IMPACT OF HYDROGEN PEROXIDE ON C. ELEGANS PUMP DURATION AND FREQUENCY

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WINTER 2024

Laboratory in Neurobiology
BIOL 389

Abstract:

This study investigates the effects of hydrogen peroxide (H2O2) on the pumping behavior of C. elegans in both wild-type and EGL-19 mutant strains. Through Electropharyngeogram recordings, we examined the pharyngeal pump duration, frequency, and inter-pump intervals. Our findings indicate that following toxic H2O2 exposure, wild-type worms first showed high pump frequency followed by a rapid decline in frequency over time. While EGL-19 mutants exhibited longer pump durations after H2O2 exposure, their pump frequency did not decline as quickly as in wild-type worms. Despite our small sample size, which limits the statistical significance of our results, the observed trends suggest intriguing avenues for further research into the neural mechanisms underlying stress responses and potential learning behaviors in C. elegans.

Introduction:

The nematode C. elegans is a very prevalent model organism in biology due to its small size and anatomical simplicity. With only 302 neurons, a well-characterized genome (over 5000 of its genes have homologs in humans), and a multitude of natural and lab-made mutants, this nematode allows for intricate neuroethological and neuropharmacological study (1).

The C. elegans nervous system is composed of two systems: the somatic nervous system and the self-contained and autonomous pharyngeal nervous system. The 20-neuron pharyngeal system is responsible for the coordination of the pharynx, a neuromuscular tube necessary for the ingestion and digestion of food in the form of bacteria (2). This independent system is particularly attractive as its small number of neurons makes it ideal for thoroughly understanding how neurons communicate to produce behaviors, for studying the role of molecules such as hormones and neurotransmitters in coordinating signals, and for developing comprehensive circuit models.

The C. elegans pharynx is made of three functional units. Following the ingestion of bacteria into the Buccal Cavity of the Corpus, bacteria are pushed along into the Isthmus through wave-like muscular contractions towards the grinder in the Terminal Bulb that delivers crushed bacteria into the intestine (2). The motor neurons essential for pharyngeal muscle contractions function by triggering action potentials that depolarize the muscle and open voltage-gated calcium channels (3). The subsequent intracellular rise in calcium concentration powers the pharynx contraction that in typical conditions, results in a pumping rate of about 3-4 Hz (4). The pumping rate and form, however, can be impacted by a variety of environmental and chemical cues such as changes in lighting, pheromone concentration, and the presence of toxic chemicals.

Notably, naturally occurring hydrogen peroxide (H2O2), one of the most common chemical threats, impacts pharyngeal pump rate (5, 6, 7). Over time, the presence of H2O2 results in a decrease and subsequent inhibition of pharyngeal pumping due to the coordination of several gustatory receptors and the I2 pharyngeal neuron (8, 9). A recent study, however, suggests that there may be more to the story. When observed closely, the presence of toxic chemicals or particles causes “burst pumping” in C. elegans that pushes unwanted material out of the mouth and back into the environment (5). An increase of calcium around the pharyngeal valve causes the anterior portion of the pm3 muscles to contract and hold open the valve for the subsequent ejection of materials during rapid pumping (5). This finding is unlike those of previous studies, which only observed pumping frequency decreases at larger timescales. The presence of this complex behavior isolated in the pharyngeal system provides a unique opportunity to study the complexity of muscle dynamics and neuronal communication in a small and accessible system.

To study the nuances of these pumping behaviors, our study explores the responses of both wild-type and genetically modified strains of C. elegans, EGL-19 gain-of-function mutants, to toxic concentrations of H2O2. EGL-19 n2368/MT6129 mutants (MT) have altered voltage-gated calcium channels that require stronger and longer depolarizations to trigger the intracellular calcium-increase-induced muscle contraction necessary for pumping behavior (10, 11). This extended depolarization is associated with myotonic conditions, resulting in defective relaxation of the terminal bulb, longer pump durations, and arrhythmic pumping patterns (12).

If rapid "burst pumping" is necessary to expel ingested toxic substances like hydrogen peroxide from the pharynx, we hypothesize that wild-type C. elegans will exhibit an initial increase in rapid pumping frequency upon H2O2 exposure, followed by a subsequent decrease and inhibition of pumping. However, we expect the EGL-19 mutants' altered calcium dynamics and prolonged pump durations to impair their ability to generate the rapid pumping necessary for effective expulsion of hydrogen peroxide. Consequently, we hypothesize that EGL-19 worms will display even longer pump durations during the spitting process, followed by a faster inhibition of pumping due to prolonged exposure to the toxic effects of H2O2. If the EGL-19 mutants cannot expel

H2O2 from their pharynx at a sufficient rate, we anticipate that they will exhibit an increased mortality rate compared to wild-type organisms when exposed to toxic concentrations of H2O2. This research not only deepens our understanding of C. elegans muscular and neural regulation but also underscores the broader significance of studying such dynamics in a simple and accessible system.

Method details:

Eletropharyngeogram Experiments:

All electropharyngeogram (EPG) recordings were completed as detailed in Electrophysiological Methods by Avery et al. (13). All worms in both control and experimental conditions (H2O2 addition) were kept in baths containing Dent's solution and 1 mM serotonin to induce pumping.

WT and Mutant Control:

Sterilized 100mm x 15 mm petri dishes were provided. Worms were washed prior to addition into the dish and individual and unique worms were used for each recording. The bath was not replaced between experimental trials and recordings. However, the baths and dishes were changed between wild-type and mutant conditions. Once the worms were properly secured in the recording pipette and exhibited regular pumping, we began an axoscope recording that terminated after either 3 minutes or until the worm escaped the recording pipette.

For the wild-type control experiments, we successfully recorded 8 worms. Sections of 80-120 ms were used for all annotation and data analysis for consistency of noiseless signals and sufficient periods of pumping. We recorded 10 EGL-19 mutant individuals for our control experiment. However, only 5 recordings were viable for further annotation and analysis due to considerable noise artifacts or the worm having escaped from the recording pipette prior to 80-120 ms of relatively consistent pumping activity.

WT and Mutant H2O2:

Worms were washed with Dent's solution containing 1mM serotonin, as was done for the control conditions (13). A 60x15mm Petri dish was used for each H2O2-addition experiment in which 2mL of the control bath was added containing about 10 works (much less than in the control experiments). After a worm was selected and secured in the recording pipette, an axoscope recording was started during a period of consistent pumping. After a few seconds of pumping, 11.3 uL of concentrated stock H2O2 was added via micropipette into the petri dish near the recording electrode and secured worm. This resulted in the dish having a .005M (5mM) H2O2 concentration as motivated by previous experiments (8, 14). The precise time of H2O2 addition was recorded for all trials for both EGL-19 mutant worms and wild-type organisms. Recordings were sustained for 3 minutes or until the worm escaped the recording electrode. All present and clear pulse peaks were annotated and analyzed both before and after H2O2 addition. We suggest that future studies leave the worms in the recording pipette for at least 45 seconds before H2O2 addition to allow for proper normalization between pre and post pulse data.

WT and Mutant Poke Test Assay:

We performed a modified liquid killing and oxidative stress assay in which C. elegans were exposed to 0.05M H2O2 and were monitored every 15 minutes post-exposure to determine whether they were alive (14). Three 15 by 150 mm petri dishes were used for the poke test experiment with one new dish per trial. As detailed in (15), worms that do not spontaneously move, respond to shaking or poking are considered dead. Worms were isolated by lining the large petri dish with 1-2 drops of worm/H2O2 solution. Isolation of worms into drops (0-3 worms per drop0 allowed for the monitoring of individual worms over time as shown in the appendix Figure 1. N=33 worms were monitored per trial (2 mutant and 1 wild-type trial) for 60 minutes for the two mutant trials and only 30 minutes for the WT worms (cut short due to time limitations). See appendix figure 1 for an image of the experimental set-up.

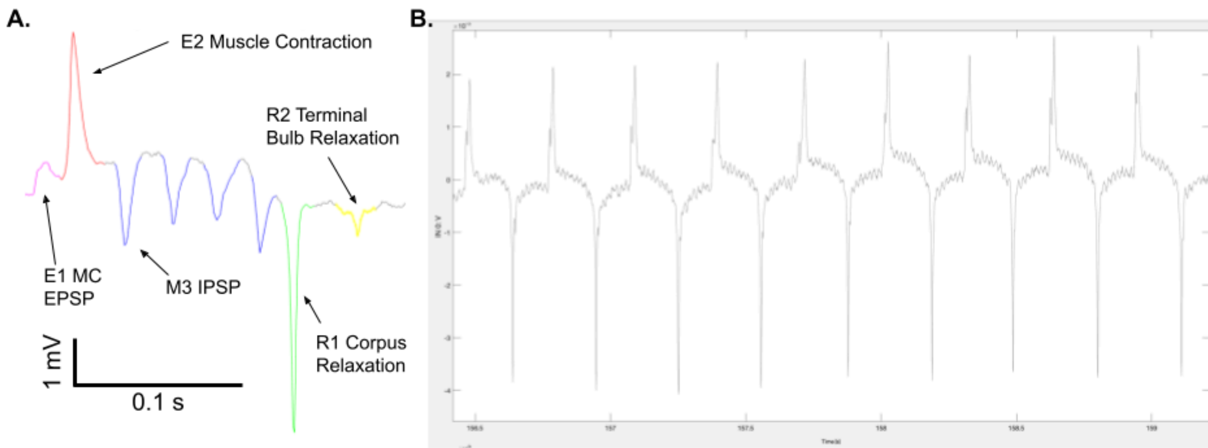

Figure 1. Electropharyngeogram Traces

(A) Detailed view of a single EPG trace from an example WT C. elegans sample in the serotonin bath. Traces are colored and labeled.
(B) Overview of the EPG Analysis AxoScope software containing an EPG trace from an example WT C. elegans sample in the serotonin bath.

Results:

To identify and quantify wild-type (WT) C. elegans pumping patterns and trends, we analyzed both individual and trains of pumps per organism in serotonin control conditions (see methods for details). We first analyzed the pump duration measured for each pump as the difference in seconds between the muscle contraction (E) and corpus relaxation (R) peaks (Figure 1. A). These were chosen due to similar measurements in previous experiments (17) and their presence in all recordings. As shown in Figure 1B, these pumps occurred in trains and rarely as individual units in the WT control samples. For each organism and sampled time range, all pump durations were recorded and plotted as shown in Figure 2A (see supplemental figure 1. for all plots). Mean pump values for the n=8 WT control worms are as follows: 0.196s, 0.162s, 0.177s, 0.143s, 0.159s, 0.188s, 0.152s, and 0.147s respectively, for a total mean pump duration of 0.165 seconds over all WT control worms. The standard deviations for the n=8 WT control worms are as follows: 0.0697s, 0.0051s, 0.0111s, 0.0308s, 0.0126s, 0.0761s, 0.0398s, and 0.0324s respectively for a mean standard deviation of 0.0347s. While the individual pump durations varied over time per individual, all pump lengths remained within a narrow duration range, with a majority of peaks centered around the population mean value indicated above (Figure 2. C). Further quantification of the data can be noted in the Appendix Table 1.

We then measured the inter-pump duration and pump frequency over time. We defined the inter-pump duration to be the difference between the R peak of a first pump and the E peak of a subsequent pump. Figure 2B shows example plots containing the inter-pump interval in seconds for all recorded pumps in the sampled time range for two worms (Figure 3. A, B). Mean inter-pump gap durations for the n=8 WT worms are as follows: 0.655s, 0.158s, 0.191s, 0.187s, 0.152s, 0.294s, 0.184s, and 0.157s for an average gap duration of 0.427 seconds over all organisms. Similarly to the kernel density estimate plot for pump durations, all inter-pump gap durations were centered

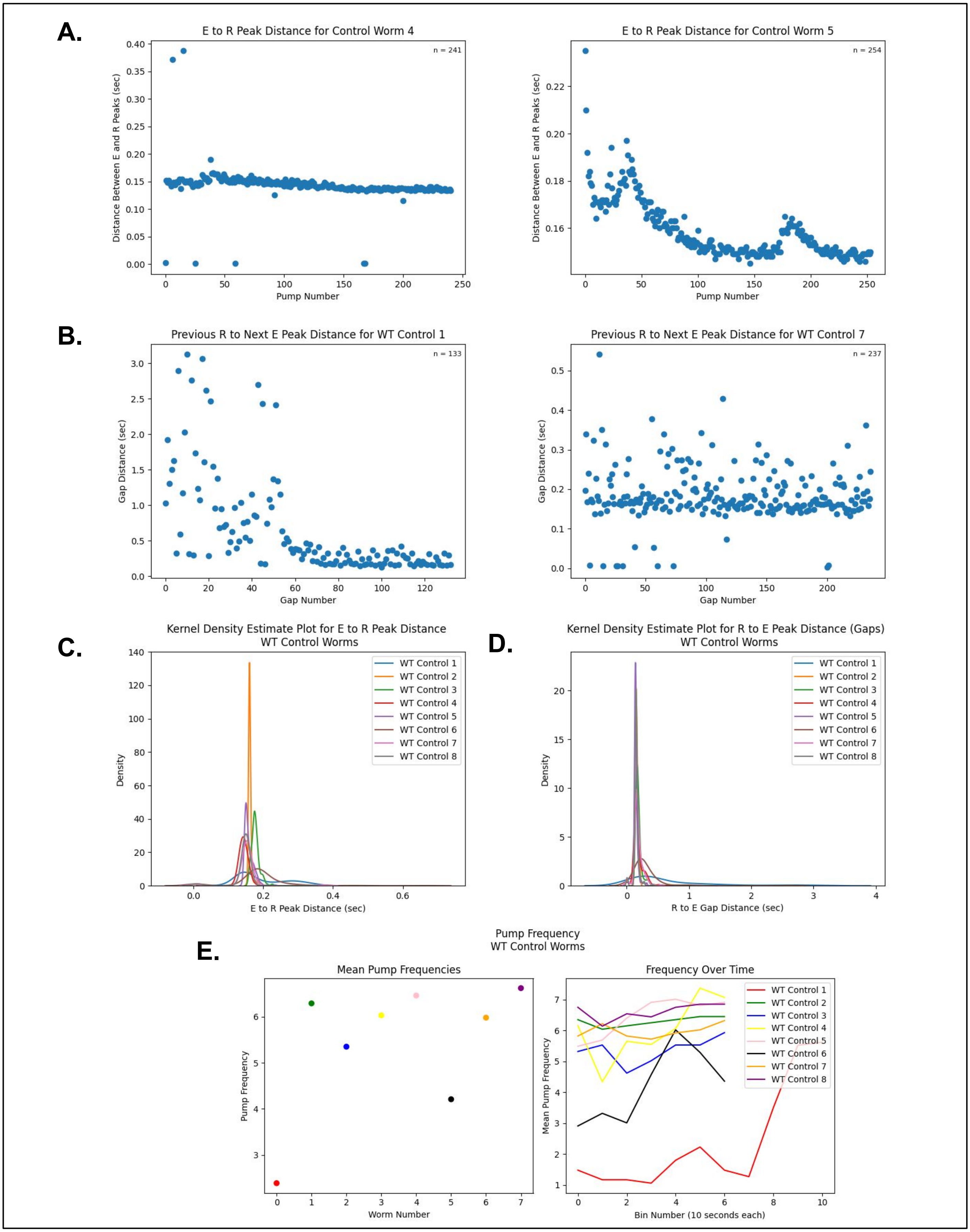

Figure 2. WT Control Worm Pump Characteristics

(A) Example distance values between E and R peaks of a single EPG trace pump from WT control worm 4 (left, n=241) and worm 5 (right, n=254) in the serotonin bath. Each blue dot represents the distance value for a single pump.
(B) Example distance values between the R peak of a previous pump and the E peak of a current pump for the EPG trace pumps from WT control worm 1 (left, n=133) and control worm 7 (right, n=237) in the serotonin bath. Each blue dot represents the gap distance value for a single pump.
(C) Kernel Density Estimate Plot for pump duration for all WT control worms in the serotonin bath.
(D) Kernel Density Estimate Plot for inter-pump gap duration for all WT control worms in the serotonin bath.
(E) Pump Frequency Mean values for each WT worm (left) and for all 10 second bins per worm EPG recording (right).

narrowly around the population mean. Further quantification of the data can be noted in Appendix Table 1.

The mean frequency was calculated by dividing the recorded time range into 10-second bins from which bin frequencies were then calculated. This was done to detect potential changes in worm pump frequency over time. Figure 2E shows the mean pump frequency per sampled worm in addition to the frequency per bin value found per organism over time. The mean control frequency for all WT organisms was 5.24 Hz while the mean per-bin frequency for all WT control organisms was 5.42 Hz. These values were slightly greater than in previous literature that noted a mean pulse frequency of 4.4 ± 0.48 Hz for wild-type organisms (17). This may be due to the presence of serotonin in our recording bath. In our data, the lowest recorded frequency was 1.06 Hz while the highest was 7.37 Hz.

We then sought to compare these above-observed values with those of WT worms in the presence of 5mM hydrogen peroxide (H2O2). Hydrogen peroxide was added to the recording bath following the immobilization of a worm head into the recording electrode. Figure 3A shows an example of the pump duration and inter-pump gap length in seconds for an example sample worm before and after the addition of H2O2 in the recording bath. Similar plots for all other organisms in this condition can be found in Supplemental Figure 2. For three worms, the pump duration over time did not significantly change between pre- and post-H2O2-addition. However, for worm number 4 (shown in Figure 3A), the post-H2O2 addition pump lengths were significantly shorter than those pre-H2O2 addition. The significant p-value of less than .05 was calculated using the Mann-Whitney U Test. For the inter-pump duration, three of our samples had

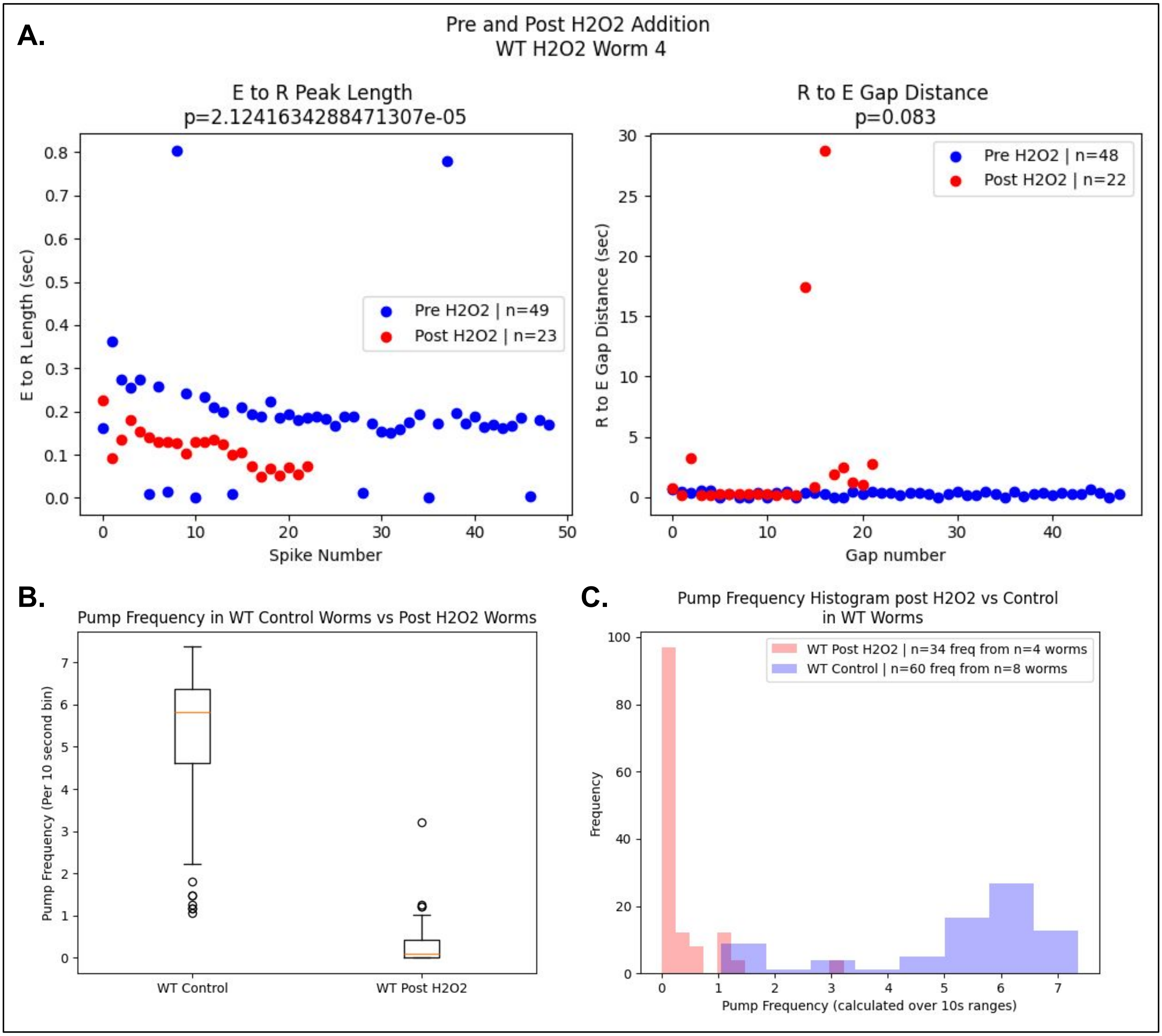

Figure 3. WT Worms After Exposure to H2O2

(A) Pre vs Post WT worm analysis. Pump length pre vs post for all WT worms in experimental condition (left). Inter-pump gap length pre vs post exposure to H2O2 for all WT worms in the experimental condition (right). Mann Whitney U Test was performed for pre and post conditions. Significant P values resulted for Pulse Length but not inter-pump gap distance.
(B) Box plots for frequencies from all 10 second bins for WT control worms (left) and WT worms after exposure to H2O2 (right).
(C) Kernel Density Estimate Plot for pump duration for all WT control worms in the serotonin bath.

significant changes in gap length between pre- and post-H2O2-addition (note however, the small pump numbers for these samples). In these samples, H2O2 addition resulted in longer and more variable inter-pump gap durations (see supplemental figure 2 for all plots).

Frequency was then analyzed between pre and post-H2O2-addition. A box plot and histogram representation can be seen in Figures 3B and 3C respectively. The pre-H2O2 mean frequencies were 2.16Hz, 0.15Hz, 6.22Hz, and 5.62Hz for each tested worm. The post-H2O2-addition mean frequencies were 0.36Hz, 0.13Hz, 0.58Hz, and 0.68Hz respectively. The seemingly decreased frequency may be due to the decrease in pumping over time or the escape of the worms in certain trials. Due to our method of sampling frequency, these large gaps of pulses (0 Hz) may disproportionately impact the mean pump frequency. It may be beneficial to find a better metric to calculate pulse frequency in order to properly compare and analyze these values. Because of our small sample size and the small number of pumps recorded before the addition of H2O2 for all samples, no significance tests were performed (see Discussion for future studies).

To work towards understanding the underlying mechanisms potentially causing these differences in pump duration and frequency, we studied EGL-19 mutant C. elegans and performed identical tests and conditions as for the wild-type worms. Figure 4. shows an overview of mutant worm pump characteristics for our control samples. The mean pump durations for mutant samples (n=5) were 0.164s, 0.149s, 0.194s, 0.154s, and 0.166s for an overall mean of 0.165 seconds. This is identical to the mean pump duration for the WT condition. The mean gap distances between pumps for the mutant samples were as follows: 0.357s, 0.234s, 0.829s, 0.196, and 0.336 for an overall mean gap value of 0.390 seconds. This is shorter than the 0.42-second mean for the WT group. Similarly to the plots in Figures 2C and 2D, Figures 4C and 4D show that the mean pump duration and inter-pump gap durations are narrowly centered around the mean. The mean frequency for each MT organism was the following: 3.6Hz, 5.69Hz, 2.07Hz, 6.12Hz. The overall mean frequency was 4.39Hz while the per-bin mean frequency across all MT organisms was 4.374 Hz. Based on the Mann U Whitney test, the difference of per-bin mean frequency values between the WT and MT control worms was significant, with a corresponding p-value of 0.008.

Analyses of H2O2 exposure to Mutant EGL-19 worms are shown in Figure 5. Before H2O2 addition, mutant worms had mean pump durations of 0.172s, 0.261s, and 0.153s while post-H2O2-addition mean pump durations were 0.140s, 0.309s, and 0.171s respectively. This difference was significant for only one of our three samples. The gap distances were of 0.373s, 0.747s, and 0.707s prior to the H2O2 addition and 0.712s, 1.19s, and 0.416s post-H2O2 addition. This change was significant for two of our three samples. Frequency values pre H2O2 addition were 4.36Hz, 2.33Hz, and 2.93Hz and 2.58Hz, 1.34Hz and 3.35Hz post H2O2 addition. As mentioned previously, we suspect that a decrease in pump frequency could be due to an inhibition of pumping which should be considered for future studies of frequency. A histogram and boxplot are shown in Figure 5B and 5C respectively to visualize possible differences in frequency between mutant controls and post-H2O2 mutant organisms. No statistical tests were run due to our small number of samples.

Finally, we completed a preliminary death over time assay for both wild-type and mutant EGL-19 C. elegans organisms to quantify the potential effects of pump duration

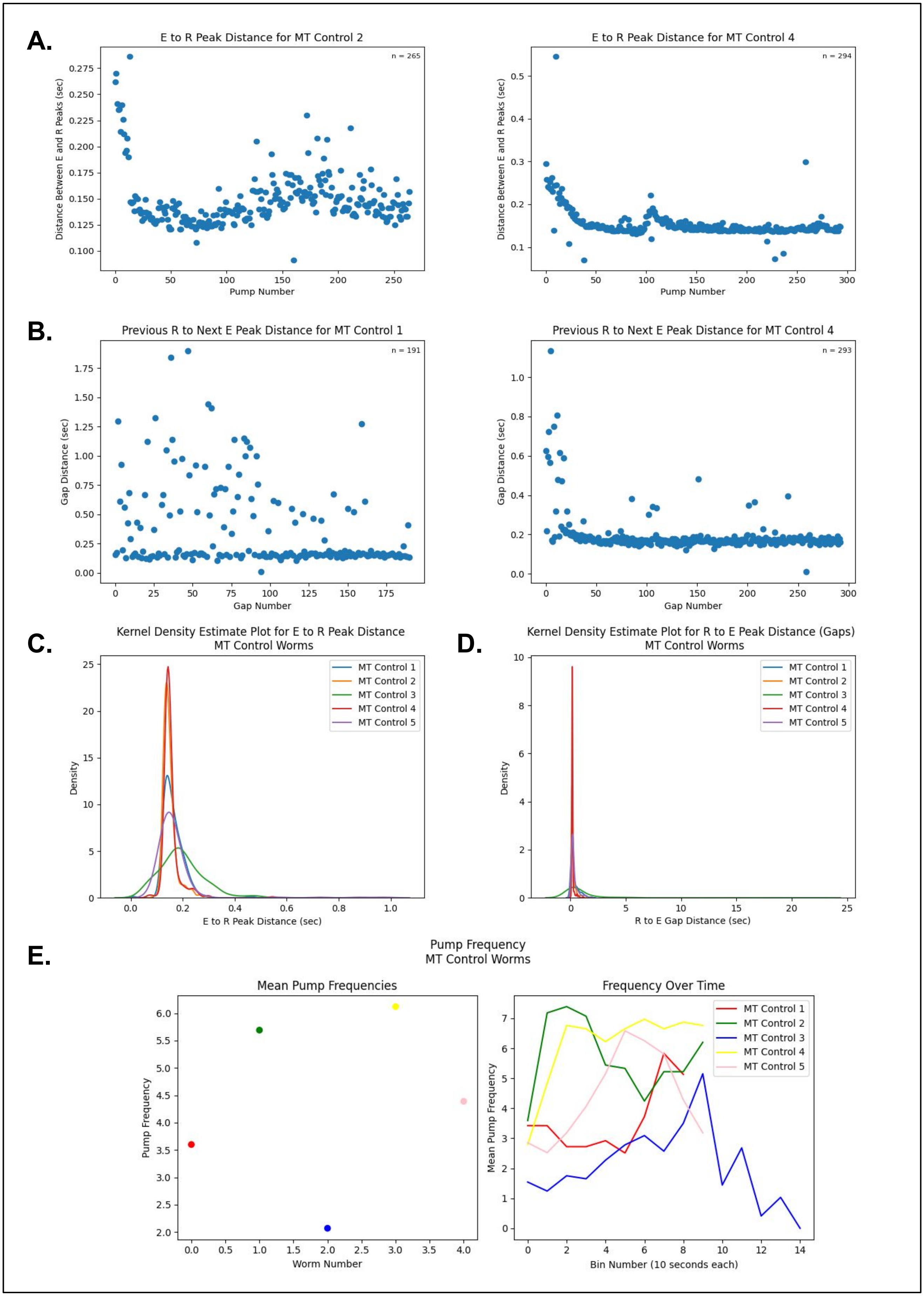

Figure 4. Mutant Control Worm Pump Characteristics

(A) Example distance values between E and R peaks of a single EPG trace pump from MT control worm 2 (left, n=265) and worm 4 (right, n=294) in the serotonin bath. Each blue dot represents the distance value for a single pump.
(B) Example distance values between the R peak of a previous pump and the E peak of a current pump for the EPG trace pumps from MT control worm 1 (left, n=191) and control worm 4 (right, n=293) in the serotonin bath. Each blue dot represents the gap distance value for a single pump.
(C) Kernel Density Estimate Plot for pump duration for all MT control worms in the serotonin bath.
(D) Kernel Density Estimate Plot for inter-pump gap duration for all MT control worms in the serotonin bath.
(E) Pump Frequency Mean values for each MT worm (left) and for all 10 second bins per worm EPG recording (right).

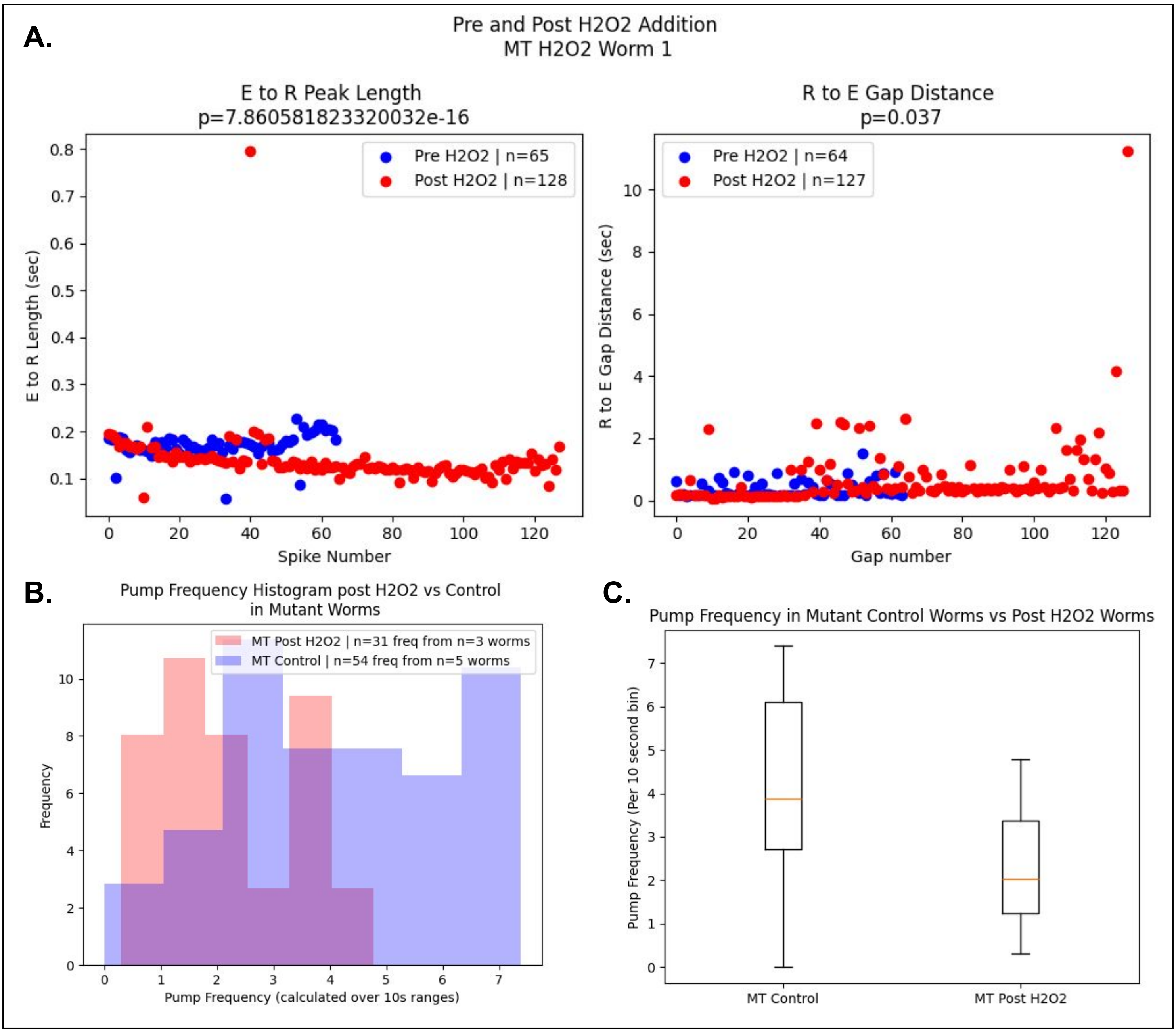

Figure 5. MT Worms After Exposure to H2O2

(A) Pre vs Post MT worm analysis. Pump length pre vs post for all MT worms in experimental condition (left). Inter-pump gap length pre vs post exposure to H2O2 for all MT worms in the experimental condition (right). Mann Whitney U Test was performed for pre and post conditions. Significant P values resulted for both pulse Length and inter-pump gap distance.

(B) Box plots for frequencies from all 10 second bins for MT control worms (left) and MT worms after exposure to H2O2 (right).

(C) Kernel Density Estimate Plot for pump duration for all MT control worms in the serotonin bath.

and frequency differences due to H2O2 presence. Worms of both types were subjected to .05M H2O2 and monitored over time. The assay was modeled from the Poke Test, as described by Park et al. (15), and modified as described in the methods section. As shown in Figure 6A, it is greatly possible that the mutant worms were affected by the toxic H2O2 at a faster rate than wild-type organisms. However, this cannot be said with certainty as a greater number of trials would need to be run over a longer period of time.

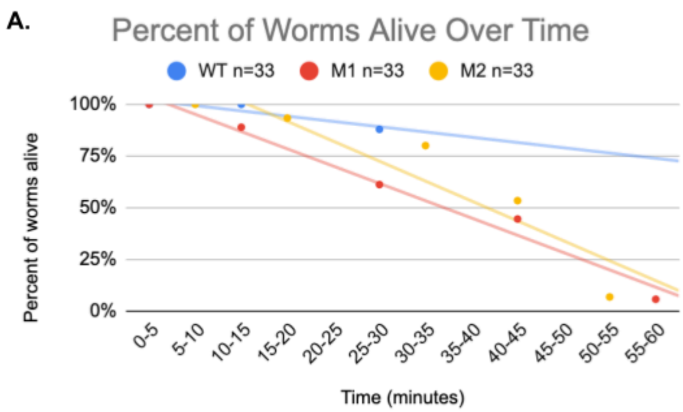

Figure 6. Death assay

(A) Percentage of worms alive over time after H2O2 addition n=33 for all trials. WT are wild-type worms while both M1 and M2 trials were completed with EGL-19 mutant worms. The single wild-type worm trial was only completed for 30 minutes as opposed to 60 for both M1 and M2 trials.

Discussion:

The findings of this study provide a preliminary look into the impact of Hydrogen peroxide on C. elegans pump characteristics. We first analyzed both wild-type (WT) and EGL-19 mutant (MT) C. elegans mean pump duration, mean inter-pump gap length, and mean pump frequency over time in control baths containing 1mM serotonin. Wild-type worms showed a mean pump duration of 165ms +- 35 ms while mutant worms had a mean pump duration of 165ms +- 58ms. This is unlike previous literature that found

EGL-19 mutant worms to have a longer pump duration and does not support our hypothesis. However, WT worms had a mean inter-pump gap length of 427 ms compared to 390 ms in MT worms. Correspondingly WT worms had a mean pump frequency of 5.42 Hz (range of 1.06 Hz to 7.37 Hz) compared to 4.37 Hz (range of 0.41 Hz to 7.39 Hz) for MT worms. This significant difference in frequency matches findings from previous literature and can be explained by the mutant's defective relaxation of the terminal bulb.

We then sought to determine the impact of H2O2 on these pump characteristics. The addition of 5mM H2O2 caused a decrease of 16.49 ms in mean pump length in WT worms and an increase of 11.23 ms in MT worms. This corresponds to our hypothesis that the addition of H2O2 would cause an increase in pump length in MT worms. However, because of our small sample size, we are unable to determine the significance of these values. The mean pump frequency across wild-type worms decreased by 3.1 Hz following the addition of H2O2. In all worms, the highest mean frequency after H2O2 addition occurred in the first 10 seconds following the toxin's addition. These frequency values were smaller than the mean pump frequency of each worm prior to H2O2 addition. It is unsure whether these can be considered "burst-like" as these values cannot be tested for significance due to small sample size and spike numbers.

Interestingly, the mean pump frequency in EGL-19 worms only decreased by 0.783 Hz between pre- and post-H2O2 addition. Additionally, we did not observe "burst" increases in pump frequency following the addition of H2O2 into the petri dish. In fact, the pump frequency remained relatively high (similar to the pre-H2O2 frequency) and only showed subsequent inhibition in one of our three samples.

While these results suggest interesting nuances, future studies should keep in mind the following limitations of our study. Importantly, our study only contained n=4 WT worms for the H2O2 condition and n=3 worms for the MT condition. This is a large limitation as we were unable to retrieve significant data between samples and H2O2 addition. Future studies should consider recording all worms at least 45 seconds prior to H2O2 addition in order to have enough pump data to compare pre- and post-H2O2 pump statistics per worm. All H2O2 recordings in our study also contained large amounts of recording noise during and immediately following H2O2 addition due to the physical presence of the micropipette and human hand. This noise was too great to properly analyze pump statistics immediately following the H2O2 addition and may have hidden important changes in pump frequency that would have resembled the “bursts” as noted in (5). Automating the addition of H2O2 into the recording bath would allow for this noise to be removed. Finally, the Poke Test Assay should be completed for longer periods with greater trials for both mutant and wild-type worms to allow for proper analysis of H2O2 impact on mortality rate over time.

These modifications to our experimental design are important and should be considered as research on pharyngeal spitting behavior and response to hydrogen peroxide in C. elegans could allow for a novel understanding of neural circuitry and its impact on behavior. For example, the light-sensitive M1 neuron, once considered to be potentially redundant, is now a critical element for specialized behaviors such as spitting, the detection of internally generated H2O2, and noxious light (7, 8). The self-contained nature of the pharyngeal nervous system in C. elegans also presents a unique opportunity for advancing our understanding of neural learning. Research has shown that C. elegans can exhibit associative learning and can modify behaviors based on environmental cues, such as inhibiting pumping in response to noxious stimuli paired with light (8, 16). Identifying a learning paradigm within the pharyngeal circuit and understanding relevant stimuli (such as hydrogen peroxide) could revolutionize our approach to studying neural adaptation and memory, providing a blueprint for exploring these processes in more complex organisms (18).

As this and recent studies continuously remind us, the study of the seemingly simple C. elegans consistently uncovers new scientific directions; our exploration and understanding is far from complete.

Resource availability:

<table>
<tr><td colspan="2"><u>Data and code availability</u>: All original code and data are in GitHub and publicly available as of April 2024 and can be accessed through the following link: https://github.com/BruneBettler/BIOL-389-C.-elegans

<u>Statistical Analysis:</u> All statistical tests used the Mann Whitney U Test completed on Python with the scipy.stats library unless specified otherwise.</td></tr>
<tr><td colspan="2"><u>Experimental model and subject details</u>:
Worms were stored and prepared before manipulation as necessitated by the BIOL 389 lab course. All worms were washed with Dent’s solution and recorded with a 1mM serotonin. A total of (33+8+4) wild-type C. elegans were studied, in addition to (66+5+3) EGL-19 mutant C. elegans for a total of 119 worms. More worms were present in the dish during recordings but were not taken into account in this study.</td></tr>
<tr><td><u>Hardware and behavior setup</u>:
- Digidata 1550B
- Assembled as described in the First EPG Lab_AMsystems amp document</td><td><u>Software</u>:
- AxoScope 10
- Matlab
- Python</td></tr>
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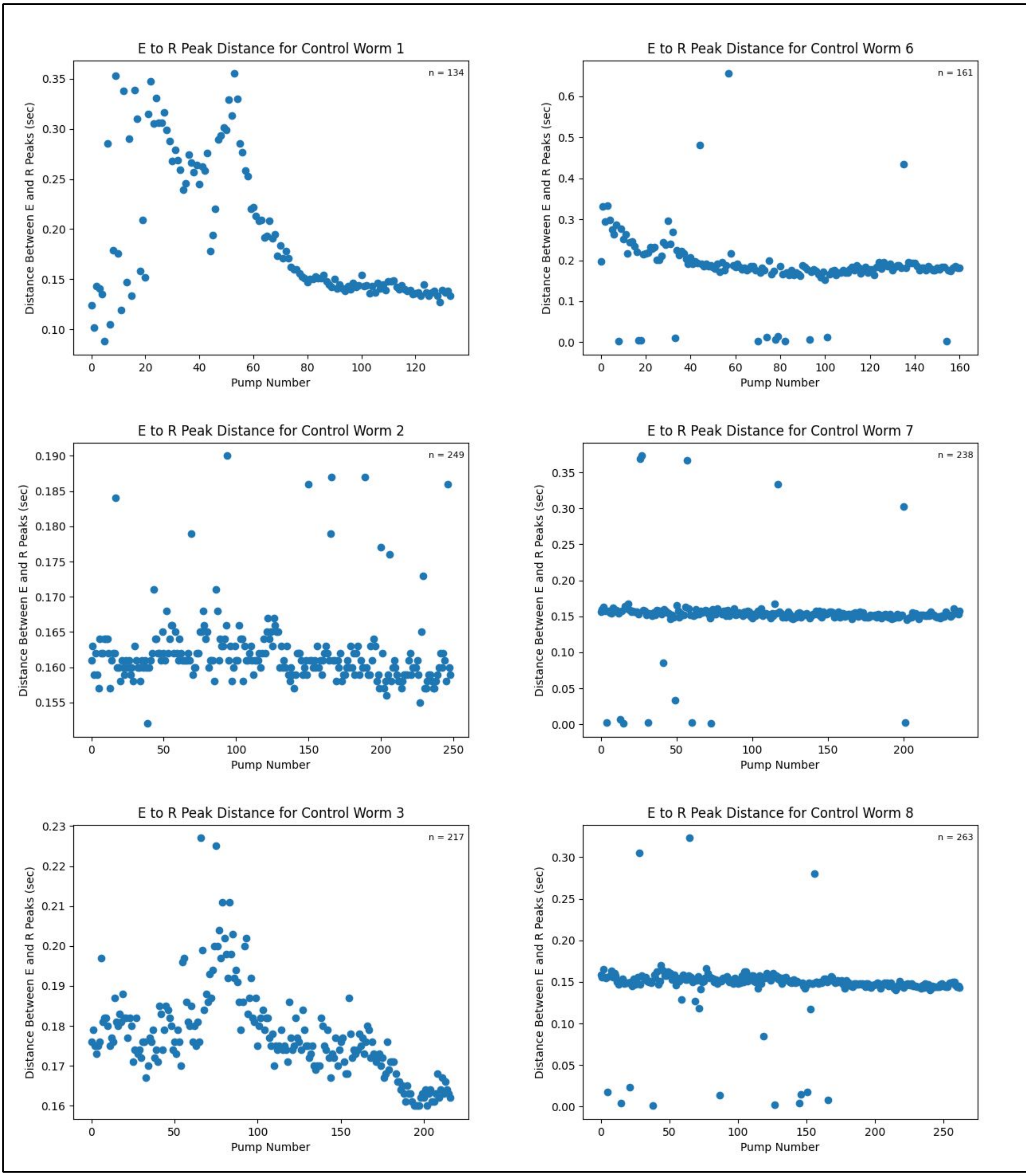
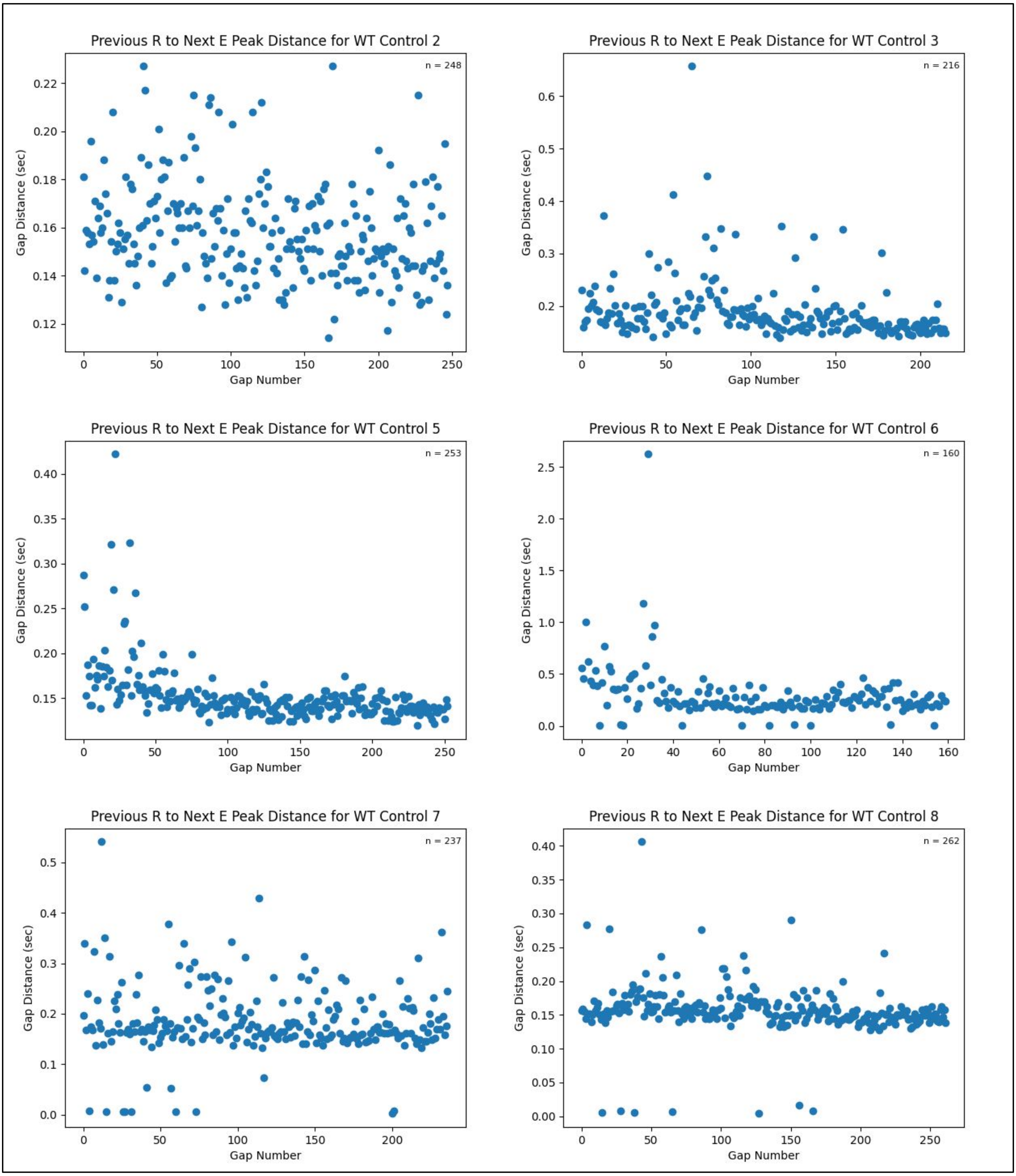


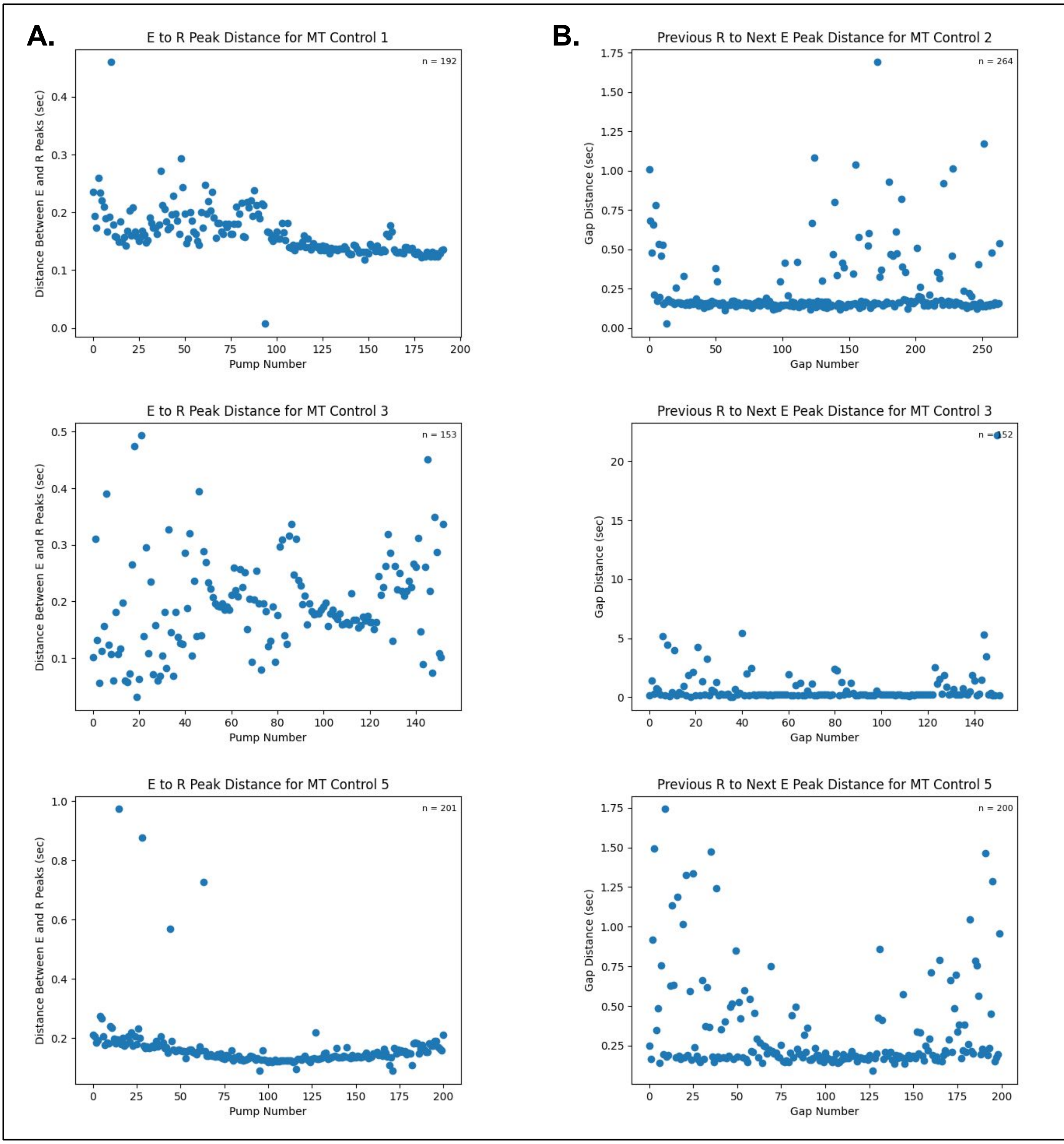
Supplementary Figure 1. WT Worms Pump Duration

All worm pump duration plots not shown in the main figures are included here.



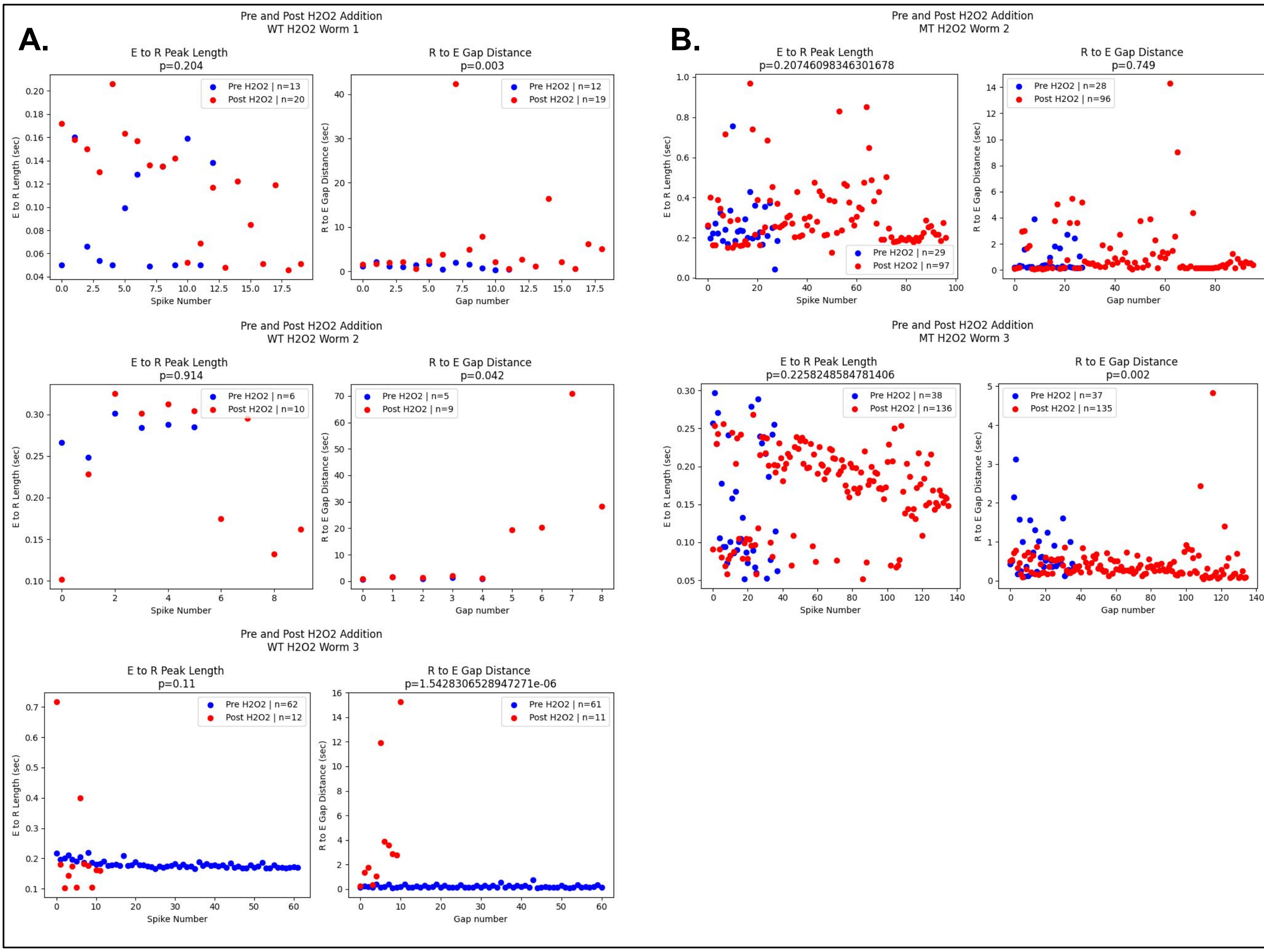
Supplementary Figure 2. WT Worm Inter-Pump Gap Duration

All worm inter-pump gap duration plots not shown in the main figures are included here.



Supplementary Figure 3. MT Worm Pump and Inter-Pump Duration

(A) All worm pump duration plots not shown in the main figures are included here.
(B) All worm inter-pump gap duration plots not shown in the main figures are included here.



Supplementary Figure 4. WT and MT Pre and Post H2O2 Addition

(A) All WT worm pre and post H2O2 addition plots not shown in the main figures are included here.
(B) All MT worm pre and post H2O2 addition plots not shown in the main figures are included here.

Supplementary Figures:

References:

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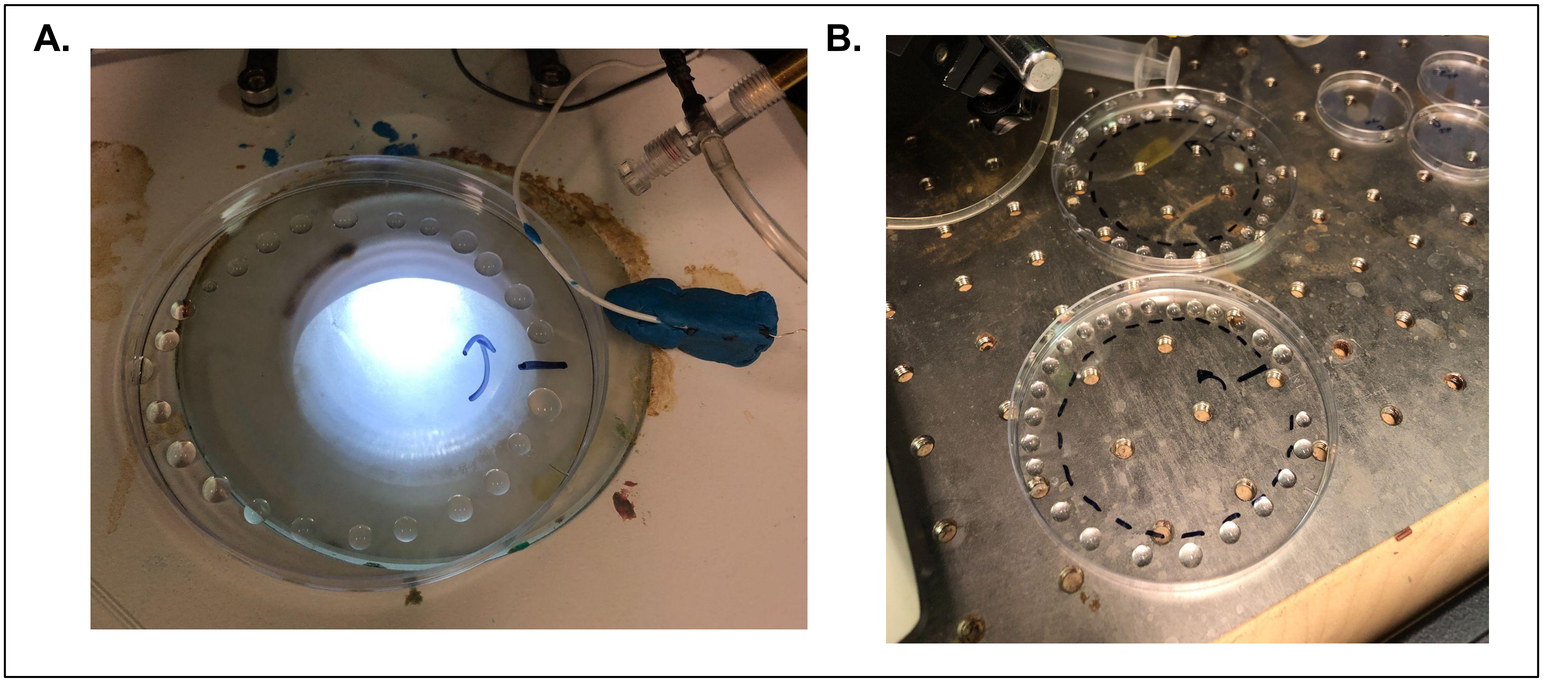
Appendix:

Figure 1: Liquid Poke and Death Test

Table 1: WT Worm Data

Table 2: Mutant Worm Data



Appendix Figure 1. Liquid Poke and Death test

(A) 15x150mm petri dish on microscope containing individual drops in which 0-3 worms were contained. This setup allowed for the same worms to be monitored over time and poked if and when necessary to determine their alive or dead status.

(B) A second view of the individual droplets of .05M H2O2 solution in which each drop contained 0-3 worms that were monitored over time.

WT CONTROL WORMS											
n=8											
WT CONTROL ER DURATION		Worm num: 0	Worm num: 1	Worm num: 2	Worm num: 3	Worm num: 4	Worm num: 5	Worm num: 6	Worm num: 7		
seconds	mean	0.1957089552	0.1620281124	0.1774608295	0.1429128631	0.1589173228	0.1875838509	0.1524201681	0.146513308		
	std	0.06965628028	0.005143250427	0.01110235518	0.03080841415	0.01258721758	0.0760902177	0.03980656924	0.03244754195		
	variance	0.004851997382	2.65E-05	0.0001232622906	0.0009491583823	0.0001584380464	0.00578972123	0.001584562955	0.001052842979		
	median	0.155	0.161	0.176	0.142	0.154	0.183	0.153	0.15		
	range	0.267	0.038	0.067	0.386	0.09	0.653	0.372	0.322		
	IQR	0.1175	0.003	0.011	0.013	0.015	0.023	0.005	0.0085		
	skewness	0.770619847	3.134388166	1.266271224	1.789750533	1.865974538	1.214150815	0.9702912748	-1.51179937		
	kurtosis	-0.825455409	11.92900738	2.900961035	34.93364681	5.434376299	10.58793065	17.17875031	15.52214059		
WT CONTROL RE GAP LENGTH		Worm num: 0	Worm num: 1	Worm num: 2	Worm num: 3	Worm num: 4	Worm num: 5	Worm num: 6	Worm num: 7		
seconds	mean	0.6545413534	0.1581693548	0.1911388889	0.1872875	0.1522252964	0.2941875	0.1838481013	0.156759542		
	std	0.7003784102	0.02060798537	0.05796685808	0.09336177757	0.03267193246	0.2521494643	0.06775696616	0.03753440412		
	variance	0.4905299175	0.0004246890609	0.003360156636	0.00871642151	0.00106745517	0.06357935234	0.004591006463	0.001408831493		
	median	0.338	0.155	0.1745	0.1575	0.146	0.2385	0.169	0.154		
	range	2.997	0.113	0.518	0.988	0.303	2.623	0.539	0.402		
	IQR	0.663	0.02525	0.03725	0.052	0.017	0.15675	0.054	0.01975		
	skewness	1.918765751	0.8830391994	3.801528818	3.465876065	4.254095881	5.519951249	0.7799188034	0.2428823455		
	kurtosis	3.010232673	0.9195784687	21.1934493	23.27126543	24.47765436	44.6476536	4.563033559	13.38729156		
WT CONTROL FREQUENCIES		Worm num: 0	Worm num: 1	Worm num: 2	Worm num: 3	Worm num: 4	Worm num: 5	Worm num: 6	Worm num: 7		
Hz	mean frequency	2.39	6.29	5.35	6.03	6.46	4.21	5.98	6.62		
	frequency per 10 second bin	1.48	6.35	5.32	6.16	5.49	2.91	5.82	6.75		
		1.17	6.04	5.53	4.34	5.69	3.32	6.22	6.14		
		1.17	6.15	4.62	5.65	6.4	3.01	5.82	6.54		
		1.06	6.25	5.02	5.55	6.91	4.57	5.72	6.44		
		1.8	6.35	5.53	6.06	7.01	6.02	5.92	6.75		
		2.23	6.45	5.53	7.37	6.81	5.29	6.02	6.85		
		1.48	6.45	5.93	7.07	6.91	4.36	6.32	6.85		
		1.27									
		3.5									
		5.52									
		5.62									
WT H2O2 FREQUENCIES		worm 0	worm 1	worm 2	worm 3			worm 0	worm 1	worm 2	worm 3
Hz	Pre mean frequency	2.16	0.15	6.22	5.62		post mean frequency	0.36	0.13	0.58	0.68
	Pre frequency per 10 second bin	[2.16] Hz	0.15 Hz	[6.34 6.09] Hz	[5.55 5.69] Hz		post frequency per 10 second bin	[1.22 0.41 0. 0. 0. 0.2 0.2 1.01 0. 0.61] Hz	[1.21 0. 0.2 0. 0.2 0. 0. 0. 0. 0. 0. 0.2 0. 0.] Hz	[1.26 0.21 0.63 0.21] Hz	[3.22 0. 0.43 0. 0. 0.43] Hz
WT H2O2 STATS	pre length ---------	pre length ---------	pre length ---------	pre length ---------		pre gap ---------	pre gap ---------	pre gap ---------	pre gap ---------		
seconds	Worm num: 0	Worm num: 1	Worm num: 2	Worm num: 3		Worm num: 0	Worm num: 1	Worm num: 2	Worm num: 3		
	Mean: 0.09138461538461556	Mean: 0.2786666666666673	Mean: 0.1802580645161292	Mean: 0.19420408163265274		Mean: 1.1484999999999999	Mean: 1.0824	Mean: 0.21511475409836045	Mean: 0.277979166666667		
	Standard Deviation: 0.044239798409504566	Standard Deviation: 0.017104255480890056	Standard Deviation: 0.012093634001021964	Standard Deviation: 0.14467457917993526		Standard Deviation: 0.5925050632695051	Standard Deviation: 0.288941931882515	Standard Deviation: 0.11757719450455718	Standard Deviation: 0.1784662070700562		
	Variance: 0.001957159763313603	Variance: 0.00029255555555555746	Variance: 0.00014625598335067454	Variance: 0.020930733860891355		Variance: 0.3510622500000003	Variance: 0.08348743999999994	Variance: 0.013824396667562472	Variance: 0.031850187065972174		
	Median: 0.06599999999999984	Median: 0.2845	Median: 0.1770000000000005	Median: 0.18599999999999994		Median: 1.1039999999999999	Median: 1.0	Median: 0.1509999999999998	Median: 0.307500000000001		
	Range: 0.11100000000000043	Range: 0.05300000000000016	Range: 0.05399999999999938	Range: 0.8020000000000014		Range: 1.806000000000001	Range: 0.8249999999999997	Range: 0.6429999999999989	Range: 0.6200000000000019		
	IQR: 0.08499999999999908	IQR: 0.016750000000000265	IQR: 0.012500000000001288	IQR: 0.0330000000000125		IQR: 0.8692499999999996	IQR: 0.3649999999999977	IQR: 0.1509999999999998	IQR: 0.1842499999999978		
	Skewness: 0.3888647729041469	Skewness: -0.6166616065095396	Skewness: 1.5453330204702707	Skewness: 2.60933804047138		Skewness: 0.14285776395977304	Skewness: 0.35835767739969526	Skewness: 2.068392456315639	Skewness: -0.1677989235189807		
	Kurtosis: -1.583463605901747	Kurtosis: -0.7236810997868268	Kurtosis: 2.009828692978103	Kurtosis: 9.331662562368235		Kurtosis: -1.1475358565773597	Kurtosis: -1.1643913046611971	Kurtosis: 5.722015843716417	Kurtosis: -0.7790381432245783		
	post length ---------	post length ---------	post length ---------	post length ---------		post gap ---------	post gap ---------	post gap ---------	post gap ---------		
	Worm num: 0	Worm num: 1	Worm num: 2	Worm num: 3		Worm num: 0	Worm num: 1	Worm num: 2	Worm num: 3		
	Mean: 0.11545000000000272	Mean: 0.23360000000000075	Mean: 0.21724999999999905	Mean: 0.11221739130434781		Mean: 5.591894736842103	Mean: 16.22511111111111	Mean: 4.095272727272728	Mean: 2.8530454545454544		
	Standard Deviation: 0.04741252471657537	Standard Deviation: 0.08000149998593555	Standard Deviation: 0.16823204460902602	Standard Deviation: 0.04225693307842748		Standard Deviation: 9.389335467179531	Standard Deviation: 21.773112534318532	Standard Deviation: 4.67168505886861	Standard Deviation: 6.677949150867373		
	Variance: 0.0022479474999998706	Variance: 0.006400239999999645	Variance: 0.02830202083333332	Variance: 0.001785648393194699		Variance: 88.15962051523546	Variance: 474.06842943209875	Variance: 21.824641289256203	Variance: 44.59500486157026		
	Median: 0.12600000000000122	Median: 0.2614999999999972	Median: 0.16800000000000104	Median: 0.125		Median: 2.1610000000000014	Median: 1.9469999999999992	Median: 2.7849999999999966	Median: 0.29650000000000176		
	Range: 0.1599999999999966	Range: 0.22299999999999898	Range: 0.6140000000000008	Range: 0.17600000000000904		Range: 41.85399999999999	Range: 70.07000000000001	Range: 15.0	Range: 28.616000000000003		
	IQR: 0.08699999999999708	IQR: 0.13799999999999324	IQR: 0.04775000000000151	IQR: 0.05950000000000344		IQR: 3.328499999999991	IQR: 19.122	IQR: 2.5444999999999993	IQR: 1.4742500000000032		
	Skewness: -0.14171371240831449	Skewness: -0.3445799296972615	Skewness: 2.1973278743231064	Skewness: 0.5508166725934276		Skewness: 3.190833561256442	Skewness: 1.6066900069712158	Skewness: 1.5053621697595299	Skewness: 3.080367900433547		
	Kurtosis: -1.1070474651785975	Kurtosis: -1.5070844222520536	Kurtosis: 3.662950432237807	Kurtosis: 0.27601465702942374		Kurtosis: 9.498593537493376	Kurtosis: 1.5599944437034798	Kurtosis: 0.7792735020228507	Kurtosis: 8.310315274687404		

MT CONTROL WORMS								
n=5								
MT CONTROL ER DURATION		Worm num: 0	Worm num: 1	Worm num: 2	Worm num: 3	Worm num: 4		
seconds		Mean: 0.1638125000000001	Mean: 0.14873207547169837	Mean: 0.19383006535947686	Mean: 0.15379591836734674	Mean: 0.16593034825870653		
		Standard Deviation: 0.04094096677432849	Standard Deviation: 0.026579178782442307	Standard Deviation: 0.08297686825074559	Standard Deviation: 0.035899034299161935	Standard Deviation: 0.09560821341295937		
		Variance: 0.0016761627604166697	Variance: 0.0007064527447490314	Variance: 0.00688516066470159	Variance: 0.0012887406636124049	Variance: 0.009140930472017984		
		Median: 0.15549999999999997	Median: 0.14099999999999824	Median: 0.18599999999999994	Median: 0.14400000000000546	Median: 0.14700000000000557		
		Range: 0.4530000000000083	Range: 0.1949999999999985	Range: 0.46199999999999974	Range: 0.47600000000000087	Range: 0.8840000000000003		
		IQR: 0.045250000000010004	IQR: 0.02199999999999136	IQR: 0.0969999999999942	IQR: 0.013750000000001705	IQR: 0.03899999999999437		
		Skewness: 2.23525519989234	Skewness: 2.226451989644603	Skewness: 0.8076678296261335	Skewness: 5.4095166778051516	Skewness: 6.416240156447536		
		Kurtosis: 14.074235002480854	Kurtosis: 6.3293337382732275	Kurtosis: 1.2620108239540233	Kurtosis: 48.707438098234654	Kurtosis: 45.063053745023325		
MT CONTROL RE GAP LENGTH		Worm num: 0	Worm num: 1	Worm num: 2	Worm num: 3	Worm num: 4		
seconds		Mean: 0.35669109947643973	Mean: 0.23446212121212096	Mean: 0.8293157894736845	Mean: 0.19606484641638244	Mean: 0.33585499999999996		
		Standard Deviation: 0.3551035154355804	Standard Deviation: 0.20478787640574123	Standard Deviation: 2.0289498658596132	Standard Deviation: 0.10733739615835151	Standard Deviation: 0.30601476104103215		
		Variance: 0.12609850667470748	Variance: 0.04193807432277314	Variance: 4.1166375581717425	Variance: 0.011521316614054894	Variance: 0.093645033975		
		Median: 0.16399999999999793	Median: 0.15899999999999537	Median: 0.2275000000000027	Median: 0.17099999999999227	Median: 0.19550000000000267		
		Range: 1.8879999999999981	Range: 1.6639999999999944	Range: 22.163999999999987	Range: 1.1239999999999988	Range: 1.6519999999999957		
		IQR: 0.3525000000000009	IQR: 0.029249999999996223	IQR: 0.41150000000000064	IQR: 0.021999999999998465	IQR: 0.19150000000000844		
		Skewness: 1.8947823349605737	Skewness: 3.4174265180200294	Skewness: 7.985656196478848	Skewness: 4.906238903756914	Skewness: 2.361806888282102		
		Kurtosis: 3.340181382841691	Kurtosis: 14.149396622152118	Kurtosis: 78.19638973580767	Kurtosis: 28.84776093594951	Kurtosis: 5.340518098915686		
MT CONTROL FREQUENCIES		Worm num: 0	Worm num: 1	Worm num: 2	Worm num: 3	Worm num: 4		
Hz	mean	mean 3.6	mean 5.69	mean 2.07	mean 6.12	mean 4.39		
	frequency per 10 second bin	[3.42 3.42 2.72 2.72 2.92 2.51 3.72 5.83 5.13] Hz	[3.59 7.18 7.39 7.07 5.44 5.33 4.24 5.22 5.22 6.2] Hz	[1.54 1.24 1.75 1.65 2.27 2.78 3.09 2.57 3.5 5.15 1.44 2.68 0.41 1.03 0.	[2.79 4.83 6.76 6.65 6.22 6.65 6.97 6.65 6.87 6.76] Hz	[2.85 2.52 3.18 4.06 5.15 6.58 6.25 5.81 4.28 3.18] Hz		
MT H2O2 FREQUENCIES		worm 0	worm 1	worm 2		worm 0	worm 1	worm 2
Hz	mean	mean 4.36	mean 2.33	mean 2.93		mean 2.58	mean 1.34	mean 3.35
	frequency per 10 second bin	Frequencies over time: [4.82 4.48 3.79] Hz	Frequencies over time: [2.23 2.44] Hz	Frequencies over time: [2.01 3.39 3.39] Hz		Frequencies over time: [4.78 3.46 1.53 2.03 2.44 3.26 3.	Frequencies over time: [1.35 2.29 1.14 0.62 0.73 2.18 1.56 1.56 1.04 0.62 0.31 0	Frequencies over time: [3.23 3.53 3.33 3.63 4.04 3.43 2.22] Hz
MT H2O2 STATS		worm 0	worm 1	worm 2		worm 0	worm 1	worm 2
Seconds		pre length ---------------	pre length ---------------	pre length ---------------		pre gap ---------------	pre gap ---------------	pre gap ---------------
		Worm num: 0	Worm num: 1	Worm num: 2		Worm num: 0	Worm num: 1	Worm num: 2
		Mean: 0.1720153846153845	Mean: 0.260862068965518	Mean: 0.15263157894736806		Mean: 0.37284375000000014	Mean: 0.747464285714285	Mean: 0.70672972972973
		Standard Deviation: 0.025905444813897758	Standard Deviation: 0.12067903401540703	Standard Deviation: 0.07949327050387202		Standard Deviation: 0.28262631129450344	Standard Deviation: 0.9371255245293927	Standard Deviation: 0.6323832933092741
		Variance: 0.0006710920710059023	Variance: 0.014563429250891765	Variance: 0.006319180055401768		Variance: 0.07987763183593757	Variance: 0.8782042487244894	Variance: 0.3999086296566834
		Median: 0.1720000000000006	Median: 0.23000000000000043	Median: 0.11049999999999738		Median: 0.2115000000000009	Median: 0.2759999999999998	Median: 0.4510000000000005
		Range: 0.16799999999999926	Range: 0.7129999999999992	Range: 0.2450000000000001		Range: 1.3699999999999974	Range: 3.740000000000002	Range: 3.0109999999999992
		IQR: 0.021000000000000796	IQR: 0.09699999999999775	IQR: 0.14950000000000152		IQR: 0.3875000000000033	IQR: 0.7914999999999994	IQR: 0.6769999999999943
		Skewness: -1.729345082707797	Skewness: 2.283031563783203	Skewness: 0.4009577777438149		Skewness: 1.5449304072104768	Skewness: 1.8762689348156043	Skewness: 1.9143574628208329
		Kurtosis: 6.155535433618876	Kurtosis: 7.451968447430231	Kurtosis: -1.4013160638362947		Kurtosis: 2.5151849196469644	Kurtosis: 2.7738242787167238	Kurtosis: 3.9646245512379306
		post length ---------------	post length ---------------	post length ---------------		post gap ---------------	post gap ---------------	post gap ---------------
		Worm num: 0	Worm num: 1	Worm num: 2		Worm num: 0	Worm num: 1	Worm num: 2
		Mean: 0.13939843750000036	Mean: 0.3093092783505159	Mean: 0.1705000000000007		Mean: 0.7121417322834642	Mean: 1.1925312499999996	Mean: 0.416429629629629
		Standard Deviation: 0.06340942603476706	Standard Deviation: 0.16188600805700956	Standard Deviation: 0.05555826300592191		Standard Deviation: 1.1649688227007255	Standard Deviation: 2.0238990111556374	Standard Deviation: 0.47838858435439685
		Variance: 0.004020755310058594	Variance: 0.026207079604634167	Variance: 0.0030867205882351913		Variance: 1.3571523578647147	Variance: 4.096167207356768	Variance: 0.22885563764060385
		Median: 0.1290000000000049	Median: 0.2610000000000028	Median: 0.1834999999999951		Median: 0.3789999999999907	Median: 0.46400000000000574	Median: 0.30299999999999727
		Range: 0.7349999999999994	Range: 0.8420000000000059	Range: 0.21599999999999397		Range: 11.175999999999988	Range: 14.238999999999983	Range: 4.784999999999997
		IQR: 0.02425000000000388	IQR: 0.18099999999998317	IQR: 0.07375000000000753		IQR: 0.5764999999999993	IQR: 1.0827499999999937	IQR: 0.34450000000000713
		Skewness: 8.679057625113142	Skewness: 1.9237003889806397	Skewness: -0.5454432154031521		Skewness: 6.214188132169107	Skewness: 3.7895070923994854	Skewness: 6.440705480044674
		Kurtosis: 86.91054998202428	Kurtosis: 3.9072840645753635	Kurtosis: -0.8196169258707005		Kurtosis: 50.510274171661884	Kurtosis: 18.441448263281057	Kurtosis: 53.37973955936017