

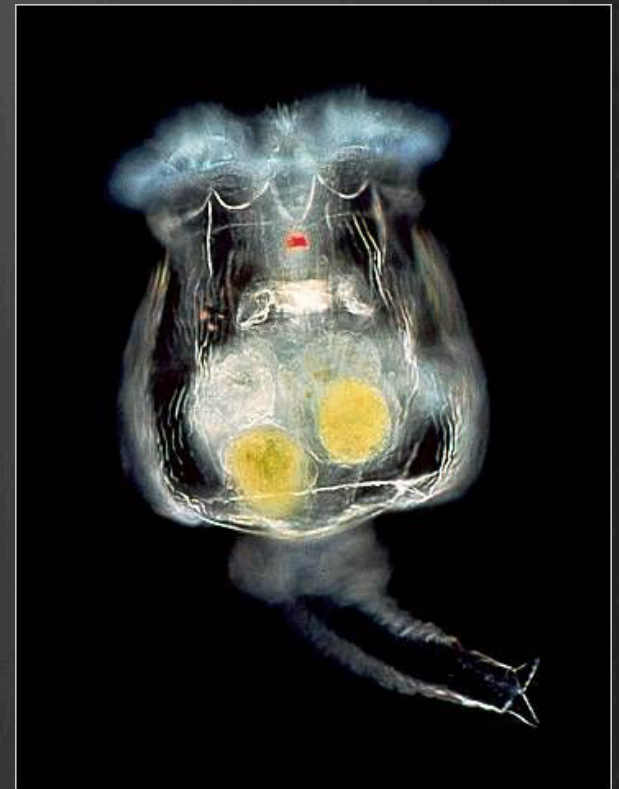
Neutral lipid metabolism of the rotifer
Brachionus plicatilis during periods of
starvation

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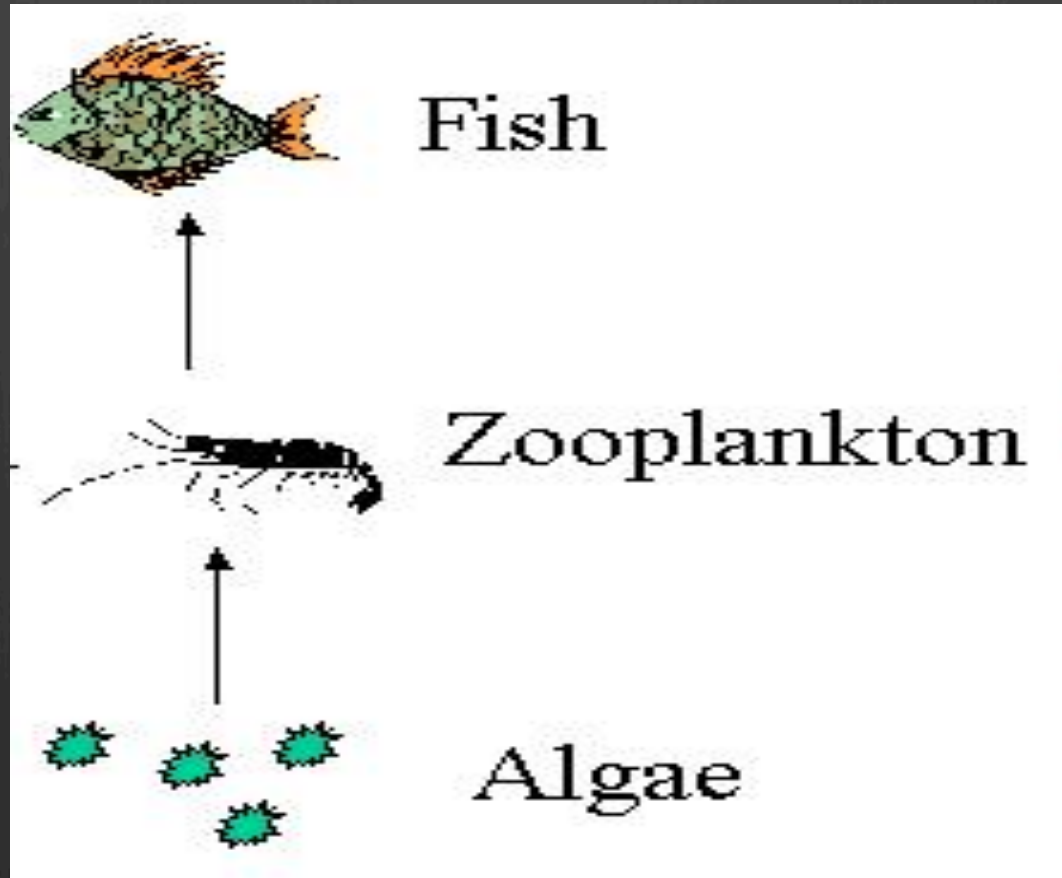
Background

- Type of zooplankton
- 0.05 to 2 mm in size
- Lifespans ~1 to 3 weeks
- Live in freshwater and saltwater
- Used as food for young fish
- Common research subject

(e.g., Wallace et al. 2015)



Background



Background

- Rotifer lipid content depends on both their metabolism and diet (Fernández & Labarta 1996)
- Neutral lipids are used for energy storage in rotifers (Frolov & Pankov 1992)
- Lipids serve a structural role in rotifers (Guisande & Serrano 1989)

Background

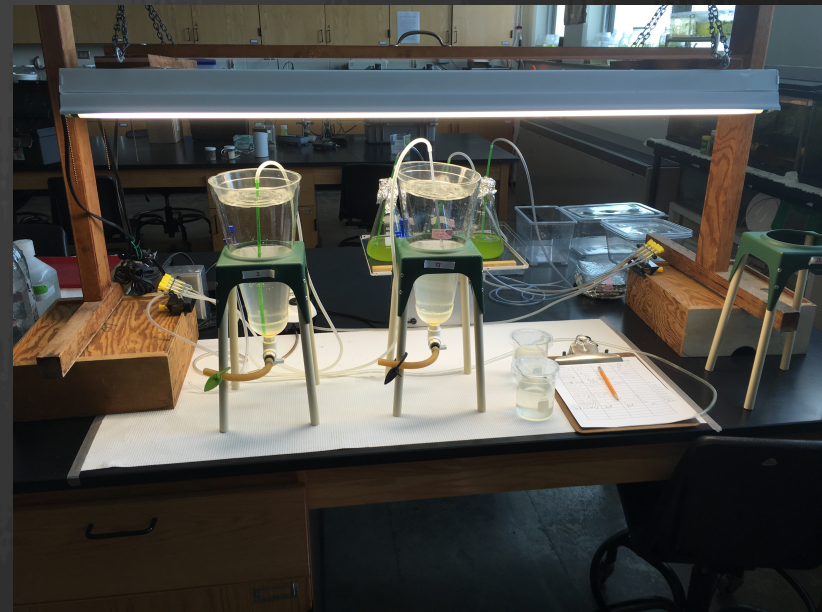
- Food sources change over the growing season: algal & bacterial species change (Wallace et al. 2015)
- Lipids are metabolized when the rotifers starve (Wilde '16)
- LCMS can be used to identify neutral lipids (Knittelfelder et al. 2014)

Goal and Hypothesis

- Goal: To examine the neutral lipid profile in the rotifer *Brachionus plicatilis* as they starve
- Hypothesis: Starved rotifers will exhibit a change in the neutral lipid concentration and profile

Methods 1: culture

- Cultured in conical vessels in 15 ppt Instant Ocean™
- Fed algae *Nannochloropsis* & Roti-Rich™ (food supplement)
- Filtration down to 180 μm , backwash into 200 mL beaker
- Starved to clear guts for 30 mins.
- Filter again & starved for 0, 24, 48, & 72 hours



Methods 2: Nile Red stain

- Filtered again, placed in 100 mL 15 ppt Instant OceanTM with 500 μ L of Nile Red alcoholic solution (60 mins.)
- Imaged 30 rotifers using Zeiss[®] microscope with Spot[®] (Insight FireWire) camera attachment
- Printed, used Zidas[®] (Carl Zeiss Incorporated) to measure lipid droplets
- Recorded into Excel[®] and performed ANOVA and Tukey-Kramer tests

Methods 2: Nile Red stain



Methods 3: lipid profile

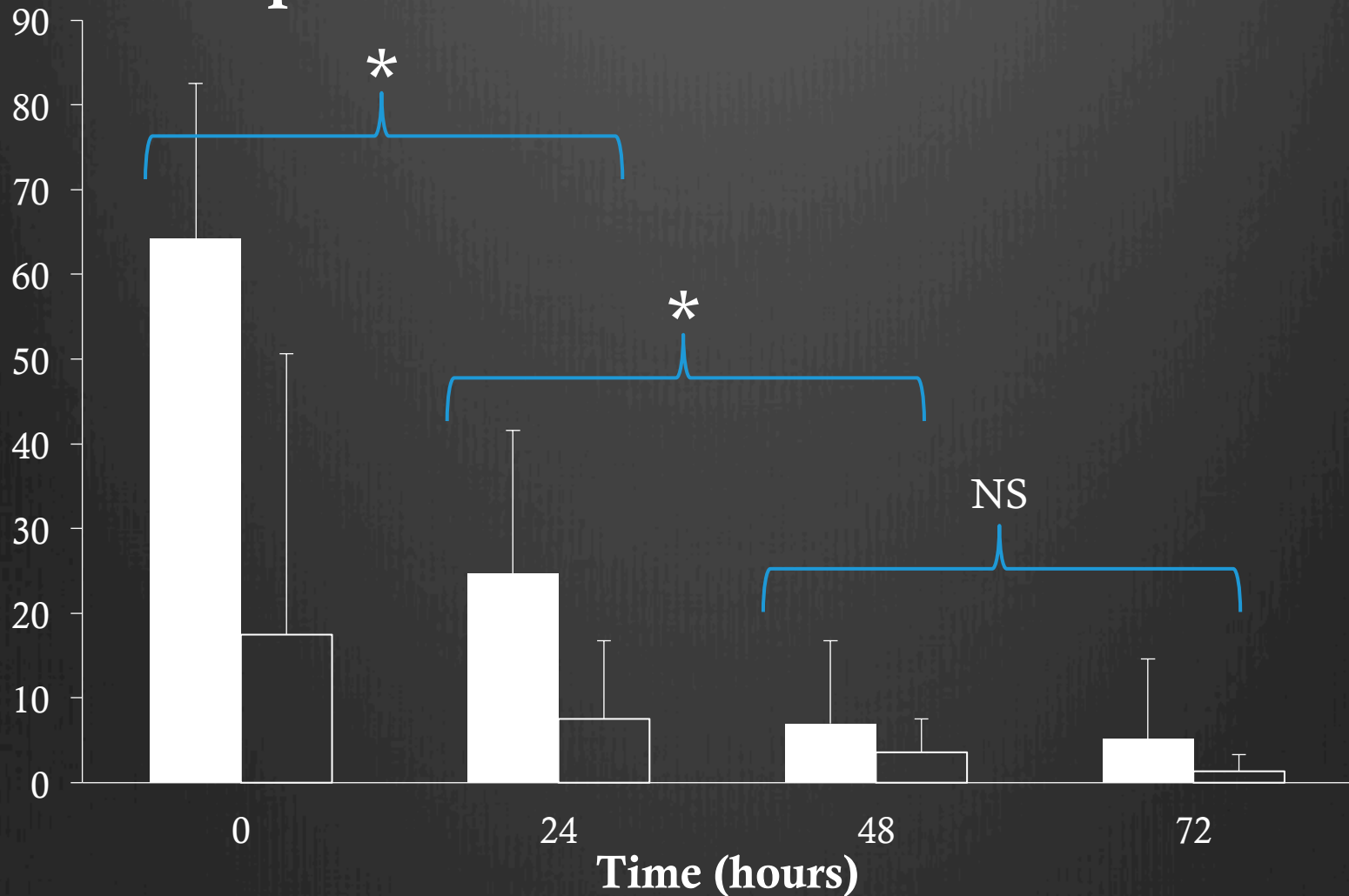
- Re-filter, suspend in 2:1 methanol:water, sonicate (0°C, 6 mins)
- Double the volume with chloroform, vortex, place on ice
- Centrifuge, isolate chloroform layer, rotavape
- Re-suspend in 2 water, 1 methanol, 1 acetonitrile
- Analyze via LCMS, use Agilent® MassHunter to identify lipids

Results

Time (hrs)	Mean Droplet Count	Mean Droplet Volume (μL)	Total Lipid Volume (μL)
0	64.23	17.49	1123.70
24	24.67	7.52	185.57
48	6.97	3.56	24.77
72	5.20	1.37	7.10

Results: Nile Red

Droplet Count and Volume vs. Time



Results: Neutral Lipids

Fatty Acid	0 hours	24 hours	48 hours	72 hours
Linoleic acid	Present	Present	Present	Not Present
Alpha-Linoleic acid	Present	Present	Not Present	Not Present
Nonadecenoic acid	Present	Present	Not Present	Not Present
Eicosadienoic acid	Not Present	Not Present	Not Present	Present
Eicosatrienoic acid	Not Present	Not Present	Present	Not Present
Eicosapentaenoic acid (EPA)	Not Present	Not Present	Present	Not Present
Docosahexaenoic acid	Not Present	Not Present	Not Present	Not Present
Arachidonic acid	Present	Not Present	Present	Not Present
Octadecatetraenoic acid	Present	Present	Present	Not Present

Discussion

- ⊗ Droplet count and volume both decreased over the starvation period
- ⊗ No significant change in droplet count or volume after 48 hours
- ⊗ Rotifers likely switch from lipids to proteins for energy as suggested by Frolov & Pankov (1992)

Conclusions

- ⊗ Neutral lipid content decreases over periods of starvation up to 48 hours
- ⊗ Fatty acid signature changes during starvation
- ⊗ Thus, rotifers use neutral lipids for energy when they starve

Future Directions

- Determine protein content of rotifers before, during, and after periods of starvation
- Repeat research with wild-caught rotifers
- Quantify death of rotifers through periods of starvation

Acknowledgments

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Questions

