Supporting information for

Feeding and growth variations affect δ13C and δ15N budgets during ontogeny in a lepidopteran larva

Samuel M. Charberet, Annick Maria, David Siaussat, Isabelle Gounand, Jérôme Mathieu

Contents

1. Food ingredients

Ingredient	% m/m
Deionized water	76.7
Soja meal	6.79
Corn flour	6.79
Germalyne	3.40
Yeast	2.55
Agar	1.20
Casein	7.19E-01
D-Glucose	6.01E-01
Ascorbic acid	5.10E-01
Benzoic acid	2.69E-01
Linseed oil	1.92E-01
Nipagin	1.16E-01
Choline chloride	5.41E-02
Formaldehyde	3.60E-02
Alpha-Tocopheryl acetate	1.59E-02
Actitetra (Oxytetracycline 50%)	9.59E-03
Ampicillin sodium salt	7.19E-03
Myo-inositol	3.61E-03
Nicotinic acid	3.21E-03
Menadione	1.62E-03
Retinyl acetate	1.30E-03
Riboflavin	7.21E-04
Pyridoxine	7.21E-04
Thiamine hydrochloride	7.21E-04
Ergocalciferol	9.02E-05
Folic acid	6.49E-05
Biotin	1.44E-05
Cobalamin	9.74E-07

Table 1 – Composition of the feed distributed to larvae, expressed as % mass/mass.

2. Isotope absorption efficiencies ratio (IAER) and C_{Ejk} / C_{Ijk} calculation

Mass spectrometer usually directly gives isotopes ratio rather than isotopic content because usually, one of the isotopes has a low concentration. Still, it is possible to compute the isotope content of egestion C_{Ejk} and intake C_{Ijk}. For carbon, ignoring the very low concentration in unstable isotopes, we have that the total carbon content is equal to the sum of the content of each stable isotope. So that, for sample s:

$$C_s = {}^{13}C_s + {}^{12}C_s$$

On the other hand the isotopic data are usually given in delta notation:

$$\delta^{13}C_s = 1000\left[\frac{{}^{13}C_s/{}^{12}C_s}{{}^{13}C_{PDB}/{}^{12}C_{PDB}} - 1\right]$$

We have thus two unknowns, ${}^{13}C_s$ and ${}^{12}C_s$, as well as two equations, enabling us to solve for the two isotopes content:

$$\frac{{}^{13}C_{PDB}}{{}^{12}C_{PDB}}\left(\frac{\delta^{13}C_s}{1000} + 1\right) = \frac{{}^{13}C_s}{{}^{12}C_s}$$

$$\frac{{}^{13}C_{PDB}}{{}^{12}C_{PDB}}\left(\frac{\delta^{13}C_s}{1000} + 1\right)(C_s - {}^{13}C_s) = {}^{13}C_s$$

$${}^{13}C_s = Cs\frac{\frac{{}^{13}C_{PDB}}{{}^{12}C_{PDB}}\left(\frac{\delta^{13}C_s}{1000} + 1\right)}{1 + \frac{{}^{13}C_{PDB}}{{}^{12}C_{PDB}}\left(\frac{\delta^{13}C_s}{1000} + 1\right)}$$

$${}^{12}C_s = \frac{C_s}{1 + \frac{{}^{13}C_{PDB}}{{}^{12}C_{PDB}}\left(\frac{\delta^{13}C_s}{1000} + 1\right)}$$

We have that $\frac{{}^{13}C_{PDB}}{{}^{12}C_{PDB}} \approx 0.0112372$, so, finally:

$${}^{12}C_s = \frac{C_s}{1 + 0.0112372\left(\frac{\delta^{13}C_s}{1000} + 1\right)}$$

Using the isotopic content, we can compute the absorption efficiency of each isotope.

3. Justification of the choice of linear models

We predicted that growth experienced during a given period at a certain rate would affect the isotopic content of the organism, that is, taking the example of carbon, which also holds for nitrogen:

$$13C_l = a.R + b$$

with $13C_l$ the 13C content in the larva, R the growth rate, a and b some constant, we should have $\delta 13C$ expressed as a function of growth rate R as follows:

$$\delta 13C_l = 1000\Big(\frac{c.a.R + b}{1 - a.R - b} - 1\Big)$$

which is a hyperbolic function of R (c here is the standard isotopes ratio constant). We should thus expect non-linearity. However, as $13C_l$ is very low, and making the approximation that for $x << 1$, $\frac{x}{x-1} \approx x$ we can model this relation using a linear approach. We nevertheless tested for non-linearity by performing generalized additive models and examining the effective degree of freedom (edf). For the two trophic fractionations and C IAER, the EDF indicate a linear dependence, with EDF roughly between 1 and 2 (table 2). We therefore chose to use linear models.

GAM formula	n	EDF	p-value	R^2
$\Delta 13C \sim$ s(GR)	92	2.03	<2e-16	0.368
$\Delta 15N \sim$ s(GR)	92	1	<2e-16	0.524
C IAER $\sim$ s(MSIR)	100	1	<2e-16	0.27

Table 2 – Generalized additive models results, with the sample size n, the effective degree of freedom (edf), p-value of the smooth term and the R^2.