What Does a Stream Ecologist Need to Know About Fatty Acids?

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Saturated Fatty Acids (SAFAs); e.g. Stearic acid (18:0)

Monounsaturated Fatty Acids (MUFAs); Oleic acid (18:1ω9)

Poly Unsaturated Fatty Acids (PUFAs)
  ω6 family
   Linoleic acid (LA; 18:2ω6)
   γ-Linolenic acid (γ-LA; 18:3ω6)
   Arachidonic acid (ARA; 20:4ω6)
  ω3 family
   α-Linolenic acid (α-LA; 18:3ω3)
   Stearidonic acid (SDA; 18:4ω3)
   Eicosapentaenoic acid (EPA; 20:5ω3)
   Docosahexaenoic acid (DHA; 22:6ω3)

"Essential Fatty Acids" (EFAs)
  The ω6 and ω3 families

Highly unsaturated fatty acids (HUFAs)
  ARA, EPA and DHA
Figure 1  General pathway of PUFA biosynthesis in eukaryotes
Stearic acid (18:0)
melting point = 70 °C

Oleic acid (18:1\(\omega9\))
melting point = 14 °C

\(\alpha\)-linolenic acid (18:3\(\omega3\))
melting point = -11 °C

DHA (22:6\(\omega3\))
MP = -45 °C
QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>SAFA</th>
<th>MUFA</th>
<th>16 PUFA</th>
<th>18 n-6</th>
<th>18 n-3</th>
<th>20 n-6</th>
<th>20 n-3</th>
<th>22 n-3</th>
<th>n-3:n-6</th>
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</thead>
<tbody>
<tr>
<td>Cyanophytes</td>
<td>9</td>
<td>58.6 ± 18.5</td>
<td>24.8 ± 16.6</td>
<td>0.0 ± 0.0</td>
<td>7.2 ± 6.5</td>
<td>7.0 ± 10.2</td>
<td>1.0 ± 2.4</td>
<td>0.6 ± 1.2</td>
<td>0.7 ± 2.1</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td>Chlorophytes</td>
<td>11</td>
<td>32.5 ± 9.5</td>
<td>27.9 ± 12.5</td>
<td>0.0 ± 0.0</td>
<td>14.4 ± 5.6</td>
<td>25.5 ± 9.7</td>
<td>0.2 ± 0.3</td>
<td>0.1 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>1.9 ± 0.9</td>
</tr>
<tr>
<td>Cryptophytes</td>
<td>9</td>
<td>28.4 ± 9.8</td>
<td>9.9 ± 5.1</td>
<td>0.1 ± 0.4</td>
<td>3.3 ± 2.4</td>
<td>39.7 ± 10.4</td>
<td>0.1 ± 0.2</td>
<td>15.1 ± 6.1</td>
<td>2.9 ± 1.8</td>
<td>16.8 ± 8.2</td>
</tr>
<tr>
<td>Bacillariophytes</td>
<td>6</td>
<td>23.8 ± 11.0</td>
<td>40.3 ± 12.8</td>
<td>9.1 ± 5.8</td>
<td>2.0 ± 1.8</td>
<td>2.9 ± 3.1</td>
<td>2.3 ± 1.7</td>
<td>16.9 ± 8.2</td>
<td>2.5 ± 3.0</td>
<td>7.6 ± 4.8</td>
</tr>
</tbody>
</table>
Phytoplankton Monocultures & Seston

DF1 (45.1%)

DF2 (32.2%)

Chloro

Crypto
“the gold standard: the ideal diet for fish larvae is the yolk of eggs or yolk sac larvae”; Sargent et al. 1999; Aquaculture 179: 217-
Normalized food quality by algae taxa

Phytoplankton Taxa

Normalized Food Quality

Diatom  Crypto  Chloro  Cyano

L&O 47: 1564-
Daphnia growth and biochemical composition responses to different food types

L&O 51: 2428-, Burns et al. ASLO 2006
Daphnia growth and biochemical composition responses to different food types

![Graphs showing growth rate and reproduction rates for Cyano, Chloro, and Crypto food types.](image)

- **Growth rate (day⁻¹)**
  - Cyano: 0.35
  - Chloro: 0.45
  - Crypto: 0.60
- **Reproduction (eggs per clutch)**
  - Cyano: 10
  - Chloro: 15
  - Crypto: 20

**n3 PUFAs (%)**
- Cyano: 10
- Chloro: 30
- Crypto: 40

**n3 HUFAs (%)**
- Cyano: 5
- Chloro: 10
- Crypto: 15

L&O 51: 2428-, Burns et al. ASLO 2006
Daphnia growth and biochemical composition responses to different food types

![Graphs showing growth rate, n3 PUFAs, n3 HUFAs, n3:n6 FA ratio, and FAs/Dry wt. (%) for Cyano, Chloro, and Crypto food types.]

L&O 51: 2428-, Burns et al. ASLO 2006
**Daphnia** growth and biochemical composition responses to different food types

- **Growth:** 3.5 x larger
- **n3 PUFAs:**
  - Cyano
  - Chloro
  - Crypto
  - Cyano: 4.5 x HUFA
  - Chloro: 4.0 x FAs
- **n3 HUFAs:**
  - Cyano
  - Chloro
  - Crypto
  - Cyano: 4.5 x HUFA
  - Chloro: 4.0 x FAs

L&O 51: 2428-, Burns et al. ASLO 2006
Outline - Controls on Zooplankton Fatty Acid Content

1) Taxonomic affiliation
2) Diet
3) Temperature
4) Food ration/starvation
5) Biochemical transformations
6) Homeostasis
The Pioneers and the Examples

Farkas & Herodek 1964 J Lipid Res 3: 369-

Lee, R.F. et al. 1971 Marine Biology 9: 99-

Farkas et al. 1984 Lipids 19: 436-

Bourdier & Amblard 1989 J Plankton Res 11, 1201-

Goulden & Place 1990 J Exp Zool 256, 168-

Graeve et al. 1994 J Exp Mar Biol Ecol 182, 97-

Weers et al. 1997 Freshwater Biol 38: 731-
Taxonomic differences in zooplankton fatty acid composition

Photo by Carol Eunmi Lee
1) Zooplankton have more PUFA than seston and carnivorous zooplankton have more PUFA than herbivorous zooplankton

2) Cladocerans accumulate EPA, whereas copepods accumulate EPA and especially DHA
Lake Washington Fatty Acids

Seston

Ravet et al., unpub.
Lake Washington Fatty Acids

Ravet et al., unpub.

Seston
Cladocerans
Copepods
Lake Washington Fatty Acids

-4 -2 0 2 4 6

Seston
Bosmina
Daphnia
Cyclops
Diaptomus
Epischura

Ravet et al., unpub.
Zooplankton tended to accumulate much higher proportions of ω3 PUFA than their diets.

Copepods preferentially accumulated DHA, cladocerans accumulated EPA, and both copepods and cladocerans accumulated 18 carbon chain ω3 PUFAs.
Discriminant Analysis by "critter"

Burns et al., SIL 2007
Discriminant Analysis by "critter"

Burns et al., SIL 2007
Discriminant Analysis by "critter"

- Function 1 (49.6%)
- Function 2 (45.5%)

Phyto cyan - Phyto chlor - Phyto cryp

Boeck. cyano - Boeck. chlor - Boeck. crypt

Burns et al., SIL 2007
Discriminant Analysis by "critter"

Function 1 (49.6%)

- Phyto cyan
- Phyto chlor
- Phyto cryp
- Boeck. cyano
- Boeck. chlor
- Boeck. cryp
- Daphnia cyan
- Daphnia chlor
- Daphnia cryp
- Ceriod. cyan
- Ceriod. chlor
- Ceriod. cryp

Burns et al., SIL 2007
Discriminant Analysis by "critter"

Function 1 (49.6%)

Function 2 (45.5%)

Phyto cyan
Boeck. cyano
Daphnia cyan
Ceriod. cyan
Phyto chlor
Boeck. chlor
Daphnia chlor
Ceriod. chlor
Phyto cryp
Boeck. crypt
Daphnia cryp
Ceriod. cryp

Burns et al., ASLO 2006
Discriminant Analysis by "critter"

- 89% correctly classified
- Discrimination due to 20n3, 22n3 & %FAs
- Misclassification between Daphnia & Ceriodaphnia

Burns et al., ASLO 2006
Taxonomic conclusions

Cladocerans accumulate almost exclusively EPA, whereas copepods accumulate both EPA and DHA - but predominantly DHA. **Why?**

Persson and Vrede suggested this is because cladocerans were fast (growing/reproducing) but “dumb”, whereas copepods are slow and “sensitive”.

**BUT why can’t DHA be used as a substrate to support fast growth/reproduction & why can’t EPA be used to form sensory tissues?** Furthermore, do copepods actually have more sensory tissue than cladocerans?
Dietary control of fatty acid composition
EPA, DHA and ARA in Daphnia (%) vs. EPA, DHA and ARA in diet (%)
EPA, DHA and ARA in *Daphnia* (%)

\[ y = 1.083x + 4.040 \]

\[ r^2 = 0.94 \]

L&O 51: 2428-, 2006
Ederington et al. 1995 DFA

Diatom

L&O 40: 860-
Ederington et al. 1995 DFA

DF2

DF1

-2 -1 0 1 2 3

Diatom
Ciliate
Copepod (field)

L&O 40: 860-
Ederington et al. 1995 DFA

- Diatom
- Ciliate
- Copepod (field)
- Copepod (starved)

DF1 vs. DF2

L&O 40: 860-
Ederington et al. 1995 DFA

- Diatom
- Ciliate
- Copepod (field)
- Copepod (starved)
- Copepod (diatom fed)
- Copepod (ciliate fed)
- Egg (diatom mat.)
- Egg (ciliate mat.)
Conclusions from Ederington data

1) *Acartia* had high 16:0 and 18:0 irrespective of diet

2) Diatoms and *Acartia* fed diatoms & their eggs had high EPA

3) Ciliates and *Acartia* fed ciliates & their eggs had high 18:1ω11 and 22:0

4) The FA composition of *Acartia* was much more strongly influenced by diet than was the FA composition of their eggs

**Caveat:** These were only 4 day incubations
Müller-Navarre 2006

DF1 (85.3%)

DF2 (14.7%)

Phyto Chloro
Phyto Crypto
Phyto Diatom

Archiv. Hydrobiol. 167: 501-
Müller-Navarra 2006

DF1 (85.3%)

DF2 (14.7%)

Phyto Chloro
Phyto Crypto
Phyto Diatom
Daphnia Chloro
Daphnia Crypto
Daphnia Diatom
Egg Chloro
Egg Crypto
Egg Diatom

Archiv. Hydrobiol. 167: 501-
Conclusions from M-N 2006 data

1) The 18:2\omega6, 18:3\omega3, 18:4\omega3, and EPA content and \omega3:\omega6 ratio of the diet was very strongly correlated with that of the Daphnia somatic tissues and eggs.

2) Somatic tissues and eggs have significantly more 18:1\omega7 and ARA, and significantly less DHA than the diets.

3) Daphnia eggs had significantly more 18:3\omega3 and 18:4\omega3, higher \omega3:\omega6 ratios and a higher total fatty acid content than does the somatic tissue.
Nanton and Castell 1998

Diatom

Chloro

Isochrysis

Yeast

Aquaculture 163: 251-
Dietary control of fatty acid composition with homeostasis
Diaptomus Saturated FAs

% SAFAs vs Observation

- LW Seston
- Diaptomus

Ravet et al., unpub.
Boeckella

Daphnia

Ceriodaphnia

Burns et al., SIL 2007
Burns et al., SIL 2007

**Boeckella**

- Graph showing the relationship between phyto n3 PUFA and Boeckella n3 PUFA (%).
- Equation: $y = 0.63x + 0.10$
- $r^2 = 0.82$

**Daphnia**

- Graph showing the relationship between phyto n3 PUFA and Daphnia n3 PUFA (%).
- Equation: $y = 0.60x + 0.14$
- $r^2 = 0.94$

**Ceriodaphnia**

- Graph showing the relationship between phyto n3 PUFA and Ceriodaphnia n3 PUFA (%).
- Equation: $y = 0.34x + 0.16$
- $r^2 = 0.88$

The graphs illustrate the percentage of phyto n3 PUFA in each species and how it correlates with the percentage of n3 PUFA in the species.
Biochemical transformations

PUFA Structures

- EPA 20:5(n-3)
- AA 20:4(n-6)
- DHA 22:6(n-3)
Lake Washington Seston

\[ \begin{align*}
\text{HUFA} & \quad \text{EPA} & \quad \text{DHA} \\
\end{align*} \]

\% $\Sigma$ FA

Observation

Ravet et al., unpub.
Seston HUFA is dominated by EPA

Ravet et al., unpub.
Seston HUFA is dominated by EPA

Diaptomus HUFA is dominated by DHA

Ravet et al., unpub.
Lake Washington Seston

![Graph showing % Σ FA vs Observation for Diaptomus and Lake Washington Seston.](image)

Diaptomus

![Graph showing % Σ FA vs Observation for Diaptomus.](image)

<table>
<thead>
<tr>
<th></th>
<th>EPA</th>
<th>DHA</th>
<th>HUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaptomus</td>
<td>0.01</td>
<td>0.62</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Ravet et al., unpub.
Lake Washington Seston

Diaptomus

\[ r^2 \]

<table>
<thead>
<tr>
<th></th>
<th>EPA</th>
<th>DHA</th>
<th>HUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaptomus</td>
<td>0.01</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>Diaptomus</td>
<td>0.91</td>
<td>0.62</td>
<td>0.86</td>
</tr>
<tr>
<td>Diaptomus</td>
<td>0.87</td>
<td>0.50</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Ravet et al., unpub.
Ravet et al., unpub.
Cold adaptation and zooplankton fatty acid composition: the homeoviscous response
### TABLE 1
Effects of Growth Temperature on FA Composition (% of total identified FAME) of *Daphnia pulex* Fed *Ankistrodesmus falcatus*

<table>
<thead>
<tr>
<th>A. <em>falcatus</em></th>
<th>DA22</th>
<th>DA11</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:0</td>
<td>nd</td>
<td>0.3</td>
</tr>
<tr>
<td>12:0</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>14:0</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>15:0</td>
<td>0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>16:0</td>
<td>20.4</td>
<td>18.6</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>1.0</td>
<td>3.3</td>
</tr>
<tr>
<td>17:0</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>18:0</td>
<td>0.5</td>
<td>8.1</td>
</tr>
<tr>
<td>18:1n-9t</td>
<td>nd</td>
<td>0.1</td>
</tr>
<tr>
<td>18:1n-9c</td>
<td>11.6</td>
<td>19.2</td>
</tr>
<tr>
<td>18:2n-6c</td>
<td>15.9</td>
<td>14.3</td>
</tr>
<tr>
<td>20:0</td>
<td>nd</td>
<td>0.3</td>
</tr>
<tr>
<td>18:3n-6</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>nd</td>
<td>0.1</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>44.3</td>
<td>22.4</td>
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<tr>
<td>21:0</td>
<td>nd</td>
<td>0.1</td>
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<td>20:2</td>
<td>nd</td>
<td>1.8</td>
</tr>
<tr>
<td>22:0</td>
<td>2.4</td>
<td>0.5</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>nd</td>
<td>0.1</td>
</tr>
<tr>
<td>22:1n-9</td>
<td>nd</td>
<td>0.3</td>
</tr>
<tr>
<td>20:3n-3</td>
<td>nd</td>
<td>0.2</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>nd</td>
<td>2.7</td>
</tr>
<tr>
<td>24:0</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>nd</td>
<td>3.1</td>
</tr>
<tr>
<td>24:1n-9</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Σn-3</td>
<td>44.3</td>
<td>25.6</td>
</tr>
<tr>
<td>Σn-6</td>
<td>17.4</td>
<td>18.2</td>
</tr>
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<td>ΣSFA</td>
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<td>31.0</td>
</tr>
<tr>
<td>ΣMUFA</td>
<td>12.9</td>
<td>23.3</td>
</tr>
<tr>
<td>ΣPUFA</td>
<td>61.7</td>
<td>45.7</td>
</tr>
</tbody>
</table>

*Results are means ± SE. Values within a row with a different superscripted letter are significantly different (P < 0.05). nd = not detected. DA22, *Daphnia pulex* cultured at 22°C; DA11, *D. pulex* cultured at 11°C; SFA, saturated FA; MUFA, monounsaturated FA.*
1) When cold adapted $18:2\omega 6$ and $18:3\omega 3$ go down,

2) $16:1\omega 7$ and EPA go up

3) Farkas et al. (1984, Lipids 19: 436-) previously noted this response for copepod FAs which became enriched with DHA during cold adaptation.
1) When chilled $18:2\omega 6$ and $18:3\omega 3$ go down,

2) $16:1\omega 7$ and EPA go up

3) Farkas et al. (1984, Lipids 19: 436-) previously noted this response for copepods FAs which became enriched with DHA during cold adaptation

4) Evidence of $18:3\omega 3$ conversion to EPA

Lipids 41: 397-
Starvation impacts on fatty acid composition
**FIG. 1.** Temporal trajectories of FA in fasting *Daphnia pulex* kept at (A) 22°C and (B) 11°C. Results are means ± SE. Significant differences between means were determined by one-way ANOVA followed by Tukey’s multiple comparison test. Values of the same FA with a different superscripted letter are significantly different (*P* < 0.05). An asterisk indicates that there was a statistically significant difference (*P* = 0.031), but the power of the performed test (0.56) was below the desired power of 0.80. ALA, α-linolenic acid; ARA, arachidonic acid; LIN, linoleic acid; MUFA, monounsaturated FA; SAFA, saturated FA.
4) When starved SAFA, MUFA and 18:3\omega3 are metabolized, &

5) 18:2\omega6, ARA and EPA are conserved
Okanagan Lake, July 2003

Fatty acids (µg/L)

n3 FAs

% n3 FAs

Contr +P +N +NP +NPμ

22 n3
20 n3
18 n3
20 n6
18 n6
MUFA
SAFA
Conclusions:

1) Copepods and cladocerans have distinct fatty acid profiles

2) Diet strongly influences the FA composition of zooplankton, especially the PUFAs and HUFAs

3) Despite strong dietary control, many zooplankton FAs stay within much narrower ranges than their diets

4) Copepods increase their DHA content and cladocerans increase their EPA content at lower temperatures

5) During starvation zooplankton selectively retain HUFA such as EPA and ARA

6) There is clear evidence of bioconversions of PUFA (e.g. 18:3ω3) to more physiologically important HUFA like EPA and DHA