FLIES IN A MINE

Exploring the Biology of Deep Underground Mining

John Hick
Merritt Lab
Laurentian University

SNOLAB Future Projects Workshop

McGill University and Génome Québec Innovation Centre
Innovation in action

Laurentian University
Université Laurentienne

Mining Environment

- Miners have to work in depths as great as 3.9 km.
- Atmospheric pressure over 20% greater than surface.
- Physically Strenuous Job

(Koven, 2010)

Physiological Effects of Mining

Physically Exhausting

(Merritt, 2016)

Hungry Faster

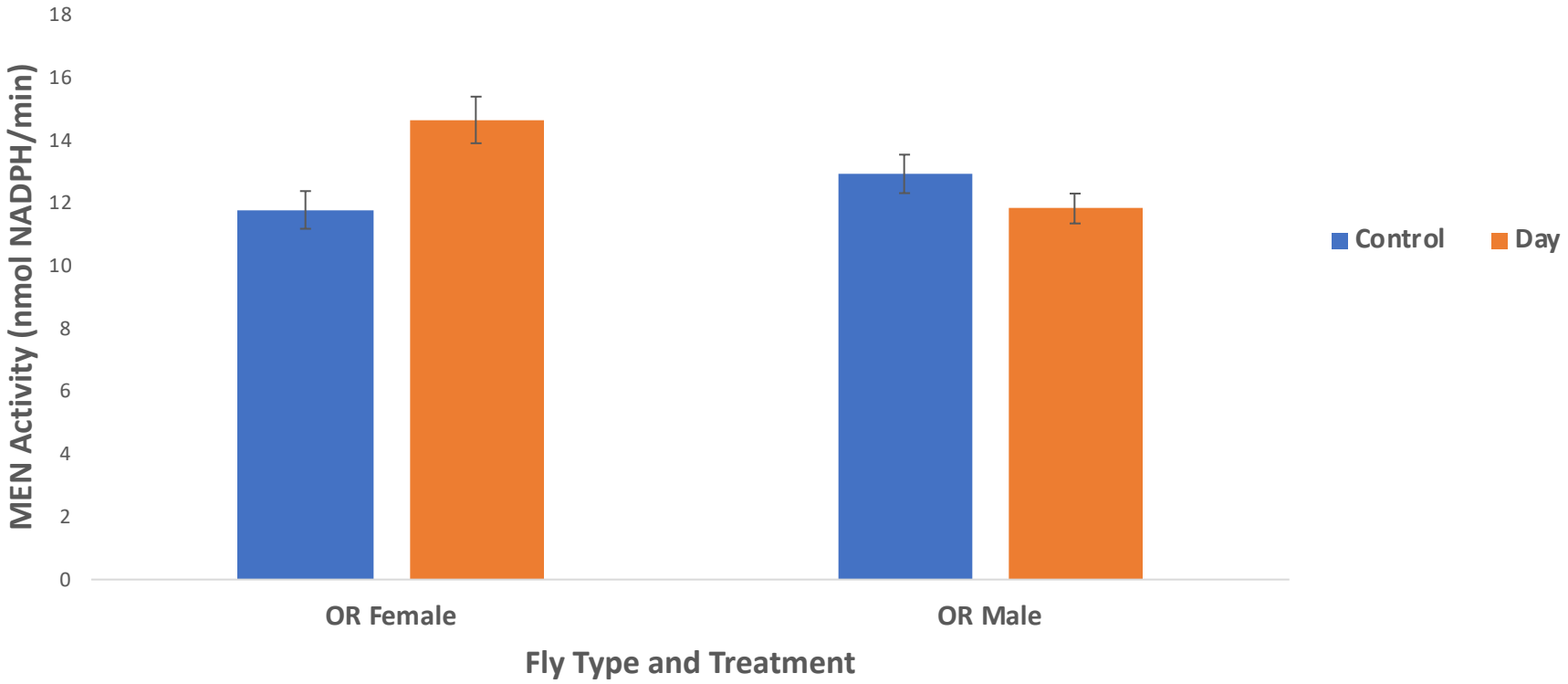
(Public Domain, 2016)

Difficulty Thinking

(Public Domain, 2016)

Beginnings of FLAME

1 Day In SNOLAB



Malic Enzyme Kinetic Assay Oregon R 1 Day Underground

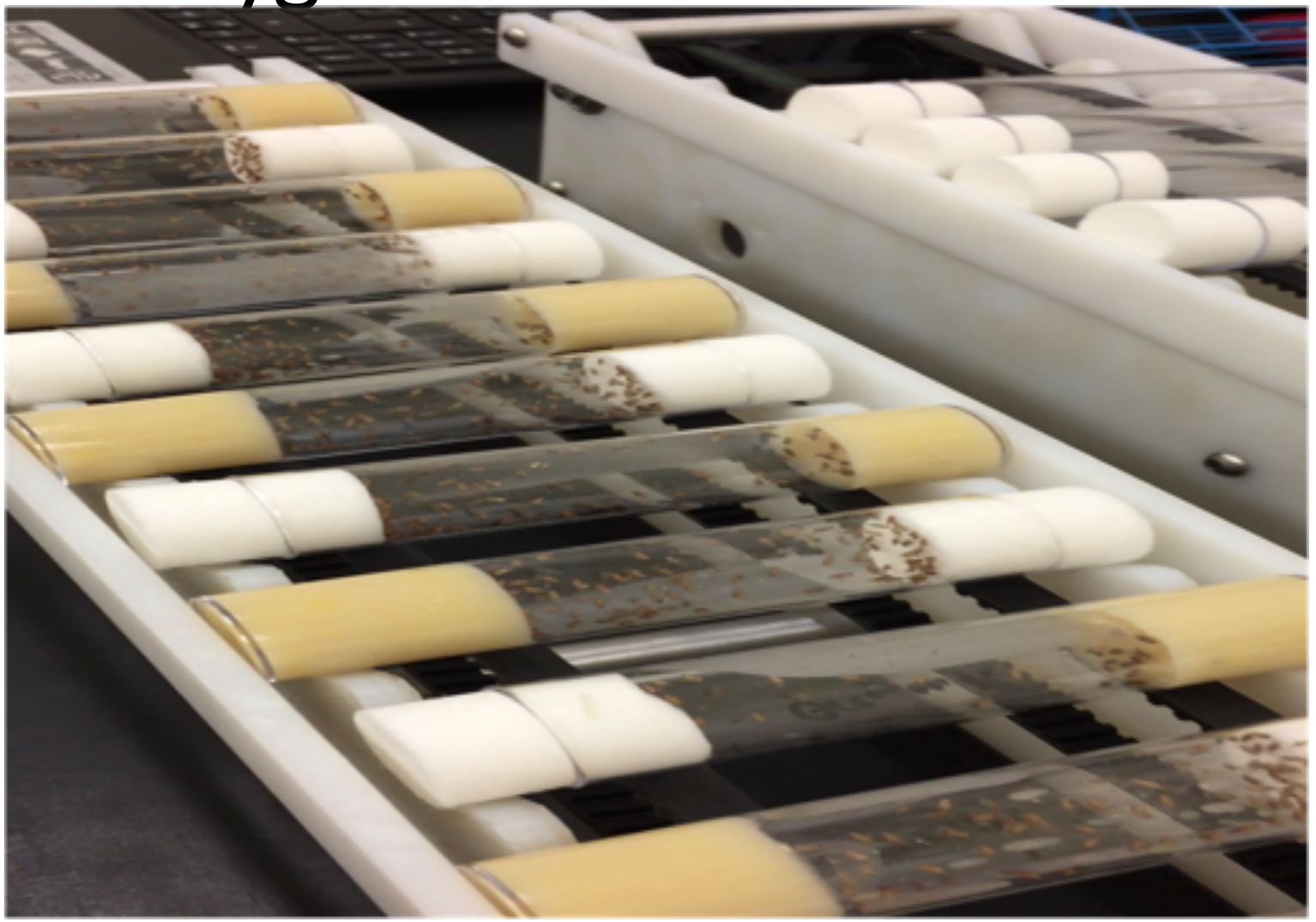
Error Bars - SE

The Fly Treadmill

Flygometer 1.0

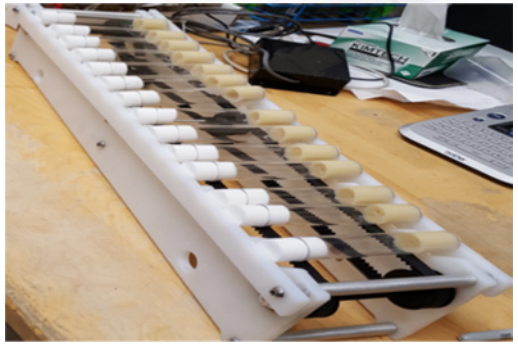
Flygometer 2.0

Flygometer 2.0 In Action



Fly Treatments

Treatment 1

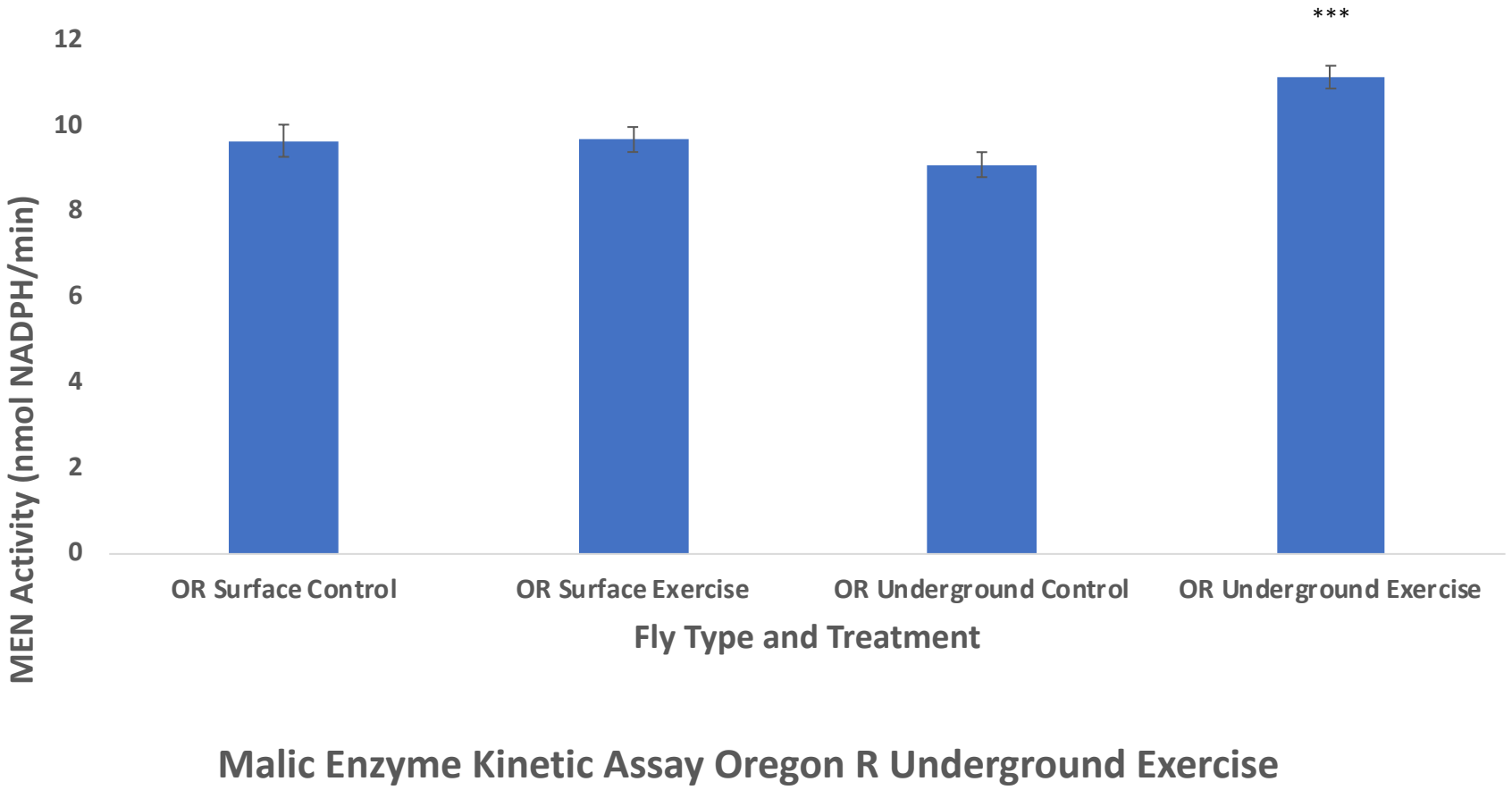


Treatment 2

Treatment 3

Treatment 4

Preliminary Data

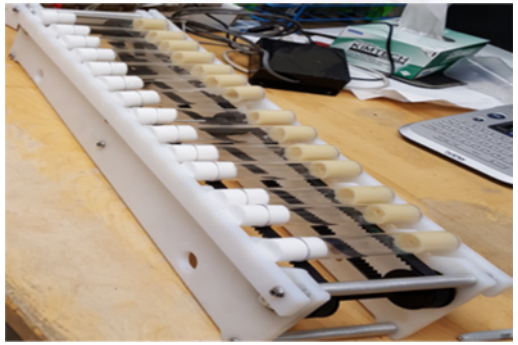


Malic Enzyme Kinetic Assay Oregon R Underground Exercise

Error Bars - SE

Fly Treatments

Treatment 1

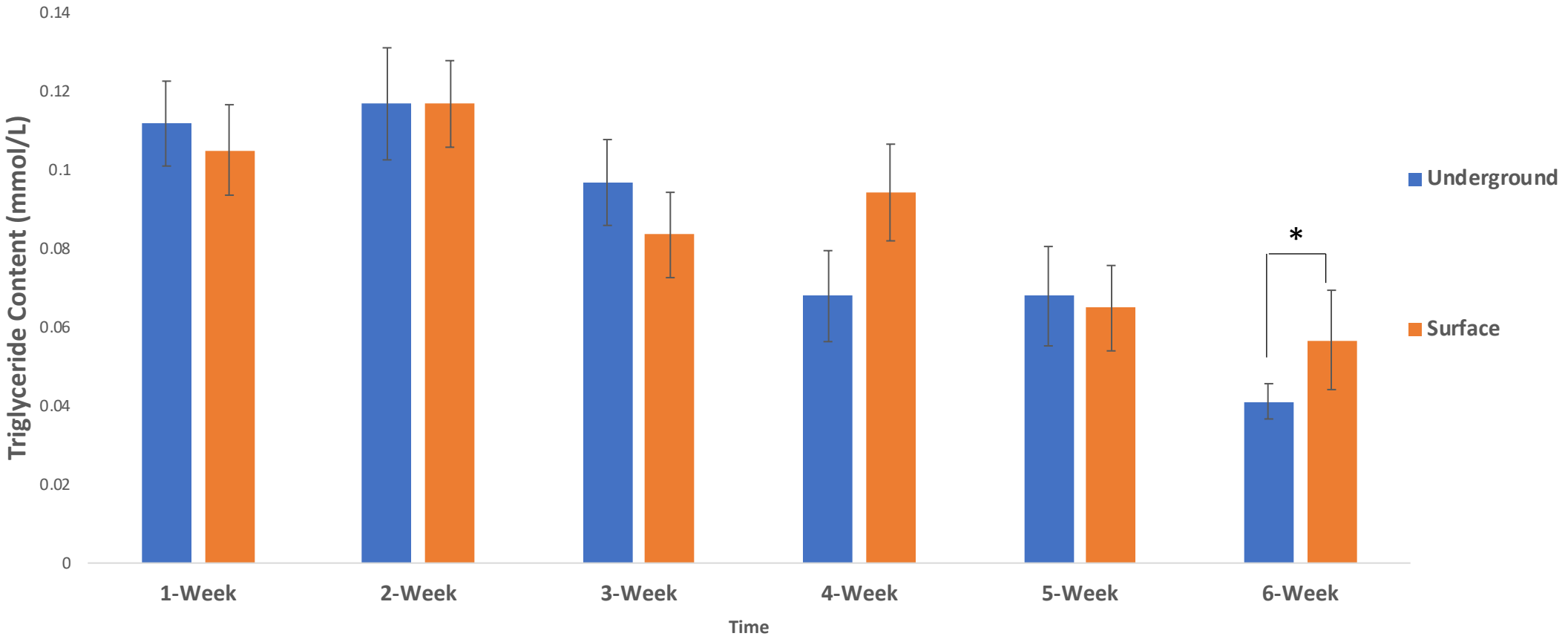


Treatment 2

Treatment 3

Treatment 4

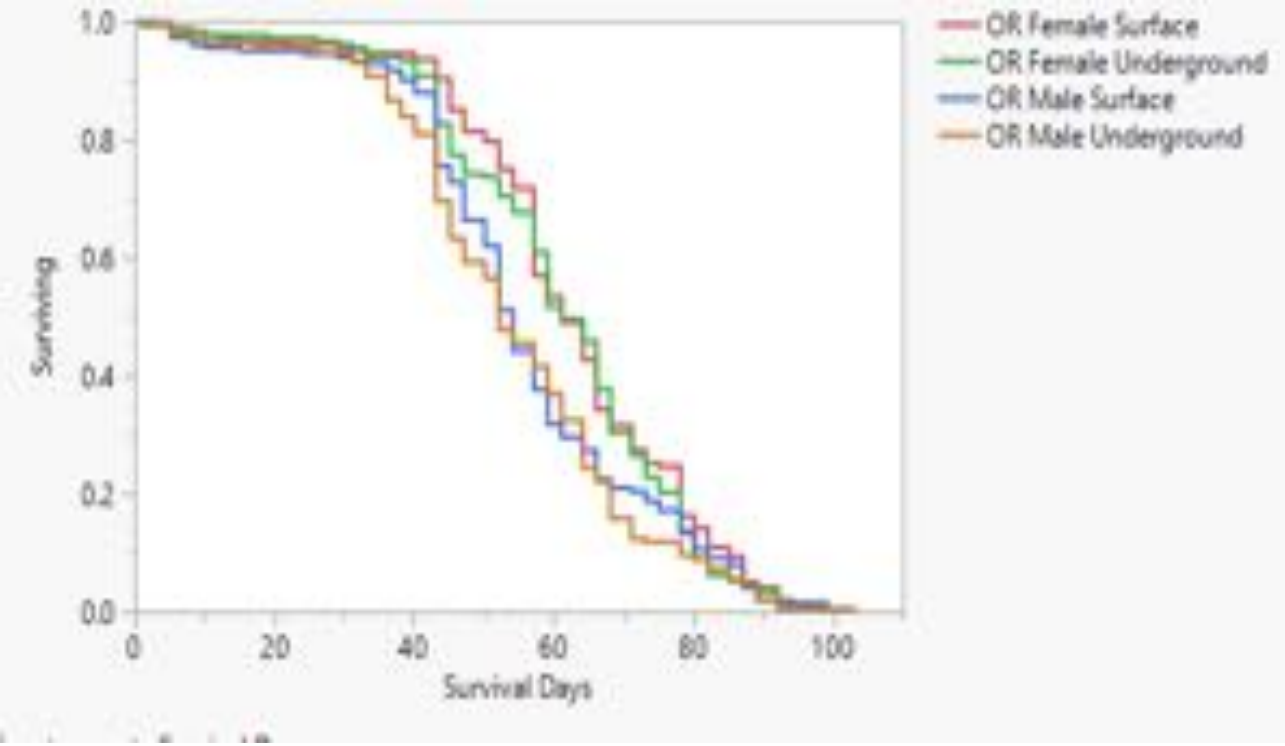
6-Week Analysis

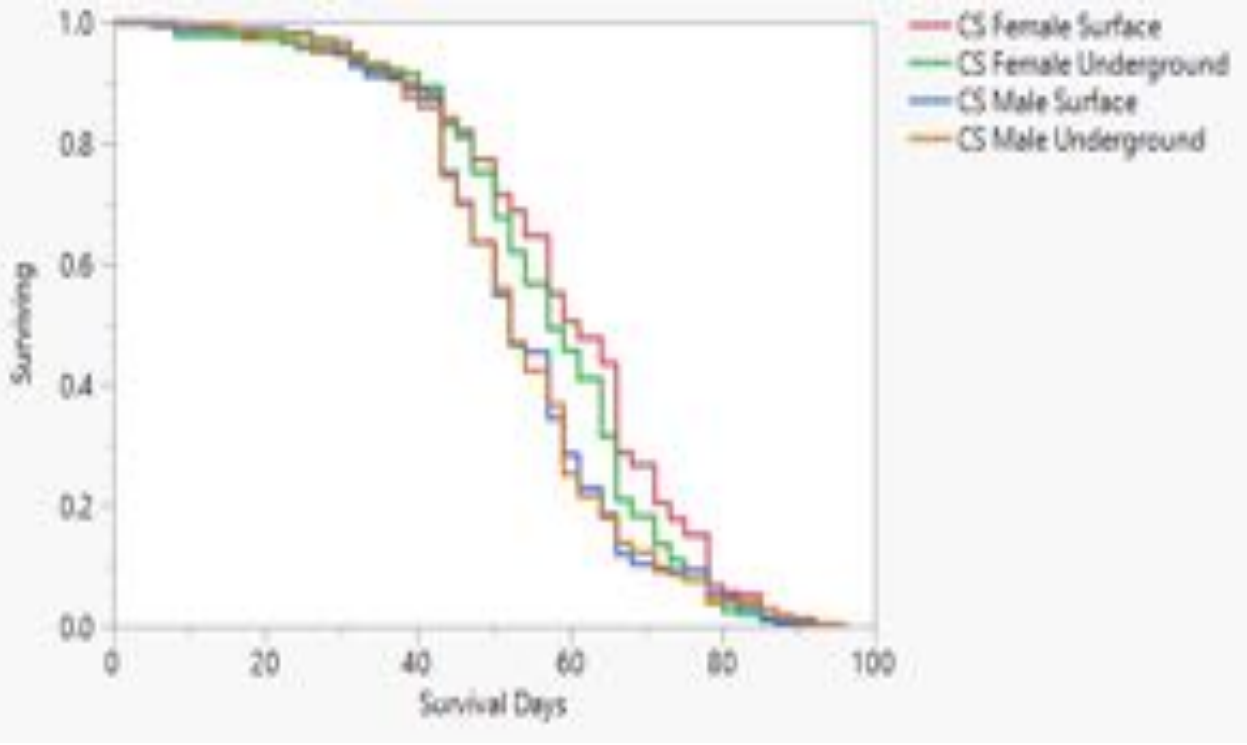


OR Male Triglyceride Content 6-Week

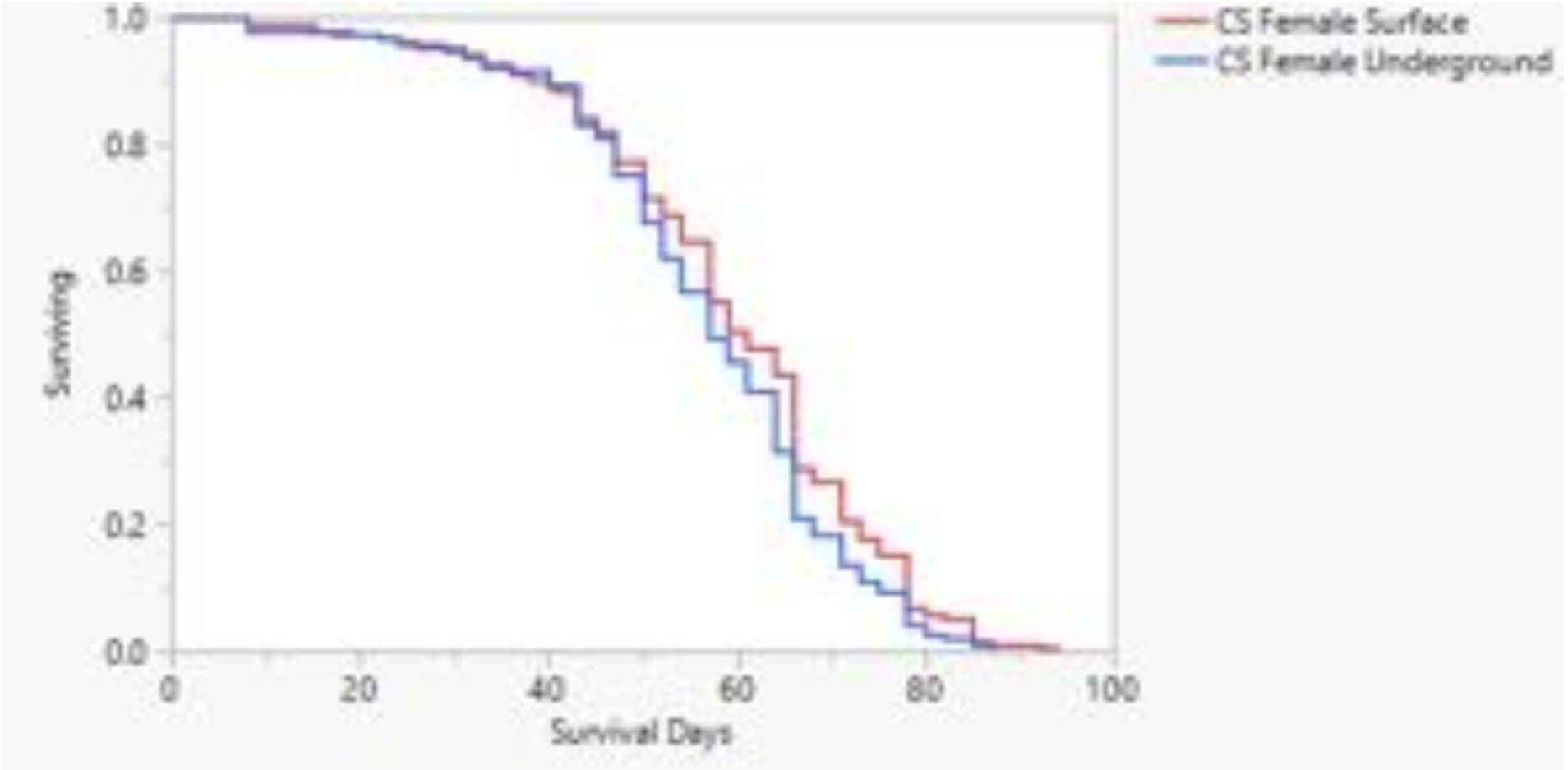
Error Bars - SE

Longevity Results



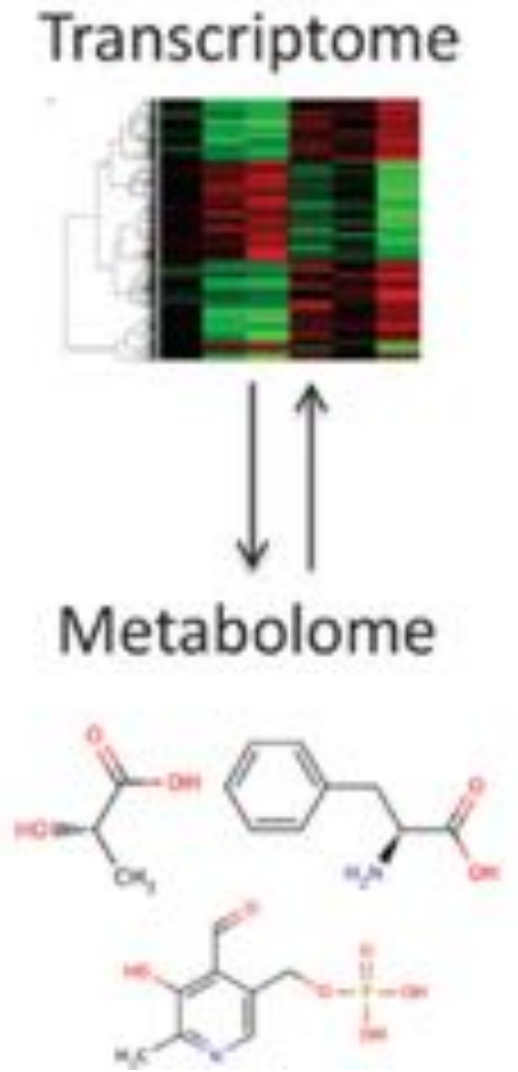


Underground Treatment Significantly Decreases CS Females Lifespan



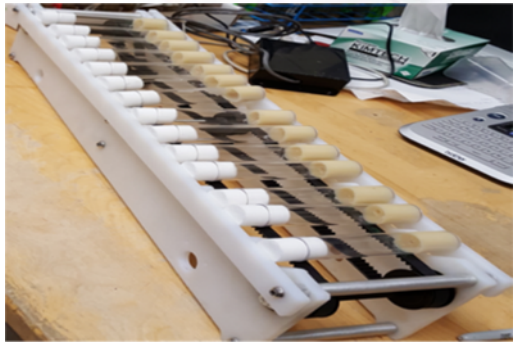
Modelling an Underground Response

- **Explore the Biology**
- **Looking for Broad Changes**



Fly Treatments

Underground Exercise



Underground Control

Surface Exercise

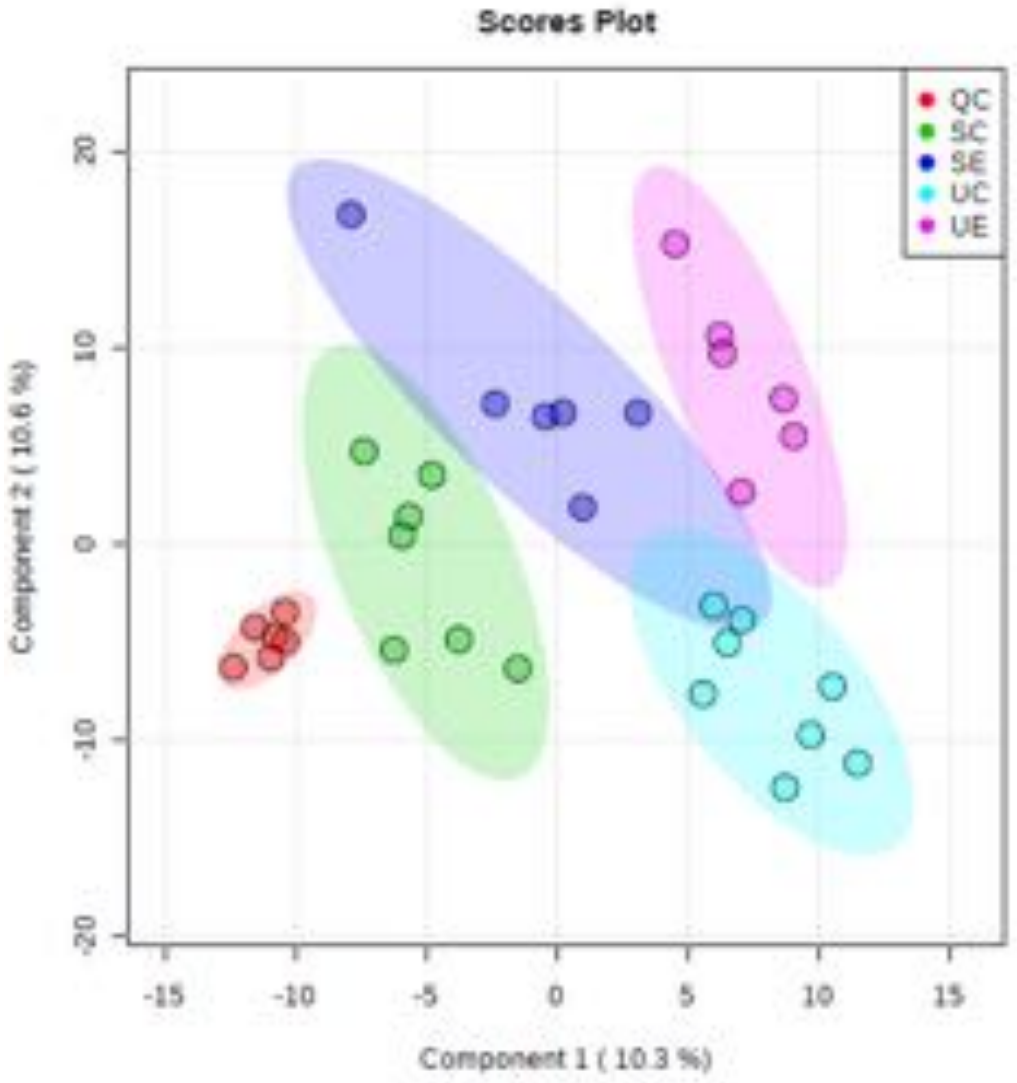
Surface Control

Monitoring Metabolism (Broad Method)

- **Broad Method:**
- **LC/MS (Liquid-chromatography/Mass Spectrometry)**
- **Separate, identify and monitor changes in concentration of metabolites**
- **Identify these metabolites**

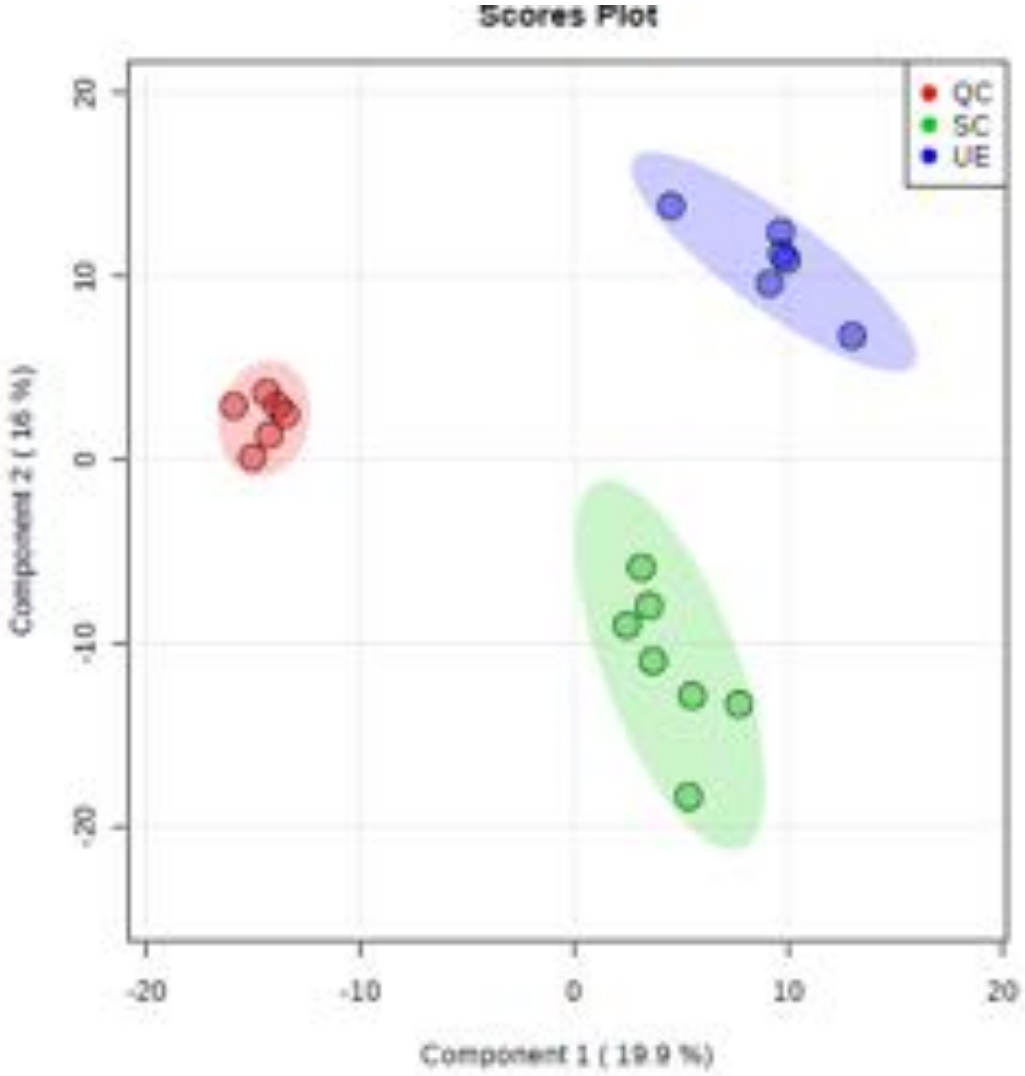
Underground Exercise Shows Largest Separation in CS Males

- **Liquid Chromatography Mass Spectrometry (LC-MS)**
- **Male Canton S**
- **Weeklong Exposure**
- **One Complete Run**
- **PCA Plot of Complete Run**



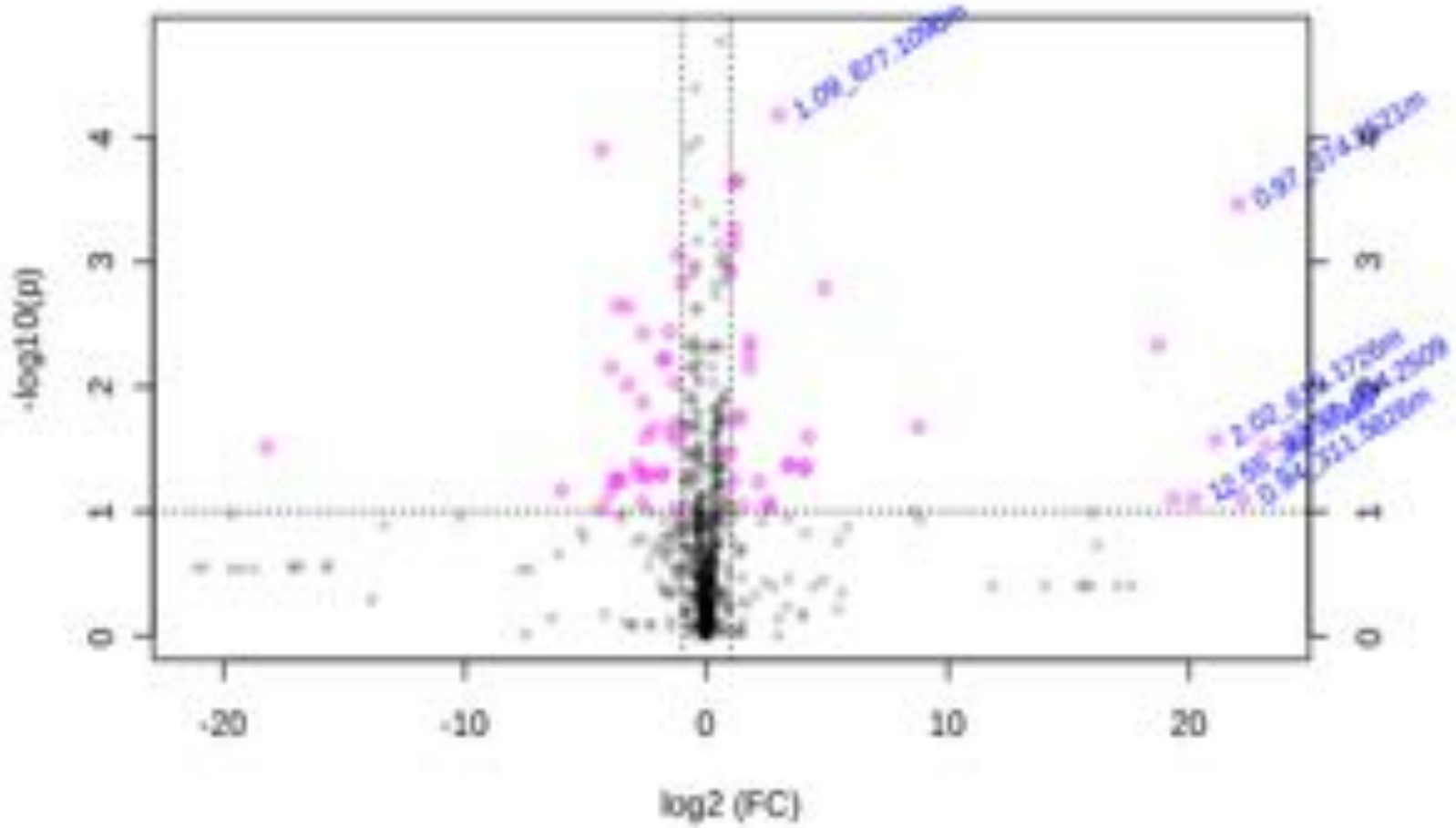
Metabolomic Shift Caused by Underground Exercise

- **LC-MS**
- **Male Canton S**
- **Weeklong Exposure**
- **Comparing Surface Control and Underground Exercise**
- **PCA Plot Comparing SC and UE**



Finding Metabolites

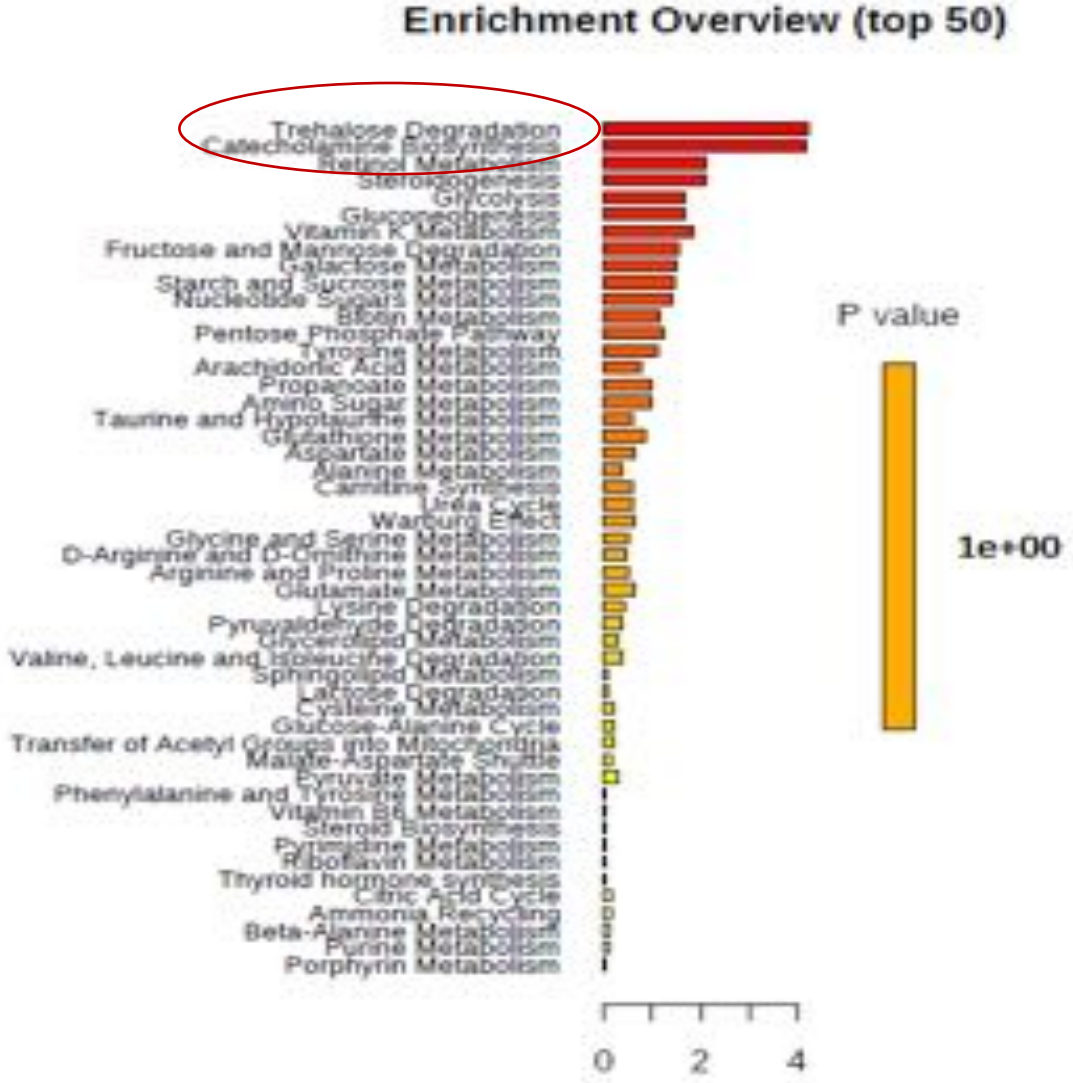
- **LC-MS**
- **Male Canton S**
- **Weeklong Exposure**
- **Fold Change vs P-value**
- **Metabolites of Interest in Pink**



Amino Acid, Carbohydrate, and Fatty Acid Metabolism Overrepresented

	Total Cmpd	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Tyrosine metabolism	30	1	3.93E-02	3.24E+00	9.51E-01	3.36E-01	0.05
Propanoate metabolism	18	1	4.91E-02	3.01E+00	1.00E+00	3.36E-01	0.00
Fructose and mannose metabolism	18	2	4.98E-02	3.00E+00	1.00E+00	3.36E-01	0.05
Galactose metabolism	26	3	5.76E-02	2.85E+00	1.00E+00	3.36E-01	0.06
Starch and sucrose metabolism	17	2	6.01E-02	2.81E+00	1.00E+00	3.36E-01	0.18
Amino sugar and nucleotide sugar metabolism	34	4	8.26E-02	2.49E+00	1.00E+00	3.53E-01	0.03
Glycolysis or Gluconeogenesis	25	2	8.84E-02	2.43E+00	1.00E+00	3.53E-01	0.10
Lysine degradation	17	1	2.99E-01	1.21E+00	1.00E+00	7.28E-01	0.00
Biotin metabolism	5	1	2.99E-01	1.21E+00	1.00E+00	7.28E-01	0.00
Glutathione metabolism	20	2	3.11E-01	1.17E+00	1.00E+00	7.28E-01	0.36
Arginine and proline metabolism	37	2	3.64E-01	1.01E+00	1.00E+00	7.28E-01	0.11
D-Arginine and D-ornithine metabolism	4	1	3.71E-01	9.92E-01	1.00E+00	7.28E-01	0.00
Aminoacyl-tRNA biosynthesis	67	5	4.09E-01	8.96E-01	1.00E+00	7.28E-01	0.00
Ascorbate and aldarate metabolism	6	1	4.57E-01	7.84E-01	1.00E+00	7.28E-01	0.00
Taurine and hypotaurine metabolism	6	1	4.72E-01	7.50E-01	1.00E+00	7.28E-01	0.00
Valine, leucine and isoleucine degradation	35	2	5.30E-01	6.34E-01	1.00E+00	7.28E-01	0.00
Pyruvate metabolism	24	1	5.60E-01	5.80E-01	1.00E+00	7.28E-01	0.17
Citrate cycle (TCA cycle)	20	1	5.60E-01	5.80E-01	1.00E+00	7.28E-01	0.07
Glycine, serine and threonine metabolism	25	1	5.60E-01	5.80E-01	1.00E+00	7.28E-01	0.00
Butanoate metabolism	21	1	5.60E-01	5.80E-01	1.00E+00	7.28E-01	0.00
Pentose phosphate pathway	19	1	5.98E-01	5.15E-01	1.00E+00	7.28E-01	0.00
Valine, leucine and isoleucine biosynthesis	13	3	6.14E-01	4.87E-01	1.00E+00	7.28E-01	0.67
beta-Alanine metabolism	13	1	6.28E-01	4.65E-01	1.00E+00	7.28E-01	0.00
Cysteine and methionine metabolism	25	2	6.45E-01	4.39E-01	1.00E+00	7.28E-01	0.00
Pantothenate and CoA biosynthesis	12	1	6.50E-01	4.31E-01	1.00E+00	7.28E-01	0.00
Alanine, aspartate and glutamate metabolism	23	2	7.05E-01	3.49E-01	1.00E+00	7.56E-01	0.15
Riboflavin metabolism	7	1	8.79E-01	1.29E-01	1.00E+00	9.12E-01	0.00
Nicotinate and nicotinamide metabolism	9	1	9.95E-01	4.81E-03	1.00E+00	9.95E-01	0.29

Carbohydrate and Stress Hormone Enriched



Underground Exercise Shows Largest Separation in CS Females

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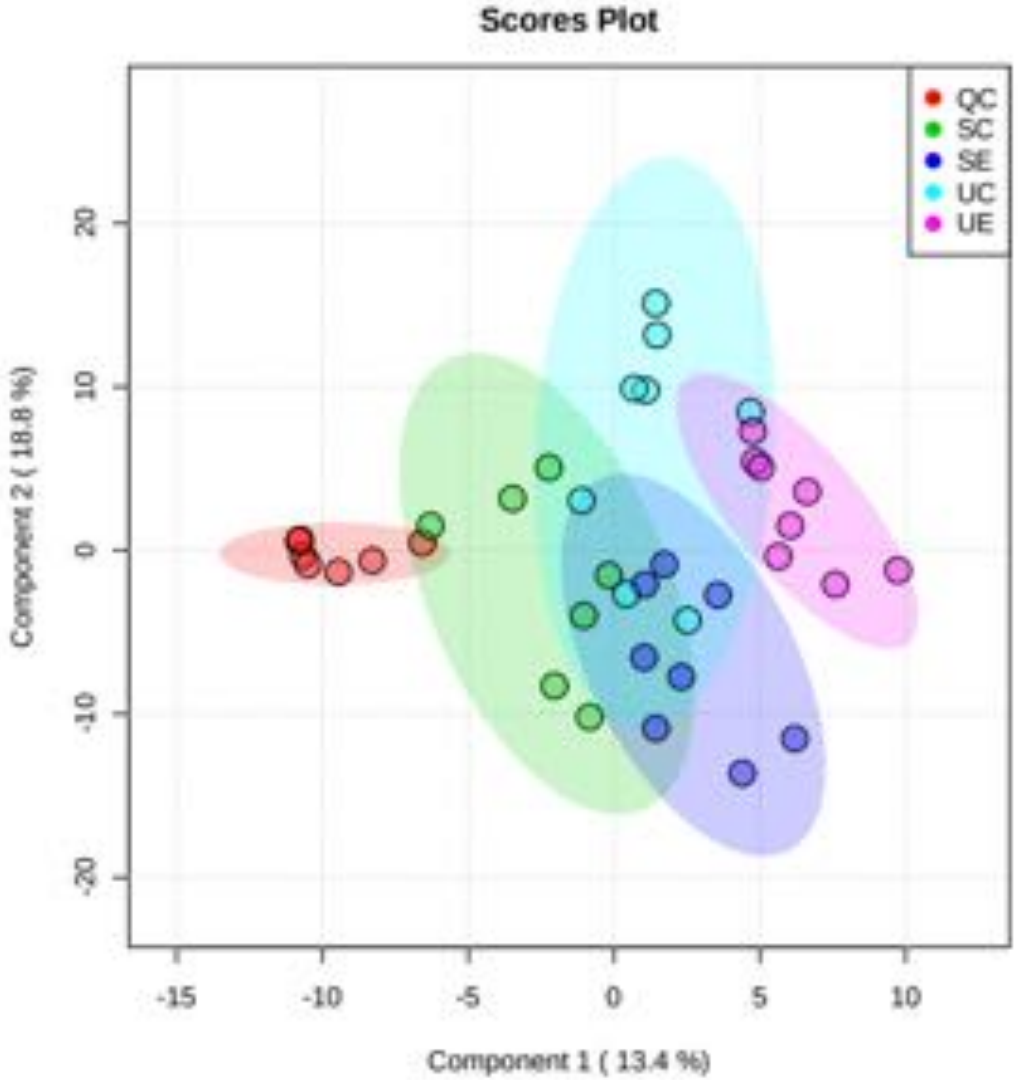

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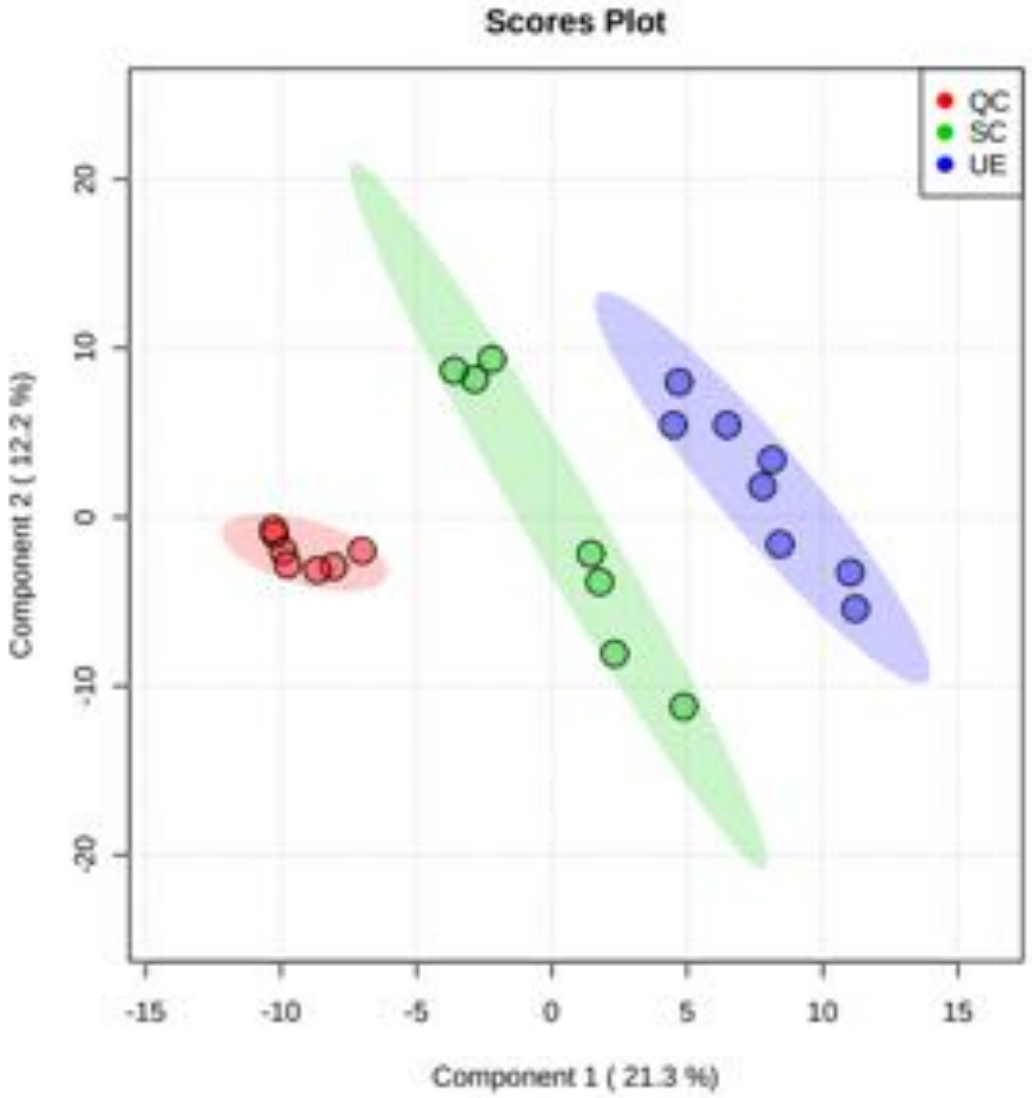

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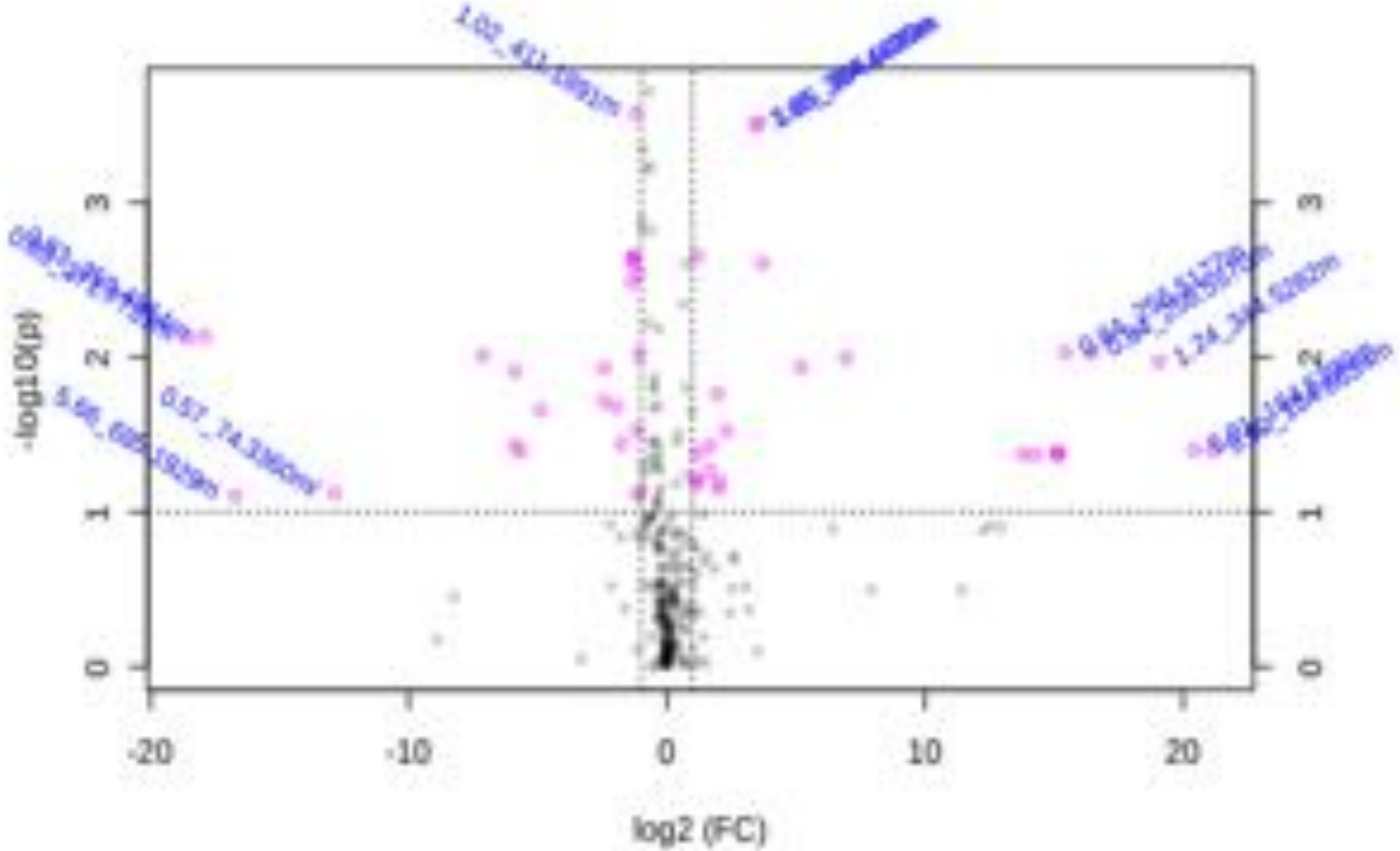
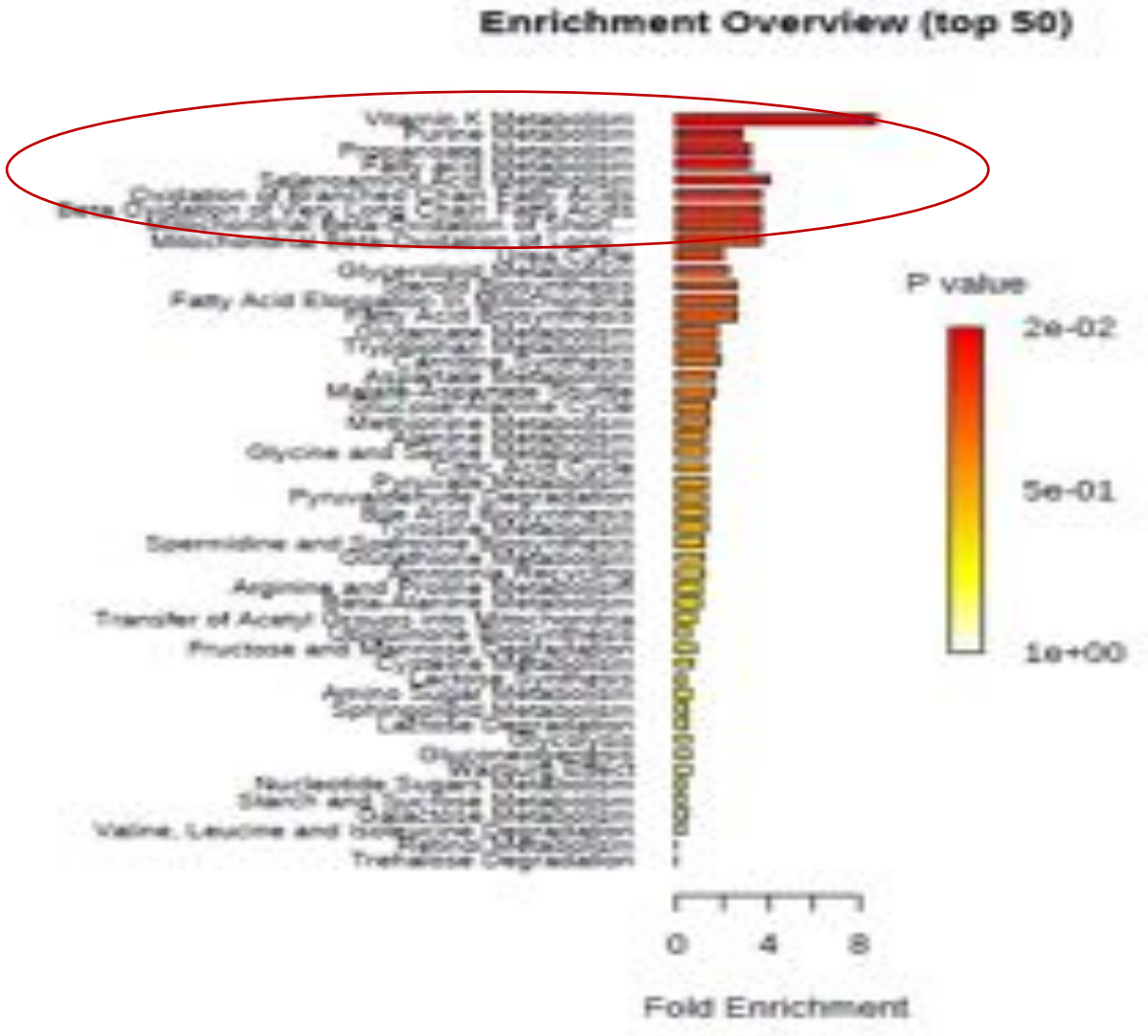


Fatty Acid and Nucleic Acid Metabolism Overrepresented

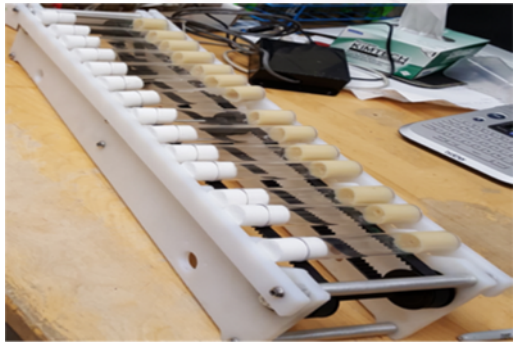
	Total Cmpd	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Propanoate metabolism	18	1	4.30E-04	7.75E+00	1.40E-02	1.04E-02	0.00
Purine metabolism	64	4	6.10E-04	7.40E+00	2.01E-02	1.04E-02	0.04
Pentose and glucuronate interconversions	16	2	5.81E-02	2.85E+00	1.00E+00	4.49E-01	0.00
beta-Alanine metabolism	13	1	6.79E-02	2.69E+00	1.00E+00	4.49E-01	0.00
Alanine, aspartate and glutamate metabolism	23	1	6.80E-02	2.69E+00	1.00E+00	4.49E-01	0.43
Fatty acid biosynthesis	38	1	1.06E-01	2.25E+00	1.00E+00	4.49E-01	0.00
Fatty acid elongation in mitochondria	27	1	1.06E-01	2.25E+00	1.00E+00	4.49E-01	0.00
Fatty acid metabolism	38	1	1.06E-01	2.25E+00	1.00E+00	4.49E-01	0.00
Aminoacyl-tRNA biosynthesis	67	6	2.11E-01	1.56E+00	1.00E+00	6.95E-01	0.00
Arginine and proline metabolism	37	3	2.23E-01	1.50E+00	1.00E+00	6.95E-01	0.28
Pyruvate metabolism	24	1	2.66E-01	1.32E+00	1.00E+00	6.95E-01	0.17
Citrate cycle (TCA cycle)	20	1	2.66E-01	1.32E+00	1.00E+00	6.95E-01	0.07
Cysteine and methionine metabolism	25	1	2.66E-01	1.32E+00	1.00E+00	6.95E-01	0.00
Tryptophan metabolism	23	1	3.02E-01	1.20E+00	1.00E+00	7.33E-01	0.00
Tyrosine metabolism	30	1	5.05E-01	6.83E-01	1.00E+00	9.55E-01	0.00
Valine, leucine and isoleucine biosynthesis	13	3	5.27E-01	6.40E-01	1.00E+00	9.55E-01	0.67
Glycine, serine and threonine metabolism	25	2	5.40E-01	6.17E-01	1.00E+00	9.55E-01	0.29
Glycolysis or Gluconeogenesis	25	2	5.43E-01	6.10E-01	1.00E+00	9.55E-01	0.10
Butanoate metabolism	21	2	5.48E-01	6.01E-01	1.00E+00	9.55E-01	0.00
Pentose phosphate pathway	19	1	5.62E-01	5.77E-01	1.00E+00	9.55E-01	0.00
Valine, leucine and isoleucine degradation	35	2	6.29E-01	4.64E-01	1.00E+00	9.94E-01	0.00
Pantothenate and CoA biosynthesis	12	2	6.48E-01	4.34E-01	1.00E+00	9.94E-01	0.00
Histidine metabolism	7	1	6.72E-01	3.97E-01	1.00E+00	9.94E-01	0.00
Amino sugar and nucleotide sugar metabolism	34	2	7.50E-01	2.87E-01	1.00E+00	9.98E-01	0.00
Starch and sucrose metabolism	17	3	7.94E-01	2.30E-01	1.00E+00	9.98E-01	0.16
Galactose metabolism	26	2	7.98E-01	2.26E-01	1.00E+00	9.98E-01	0.06
Glutathione metabolism	20	4	8.08E-01	2.13E-01	1.00E+00	9.98E-01	0.12
Fructose and mannose metabolism	18	1	8.66E-01	1.44E-01	1.00E+00	9.98E-01	0.00
D-Glutamine and D-glutamate metabolism	5	1	9.12E-01	9.23E-02	1.00E+00	9.98E-01	1.00
Cyanoamino acid metabolism	6	1	9.83E-01	1.76E-02	1.00E+00	9.98E-01	0.00
Methane metabolism	9	1	9.83E-01	1.76E-02	1.00E+00	9.98E-01	0.00
Porphyrin and chlorophyll metabolism	28	2	9.93E-01	7.19E-03	1.00E+00	9.98E-01	0.00
Nitrogen metabolism	7	2	9.93E-01	7.19E-03	1.00E+00	9.98E-01	0.00
Phenylalanine metabolism	10	2	9.98E-01	2.15E-03	1.00E+00	9.98E-01	0.00

Vitamin K, Fatty Acid, and Nucleic Acid Metabolism Enriched



Fly Treatments

Underground Exercise



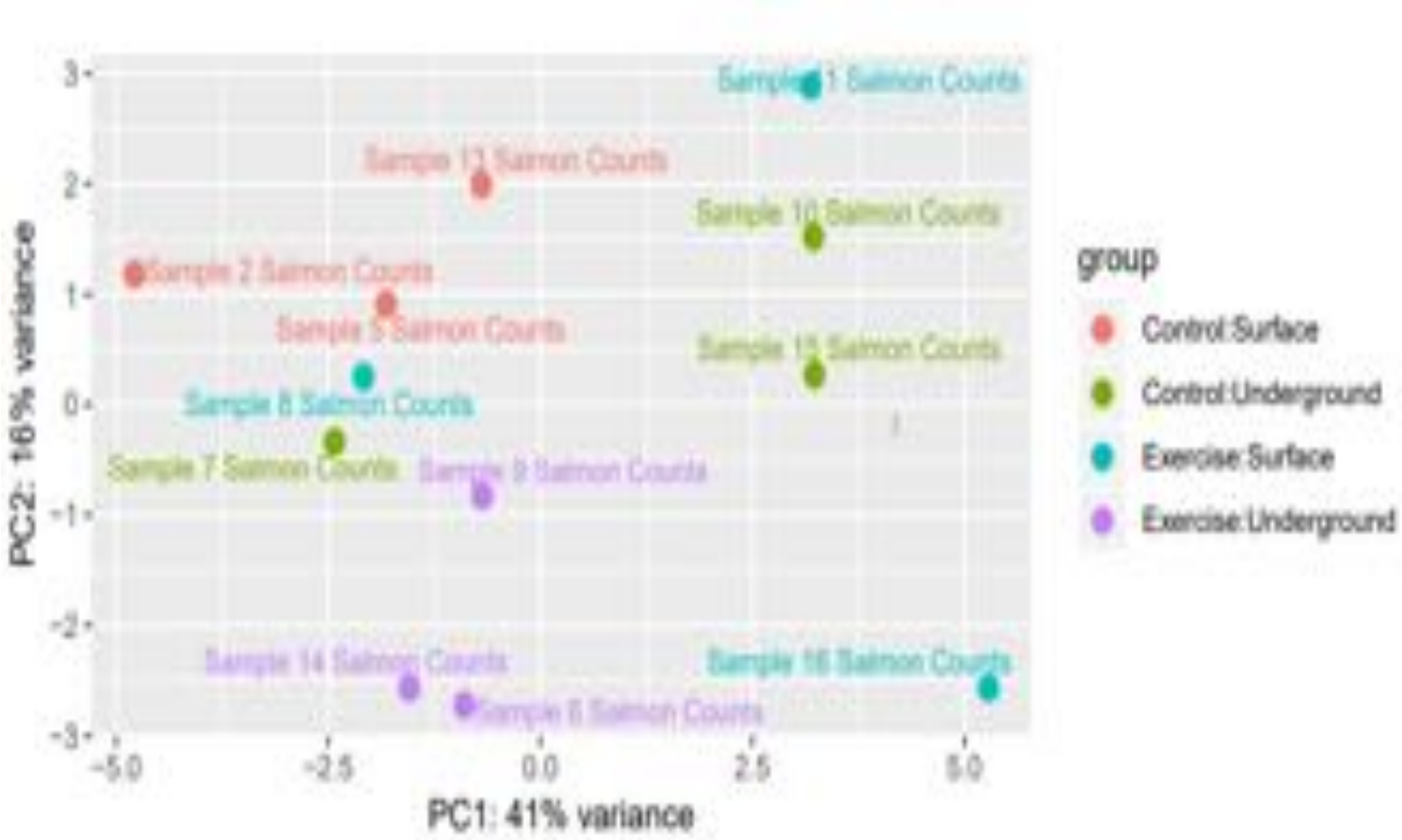
Underground Control

Surface Exercise

Surface Control

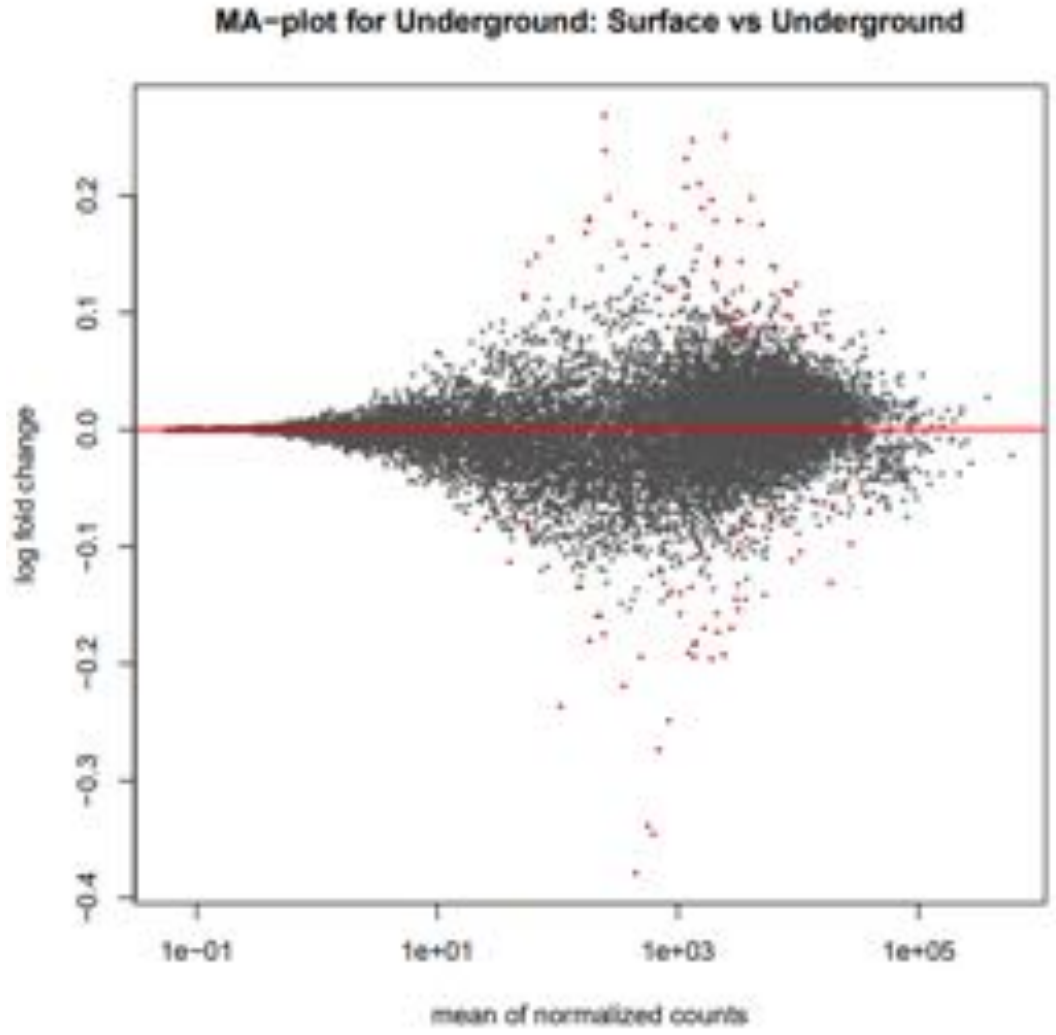
Underground Exercise Shows Transcriptomic Shift in CS Females

- **Illumina NovaSeq mRNAseq 100 bp paired end reads**
- **Female Canton S**
- **Weeklong Exposure**
- **PCA Plot Comparing Treatments**
- **Largest Shift Between Surface Control and Underground Exercise**



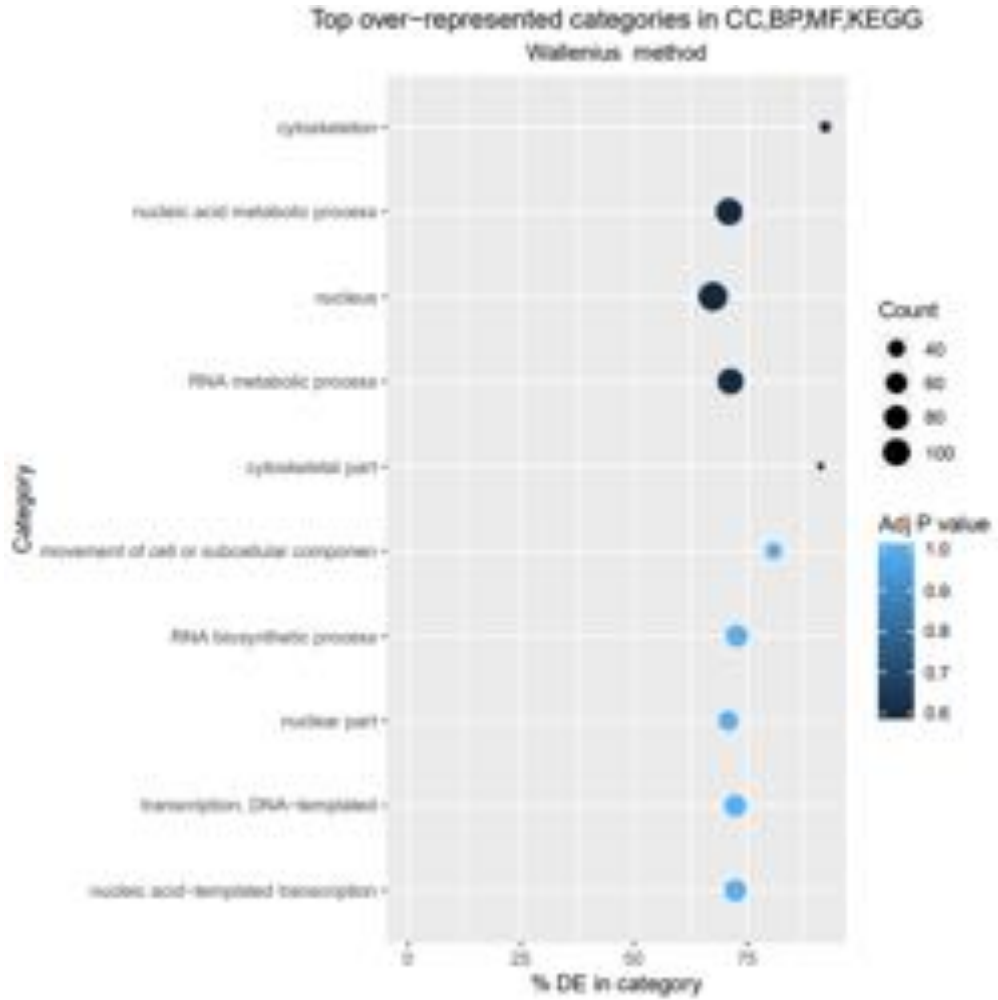
Gene Expression Changes in an Underground Environment

- **Illumina NovaSeq mRNAseq 100 bp paired end reads**
- **Female Canton S**
- **Weeklong Exposure**
- **MA Plot Comparing Surface and Underground Gene Expression**
- **Gene Expression Levels Changing Between Surface and Underground Treatments**



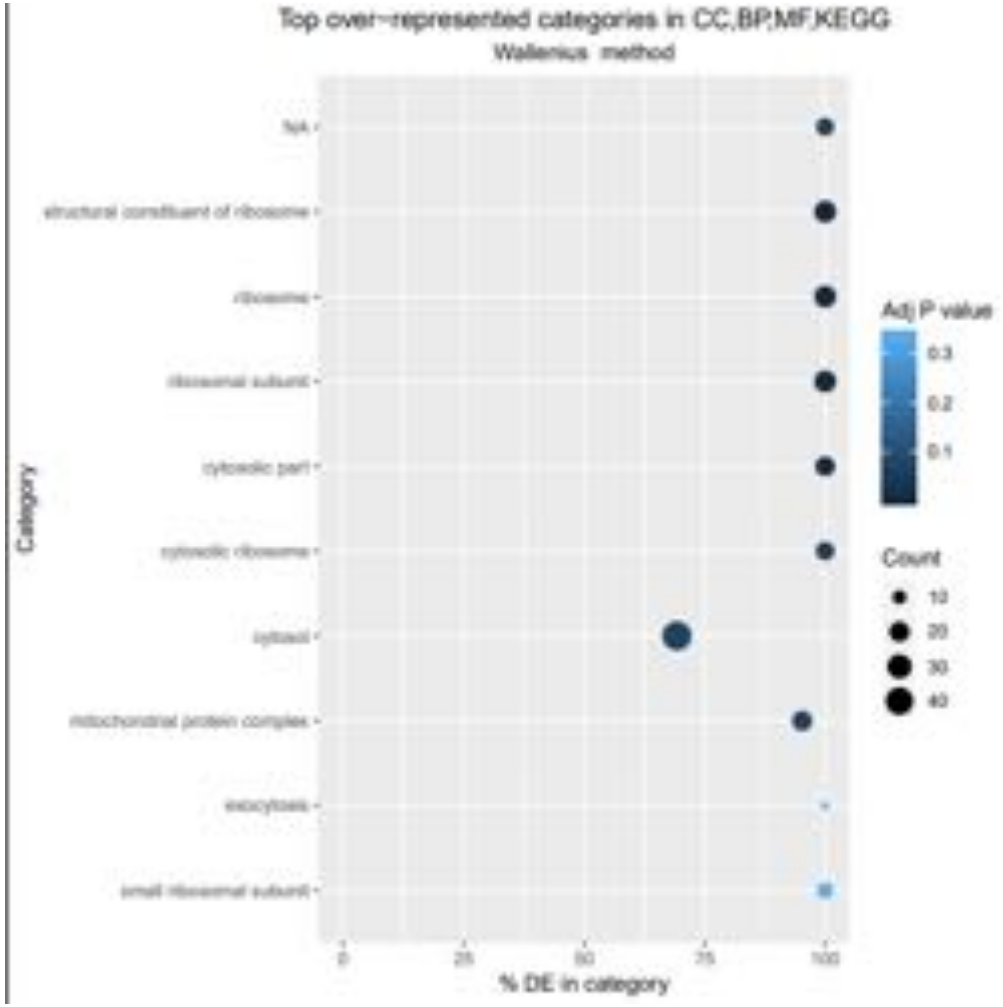
Gene Ontology Analysis Comparing Surface to Underground

- **Gene Ontology Analysis**
- **Canton S Female**
- **Weeklong Exposure**
- **Surface to Underground Comparison**
- **Not Significantly Overrepresented Upregulated Pathways**



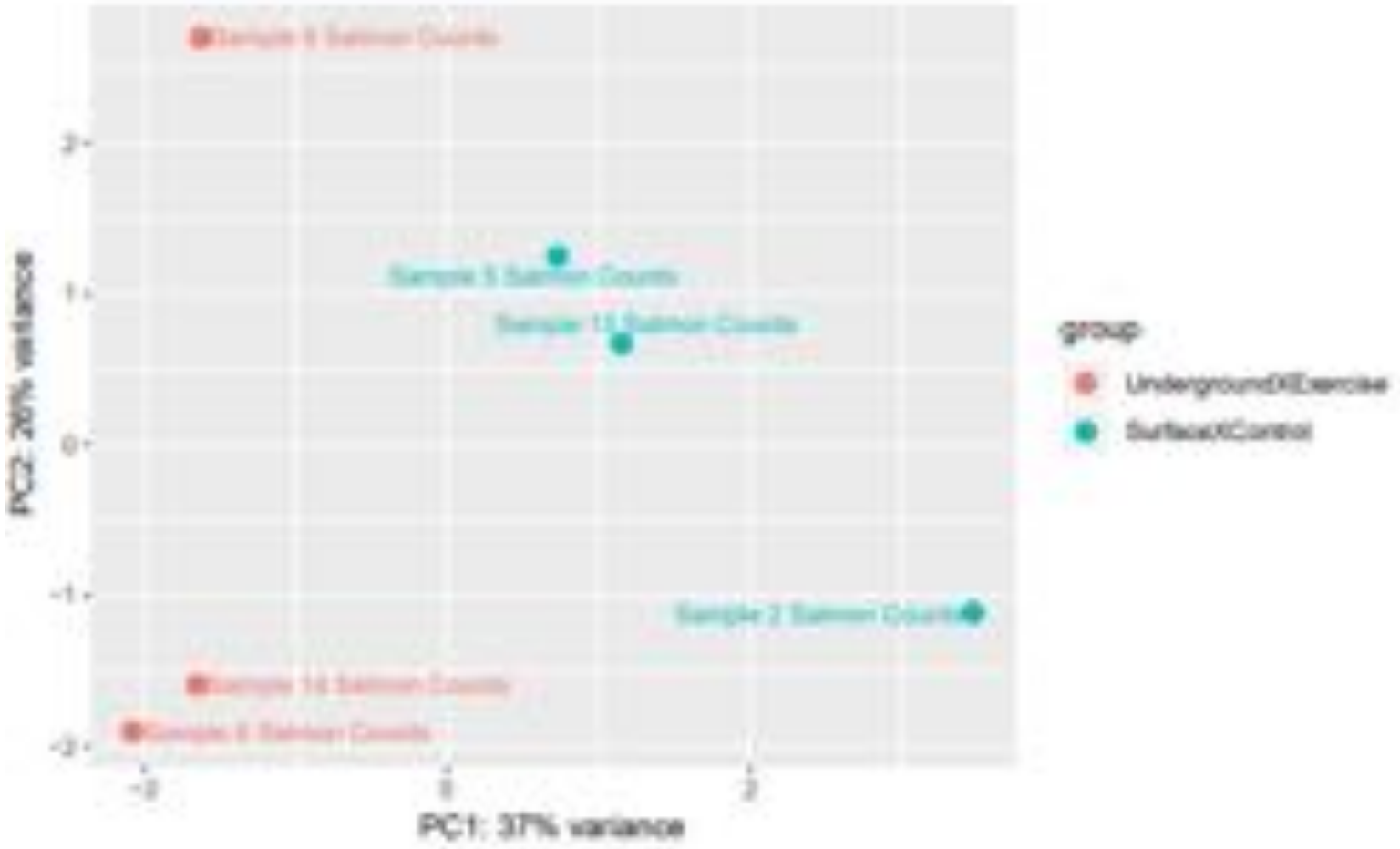
Gene Ontology Analysis Comparing Surface to Underground

- **Gene Ontology Analysis**
- **Canton S Female**
- **Weeklong Exposure**
- **Surface to Underground Comparison**
- **Not Significantly Overrepresented Downregulated Pathways**



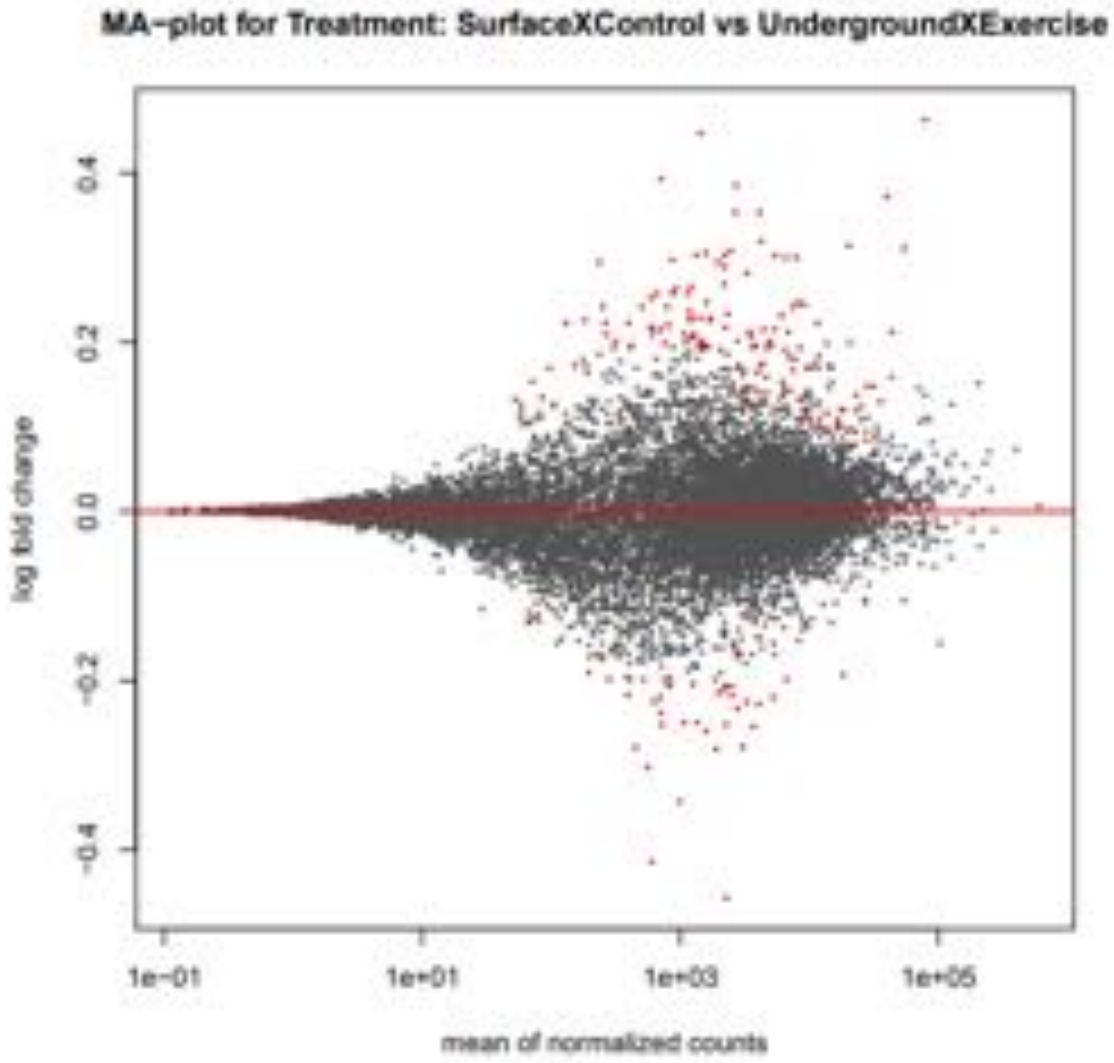
Transcriptomic Shift Caused by Underground Exercise

- **Illumina NovaSeq mRNAseq 100 bp paired end reads**
- **Female Canton S**
- **Weeklong Exposure**
- **PCA Plot Comparing Treatments**
- **Surface Control vs Underground Exercise**



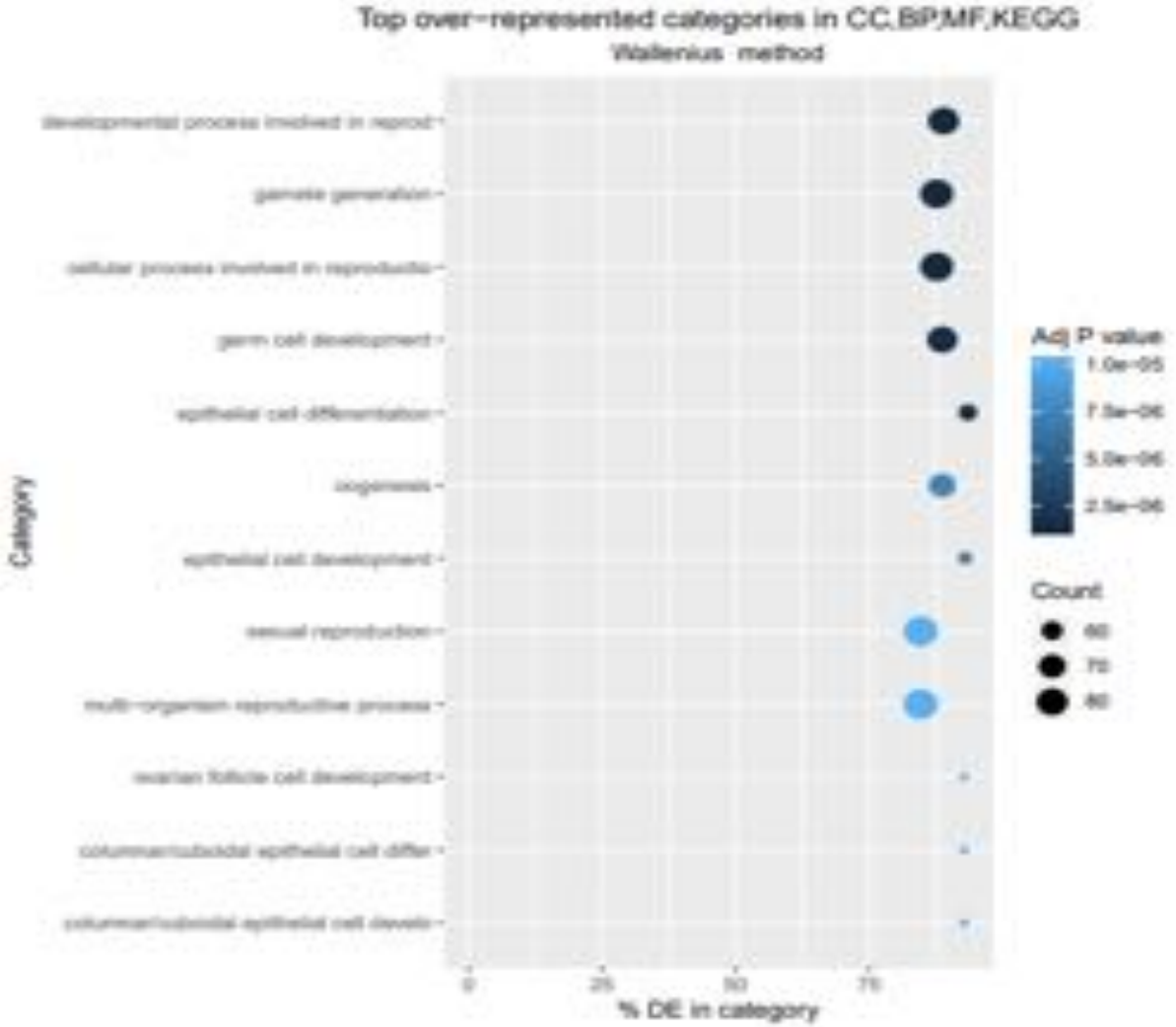
Underground Exercise had the Largest Change in Gene expression

- **Illumina NovaSeq mRNAseq 100 bp paired end reads**
- **Female Canton S**
- **Weeklong Exposure**
- **MA Plot Comparing Surface Control and Underground Exercise**
- **Gene Expression Levels Changing Between Surface Control and Underground Exercise**



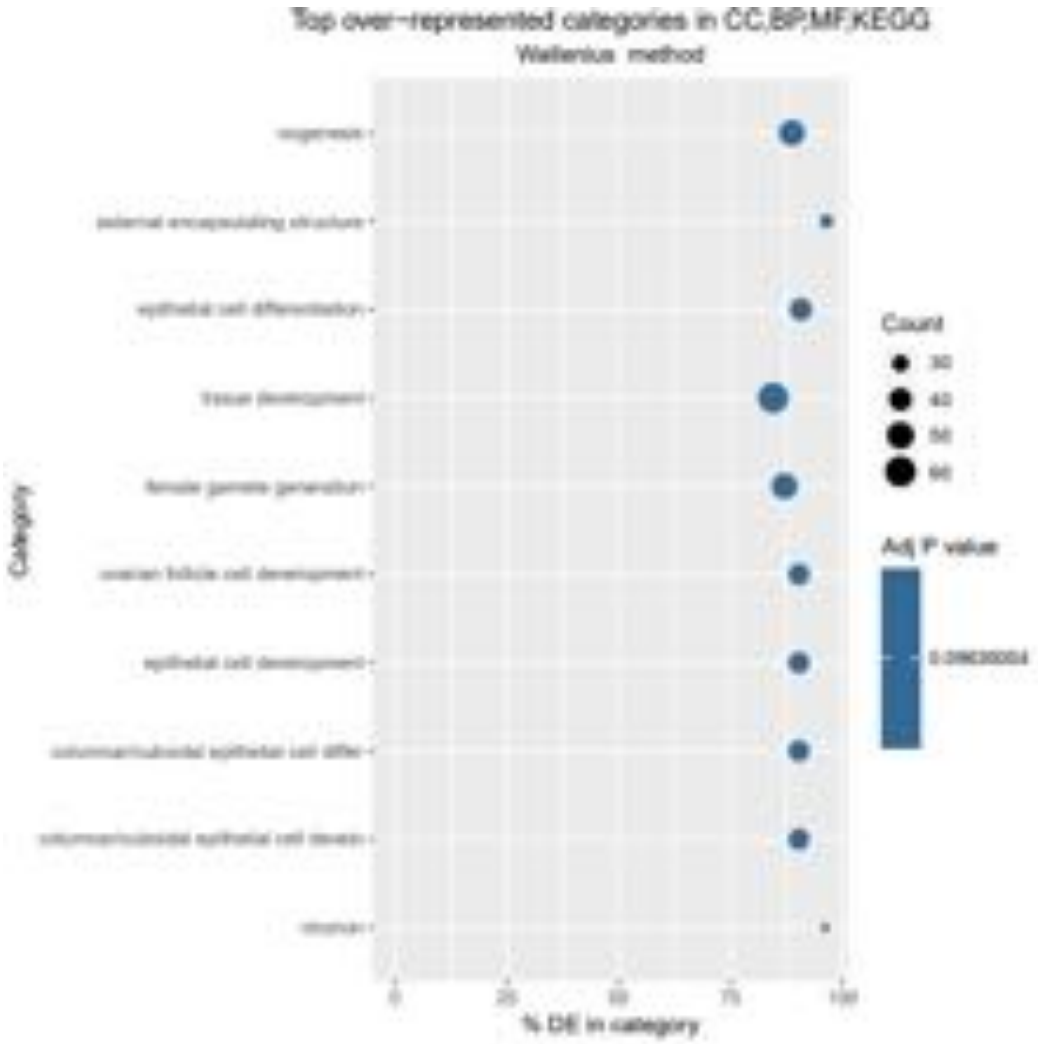
Underground Exposure Upregulates Reproductive Gene Pathways

- **Gene Ontology Analysis**
- **Canton S Female**
- **Weeklong Exposure**
- **Surface Control vs Underground Exercise**
- **Over-represented Categories in Female Reproductive Processes**



Without Exercise Changes Not as Pronounced

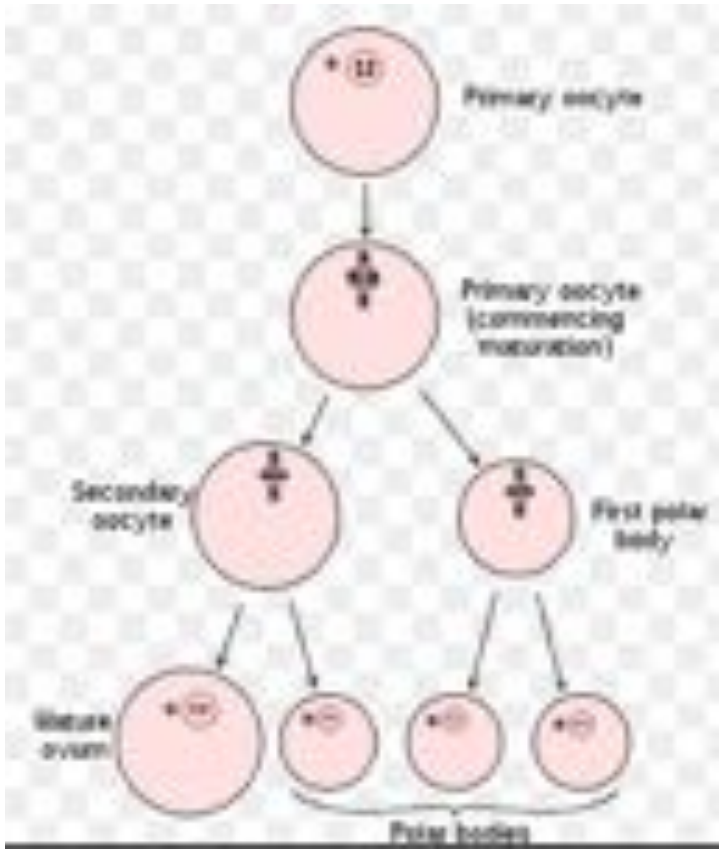
- **Gene Ontology Analysis**
- **Canton S Female**
- **Weeklong Exposure**
- **Surface Control vs Underground Control**
- **Over-represented Categories in Female Reproductive Processes**



Upregulated Gene Pathways

- **Female gamete generation**
- **Oogenesis**
- **Chorion**
- **Vitelline Membrane**
- **Egg Cell Development**

(Landis *et al.* 1997)



Why Might This Be Interesting?

A direct test of the effects of changing atmospheric pressure on the mating behavior of *Drosophila melanogaster*

Christopher J. Austin · Christopher G. Guglielmo · Amanda J. Moehring

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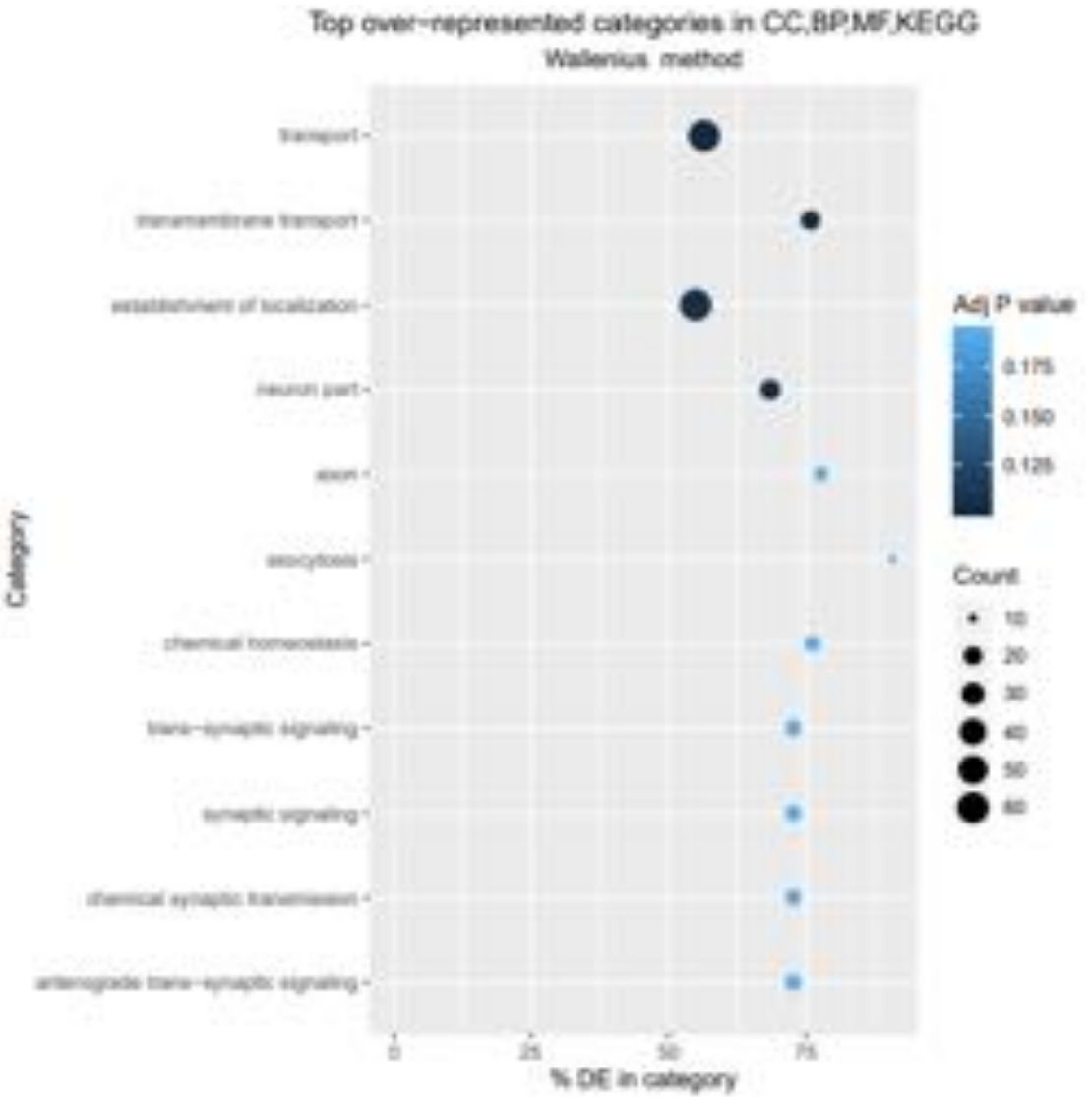
Abstract Changes in weather can be catastrophic for small insects. As such, it would be highly adaptive for insects to be able to sense when a weather front is approaching and respond appropriately. While correlative and anecdotal evidence exists that flies behaviorally respond to changes in barometric pressure, which indicate variation in weather, a direct test has yet to be performed. Here, we subject multiple strains of *Drosophila melanogaster* to changes in barometric pressure within a hypobaric chamber and measure male courtship and female receptivity. Since this species has a long copulation duration, copulating when adverse weather is approaching could subject both males and females to potentially lethal conditions. As predicted, some flies reduced their mating activity when exposed to a change in pressure that indicated imminent adverse weather. Surprisingly, however, some flies instead increased their mating activity; the behavioral response depended upon the strain's native population location and intra-population variation, demonstrating that there is genetic variation for the behavioral response. This indicates that flies are able to anticipate weather patterns and change their behavior depending on the barometric pressure they experience, but that the form of behavioral response varies both within and between populations.

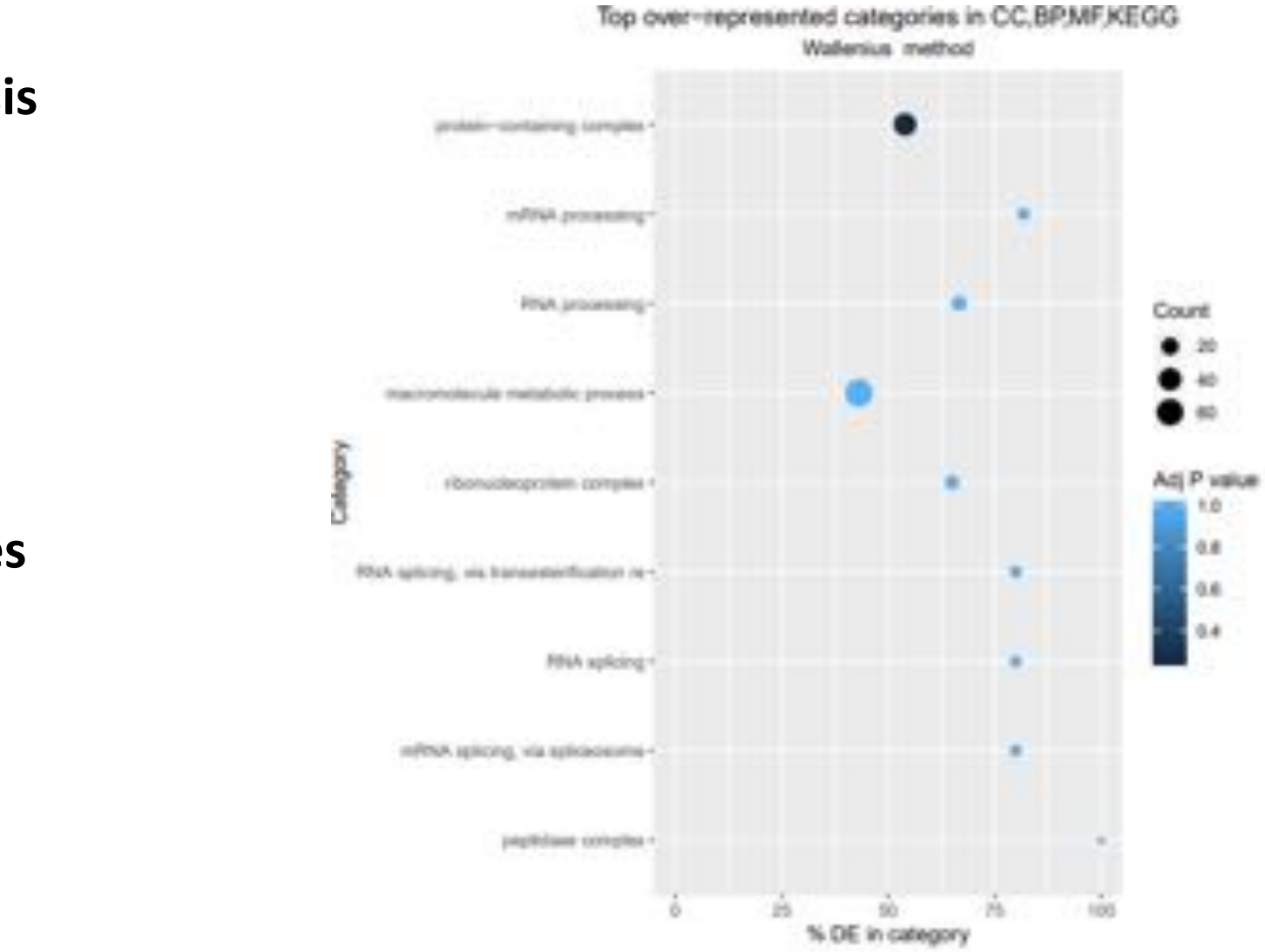
Underground Exposure Downregulates Gene Pathways

- **Gene Ontology Analysis**
- **Canton S Female**
- **Weeklong Exposure**
- **Surface Control vs Underground Exercise**
- **Over-represented Categories in Transportation and Signalling**



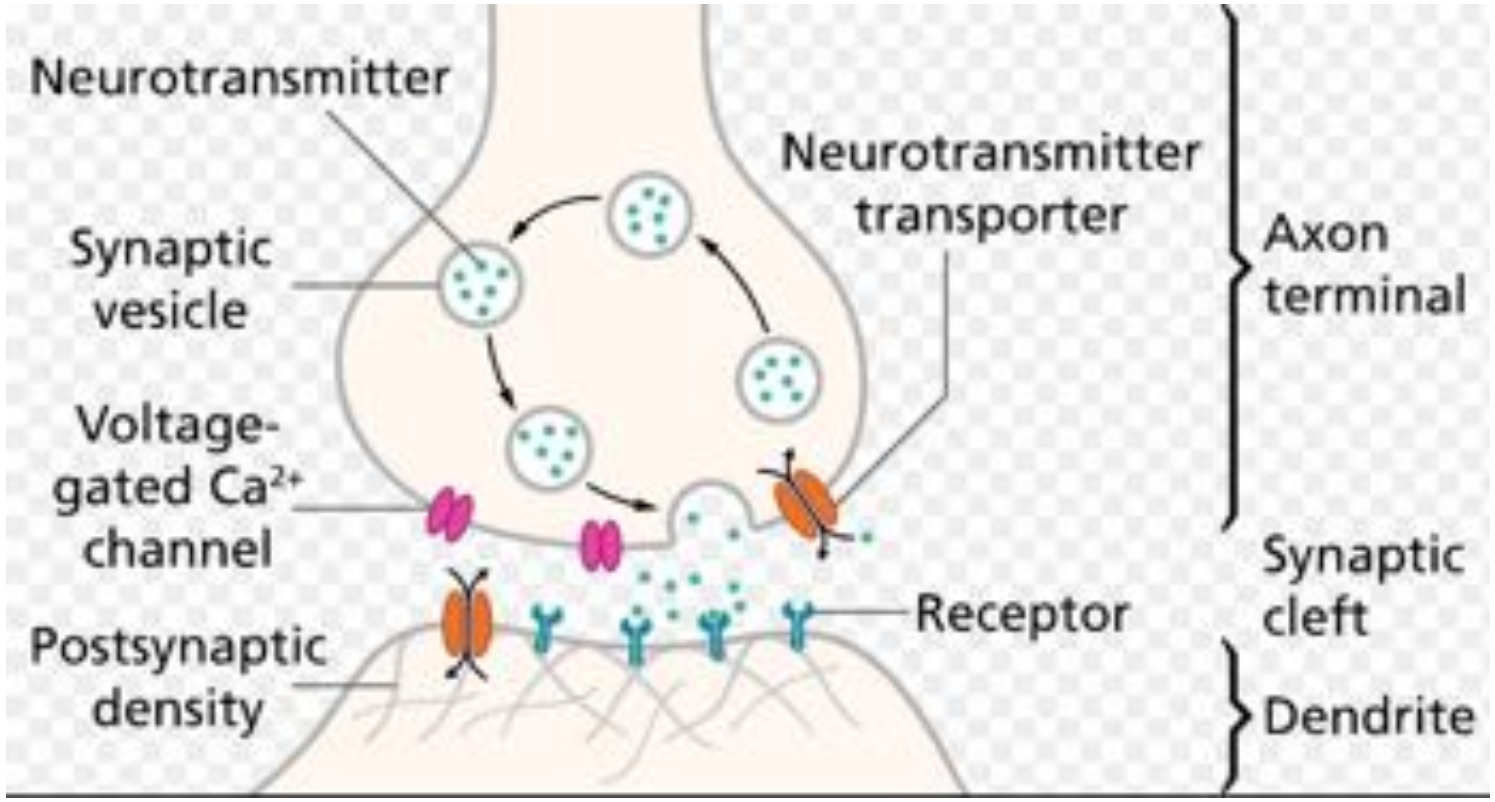
Changes Different Without Exercise

- **Gene Ontology Analysis**
- **Canton S Female**
- **Weeklong Exposure**
- **Surface Control vs Underground Control**
- **No Significant Over-represented Categories**



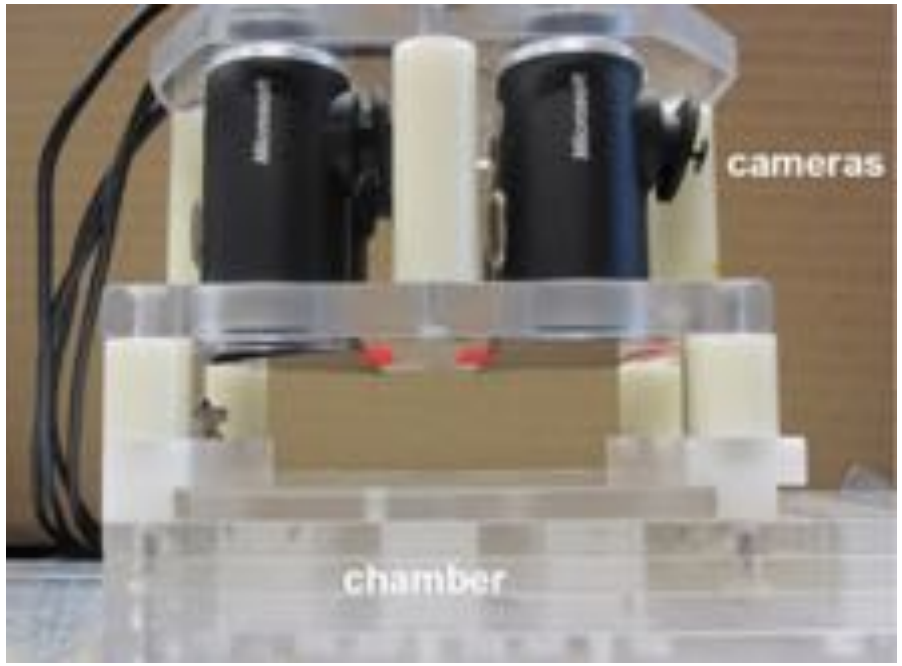
Downregulated Gene Pathways

- **Synaptic Signalling**
- **Chemical Synaptic Transmission**
- **Calcium Regulated Exocytosis**



How to Test This?

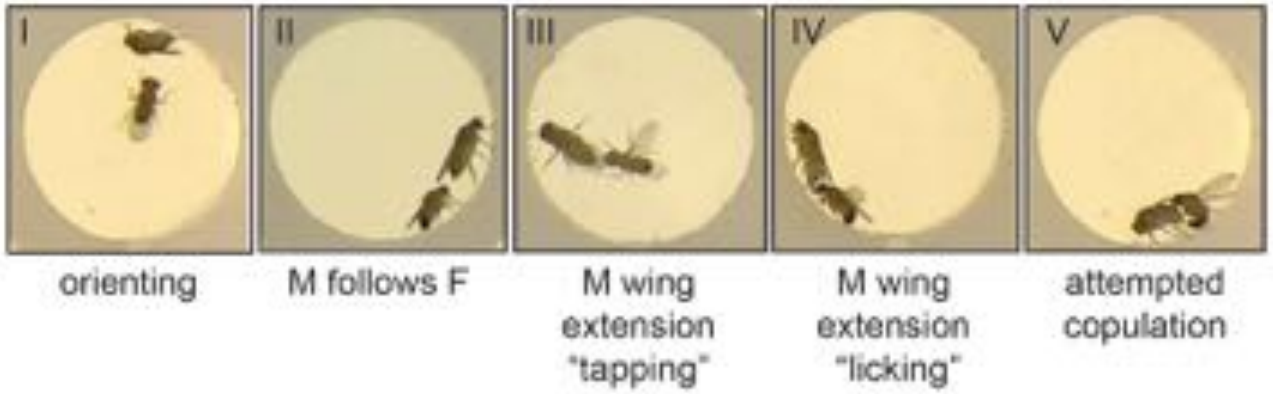
Egg Laying Assays

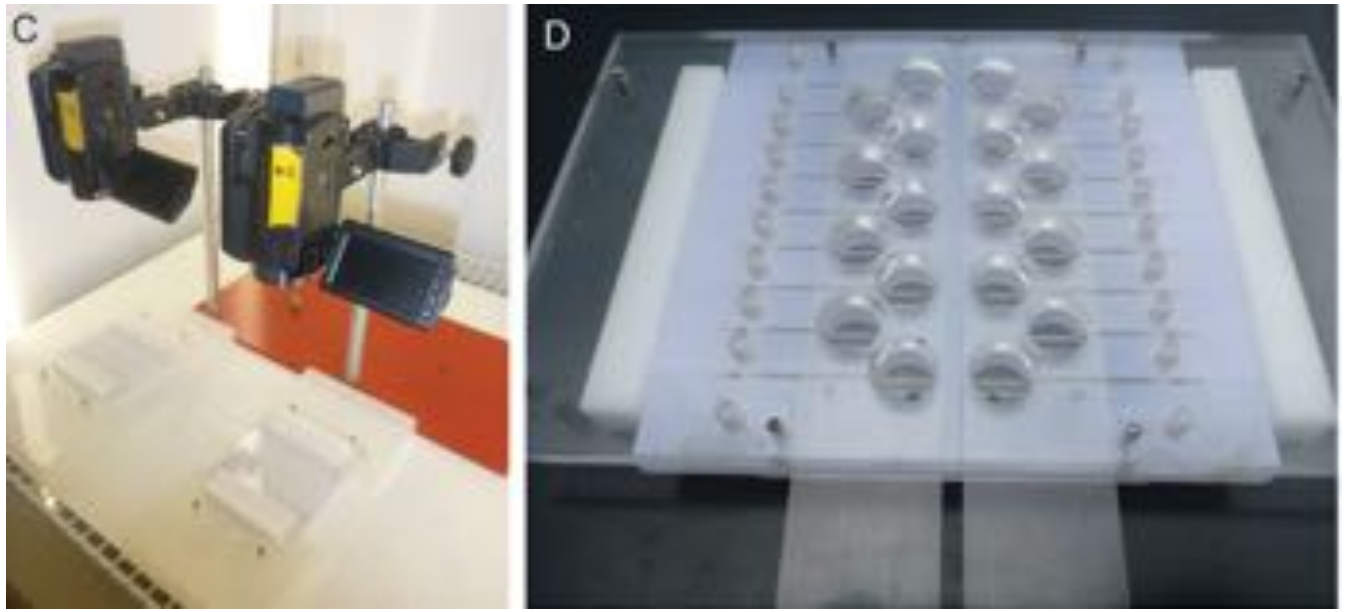


(Yang *et al.* 2016)

(Yang *et al.* 2016)

Reproductive Behaviour

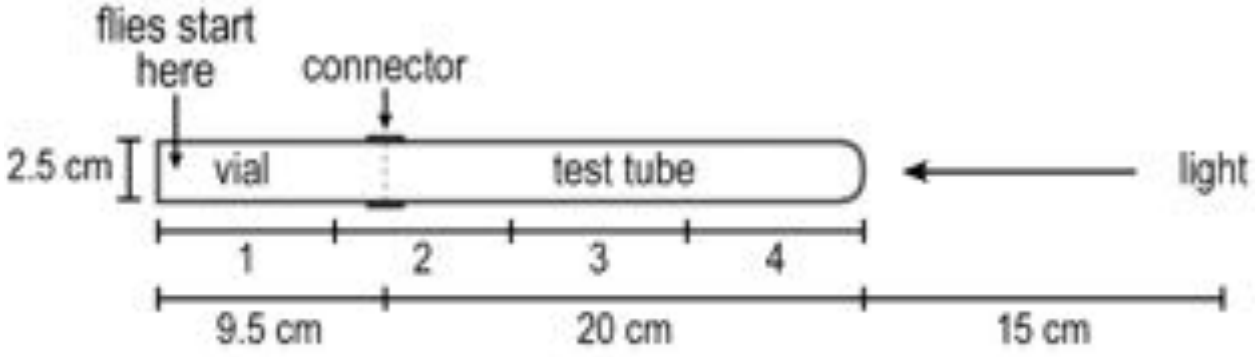




(Koemans *et al.* 2017)

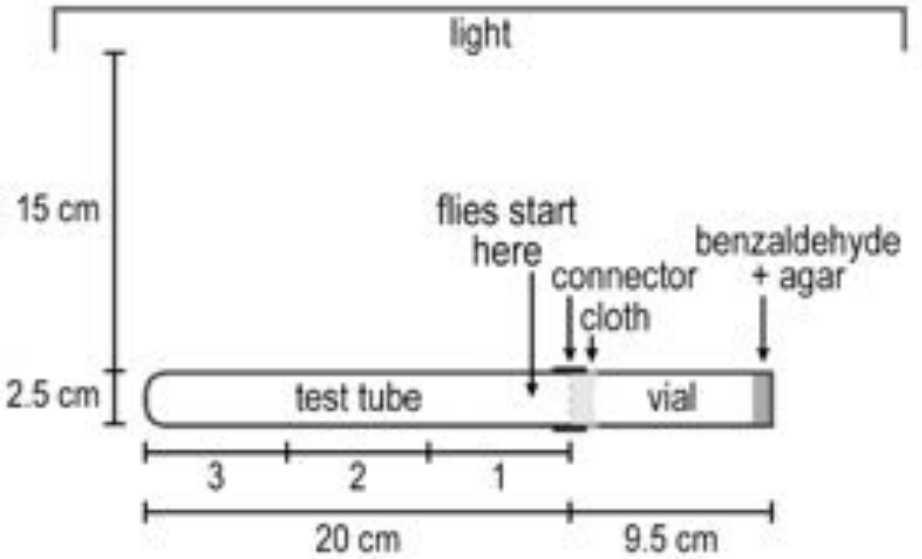
Behaviour Tests

Phototaxis Assay



(Vang, Medvedev and Adler, 2012)

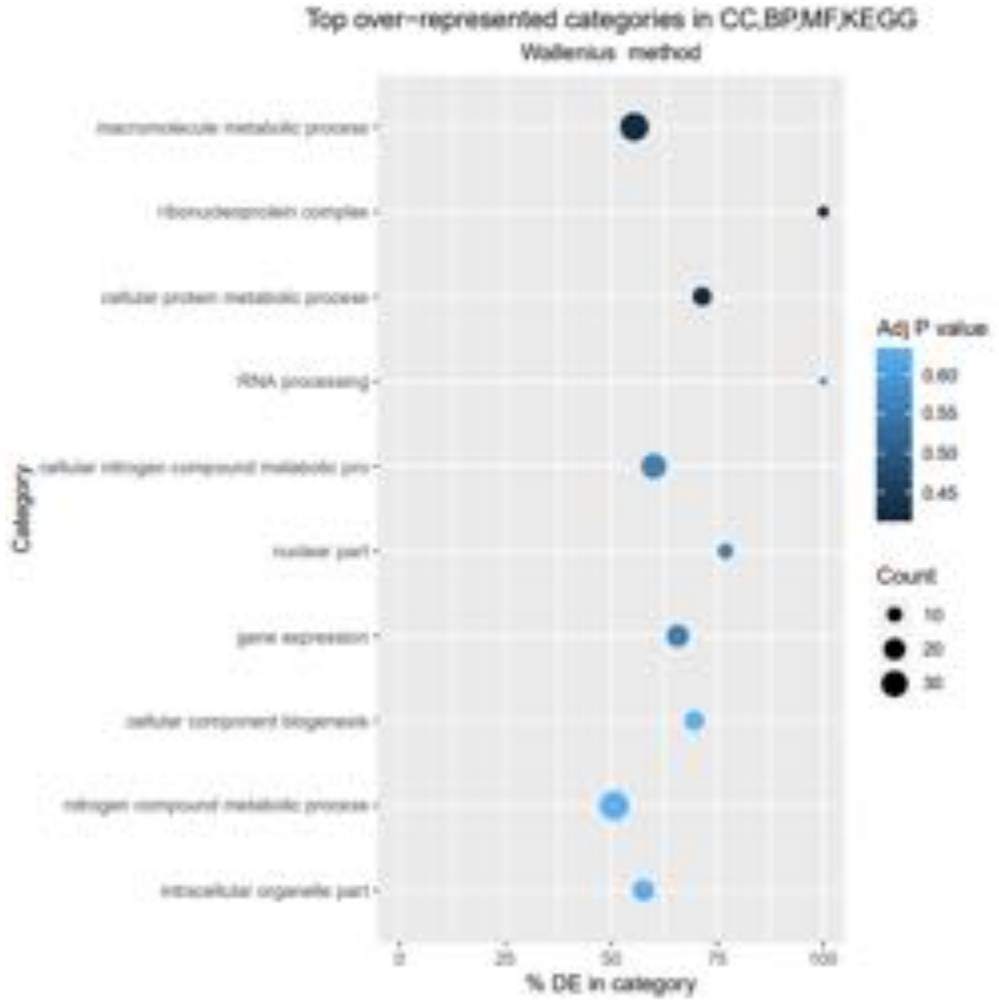
Chemotaxis Assay



(Vang, Medvedev and Adler, 2012)

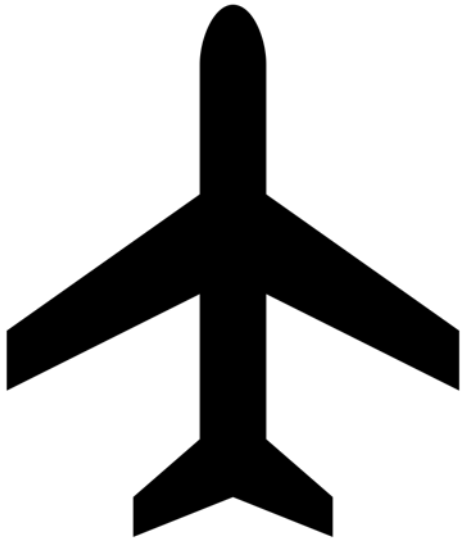
Gene Ontology Underground Control vs Underground Exercise

- **Gene Ontology Analysis**
- **Canton S Female**
- **Weeklong Exposure**
- **Underground Control vs Underground Exercise**
- **Over-represented Categories in Carbohydrate Metabolism**



Questions Arising

- **How Male Transcriptome differently affected?**
- **Do these changes translate to behavioural changes?**



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Innovation in action

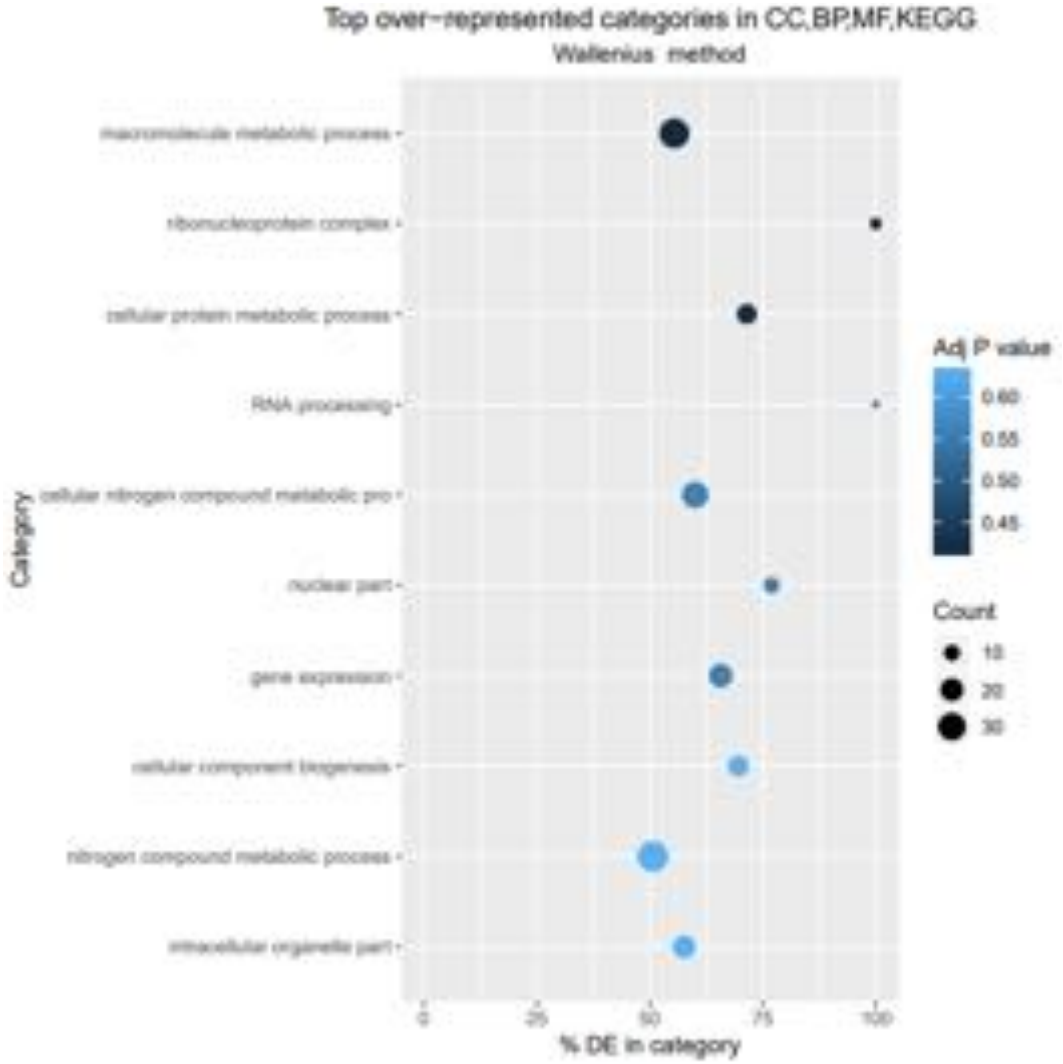
SNOLAB

NSERC CRSNG

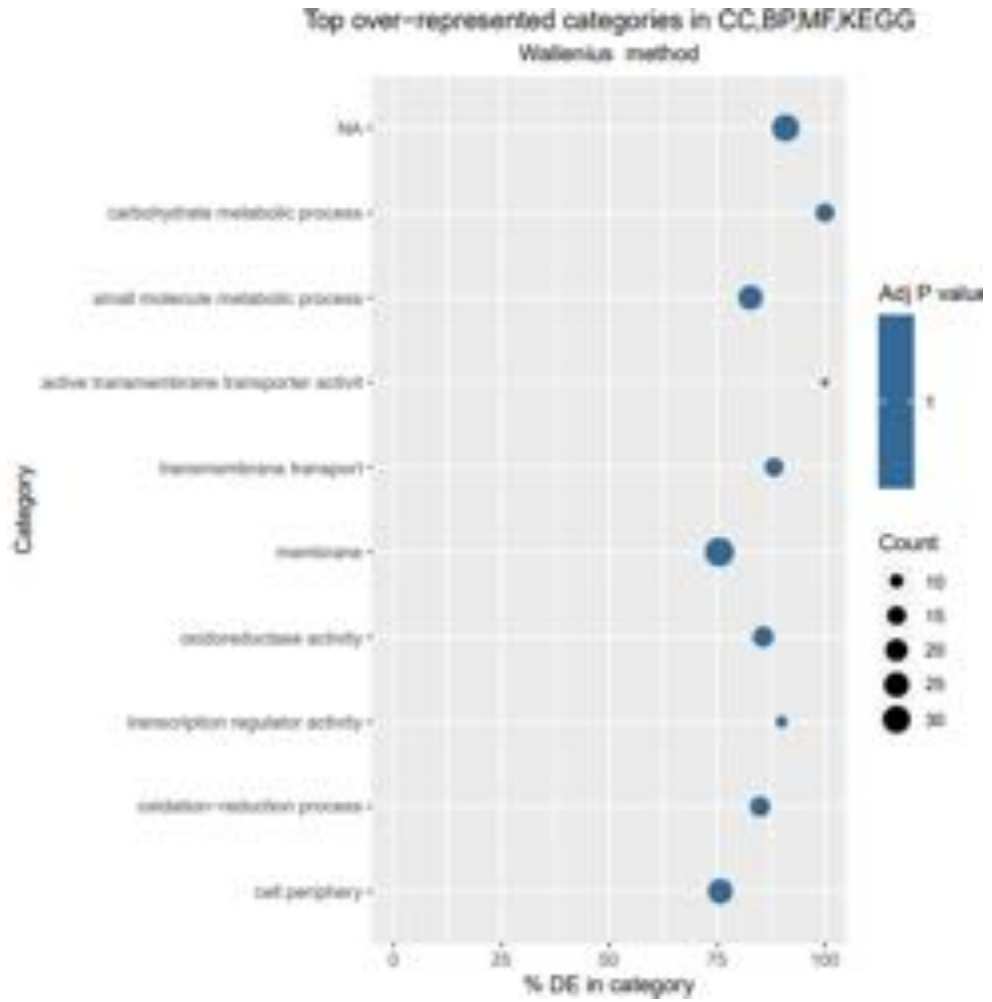
Laurentian University Université Laurentienne

Canada Research Chairs / Chaires de recherche du Canada

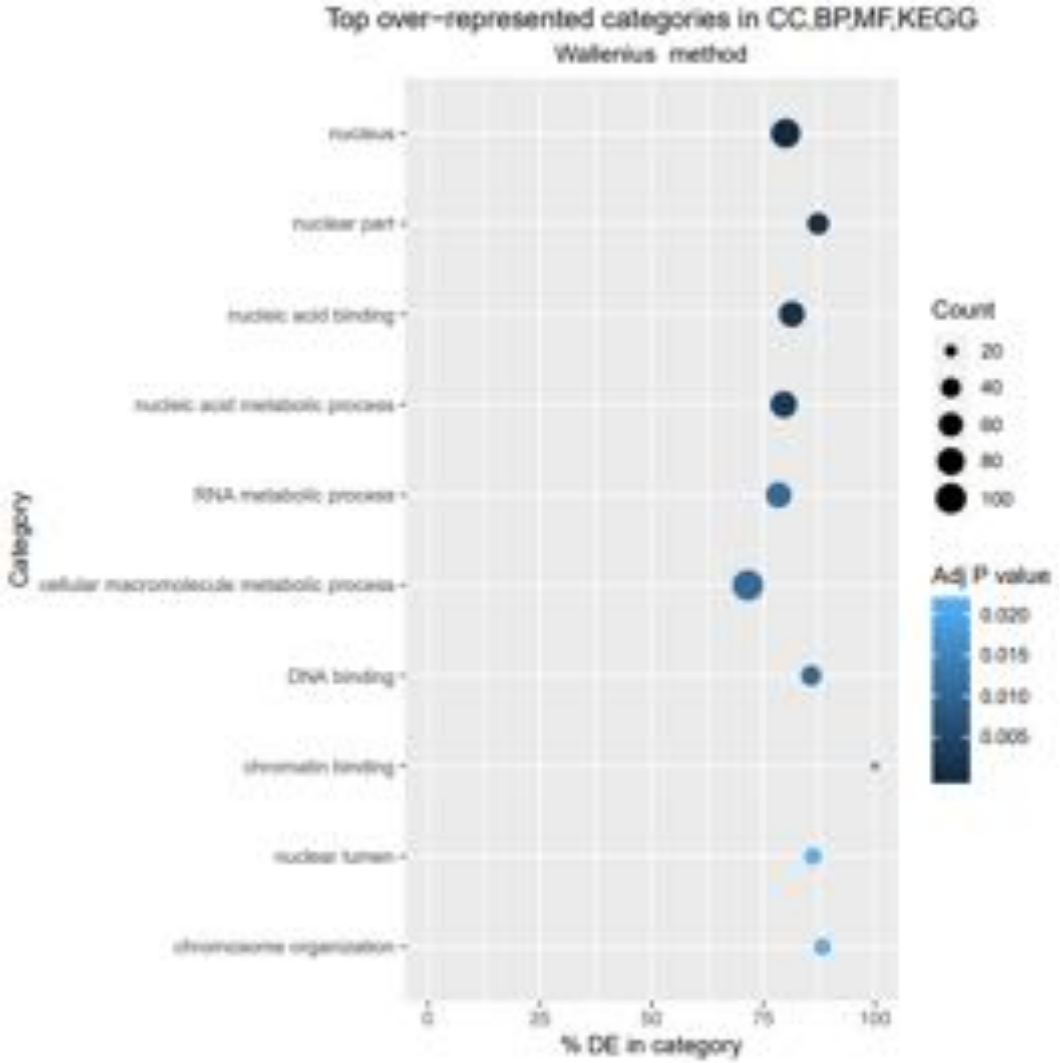
Underground Control vs Underground Exercise Upregulated



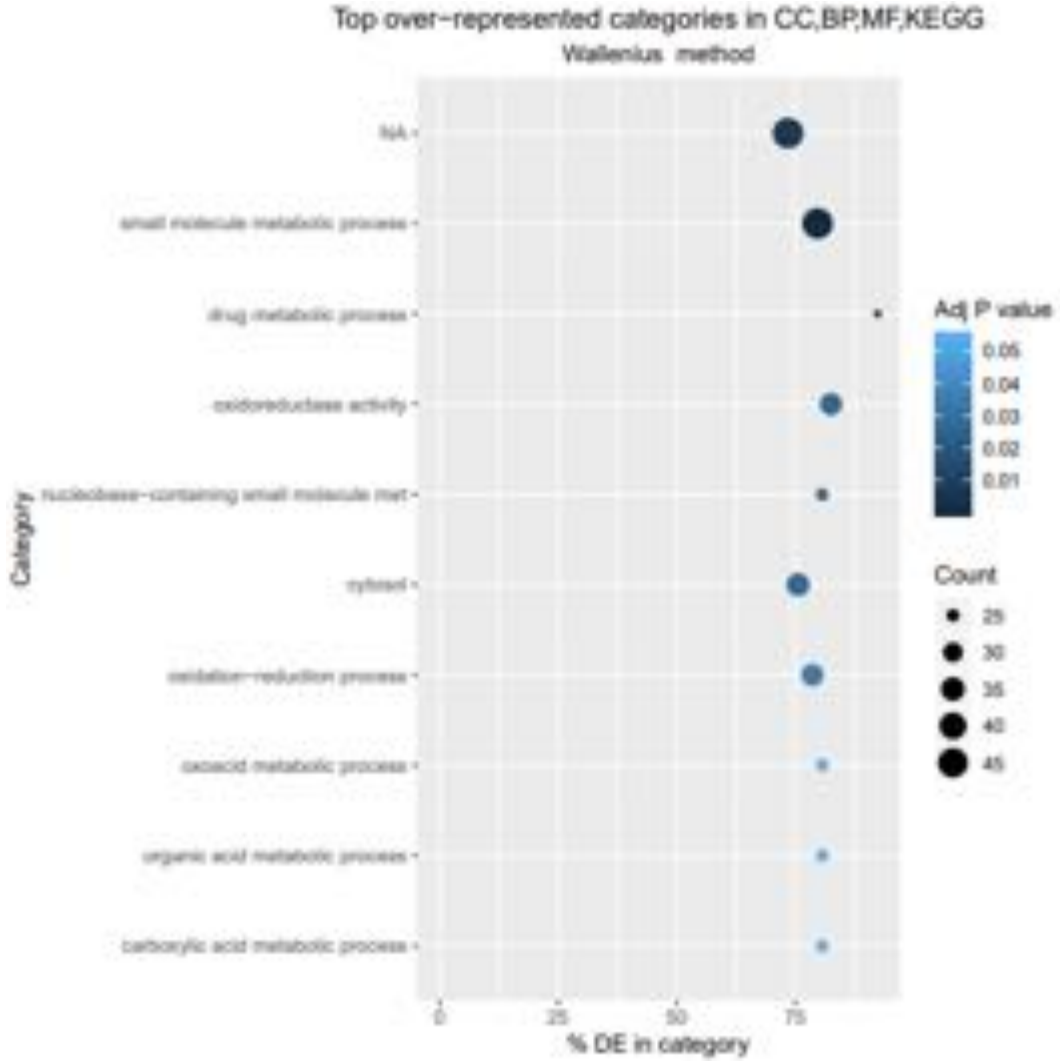
Underground Control vs Underground Exercise Downregulated



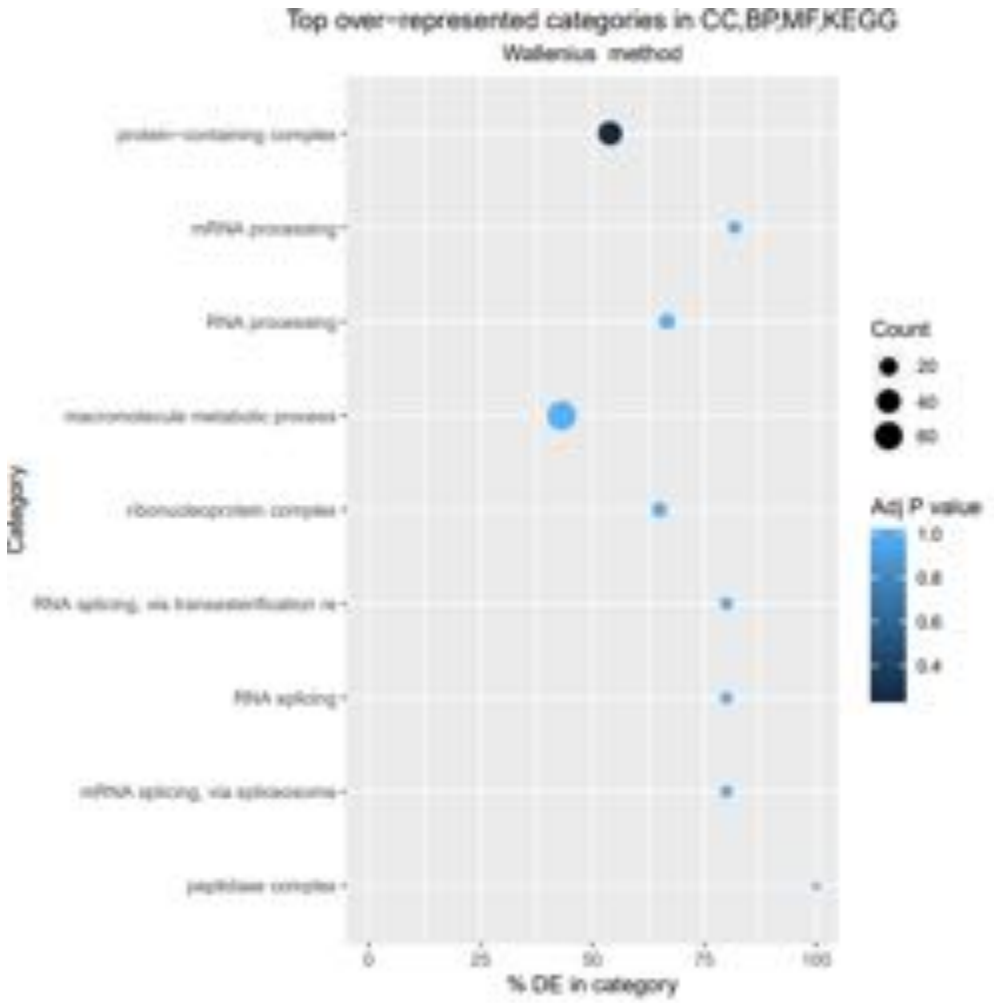
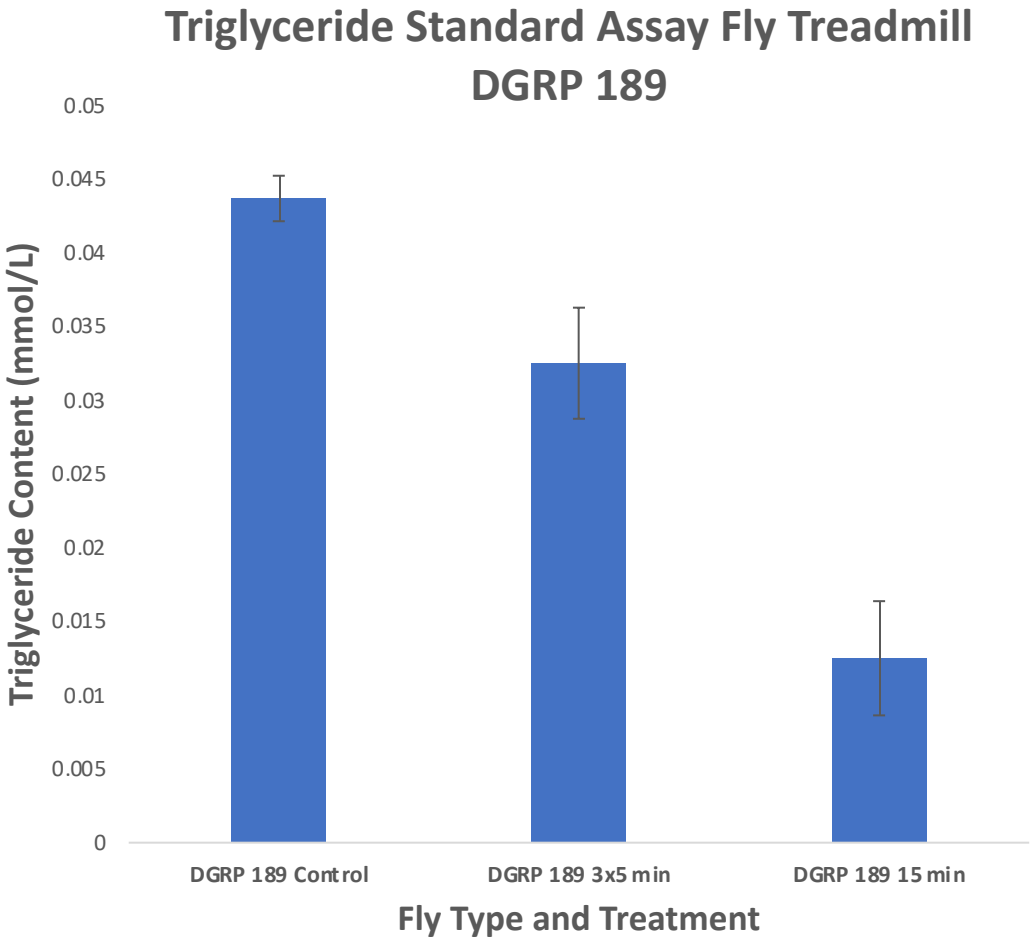
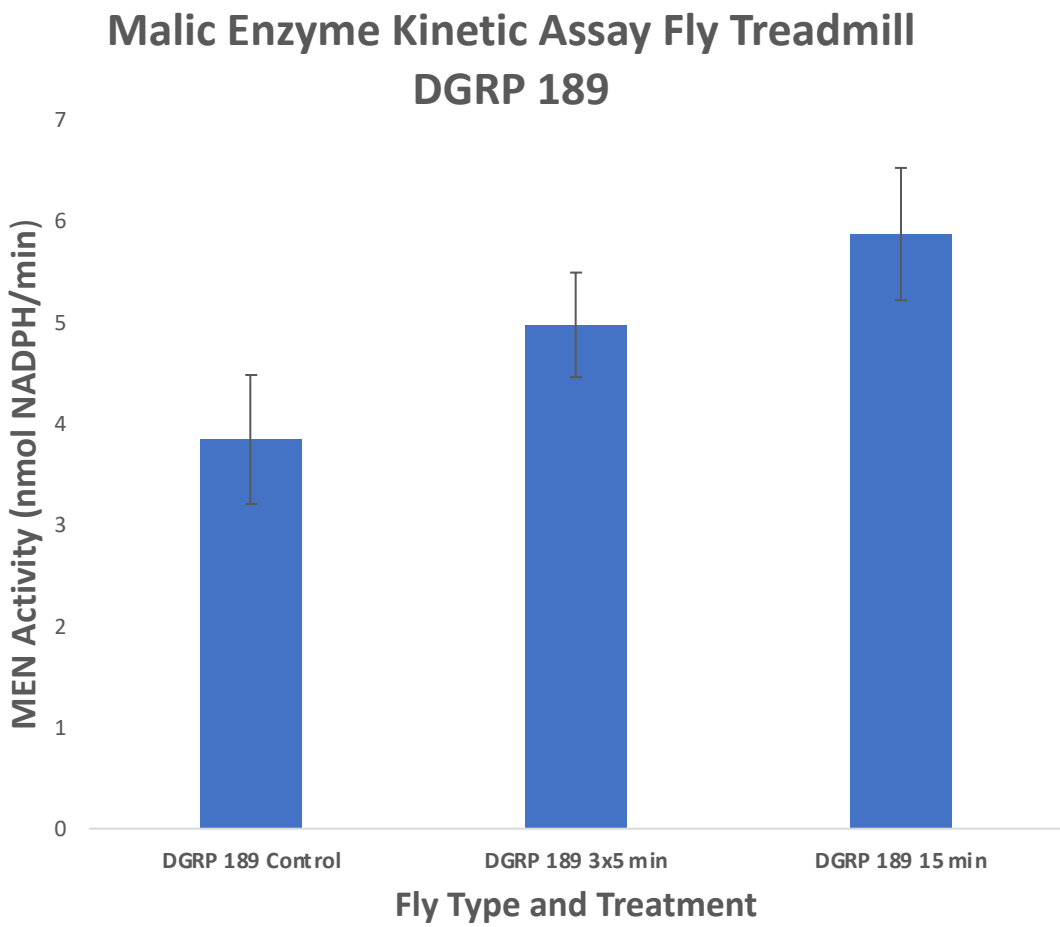
Surface Exercise vs Underground Exercise Upregulated



Surface Exercise vs Underground Exercise Downregulated



Surface Control vs Underground Control Downregulated



Does The Flygometer Work?