

# 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 (18:1 $\omega$ 9)

Poly Unsaturated Fatty Acids (PUFAs)

$\omega$ 6 family

Linoleic acid (LA; 18:2 $\omega$ 6)

$\gamma$ -Linolenic acid ( $\gamma$ -LA; 18:3 $\omega$ 6)

Arachidonic acid (ARA; 20:4 $\omega$ 6)

$\omega$ 3 family

$\alpha$ -Linolenic acid ( $\alpha$ -LA; 18:3 $\omega$ 3)

Stearidonic acid (SDA; 18:4 $\omega$ 3)

Eicosapentaenoic acid (EPA; 20:5 $\omega$ 3)

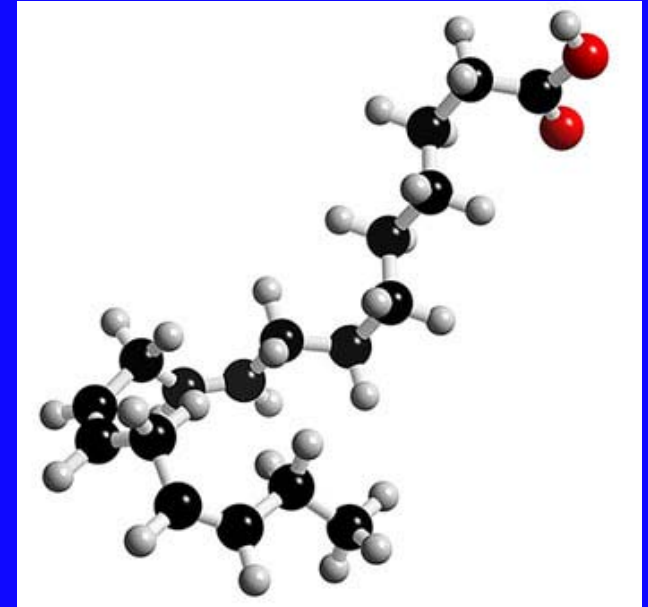
Docosahexaenoic acid (DHA; 22:6 $\omega$ 3)

"Essential Fatty Acids" (EFAs)

The  $\omega$ 6 and  $\omega$ 3 families

Highly unsaturated fatty acids (HUFAs)

ARA, EPA and DHA



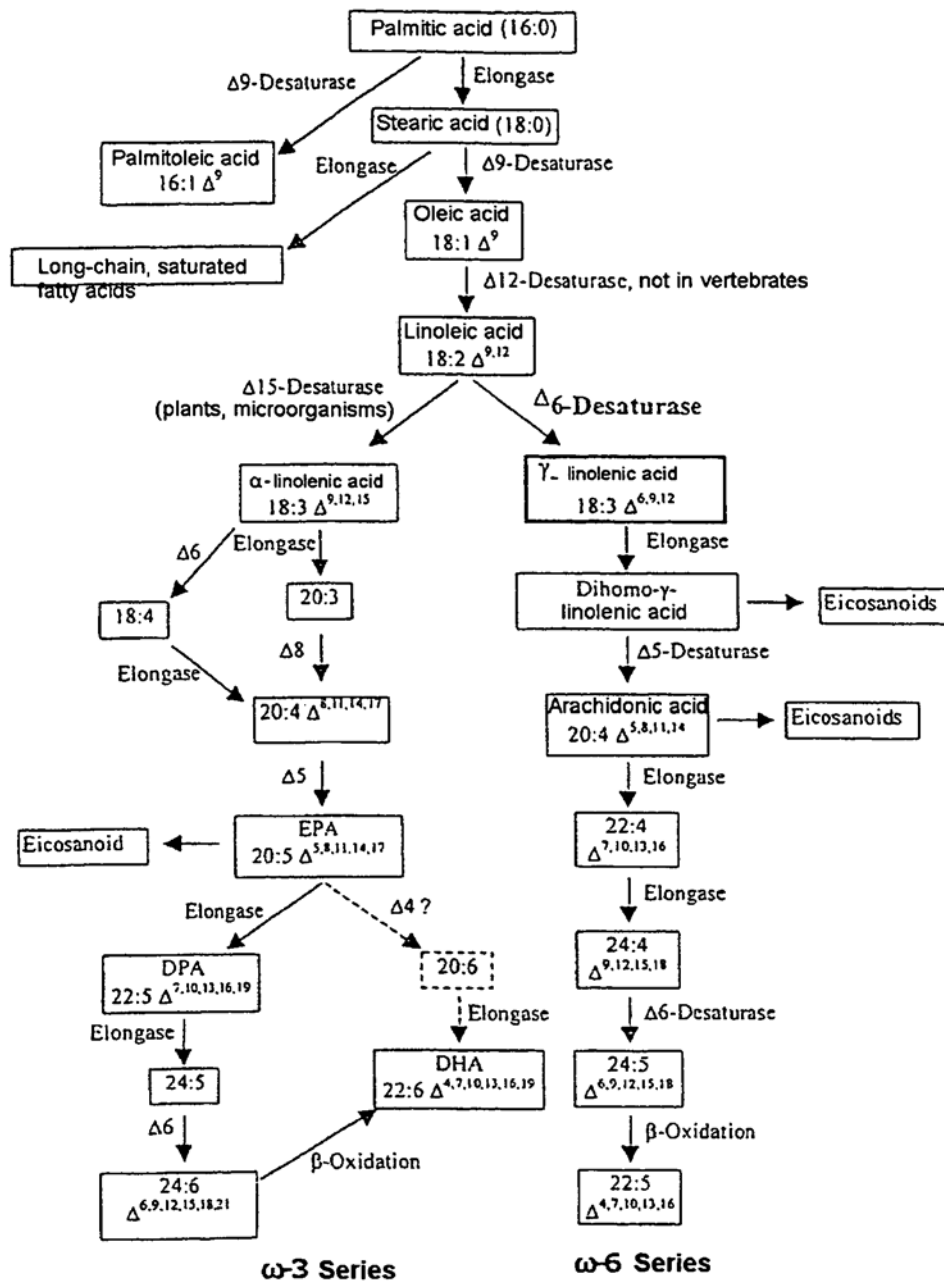
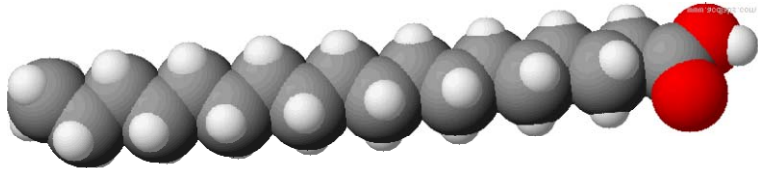


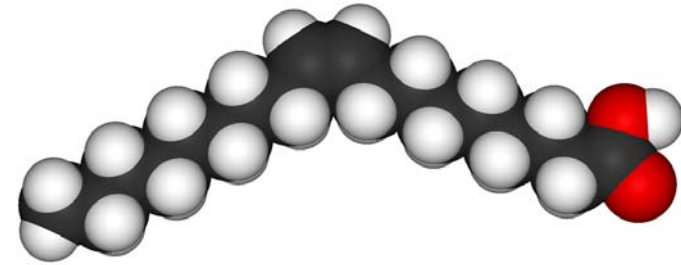
Figure 1

General pathway of PUFA biosynthesis in eukaryotes

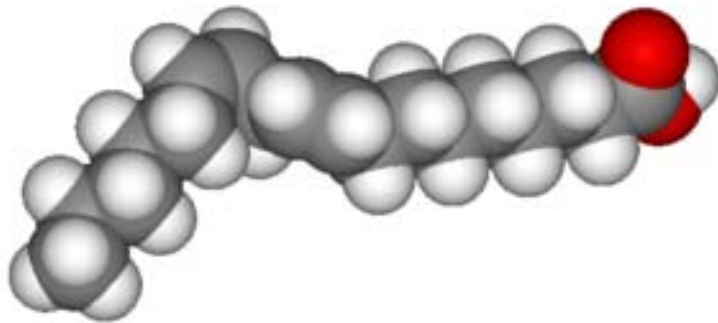
Stearic acid (18:0)  
melting point = 70 °C



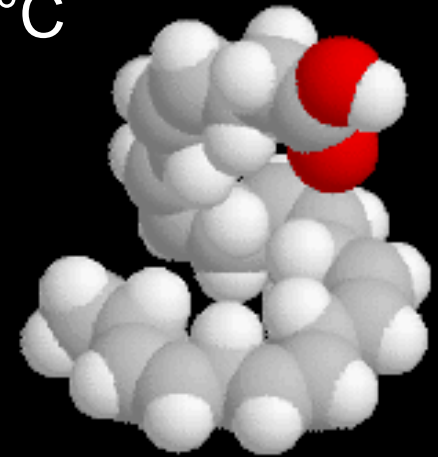
Oleic acid (18:1 $\omega$ 9)  
melting point = 14 °C



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



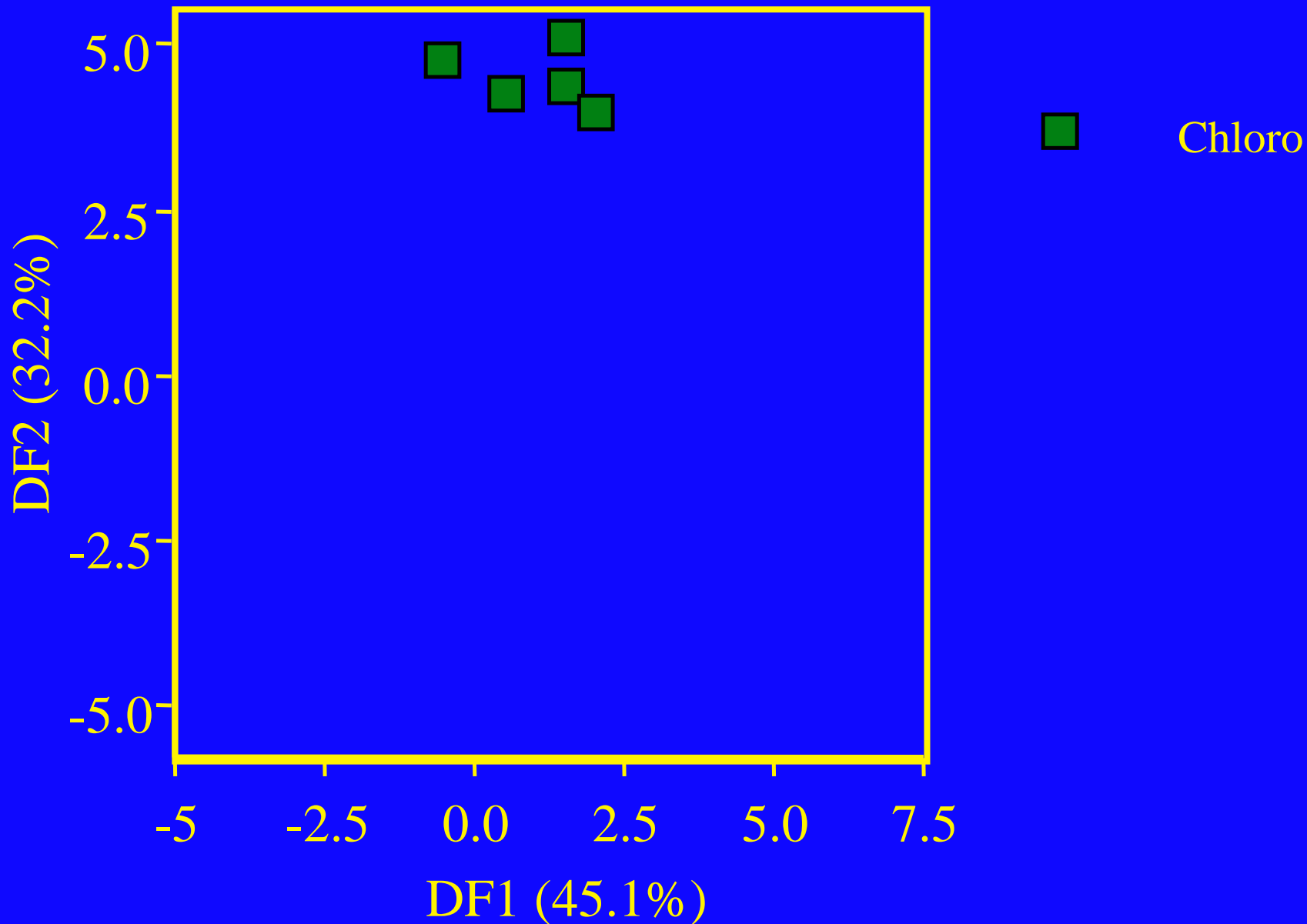
DHA (22:6 $\omega$ 3)  
MP = -45 °C



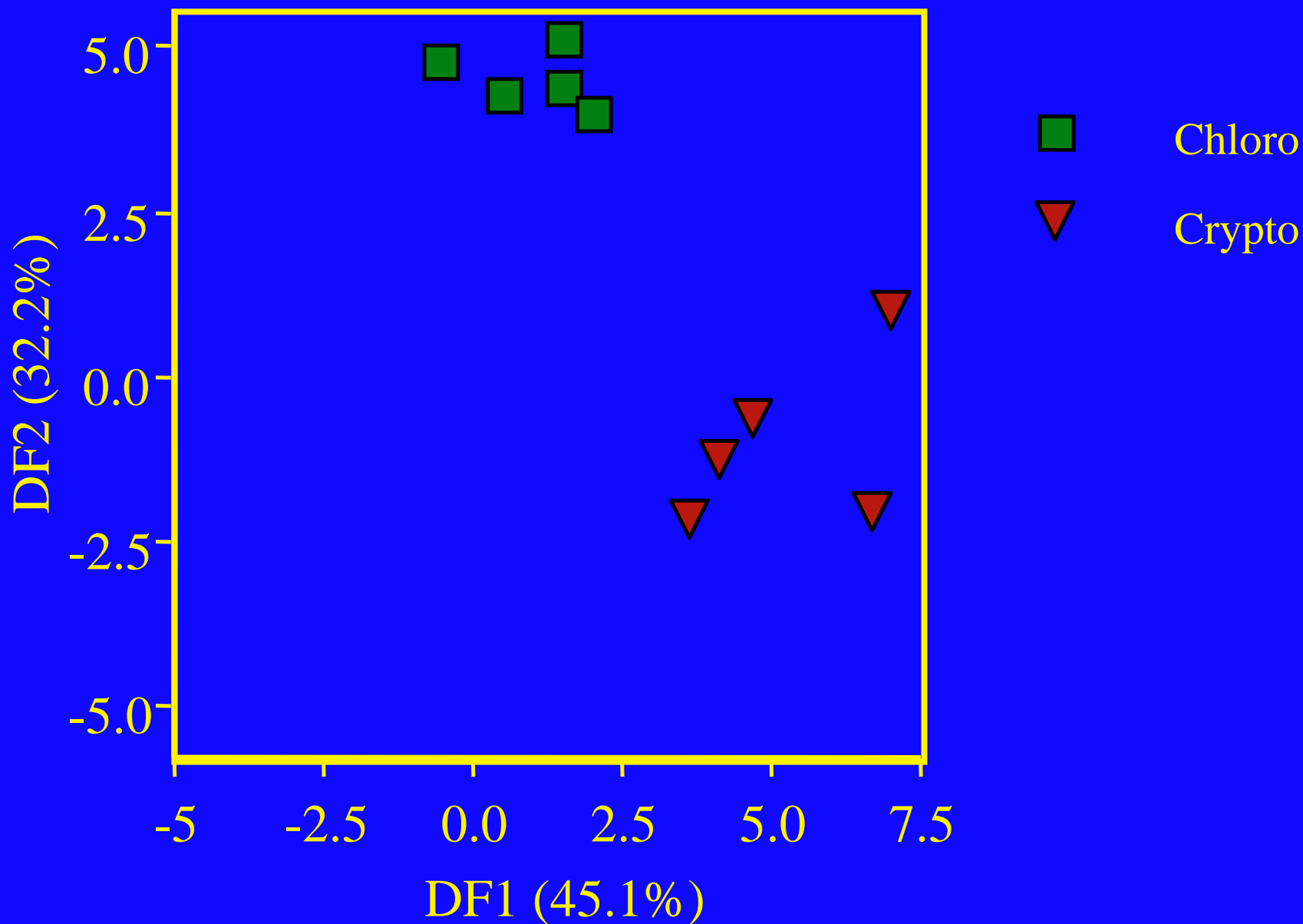
QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

Group	n	SAFA	MUFA	16 PUFA	18 n-6	18 n-3	20 n-6	20 n-3	22 n-3	n-3:n-6
Cyanophytes	9	58.6 ± 18.5	24.8 ± 16.6	0.0 ± 0.0	7.2 ± 6.5	7.0 ± 10.2	1.0 ± 2.4	0.6 ± 1.2	0.7 ± 2.1	1.0 ± 1.0
Chlorophytes	11	32.5 ± 9.5	27.3 ± 12.5	0.0 ± 0.0	14.4 ± 5.6	25.5 ± 9.7	0.2 ± 0.3	0.1 ± 0.2	0.0 ± 0.0	1.9 ± 0.9
Cryptophytes	9	28.4 ± 9.8	9.9 ± 5.1	0.1 ± 0.4	3.3 ± 2.4	39.7 ± 10.4	0.1 ± 0.2	15.1 ± 6.1	2.9 ± 1.8	16.8 ± 8.2
Bacillariophytes	6	23.8 ± 11.0	40.3 ± 12.8	9.1 ± 5.8	2.0 ± 1.8	2.9 ± 3.1	2.3 ± 1.7	16.9 ± 8.2	2.5 ± 3.0	7.6 ± 4.8

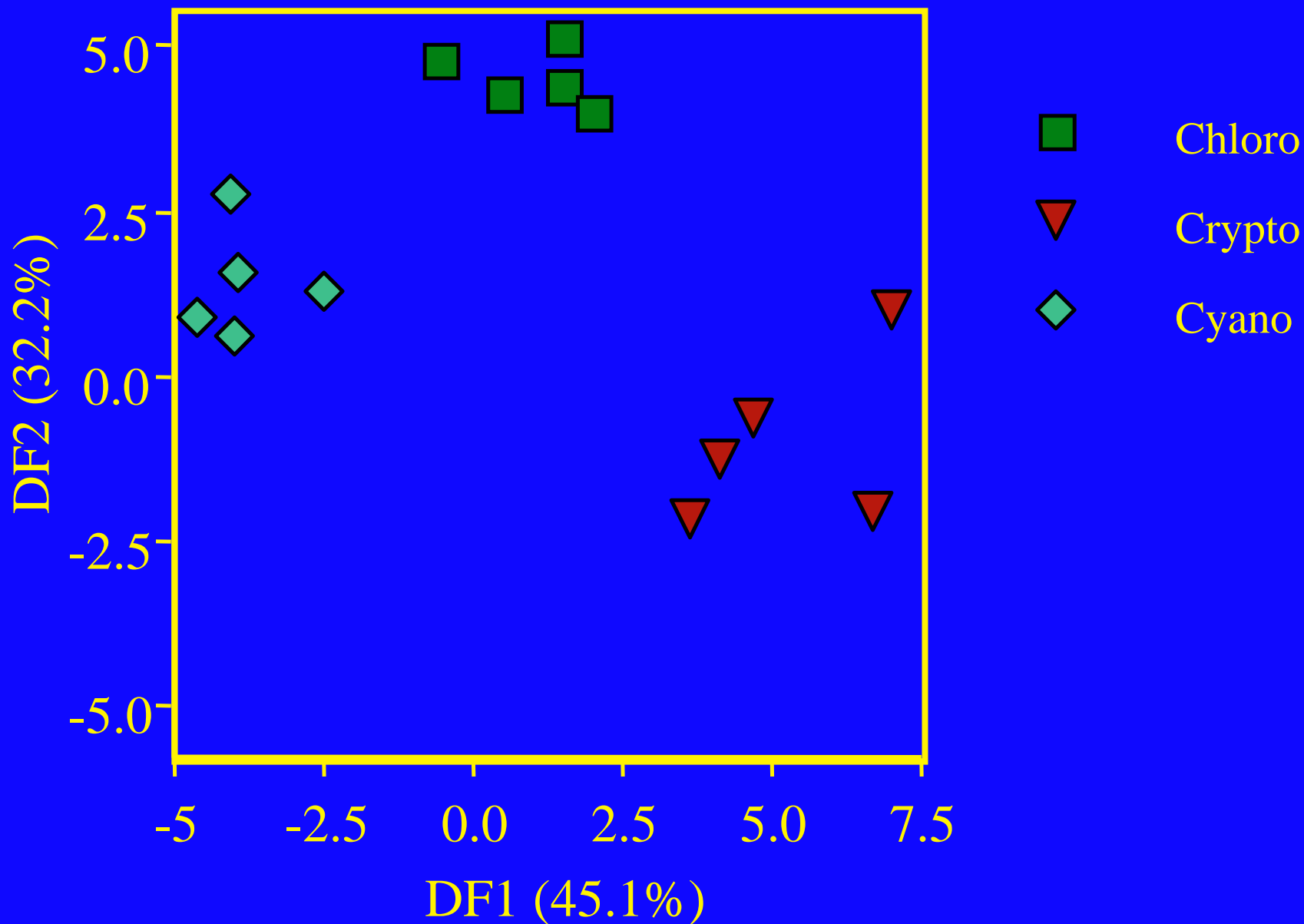
# Phytoplankton Monocultures & Seston



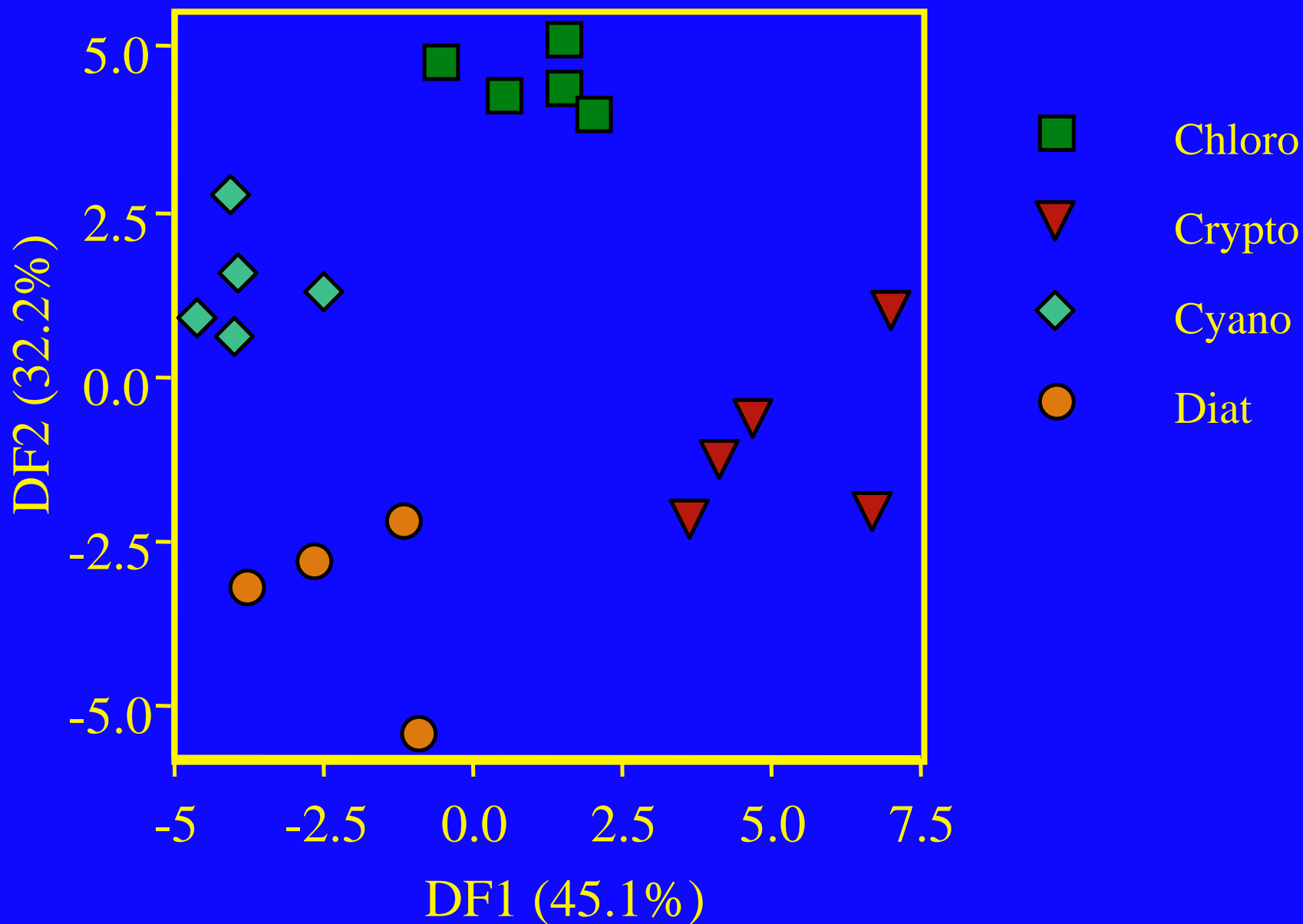
# Phytoplankton Monocultures & Seston



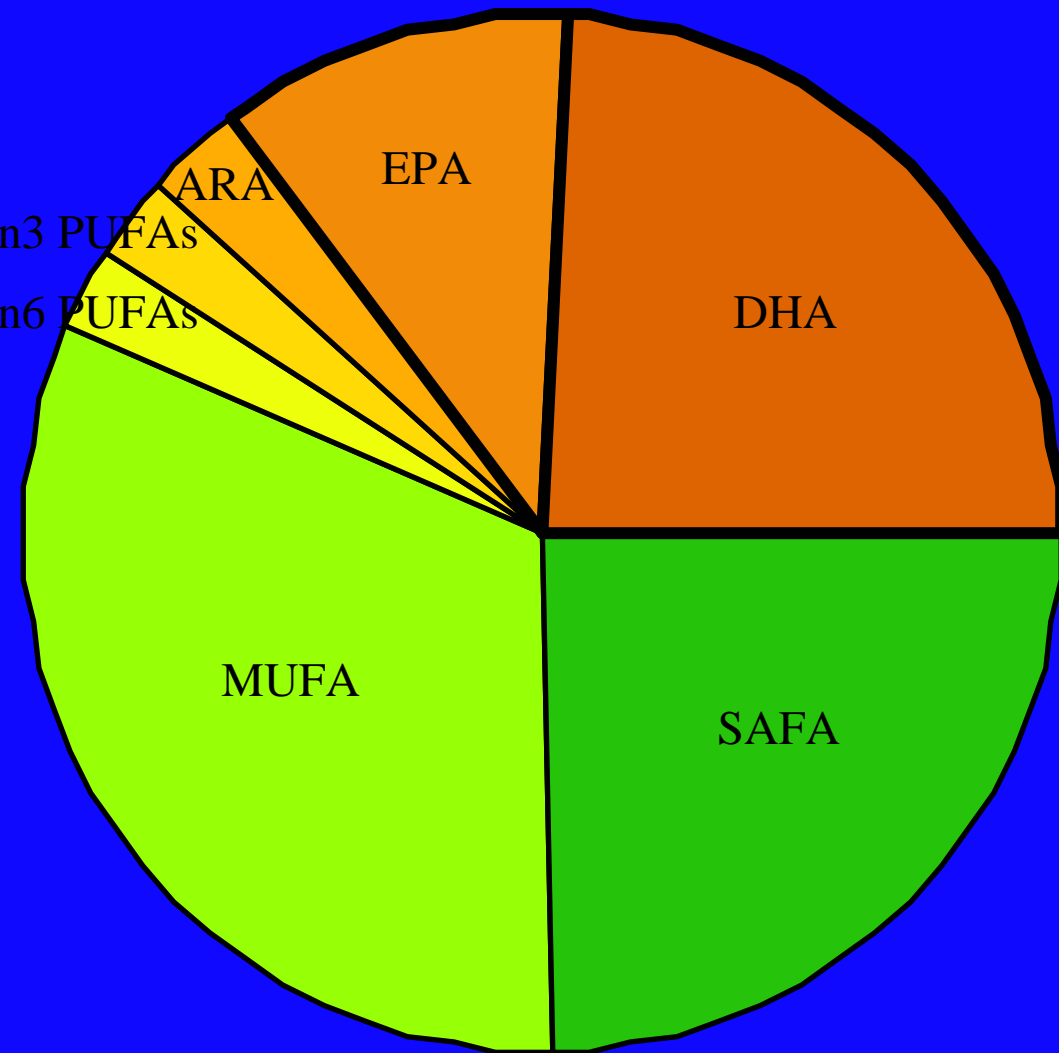
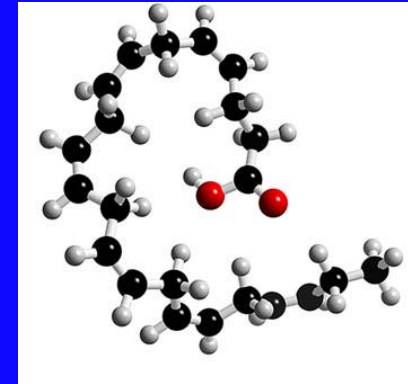
# Phytoplankton Monocultures & Seston

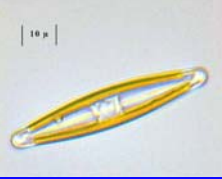


# Phytoplankton Monocultures & Seston

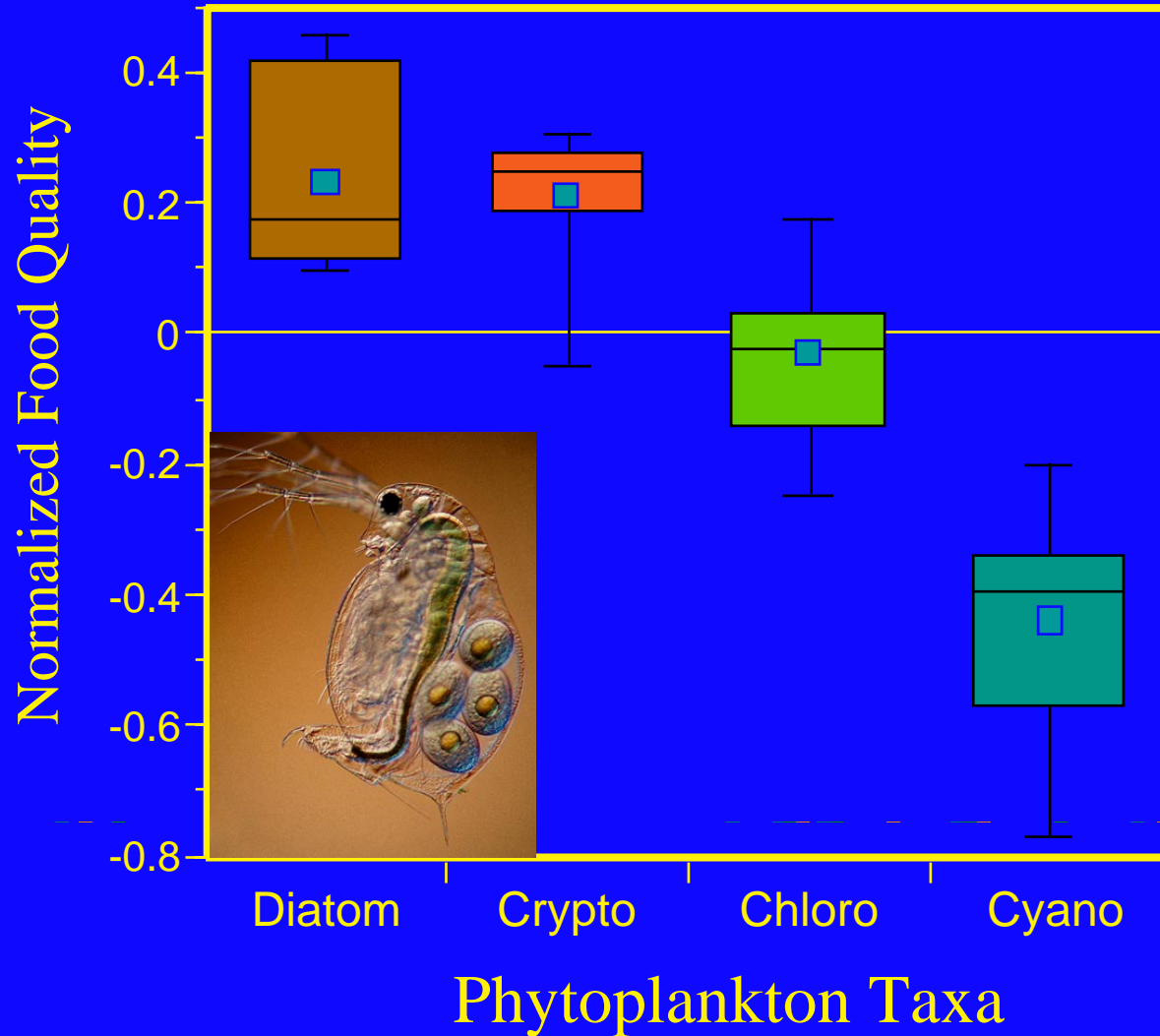


“the gold standard: the ideal diet for fish larvae is the yolk of eggs or yolk sac larvae”; Sargent et al. 1999; Aquaculture 179: 217-

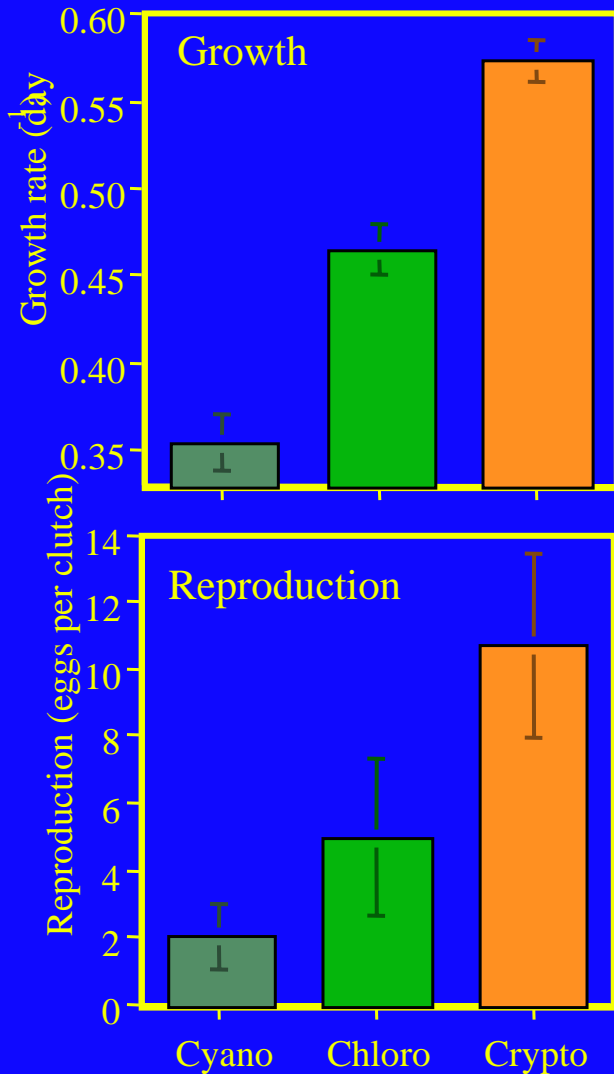




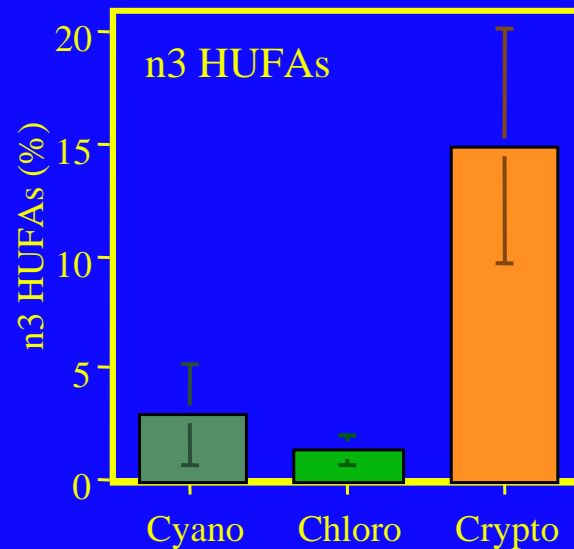
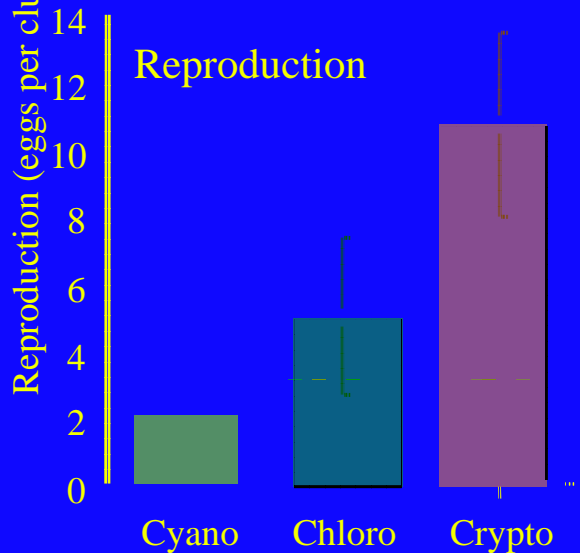
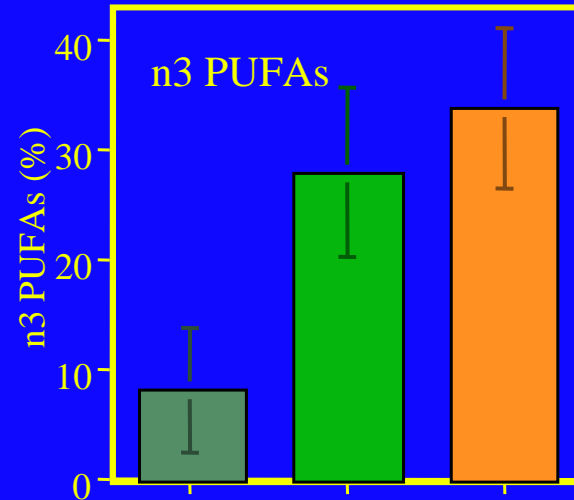
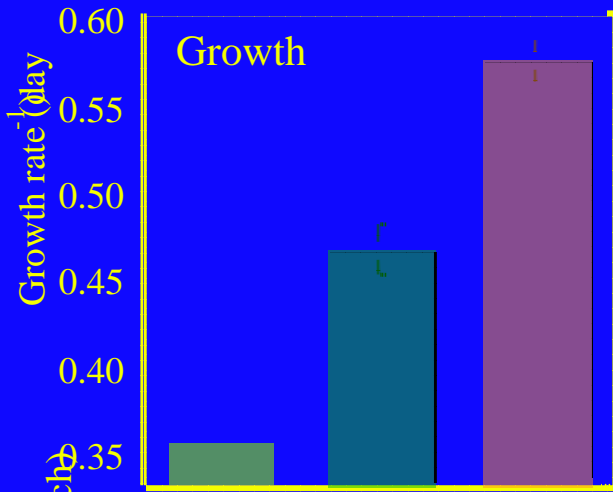
## Normalized food quality by algae taxa



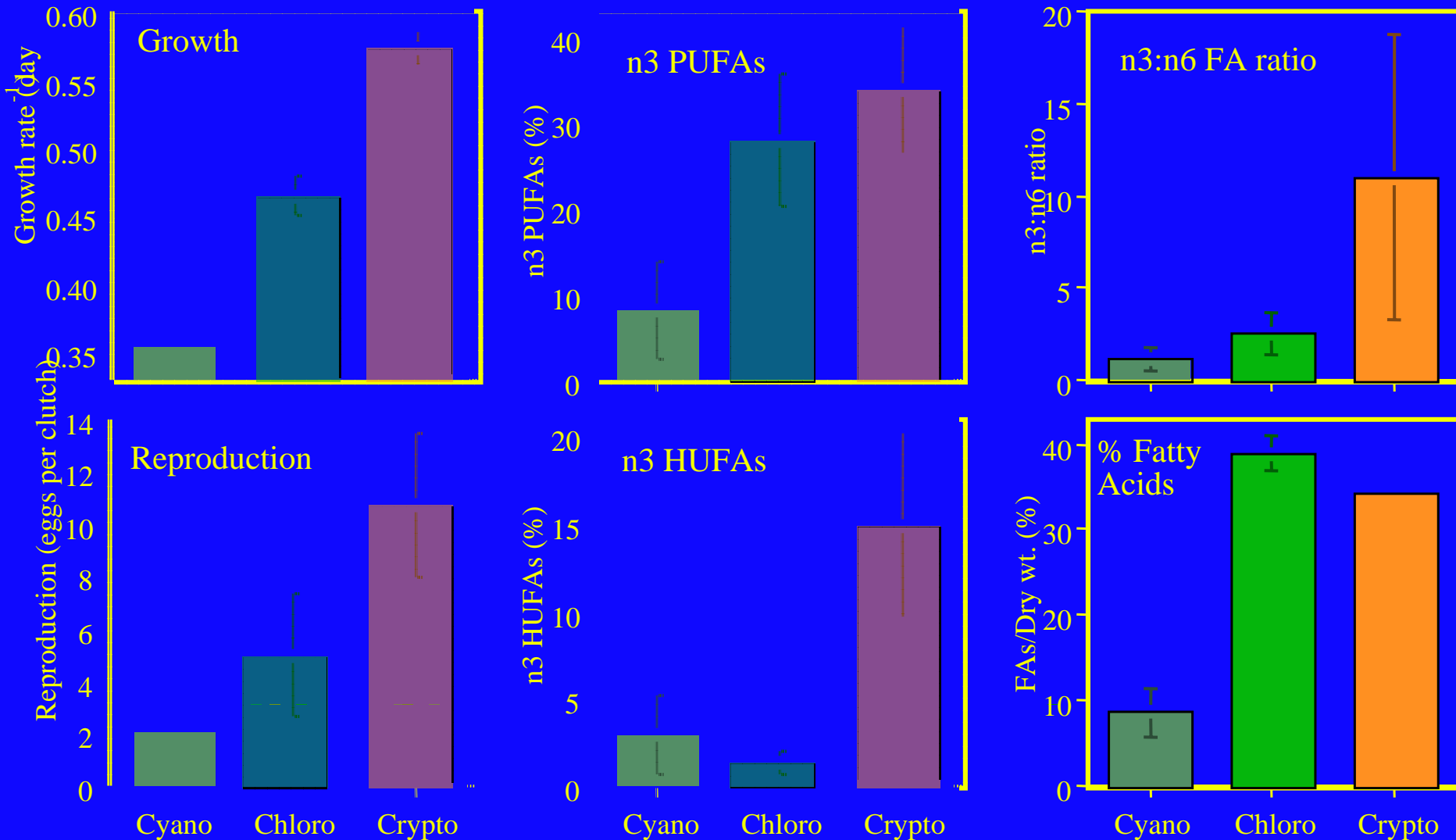
# *Daphnia* growth and biochemical composition responses to different food types



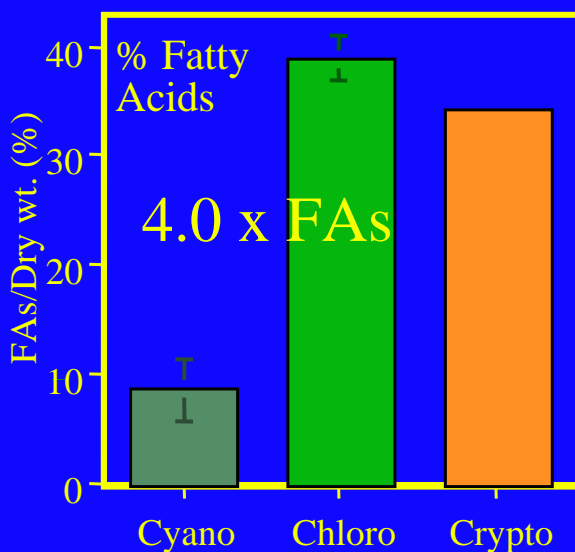
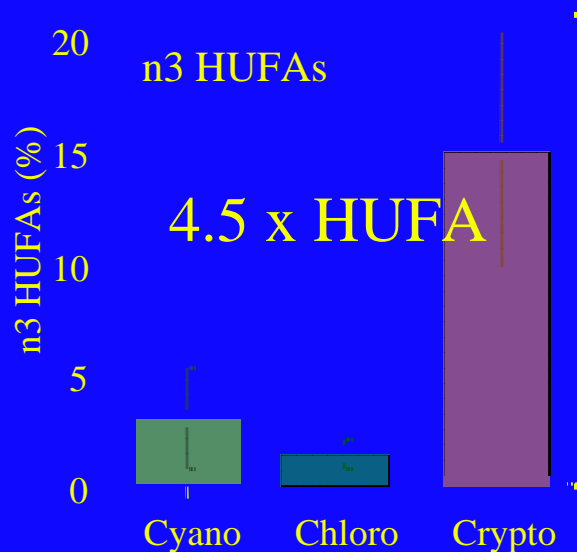
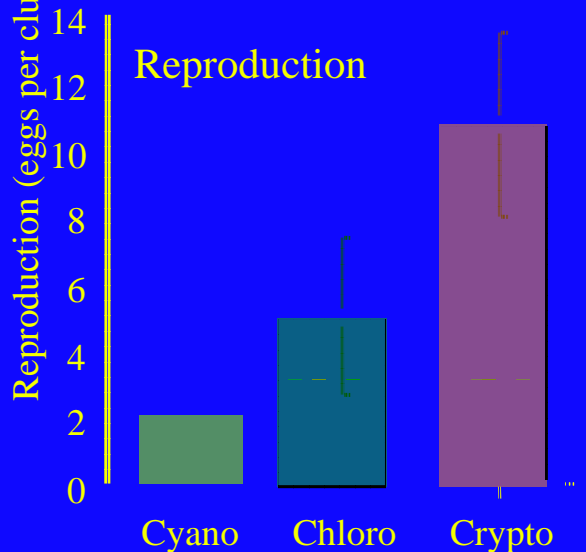
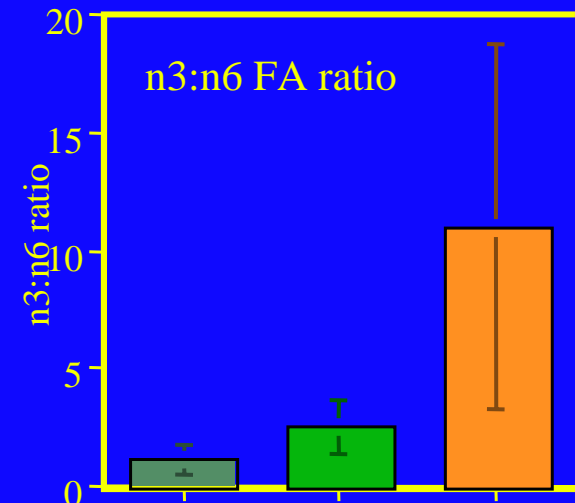
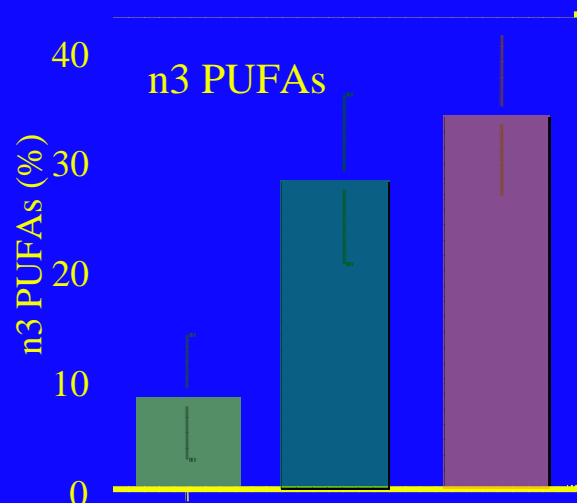
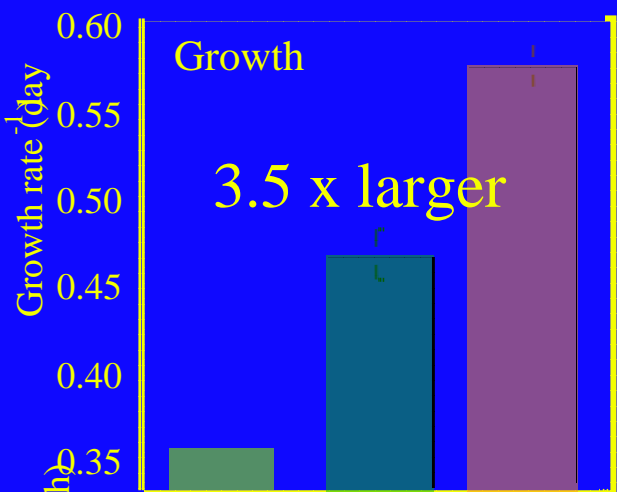
# *Daphnia* growth and biochemical composition responses to different food types



# *Daphnia* growth and biochemical composition responses to different food types



# *Daphnia* growth and biochemical composition responses to different food types



# 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

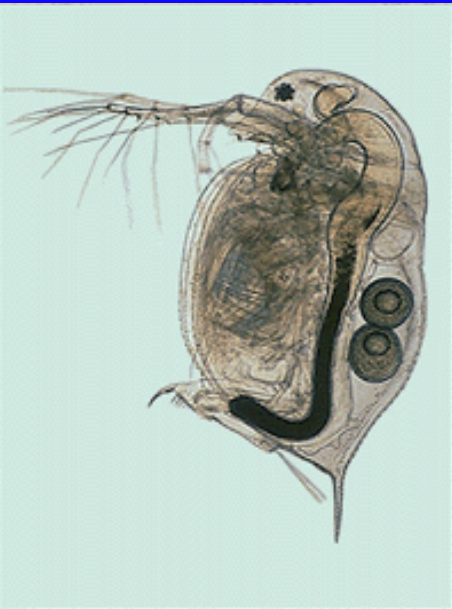
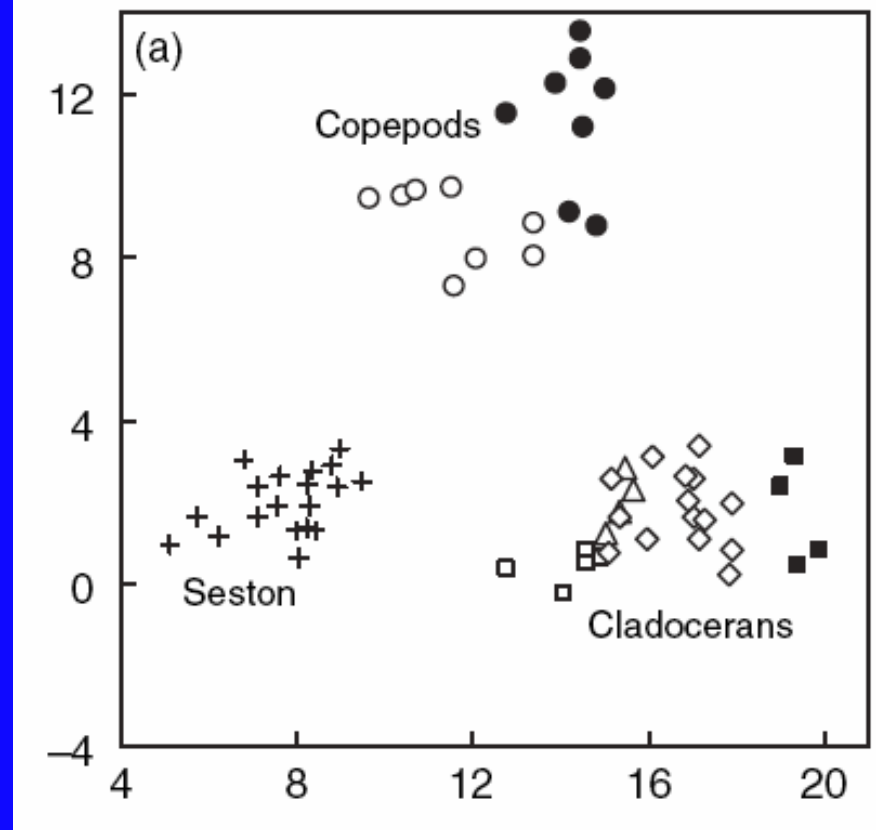
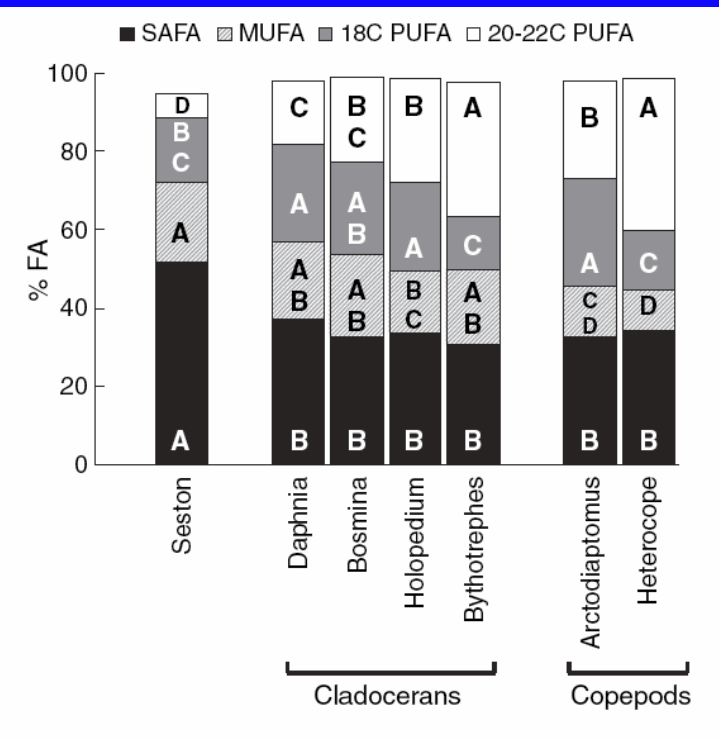


Photo by Carol Eunmi Lee

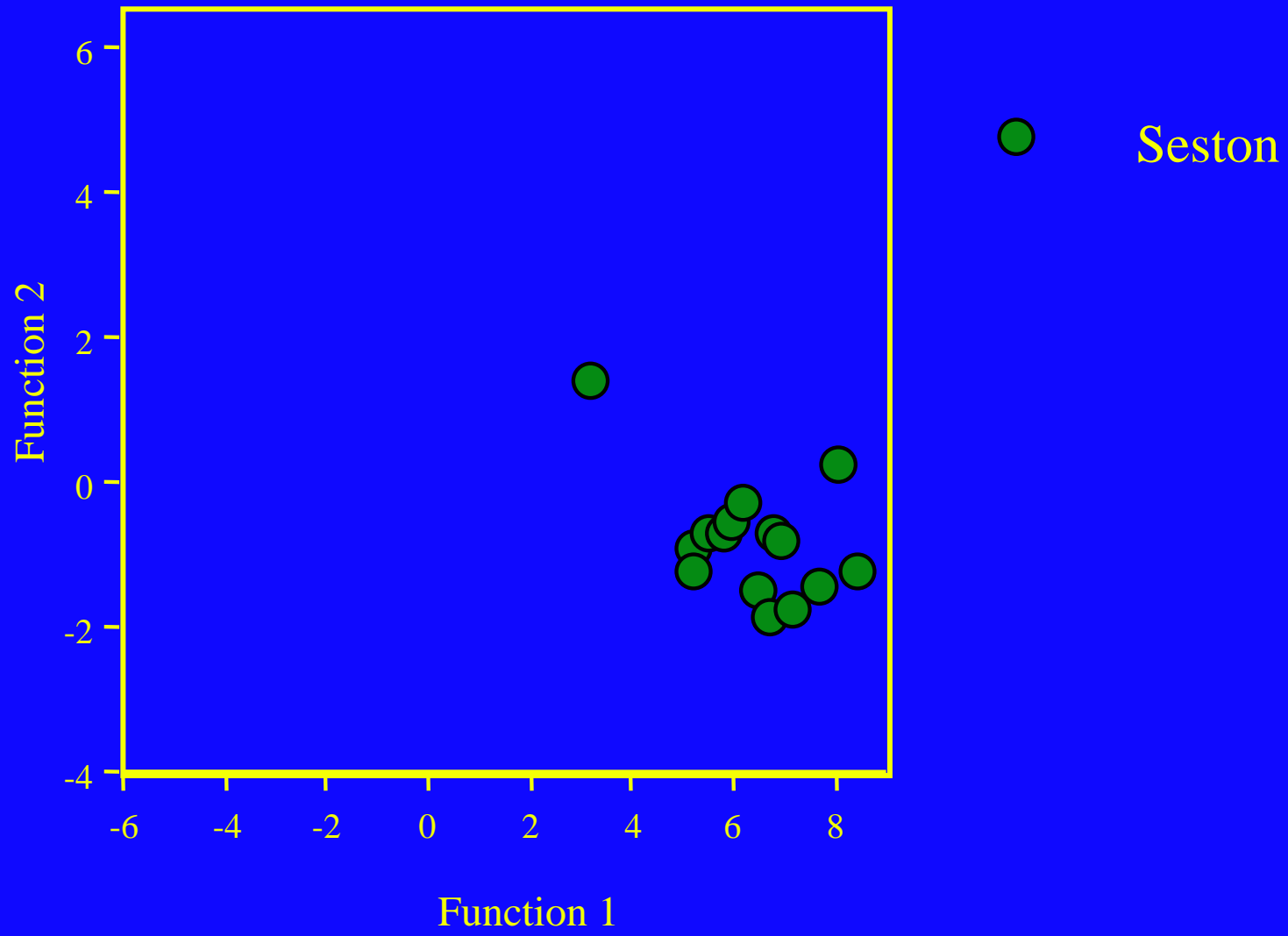
# Persson and Vrede 2006



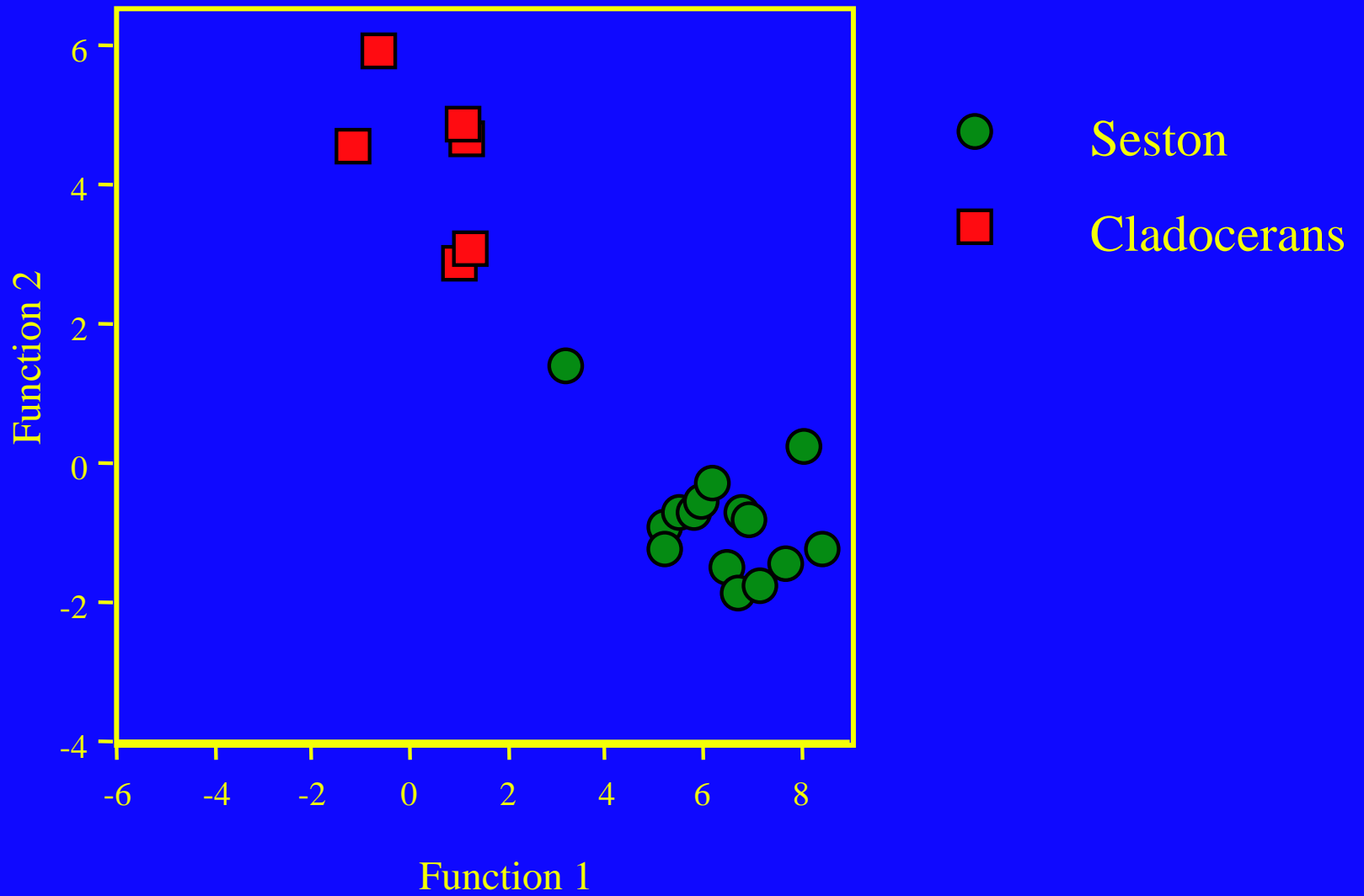
1) Zooplankton have more PUFA than seston and carnivorous zooplankton have more PUFA than herbivorous zooplankton

2) Cladocerans accumulate EPA, whereas copepods accumulate EPA and especially DHA

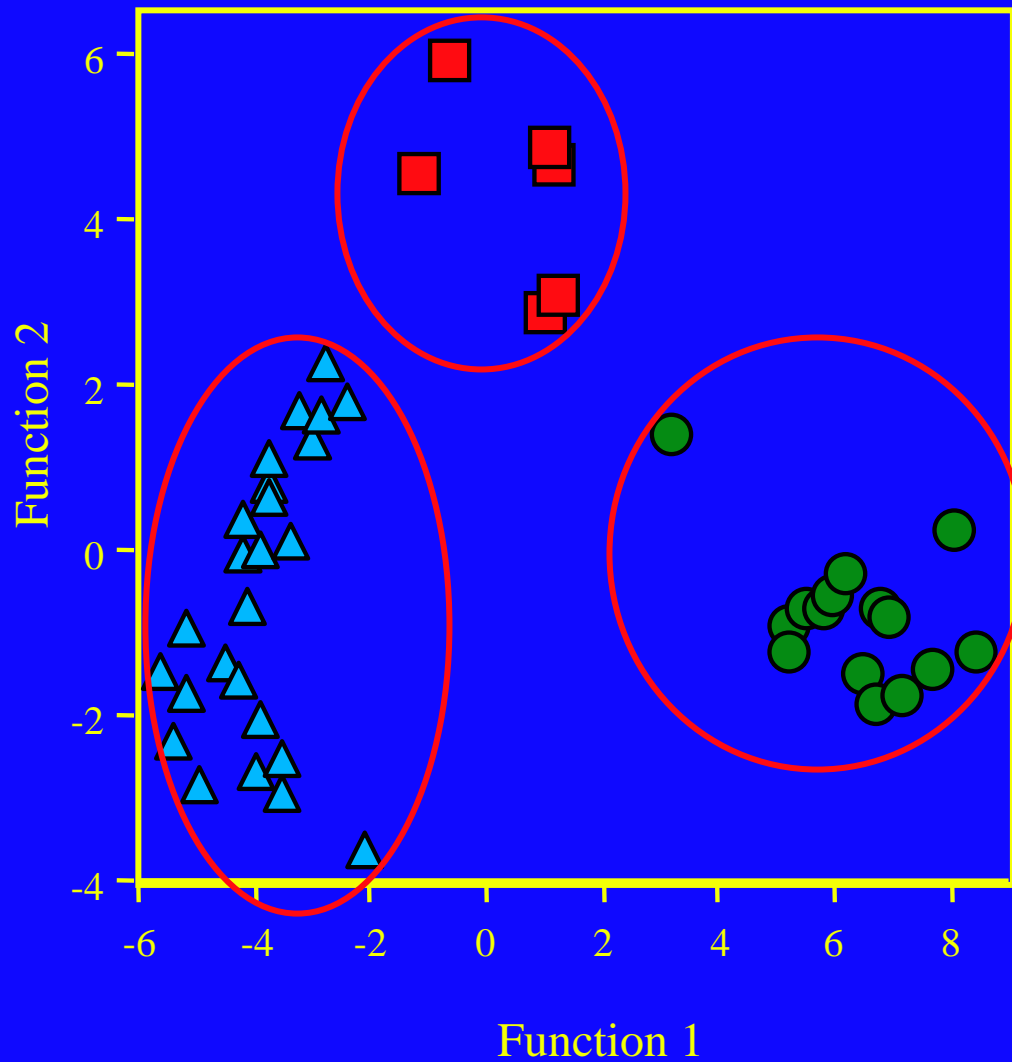
# Lake Washington Fatty Acids



# Lake Washington Fatty Acids

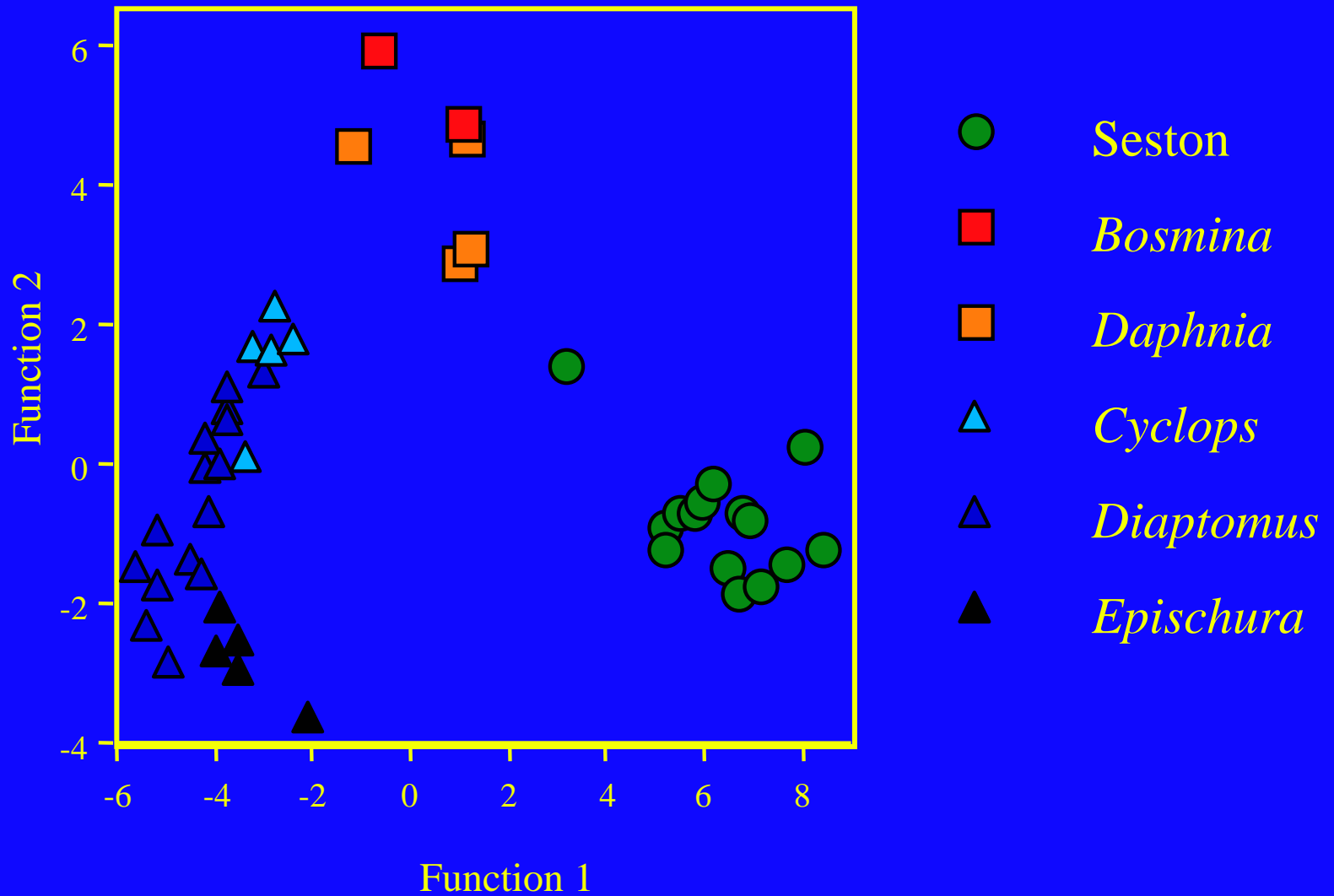


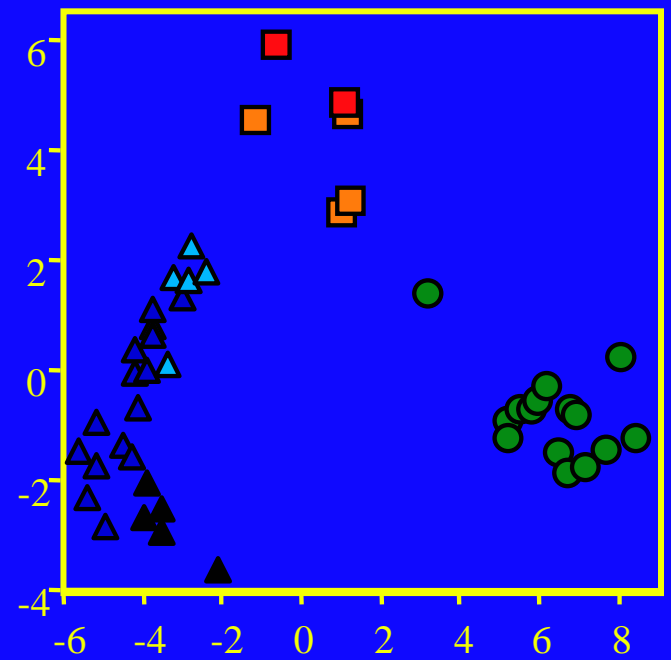
# Lake Washington Fatty Acids



- Seston
- Cladocerans
- ▲ Copepods

# Lake Washington Fatty Acids

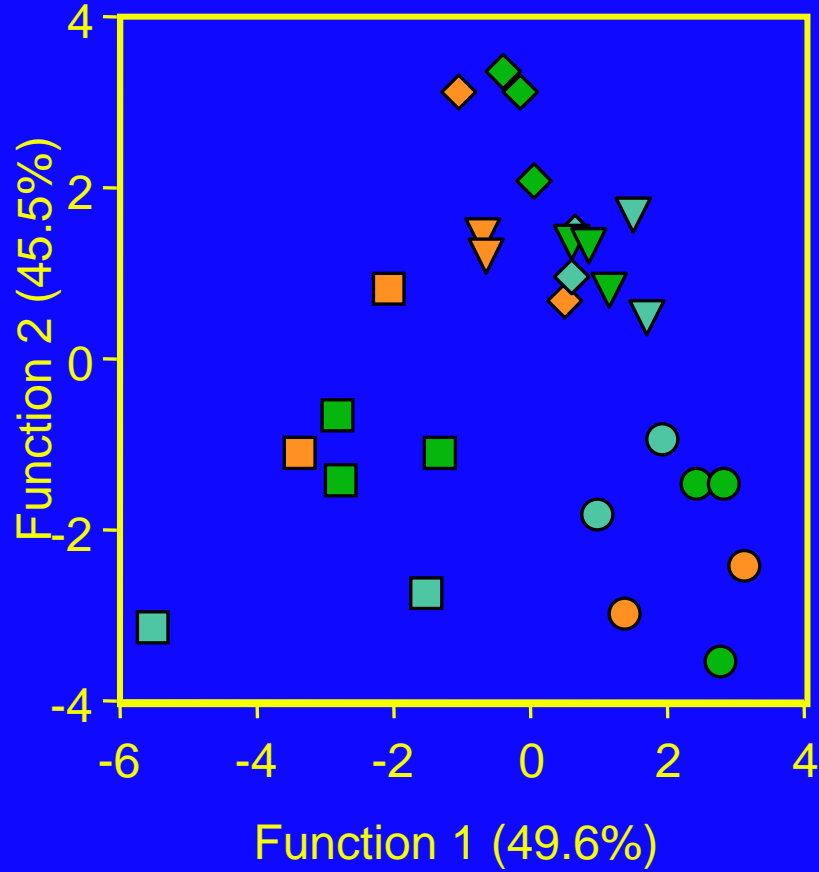




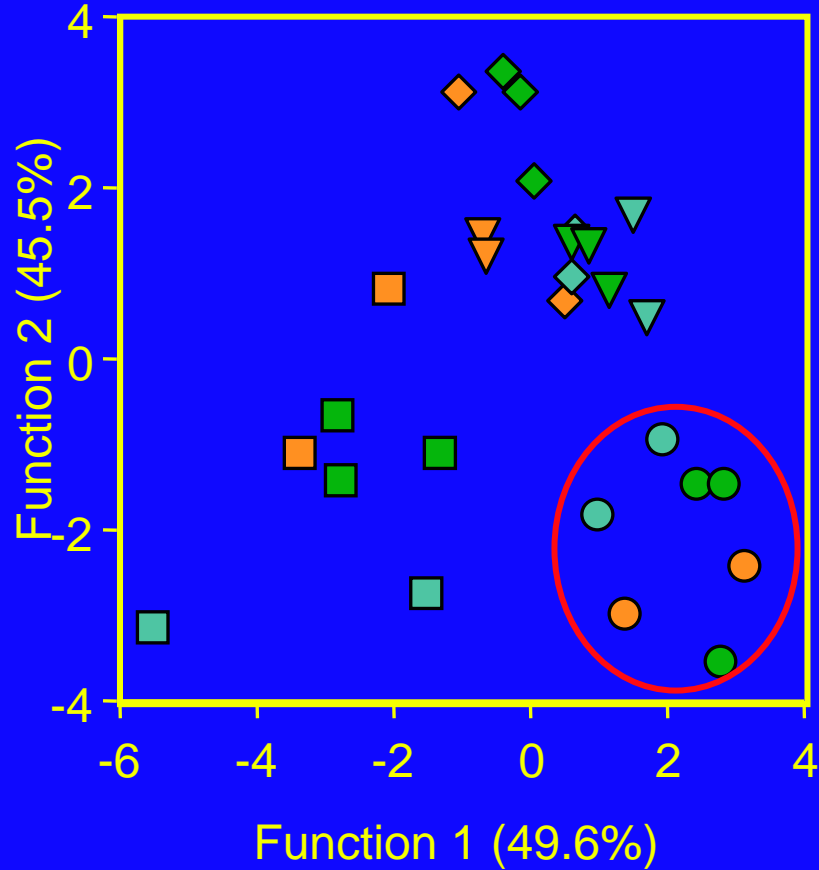
Zooplankton tended to accumulate much higher proportions of  $\omega$ 3 PUFA than their diets.

Copepods preferentially accumulated DHA, cladocerans accumulated EPA, and both copepods and cladocerans accumulated 18 carbon chain  $\omega$ 3 PUFAs.

Discriminant Analysis by "critter"



# Discriminant Analysis by "critter"



Phyto cyan

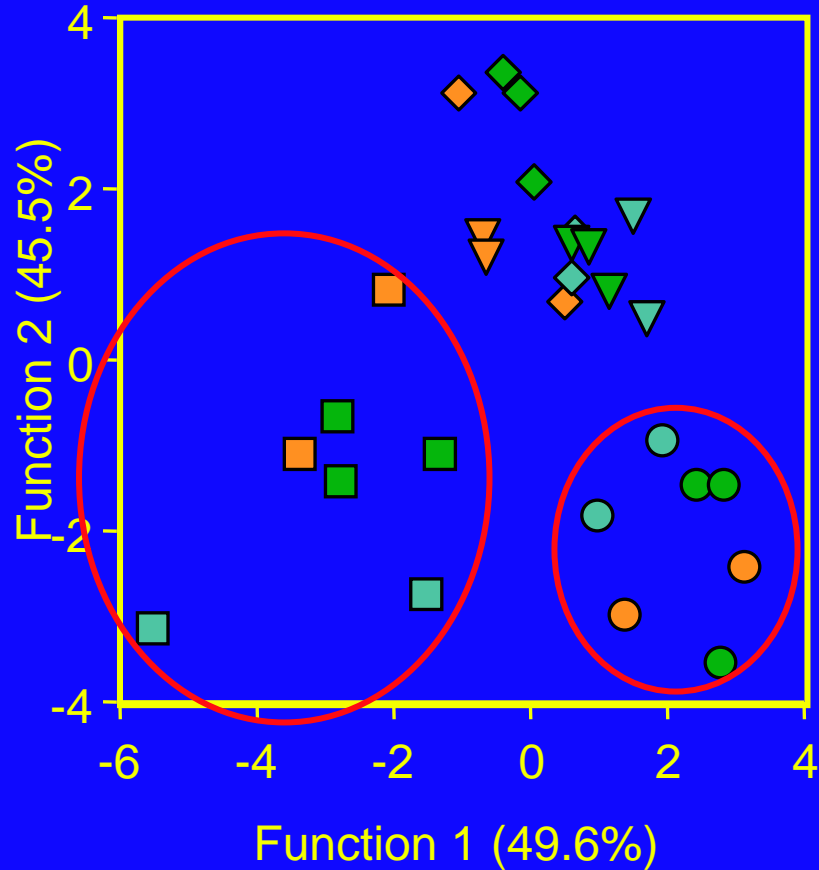


Phyto chlor



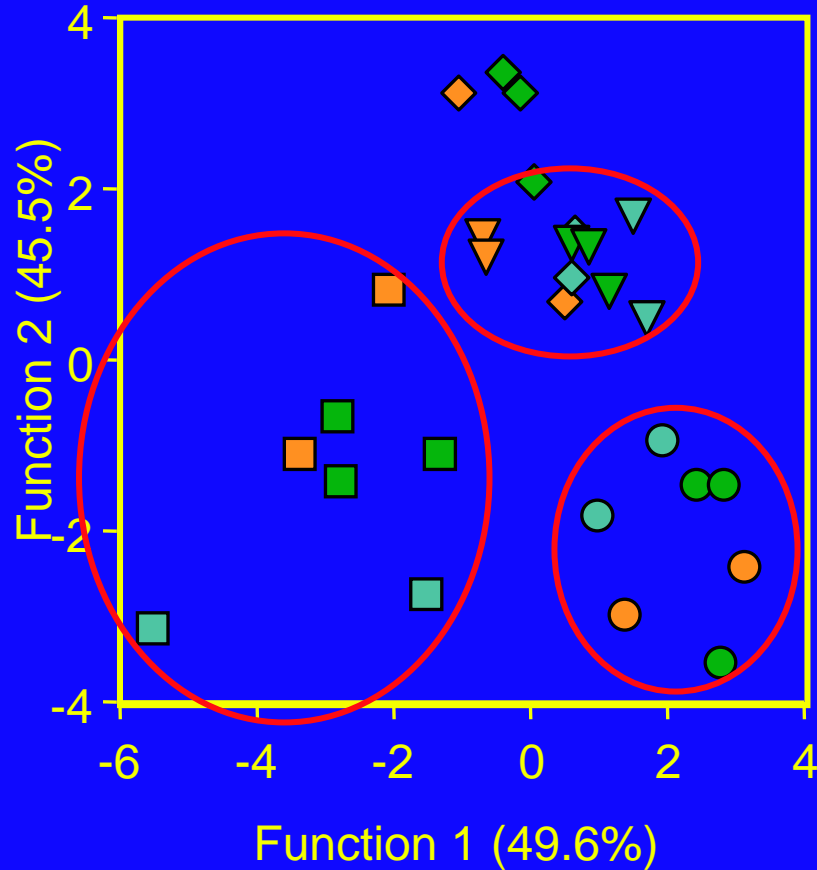
Phyto cryp

# Discriminant Analysis by "critter"



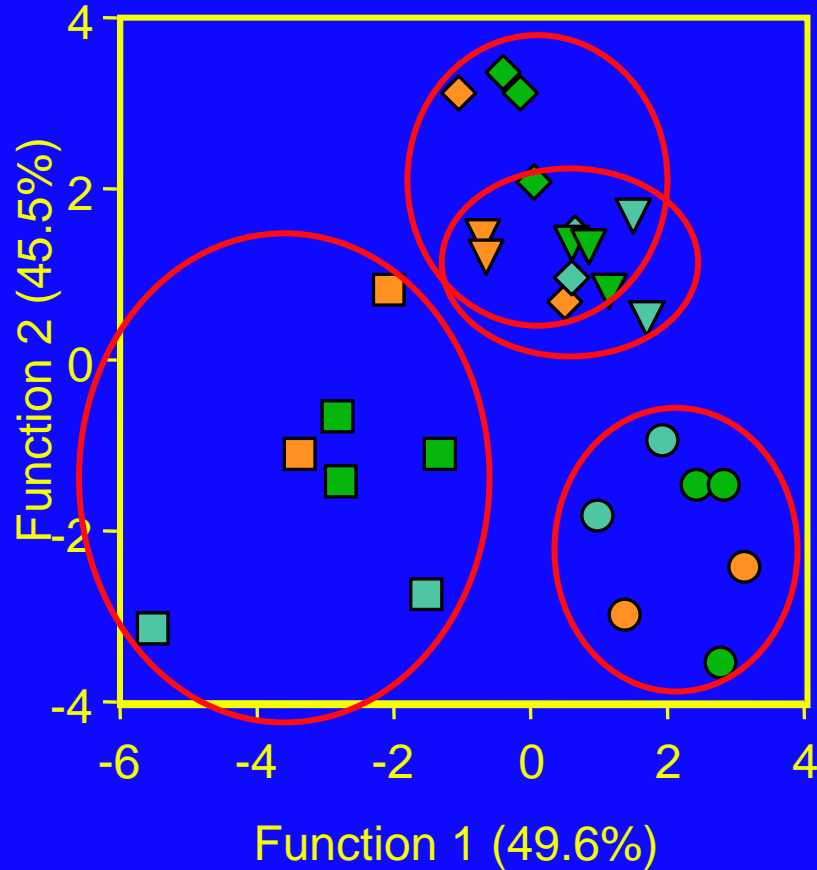
- Phyto cyan
- Phyto chlor
- Phyto cryp
- Boeck. cyano
- Boeck. chlor
- Boeck. crypt

# Discriminant Analysis by "critter"



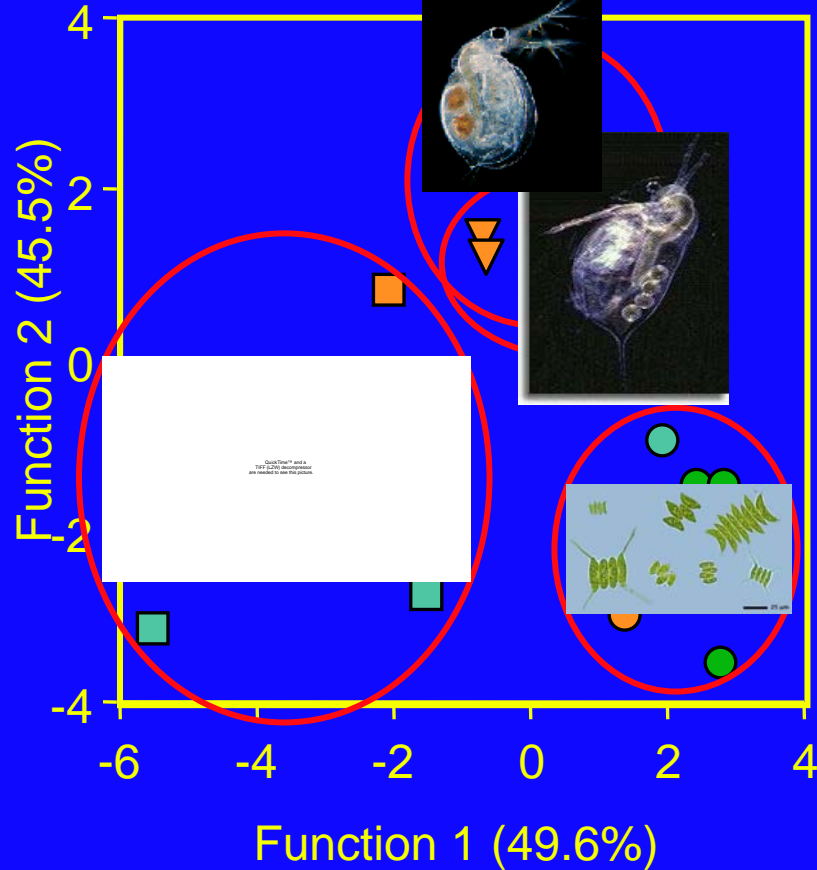
- |   |              |   |               |   |              |
|---|--------------|---|---------------|---|--------------|
| ● | Phyto cyan   | ● | Phyto chlor   | ● | Phyto cryp   |
| ■ | Boeck. cyano | ■ | Boeck. chlor  | ■ | Boeck. crypt |
| ▼ | Daphnia cyan | ▼ | Daphnia chlor | ▼ | Daphnia cryp |

# Discriminant Analysis by "critter"



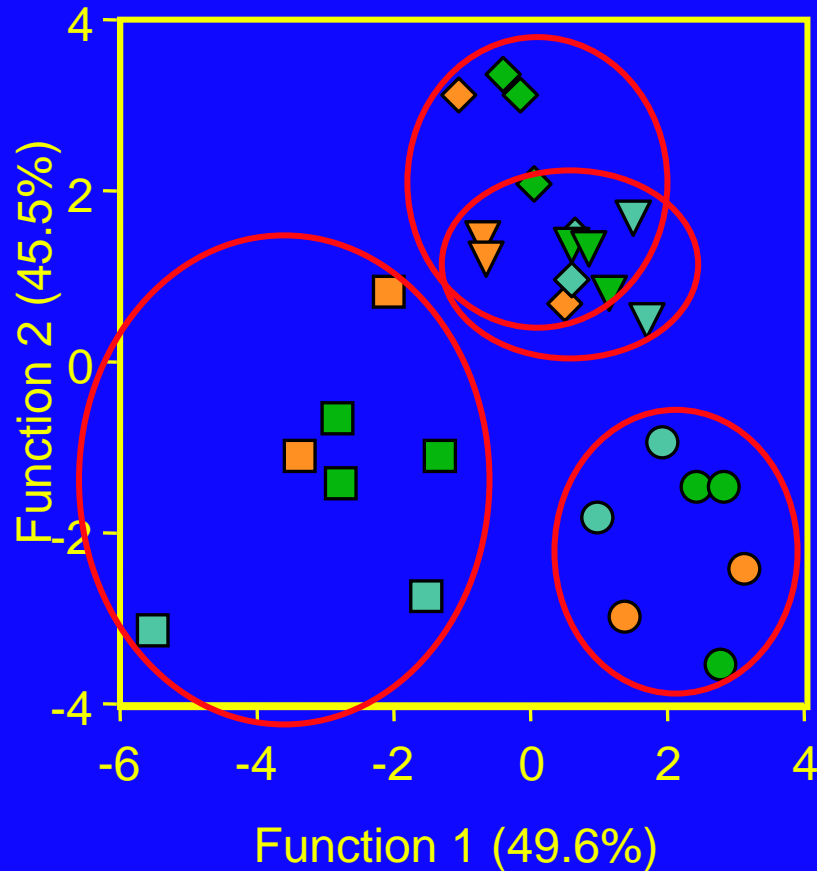
- |   |              |   |               |   |              |
|---|--------------|---|---------------|---|--------------|
| ● | Phyto cyan   | ● | Phyto chlor   | ● | Phyto cryp   |
| ■ | Boeck. cyano | ■ | Boeck. chlor  | ■ | Boeck. crypt |
| ▼ | Daphnia cyan | ▼ | Daphnia chlor | ▼ | Daphnia cryp |
| ◆ | Ceriod. cyan | ◆ | Ceriod. chlor | ◆ | Ceriod. cryp |

# Discriminant Analysis by "critter"



- |   |              |   |               |   |              |
|---|--------------|---|---------------|---|--------------|
| ● | Phyto cyan   | ● | Phyto chlor   | ● | Phyto cryp   |
| ■ | Boeck. cyano | ■ | Boeck. chlor  | ■ | Boeck. cryp  |
| ▼ | Daphnia cyan | ▼ | Daphnia chlor | ▼ | Daphnia cryp |
| ◆ | Ceriod. cyan | ◆ | Ceriod. chlor | ◆ | Ceriod. cryp |

## Discriminant Analysis by "critter"



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

●	Phyto cyan	●	Phyto chlor	●	Phyto cyp
■	<i>Boeck. cyano</i>	■	<i>Boeck. chlor</i>	■	<i>Boeck. cyp</i>
▼	<i>Daphnia cyan</i>	▼	<i>Daphnia chlor</i>	▼	<i>Daphnia cyp</i>
◆	<i>Ceriod. cyan</i>	◆	<i>Ceriod. chlor</i>	◆	<i>Ceriod. cyp</i>

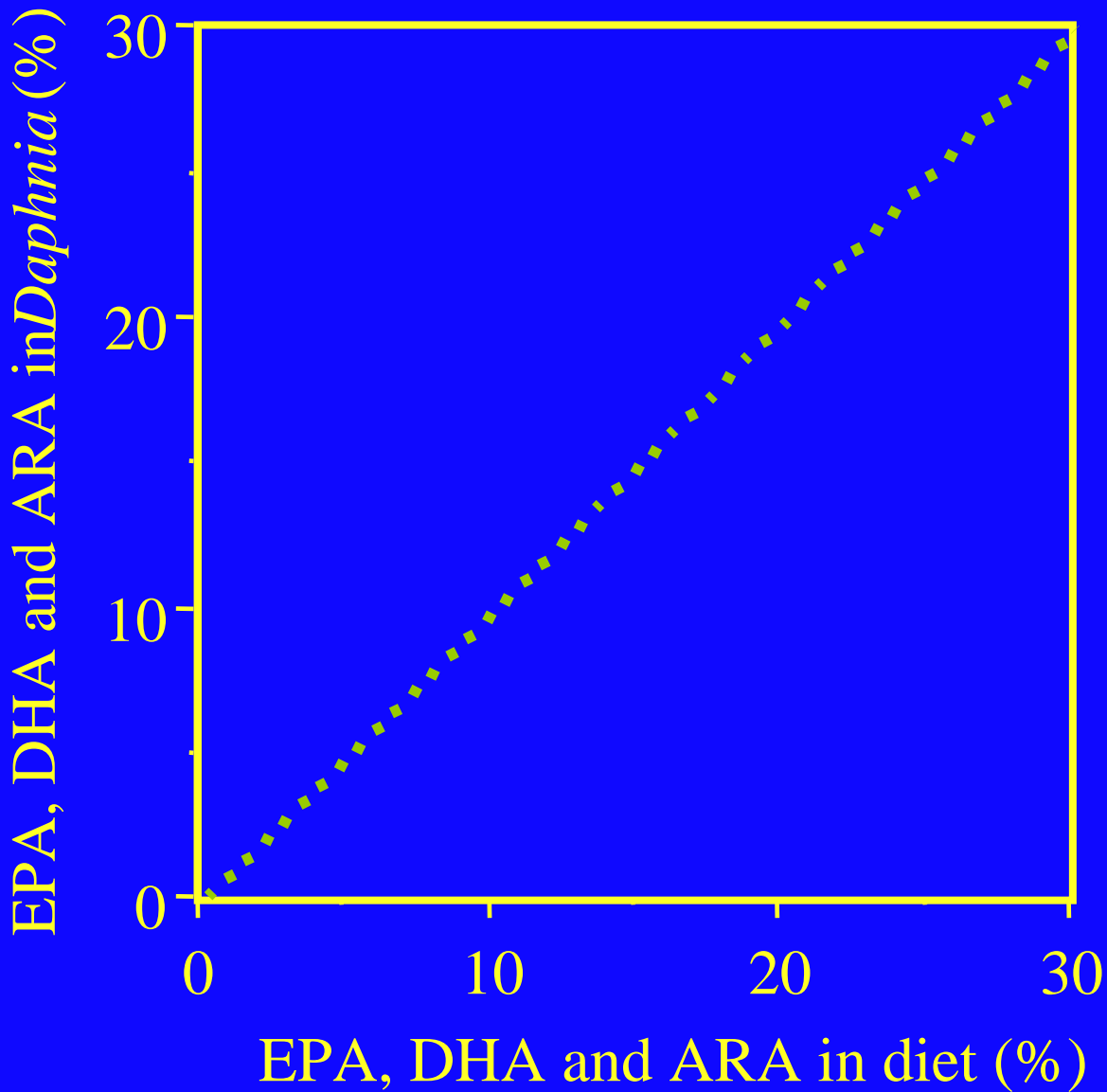
# Taxonomic conclusions

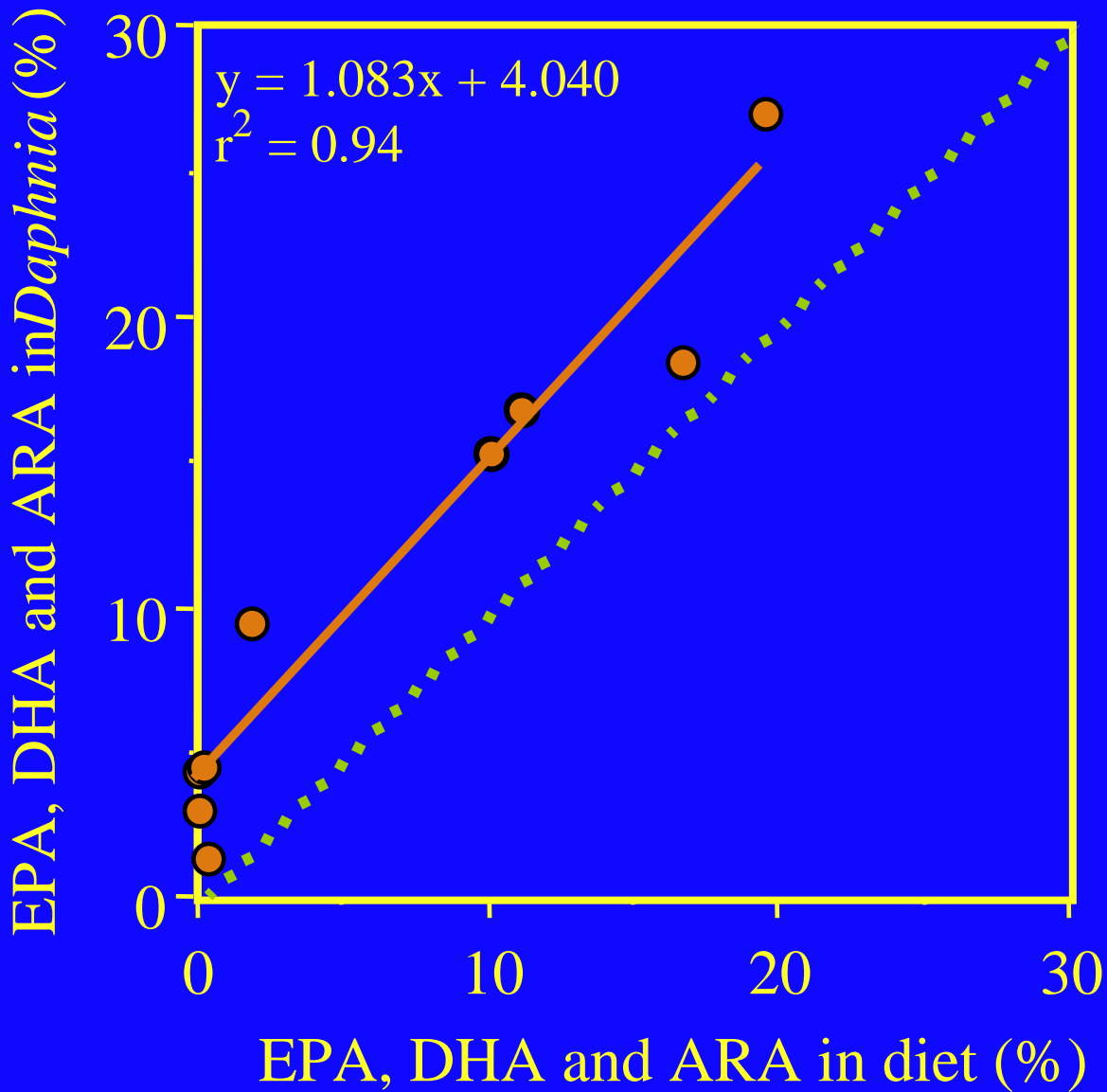
Cladocerans accumulate almost exclusively EPA, whereas copepods accumulate both EPA and DHA - but predominantly DHA. Why?

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

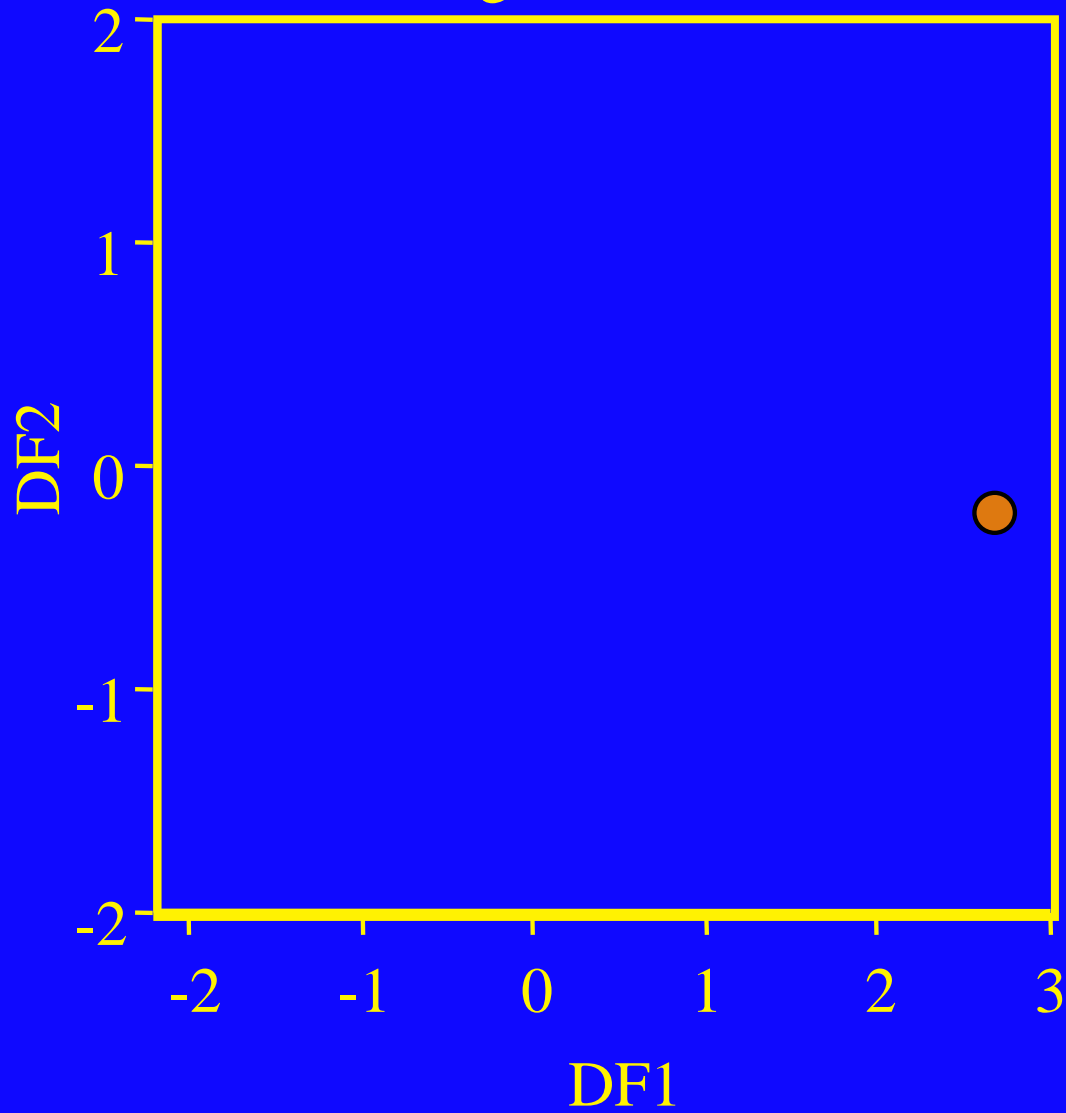
**BUT** why can't DHA be used as a substrate to support fast growth/reproduction & why can't EPA be used to form sensory tissues? Furthermore, do copepods actually have more sensory tissue than cladocerans?

# Dietary control of fatty acid composition

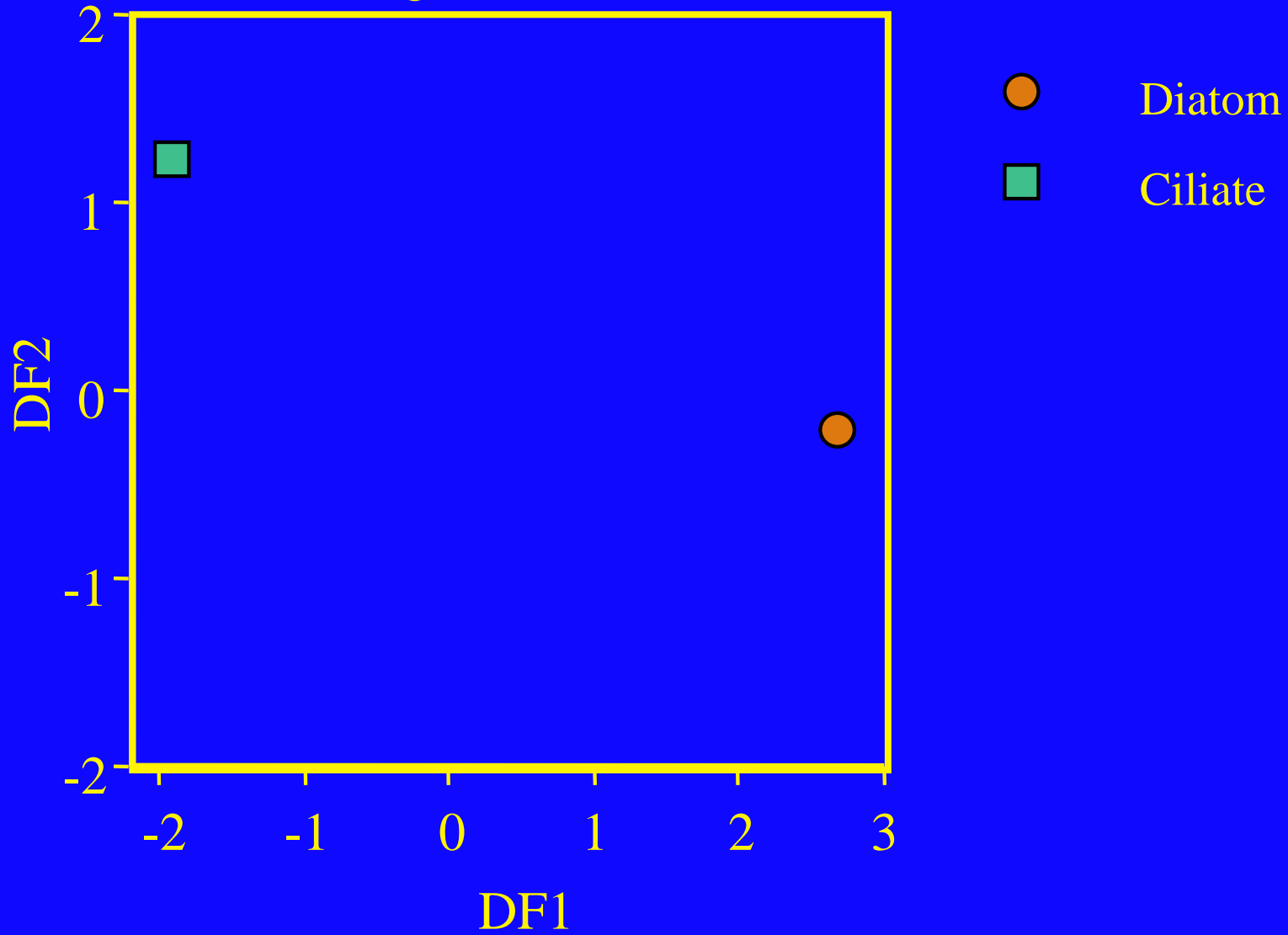




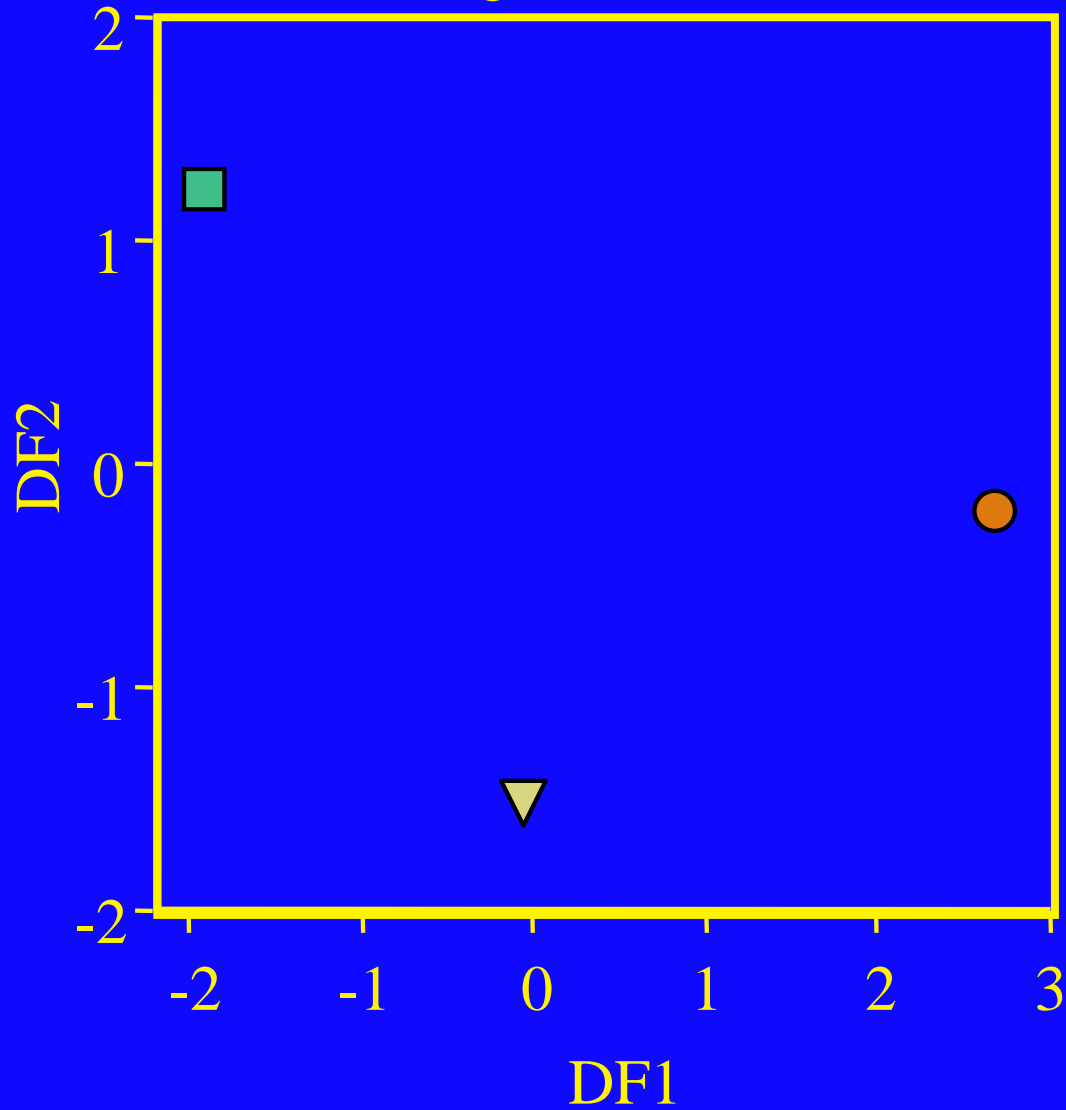
# Ederington et al. 1995 DFA



# Ederington et al. 1995 DFA

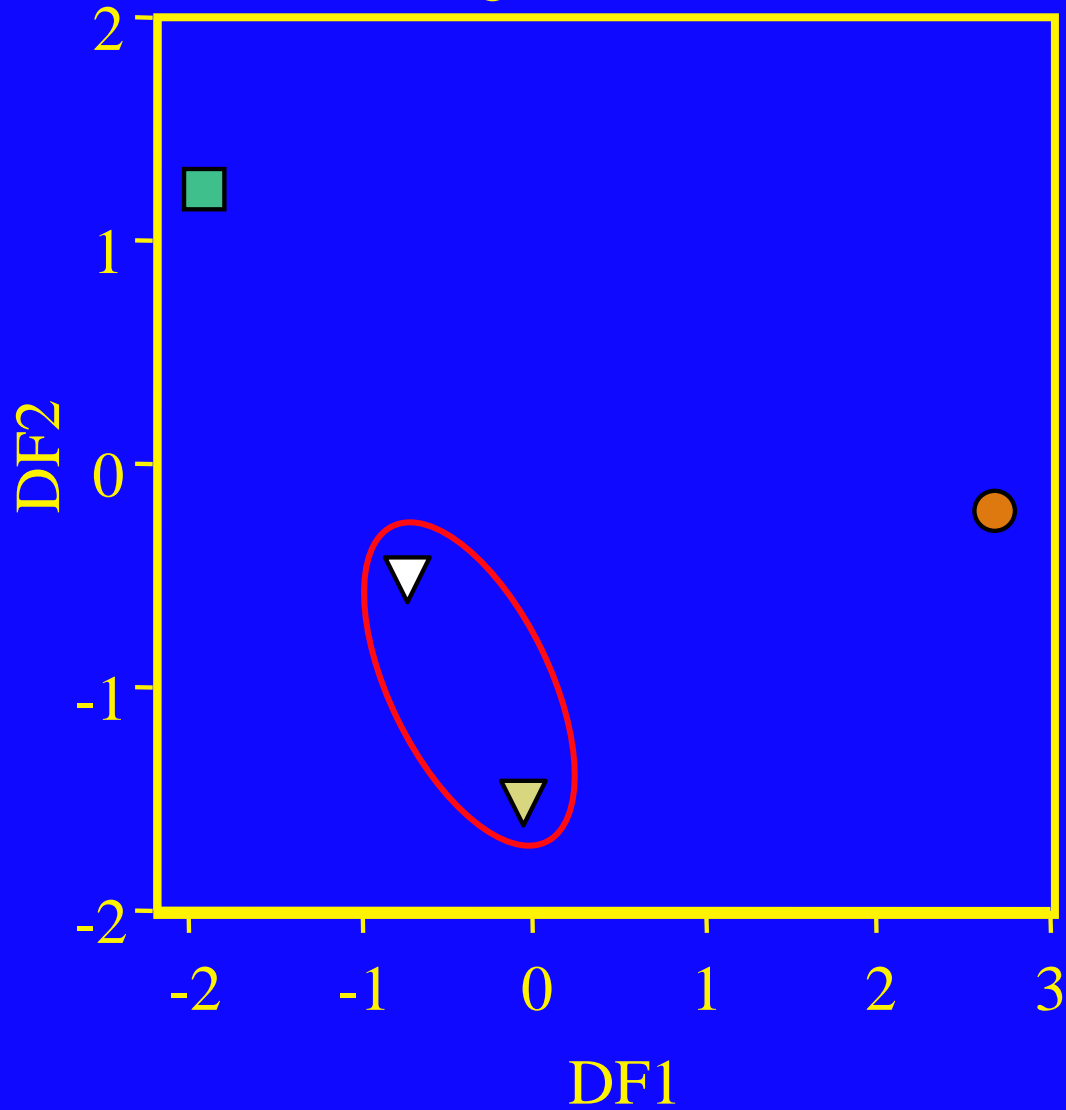


# Ederington et al. 1995 DFA



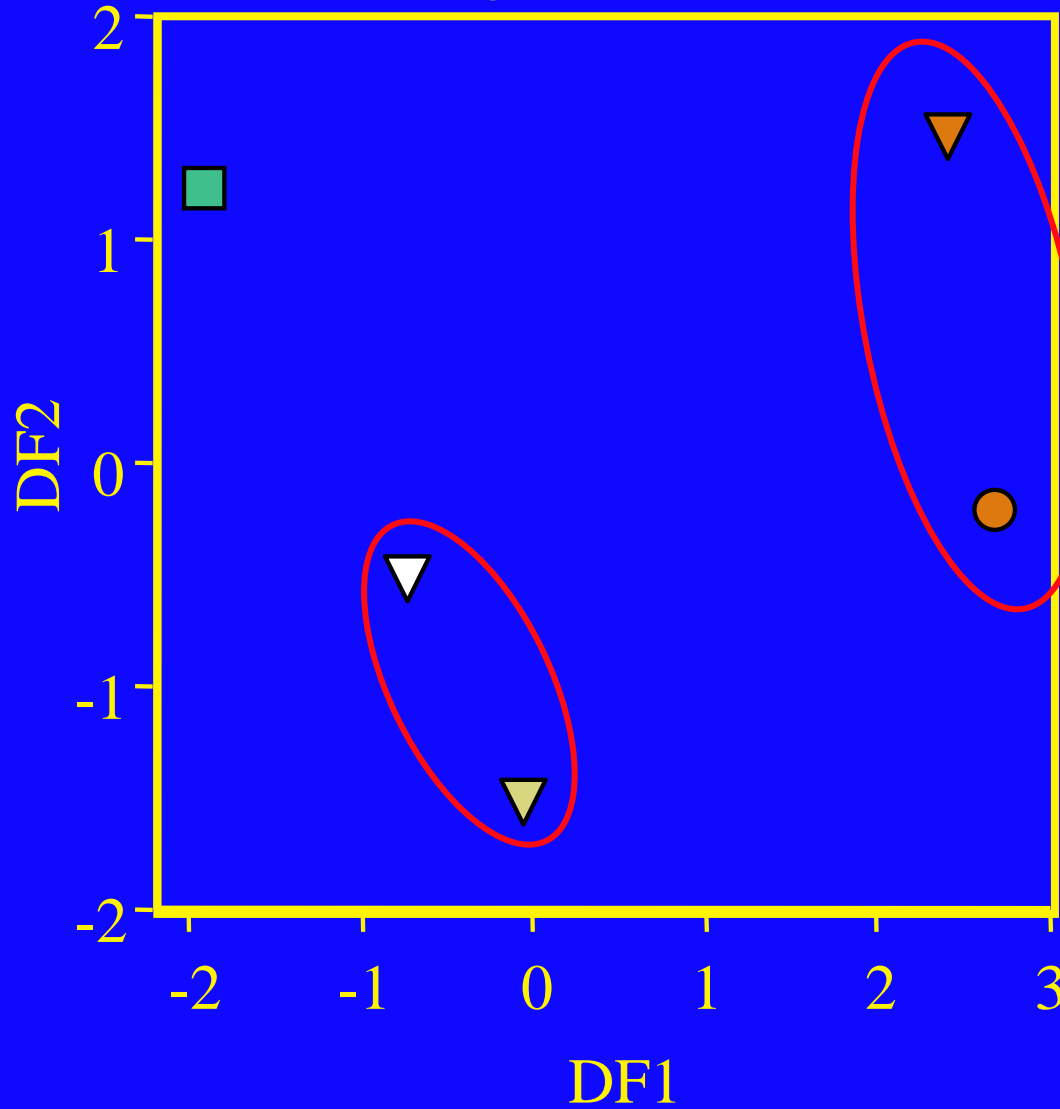
- Diatom
- Ciliate
- ▼ Copepod (field)

# Ederington et al. 1995 DFA



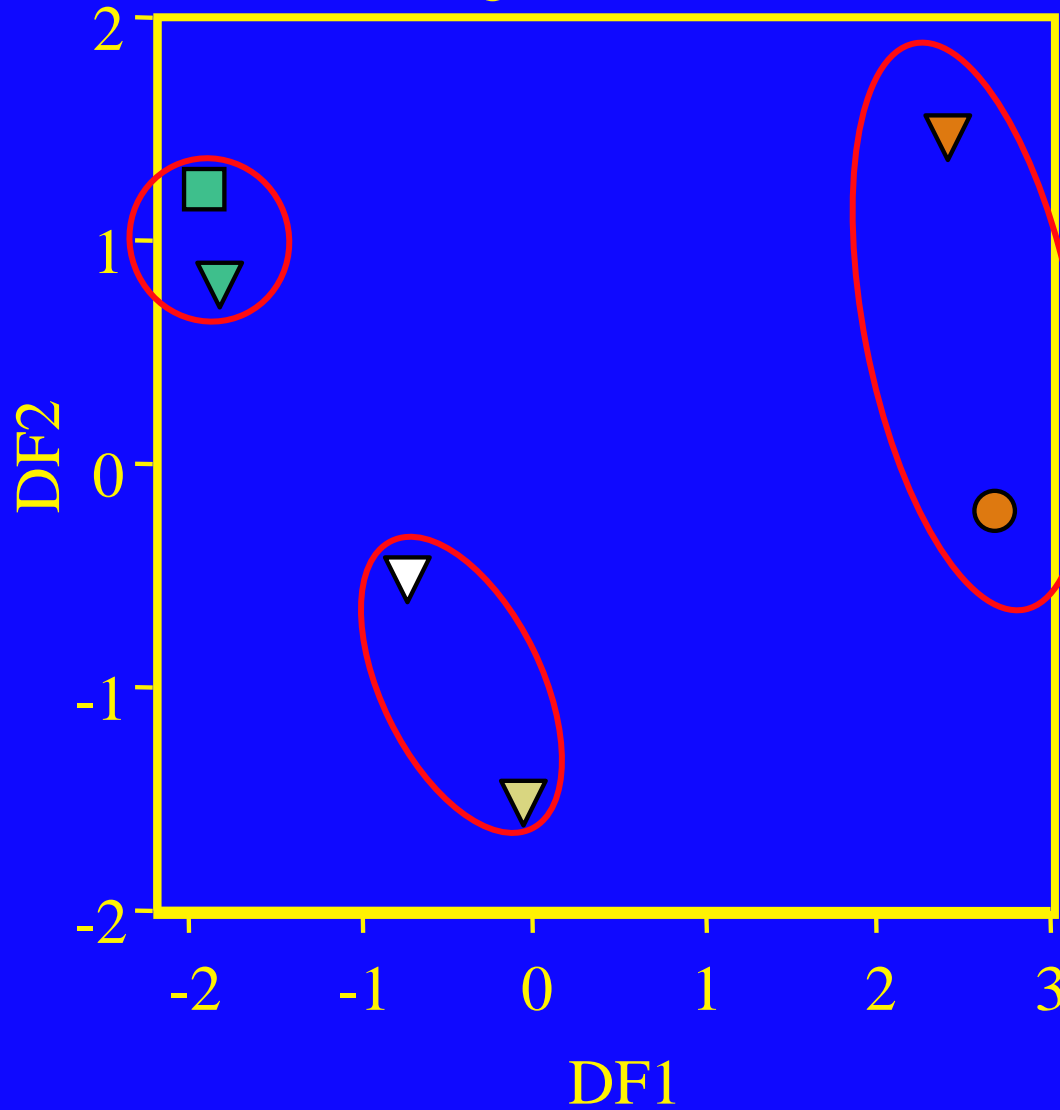
- Diatom
- Ciliate
- ▼ Copepod (field)
- ▼ Copepod (starved)

# Ederington et al. 1995 DFA



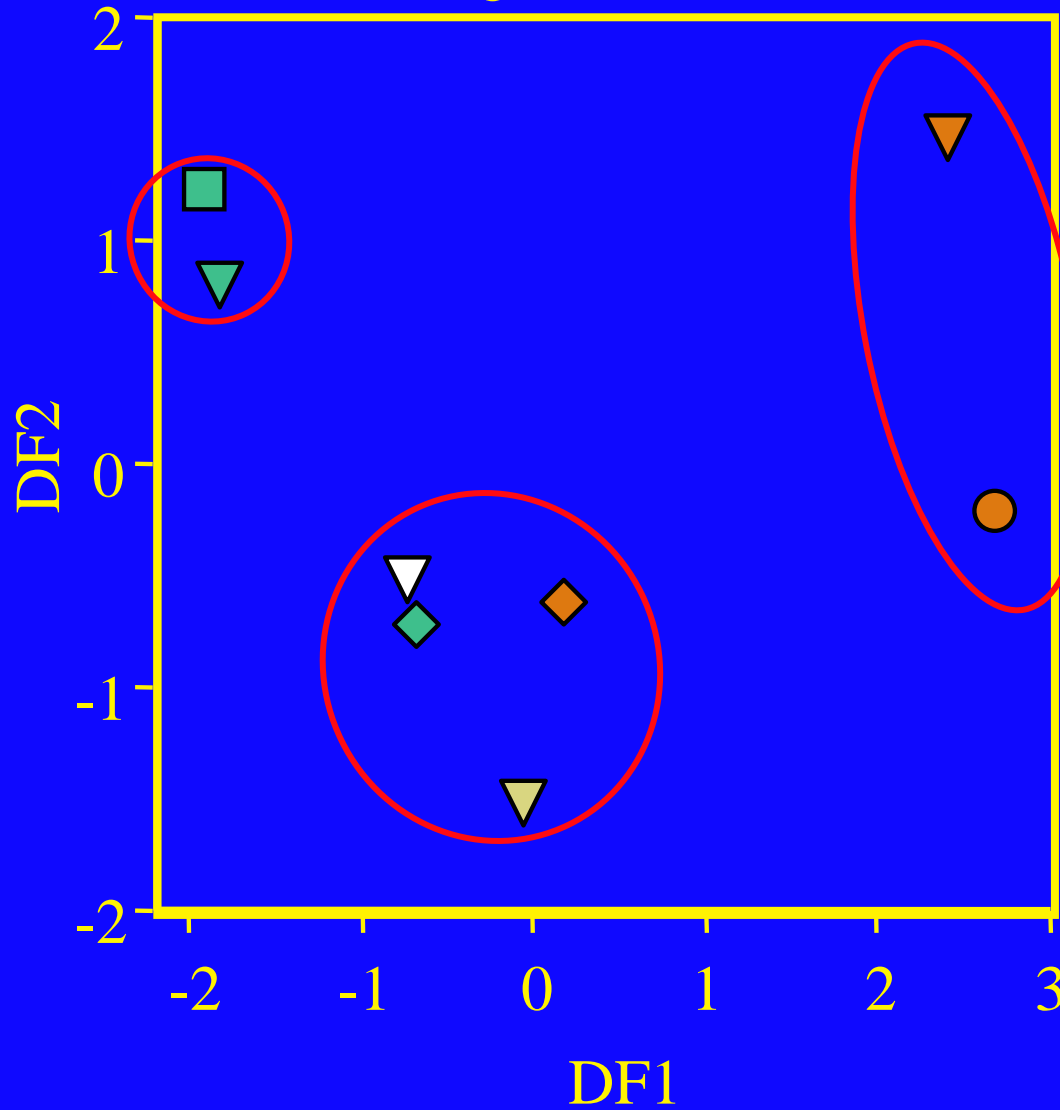
- Diatom
- Ciliate
- ▼ Copepod (field)
- ▼ Copepod (starved)
- ▼ Copepod (diatom fed)

# Ederington et al. 1995 DFA



- Diatom
- Ciliate
- ▼ Copepod (field)
- ▼ Copepod (starved)
- ▼ Copepod (diatom fed)
- ▼ Copepod (ciliate fed)

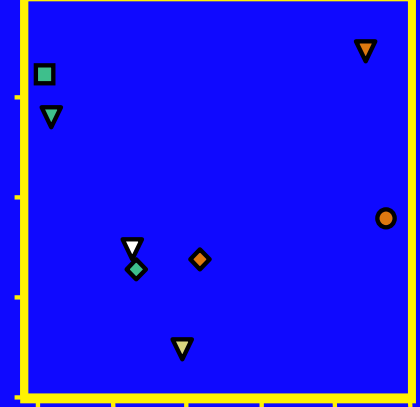
# Ederington et al. 1995 DFA



- 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



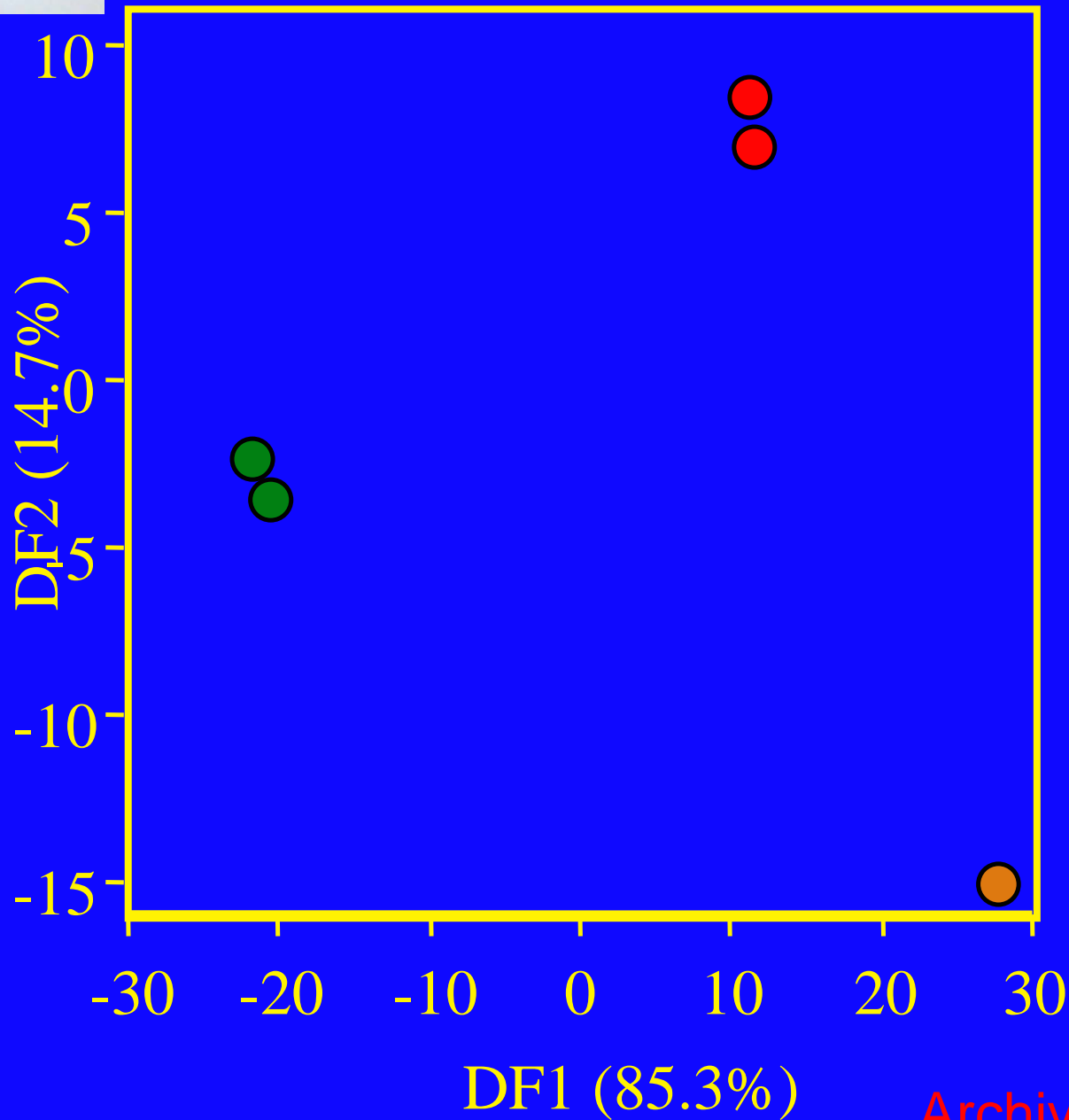
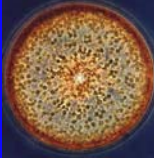
2) Diatoms and *Acartia* fed diatoms & their eggs had high EPA

3) Ciliates and *Acartia* fed ciliates & their eggs had high 18:1 $\omega$ 11 and 22:0

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

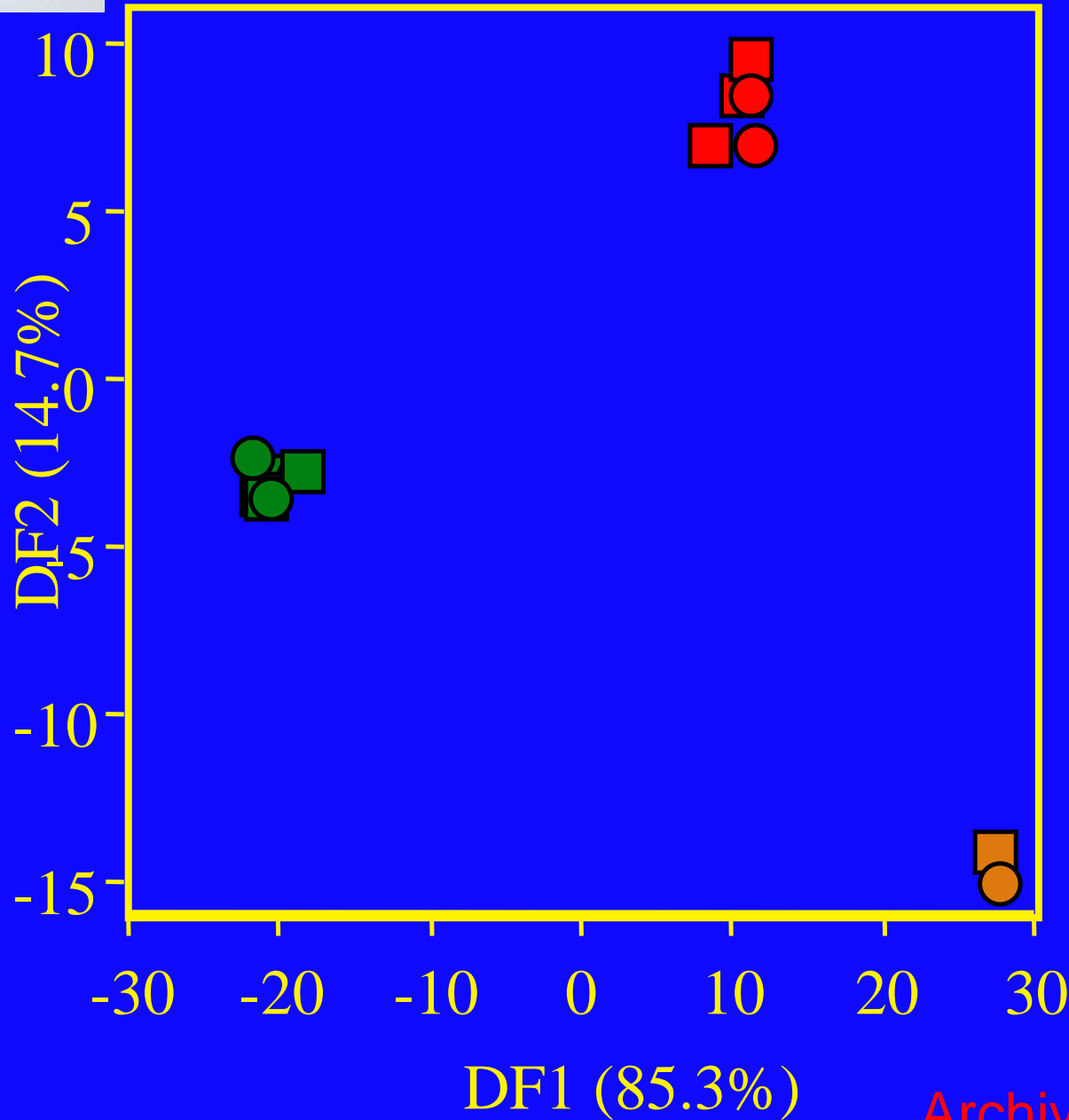
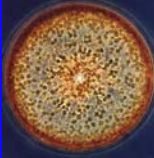
**Caveat:** These were only 4 day incubations

# Müller-Navarra 2006

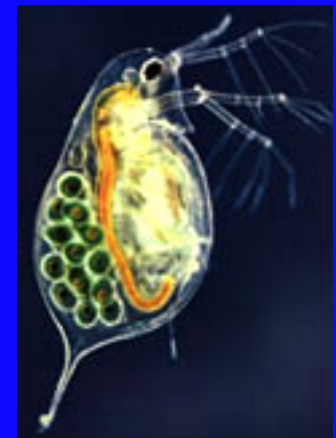


- Phyto Chloro
- Phyto Crypto
- Phyto Diatom

# Müller-Navarra 2006



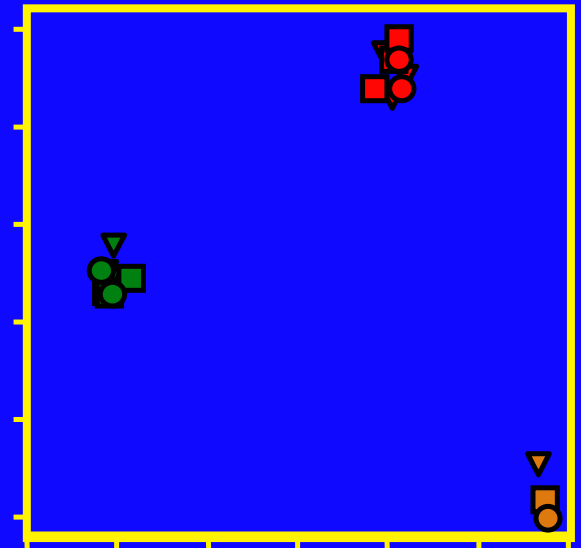
- Phyto Chloro
- Phyto Crypto
- Phyto Diatom
- *Daphnia* Chloro
- *Daphnia* Crypto
- *Daphnia* Diatom





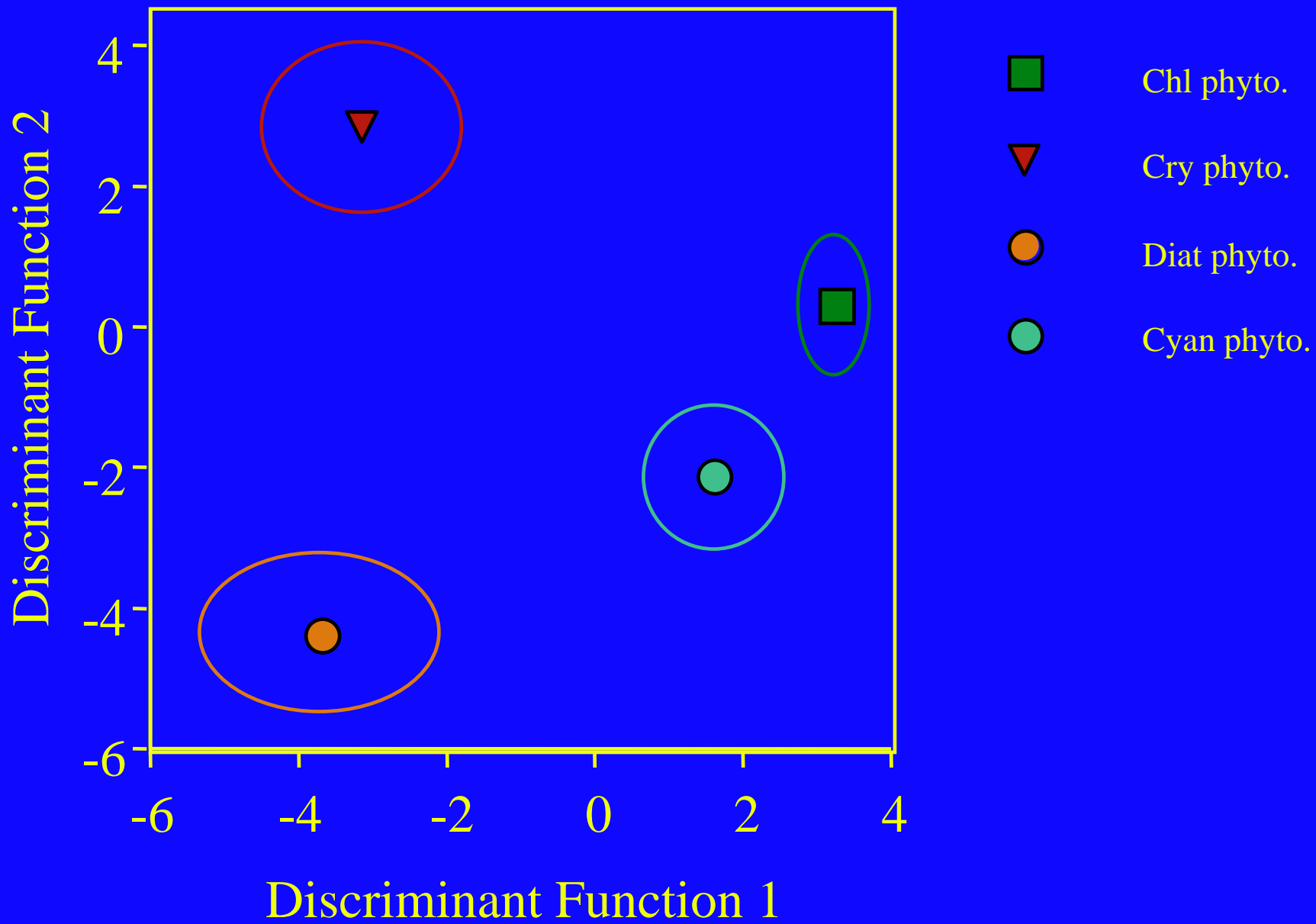
# Conclusions from M-N 2006 data

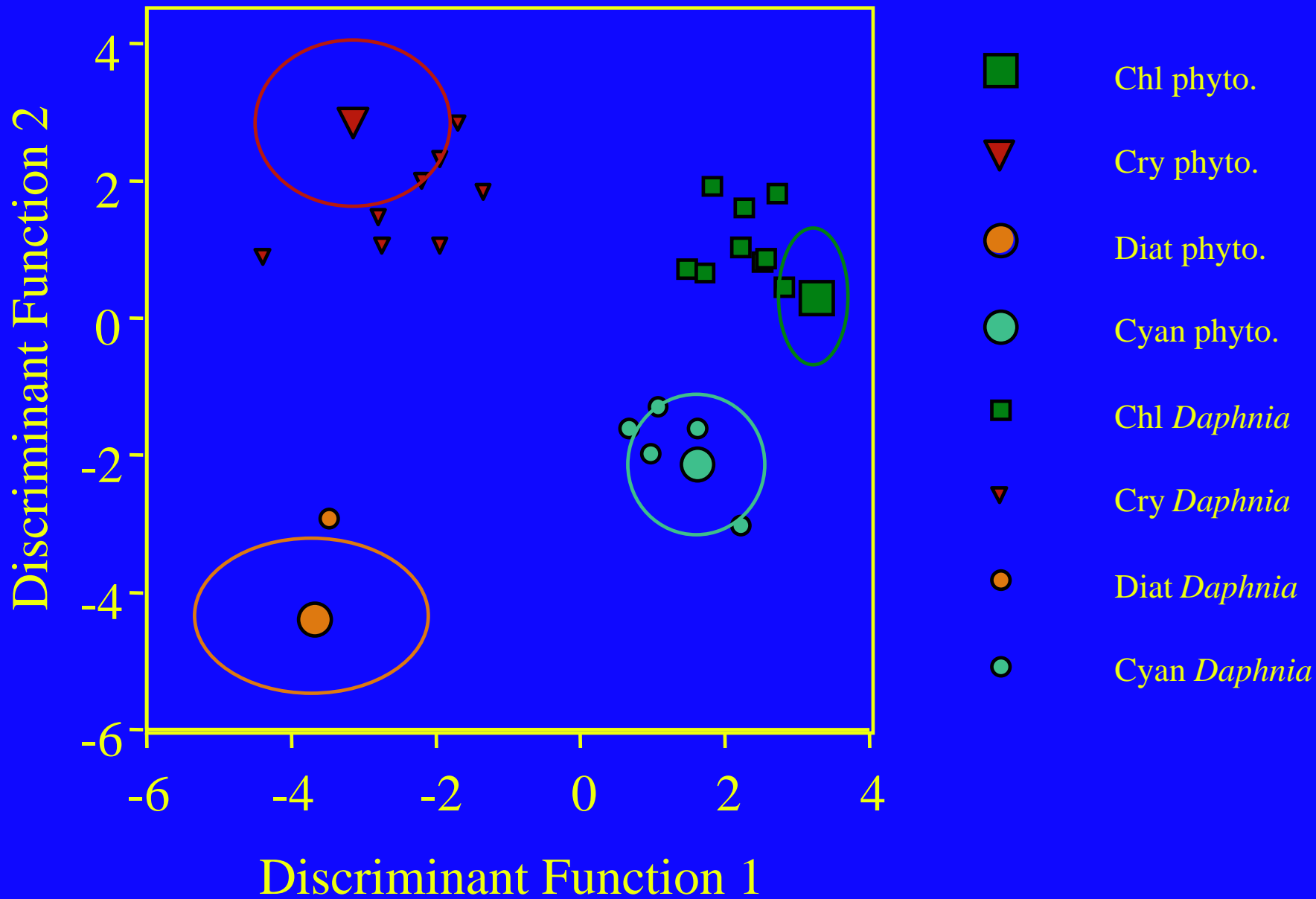
1) The 18:2 $\omega$ 6, 18:3 $\omega$ 3, 18:4 $\omega$ 3, and EPA content and  $\omega$ 3: $\omega$ 6 ratio of the diet was very strongly correlated with that of the *Daphnia* somatic tissues and eggs



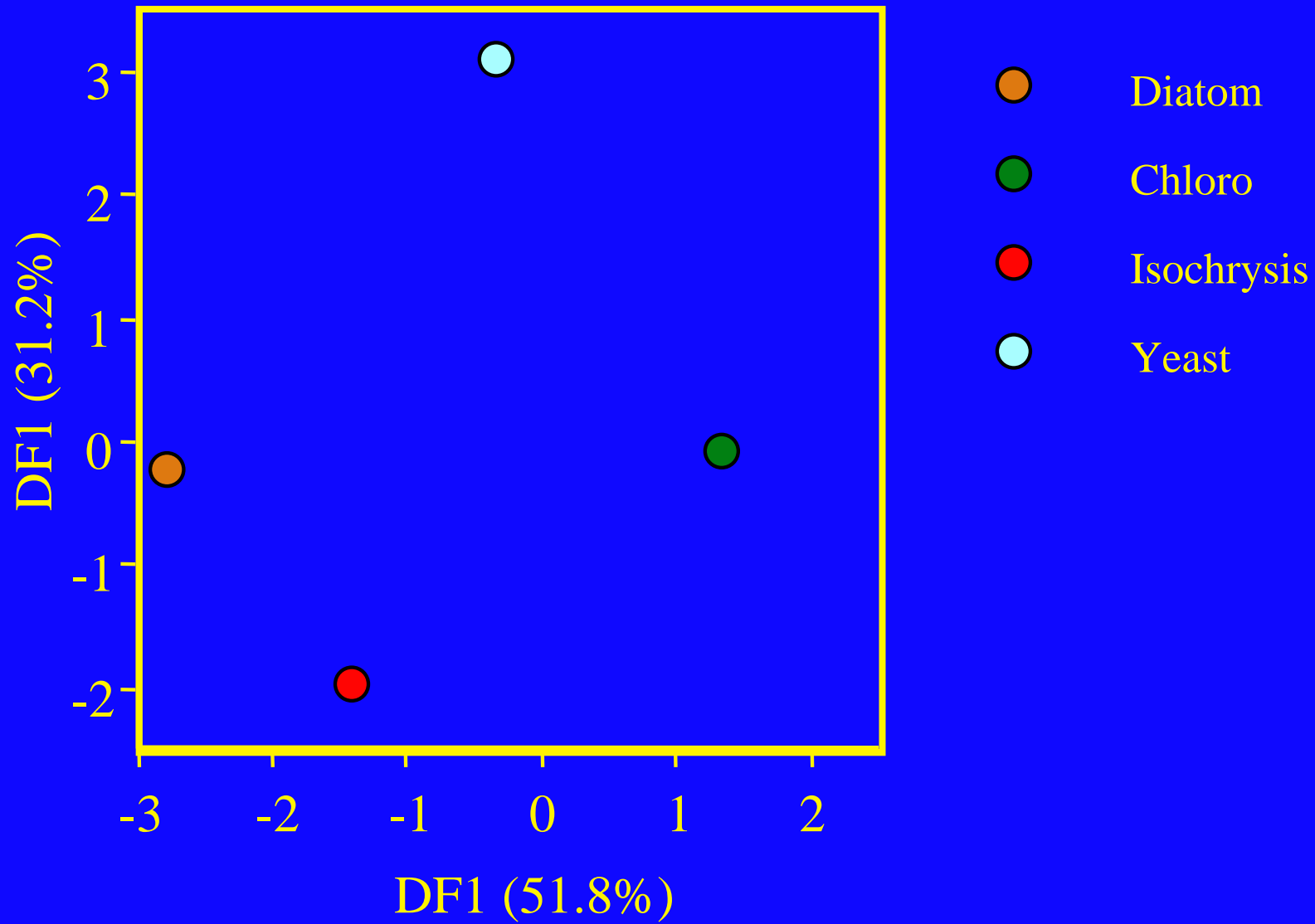
2) Somatic tissues and eggs have significantly more 18:1 $\omega$ 7 and ARA, and significantly less DHA than the diets

3) *Daphnia* eggs had significantly more 18:3 $\omega$ 3 and 18:4 $\omega$ 3, higher  $\omega$ 3: $\omega$ 6 ratios and a higher total fatty acid content than does the somatic tissue

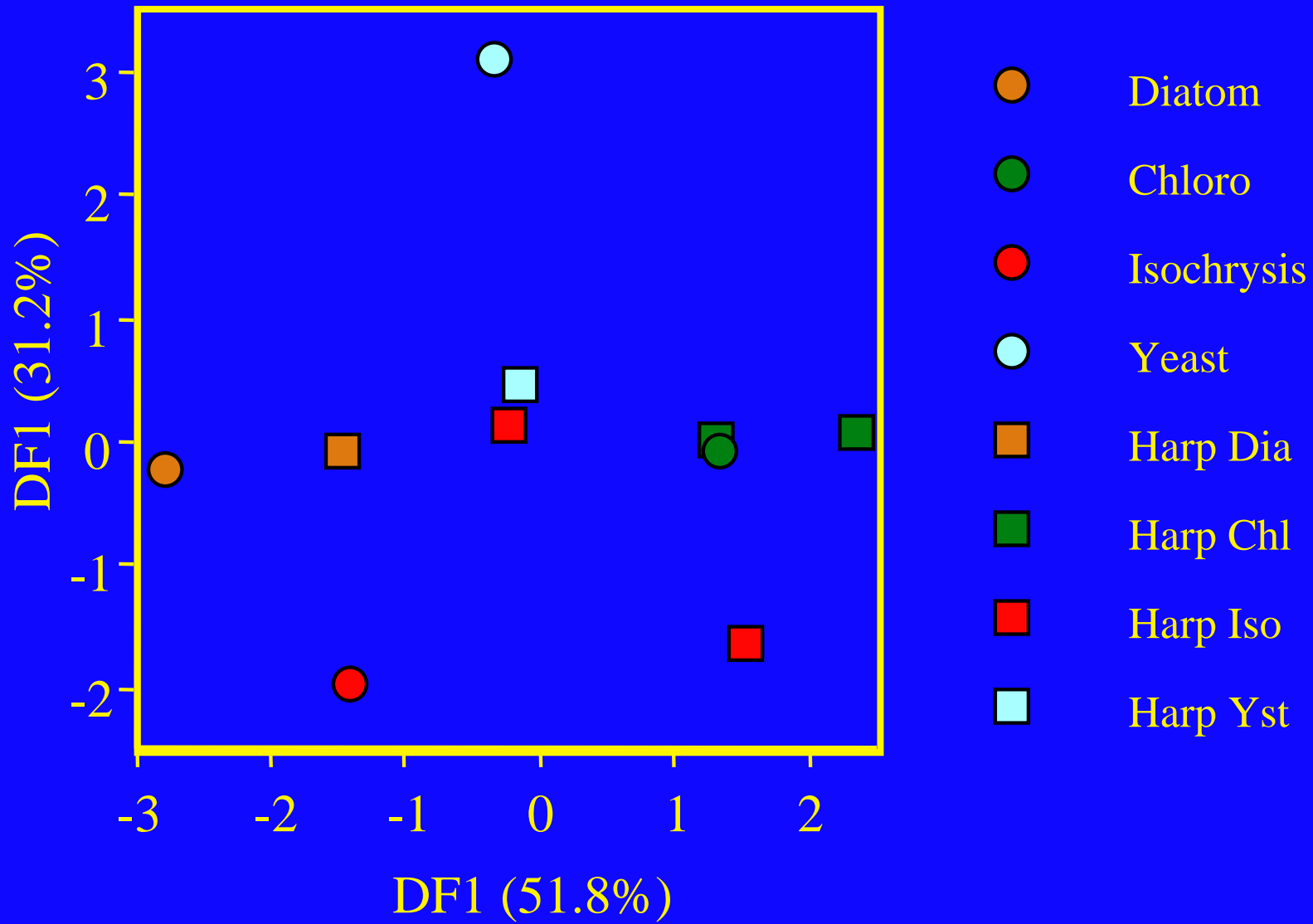




# Nanton and Castell 1998

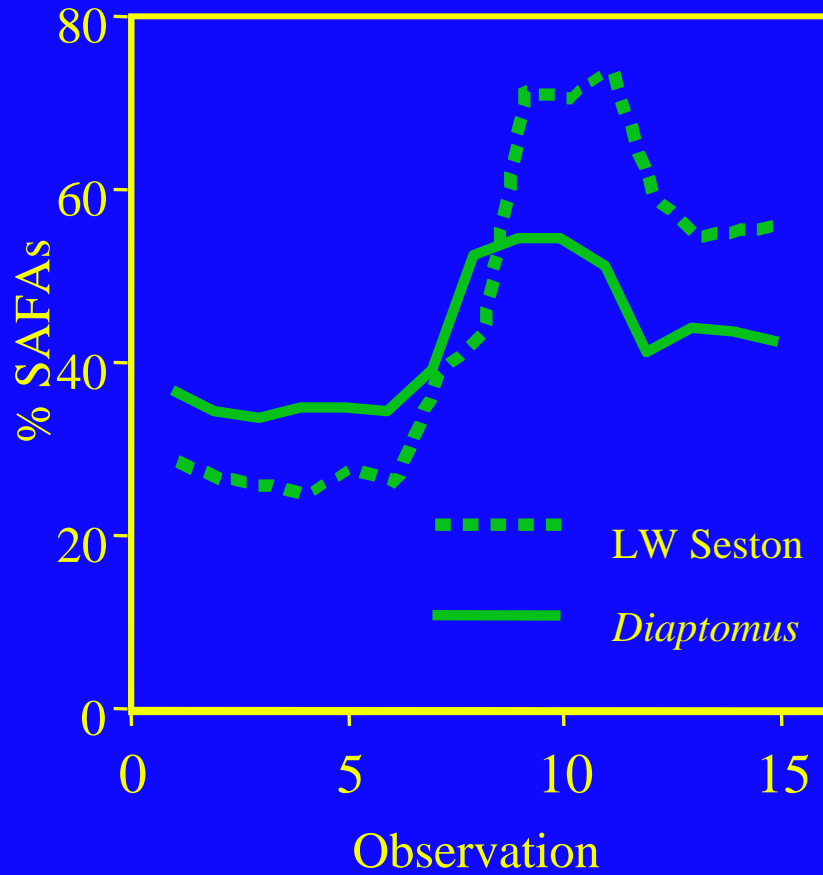


# Nanton and Castell 1998

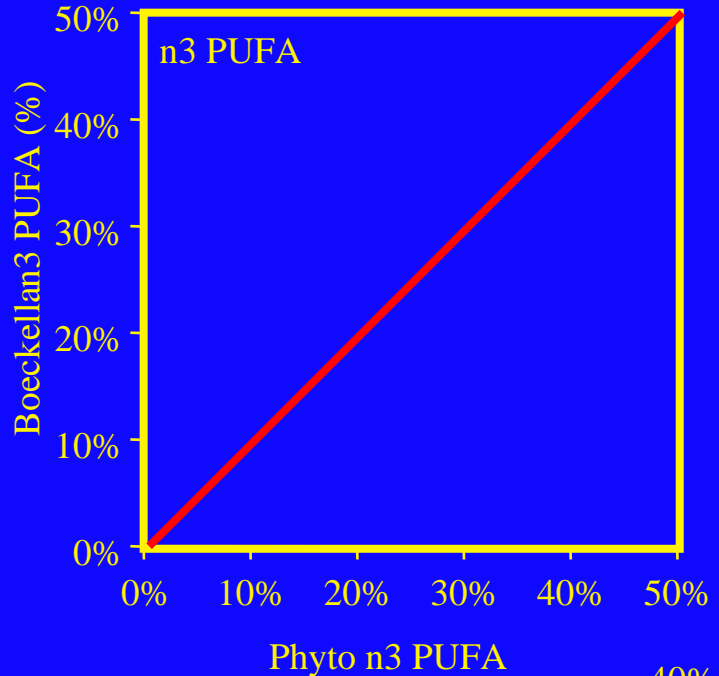


Dietary control of fatty acid  
composition with homeostasis

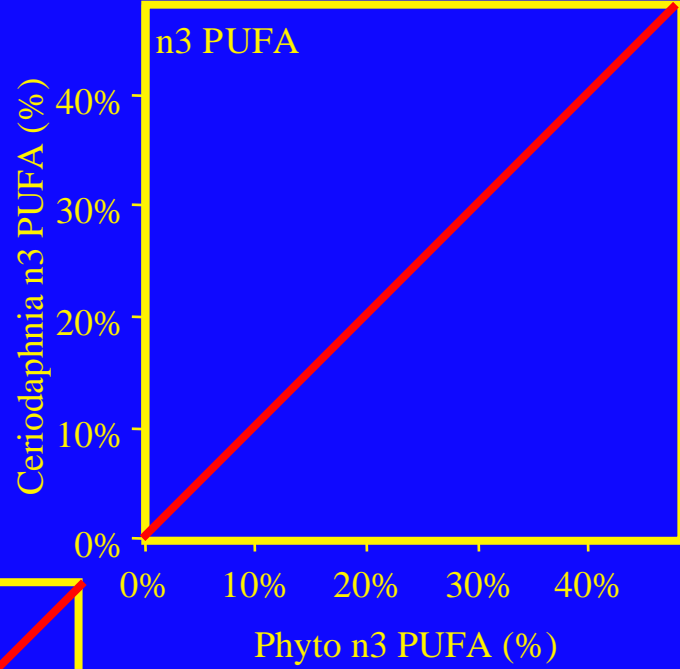
*Diaptomus* Saturated FAs



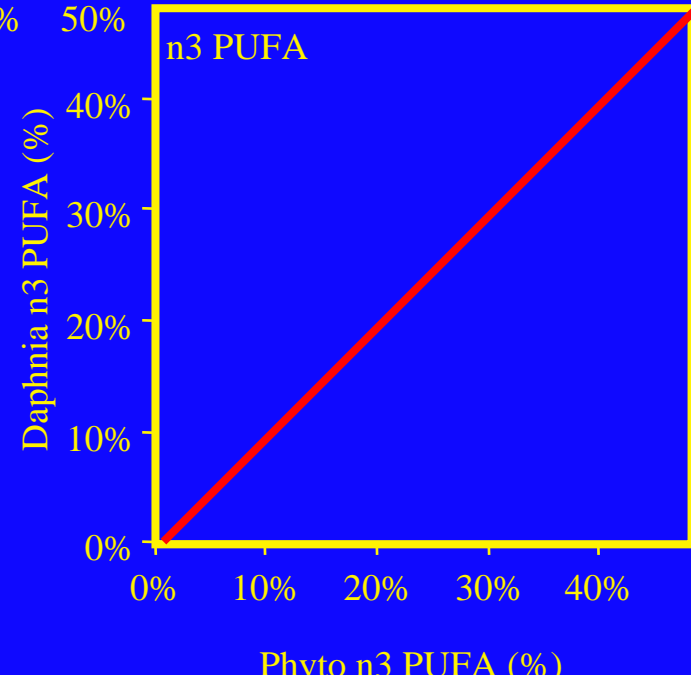
# Boeckella



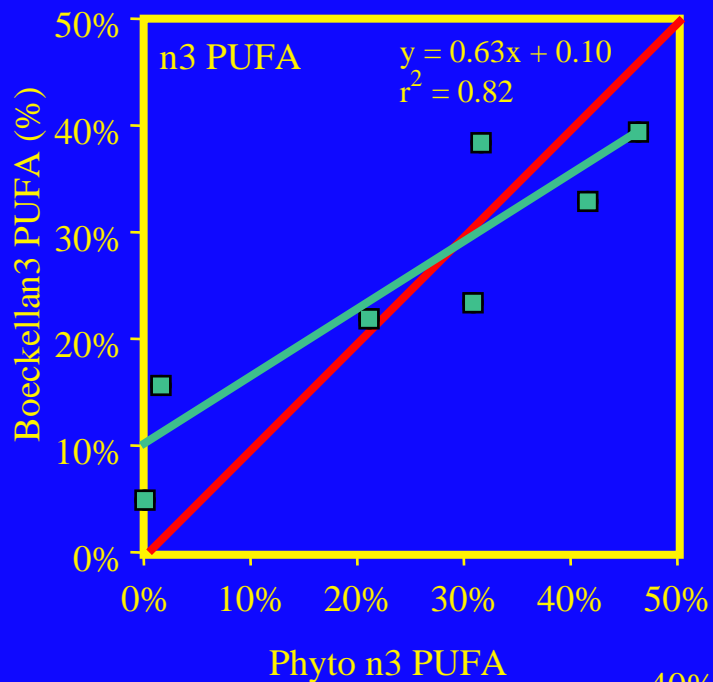
# Ceriodaphnia



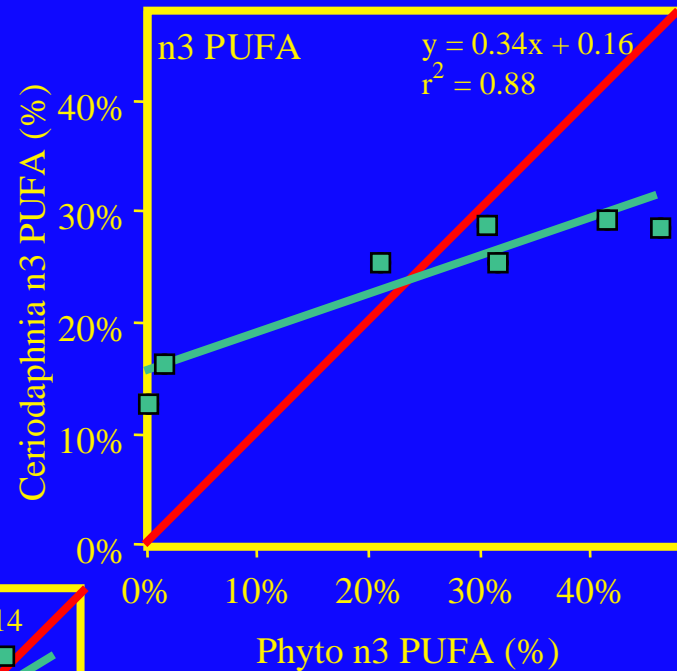
# Daphnia



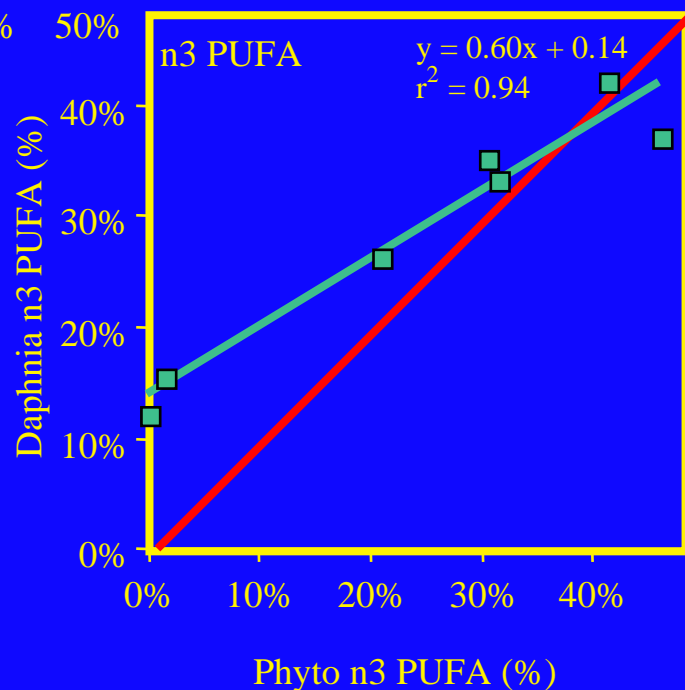
## Boeckella



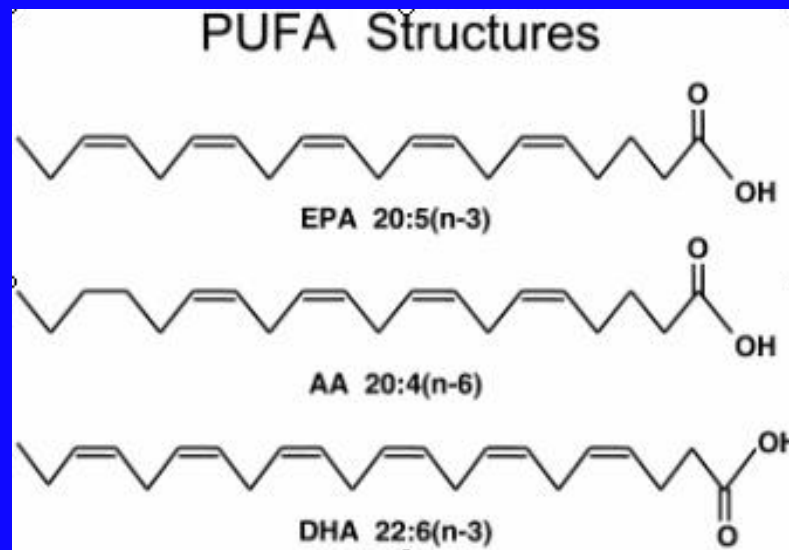
## Ceriodaphnia



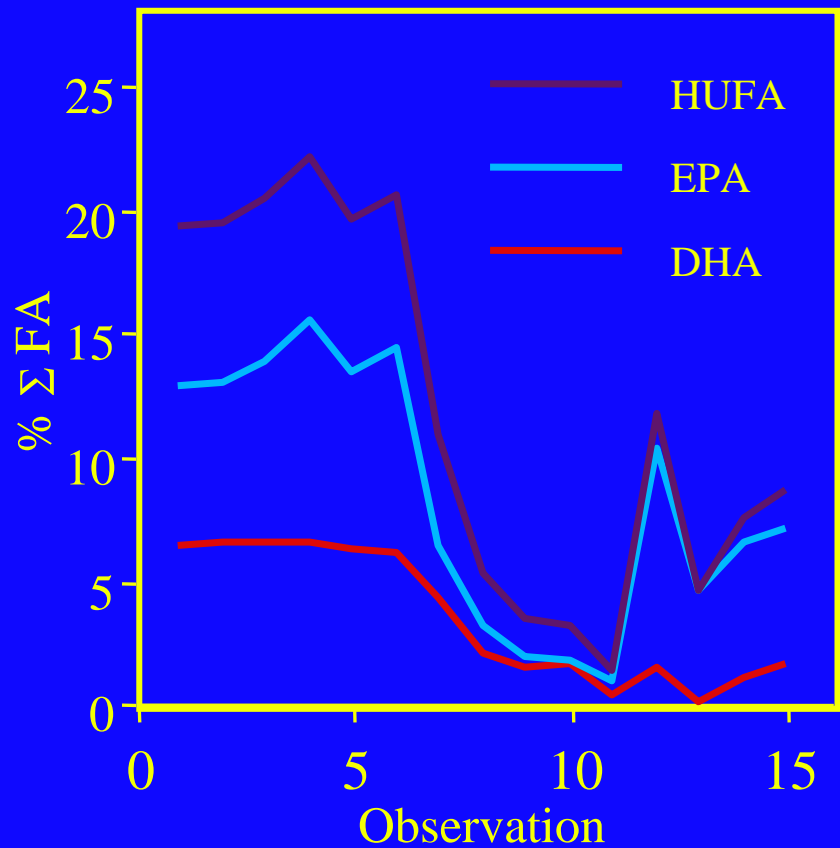
## Daphnia



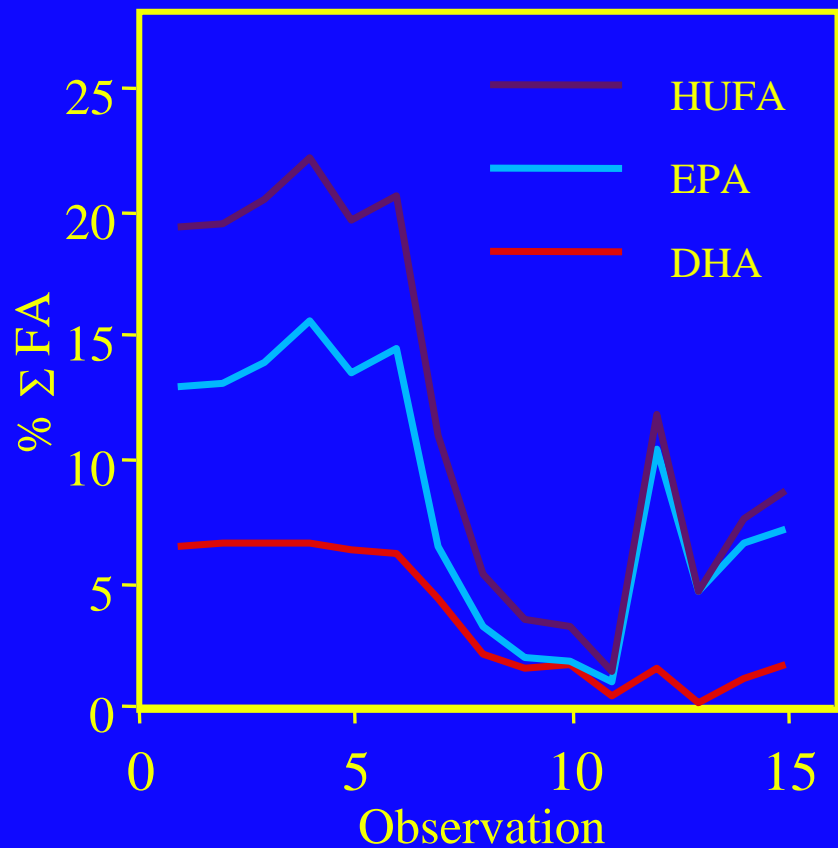
# Biochemical transformations



# Lake Washington Seston

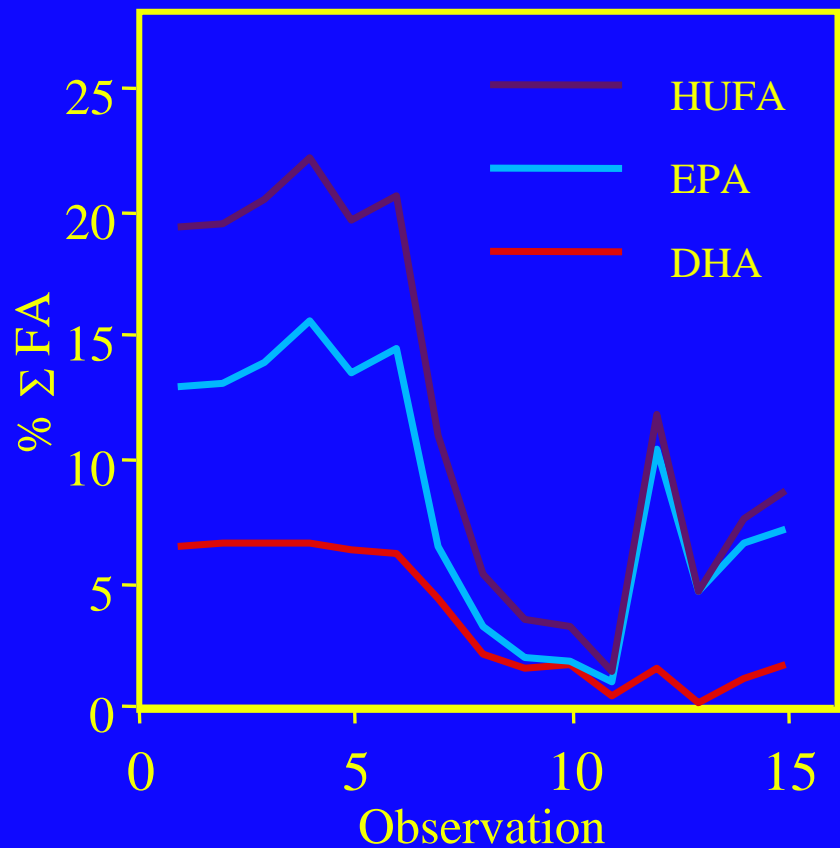


## Lake Washington Seston



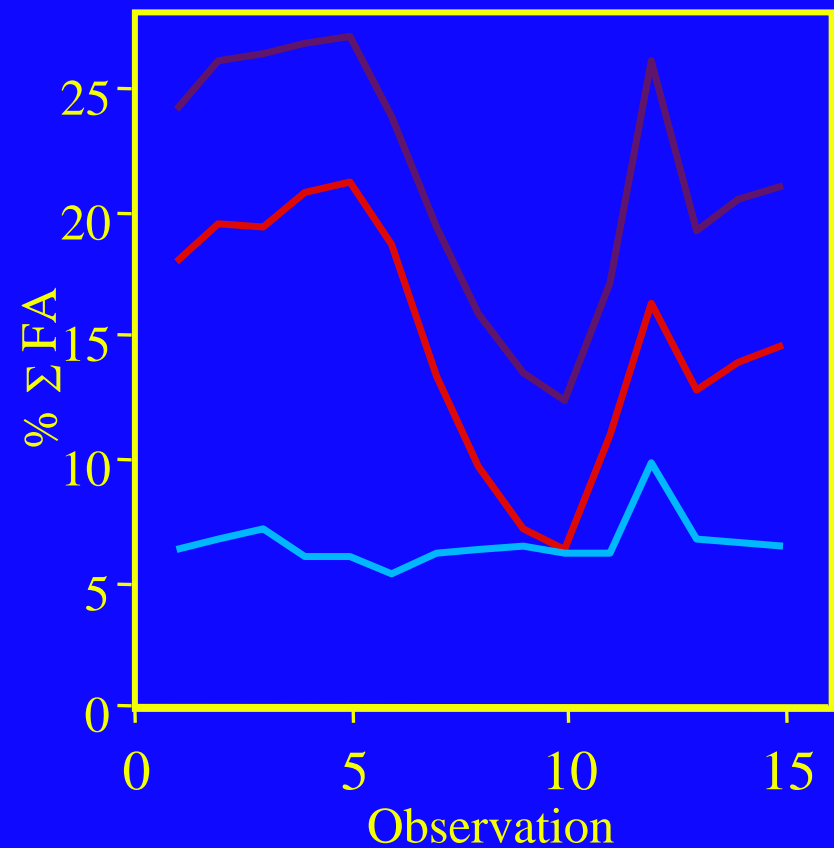
Seston HUFA is dominated by EPA

Lake Washington Seston



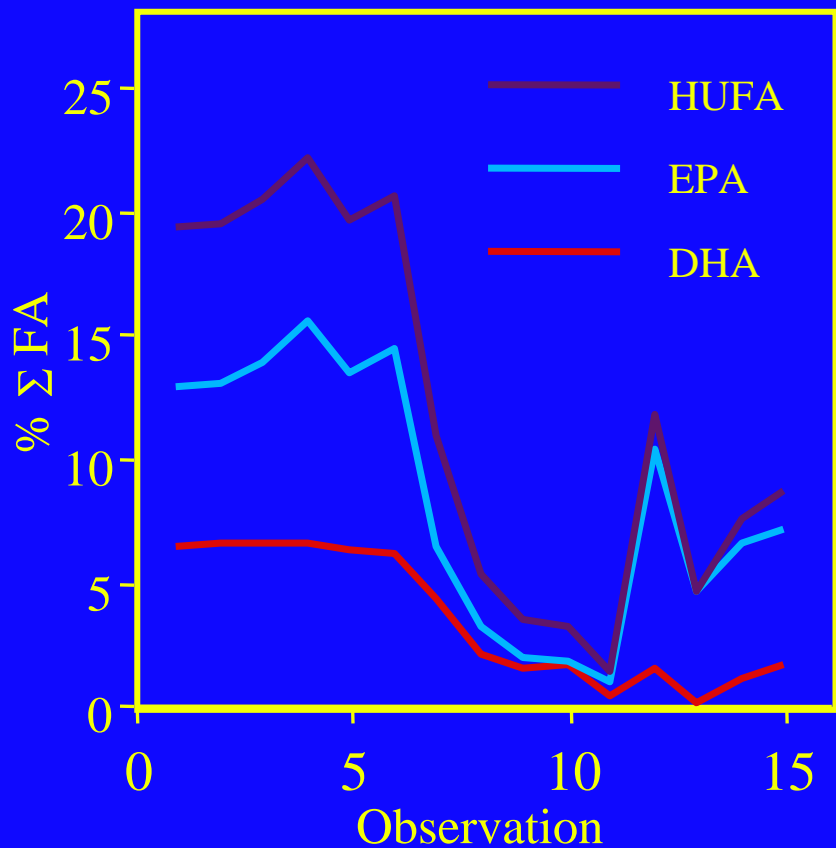
Seston HUFA is dominated by EPA

*Diaptomus*

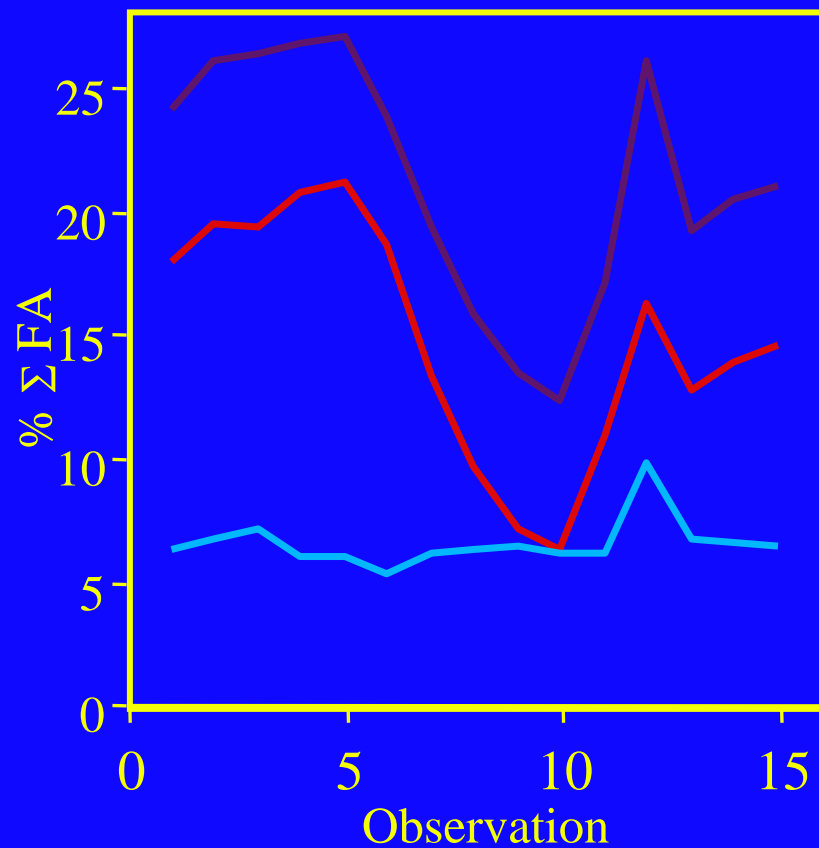


*Diaptomus* HUFA is dominated by DHA

Lake Washington Seston

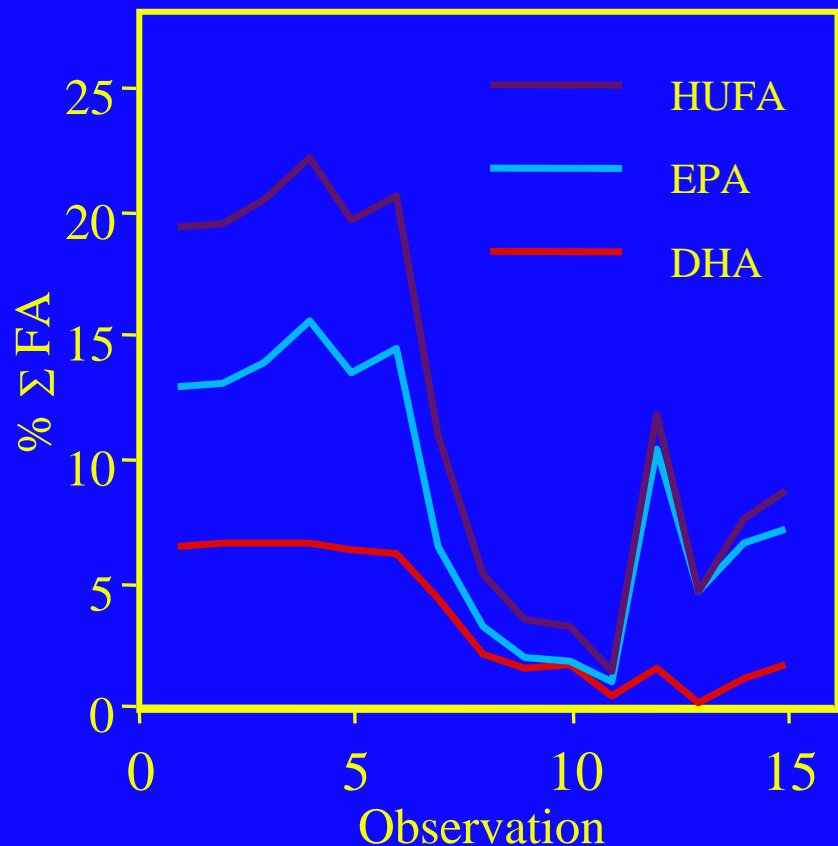


*Diaptomus*

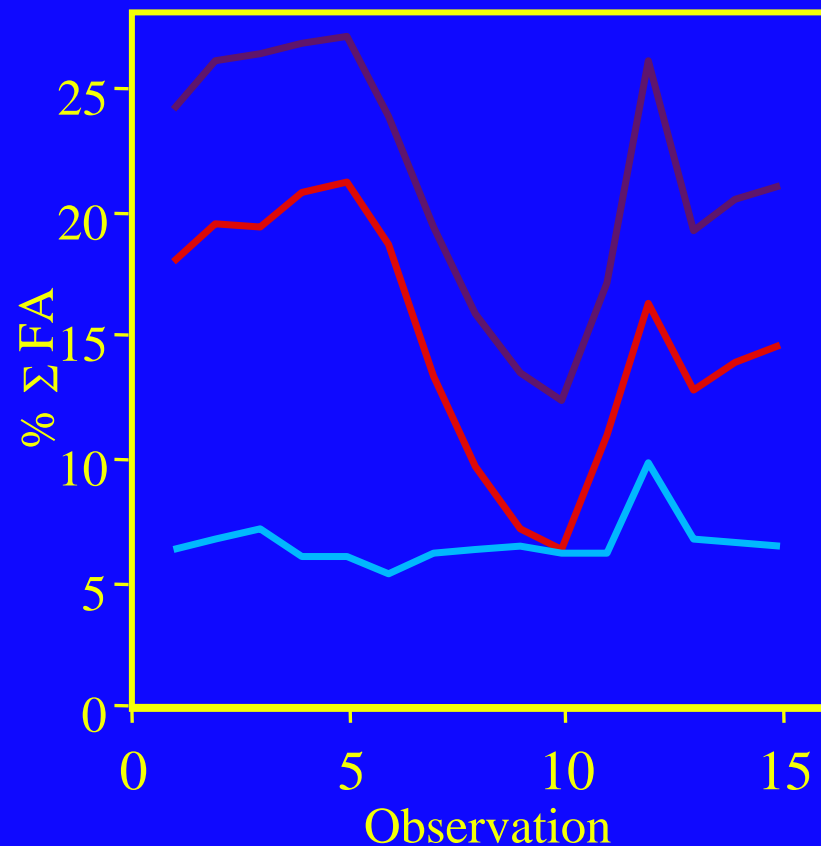


	$r^2$	Seston EPA	Seston DHA	Seston HUFA
Diaptomus EPA		0.01		
Diaptomus DHA			0.62	
Diaptomus HUFA				0.78

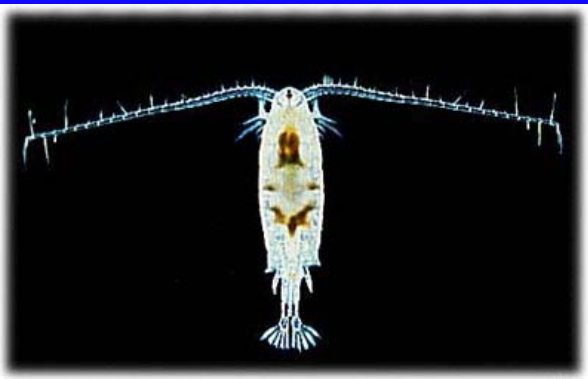
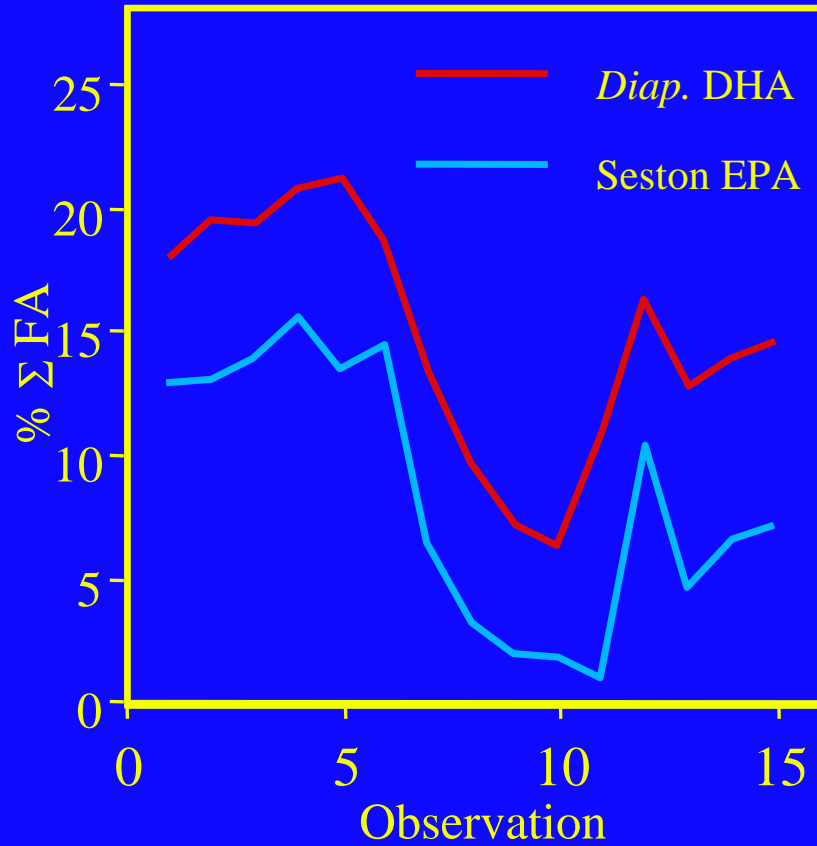
Lake Washington Seston



*Diaptomus*



	$r^2$	Seston EPA	Seston DHA	Seston HUFA
Diaptomus	EPA	0.01	0.06	0.00
Diaptomus	DHA	0.91	0.62	0.86
Diaptomus	HUFA	0.87	0.50	0.78



**Ravet et al., unpub.**

# Cold adaptation and zooplankton fatty acid composition: the homeoviscous response

# Schlechtriem et al. 2006

**TABLE 1**  
Effects of Growth Temperature on FA Composition (% of total identified FAME) of *Daphnia pulex* Fed *Ankistrodesmus falcatus*

	<i>A. falcatus</i>	DA22		DA11	
		Mean	SE	Mean	SE
11:0	nd	nd		0.3	0.2
12:0	0.6	0.2	0.0 <sup>a</sup>	1.0	0.2 <sup>a</sup>
14:0	0.3	1.4	0.1 <sup>a</sup>	1.7	0.1 <sup>a</sup>
15:0	0.1	0.8	0.0 <sup>a</sup>	0.5	0.0 <sup>b</sup>
16:0	20.4	18.6	0.5 <sup>a</sup>	19.1	0.6 <sup>a</sup>
16:1n-7	1.0	3.3	0.1 <sup>b</sup>	13.0	0.4 <sup>a</sup>
17:0	0.1	0.9	0.0 <sup>a</sup>	1.1	0.0 <sup>a</sup>
18:0	0.5	8.1	0.5 <sup>a</sup>	6.2	1.7 <sup>a</sup>
18:1n-9t	nd	0.1	0.0 <sup>a</sup>	0.1	0.0 <sup>a</sup>
18:1n-9c	11.6	19.2	0.5 <sup>b</sup>	23.2	0.5 <sup>a</sup>
18:2n-6c	15.9	14.3	0.3 <sup>a</sup>	6.8	1.2 <sup>b</sup>
20:0	nd	0.3	0.1	nd	
18:3n-6	1.4	1.2	0.0 <sup>a</sup>	0.7	0.0 <sup>b</sup>
20:1n-9	nd	0.1	0.0	nd	
18:3n-3	44.3	22.4	0.3 <sup>a</sup>	11.3	0.2 <sup>b</sup>
21:0	nd	0.1	0.0	nd	
20:2	nd	1.8	0.2	nd	
22:0	2.4	0.5	0.1	nd	
20:3n-6	nd	0.1	0.0 <sup>a</sup>	0.1	0.0 <sup>a</sup>
22:1n-9	nd	0.3	0.0	nd	
20:3n-3	nd	0.2	0.0	nd	
20:4n-6	nd	2.7	0.3 <sup>a</sup>	2.1	0.1 <sup>a</sup>
24:0	0.9	0.2	0.0	nd	
20:5n-3	nd	3.1	0.2 <sup>a</sup>	12.7	0.4 <sup>b</sup>
24:1n-9	0.2	0.3	0.3	nd	
Σn-3	44.3	25.6	0.3 <sup>a</sup>	24.0	0.6 <sup>a</sup>
Σn-6	17.4	18.2	0.5 <sup>a</sup>	9.7	1.3 <sup>b</sup>
ΣSAFA	25.4	31.0	1.0 <sup>a</sup>	30.0	2.2 <sup>a</sup>
ΣMUFA	12.9	23.3	0.4 <sup>b</sup>	36.3	0.4 <sup>a</sup>
ΣPUFA	61.7	45.7	0.5 <sup>a</sup>	33.7	1.8 <sup>b</sup>

<sup>a</sup>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; SAFA, saturated FA; MUFA, monounsaturated FA.



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1) When cold adapted 18:2ω6 and 18:3ω3 go down,

2) 16:1ω7 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

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20:3n-6	nd	0.1	0.0 <sup>a</sup>	0.1	0.0 <sup>a</sup>
22:1n-9	nd	0.3	0.0	nd	
20:3n-3	nd	0.2	0.0	nd	
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20:5n-3	nd	3.1	0.2 <sup>a</sup>	12.7	0.4 <sup>b</sup>
24:1n-9	0.2	0.3	0.3	nd	
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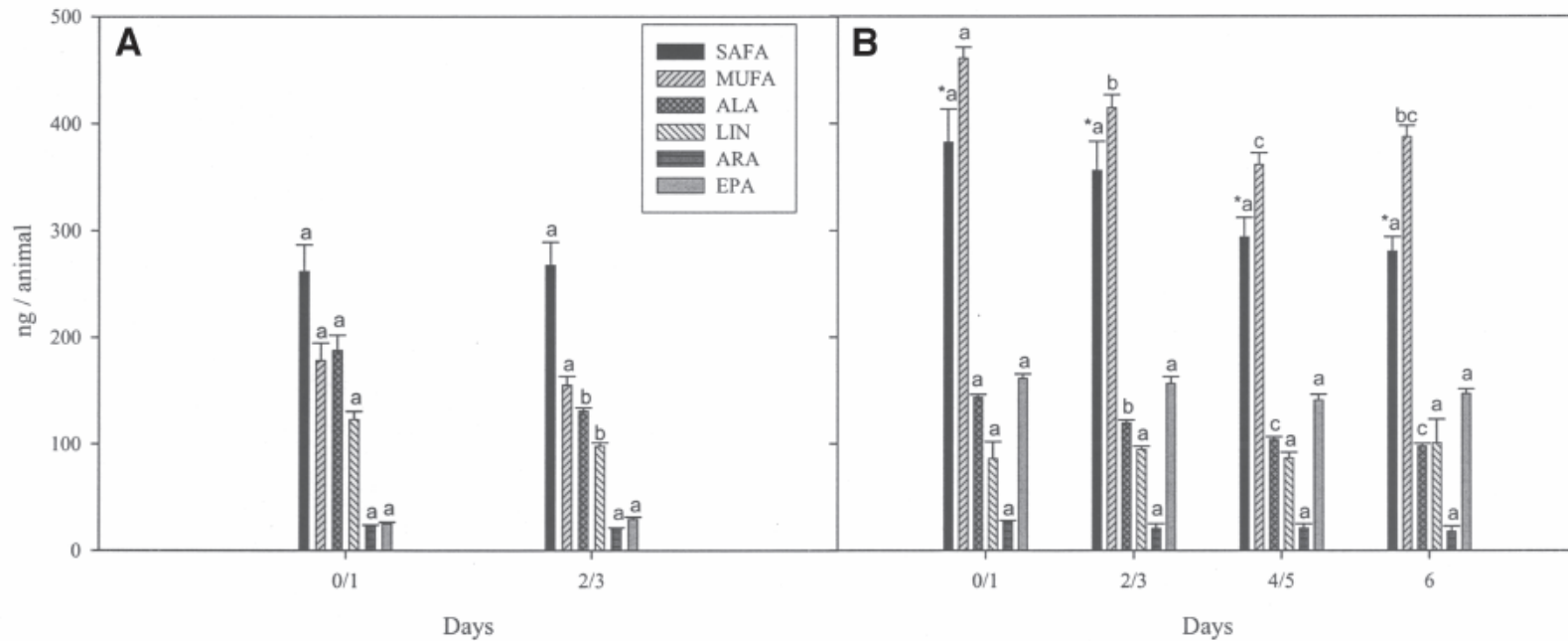
2) 16:1ω7 and EPA go up

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4) Evidence of 18:3ω3 conversion to EPA

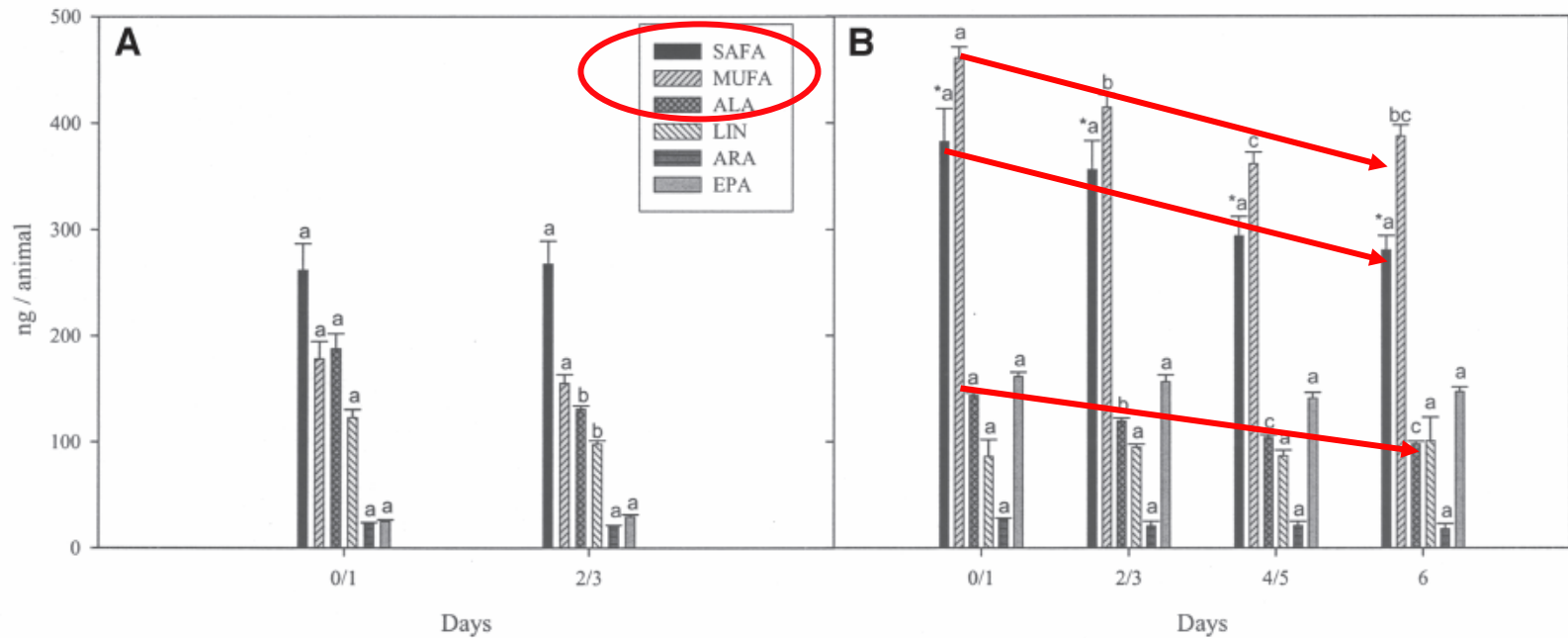
# Starvation impacts on fatty acid composition

# Schlechtriem et al. 2006



**FIG. 1.** Temporal trajectories of FA in fasting *Daphnia pulex* kept at (A) 22 and (B) 11°C. Results are means  $\pm$  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,  $\alpha$ -linolenic acid; ARA, arachidonic acid; LIN, linoleic acid; MUFA, monounsaturated FA; SAFA, saturated FA.

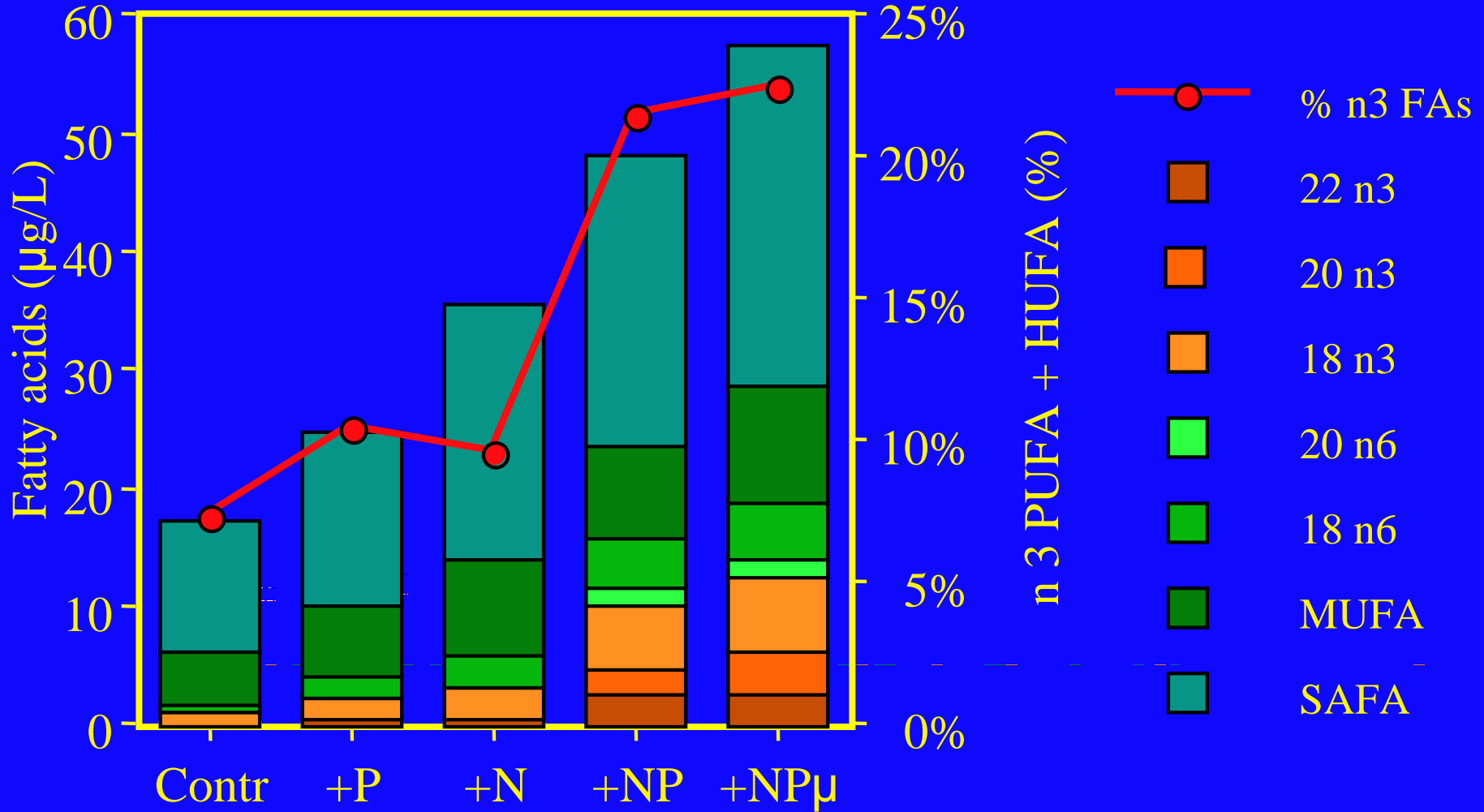
# Schlechtriem et al. 2006



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- 4) When starved SAFA, MUFA and 18:3 $\omega$ 3 are metabolized, &
- 5) 18:2 $\omega$ 6, ARA and EPA are conserved

# Okanagan Lake, July 2003



# 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. 18:3 $\omega$ 3) to more physiologically important HUFAs like EPA and DHA





