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

**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
Ingestion and protein turnover using enriched feeds

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

![15N labelled algae](image1)

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)**


See also Conceição *et al* (2007) for review
Longer term natural isotope signature changes

δ\(^{15}\)N \(\text{‰}\) 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

Tissue isotopic signature changes with diet

Discrimination factor

\[ \Delta^{13}C = \delta^{13}C_{\text{consumer}} - \delta^{13}C_{\text{diet}} \]

Rate of change

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

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

Artemia vs inert diet*

85-90% vs 10-15%
69-76% vs 23-31%

\[ \delta^{13}C = \text{linear equation} \]

Two source mixing model, adapted from Fry (2006)

*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)
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 analysis 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)


