Supplementary Material for:

Measuring *C. elegans* spatial foraging and food intake using bioluminescent bacteria

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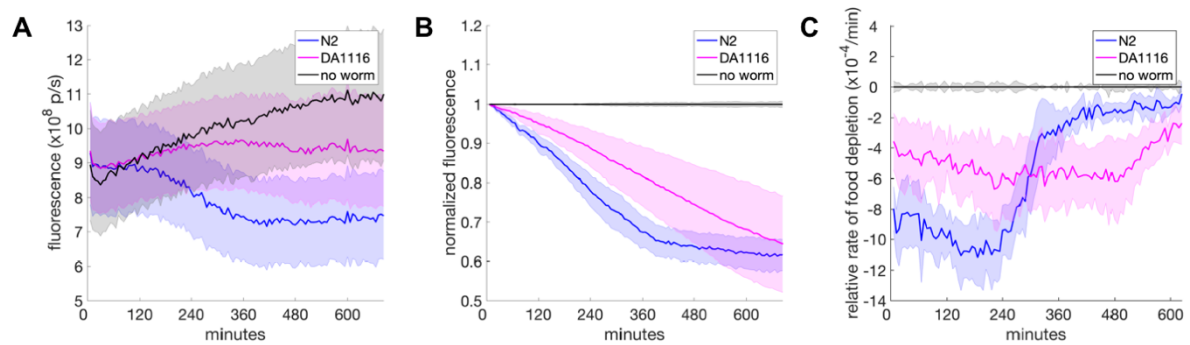
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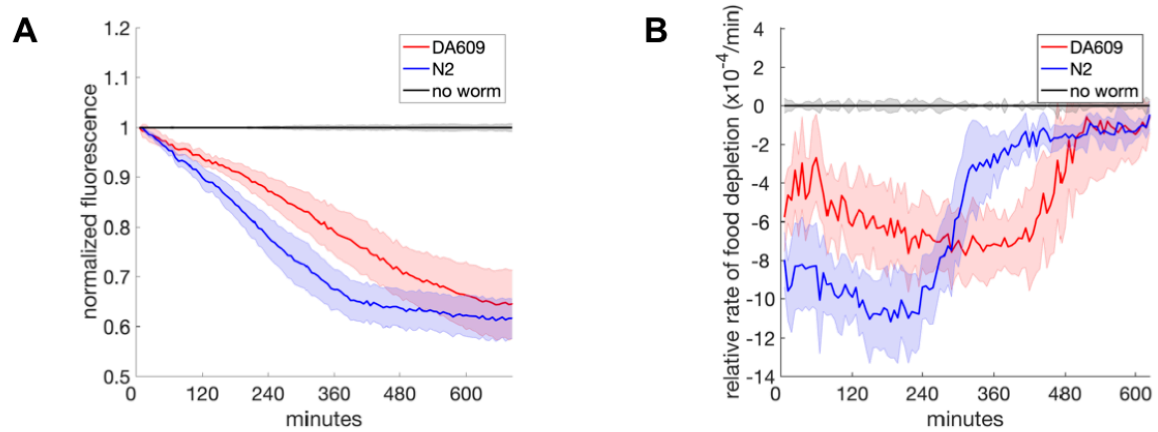United Kingdom

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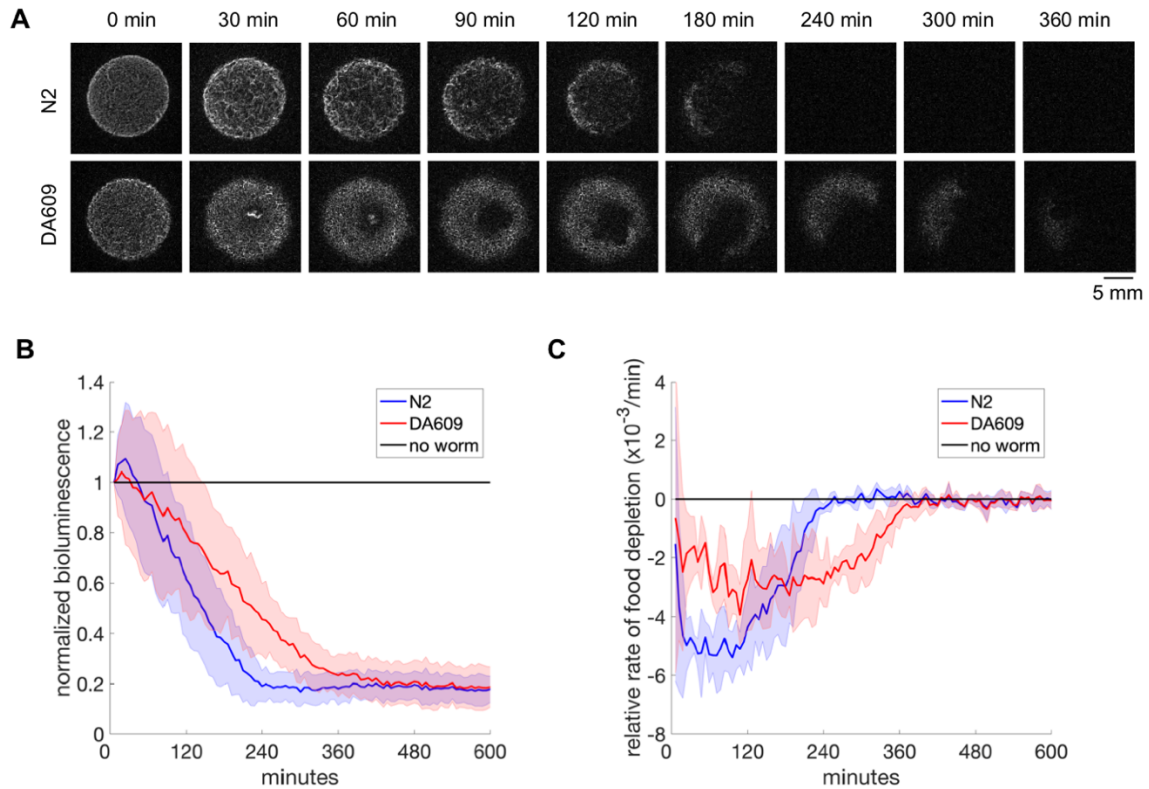
Supplementary Figures

Supplementary Figure S1. Fluorescence signal from population feeding experiments of N2 and DA1116 worms. A) Raw signal. **B**) Normalized signal (normalized to the starting signal and then to the no-worm control signal). **C**) Derivative of the normalized signal calculated over a 60-minute window. Forty N2 (blue) worms, forty DA1116 (magenta) worms, or no-worm control (black) experiments were performed on a 20 µL OP50-GFP lawn. 0.5 second exposure measurements were read every 6 minutes using 465 nm excitation and 520 nm emission filters. All samples shown were imaged simultaneously. Here n = 11 for N2, n = 10 for DA1116, n = 5 for control, pooled from four independent sets of experiments; error bars represent ±1 SD.
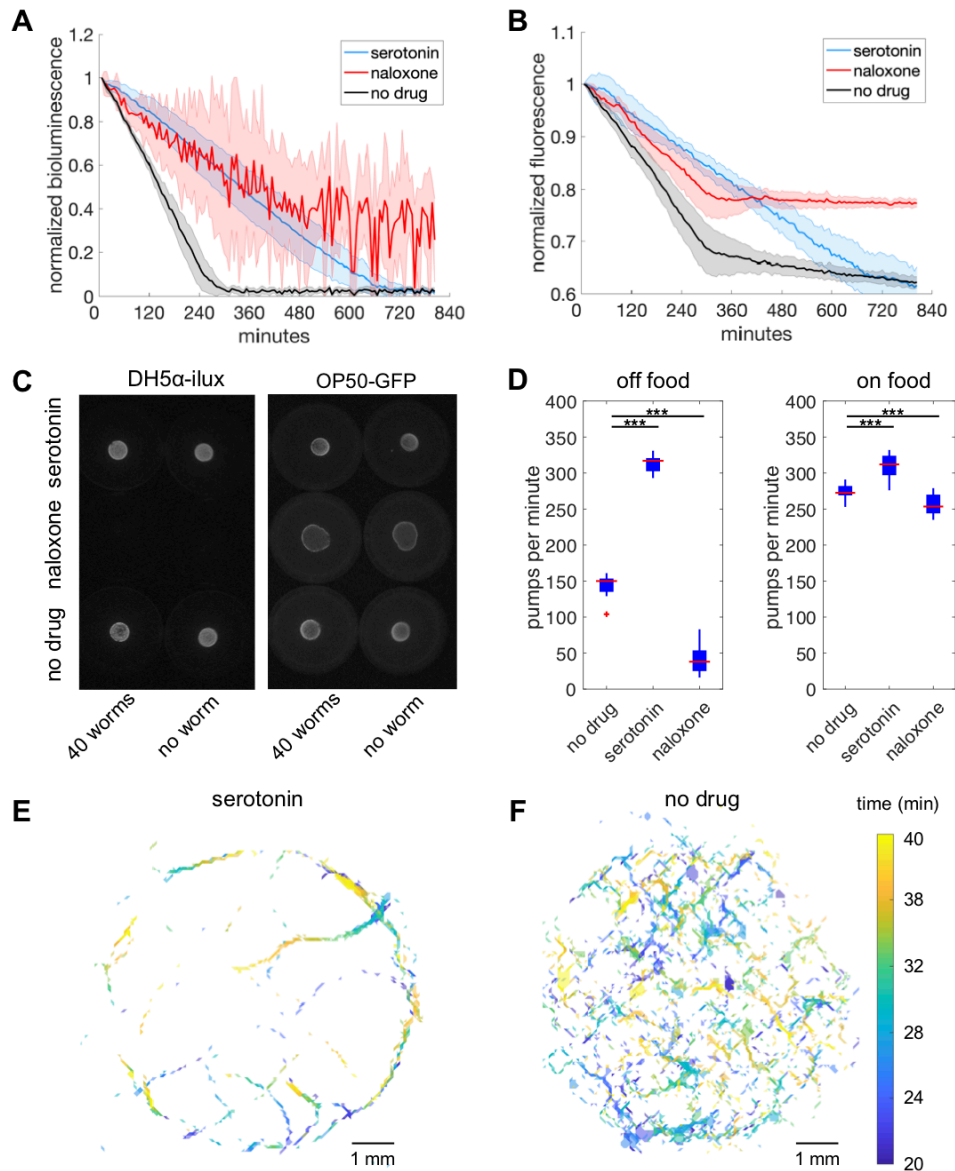
Supplementary Figure S2. The effect of drug treatments on *C. elegans* feeding rates and foraging. Forty pre-starved N2 worms were subject to serotonin-, naloxone-, or no-drug mock treatment for 1 hour on food before feeding was measured. **A-B**) Normalized signal from experiments on DH5α-ilux (A) or OP50-GFP (B) bacterial lawn following treatment with serotonin (blue), naloxone (red), or no-drug mock control (black). For A), 1 second exposure measurements were read every 6 minutes from the start of re-feeding. n = 6 for each condition, pooled between three independent sets of experiments, error bars represent ±1 SD. For B), 0.5 second exposure measurements were read every 6 minutes from the start of re-feeding using 465 nm excitation and 520 nm emission filters. n = 6 for each condition, pooled between three independent sets of experiments, error bars represent ±1 SD. **C**) Snapshots of the signal level at the end of 1-hour drug exposure, which is the start of the feeding measurements. **D**) Pharyngeal pumps per minute of mock-, serotonin-, and naloxone-treated worms. Drug treatment was performed either off (left) or on (right) food, and pumping was scored for 60 seconds. For the boxplots in (D), the red horizontal line indicates the median, the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively, and the whiskers extend to the most extreme data points excluding the outliers, which are plotted individually using the red '+' symbol. n = 9 for no-drug off food, n = 11 for serotonin off food, n = 5 for naloxone off food, n = 10 for no-drug on food, n = 10 for serotonin on food, n = 10 for naloxone on food. ***: $p < 0.01$, ns: $p > 0.05$, two-sample t-test of the replicate means. **E-F**) Worm positions on the circular bacterial lawn during the 20-40 min imaging window following serotonin- (E) or mock- (F) treatment. Different colors indicate time progression according to the color bar.
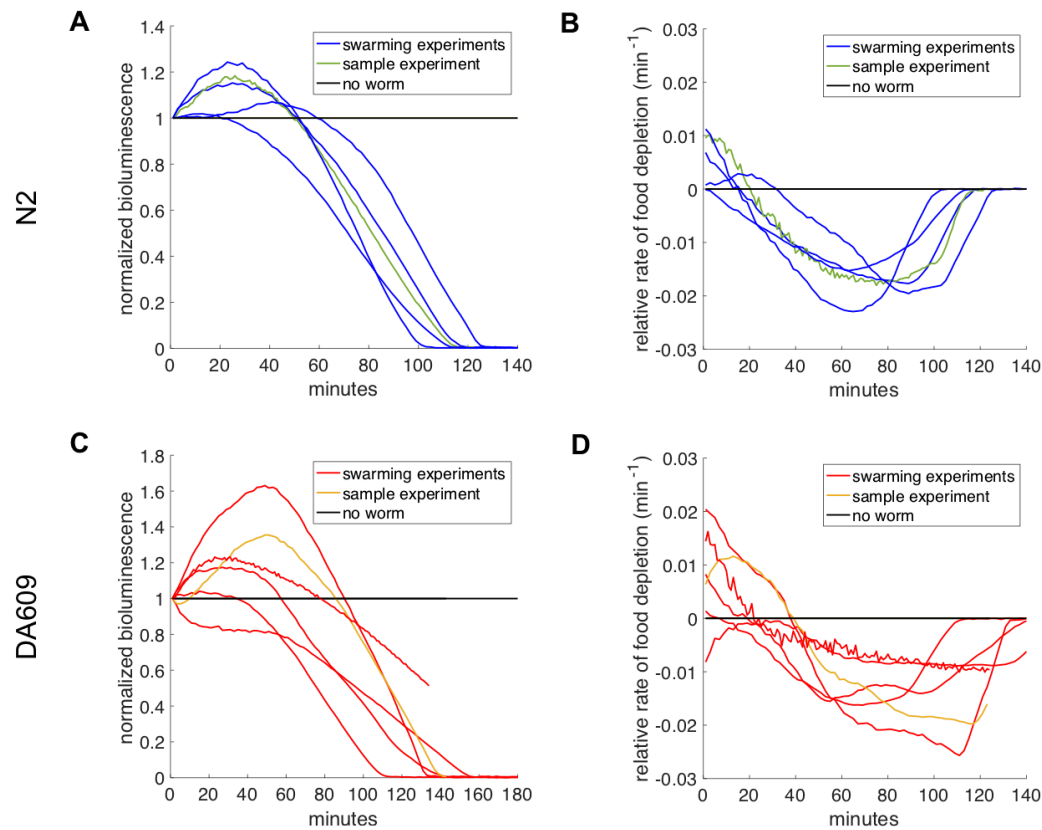
Supplementary Figure S3. Fluorescence signal from population feeding experiments of N2 and DA609 worms. A) Normalized signal. **B**) Derivative of the normalized signal calculated over a 60-minute window. Forty DA609 (red) or N2 (blue) worms or no-worm control (black) experiments were performed on a 20 μL OP50-GFP lawn. 0.5 second exposure measurements were read every 6 minutes using 465 nm excitation and 520 nm emission filters. All samples were imaged simultaneously. Here n = 11 for N2, n = 10 for DA609, n = 5 for control, pooled from four independent sets of experiments; error bars represent ±1 SD.

Supplementary Figure S4. Bioluminescence signal from population feeding experiments of N2 and DA609 worms, using a commercial-grade digital SLR camera for imaging. **A**) A series of snapshots contrasting the spatial pattern of food depletion in N2 (top) and DA609 (bottom) population feeding experiments. **B**) Normalized signal, and **C**) derivative of the normalized signal calculated over a 60-minute window. Forty DA609 (red) or N2 (blue) worms or no-worm control (black) experiments were performed on a 20 μL DH5α-ilux lawn. Thirty second exposure measurements were read every 6 minutes. n = 4 for each condition, pooled between four independent sets of experiments; error bars represent ±1 SD. Note that these feeding experiments were performed at 22°C rather than the usual 20°C, which may have resulted in faster food depletion by both worm strains (compare to Figure 3A-B).
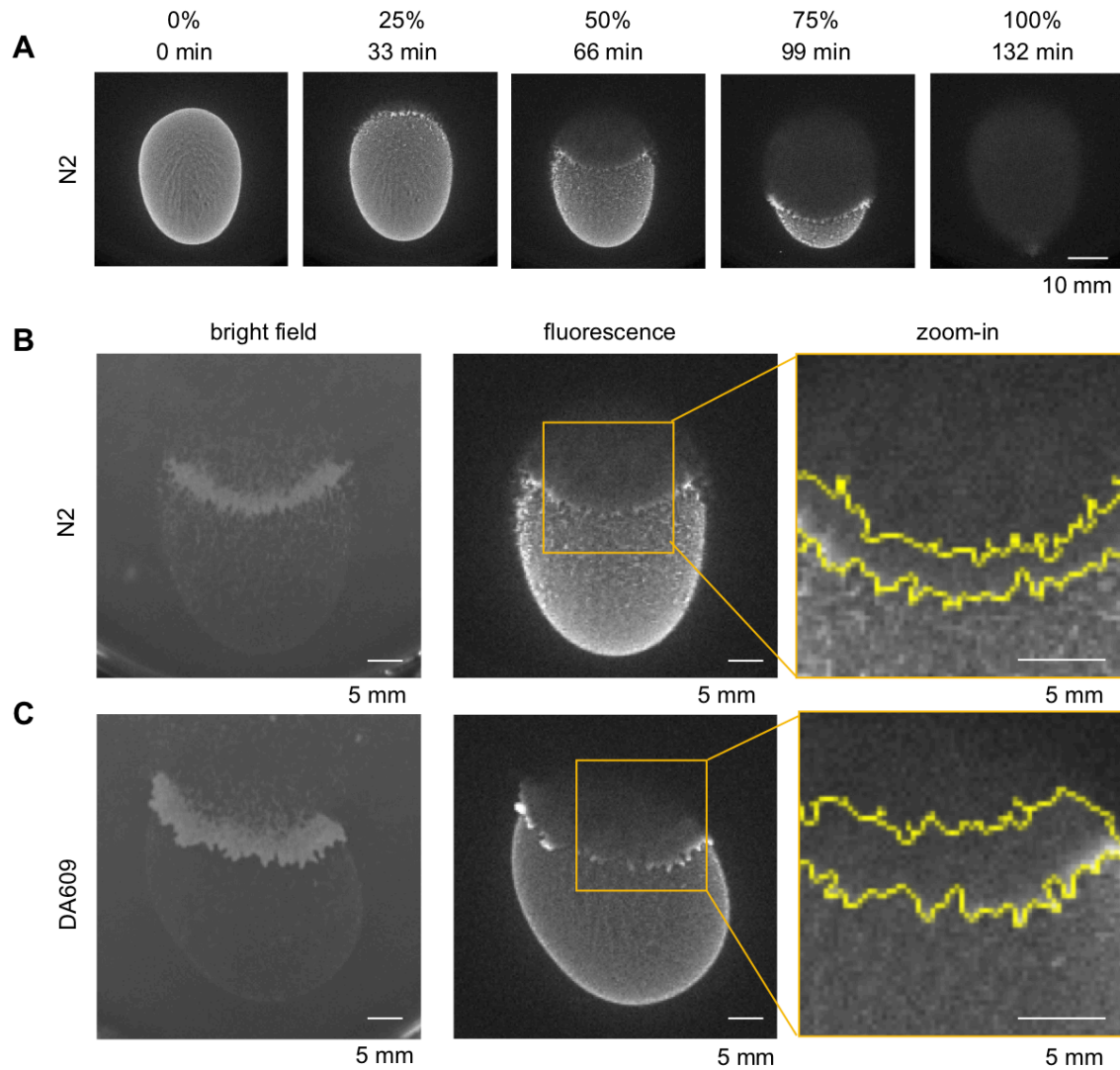
Supplementary Figure S5. Fluorescence signal from large population swarming experiments.
A few thousand age-synchronized worms were allowed to feed and swarm over a 500 µL OP50-GFP
lawn. **A**) Snapshots of a representative N2 swarming experiment on a OP50-GFP lawn, with time
progression to total food depletion indicated at the top. **B-C**) Sample snapshots from N2 (B) and
DA609 (C) swarming experiments, showing bright field (left) and fluorescence (middle) channels. The
boxes in the middle panels are zoomed in and displayed on the right. The worm front outlines (yellow
lines, right) were automatically extracted from the bright field channel and overlaid onto fluorescence
images.

Supplementary Figure S6. Bioluminescence signal from large population swarming experiments. A-B) Normalized signal (A) and derivative of the normalized signal calculated over a 10-minute window (B) for N2 swarming experiments. Each line is an independent replicate, green lines show results from the sample experiment shown in Figure 4B and Movie 2. **C-D)** Normalized signal (C) and derivative of the normalized signal calculated over a 10-minute window (D) for DA609 swarming experiments. Each line is an independent replicate, orange lines show results from the sample experiment shown in Figure 4C and Movie 4. Note that we show replicates as individual lines here rather than pooling between the experiments, because the details of the feeding rates depend on initial conditions such as the precise worm number, the geometry of the food patch, and how the worms arrive at the patch boundary, which are difficult to control in large population swarming experiments.