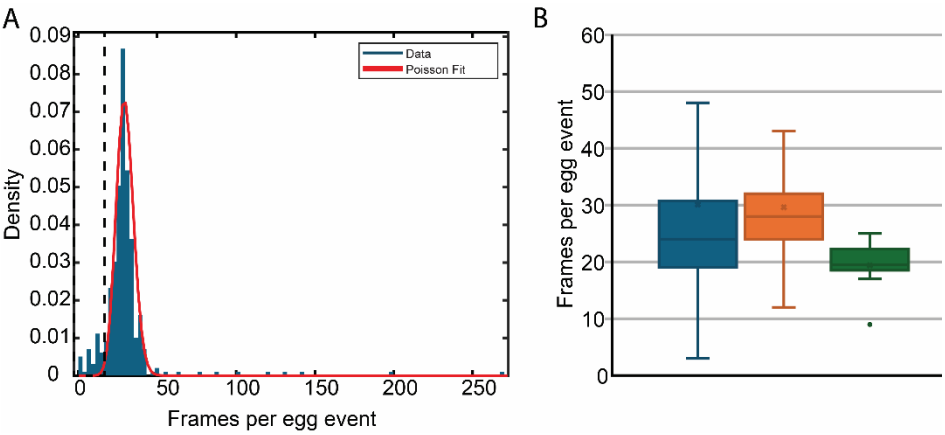

Fig. S8 Frame counts per egg event (A) A histogram for the number of frames captured for each egg-event is plotted along with a Poisson fit. (B) Data for the distribution of frames per egg from three independent experiments.

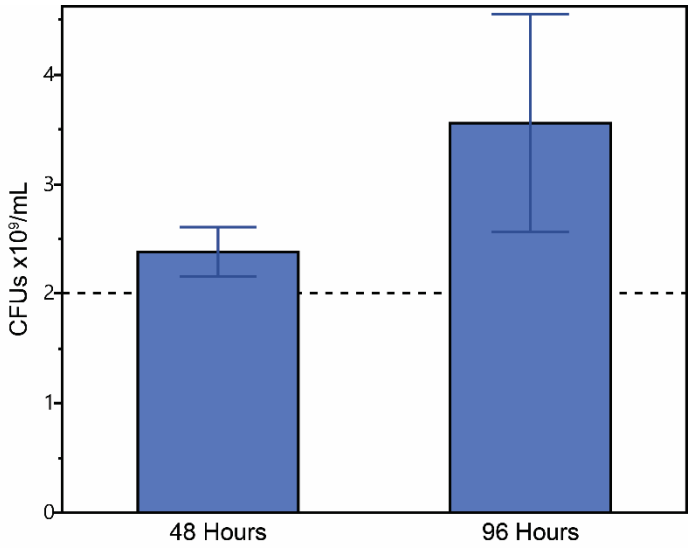

Fig. S9 Food concentration over time in the Egg-Counter The concentration of bacteria exiting the microfluidic Egg-Counter was determined by serial dilution and CFU counting. The bacterial solution was made at 2.0x109 cells (dashed line) at the start of the experiment (hour zero). The outflow was collected from the chip at 48 and 96 hours and diluted 1:1,000,000. We then plated 100 µL samples on LB plates without antibiotics and incubated at 37C. CFU counts were then determined by colony counting. At 48 hours, the minimum concentration measured was 1.75 x109 CFUs, and the maximum was 2.74 x109 CFUs. Presented are the mean and 95% confidence intervals for from replicates pooled from two biological replicates.

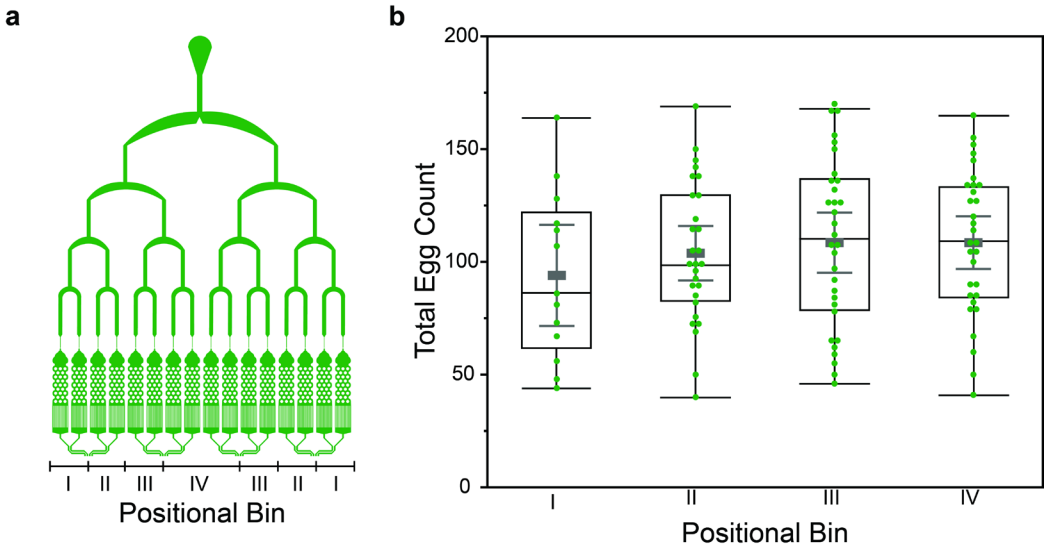

Fig. S10 Analysis of positional effects within the egg counter. (a) the data presented in the manuscript were binned by lane position relative to the outer (position I) and inner (position IV) portions of the device. (b) Reproductive output within each positional bin does not show positional effects (p=.57).

References

1. Banse, S. A. *et al.* Egg Counter: Schematics and Build Guides. *figshare* (2022) doi:10.6084/m9.figshare.c.6122667.

2. Banse, S. A. *et al.* Egg Counter: Software. *figshare* (2022) doi:10.6084/m9.figshare.c.6122634.

3. Banse, S. A. *et al.* Egg Counter: Data and Analysis. *figshare* (2022) doi:10.6084/m9.figshare.c.6122682.

4. Waggoner, L. E., Zhou, G. T., Schafer, R. W. & Schafer, W. R. Control of alternative behavioral states by serotonin in *Caenorhabditis elegans*. *Neuron* **21**, 203–14 (1998).

5. Zhou, G. T., Schafer, W. R. & Schafer, R. W. A three-state biological point process model and its parameter estimation. *IEEE Trans. Signal Process.* **46**, 2698–2707 (1998).