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Hemolymph chemistry profiles and fatty acid analyses as tools for evaluating freshwater mussel health

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U.S. Department of the Interior
U.S. Geological Survey

Road map…

- Development of biomonitoring techniques
  - Effects on survival
- Hemolymph chemistry profiles
  - Thermal exposure, CO_2 exposure
- Fatty acids
  - A New Frontier

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What can biomonitoring tell us?

- Tissue samples —> energy stores, genetic analysis
- Hemolymph samples akin to human blood tests
    - E.g. cholesterol, blood sugar, white blood cells

HEALTHCHEK PLUS	CBC COMPREHENSIVE		
Test	Result	Reference Range	Flag
WBC	7.9	4.9 - 17.6 K/uL	
RBC	**5.31**	**5.39 - 8.70 M/uL**	**L**
HGB	**13.3**	**13.4 - 20.7 g/dL**	**L**
HCT	**36.4**	**38.3 - 56.5 %**	**L**
MCV	69	59 - 76 fL	
MCH	25.0	21.9 - 26.1 pg	
MCHC	36.5	32.6 - 39.2 g/dL	

- Inform how sub-acute stresses may impact the health of mussel populations

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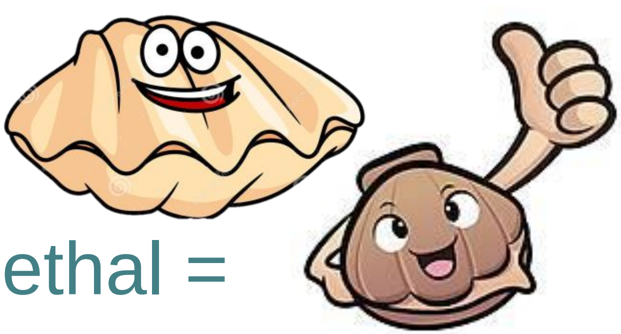
Non-lethal metrics

- Essential when working with imperiled species such as freshwater mollusks

Non-lethal =



Lethal =

- Assessment of extraction techniques reaches back over two decades…

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Tissue extraction effects

- No effect on survival of a 1 cm^2 mantle biopsy (~34 mg wet weight) after 1 year (Berg et al. 1995)
  - *Quadrula quadrula, Actinonaias ligamentina*

- No effect on survival of a 5-10 mg foot biopsy after 1.5 years (Naimo et al. 1998)
  - *Amblema plicata*

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Hemolymph extraction effects

- No effect on survival of 0.5 mL hemolymph extraction (anterior adductor muscle) after 3 mo (Gustafson et al. 2005)
  - *Elliptio complanata*

- No effect on survival of 0.1-0.2 mL hemolymph extraction after 8 weeks (McCartney et al. 2009)
  - *Elliptio waccamawensis, Lampsilis fullerkati*

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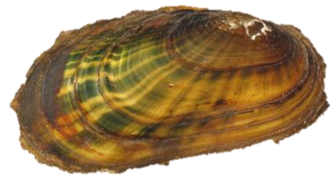
Tandem tissue extraction

- *Elliptio crassidens* (large), *Villosa vibex* (small)



- Treatments
  - Control
  - Hemolymph (0.5 mL w/ 25 gauge needle)
  - Foot biopsy (~15 mg)
  - Hemolymph & foot biopsy
- Long-term survival
  - Floating baskets in pond
  - *E. crassidens* = 945 d, *V. vibex* = 820 d

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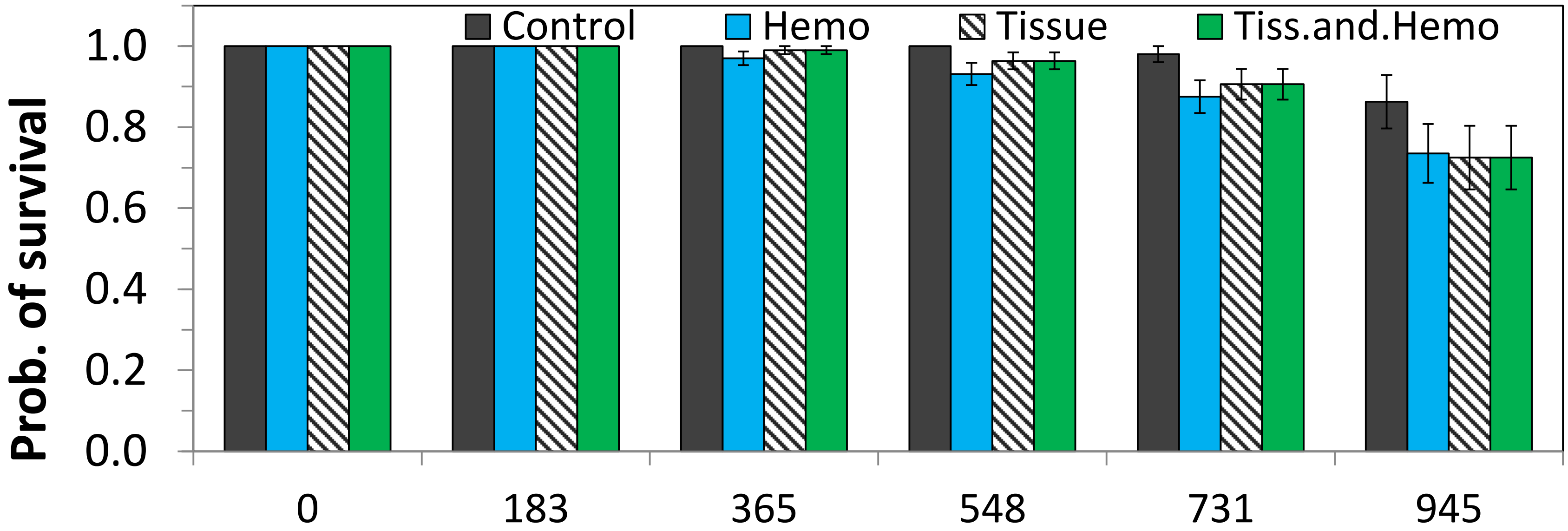
E. crassidens



Survival conclusions

- Minimal effect on survival of large-bodied species
- Reduced survival in small-bodied species
  - Pond conditions unfavorable?
- Important to monitor for prolonged duration (i.e. >1yr)

Regenerated foot tissue, *V. vibex*
3 months post-biopsy, currently brooding

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Hemolymph history

- Foundational hemolymph work
  - Gustafson et al. (2005)
- Thermal stress exposures/dewatering/low flows
  - Archambault et al. 2013
  - Fritts et al. 2015a,b
- Elevated carbon dioxide
  - Hannan et al. 2016a,b,c

Vol. 65: 159–165, 2005 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published June 30

Evaluation of a nonlethal technique for hemolymph collection in *Elliptio complanata*, a freshwater bivalve (Mollusca: Unionidae)

Lori L. Gustafson[1,2,3,8], Michael K. Stoskopf[1,3,5], Arthur E. Bogan[1,4], William Showers[1,6], Thomas J. Kwak[1,7], Shane Hanlon[2,9], Jay F. Levine[1,2,*]

Vol. 65: 167–176, 2005 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published June 30

Reference ranges for hemolymph chemistries from *Elliptio complanata* of North Carolina

Lori L. Gustafson[1,2,3,8], Michael K. Stoskopf[1,3,4], William Showers[1,5], Greg Cope[1,4], Chris Eads[2], Richard Linnehan[1,3,7], Thomas J. Kwak[1,6], Beth Andersen[5], Jay F. Levine[1,2,*]

Marine and Freshwater Behaviour and Physiology, 2013
Vol. 46, No. 4, 229–250, http://dx.doi.org/10.1080/10236244.2013.805891
Taylor & Francis
Taylor & Francis Group

Burrowing, byssus, and biomarkers: behavioral and physiological indicators of sublethal thermal stress in freshwater mussels (Unionidae)

Jennifer M. Archambault[a]*, W. Gregory Cope[b] and Thomas J. Kwak[c]

1456
NRC Research Press
ARTICLE

Evaluation of methods for assessing physiological biomarkers of stress in freshwater mussels[1]

Andrea K. Fritts, James T. Peterson, Peter D. Hazelton, and Robert B. Bringolf

1460
NRC Research Press
ARTICLE

Nonlethal assessment of freshwater mussel physiological response to changes in environmental factors[1]

Andrea K. Fritts, James T. Peterson, Jason M. Wisniewski, and Robert B. Bringolf

1538
NRC Research Press
ARTICLE

Physiological effects of short- and long-term exposure to elevated carbon dioxide on a freshwater mussel, *Fusconaia flava*

Kelly D. Hannan, Jennifer D. Jeffrey, Caleb T. Hasler, and Cory D. Suski

Conservation Physiology
Volume 4 • 2016
10.1093/conphys/cow066
SEB Society for Experimental Biology
Research article

Physiological responses of three species of unionid mussels to intermittent exposure to elevated carbon dioxide

Kelly D. Hannan, Jennifer D. Jeffrey, Caleb T. Hasler and Cory D. Suski*

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Decoding hemolymph

- Reference ranges for *Elliptio complanata* in NC
  - Gustafson et al. 2005

Parameter	Mussels	Lower ref. limit	Lower limit 90% CI	Upper ref. limit	Upper limit 90% CI
Weight (g)	380	18.8	(17.0, 21.5)	104.6	(96.0, 113.3)
Length (mm)	380	54	(52, 55)	94	(90, 95)
Density (mg mm^{-3})	380	0.58	(0.51, 0.59)	0.77	(0.75, 0.79)
Glucose (mg dl^{-1})	372	<2	(<2, <2)	4	(4, 5)
Phosphorus (mg dl^{-1})	374	<0.3	(<0.3, <0.3)	0.9	(0.9, 1.0)
Calcium (mg dl^{-1})	375	13.1	(12.5, 13.8)	23.7	(22.7, 25.0)
Magnesium (mg dl^{-1})	374	1.6	(1.5, 1.9)	3.8	(3.7, 4.0)
AST (U l^{-1})	374	<4	(<4, <4)	38	(27, 42)
Ammonia (μmol l^{-1})	380	<10	(<10, <10)	138	(111.2, 198.8)
Bicarbonate (mmol l^{-1})	375	<5	(<5, 5)	12	(11, 13)
Protein (mg dl^{-1})	378	19.5	(13.3, 22.5)	142.8	(130.1, 160.1)
Cell count (cells μl^{-1})	377	250	(170, 300)	2300	(2020, 2900)
Glycogen (mg g^{-1})	78	47	(36, 57)	176	(155, 187)

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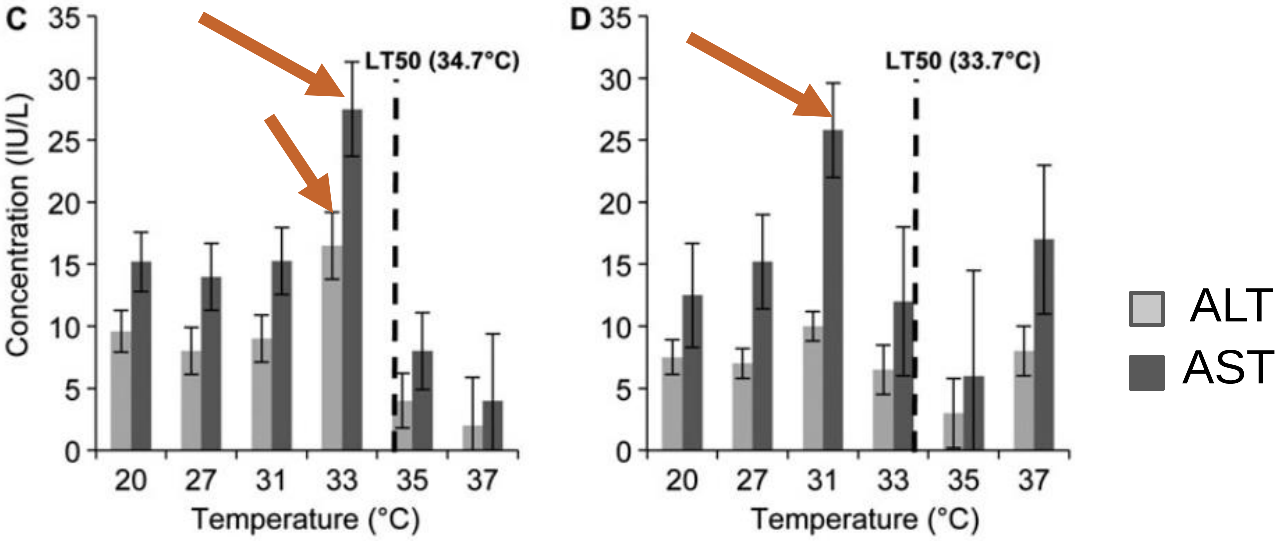
Decoding hemolymph: enzymes

- Elevated enzymes may indicate tissue damage
- ALT and AST—nonmonotonic response to thermal stress in multiple mussel species
  - highest levels at temps just below LT50s



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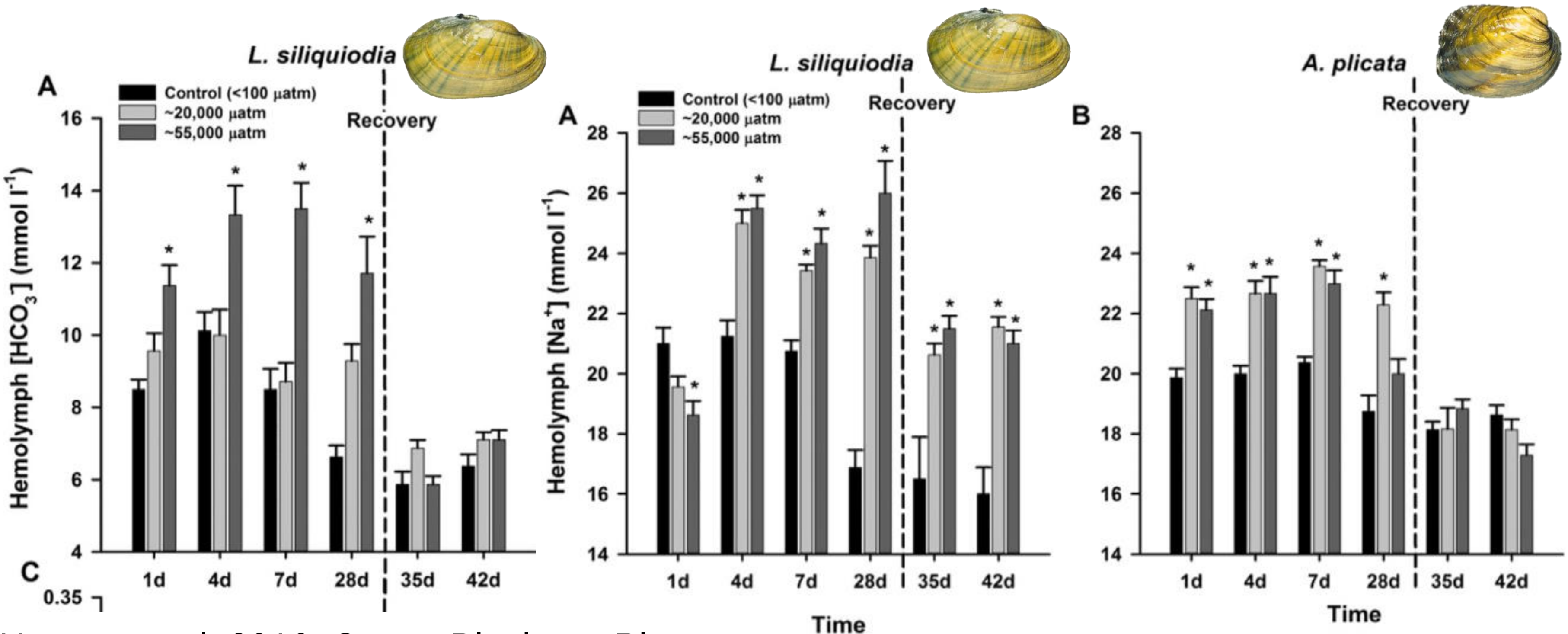
Archambault et al. 2013

Decoding hemolymph: ions

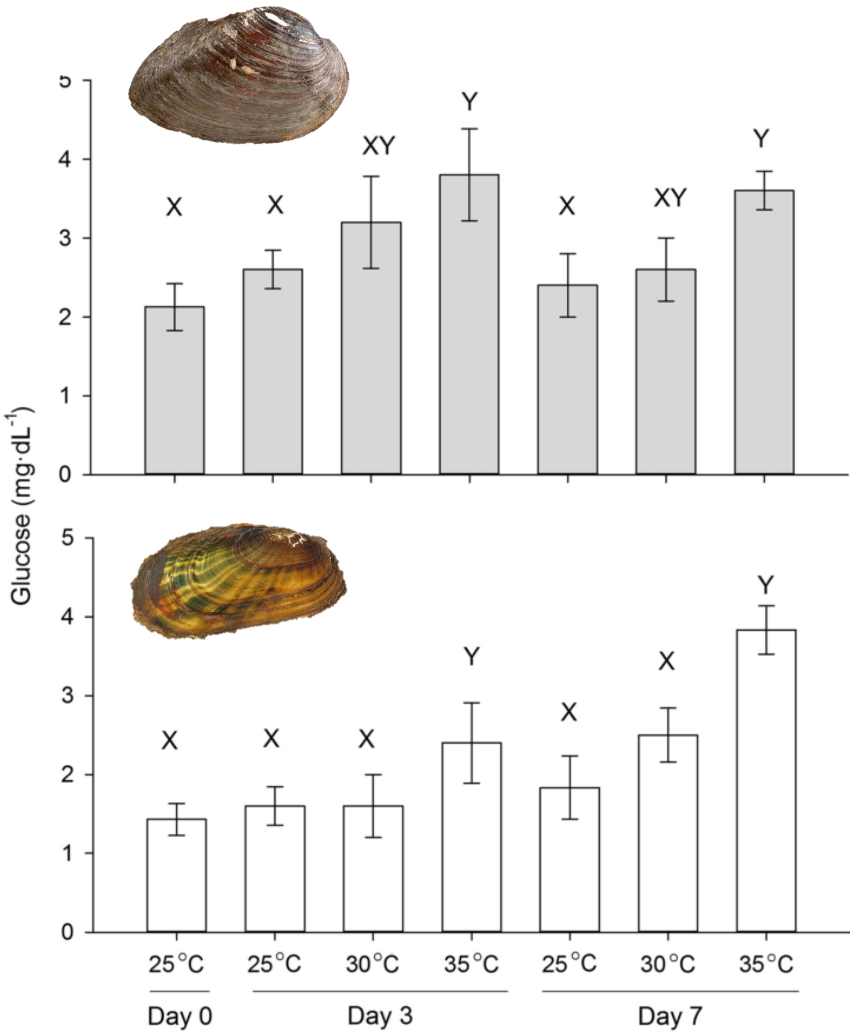
- Ions change to mediate acidosis (⇩pH, ⇧H^+)
  - Elevated Ca^+, Na^+, HCO_3^-, decreased Cl^-
    - Nice description in Hannan et al. 2016 CJFAS
    - Similar response in both thermal and $p$$CO_2$ exposure



Hannan et al. 2016, Comp. Biochem. Phys

Decoding hemolymph: glucose

- Elevated glucose
  - catabolism of glycogen to supplement glucose during stress events



Fritts et al. 2015

Hemolymph synthesis

- Similar responses in other aquatic inverts
  - Oyster, Asian clam, isopod, crab, crayfish

- May indicate a generalized response to stress
- Smaller individuals may be more susceptible to stress events (Fritts et al. 2015, field study)

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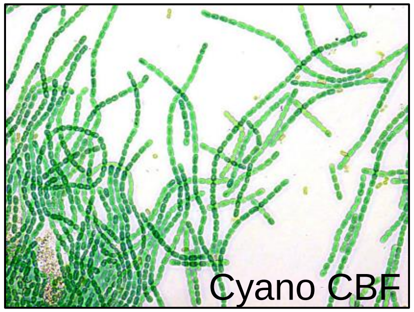
Hemolymph future work

- May vary…
  - under different types of stressors
  - among individuals of different size, sex, species
- Need to identify optimal ranges

Fatty acid research

- Fatty acids, survival, growth of mussels over riverine gradient with variable cyanobacteria

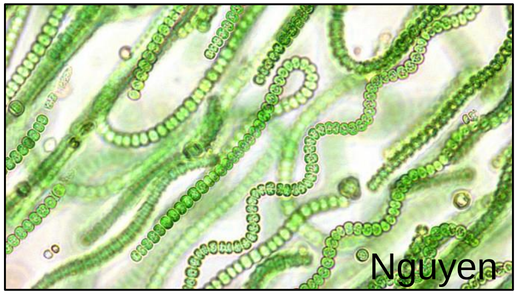


PLOS | ONE

RESEARCH ARTICLE

Effects of food resources on the fatty acid composition, growth and survival of freshwater mussels

Michelle R. Bartsch[1]*, Lynn A. Bartsch[1], William B. Richardson[1], Jon M. Vallazza[1], Brenda Moraska Lafrancois[2]



- Mussel fatty acid profiles over a large spatial gradient in Midwest
  - Potential competition w/ bigheaded carps

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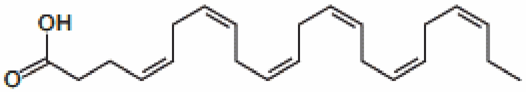
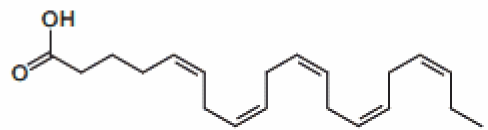
Fatty acid future work

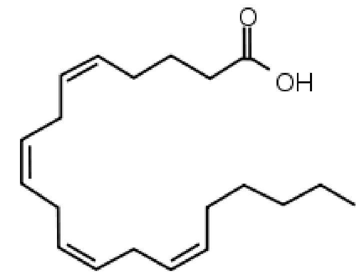
- Additional work needed to identify optimal concentrations of specific fatty acids
  - e.g. EPA, DHA, ARA



DHA (22:6n-3)



EPA (20:5n-3)



ARA (20:4n-6)

- Evaluate how fatty acids respond to controlled stress exposures

Vs.

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Questions?
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