APPLICATION OF NATURAL STABLE ISOTOPES IN LARVAL NUTRITION STUDIES

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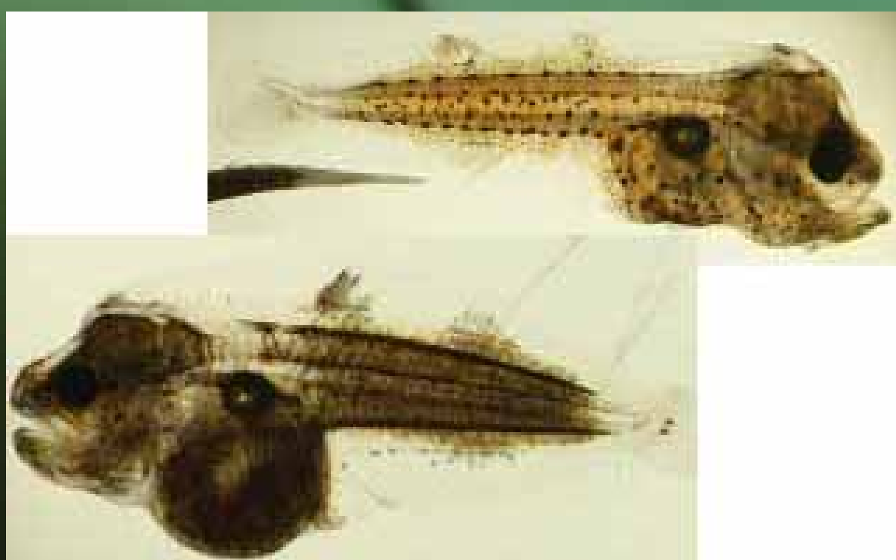


Stable isotopes applications in larval nutrition

Ratios of $^{13}C/^{12}C$ & $^{15}N/^{14}N$:
proxies for organic matter assimilation

Safe: alternative to radiolabels

Enriched: short term tracer studies
or
Natural: longer studies under normal feeding

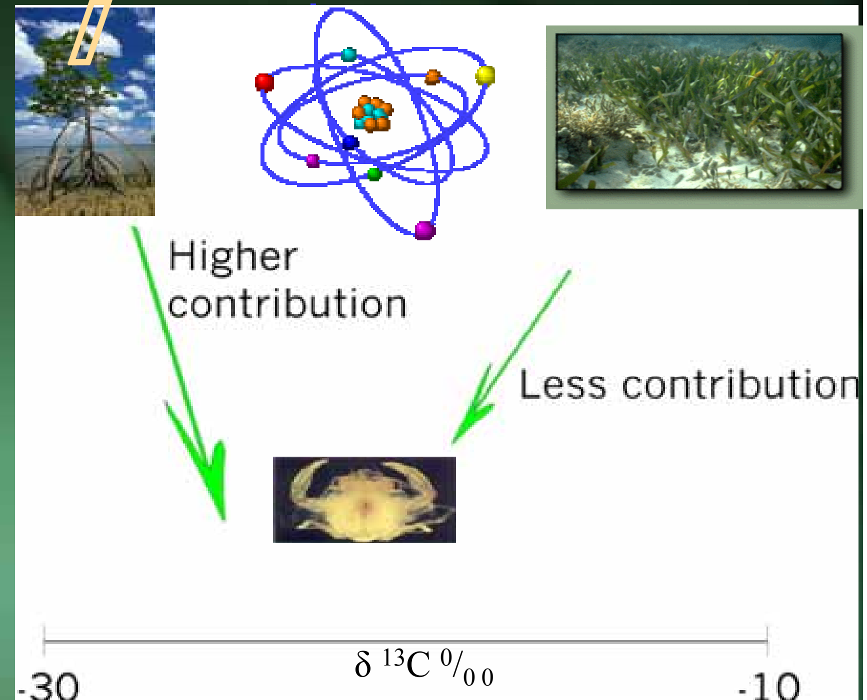
Tissue changes with time:
ingestion & turnover rates

Mixing models: contribution to growth from mixed food sources.

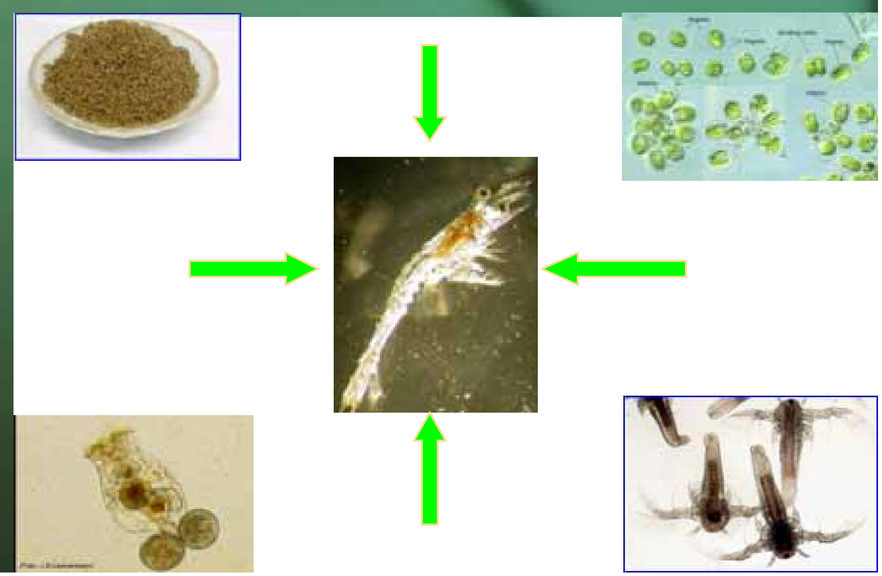
Compound specific analysis:
amino acid utilisation

You are what you eat

Widely used in ecological studies:



Hatcheries as mescosms:



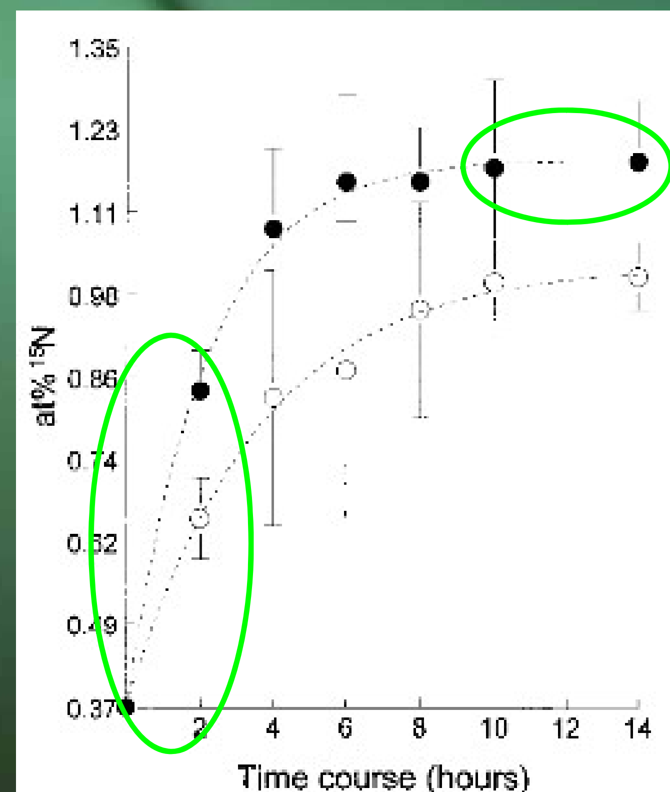
Ingestion and protein turnover using enriched feeds

eg **Short term** studies with **enriched** foods using flooding dose models developed for radiolabelled single amino acids*

^{15}N labelled algae

Rotifers fed 24h

Turbot larvae fed 14h



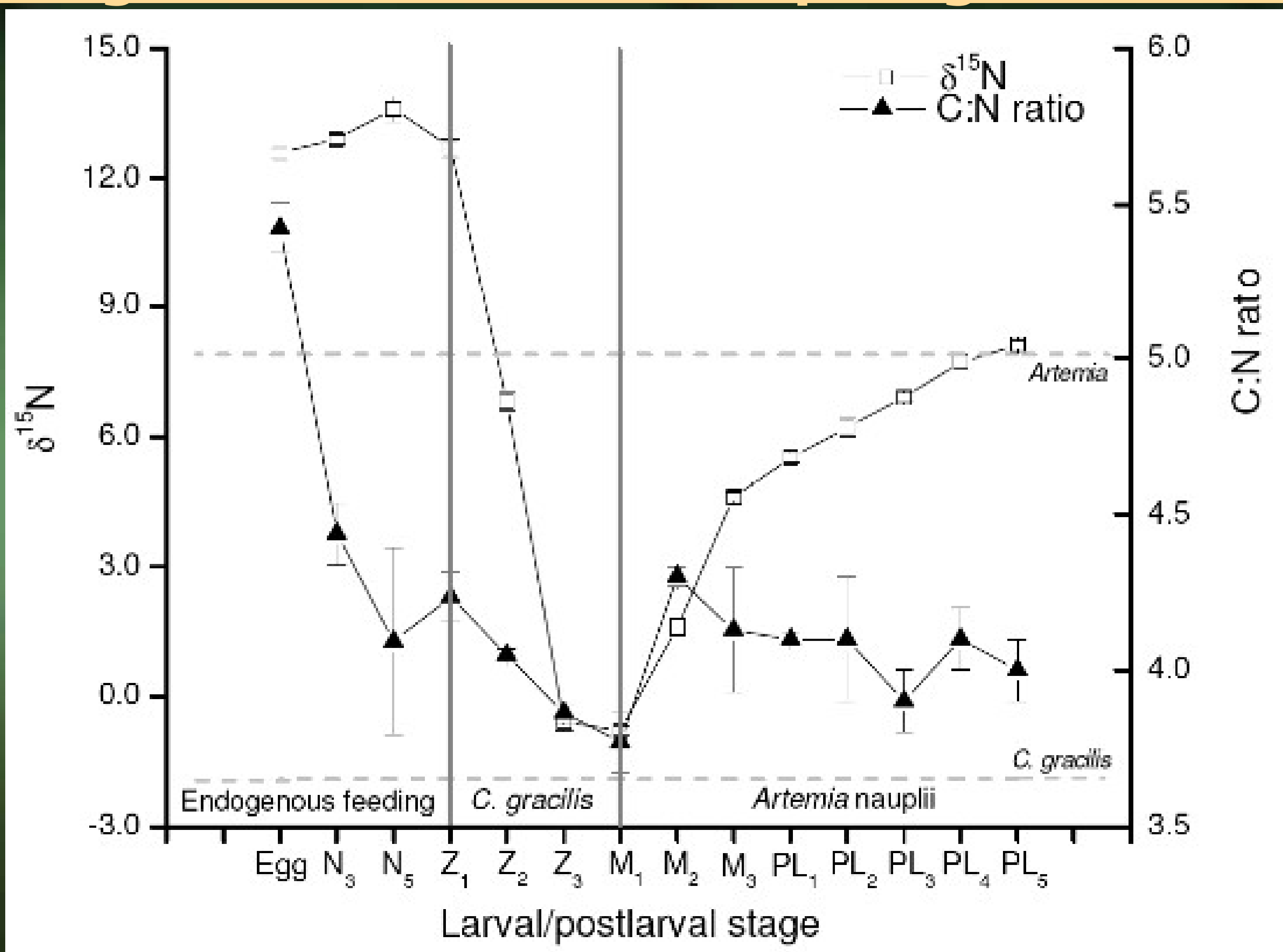
Allows estimation of
(i) ingestion rate
(ii) protein synthesis & degradation
(iii) comparison of dietary treatments

(Conceição *et al* 2001)

*Garlick *et al* 1980, Houlihan *et al* 1988

See also Conceicão *et al* (2007) for review

Longer term natural isotope signature changes



$\delta^{15}N$ ‰ and C:N ratios in *Litopenaeus vannamei* during larval development. Larvae were fed only on *C. gracilis* (zoea stages) and *Artemia* (mysis stages). Horizontal dotted lines represent isotopic signatures of foods.

(Gamboa-Delgado & Le Vay, under review)

Hatcheries as simple controlled food webs

Limited number of sources

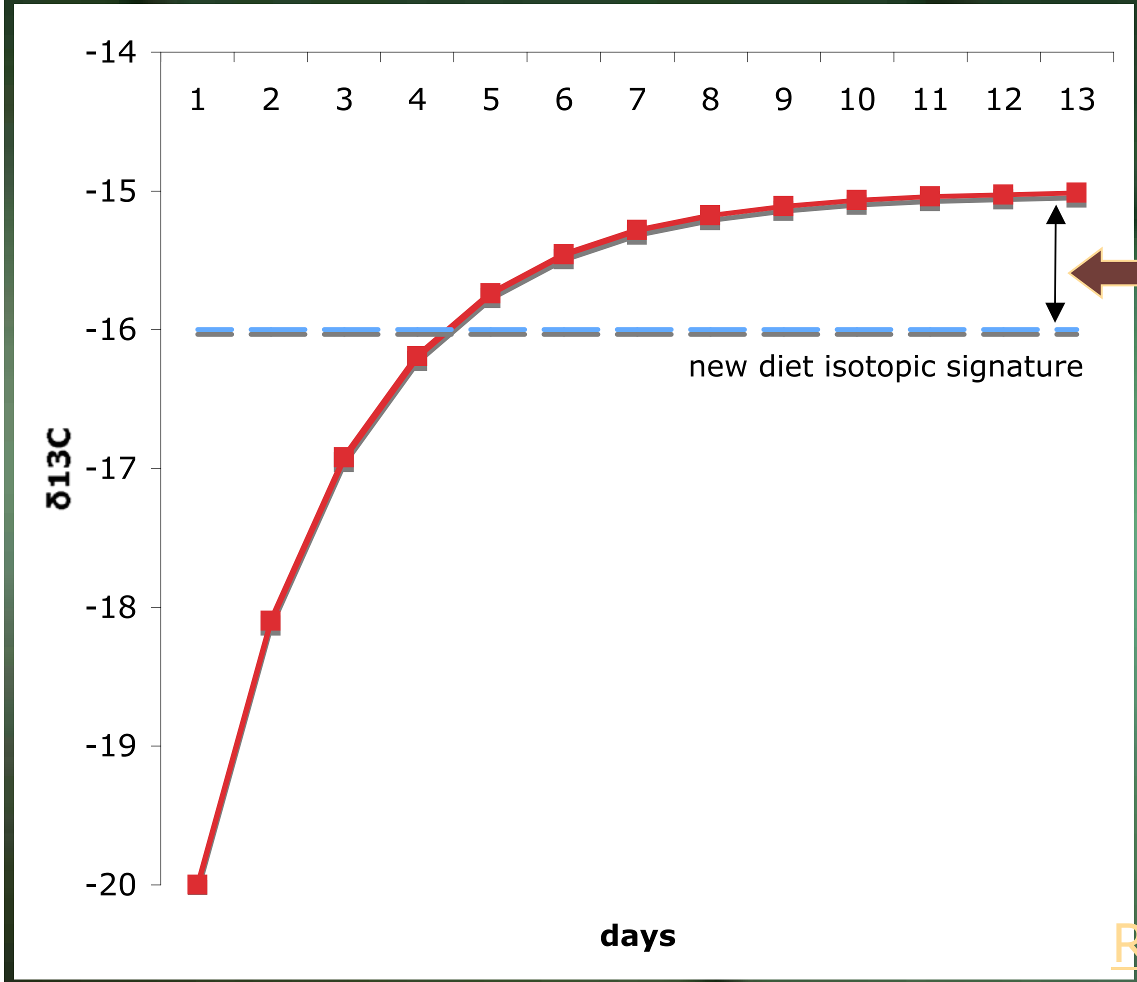
Selected for isotopic composition

Typical feeds under normal rearing conditions

eg Shlechtreim et al (2004)

Jomori et al (2008)

Tissue isotopic signature changes with diet



Discrimination factor

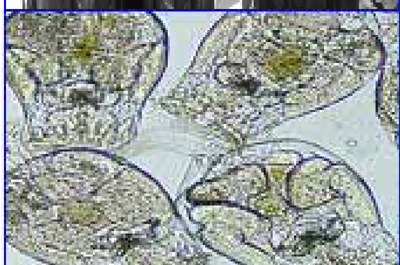
$\Delta^{13}C = \delta^{13}C_{consumer} - \delta^{13}C_{diet}$

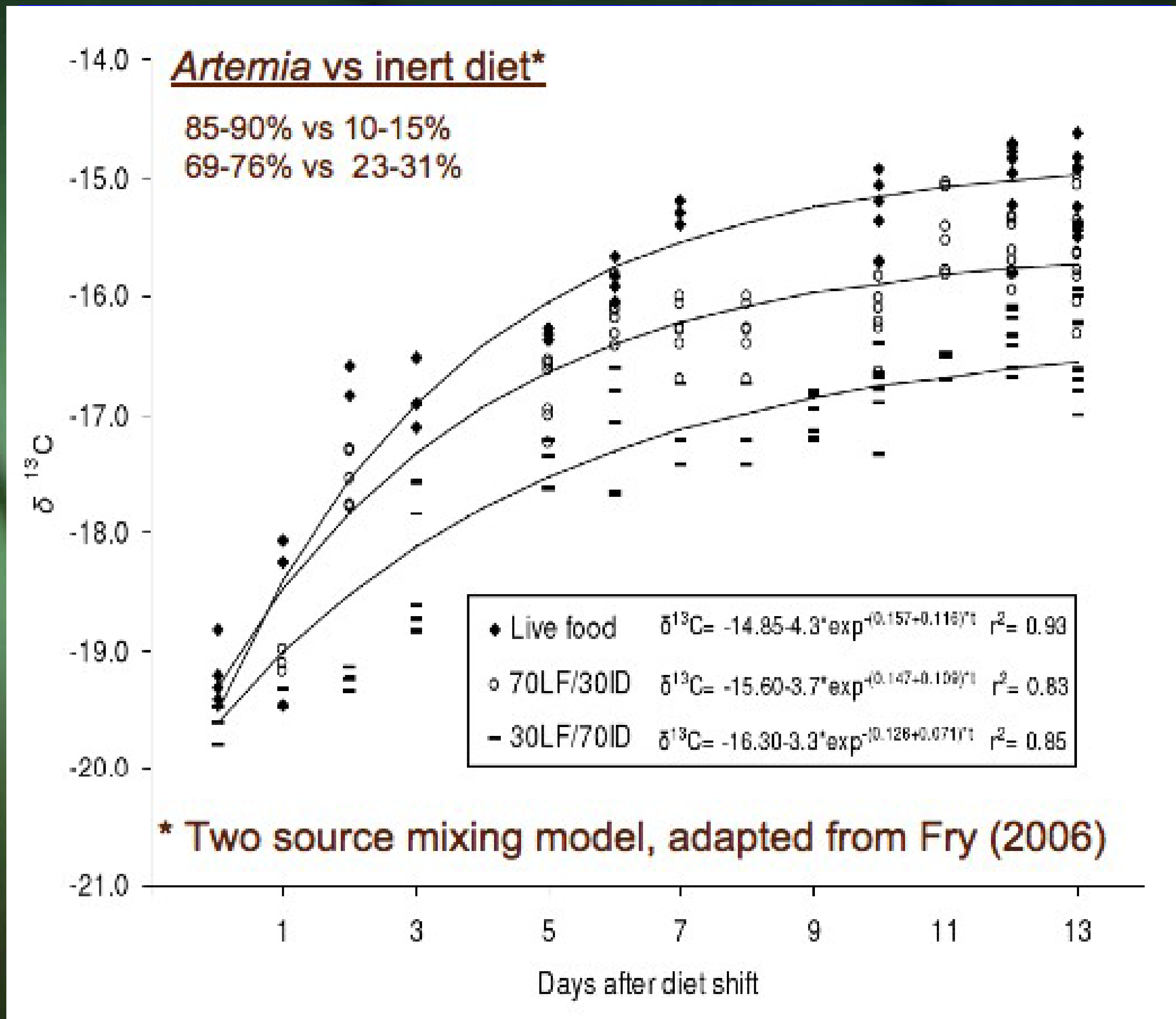
Rate of change

$$\delta^{13}C_t = \delta^{13}C_{ass} + (\delta^{13}C_{initial} - \delta^{13}C_{ass})e^{-(k+m)\cdot t}$$

Hesslein *et al* 1993, modified from Fry & Arnold (1982)

Contribution of live & inert feeds to tissue growth in Sole larvae*





*Gamboa Delgado et al 2008

Atlantic Arc Aquaculture Group

Nutrient assimilation and sources - application to compound feed components

Comparing 50% & 90% fishmeal replacement with soy protein

Soy-protein based diet:

$\delta^{13}C$ -25.36 ‰

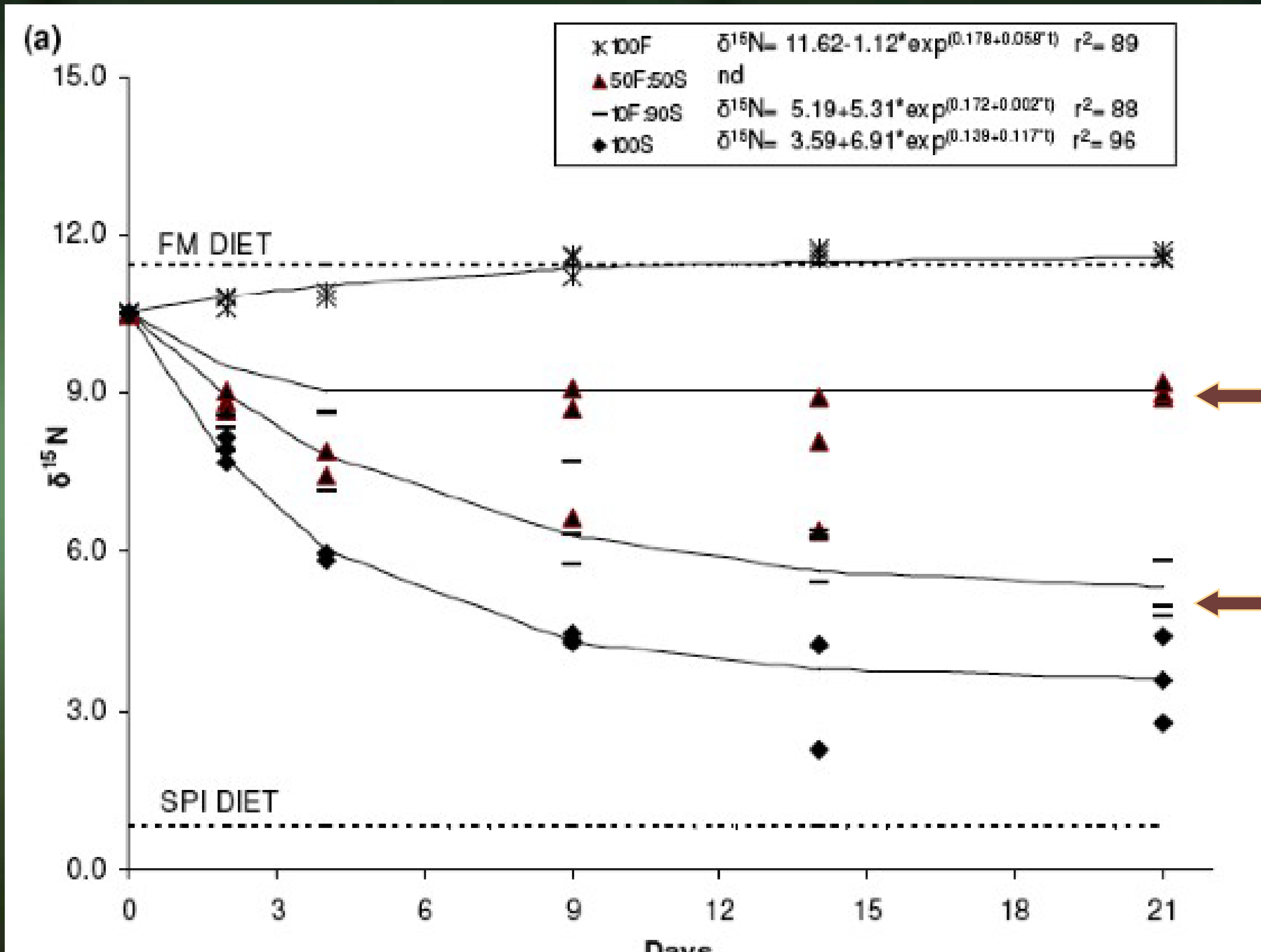
$\delta^{15}N$ 3.15 ‰

Fishmeal based diet :

$\delta^{13}C$ -20.74 ‰

$\delta^{15}N$ 9.26 ‰

Gamboa Delgado & Le Vay (2009)



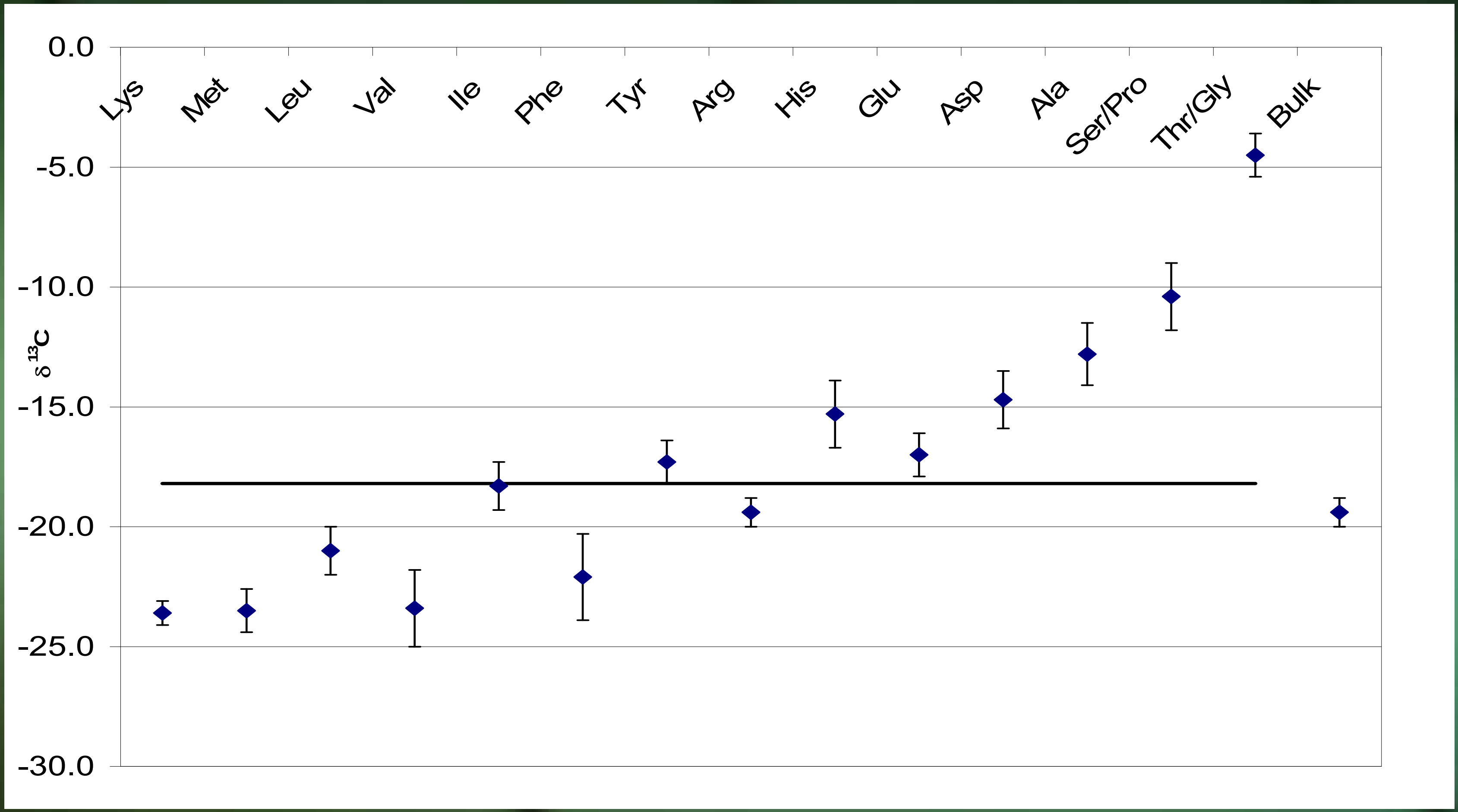
N sources:
fish:soya

Obs 69:31
Exp 50:50

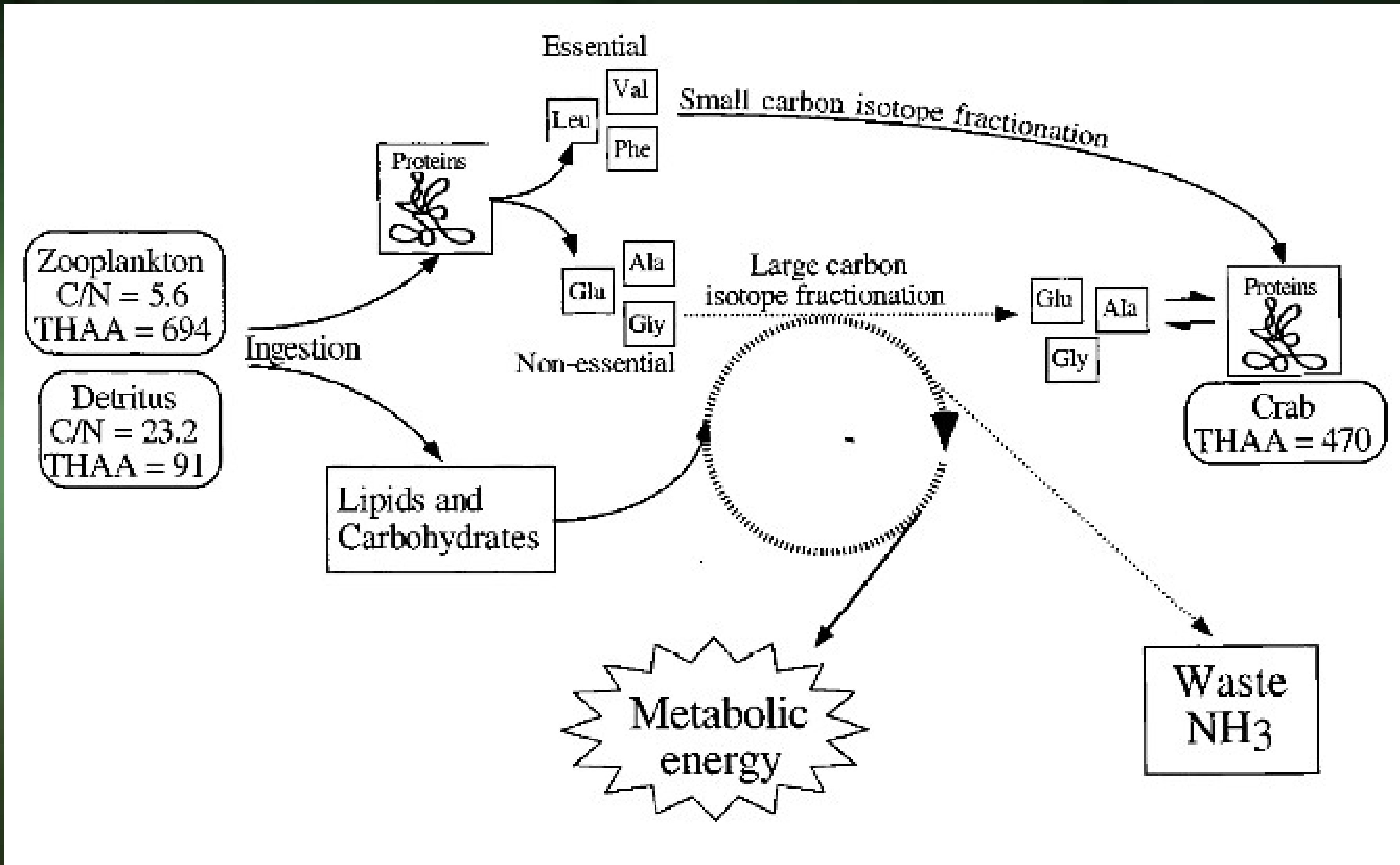
Obs 32:68
Exp 10:90

Nitrogen isotopic changes (‰) in muscle tissue of PL *L. vannamei* fed diets based on fish meal (FM) and soy protein isolate (SPI)

But.. individual amino acids behave differently



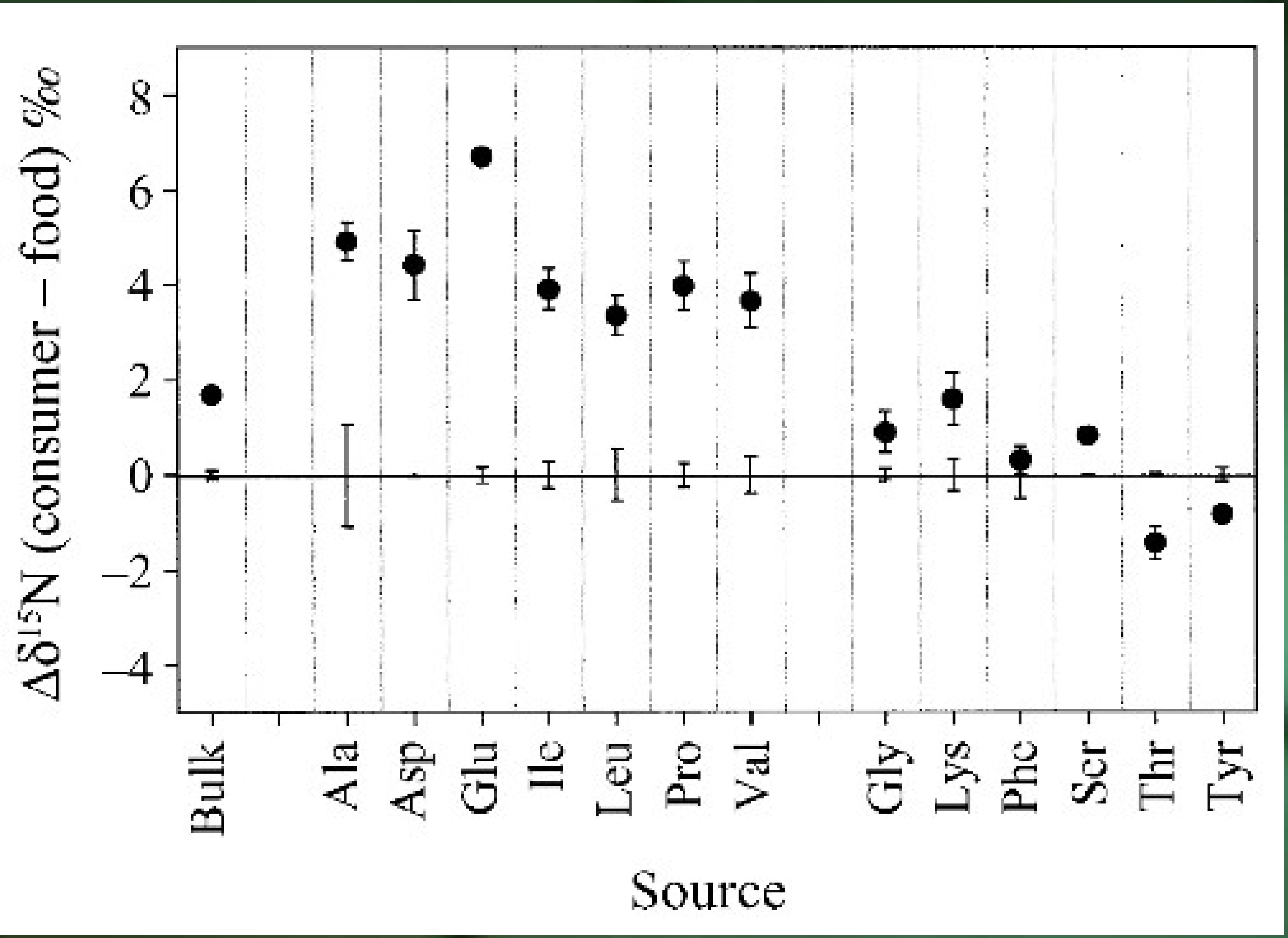
$\delta^{13}C$ of tissue amino acids following acid hydrolysis of marine shrimp tail muscle protein (750µg/ml) using a Dionex ICS3000 strong anion exchange chromatography system interfaced to a GVI Liquiface and Isoprime IRMS. (Preston, unpublished data)



Isotope fractionation of C associated with the tricarboxylic acid (TCA) cycle and other metabolic processes.

Frantle *et al* (1999)

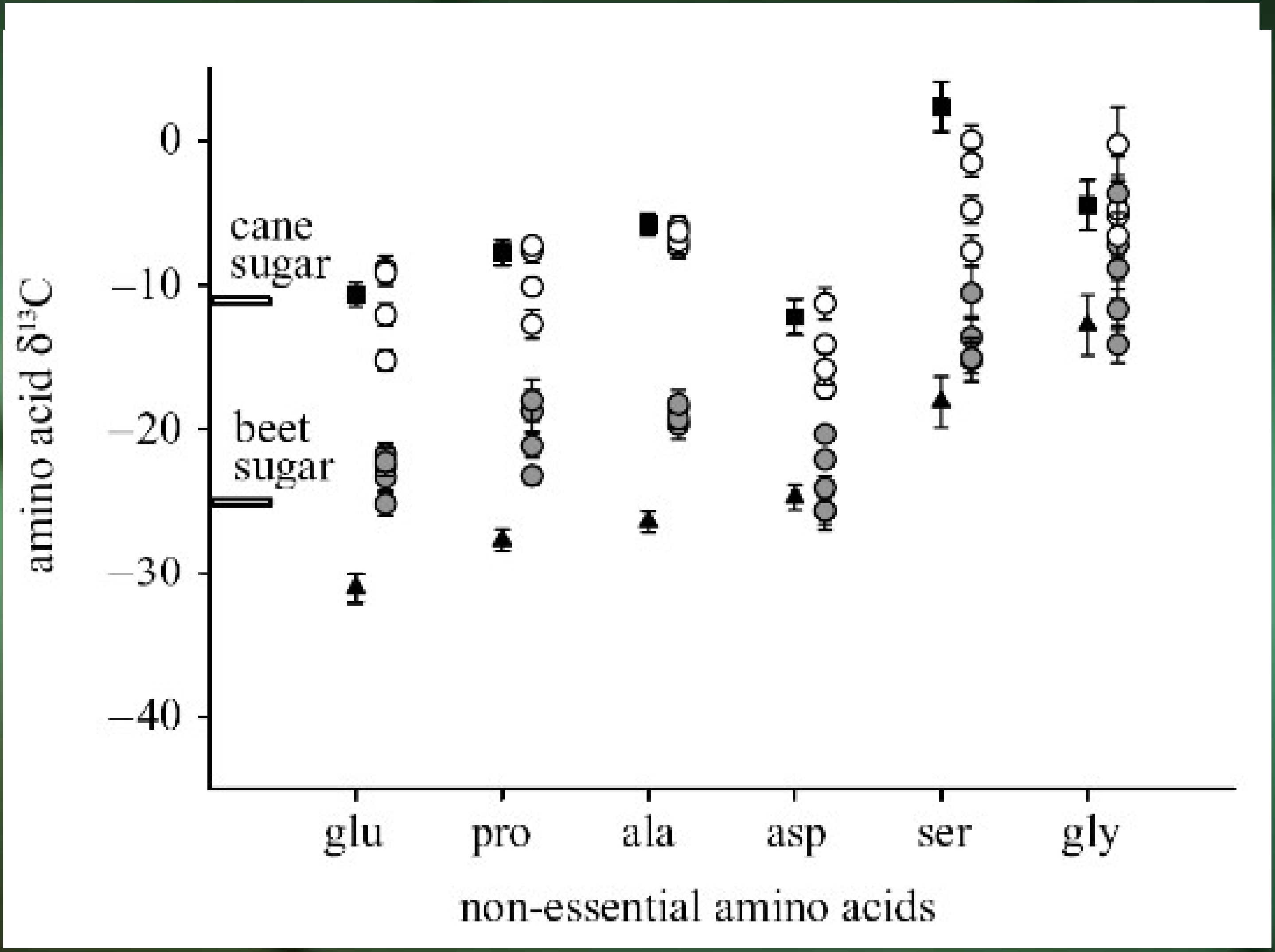
$\Delta^{15}N$ pattern less clear; also related to number of transamination steps



$\Delta^{15}N$ for bulk material and individual amino acids between *Brachionus plicatilis* and its food source (*Tetraselmis suecica*).

McLelland and Montoya (2002

Example from insects: pollen vs nectar as sources of carbon for amino acid synthesis sources of carbon in the butterfly *Heliconius**



Essential and non-essential amino acid $\delta^{13}C$ from pollen, larval host plant and eggs.

*O'Brien at al (2003)

Where next....

More on dietary sources and utilisation of inert feeds optimising co-feeding, inert feed ingestion and digestibility

Refinement of compound specific anaysis using LC-IRMS
(eg McCullagh et al 2008)

Amino-acid requirements with development/growth
(eg Berthold et al 1993)

Bioavailability of individual amino acids
(eg Saavedra et al 2007)

Utilisation of feed components
eg (Gamboa-Delgado & Le Vay 2009)

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