Bradford Hull

Lifespan Analysis of Worms

Consultation: September 12th, 2024
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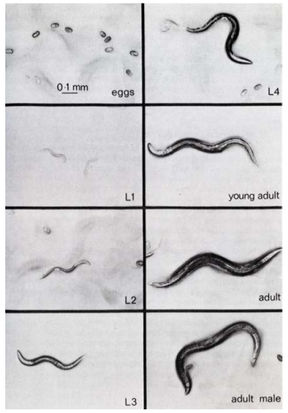
Background

Client: Bradford Hull

- 5th year PhD student in MCB
- Studying aging in worms

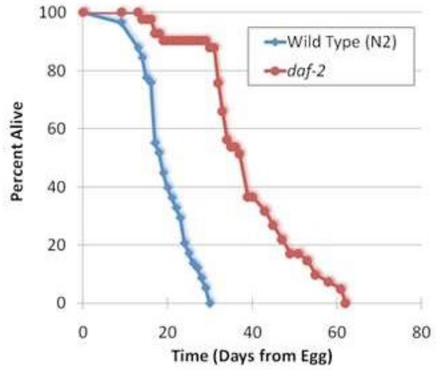


A

Time (Days from Egg)	Number Dead: Wild Type (N = 58)	Number Dead: *daf-2* (N = 41)	Percent Alive: Wild Type (N = 58)	Percent Alive: *daf-2* (N = 41)
0	0	0	100	100
9	2	0	97	100
13	5	0	88	100
14	2	1	84	98
15	4	0	78	98
16	1	0	76	98
17	12	2	55	93
18	2	0	52	93
19	4	1	45	90
20	3	0	40	90
21	2	0	36	90
22	2	0	33	90
23	2	0	29	90
24	5	0	21	90
25	2	0	17	90
26	2	0	14	90
27	1	0	12	90
28	2	0	9	90
29	2	0	5	90
30	3	1	0	88

B

Time (Days from Egg)	Number Dead: Wild Type (N = 58)	Number Dead: *daf-2* (N = 41)	Percent Alive: Wild Type (N = 58)	Percent Alive: *daf-2* (N = 41)
31		0		88
32		5		76
33		4		66
34		4		56
35		1		54
36		0		54
37		1		51
39		6		37
40		0		37
43		2		32
45		2		27
47		2		22
49		2		17
51		0		17
53		1		15
55		2		10
58		1		7
61		1		5
62		2		0



Lifespan data & K-M curve from provided example paper

Sutphin GL, Kaeberlein M. Measuring Caenorhabditis elegans life span on solid media. J Vis Exp. 2009 May 12;(27):1152. doi: 10.3791/1152. PMID: 19488025; PMCID: PMC2794294.

Summary of the Problem

- Bradford wants to compare lifespans of worms that are all drawn from the same batch of eggs placed in different media
  - Experimental Units
    - Agar culture (individual plates)
      - ~40 worms per plate
    - Liquid culture (reservoir)
      - ~5000 worms per reservoir
- Treatments are “stressors” added to each media, and he wants to evaluate the effects of stressors in each media on worm lifespans
- He has collected data and wants to perform statistical analyses of the effects of treatments both within media as well as between media to determine if different.
- There were differences between how he was able to capture lifespan data between the liquid and agar media due to some measurement challenges.
- His primary concerns were whether the experimental procedures were adequate to carry out his intended analyses.
  - Prefers Kaplan-Meier if feasible with the data he captured

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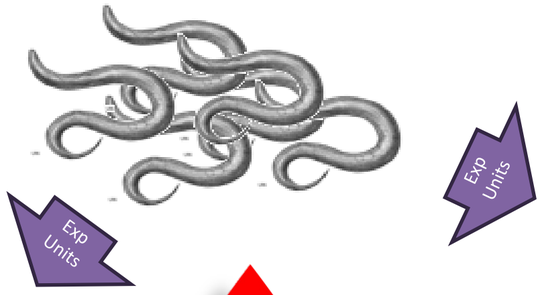
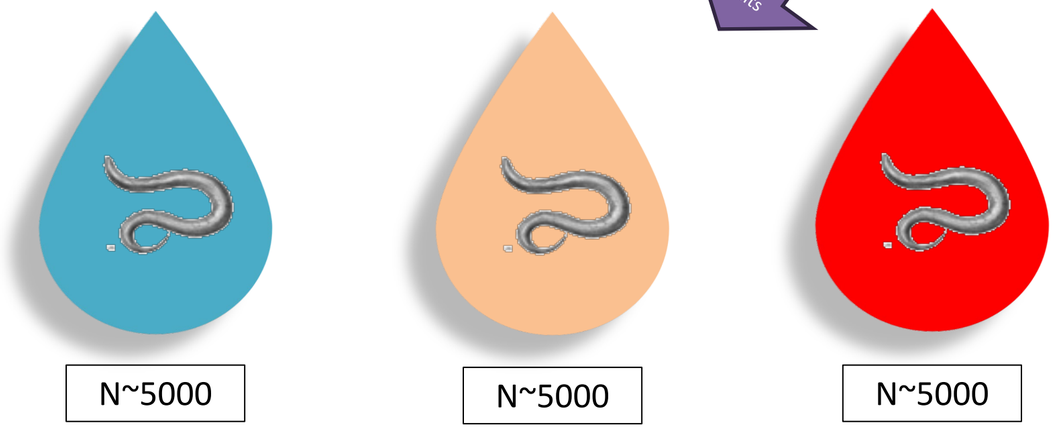
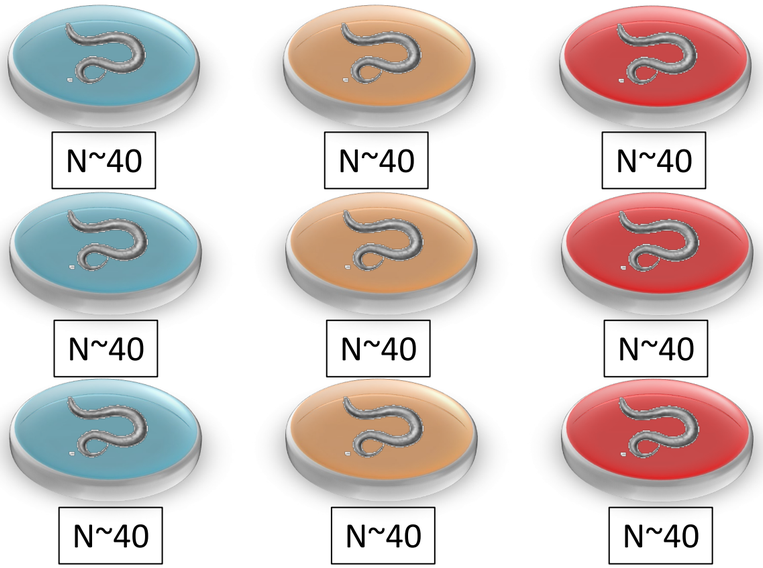
Treatments (stressors)

- **Control (no stressor)**
- **Copper**
- **DTT (dithiothreitol)**





Media: **Liquid culture** (reservoirs)



Media: **Agar culture** (plates)

Experiment

- Experimental units were made using eggs from the same batch/population, started at same time
- Data is recorded every **two days** for all experimental units
  - **Agar** – raw **number of alive remaining** are recorded
  - **Liquid** - ~150mL sampled from each and **number of alive/dead** recorded (sample *is discarded*, i.e. sampling *without replacement.*
    - **Three replicates** are performed per experimental unit per timestep (matches number Agar replicates)

Additional Information

- Number of worms per experimental unit & number of replicates determined by a power analysis in MCB for this type of experiment.
- The **R** package `survival` was used for analysis of Agar data

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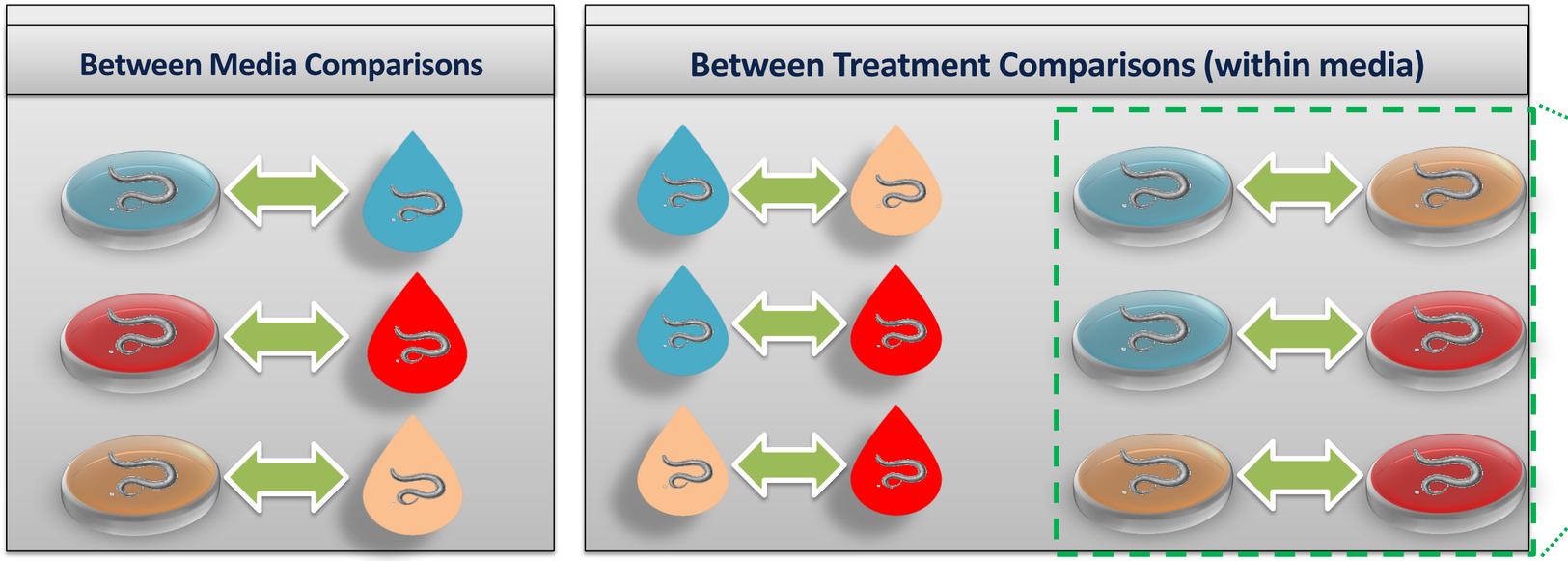

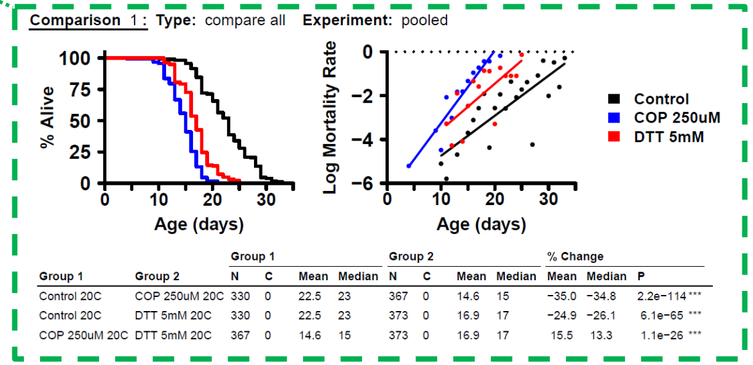

		Group 1				Group 2				% Change		
Group 1	Group 2	N	C	Mean	Median	N	C	Mean	Median	Mean	Median	P
Control 20C	COP 250uM 20C	330	0	22.5	23	367	0	14.6	15	−35.0	−34.8	2.2e−114 ***
Control 20C	DTT 5mM 20C	330	0	22.5	23	373	0	16.9	17	−24.9	−26.1	6.1e−65 ***
COP 250uM 20C	DTT 5mM 20C	367	0	14.6	15	373	0	16.9	17	15.5	13.3	1.1e−26 ***

Agar treatment comparisons have already been performed

Consultant Next Steps

- *Determine if data that has been captured can be applied to the Kaplan-Meier statistical analysis framework to perform these comparisons*
- *Can we recommend K-M, and if we cannot then what is our recommendation?*
- Based on our analysis of the provided data and experimental procedure details
  - Agar media data ***are*** suitable for K-M
  - Liquid media data ***are not*** suitable for K-M
- **Next steps**
  - Determine if/how there is an adequate statistical approach to analyze liquid lifespan data
  - If so, can we do all of desired comparisons?

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