East Tennessee State University

Digital Commons @ East Tennessee State University

Appalachian Student Research Forum & Jay S. Boland Undergraduate Research Symposium

2022 Boland Symposium Schedule

Apr 6th, 9:30 AM - 9:45 AM

Severe Hypoxia Alters Metabolism in Daphnia by Inducing Gluconeogenesis

Morad Malek
East Tennessee State University

Lev C. Yampolsky
East Tennessee State University

Follow this and additional works at: https://dc.etsu.edu/asrf

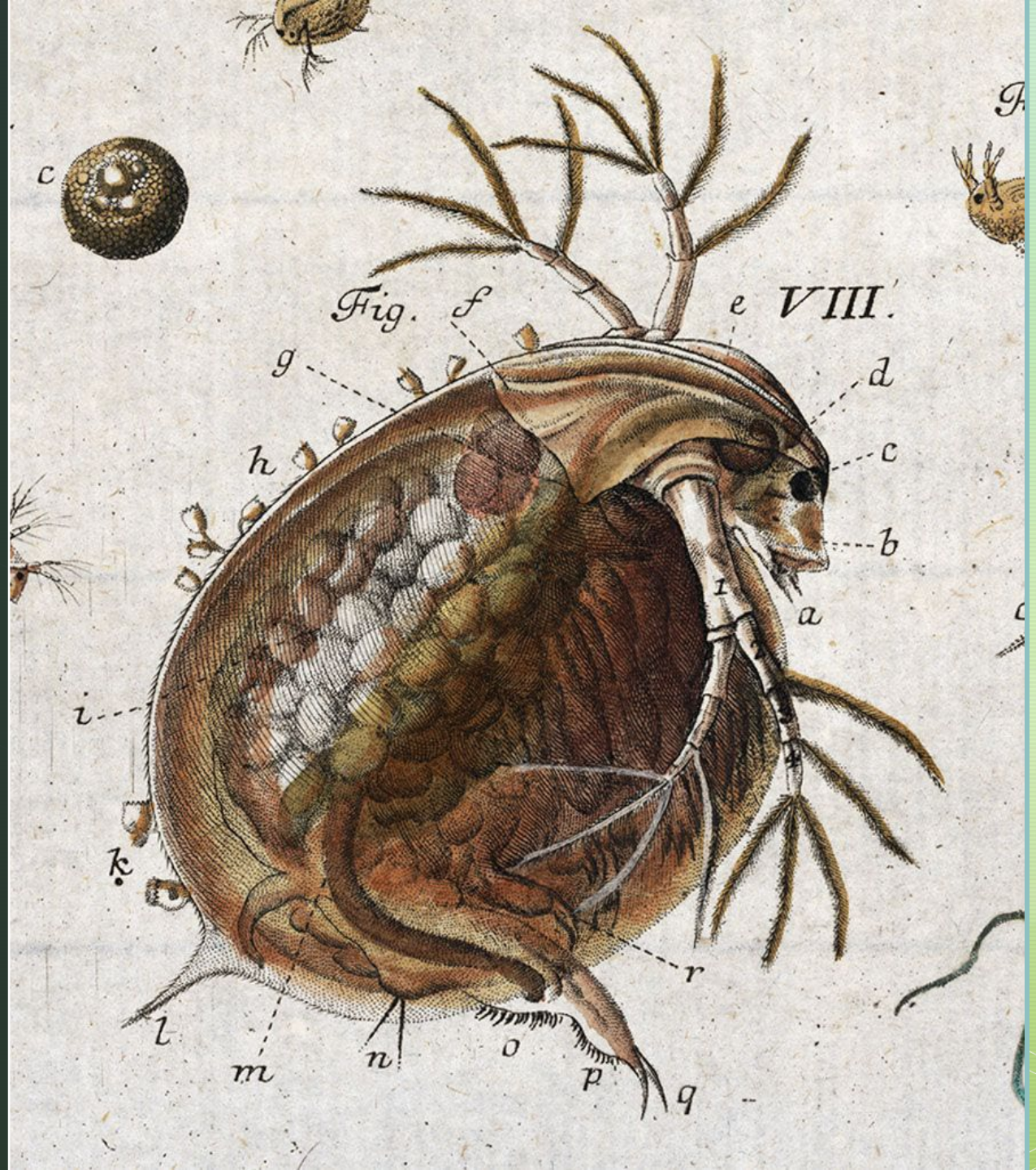
Malek, Morad and Yampolsky, Lev C., "Severe Hypoxia Alters Metabolism in Daphnia by Inducing Gluconeogenesis" (2022). *Appalachian Student Research Forum & Jay S. Boland Undergraduate Research Symposium*. 44.
https://dc.etsu.edu/asrf/2022-boland/schedule/44

This Oral Presentation is brought to you for free and open access by the Events at Digital Commons @ East Tennessee State University. It has been accepted for inclusion in Appalachian Student Research Forum & Jay S. Boland Undergraduate Research Symposium by an authorized administrator of Digital Commons @ East Tennessee State University. For more information, please contact digilib@etsu.edu.

Severe Hypoxia Induces Gluconeogenesis in *Daphnia*

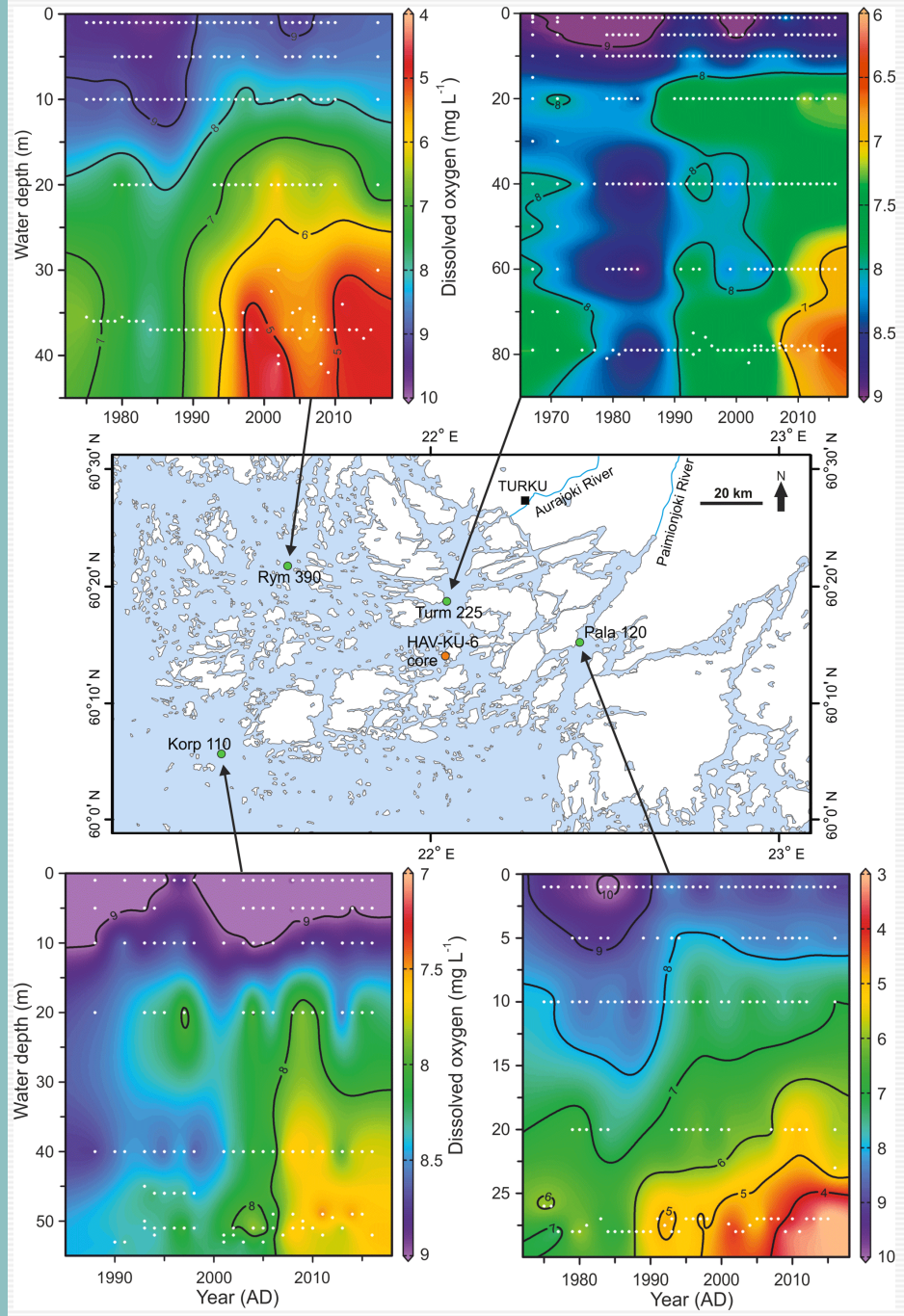
Morad Malek

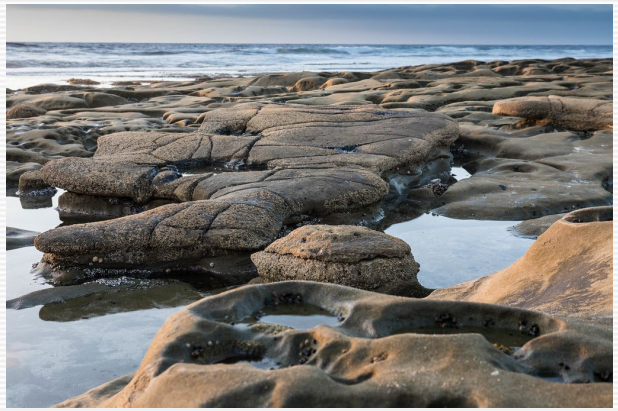
Dr. Lev Yampolsky's Lab



Introduction: Hypoxia

- Hypoxia is an ecological constraint on aquatic organisms
- O_2 concentrations in aquatic habitats can fluctuate from 300% to near complete anoxia
- We want to understanding the effects of hypoxia on life - including adaption.

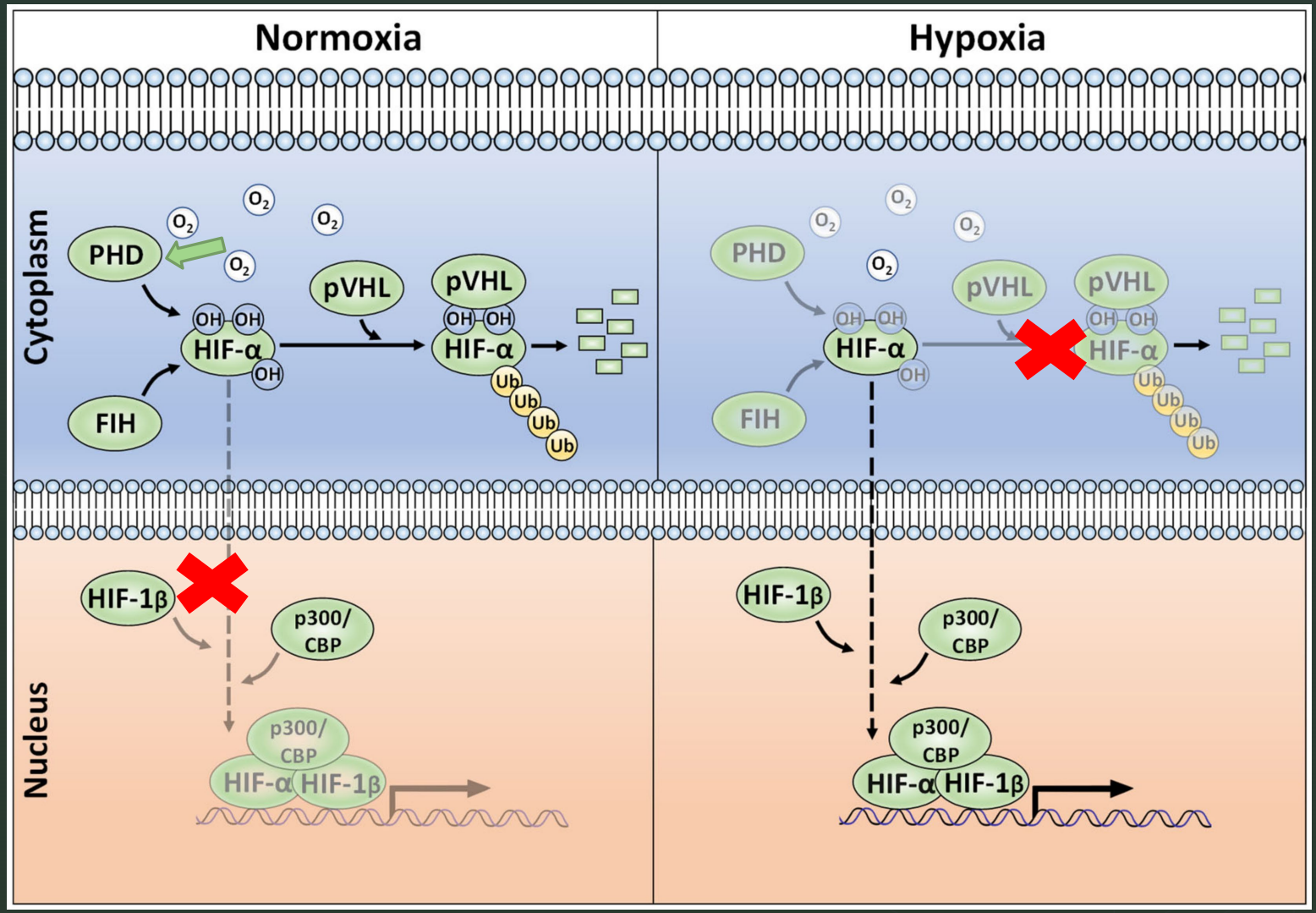




CC BY-SA-NC CC BY-NC

Genetic Responses to Hypoxia

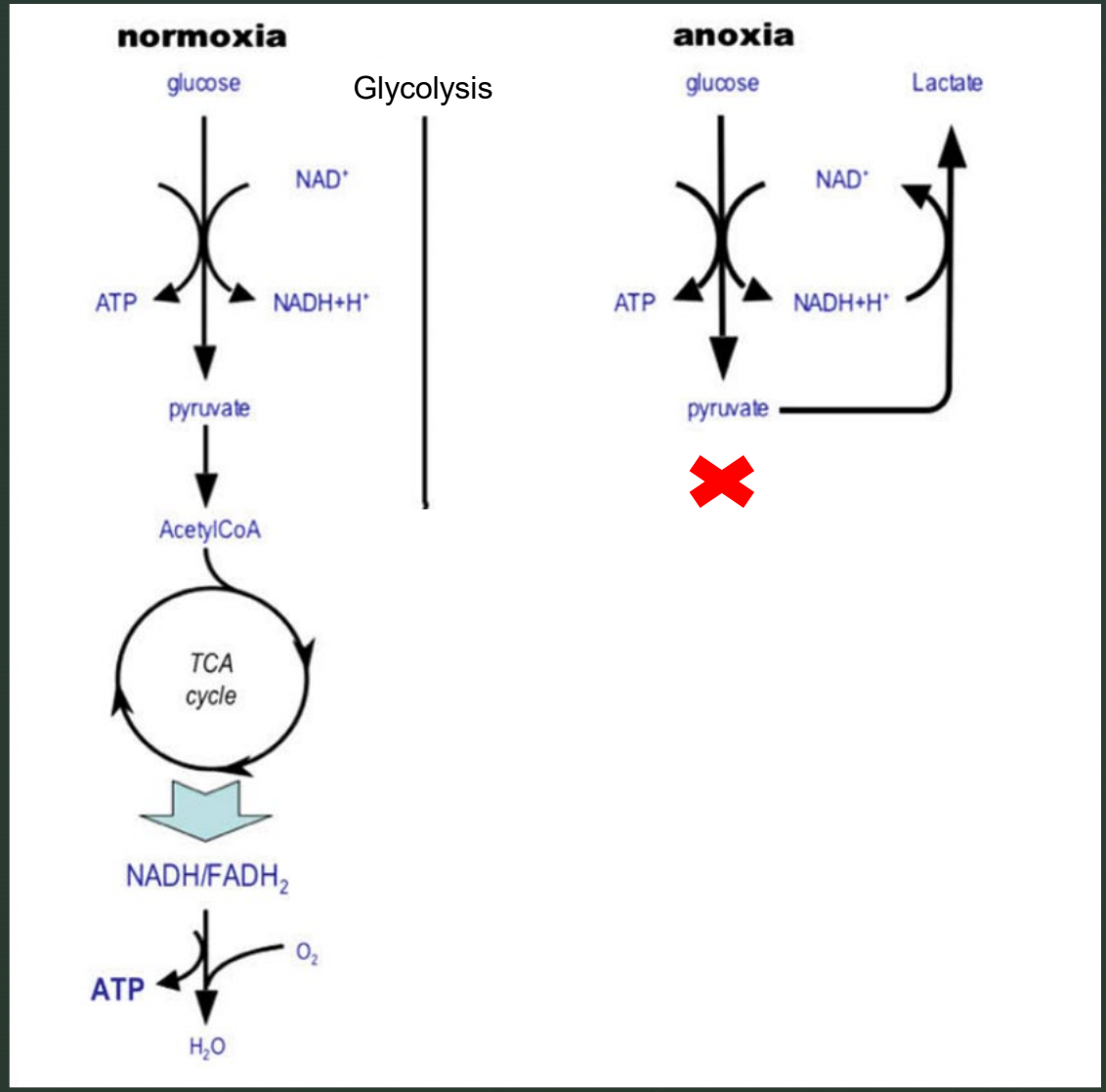
- Hypoxia inducible factors (HIFs) are the main hypoxia response genes
- Once activated, HIFs alter the transcription of hundreds of down-stream genes
- Genes involved in metabolic compensation, O2 delivery…what else?



https://doi.org/10.3390/ijms22010268

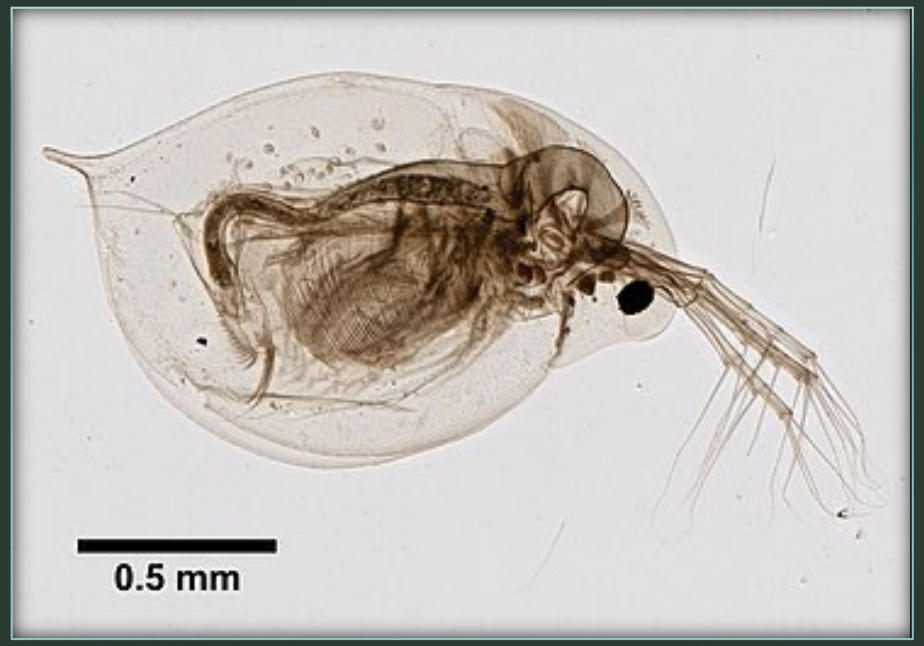
Metabolic Changes During Hypoxia

- Oxidative phosphorylation stops
- Glycolysis takes over to produce energy, with pyruvate made that is converted to lactate
- This is bad because the body will loss significant amounts of ATP



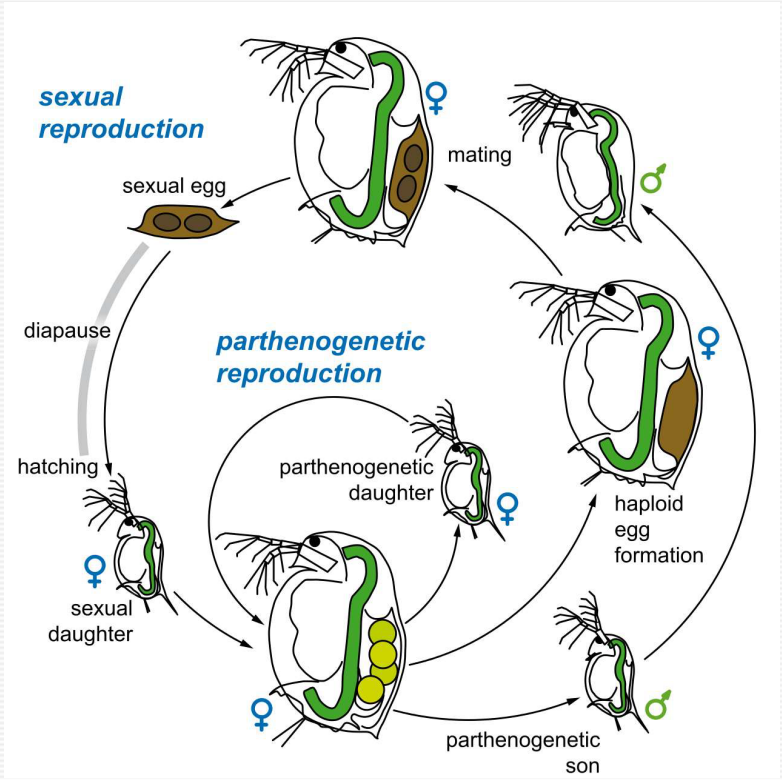
Research Questions:

- What metabolic changes occur during severe hypoxia?
- Are these metabolic changes adaptive do they show genotype-by-environment interaction?
- Used *Daphnia magna* as a model organism for an RNA-seq and survival in hypoxia experiment



Methods: *Daphnia*

- *Daphnia magna* are freshwater micro-crustaceans
- They can reproduce identical female clones of themselves
- We used 4 clones: 2 intermittent hypoxia prone, 2 not prone

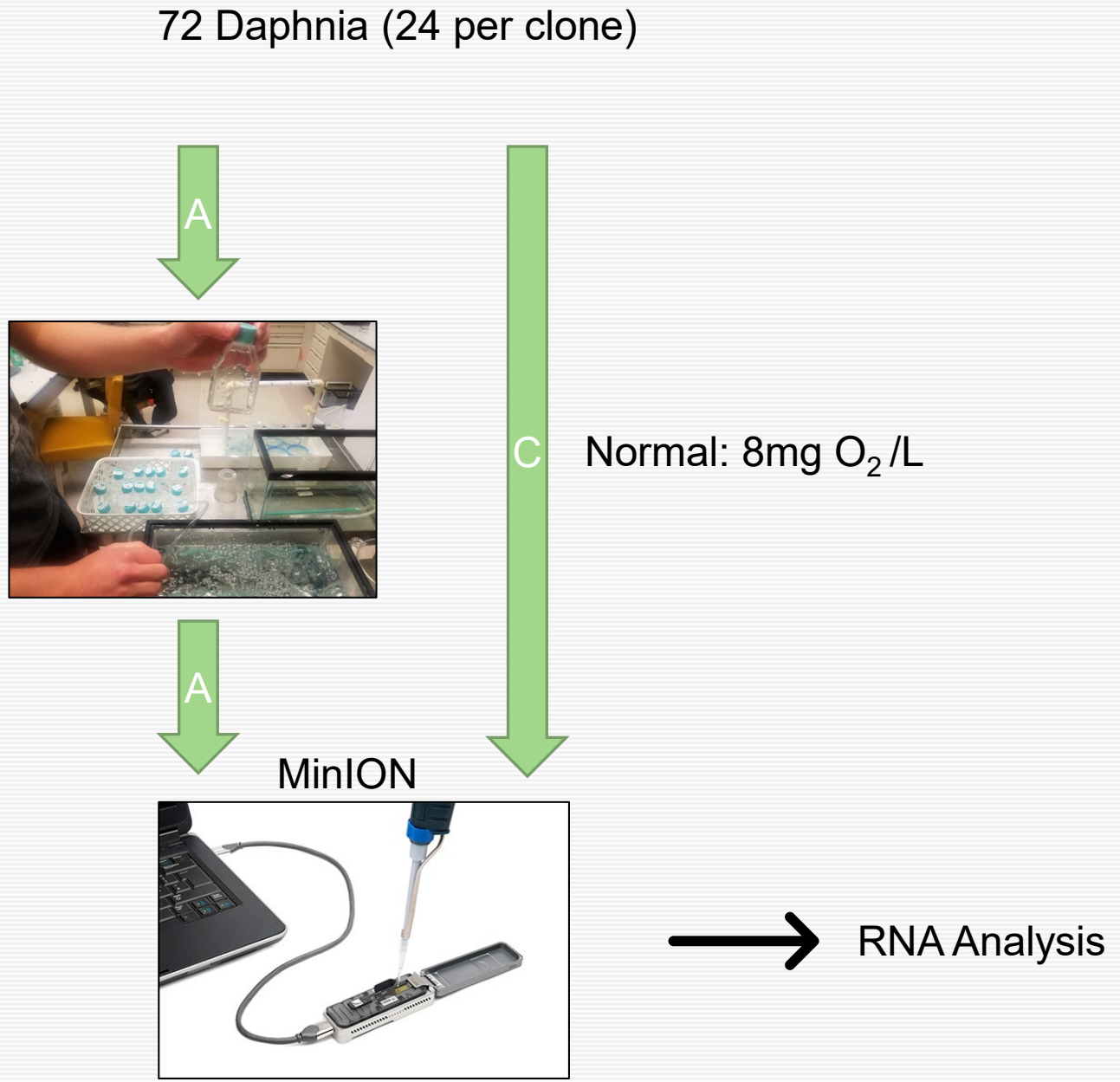


CLONE ID	LOCATION	HABITAT
IL-M1-8	Jerusalem, Israel	Intermittent mediterranean pond
FI-FSP1-16-2	Suur-Pellinki, Finland	Intermittent summer rock pool
GB-EL75-69	London, UK	Permanent pond
HU-K-6	Hungary	Permanent lake

CC BY-SA CC BY-NC

Methods: Acute Severe Hypoxia

- We reared our 4 clones to normal O_2 (8mg O_2/L)
- Exposed to 12h of acute hypoxia (1 mg O_2/L) – Control kept at normal
- Removed some clones for RNA seq and analysis using MinION

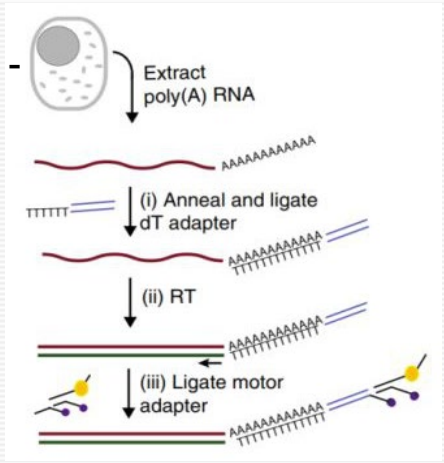


Methods:
RNA seq & analysis

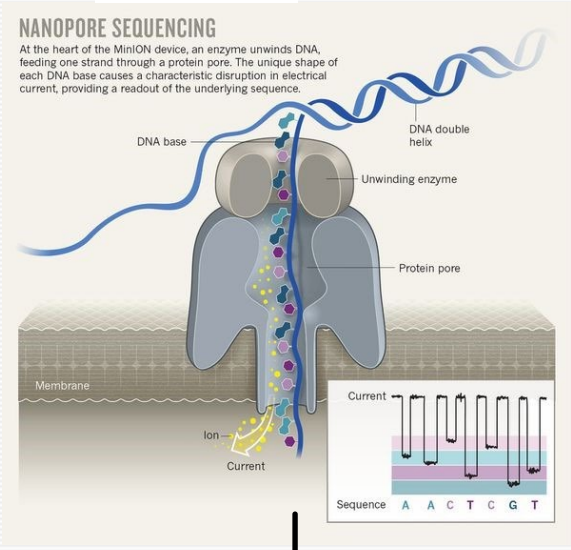
- Extracted RNA from full *Daphnia,* then sequenced copy-DNA and aligned them to reference transcriptome

- Used gene ontology analysis GO (gene clusters) to see what gene pathways are up/down-regulated

Clones ->



(iv) Load and read



Raw read data

Align reads to reference transcriptome

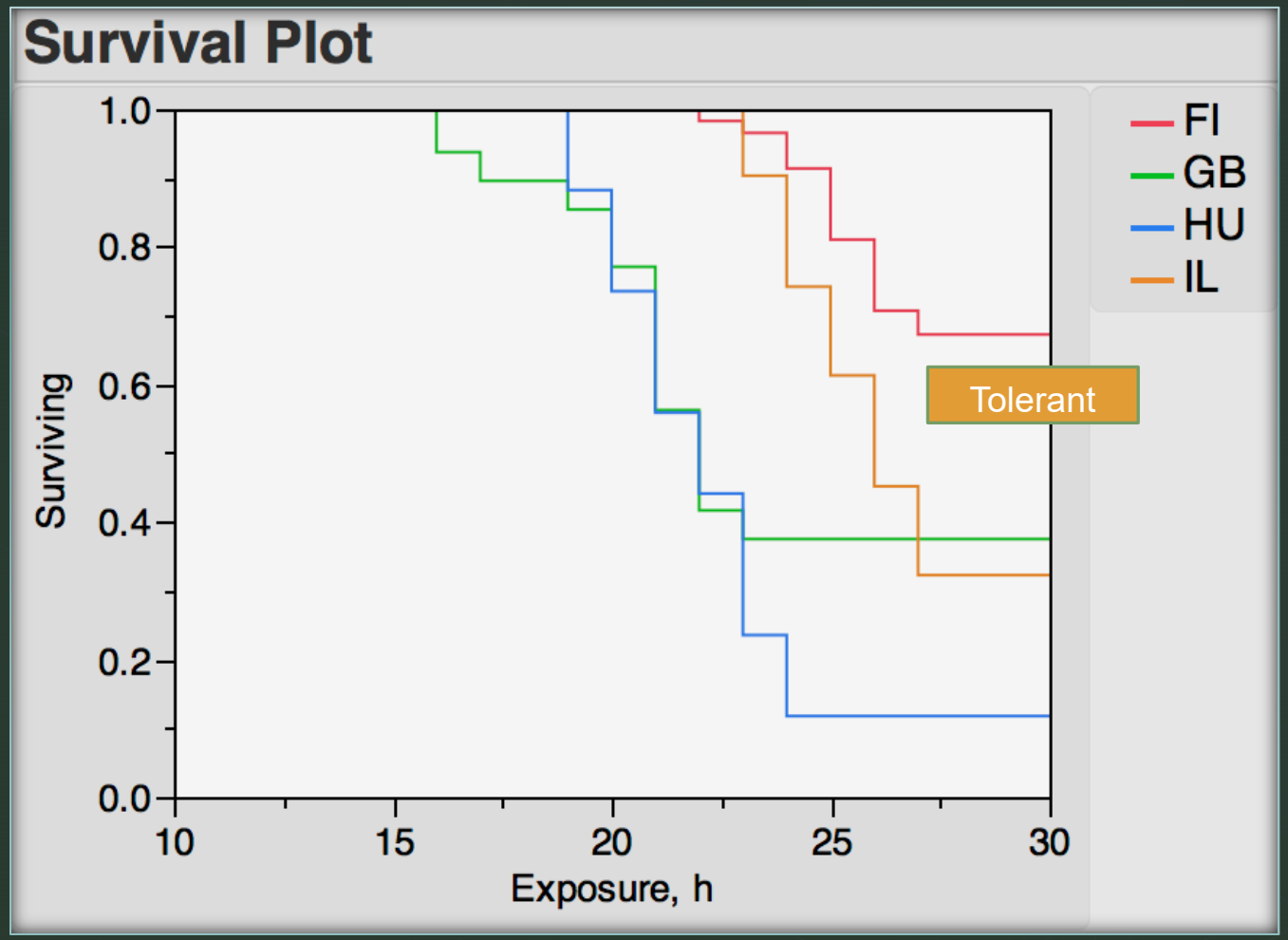
GO lists

Statistical tests:
ANOVA, likelihood ratio test,
Principle component analysis…

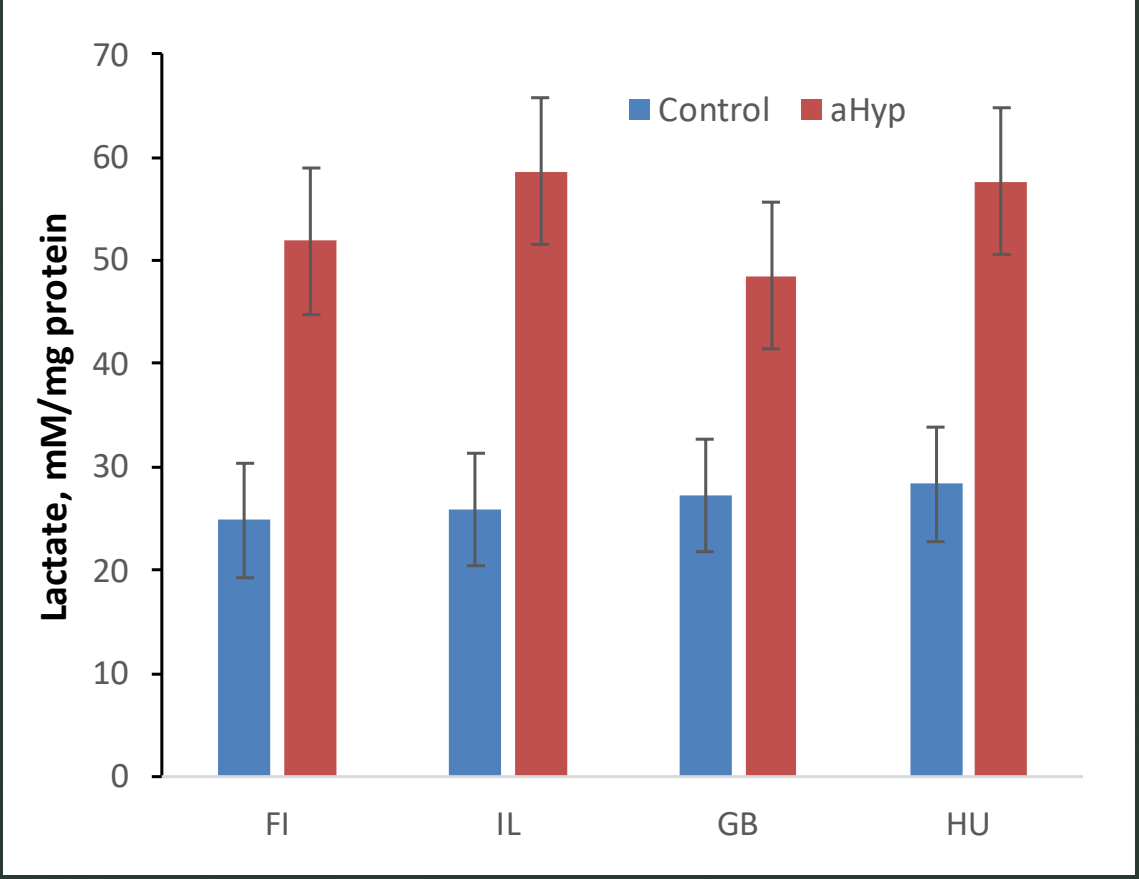
CC BY-SA CC BY-NC

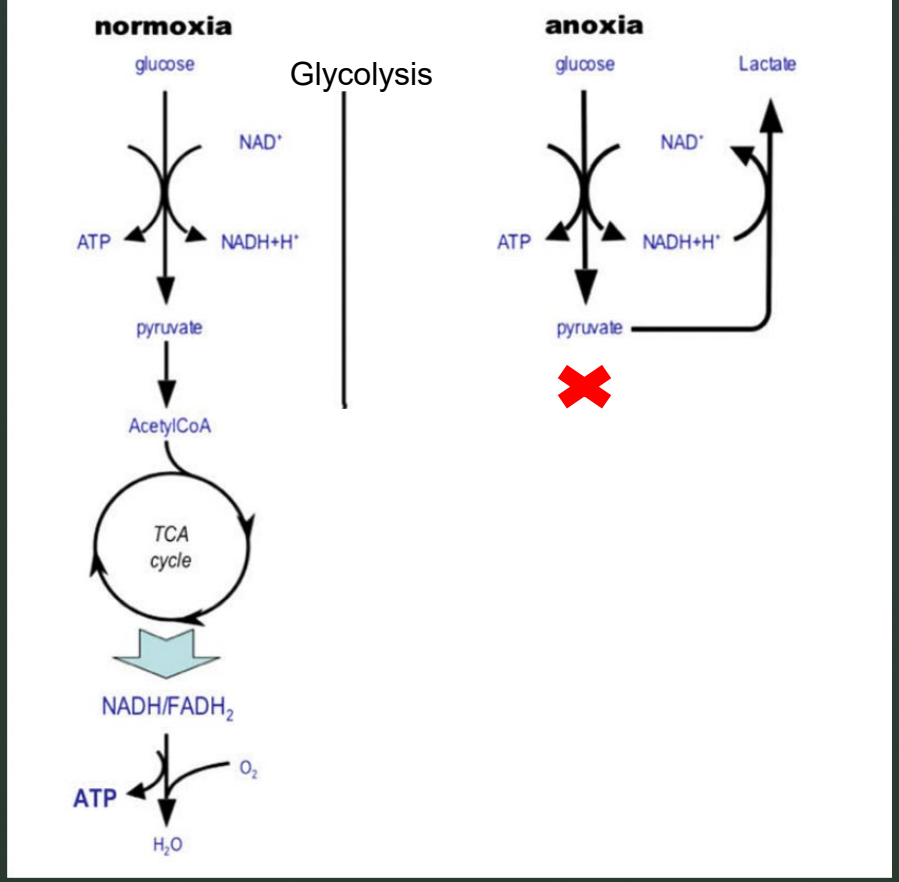
Results: Hypoxia Survival

- The clones originating from hypoxia waters generally have better survival in severe hypoxia
- However, results are limited by only having 4 clones



Lactate Response to Hypoxia

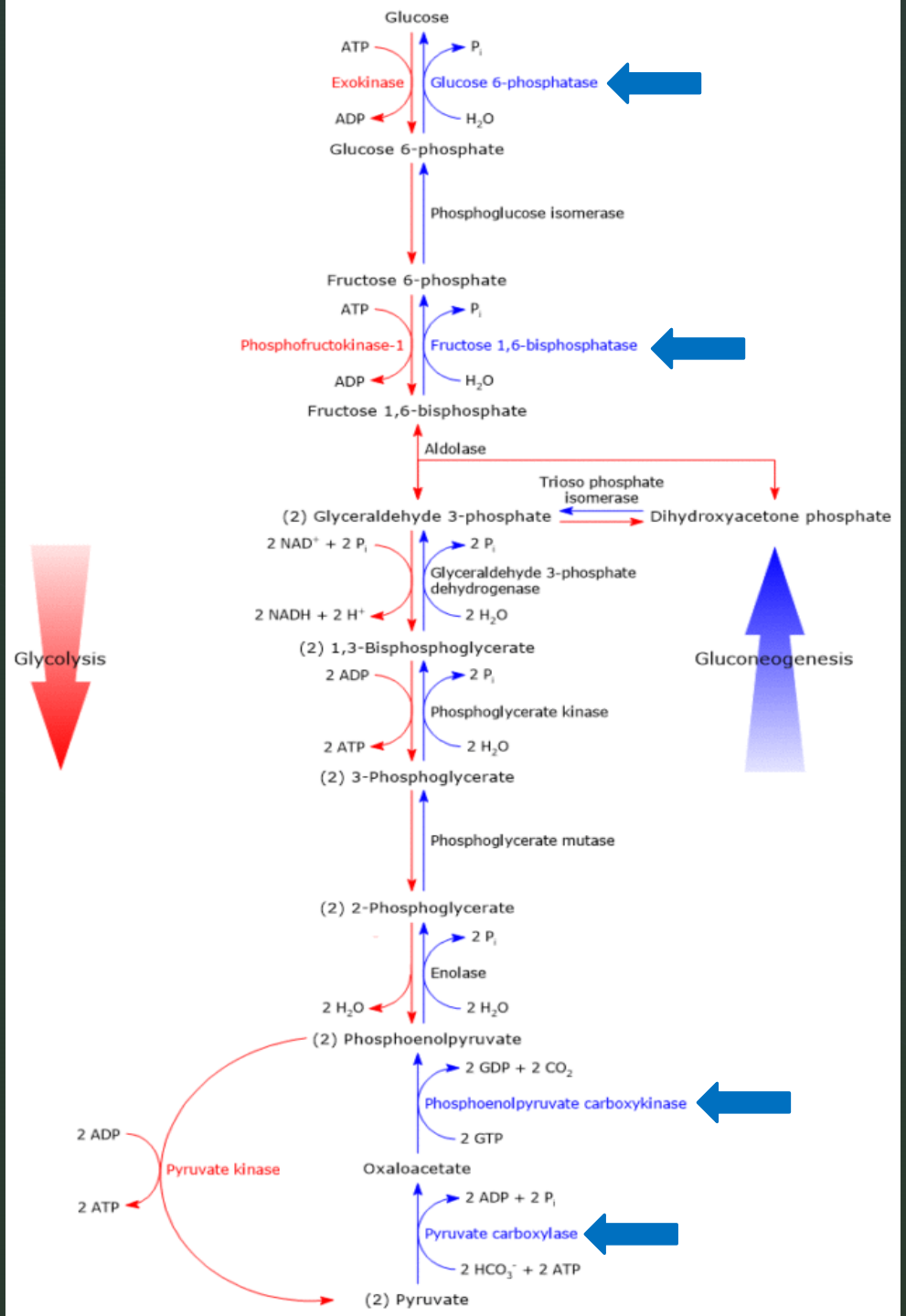




- Not surprising, but what else can pyruvate do?

Gluconeogenesis (GNG)

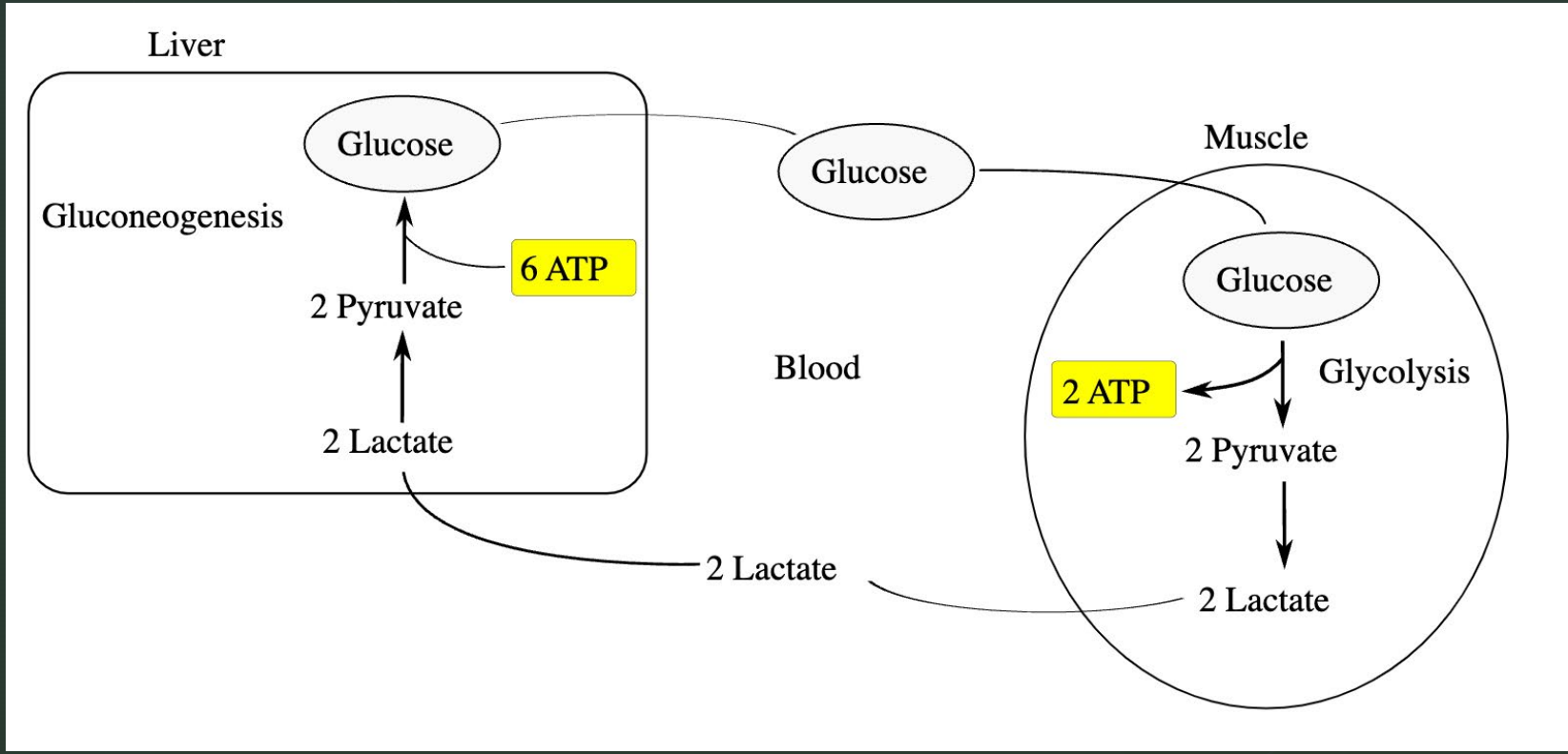
- GNG is the opposite of glycolysis.
- The pathway is mostly the same as glycolysis with “gates”



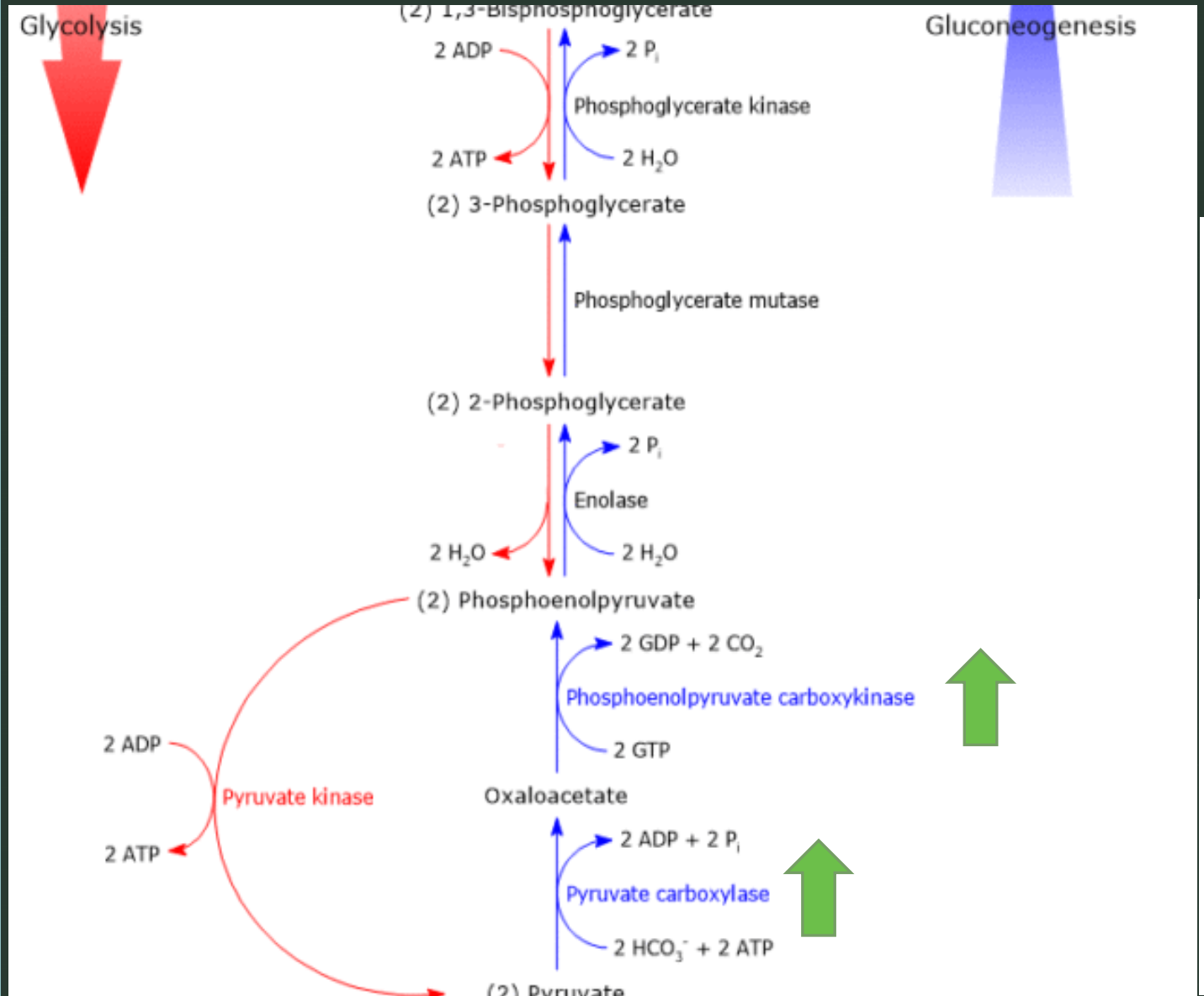
CC BY-SA

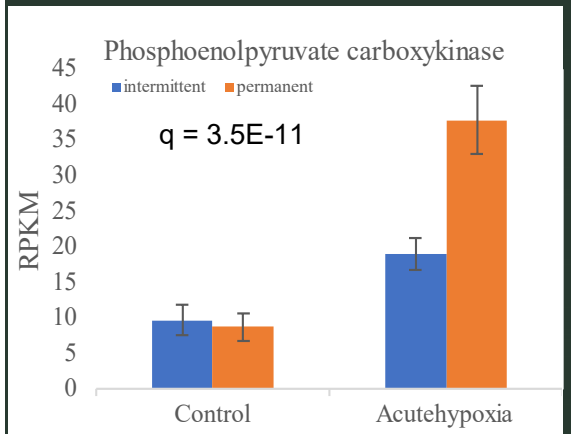
Cori Cycle

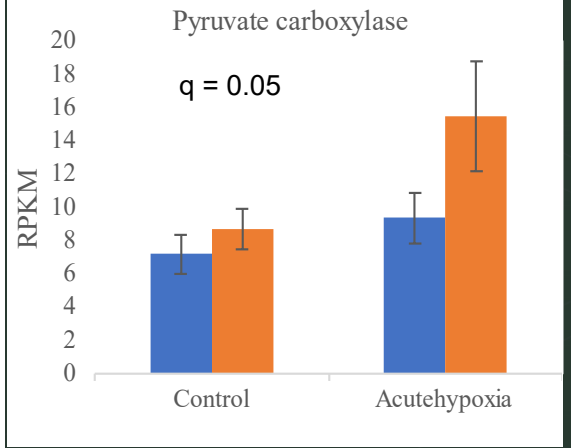
Why would both pathways occur at the same time?



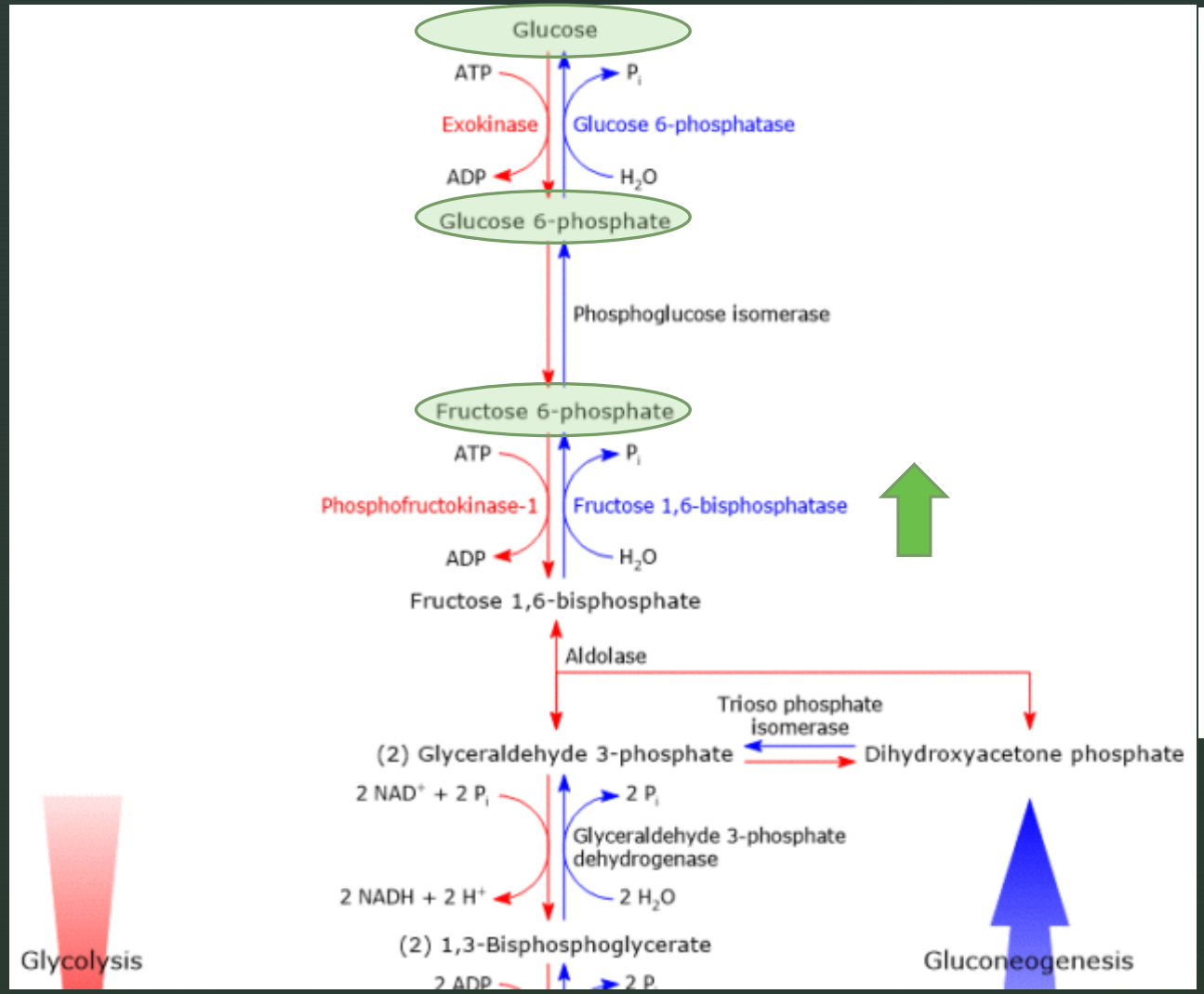
- We see significant up-regulation in (3/4) key GNG transcripts

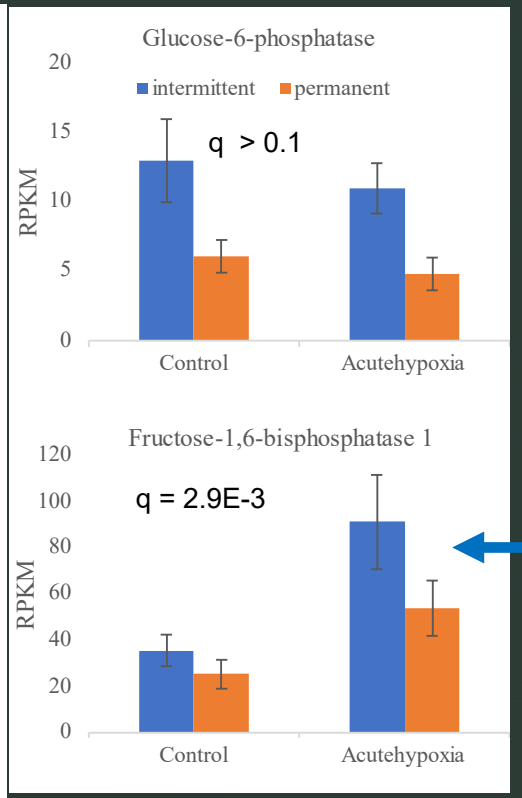






CC BY-SA

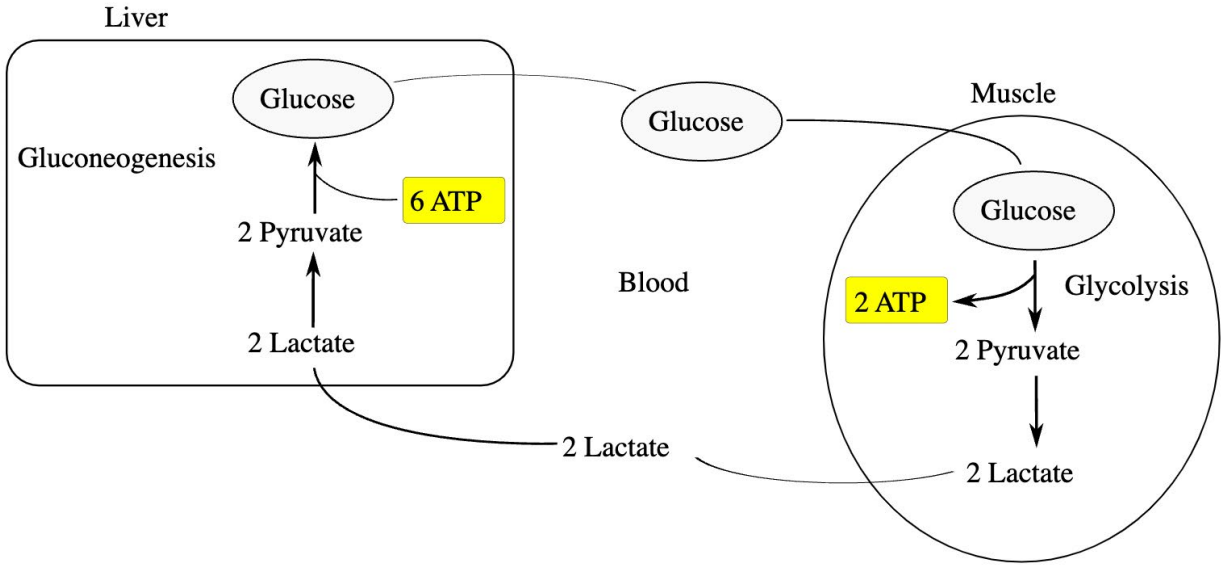




Fructose-1,6-bisphophate is the only one for hypoxia tolerant clones that is more up-regulated

- Possible protective effect

Conclusions:



- Hypoxia tolerance appears to show genotype-by-environment interactions – but more clones needed
- Overall, we can add GNG to the list of possible metabolic compensation changes that occur during hypoxia.
- Cori cycle activation (compartmentalized) by HIFs
- Could be conserved across invertebrates Vora et al. (2021)

Future work:

Expand the number of clones to 24

Full meta-omics analysis – metabolites, etc.

Tissue specific RNA sequencing

Any Questions?

Acknowledgments

- Dr. Lev Yampolsky, Cora Anderson, Millicent Ekwudo, and Taraysha Moore
- ETSU student faculty collaboration grant for funding & summer stipend
- Dieter Ebert and Peter Fields, Basel Univ., Switzerland for the most recent Daphnia reference transcriptome and Dr. Kumar for lab equipment

CC BY-NC-ND