Using stable isotopes to estimate trophic position

Loïc MICHEL – loicnmichel@gmail.com
Course "Etude des isotopes stables et applications au milieu marin"
What’s trophic position?

Trophic: relating to feeding and nutrition. From Greek τροφή (trophē): food, growth

Nyssen, 1997
What’s trophic position?

Trophic

Food chain: a succession of organisms in an ecological community that are linked to each other through the transfer of energy and nutrients.

Illustration from "Book of the animals", Al-Jahiz (776-869, Iraq). First known mention of the food chain concept.
Trophic position: the level at which a consumer is found in its food chain.
What’s trophic position?

In real-world ecosystems, consumers feed at multiple trophic levels: trophic position is not a discrete number, it is a continuous variable.

<table>
<thead>
<tr>
<th>Code</th>
<th>Species group</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH</td>
<td>Teleost fishes</td>
</tr>
<tr>
<td>CEPH</td>
<td>Cephalopods (squids, octopuses)</td>
</tr>
<tr>
<td>MOL</td>
<td>Molluscs (excluding cephalopods)</td>
</tr>
<tr>
<td>CR</td>
<td>Decapod crustaceans (shrimps, crabs, prawns, lobsters)</td>
</tr>
<tr>
<td>INV</td>
<td>Other invertebrates (all invertebrates except molluscs, crustaceans, and zooplankton)</td>
</tr>
<tr>
<td>ZOO</td>
<td>Zooplankton (mainly euphausids “krill”)</td>
</tr>
<tr>
<td>BIR</td>
<td>Seabirds</td>
</tr>
<tr>
<td>REP</td>
<td>Marine reptiles (sea turtles and sea snakes)</td>
</tr>
<tr>
<td>MAM</td>
<td>Marine mammals (cetaceans, pinnipeds, mustelids)</td>
</tr>
<tr>
<td>CHON</td>
<td>Chondrichthyan fishes (sharks, skates, rays, and chimaerids)</td>
</tr>
<tr>
<td>PL</td>
<td>Plants (marine plants and algae)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>N</th>
<th>FISH</th>
<th>CEPH</th>
<th>MOL</th>
<th>CR</th>
<th>INV</th>
<th>ZOO</th>
<th>BIR</th>
<th>REP</th>
<th>MAM</th>
<th>CHON</th>
<th>PL</th>
<th>Trophic level</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Carcharhinus acronotus</em></td>
<td>1</td>
<td>13</td>
<td>98.2</td>
<td>0.0</td>
<td>0.6</td>
<td>1.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>4.2</td>
</tr>
<tr>
<td><em>C. albimarginatus</em></td>
<td>2</td>
<td>15</td>
<td>75.0</td>
<td>12.5</td>
<td>0.0</td>
<td>0.0</td>
<td>6.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>6.3</td>
<td>4.2</td>
</tr>
<tr>
<td><em>C. altimus</em></td>
<td>3</td>
<td>22</td>
<td>43.3</td>
<td>13.4</td>
<td>0.0</td>
<td>3.3</td>
<td>3.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>36.7</td>
<td>4.3</td>
</tr>
<tr>
<td><em>C. amblyrhyynchoides</em></td>
<td>2</td>
<td>164</td>
<td>89.3</td>
<td>2.9</td>
<td>0.0</td>
<td>4.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2.9</td>
<td>0.0</td>
<td>4.2</td>
</tr>
<tr>
<td><em>C. amblyrhyynchus</em></td>
<td>9</td>
<td>253</td>
<td>69.2</td>
<td>16.6</td>
<td>0.0</td>
<td>12.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>28.0</td>
<td>4.1</td>
</tr>
<tr>
<td><em>C. amboinensis</em></td>
<td>3</td>
<td>136</td>
<td>56.3</td>
<td>5.6</td>
<td>2.0</td>
<td>7.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.7</td>
<td>0.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Why study trophic position?

Trophic position...

- is a single metric (easy to handle)
- allows estimation of energy flow through ecological communities
- is a simple way to compare organisms’ functional roles in natural ecosystems...
- ...yet can take into account complex and important processes (e.g. omnivory)

⇒ Trophic position is commonly used in trophic ecology
How to study trophic position?

Classical methods: *in situ* feeding observations and gut content analysis

Image: Julie-Anne O’Neill / National Geographic
Classic methods have limitations

- **Time-consuming**: representative sampling hard to achieve
- **Direct observations**: observer effect
- **Gut contents**: items can have different digestibility
- **Only provide a "snapshot" of the diet**
- **Info about ingestion**, but what about assimilation, and therefore energy and organic matter transfer?
Classic methods have limitations

- **Time-consuming**: representative sampling hard to achieve
- **Direct observations**: observer effect
- **Gut contents**: items can have different digestibility
- **Only provide a "snapshot" of the diet**

To overcome those limitations, classic methods can be complemented by integrative trophic markers, such as stable isotope ratios.
Due to the complex steps in digestion of proteins, the heavy nitrogen stable isotope \(^{15}\text{N}\) undergoes enrichment from diet to consumer tissue.

**ISOTOPE EFFECTS IN METABOLISM OF \(^{14}\text{N}\) AND \(^{15}\text{N}\) FROM UNLABELED DIETARY PROTEINS**

O. H. Gaebler, Trieste G. Vitti, and Robert Vukmirovich

Stable isotopes and trophic position

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$\delta^{15}$N follows a stepwise increase pattern along food chains.

This increase (trophic enrichment) is predictable.

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**Stepwise enrichment of $^{15}$N along food chains: Further evidence and the relation between $\delta^{15}$N and animal age**

Masao Minagawa* and Eitaro Wada
Geochimica et Cosmochimica Acta Vol. 48, pp. 1135-1140

If you measure the $\delta^{15}$N of a consumer and of the baseline item supporting it, you can infer the number of steps between the consumer and the food web baseline, i.e. its trophic position.
Stable isotopes and trophic position

Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes

Gilbert Cabana & Joseph B. Rasmussen
NATURE · VOL 372 · 17 NOVEMBER 1994

\[ TP = \frac{\delta^{15}N_{\text{Cons}} - \delta^{15}N_{\text{Base}}}{3.4} + 1 \]

With
\[ \delta^{15}N_{\text{Cons}} = \delta^{15}N \text{ of consumer of interest (here, lake fish)} \]
\[ \delta^{15}N_{\text{Base}} = \delta^{15}N \text{ of baseline supporting this consumer (here, zooplankton)} \]
3.4 = Mean trophic enrichment factor
1 = Trophic position of the baseline
Problem: In some ecosystems, isotopic composition of the baseline is highly variable temporally and/or spatially.

3.4 = Mean trophic enrichment factor
1 = Trophic position of the baseline
**Stable isotopes and trophic position**

Comparison of aquatic food chains using nitrogen isotopes

(food web/trophic level/sewage/eutrophication/nutrient cycling)

**Gilbert Cabana*† and Joseph B. Rasmussen**

\[
TP = \frac{\delta^{15}N_{Cons} - \delta^{15}N_{Prim}}{3.4} + 2
\]

With

\[\delta^{15}N_{Cons} = \delta^{15}N \text{ of consumer of interest}\]
\[\delta^{15}N_{Prim} = \delta^{15}N \text{ of a primary consumer belonging to the same food web}\]
3.4 = Mean trophic enrichment factor
2 = Trophic position of the primary consumer
Real-world food webs

Food webs are complex and dynamic...

Animals seldom (if ever) depend on a single baseline item

▲ In theory

In the real world ▼▼

Source: bas.ac.uk

Stable isotopes and trophic position

For fish feeding mostly in the littoral zone: the littoral baseline will be more important
For fish feeding mostly in the pelagic zone: the pelagic baseline will be more important
Stable isotopes and trophic position

If, for a single baseline

\[ TP = \frac{\delta^{15}N_{\text{Cons}} - \delta^{15}N_{\text{Base}}}{\Delta^{15}N} + \lambda \]

With

- \( \delta^{15}N_{\text{Cons}} = \delta^{15}N \) of consumer
- \( \delta^{15}N_{\text{Base}} = \delta^{15}N \) of "baseline"
- \( \Delta^{15}N = \) Trophic enrichment factor
- \( \lambda = \) Trophic position of "baseline"
Stable isotopes and trophic position

TP = \frac{\delta^{15}N_{\text{Cons}} - (\alpha \cdot \delta^{15}N_{B1} + (1 - \alpha) \cdot \delta^{15}N_{B2})}{\Delta^{15}N} + \lambda

With

- \delta^{15}N_{\text{Cons}} = \delta^{15}N \text{ of consumer}
- \delta^{15}N_{B1} = \delta^{15}N \text{ of "baseline" 1}
- \delta^{15}N_{B2} = \delta^{15}N \text{ of "baseline" 2}
- \Delta^{15}N = \text{Trophic enrichment factor}
- \lambda = \text{Trophic position of "baselines"}
- \alpha = \text{Contribution of baseline 1 to the diet of the consumer}
Stable isotopes and trophic position

Considering no isotopic fractionation of carbon:

\[ \delta^{13}C_{\text{Cons}} = \alpha . \delta^{13}C_{B1} + (1 - \alpha) . \delta^{13}C_{B2} \]
Using a suitable $\Delta^{15}\text{N}$ is important

3.4
Using a suitable $\Delta^{15}N$ is important

Literature analysis to pick values that make sense in the context of your study

Take into account environment, taxon, prey nature, etc.
Using a suitable $\Delta^{15}N$ is important


Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur

James H. McCutchan Jr, William M. Lewis Jr, Carol Kendall and Claire C. McGrath
Using a suitable $\Delta^{15}$N is important.

Table 3. Mean (± SE) estimates of trophic shift for C, N, and S; estimates for fluid-feeding consumers are excluded. Results of the Student’s t-test are given for each comparison. Statistically significant differences (p < 0.05) are indicated by *. High-protein diets include animal and microbial diets; low-protein diets include plant and algal diets.

<table>
<thead>
<tr>
<th>Consumer</th>
<th>$\Delta^{15}$C</th>
<th></th>
<th>$\Delta^{15}$N</th>
<th></th>
<th>$\Delta^{34}$S</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trophic shift</td>
<td>t-test</td>
<td>Trophic shift</td>
<td>t-test</td>
<td>Trophic shift</td>
<td>t-test</td>
</tr>
<tr>
<td>All animals</td>
<td>+0.5 ± 0.13 (102)</td>
<td></td>
<td>+2.3 ± 0.18 (73)</td>
<td></td>
<td>+0.5 ± 0.56 (12)</td>
<td></td>
</tr>
<tr>
<td>Diet type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular plants</td>
<td>+0.4 ± 0.28 (34)</td>
<td>t = 0.39;</td>
<td>+2.4 ± 0.42 (19)</td>
<td>t = 0.34;</td>
<td>-0.9 ± 0.61 (6)</td>
<td>t = 3.83;</td>
</tr>
<tr>
<td>All other diets</td>
<td>+0.5 ± 0.14 (68)</td>
<td>p = 0.70</td>
<td>+2.2 ± 0.20 (54)</td>
<td>p = 0.73</td>
<td>+1.9 ± 0.42 (6)</td>
<td>p = 0.003*</td>
</tr>
<tr>
<td>Protein content</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>+0.6 ± 0.16 (44)</td>
<td>t = 1.10;</td>
<td>+2.4 ± 0.22 (38)</td>
<td>t = 0.61;</td>
<td>+1.9 ± 0.51 (5)</td>
<td>t = 2.80;</td>
</tr>
<tr>
<td>Low</td>
<td>+0.5 ± 0.19 (58)</td>
<td>p = 0.27</td>
<td>+2.2 ± 0.30 (35)</td>
<td>p = 0.54</td>
<td>-0.5 ± 0.65 (7)</td>
<td>p = 0.019*</td>
</tr>
<tr>
<td>Metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poikilotherms</td>
<td>+0.4 ± 0.14 (91)</td>
<td>t = 1.13;</td>
<td>+2.3 ± 0.20 (65)</td>
<td>t = 0.45;</td>
<td>+0.5 ± 0.56 (12)</td>
<td></td>
</tr>
<tr>
<td>Homeotherms</td>
<td>+0.9 ± 0.37 (11)</td>
<td>p = 0.26</td>
<td>+2.0 ± 0.38 (8)</td>
<td>p = 0.66</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nitrogenous waste</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonia</td>
<td>+0.4 ± 0.18 (49)</td>
<td>t = 0.71;</td>
<td>+2.3 ± 0.28 (32)</td>
<td>t = 0.14;</td>
<td>+1.9 ± 0.51 (5)</td>
<td>t = 2.80;</td>
</tr>
<tr>
<td>Urea/uric acid</td>
<td>+0.5 ± 0.19 (53)</td>
<td>p = 0.48</td>
<td>+2.3 ± 0.24 (41)</td>
<td>p = 0.89</td>
<td>-0.5 ± 0.65 (7)</td>
<td>p = 0.019*</td>
</tr>
<tr>
<td>Environment</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Aquatic</td>
<td>+0.4 ± 0.17 (50)</td>
<td>t = 0.58;</td>
<td>+2.3 ± 0.28 (33)</td>
<td>t = 0.12;</td>
<td>+1.9 ± 0.51 (5)</td>
<td>t = 2.80;</td>
</tr>
<tr>
<td>Terrestrial</td>
<td>+0.5 ± 0.19 (52)</td>
<td>p = 0.56</td>
<td>+2.3 ± 0.24 (40)</td>
<td>p = 0.90</td>
<td>-0.5 ± 0.65 (7)</td>
<td>p = 0.019*</td>
</tr>
<tr>
<td>Analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole organism</td>
<td>+0.3 ± 0.14 (84)</td>
<td>t = 2.93;</td>
<td>+2.1 ± 0.21 (58)</td>
<td>t = 1.92;</td>
<td>-0.5 ± 0.65 (7)</td>
<td>t = 2.80;</td>
</tr>
<tr>
<td>Muscle</td>
<td>+1.3 ± 0.30 (18)</td>
<td>p = 0.004*</td>
<td>+2.9 ± 0.32 (15)</td>
<td>p = 0.090</td>
<td>+1.9 ± 0.51 (5)</td>
<td>p = 0.019*</td>
</tr>
<tr>
<td>Lipid removal (muscle)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid removed</td>
<td>+1.8 ± 0.29 (5)</td>
<td>t = 1.17;</td>
<td>+3.2 ± 0.43 (3)</td>
<td>t = 0.46;</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>+1.1 ± 0.35 (13)</td>
<td>p = 0.26</td>
<td>+2.8 ± 0.40 (12)</td>
<td>p = 0.065</td>
<td>+1.9 ± 0.51 (5)</td>
<td></td>
</tr>
<tr>
<td>Acidification (whole)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>+0.5 ± 0.17 (62)</td>
<td>t = 2.11;</td>
<td>+2.4 ± 0.24 (36)</td>
<td>t = 2.82;</td>
<td>-0.8 ± 0.81 (5)</td>
<td>t = 0.64;</td>
</tr>
<tr>
<td>Acidified</td>
<td>-0.2 ± 0.21 (22)</td>
<td>p = 0.038*</td>
<td>+1.1 ± 0.29 (15)</td>
<td>p = 0.007*</td>
<td>+0.2 ± 1.25 (2)</td>
<td>p = 0.55</td>
</tr>
</tbody>
</table>
Stable isotope ratios of consumers and baseline items, as well as trophic enrichment factors, are variable: natural variability + analytical error.
Stable isotope ratios of consumers and baseline items, as well as trophic enrichment factors, are variable: natural variability + analytical error.

A Bayesian model would allow to explicitly incorporate dispersion in trophic position estimates.
Bayesian estimation of trophic position

TROPHICPOSITION, an R package for the Bayesian estimation of trophic position from consumer stable isotope ratios

Claudio Quezada-Romegliulli1,2 | Andrew L. Jackson3 | Brian Hayden4,5 | Kimmo K. Kahilainen5,6 | Christelle Lopes7 | Chris Harrod1,2,8
Bayesian estimation of trophic position

APPLICATION

TROPHIC POSITION, an R package for the Bayesian estimation of trophic position from consumer stable isotope ratios

Claudio Quezada-Romegialli\textsuperscript{1,2} | Andrew L. Jackson\textsuperscript{3} | Brian Hayden\textsuperscript{4,5} | Kimmo K. Kahlilainen\textsuperscript{5,6} | Christelle Lopes\textsuperscript{7} | Chris Harrod\textsuperscript{1,2,8}

- Input data: $\delta^{15}N_{\text{Cons}}, \delta^{15}N_{\text{Base}}, \Delta^{15}N$ (optional: $\delta^{13}C_{\text{Cons}}, \delta^{13}C_{\text{Base}}, \Delta^{13}C$)
- Takes into account variability of SI ratios and TEFs
- Can be used for one or two baselines
- If two baselines: use of a mixing model to estimate $\alpha$ (you need carbon data)
- Output: distribution of solutions (credibility intervals)
- Allows comparisons of distributions
Bayesian estimation of trophic position

"Junk in, junk out" paradigm
Bayesian estimation of trophic position

"Junk in, junk out" paradigm

Bad data → Good model → Bad results
Bayesian estimation of trophic position

"Junk in, junk out" paradigm

Bad data → Good model → Bad results

Good data → Bad model → Bad results
Bayesian estimation of trophic position

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Good data → Bad model → Bad results

Good data → Good model → Good results
Bayesian estimation of trophic position

"Junk in, junk out" paradigm

Bad data → Good model → Bad results

Good data → Bad model → Bad results

When using tRophicPosition: you control the input data, but also the model (choice of baselines, model parameters)!

Good data → Good model → Good results
Using amino acid $\delta^{15}\text{N}$ to estimate TP

Nitrogen isotopic fractionation is linked with protein metabolism, but not all amino acids are affected in the same way...
Using amino acid $\delta^{15}\text{N}$ to estimate TP

Nitrogen isotopic fractionation is linked with protein metabolism, but not all amino acids are affected in the same way...

Essential amino acids (e.g. phenylalanine) cannot be synthesized by animals
Nitrogen isotopic fractionation is linked with protein metabolism, but not all amino acids are affected in the same way...

**Essential** amino acids (e.g. phenylalanine) cannot be synthesized by animals.

**Non-essential** amino acids (e.g. glutamic acid) can be synthesized by animals, and are involved in many metabolic reactions.
Using amino acid $\delta^{15}$N to estimate TP

Nitrogen isotopic fractionation is linked with protein metabolism, but not all amino acids are affected in the same way...

Trophic amino acids (x): undergo strong trophic fractionation. Their $\delta^{15}$N increase with each trophic level.

Source amino acids (y): undergo little trophic fractionation. Their $\delta^{15}$N reflect the one of the food web baseline.

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LIMNOLOGY
and
OCEANOGRAPHY: METHODS

Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids

Yoshito Chikaraishi*, Nanako O. Ogawa†, Yuichiro Kashiyama‡, Yoshinori Takano‡, Hisami Suga†, Akiko Tomitani†, Hideaki Miyashita‡, Hiroshi Kitazato‡, and Naohiko Ohkouchi†
Using amino acid $\delta^{15}N$ to estimate TP

Net $\delta^{15}N$ difference between trophic and source amino acids can be used to calculate trophic position

Trophic amino acids (x): undergo strong trophic fractionation. Their $\delta^{15}N$ increase with each trophic level.

Source amino acids (y): undergo little trophic fractionation. Their $\delta^{15}N$ reflect the one of the food web baseline.

**LIMNOLOGY and OCEANOGRAHY: METHODS**

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Using amino acid $\delta^{15}N$ to estimate TP

$$TP = \frac{\delta^{15}N_x - \delta^{15}N_y - \beta_{x,y}}{\Delta_x - \Delta_y} + 1$$

With

$\delta^{15}N_x = \delta^{15}N$ of trophic amino acid(s)
$\delta^{15}N_y = \delta^{15}N$ of source amino acid(s)
$\beta_{x,y} =$ Net $\delta^{15}N$ difference between trophic and source amino acids in primary producers
$\Delta_x =$ Trophic enrichment factor for trophic amino acid(s)
$\Delta_y =$ Trophic enrichment factor for source amino acid(s)
Using amino acid $\delta^{15}N$ to estimate TP

$$TP = \frac{\delta^{15}N_x - \delta^{15}N_y - \beta_{x,y}}{\Delta_x - \Delta_y} + 1$$

With

$\delta^{15}N_x = \delta^{15}N$ of trophic amino acid(s)

$\delta^{15}N_y = \delta^{15}N$ of source amino acid(s)

$\beta_{x,y} = $ Net $\delta^{15}N$ difference between trophic and source amino acids in primary producers

$\Delta_x = $ Trophic enrichment factor for trophic amino acid(s)

$\Delta_y = $ Trophic enrichment factor for source amino acid(s)

+ : No need to identify isotopic baseline, nor to sample or analyse it. The isotopic composition of this baseline is inferred from consumer’s tissues.

- : Sufficient knowledge of digestive metabolism in the studied species, and of associated trophic fractionation patterns, is required.
Thanks for your attention

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