Stable Isotopes, Fatty Acids and Compound Specific Stable Isotopes of Fatty Acids as biomarkers

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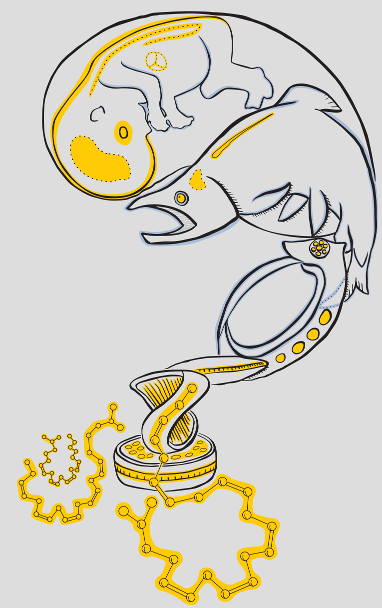
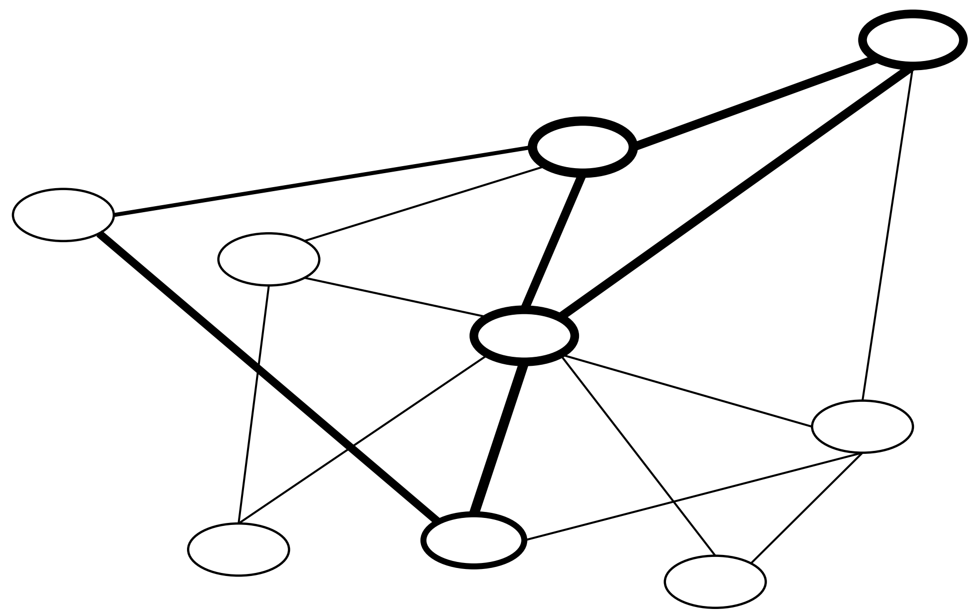


Brisbane, Dec 10, 2018

Questions in food web ecology

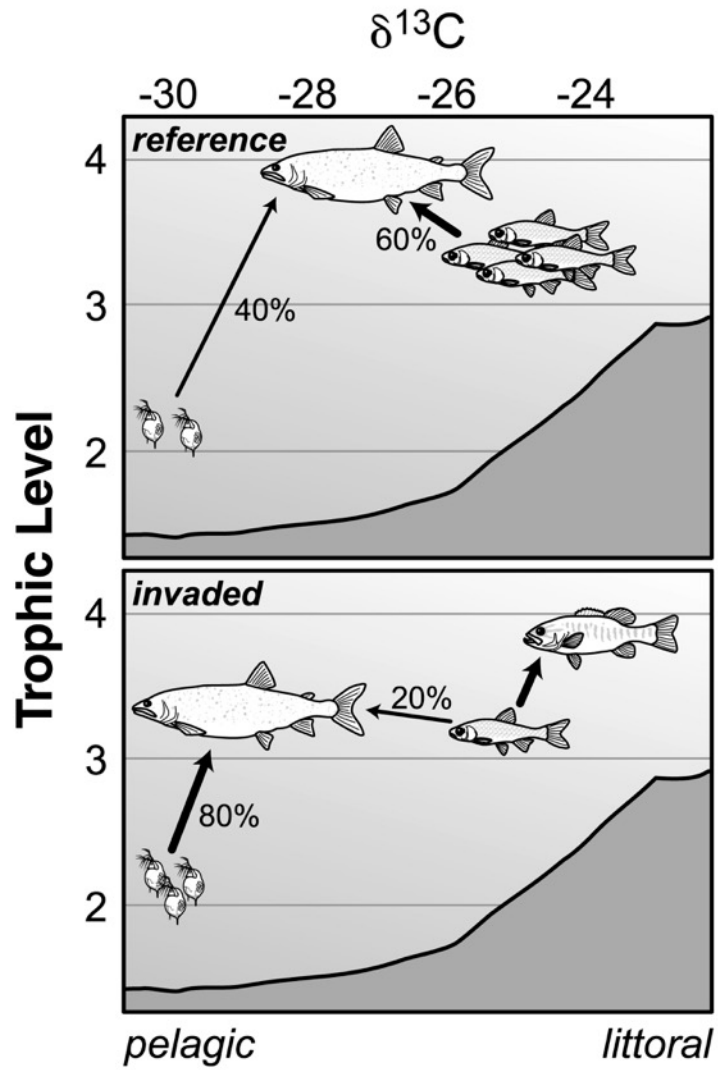
- Why is it ecologically important to understand **diet – consumer interactions**?
- How important are **spatial/temporal differences** in food web ecology?
- How important is **biodiversity** in conveying and retaining dietary nutrients?

... and how do **lipids and stable isotopes** fit into such questions?



‘The fish case’:
Why is it ecologically important to understand what fish are feeding on?

- **Fish biomass** (individuals)
- **Fish abundance** (community)
- **Food web connectedness →** provision of dietary energy
  - Somatic growth
  - Reproduction
  - Survival



Vander Zanden et al. (1999)

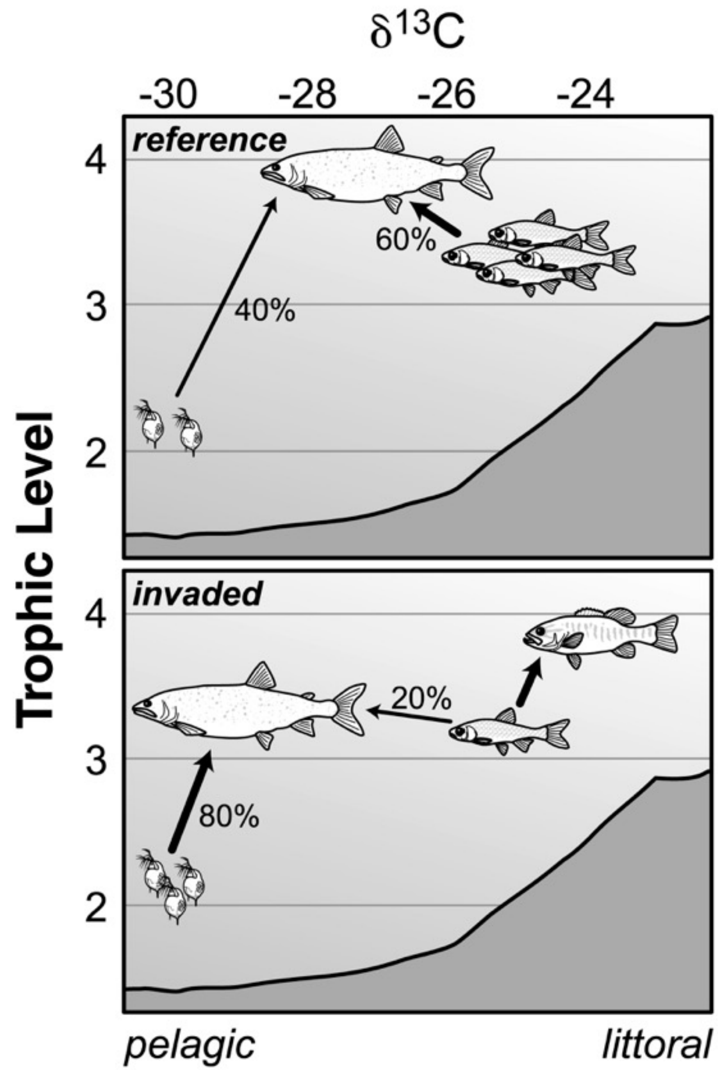
Question:
Why is it ecologically important to understand what fish are feeding on?

- $\delta^{13}C$ values – 'sources'
- $\delta^{15}N$ values – 'trophic position'

≠ **Dietary quality or quantity**

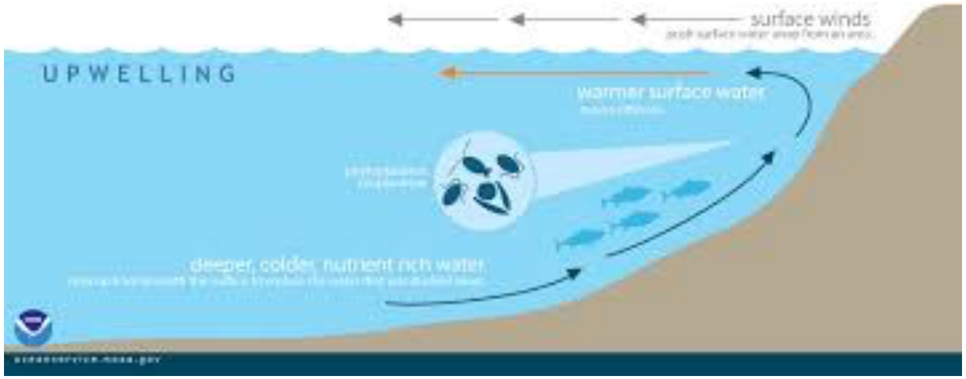
→ **Dietary energy required!**

→ Lipids, ...



Vander Zanden et al. (1999)

Different diet sources – different dietary quantity and quality



Ocean upwelling

Ocean eutrophication

Polar ecosystems

Different diet sources – different dietary quantity and quality

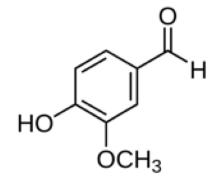
Water holes

Murray-Darling Basin

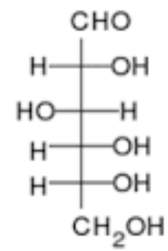
Continuum ↓

Different trophic status – different dietary energy (kcal g^{-1})

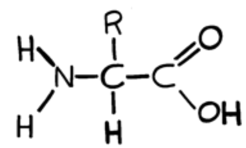
- **Fiber** (undegradable) **1.9 kcal g^{-1}**
  - Lignin
  - Cellulose
  - Hemicellulose



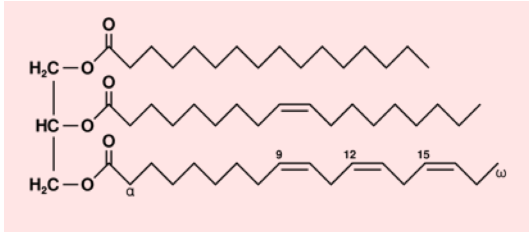
- **Sugars** **4.1 kcal g^{-1}**



- **Proteins** **4.1 kcal g^{-1}**



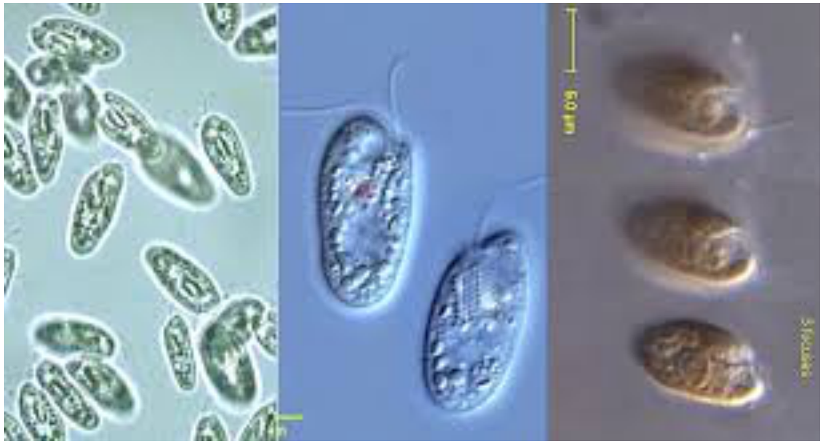
- **Lipids** **9.3 kcal g^{-1}**



Triacylglycerol

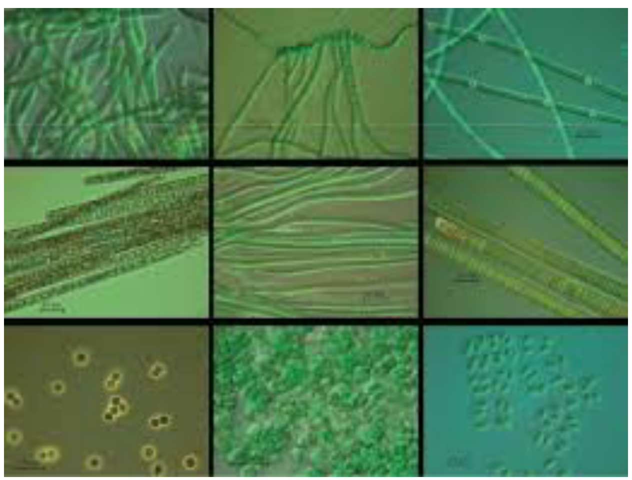
Different trophic status – different dietary quality

Oligotrophic ecosystems



Rich in omega-3 (n-3) PUFA
22:6n-3
20:5n-3
18:4n-3
18:3n-3
Sterols

Eutrophic ecosystems

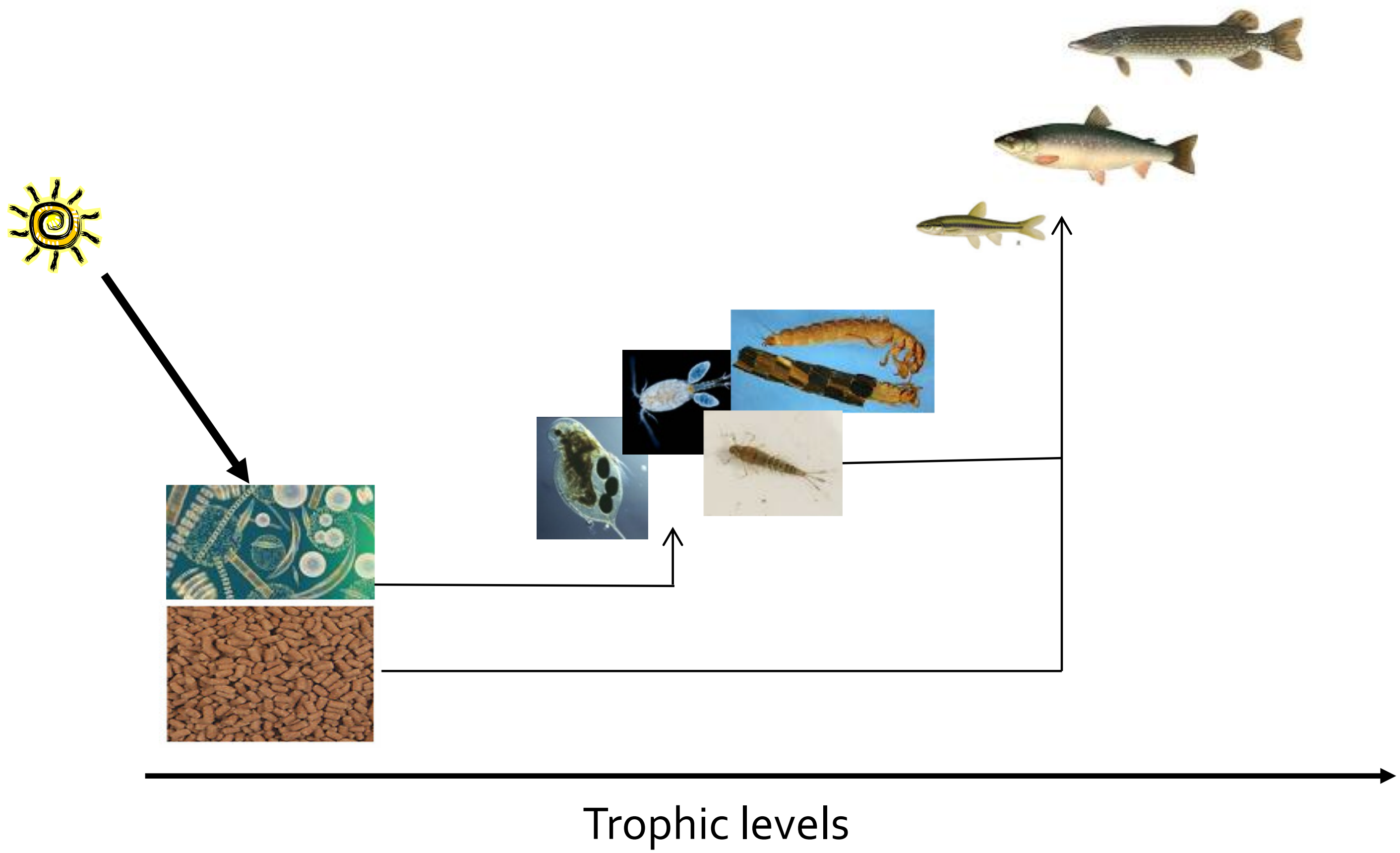


Rich in
- SAFA
- Bacterial FA

Poor in n-3 PUFA
Poor in sterols

Carbon retention in consumers

- How are carbon sources retained in aquatic consumers?
- How much carbon is there in consumers?



Energy retention across food webs – VERY POOR!

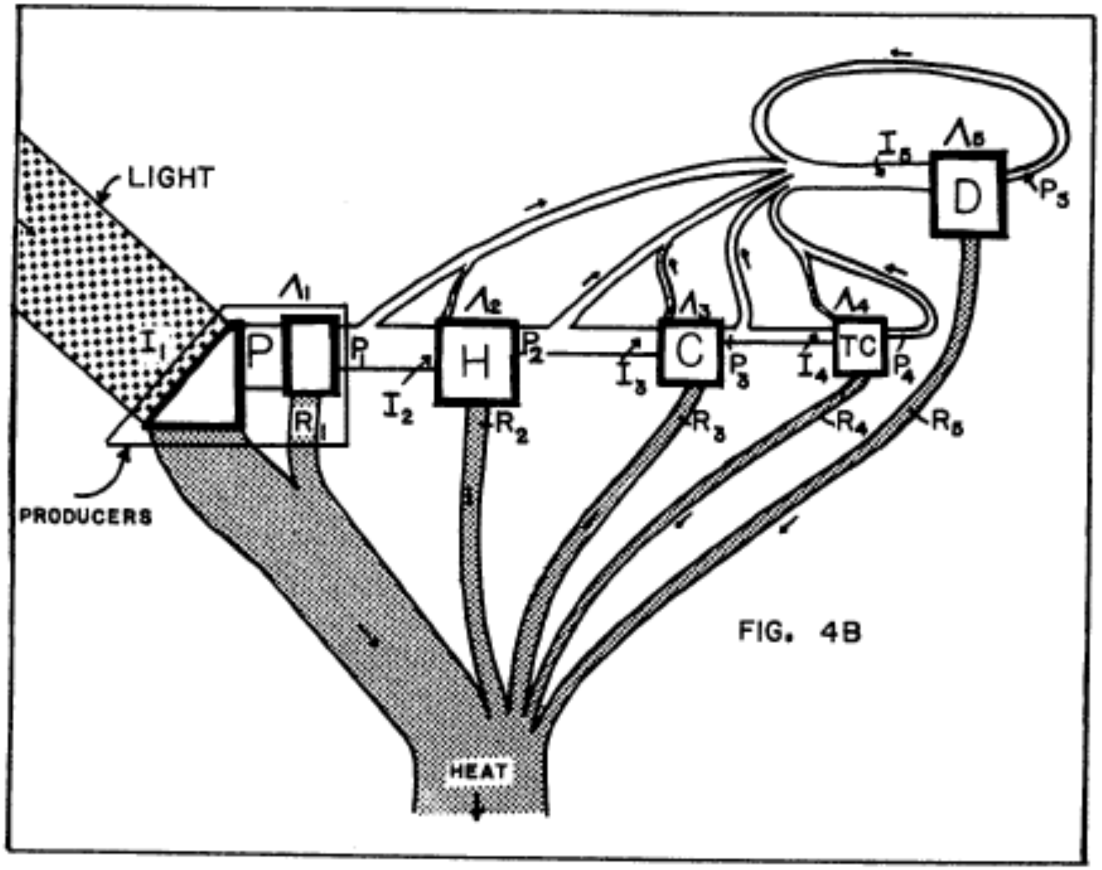


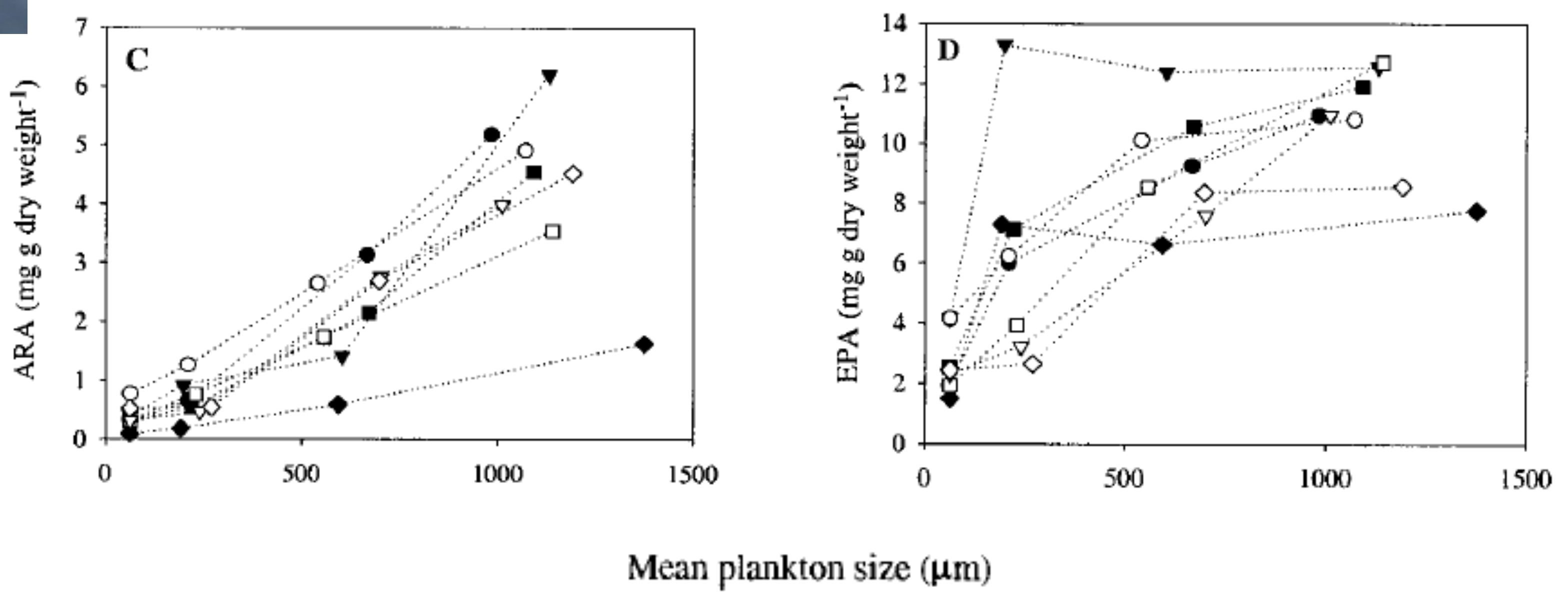
FIG. 4B

5-40 % per trophic level

Odum, H.T. 1956. Efficiencies, size of organisms, and community structure. *Ecology 37, 592-597.*

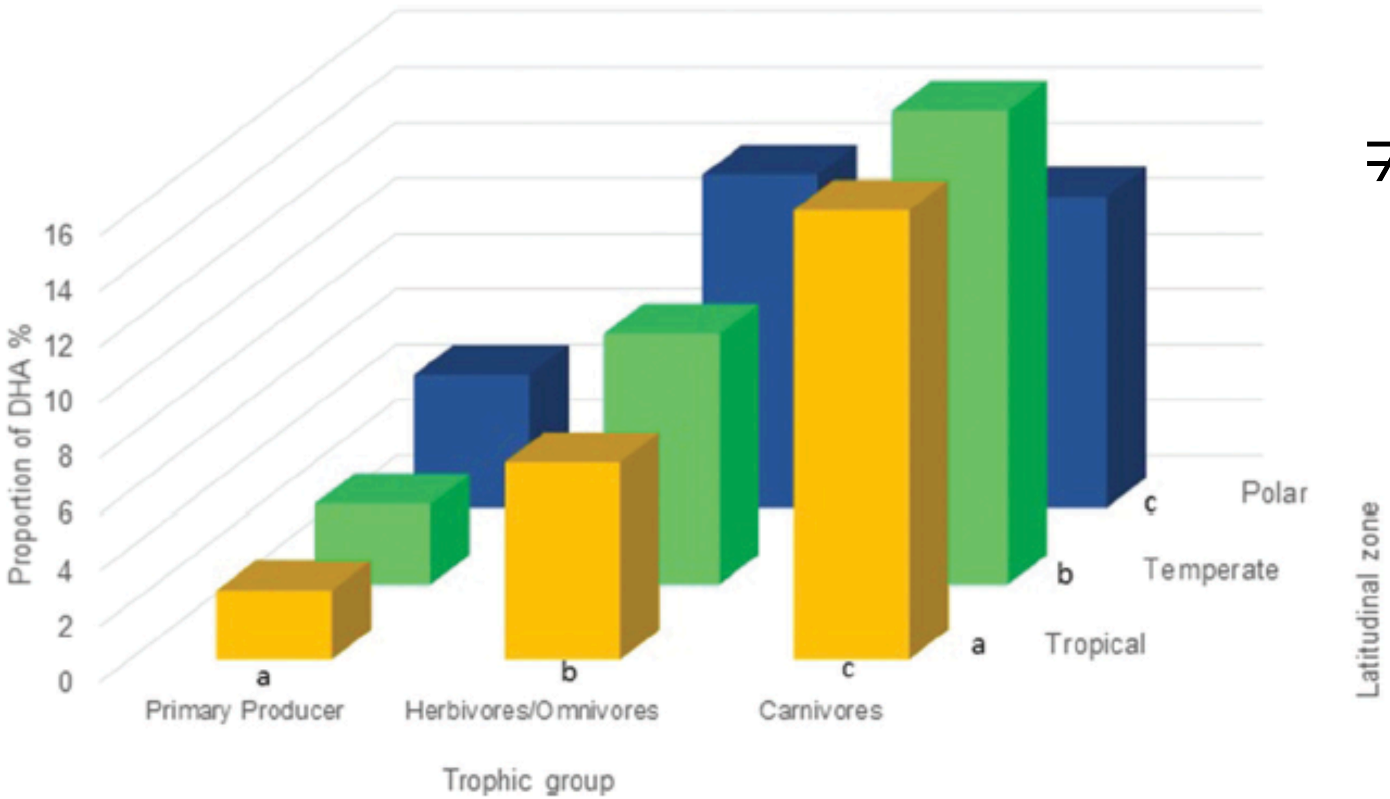
BUT, not all carbon is equal – The case of some PUFA

Carbon bioaccumulation per unit biomass – as **PUFA**



Kainz et al. (2004) L&O 49

DHA (%) in marine organisms across different trophic levels



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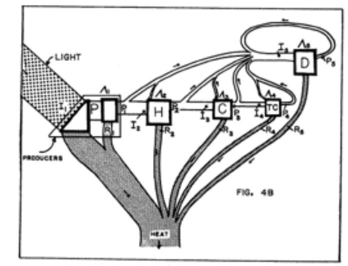
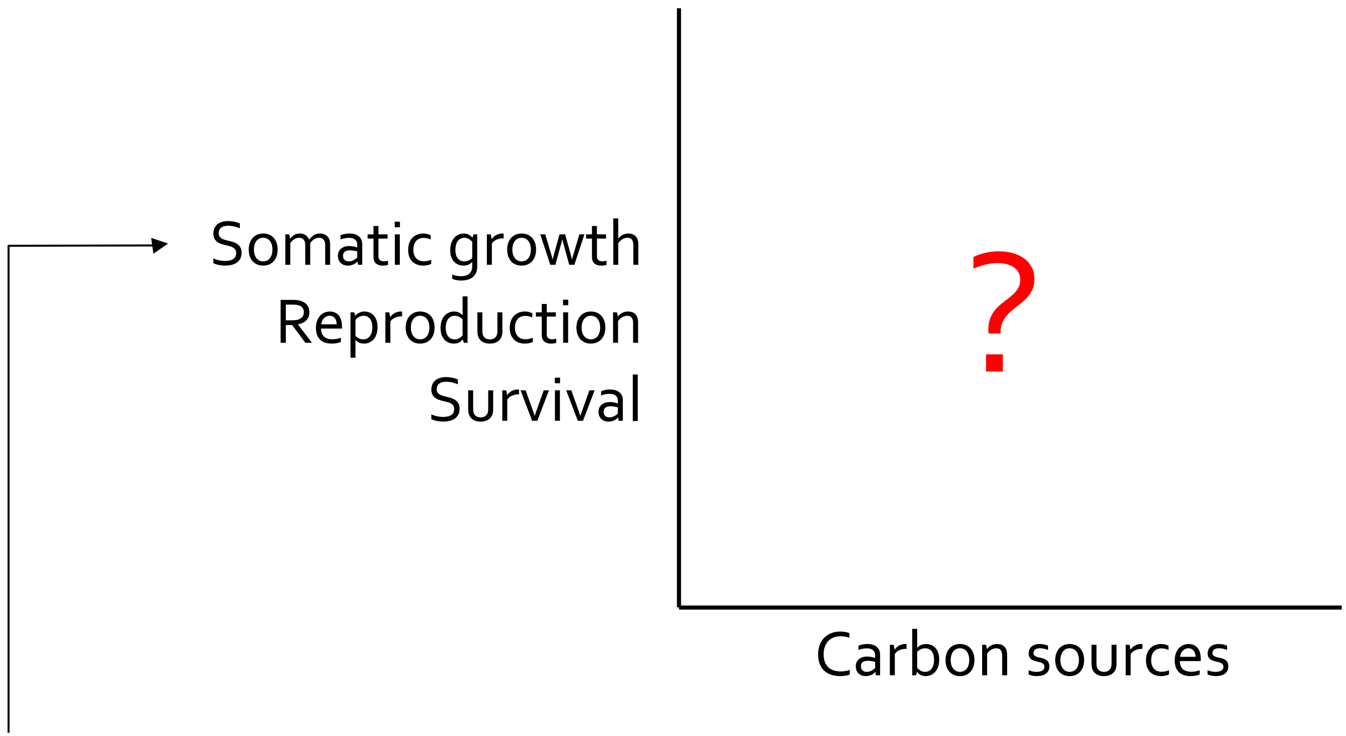


Colombo et al. (2017) Environ. Rev. 25

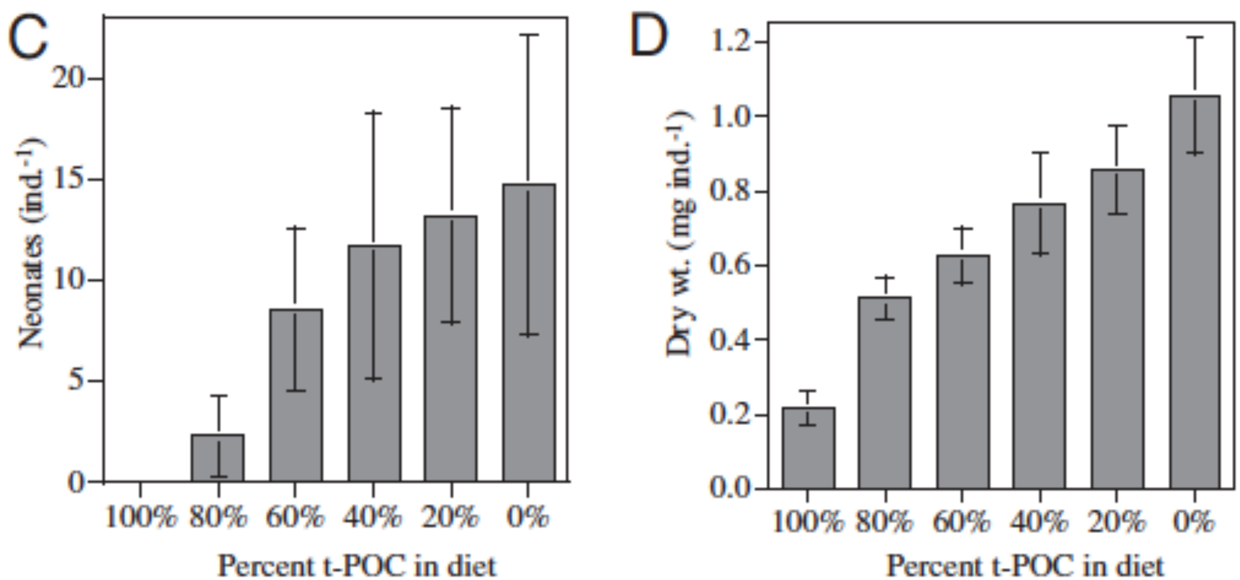
Functional response of different carbon sources



Woods (RNA:DNA)/Marshall research

Functional response of different carbon sources

<u>Terrestrial carbon</u> supports
- <u>very little</u> reproduction in zooplankton (*Daphnia*),
- and <u>barely</u> somatic growth

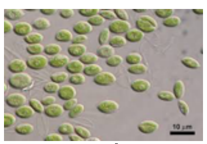


Brett et al. (2009) PNAS 106

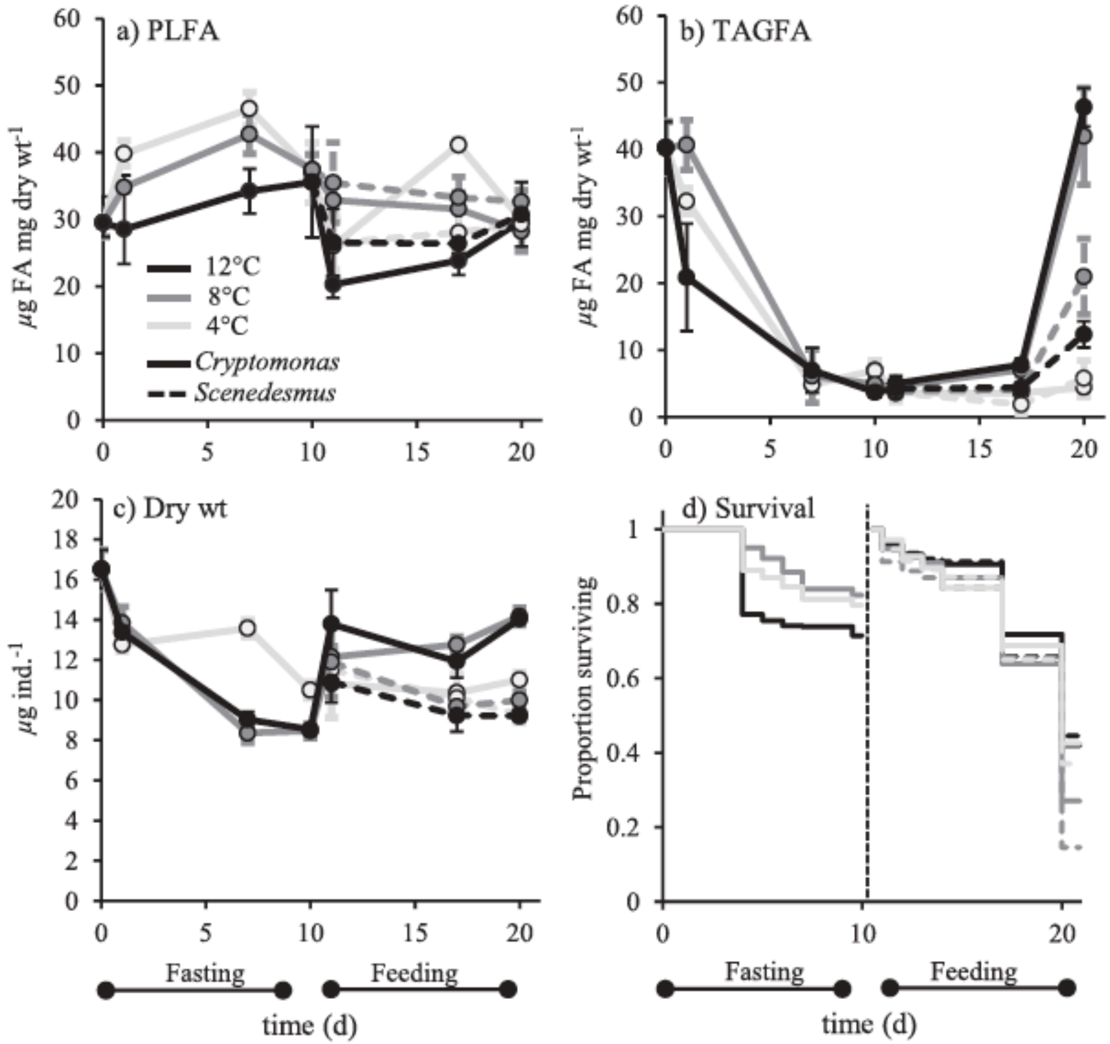
How do copepods respond to different temperature and diets?

Eudiaptomus gracilis

Cryptomonas ozolinii



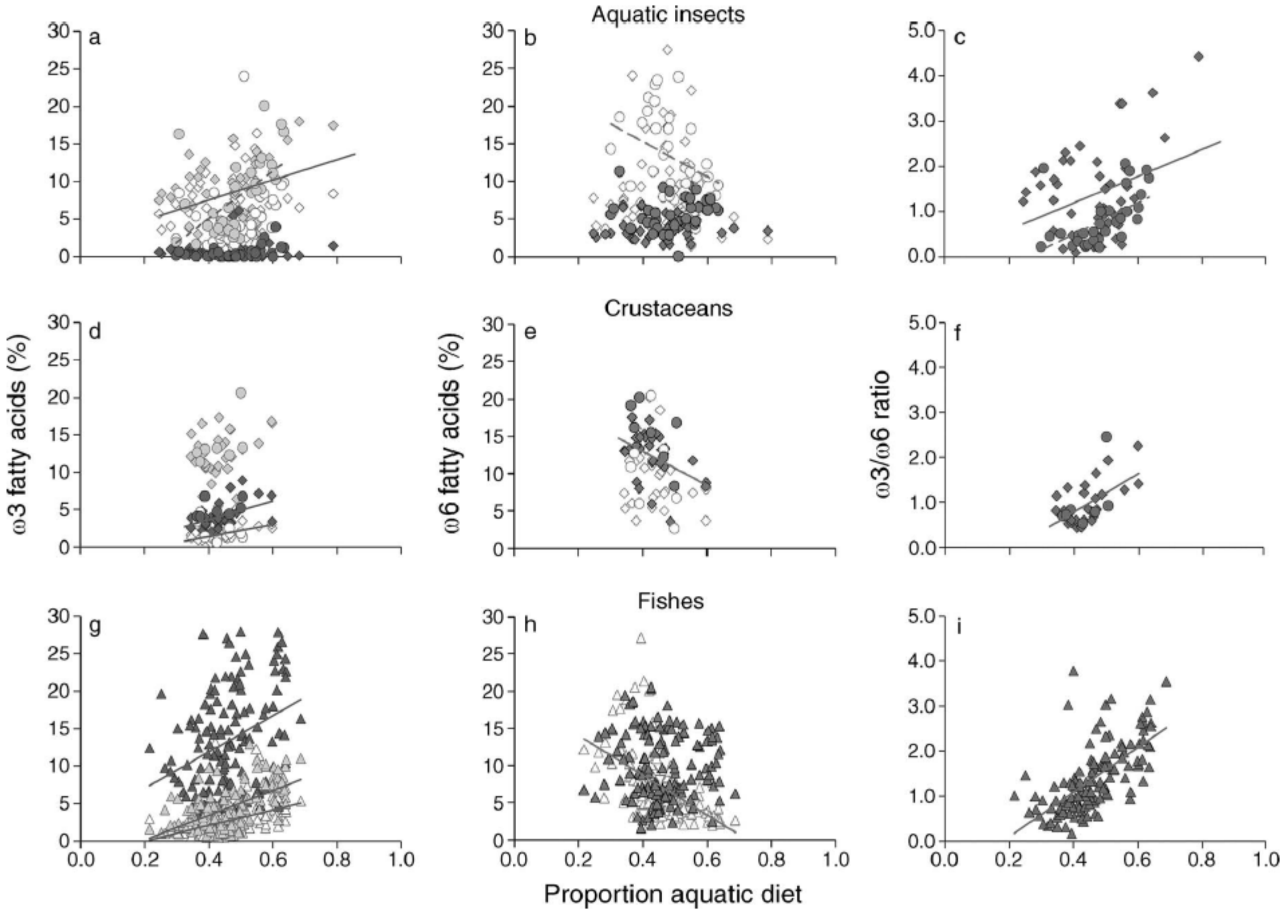
Scenedesmus obliquus



Koussoroplis et al. (2014) L&O 59(3)

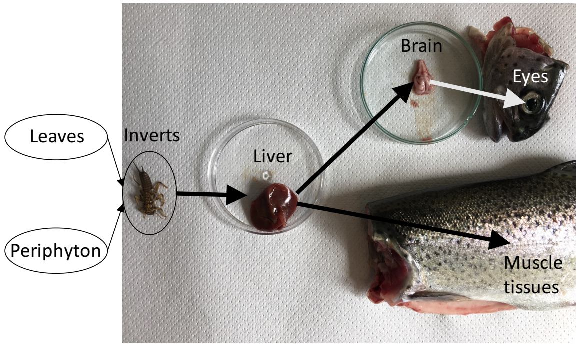
Functional response of different carbon sources

Life in very warm water holes (Australia) –
Omega-3 PUFA preferentially retained in consumers



Jardine et al. (2015) Ecology 96

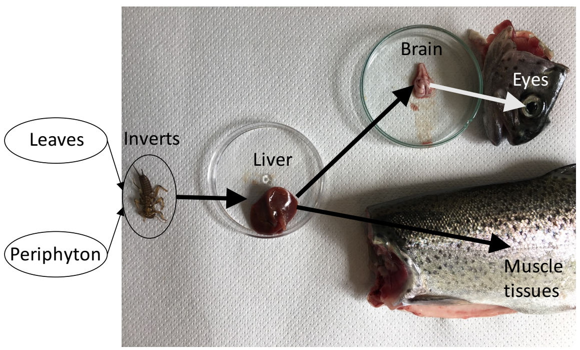
Biomarkers – stable isotopes and fatty acids



Per tissue

- $\delta^{13}C$ values – ‘bulk carbon sources’
- $\delta^{15}N$ values – ‘~ trophic position’

Biomarkers – stable isotopes and fatty acids



Per tissue

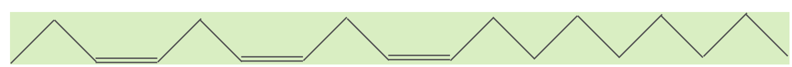
- $\delta^{13}C$ values – 'bulk carbon sources'
- $\delta^{15}N$ values – '~ trophic position'

<u>**Source-specific markers:**</u>

- **Terrestrial sources:**

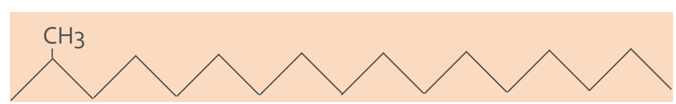
C24:0
Lignoceric acid

- **Algal/plant sources:**

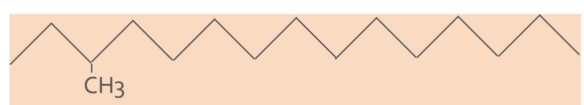


C18:3n-3
Alpha-linoleic acid

- **Bacterial sources**

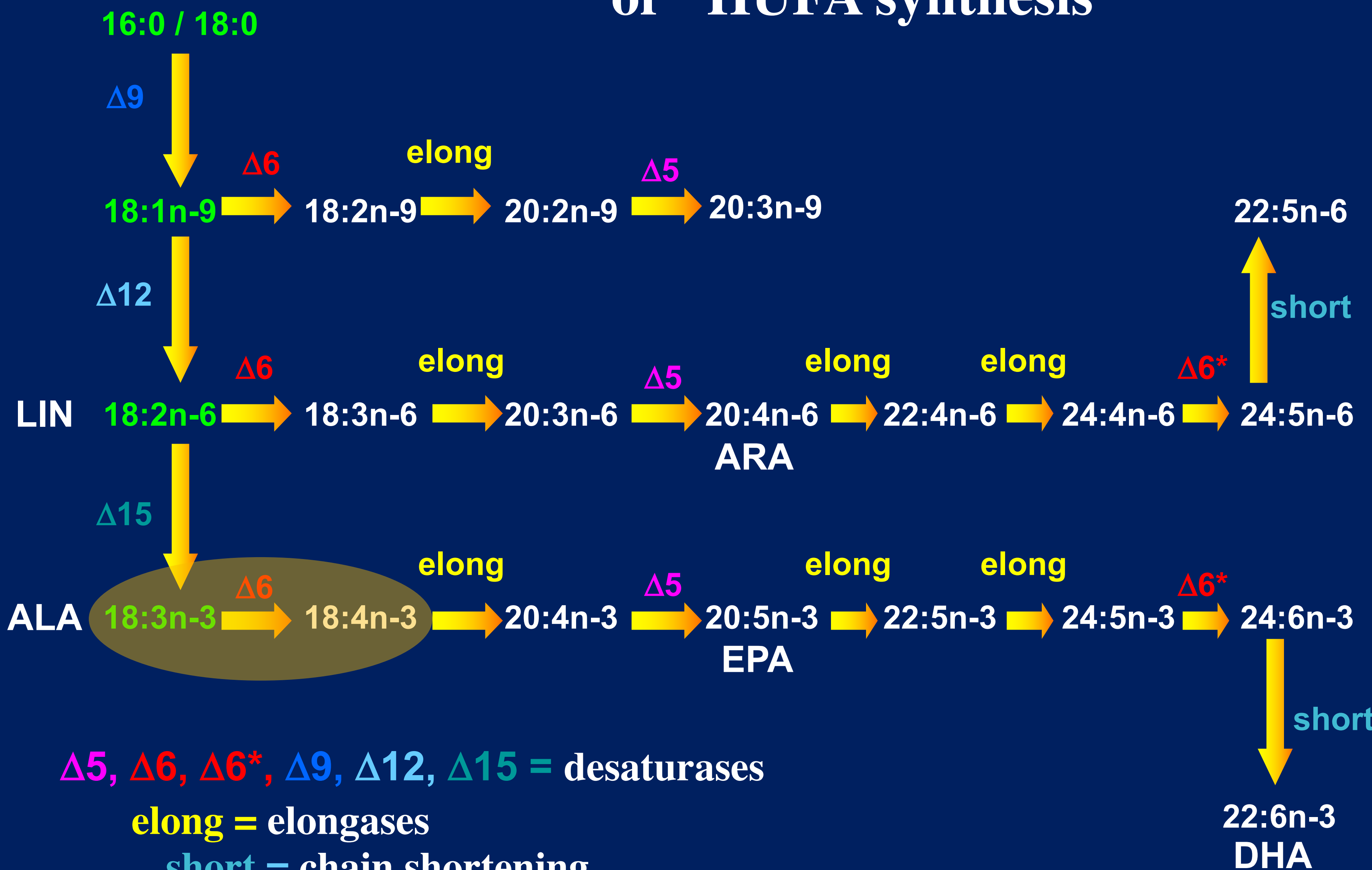


iso-C17:0



anteiso-C15:0

Pathways of fatty acid desaturation and elongation or "HUFA synthesis"



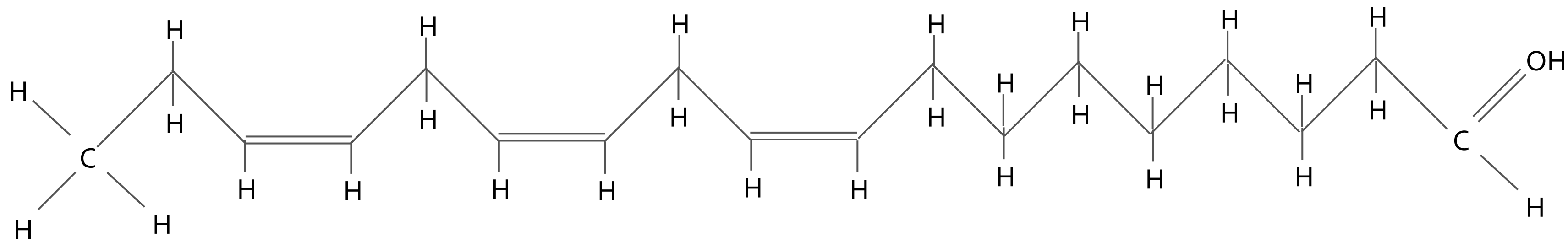
Δ5, Δ6, Δ6*, Δ9, Δ12, Δ15 = desaturases

elong = elongases

short = chain shortening

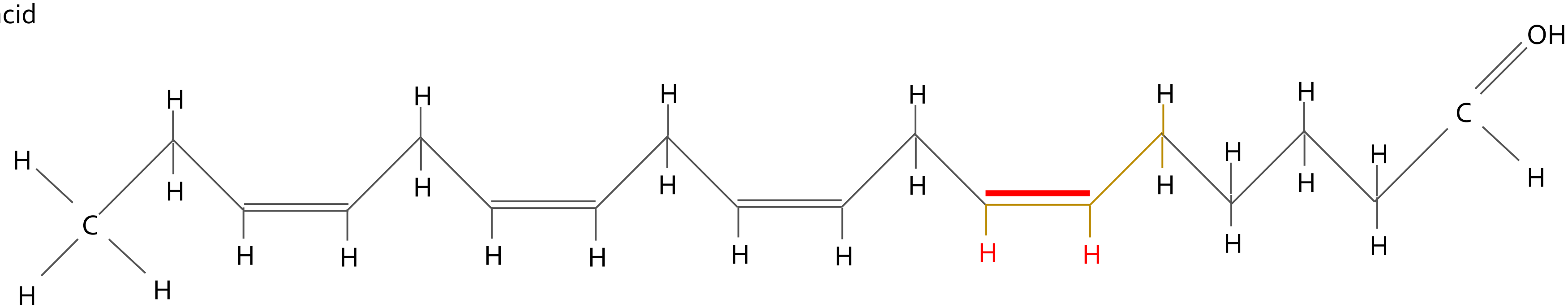
Biomarkers – stable isotopes of fatty acids

C18:3n-3
Alpha-linoleic acid



C = 18
H = 31
O = 1

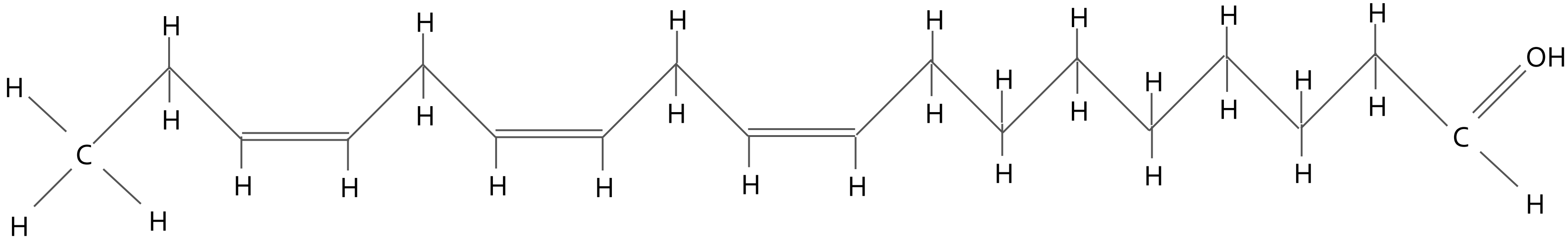
C18:**4**n-3
Steradonic acid



C = 18
H = **29 → loss of 2 hydrogens (Δ6-desaturase)**
O = 1

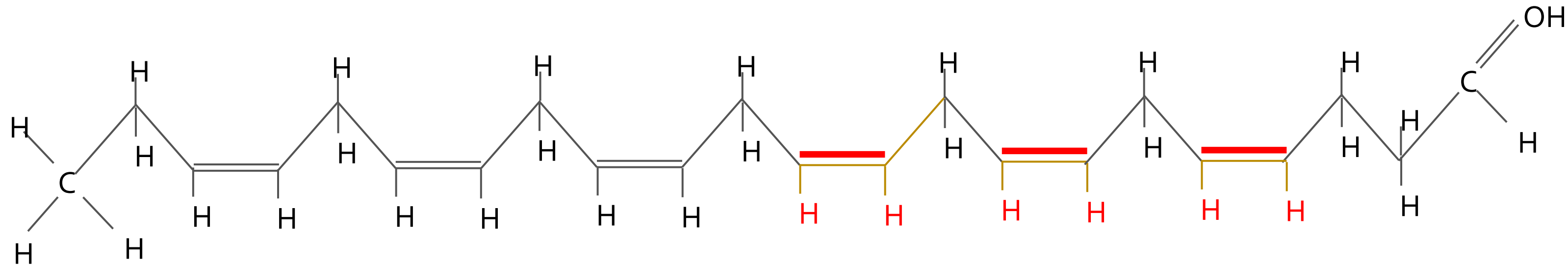
Biomarkers – stable isotopes of fatty acids

C18:3n-3
Alpha-linoleic acid



C = 18
H = 31
O = 1

C22:6n-3
Docosahexaenoic acid



C = 22 → addition of 4 carbons (elongase, incl. β-oxidation)
H = 33 → addition 2 hydrogens (via Δ6, Δ5-desaturases, β-oxi)
O = 1

Biomarkers – stable isotopes of fatty acids

Questions:

- Are there isotopic differences between terrestrial and aquatic diet sources?

  → yes there are, but sometimes very small (Fry and others).

- Are there isotopic differences in fatty acids among various diet sources?

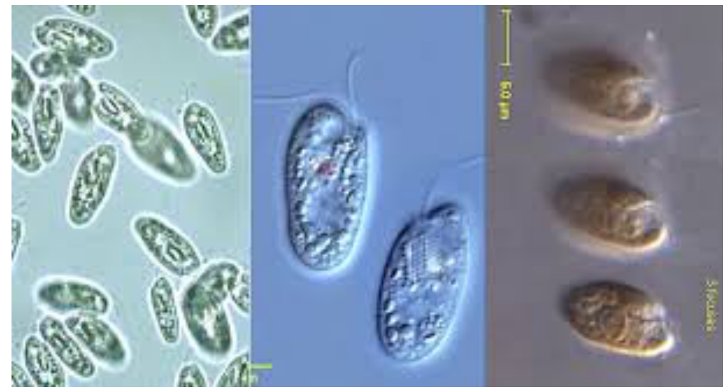
  → Let's see …

Biomarkers – bulk stable isotopes

Terrestrial source

OR

Algal source

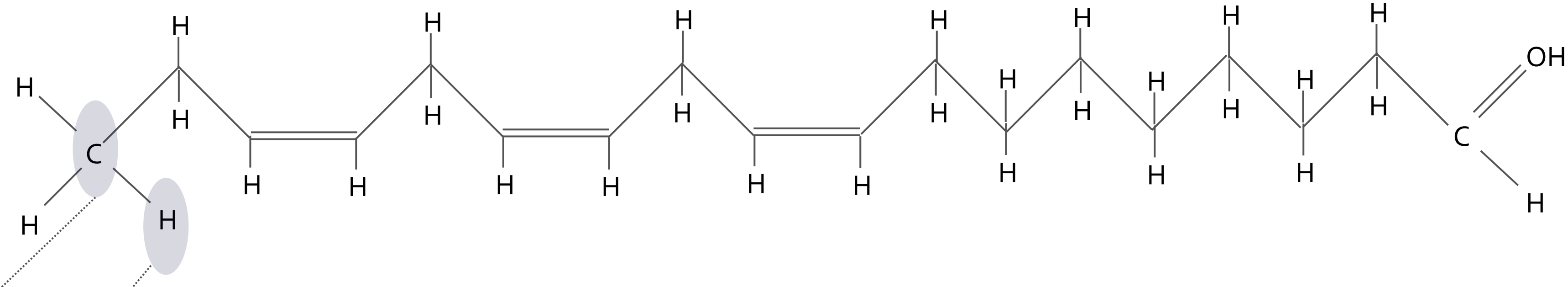


bulk $\delta^{13}C$ = -24 to 27 ‰
bulk $\delta^{2}H$ = big range

bulk $\delta^{13}C$ = -24 to -33 ‰
bulk $\delta^{2}H$ = big range

Biomarkers – stable isotopes of fatty acids

C18:3n-3
Alpha-linoleic acid



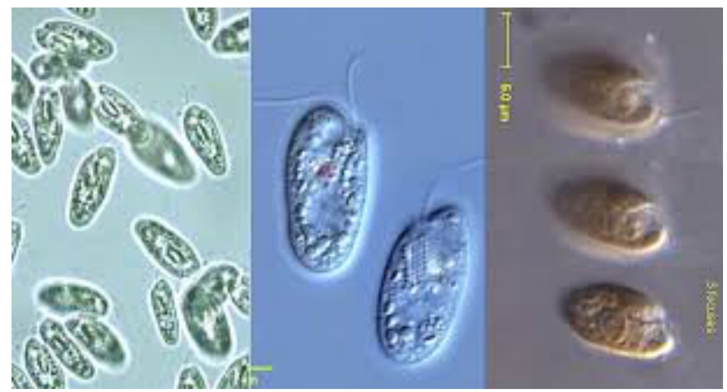
$\delta^{13}C$ (‰)

$\delta^{2}H$ (‰)

Terrestrial source

OR

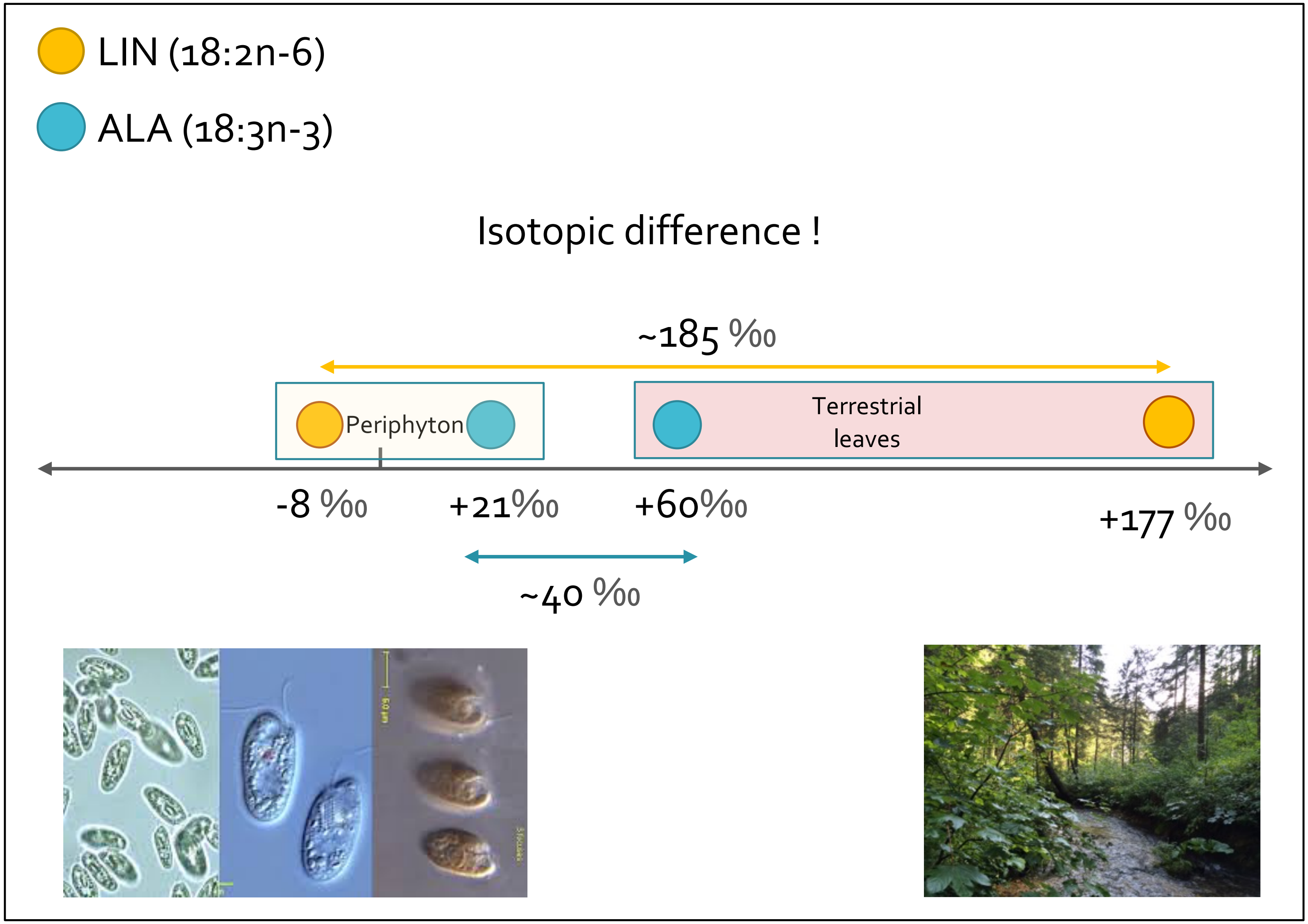
Algal source

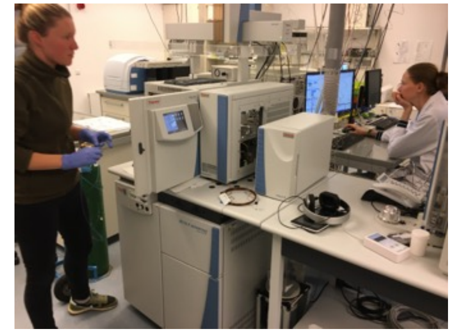


Compound-specific $\delta^{13}C$ = ‰ **??**
Compound-specific $\delta^{2}H$ = ‰ **??**
(O … we exclude for now)

Compound-specific $\delta^{13}C$ = ‰ **??**
Compound-specific $\delta^{2}H$ = ‰ **??**
(O … we exclude for now)

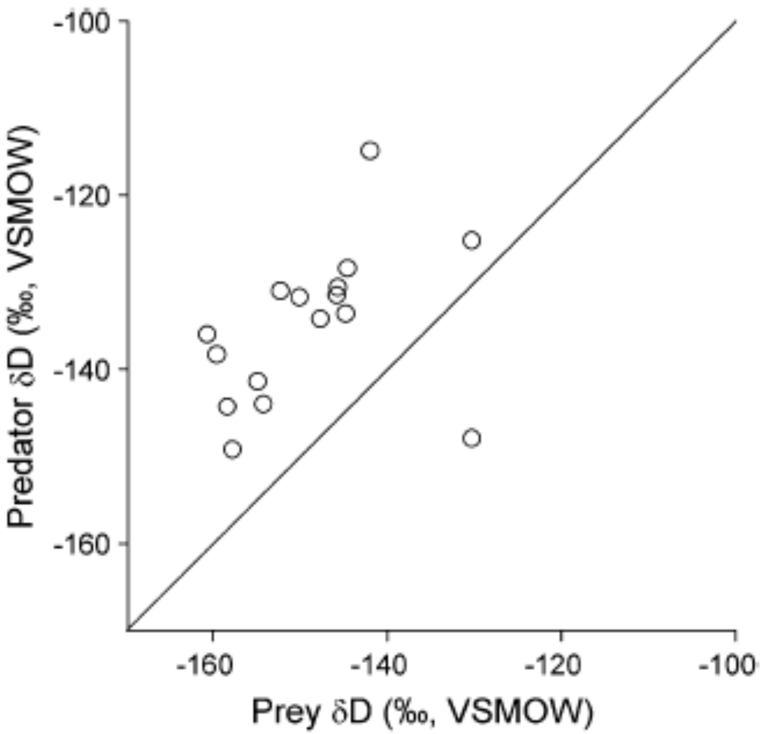
Compound-Specific Stable Isotopes (CSSI): δ^2H of fatty acids in aquatic food web research … promising!





GC-IRMS ($\delta^{13}C$, δ^2H, …)

Biomarkers – stable hydrogen isotopes (bulk)



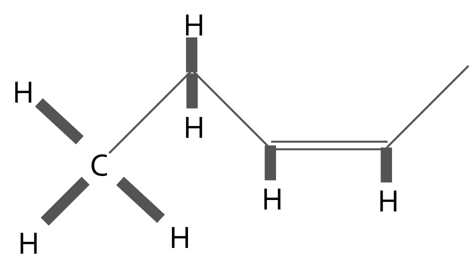
Isotopic H-fractionation during trophic transfer

→ H-enrichment from prey to predator

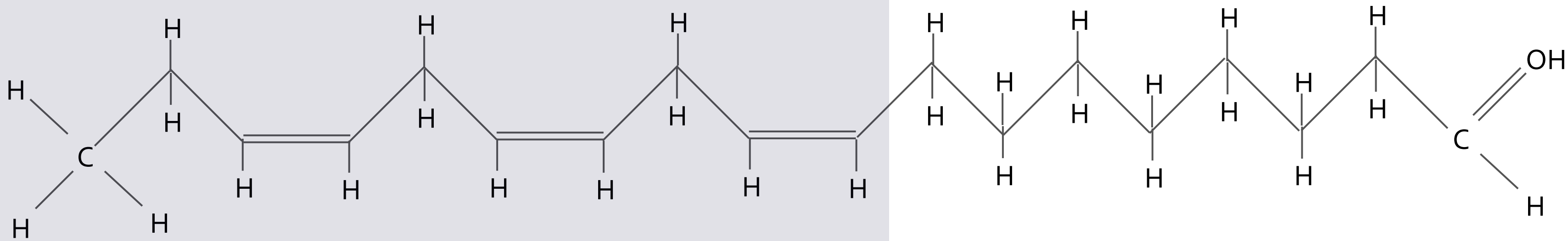
Solomon et al. (2009), Oecologia 161

Biomarkers – stable isotopes of fatty acids

- No isotopic fractionation → **C-H bonds** are irreplaceable (**do not exchange**)



Biomarkers – stable isotopes of fatty acids



This part of the acyl chain remains unchanged

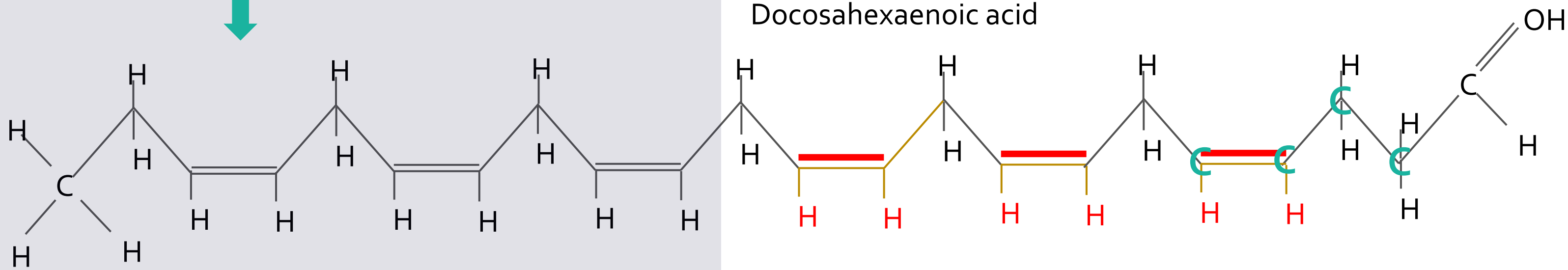
C18:3n-3

Alpha-linoleic acid

C = 18
H = 31
O = 1

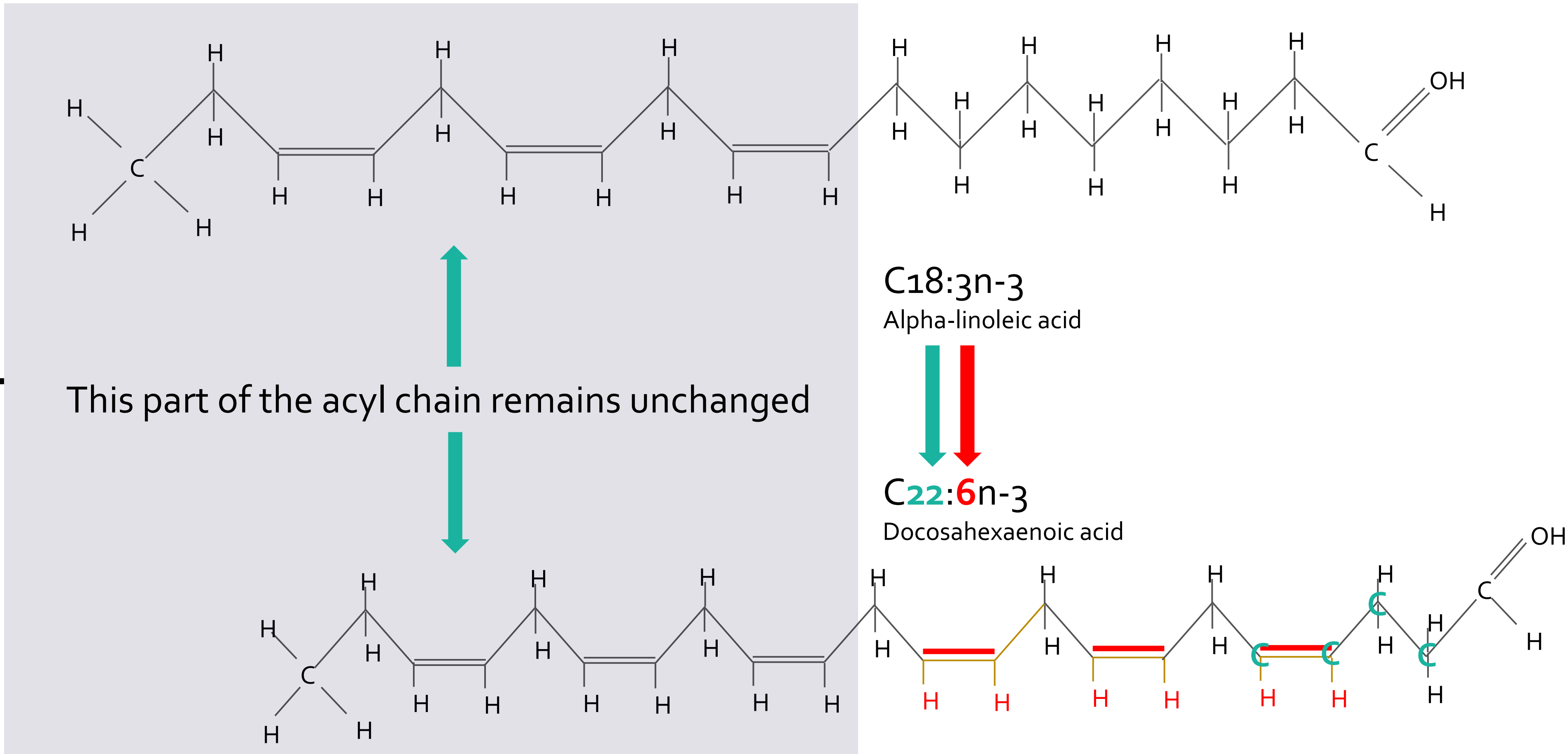
C22:6n-3

Docosahexaenoic acid



C = 18
H = 29 → **loss of 2 hydrogens (Δ6-desaturase)**
O = 1

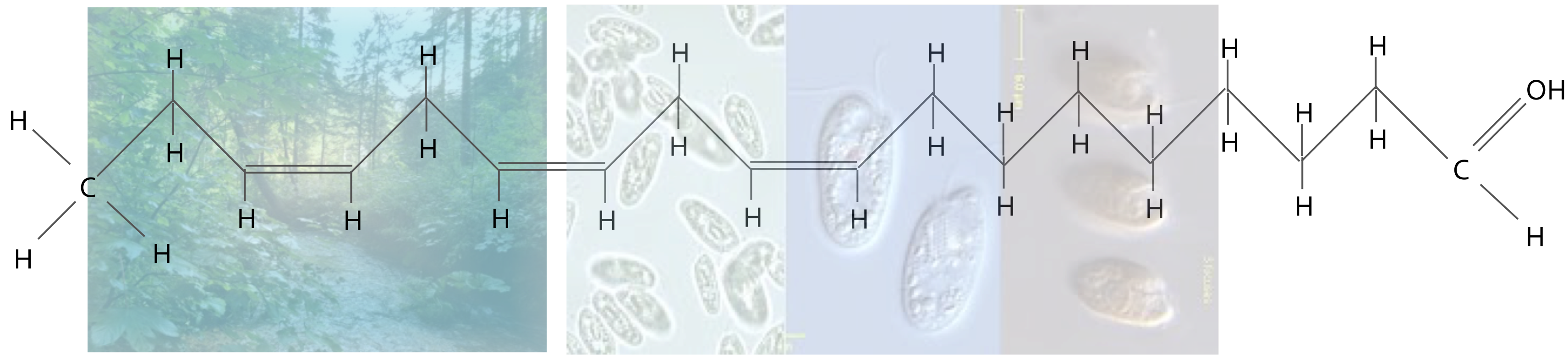
Biomarkers – stable isotopes of fatty acids



- No isotopic fractionation in this part, →
- Essential fatty acids (LIN and ALA) in consumers contain original source SI values

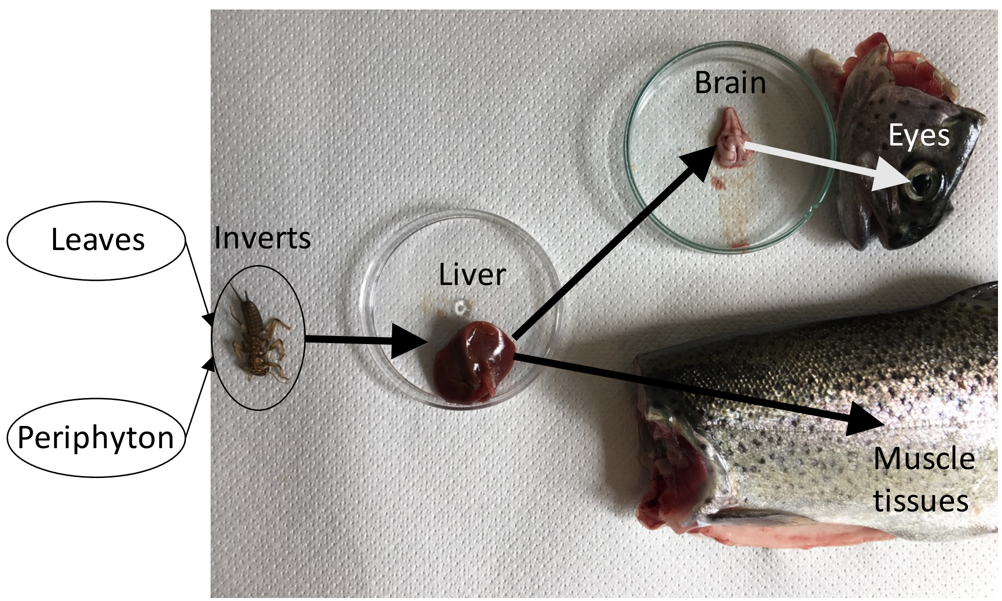
Biomarkers – stable isotopes of fatty acids

C18:3n-3
Alpha-linoleic acid



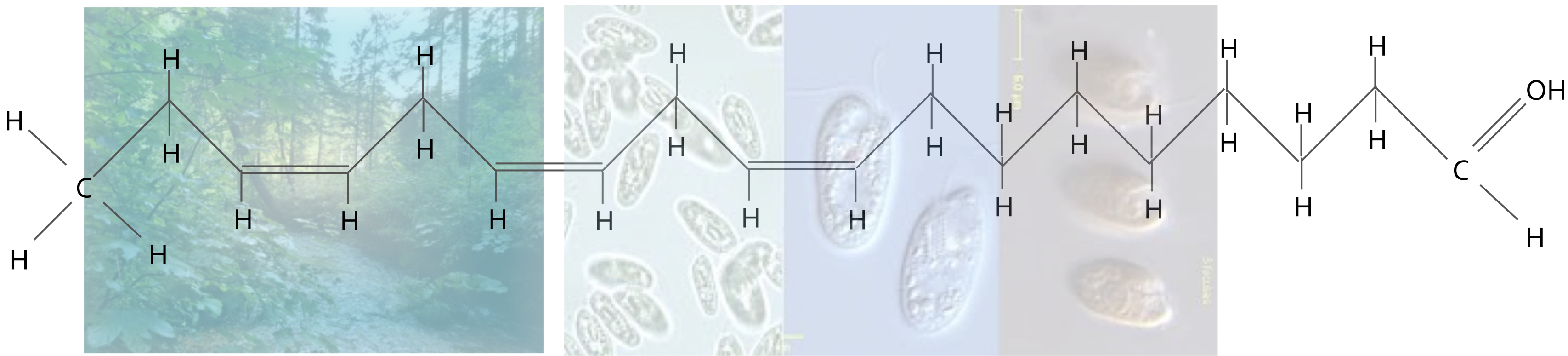
$\delta^{13}C_{terr}$
$\delta^{2}H_{terr}$

$\delta^{13}C_{auto}$
$\delta^{2}H_{auto}$

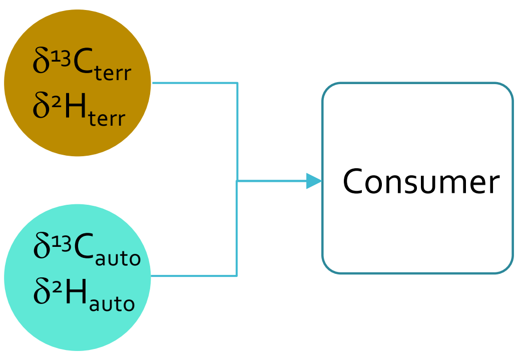


Biomarkers – stable isotopes of fatty acids

C18:3n-3
Alpha-linoleic acid



Using the 2-source mixing model of the same compound:
%C(S2) = (C – S1) / (S2 – S1)



Ecdyonurus	rinne18	rinne24	rinne26
	si		
s1	-29.92467	-29.92467	-29.92467
s2	-2.8710545	-0.1709925	-1.7840543
c	-20.828855	-17.692756	-20.46017
s2%	**0.33621438**	**0.41110594**	**0.33632879**

S1 = Source 1 of essential fatty acid A (e.g., terrestrial)
S2 = Source 2 of essential fatty acid A (e.g., algae)
C : Consumer at any trophic level
s2% = % of S2 in consumer

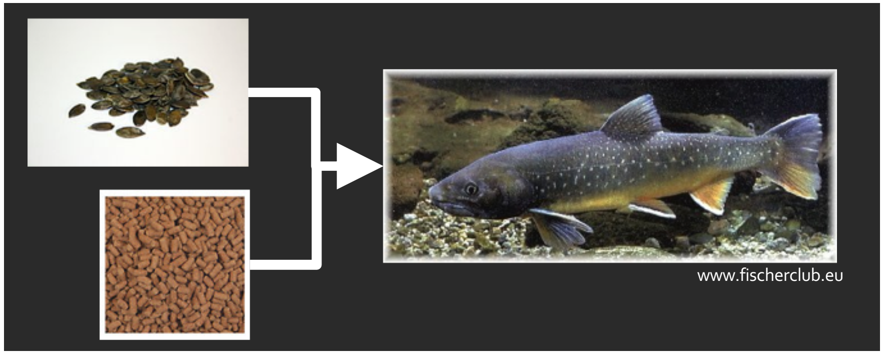
‘All diets are equal, but some are more equal than others’

The Fish Farm

How do fish respond to different dietary carbon sources?

Fish feeding experiments on Alpine charr (*Salvelinus alpinus*)

	Feed 1	Feed 2	Feed 3	Feed 4
Fish meal	High	Medium	Medium	Low
Fish oil	High	High	Low	Low

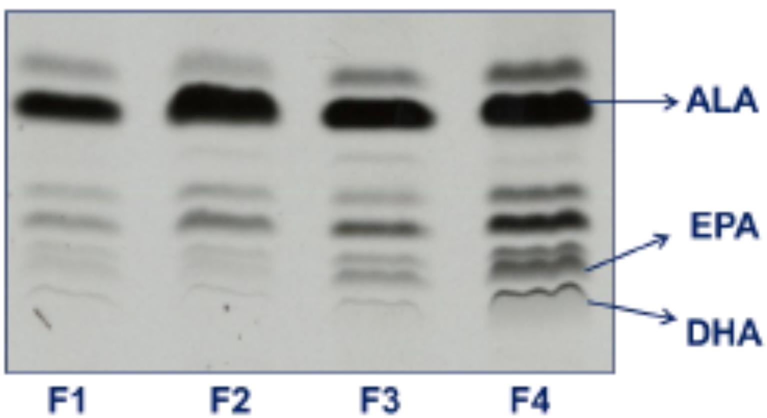


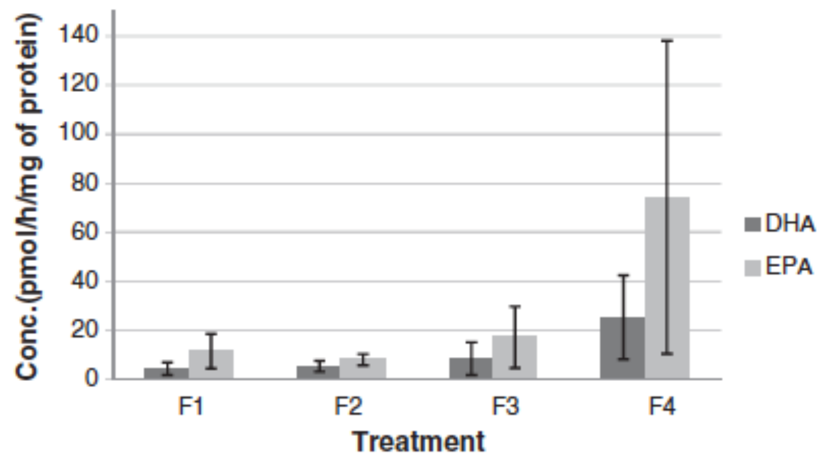
Murray et al. (2014) Aquaculture

Functional response of different carbon sources

Hepatocytes can convert (upgrade) dietary short-chain PUFA …

^{14}C-ALA (18:3n-3) added to hepatocytes





Murray et al. (2014) Aquaculture

Fig. 4. Production (mean ± SD) of EPA and DHA from ^{14}C-labelled ALA by isolated hepatocytes from Arctic charr fed diets containing decreasing amounts of fish meal and fish oil.

Functional response of different carbon sources

… but it comes at a cost → lower somatic fish growth

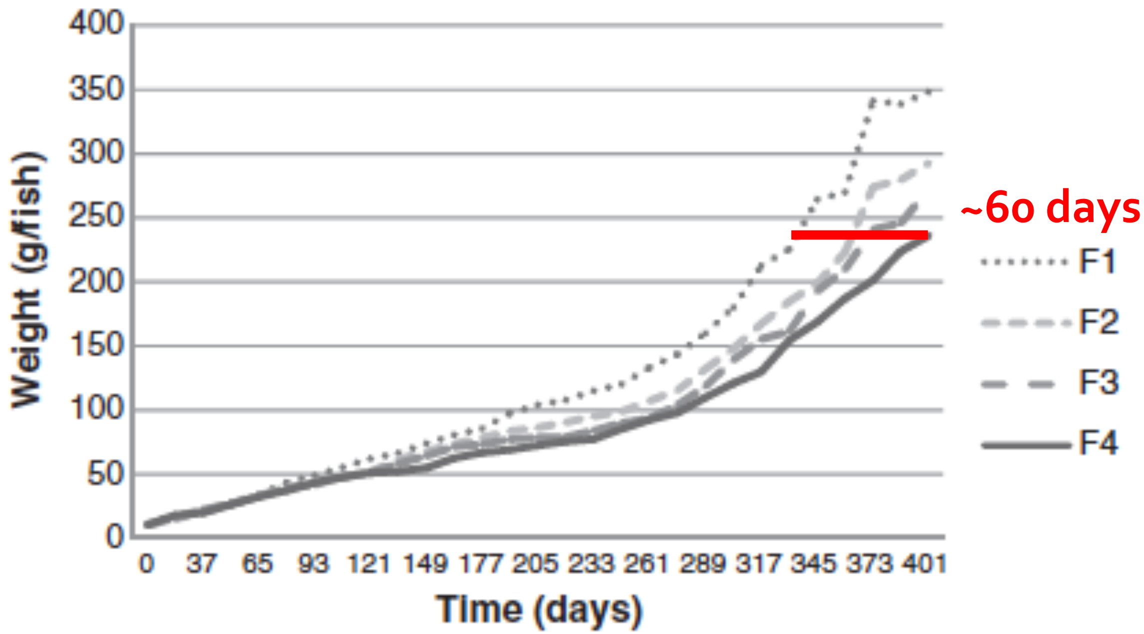
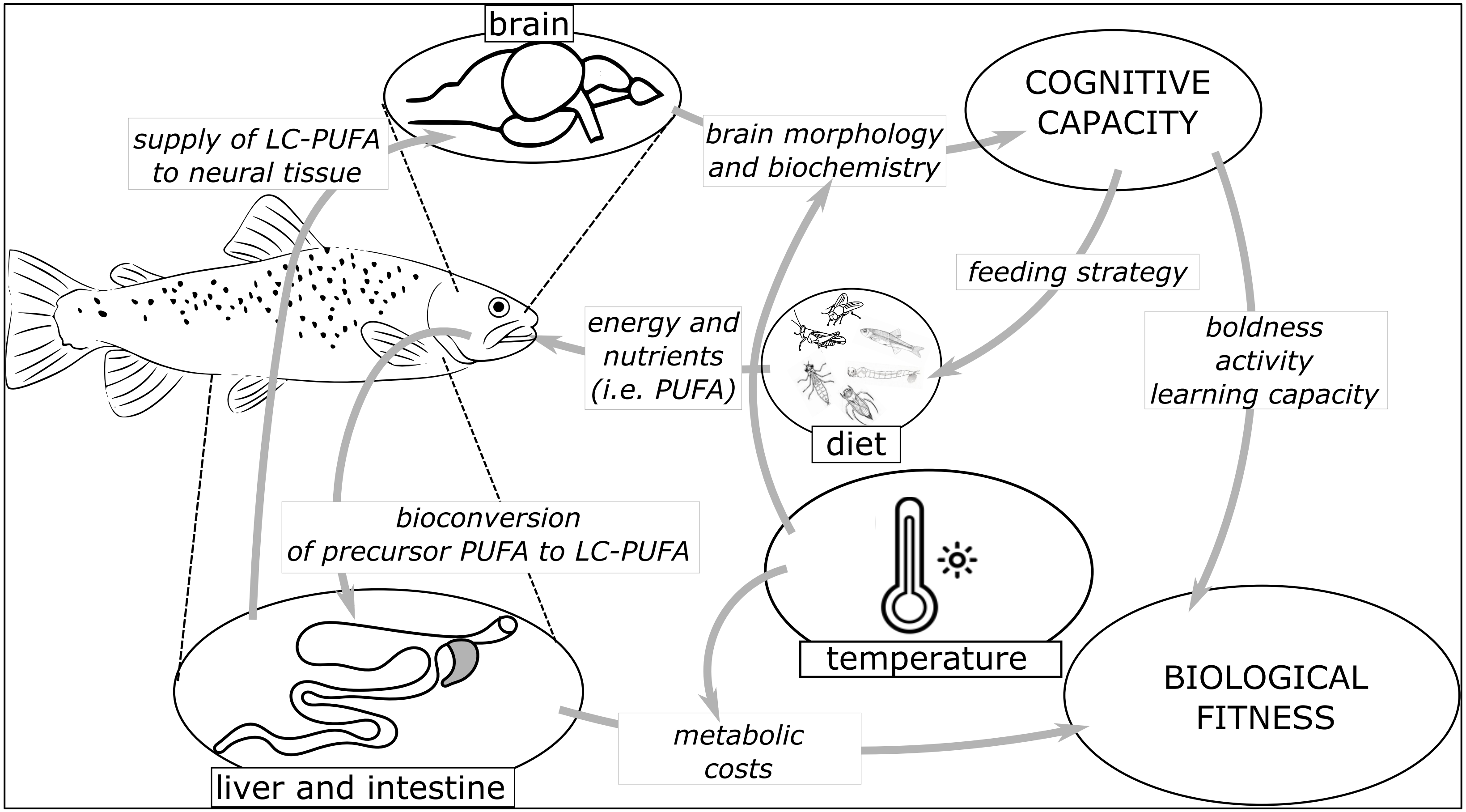


Fig. 1. Average biomass of Arctic charr fed diets containing decreasing concentrations of FM and FO and increasing concentrations of pumpkin kernel cake and rapeseed oil (F1 → F4).

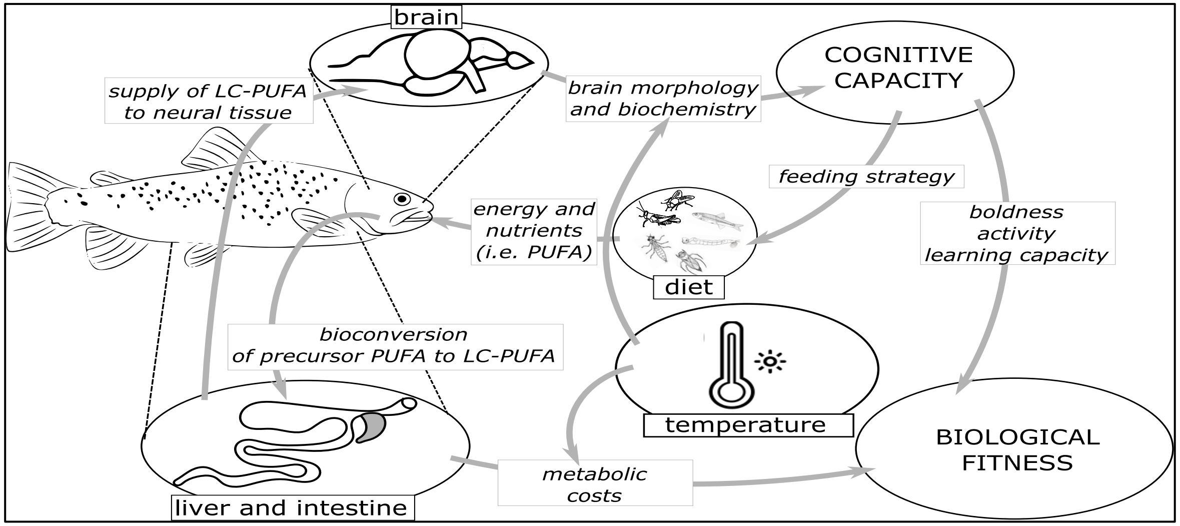
Murray et al. (2014) Aquaculture

Question:
What if there is no dietary DHA for fish in stream ecosystems?

Diet links to functions: use of dietary energy in organisms



Functions: require specific dietary energy



Question:

What if diet quality is lower than consumers requirements?

Objective:

To evaluate how required energy (lipids), <u>not supplied by diet</u>, makes it to consumers

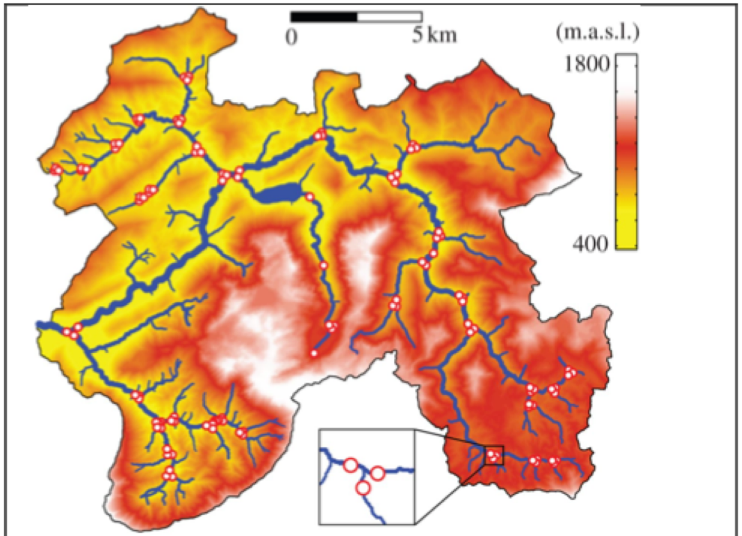
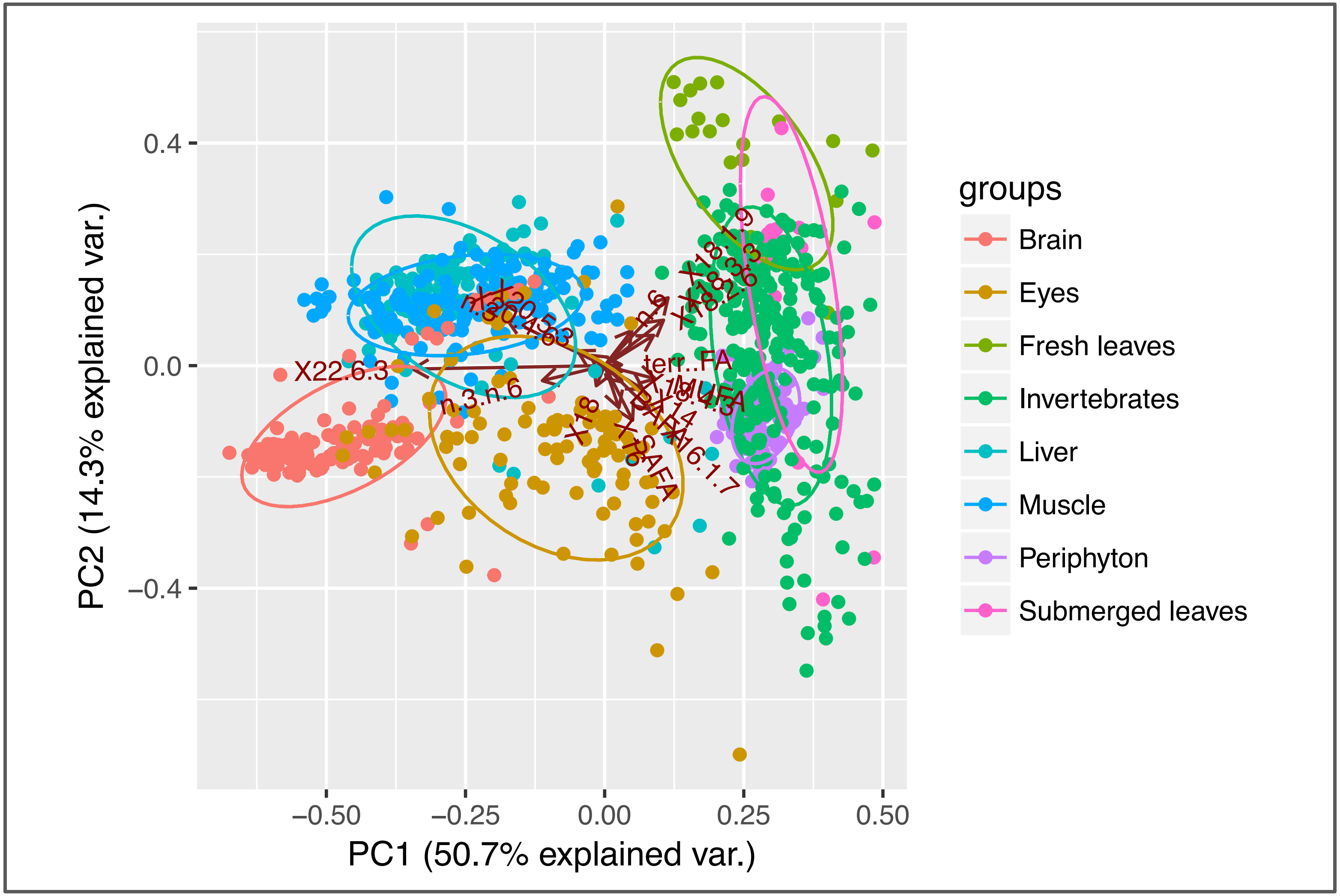
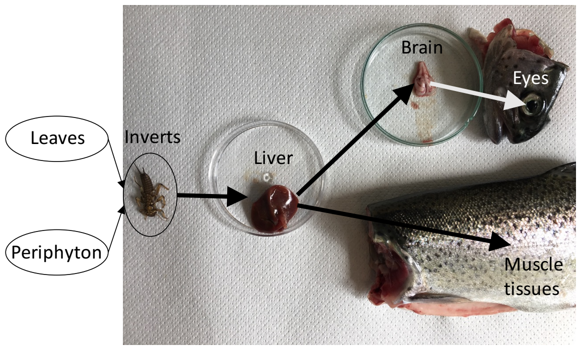


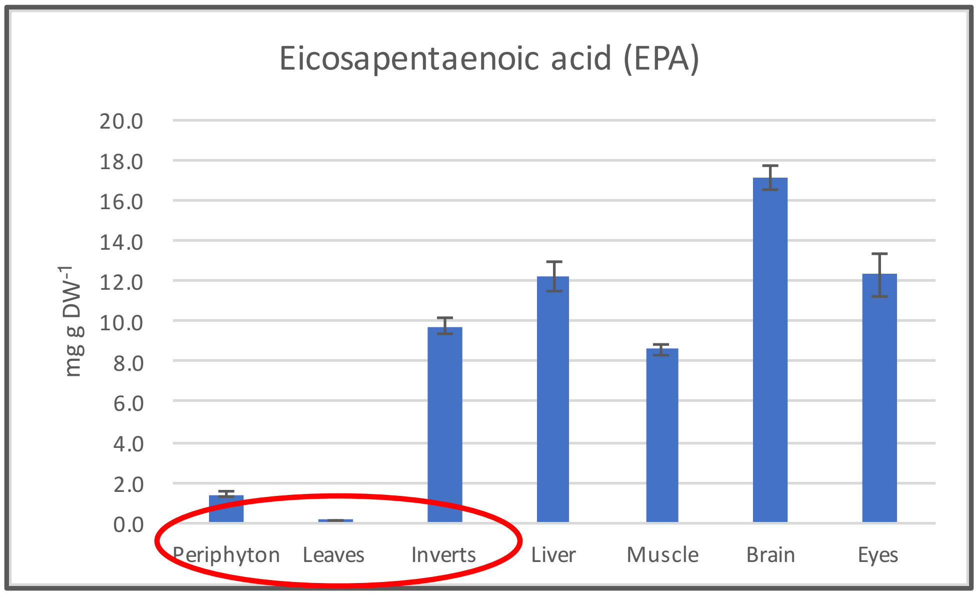
Fig. 1. Stream catchment of River Ybbs, Lower Austria, with regularly sampled sites.
Courtesy: K. Besemer

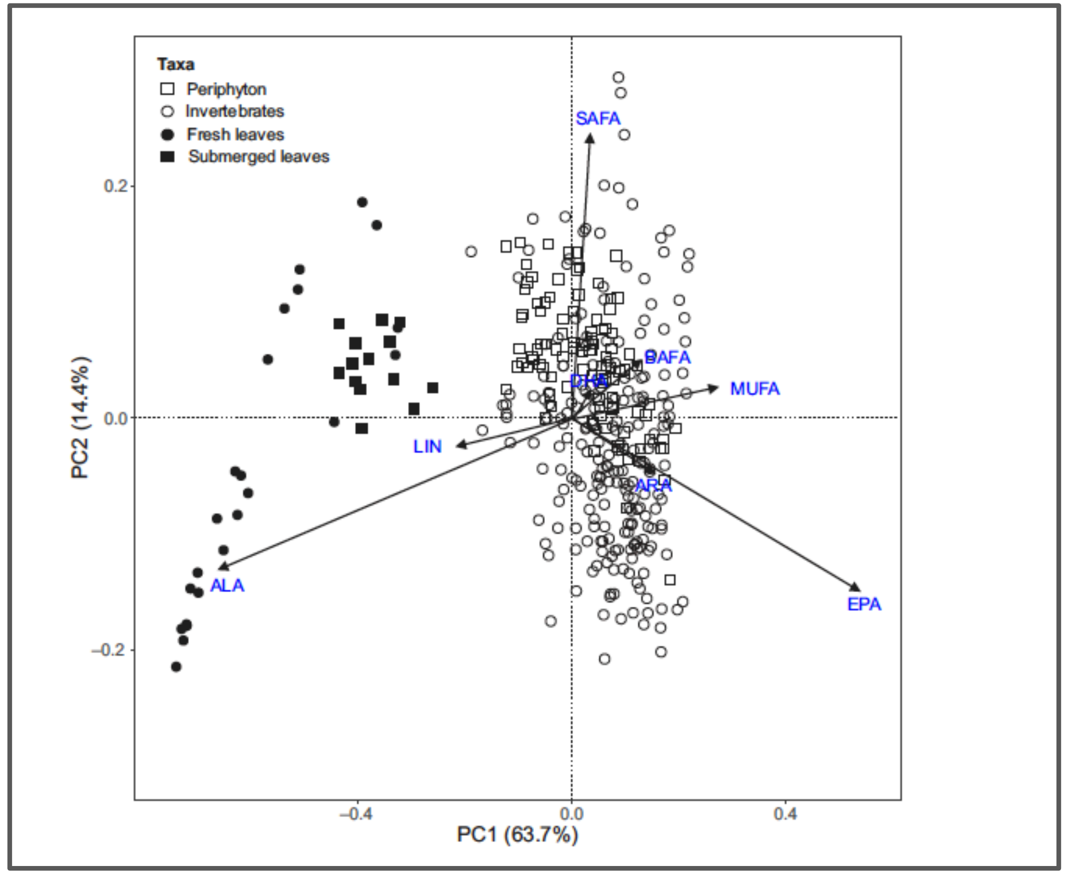
Functions: require specific dietary energy



Omega-3 C20 fatty acids – from leaves to fish

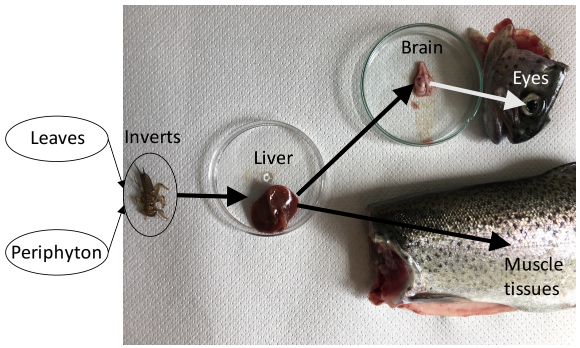


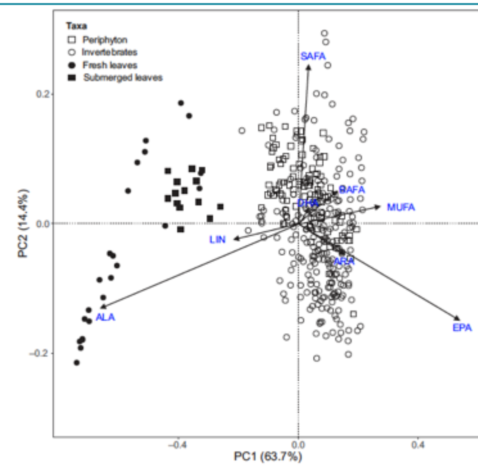


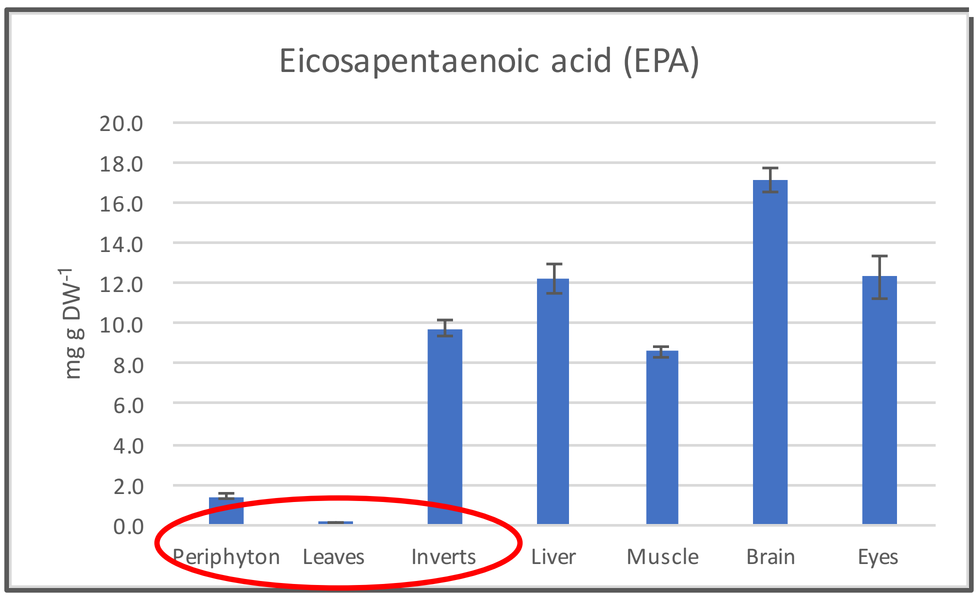


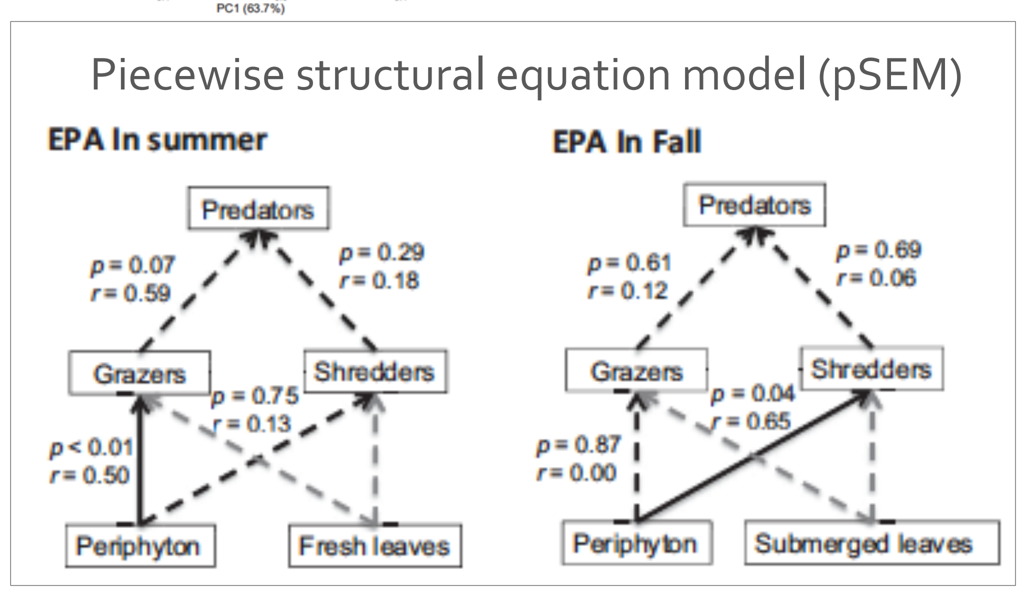
Guo et al. (2018) L&O

Omega-3 C20 fatty acids – from leaves to fish



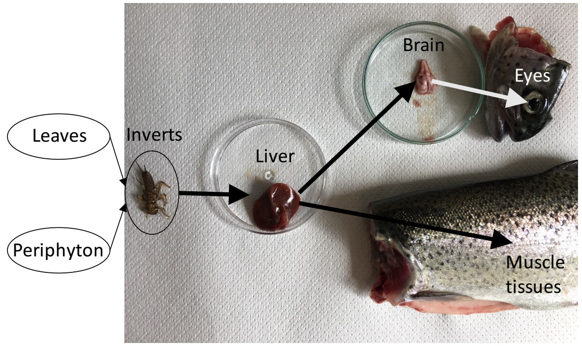


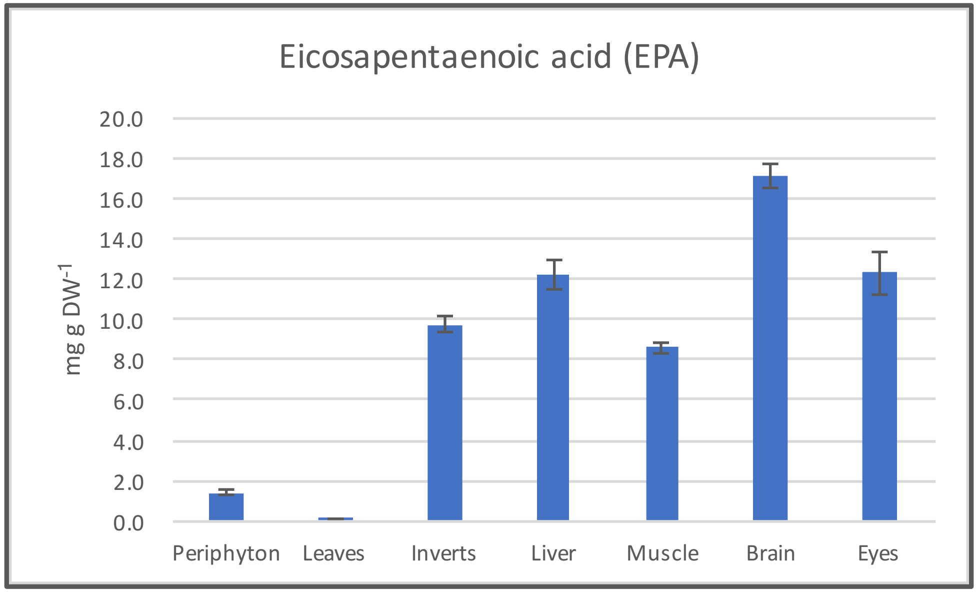


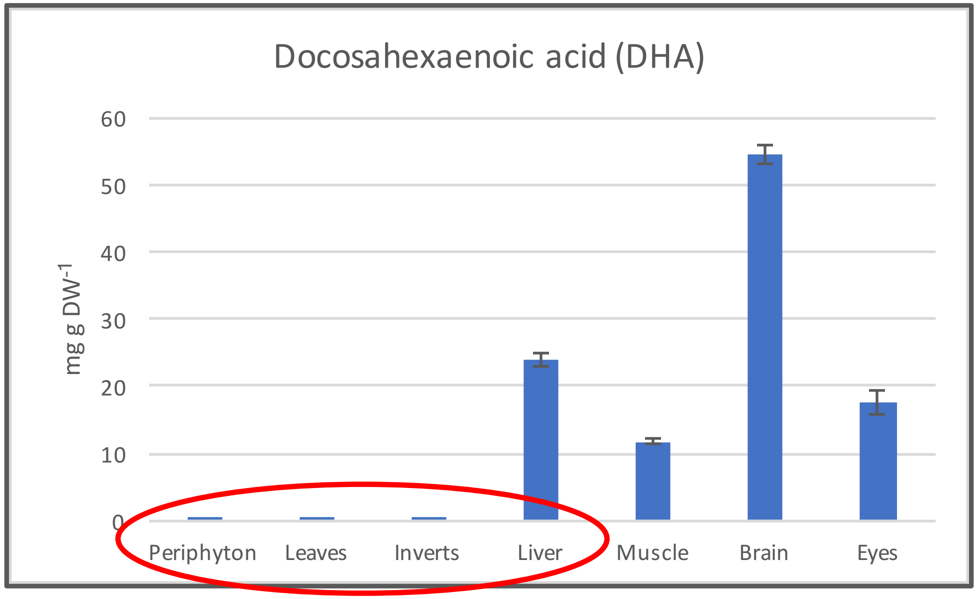


Guo et al. (2018) L&O

Omega-3 C20 fatty acids – from leaves to fish



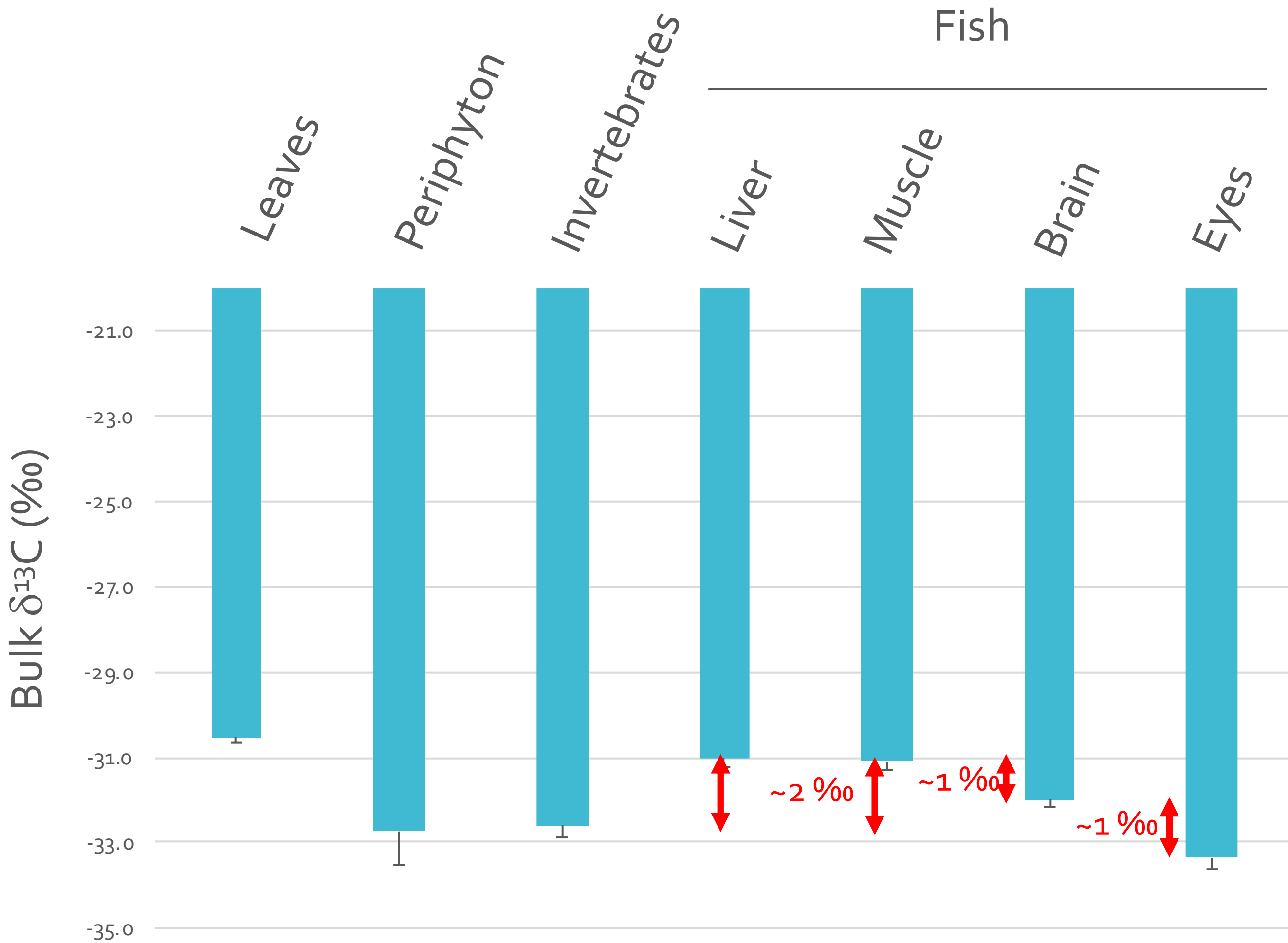




Question:

Does fatty acid reworking entail isotopic C-fractionation?

Carbon reworking (bulk δ¹³C): resources → fish organs



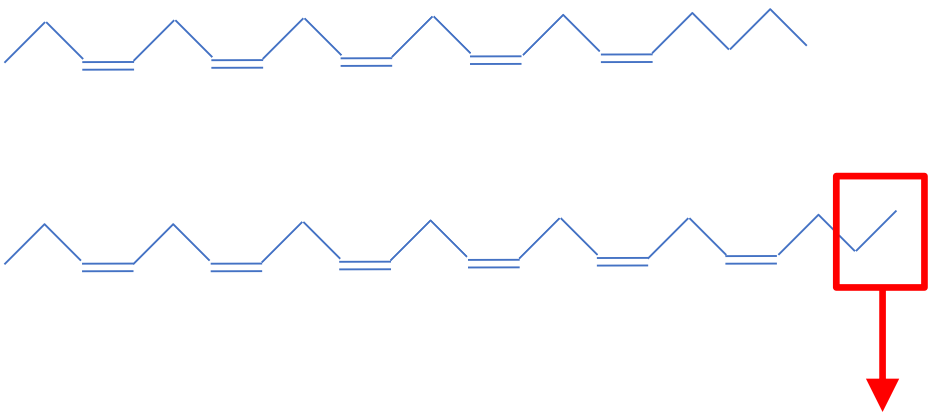
CSSI: DHA synthesis in fish results in lighter δ^{13}C values ?

Fatty acid anabolism:

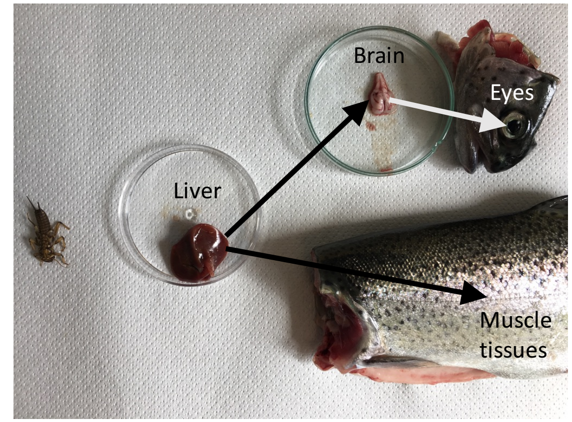
EPA

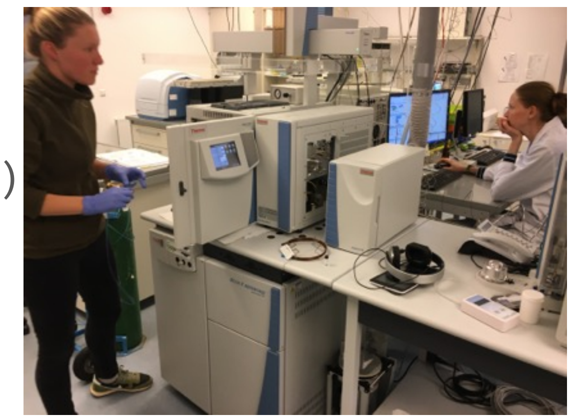
+2 C

DHA



Retention of lighter C (^{12}C addition)
→ Lighter δ^{13}C values

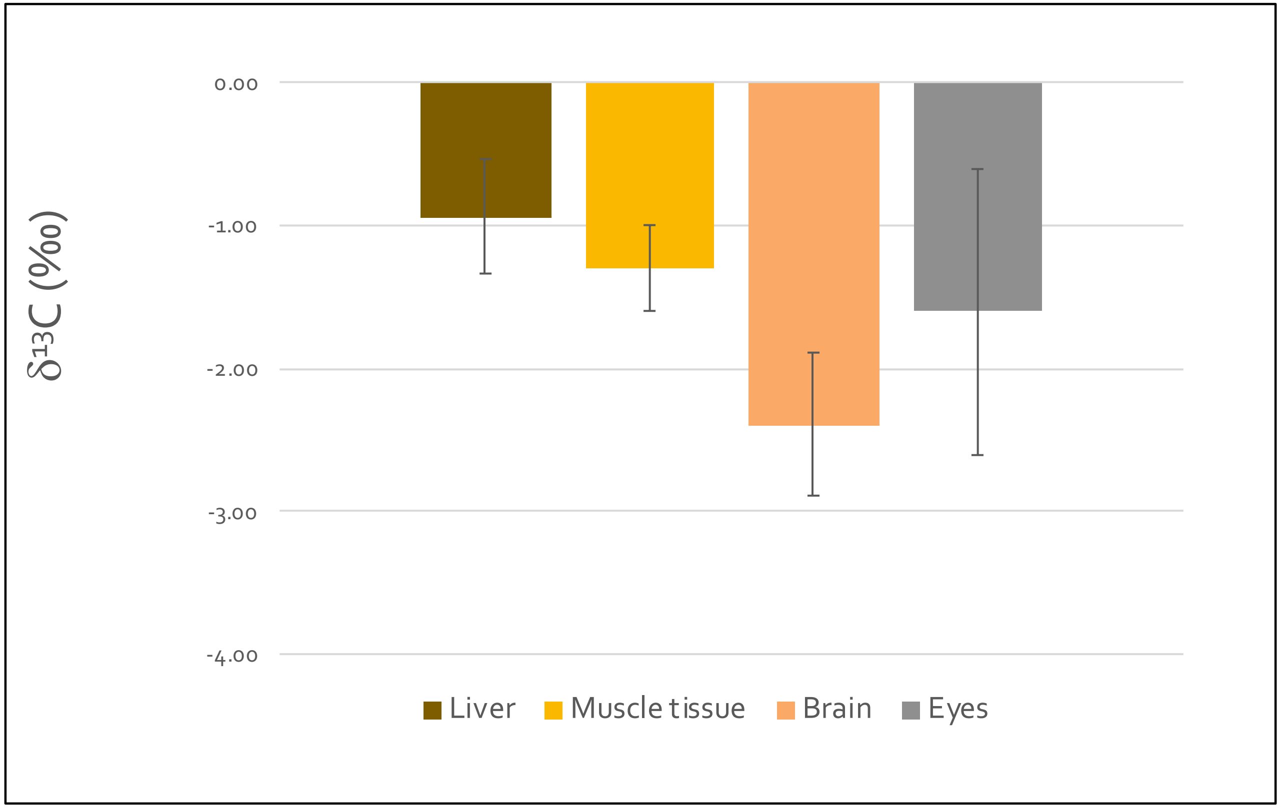


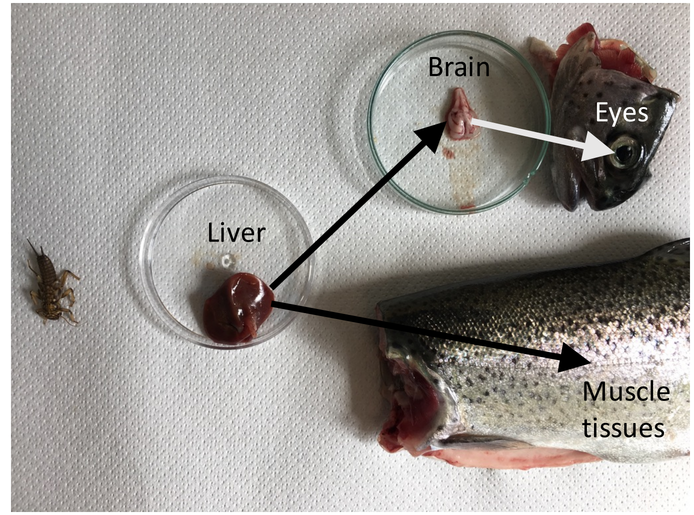


GC-IRMS (δ^{13}C, δ^{2}H, ...)

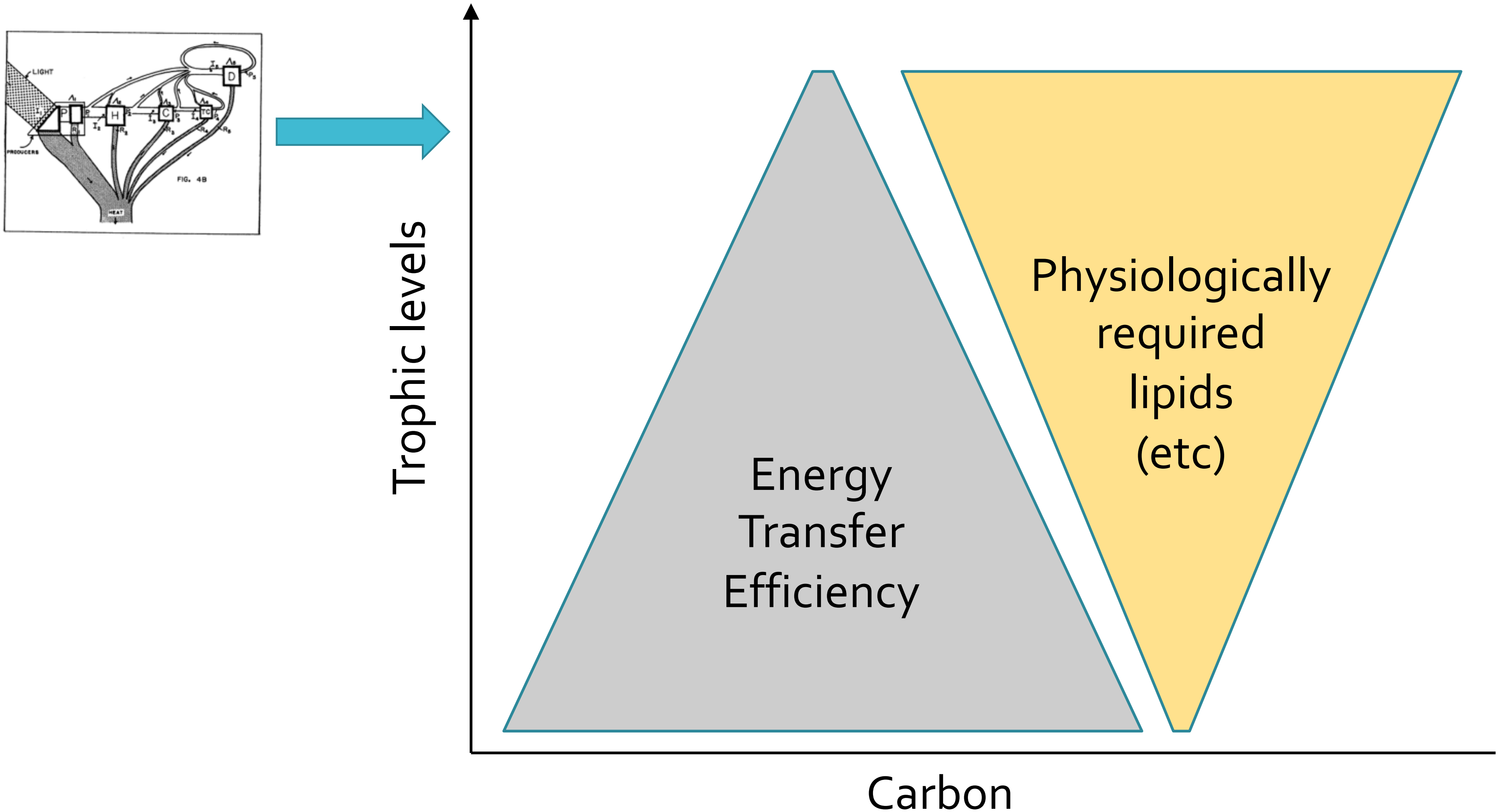
Stable Carbon Isotope fractionation: EPA → DHA synthesis

→ Carbon depletion for DHA synthesis





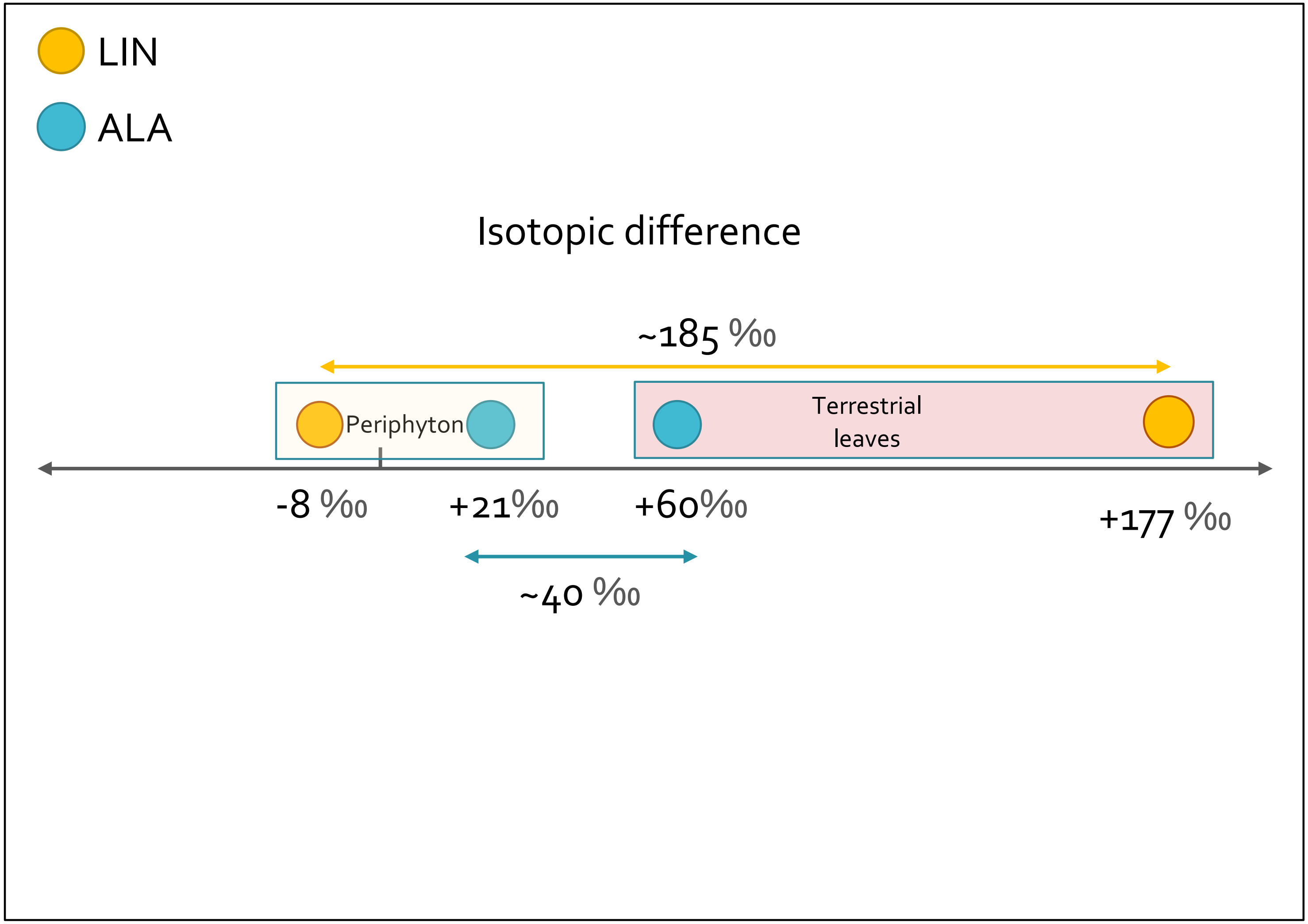
A look back – 'Classical energy transfer efficiency'
A look ahead – 'Essential dietary nutrients transfer'



Thanks to many!

WasserCluster Lunz – Biologische Station
Donau-Universität Krems, Austria
www.kainzlab.com

Compound-specific Stable Isotopes:
δ^2H of fatty acids in aquatic food web research … promising!



A look ahead – Are we losing n-3 PUFA with changing fish taxa?

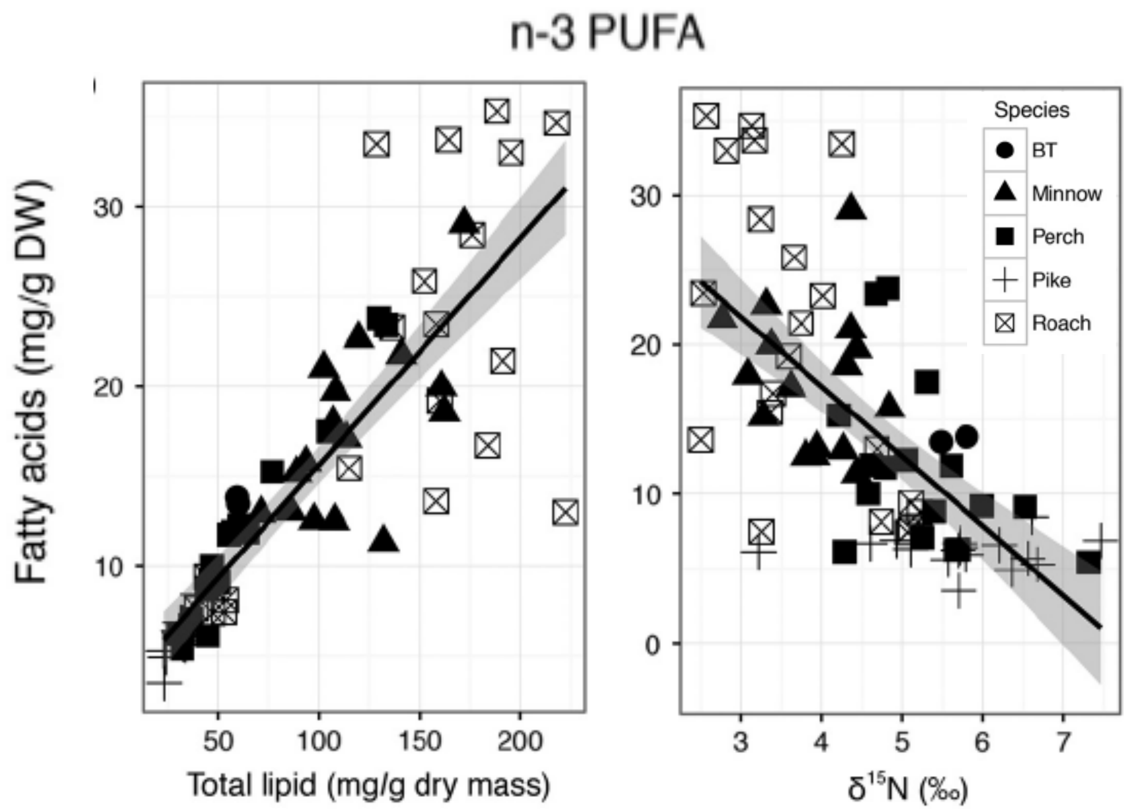
Salmonids

↓

Percids

↓

Cyprinids

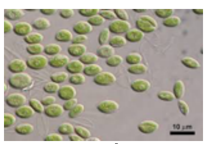


Kainz et al. (2017) Ecosphere 8

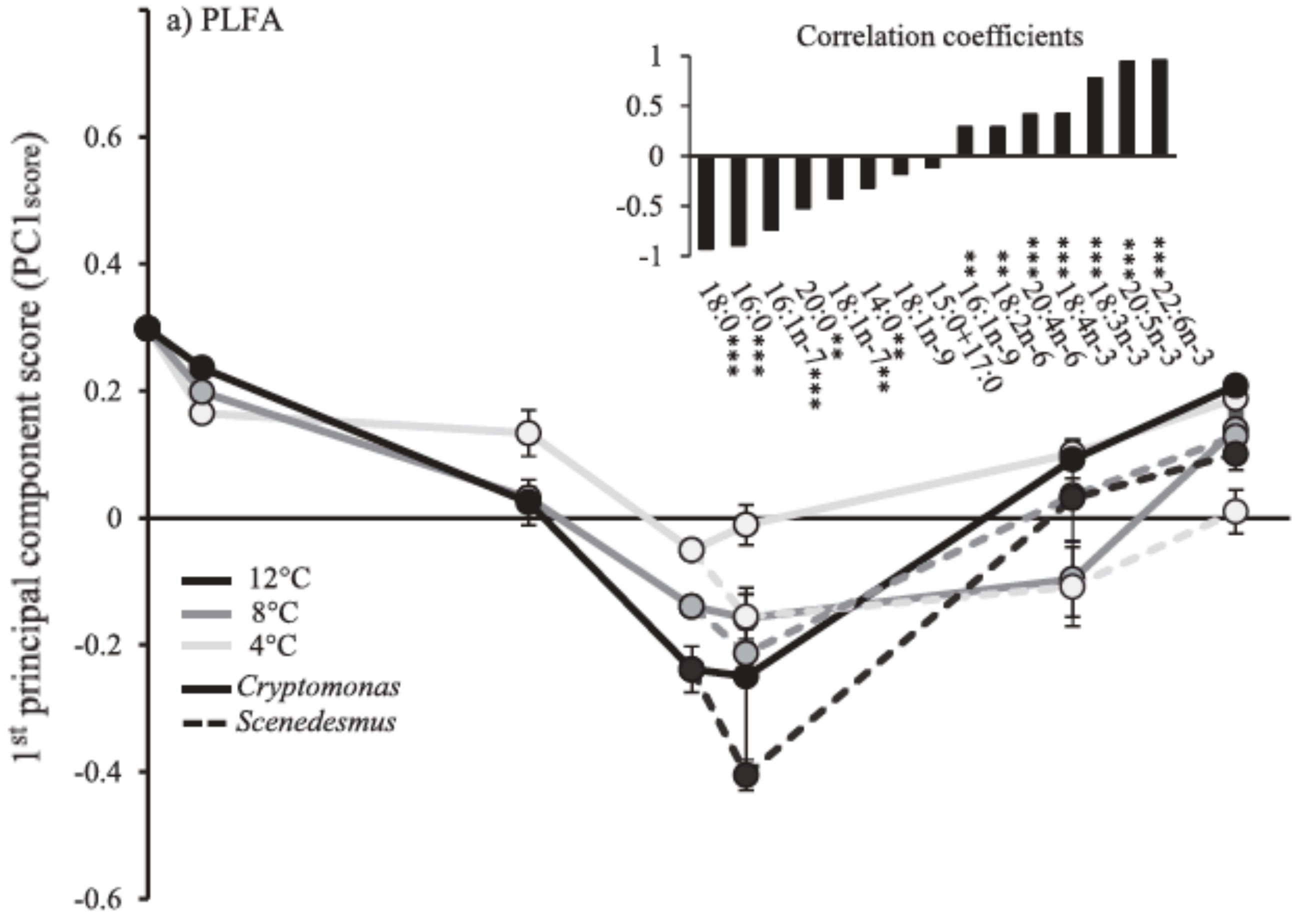
How do copepods respond to different temperature and diets?

Eudiaptomus gracilis

Cryptomonas ozolinii



Scenedesmus obliquus



Koussoroplis et al. (2014) L&O 59(3)

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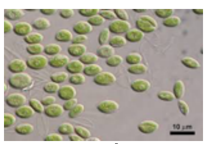
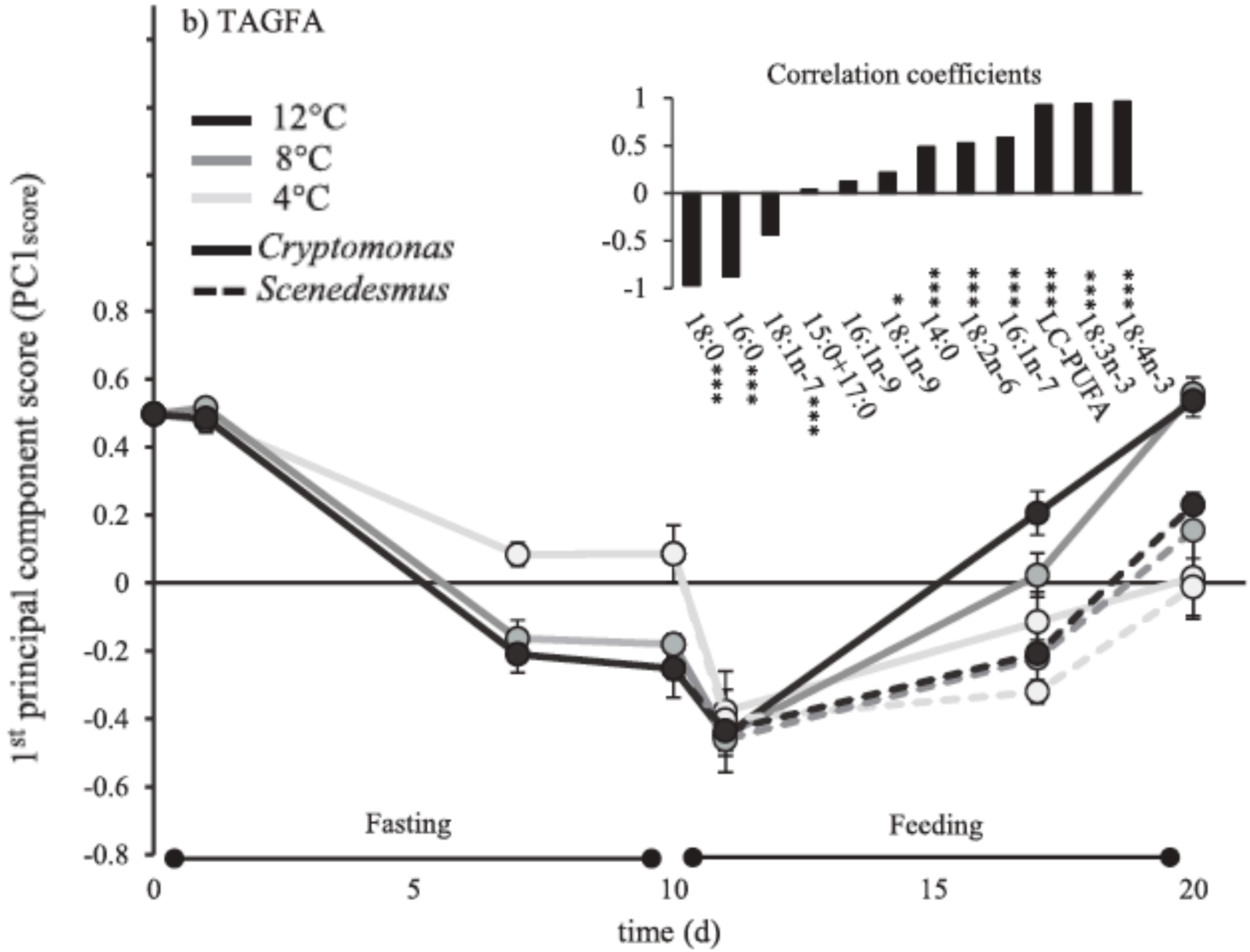


Scenedesmus obliquus



Koussoroplis et al. (2014) L&O 59(3)