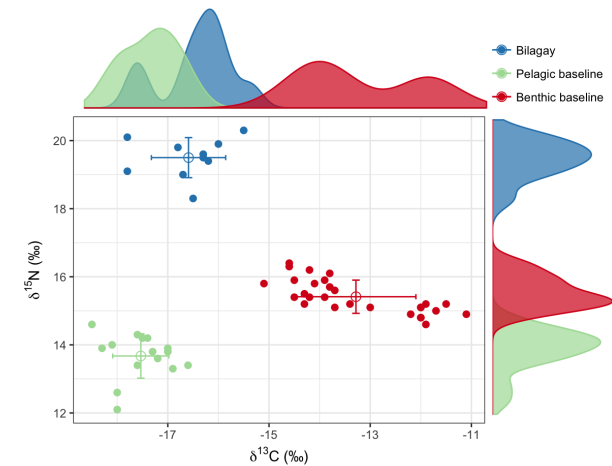
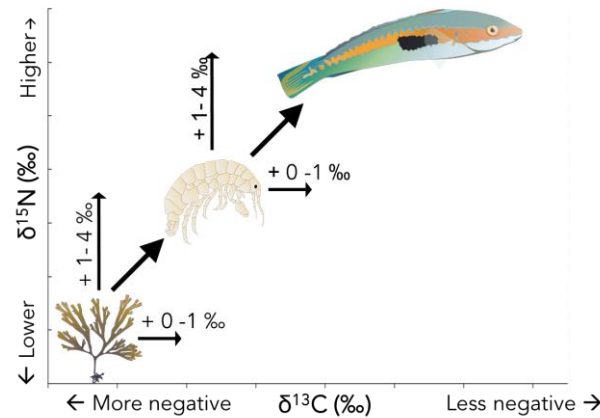
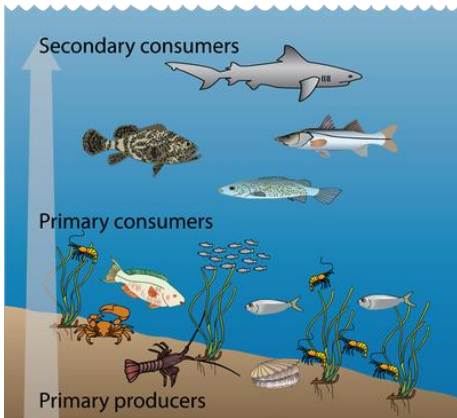


Using stable isotopes to estimate trophic position

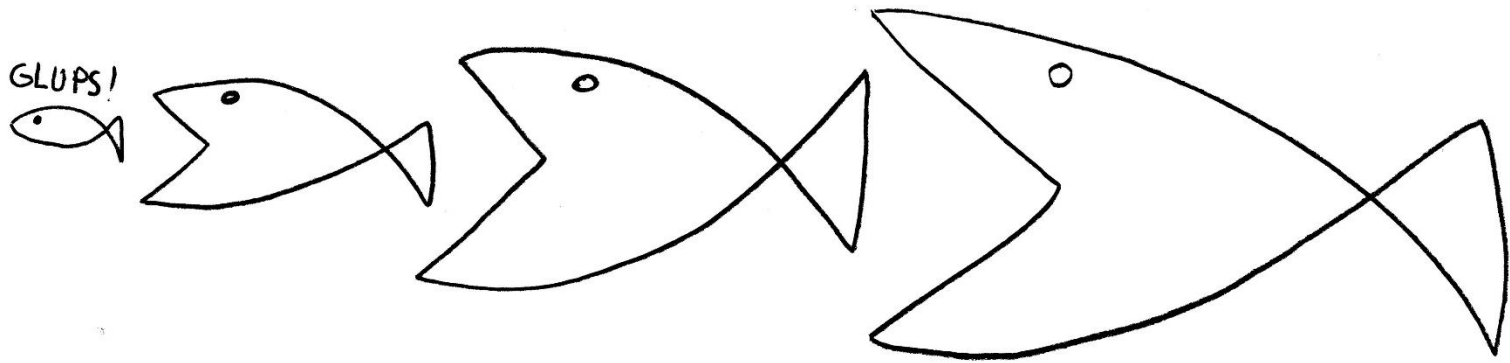


Loïc MICHEL – loicnmichel@gmail.com

Course "Etude des isotopes stables et applications au milieu marin"

What's trophic position?

Trophic: relating to feeding and nutrition. From Greek τροφή (trophê): 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.

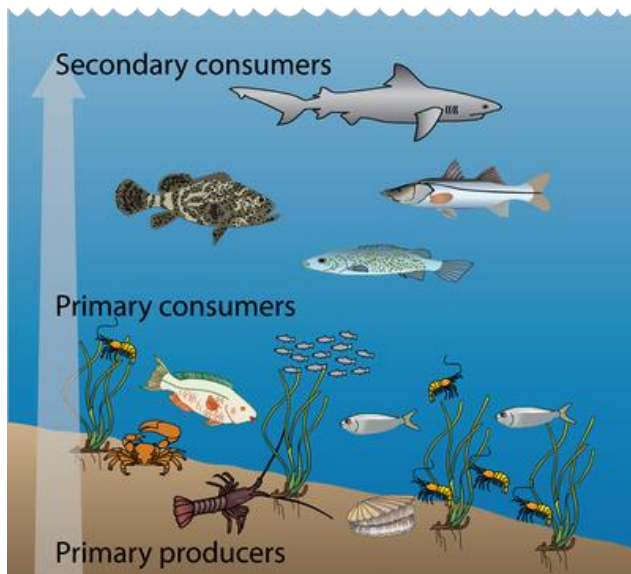


Illustration from "Book of the animals", Al-Jahiz (776-869, Iraq). First know mention of the food chain concept.

What's trophic position?

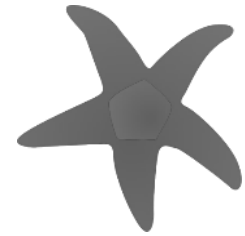
Trophic

Food chain

Trophic position: the level at which a consumer is found in its food chain

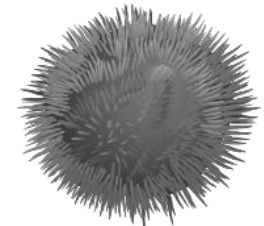
Secondary consumers

TP = 3



Primary consumers

TP = 2

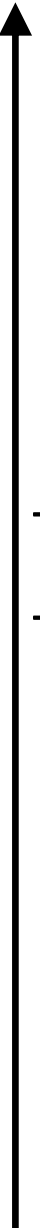


Primary producers

TP = 1



Trophic position



What's trophic position?

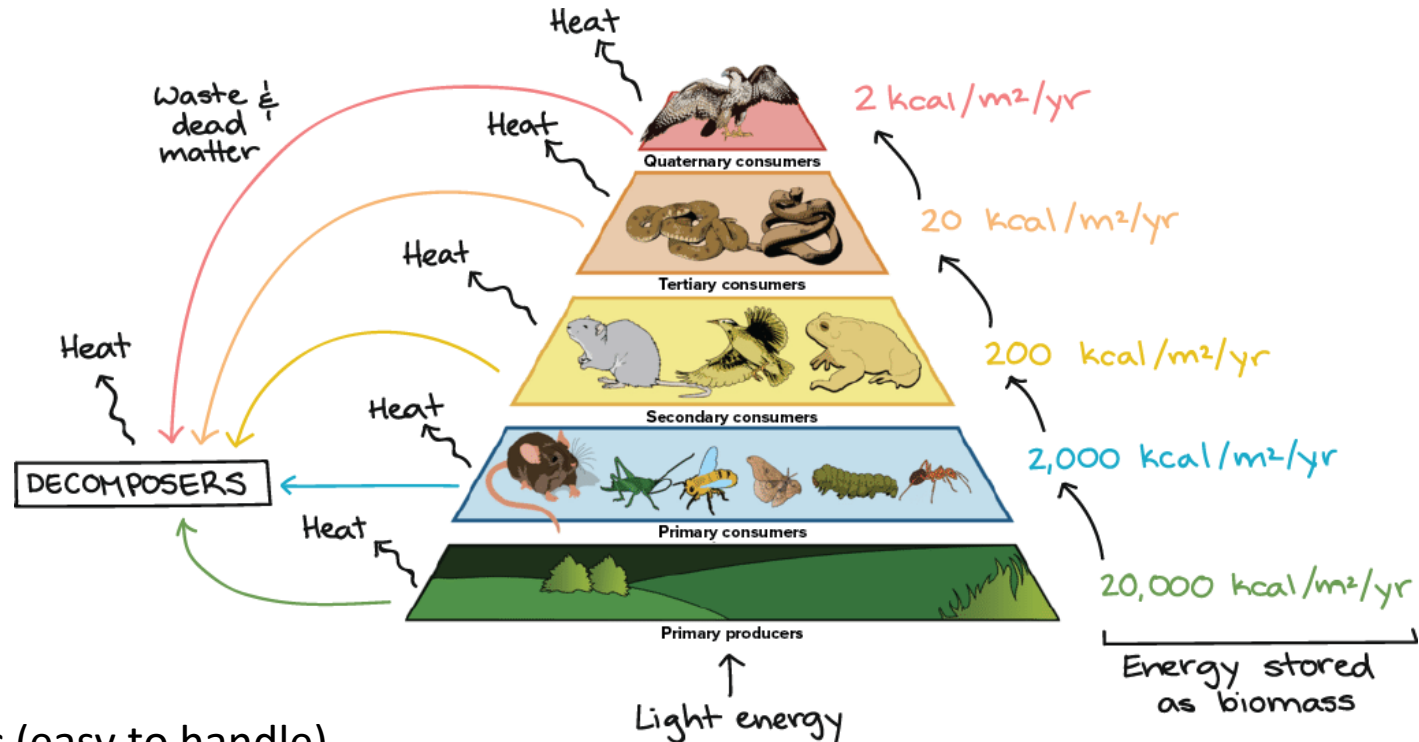
In real-world ecosystems, consumers feed at multiple trophic levels: trophic position is not a discrete number, it is a continuous variable



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

Species	n	N	FISH	CEPH	MOL	CR	INV	ZOO	BIR	REP	MAM	CHON	PL	Trophic level
Carcharhiniformes														
Carcharhinidae														
<i>Carcharhinus acronotus</i>	1	13	98.2	0.0	0.6	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2
<i>C. albimarginatus</i>	2	15	75.0	12.5	0.0	0.0	6.3	0.0	0.0	0.0	0.0	6.3	0.0	4.2
<i>C. altimus</i>	3	22	43.3	13.4	0.0	3.3	3.3	0.0	0.0	0.0	0.0	36.7	0.0	4.3
<i>C. amblyrhynchoides</i>	2	164	89.3	2.9	0.0	4.9	0.0	0.0	0.0	0.0	0.0	2.9	0.0	4.2
<i>C. amblyrhynchos</i>	9	253	69.2	16.6	0.0	12.7	0.0	0.0	0.0	0.0	0.0	0.0	1.4	4.1
<i>C. amboinensis</i>	3	136	56.3	5.6	2.0	7.4	0.0	0.0	0.0	0.0	0.7	28.0	0.0	4.3

Why study trophic position?



Trophic position...

- is a single metric (easy to handle)
- allows estimation of energy flow through ecological communities (e.g. food chain length)
- 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



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?

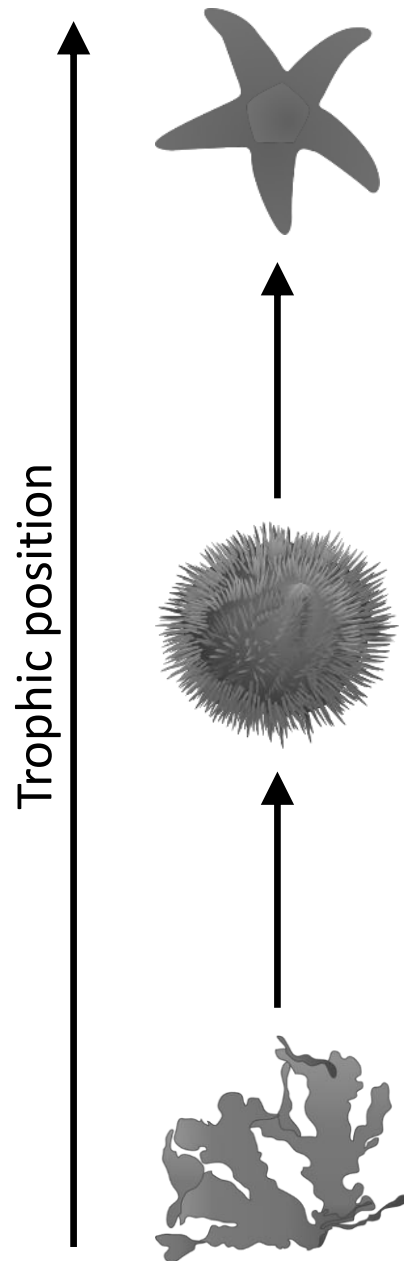


Classic methods have limitations

- **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**



Stable isotopes and trophic position



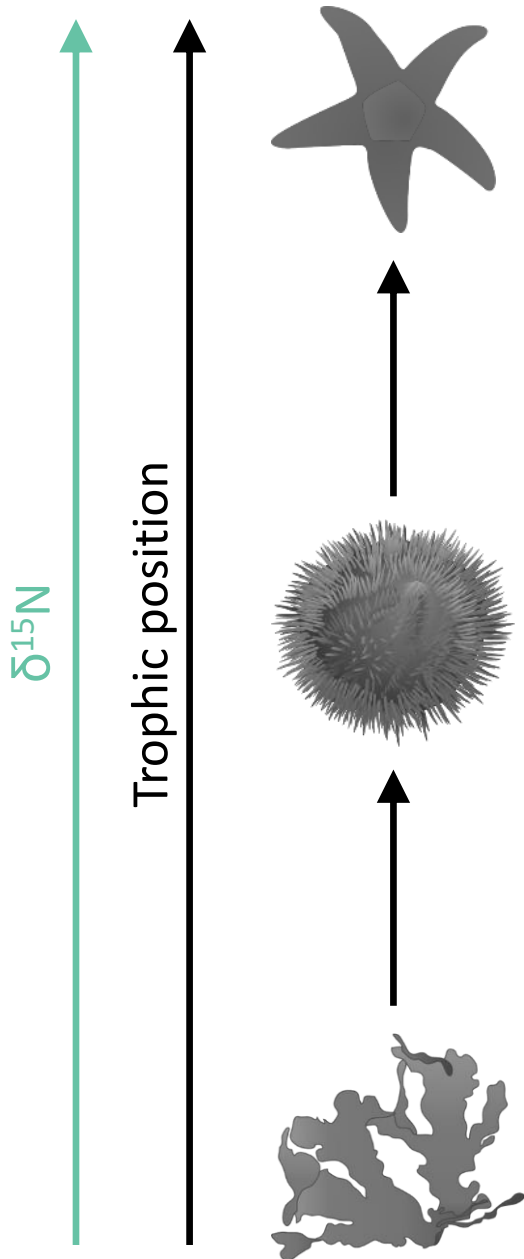
Due to the complex steps in digestion of proteins, the heavy nitrogen stable isotope (^{15}N) undergoes enrichment from diet to consumer tissue

ISOTOPE EFFECTS IN METABOLISM OF ^{14}N AND ^{15}N FROM UNLABELED DIETARY PROTEINS¹

O. H. GAEBLER, TRIESTE G. VITTI,² AND ROBERT VUKMIROVICH

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

This increase (trophic enrichment) is predictable



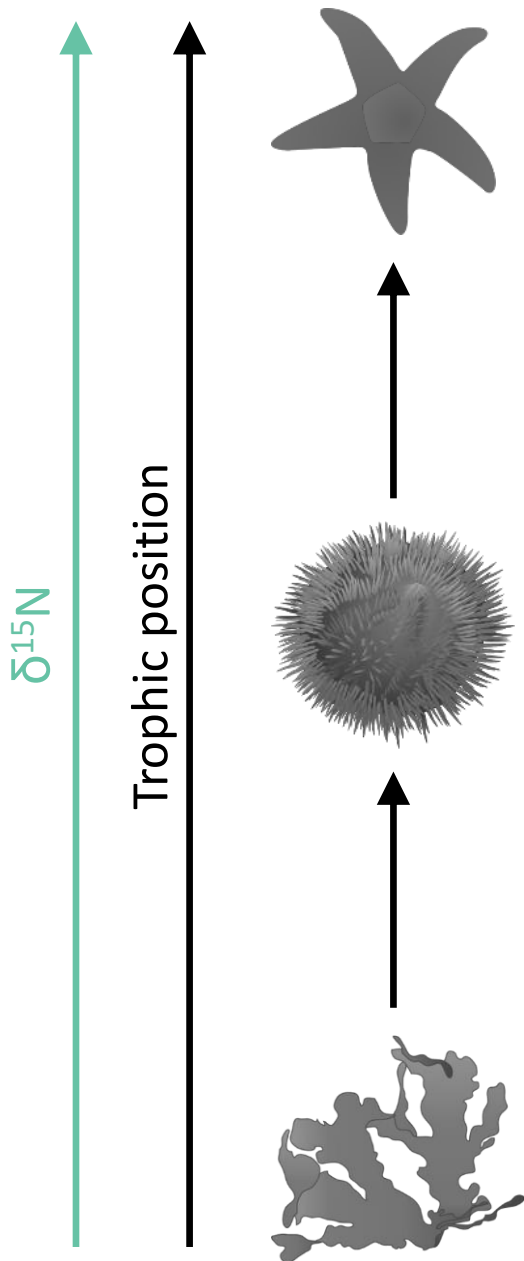
Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age

MASAO MINAGAWA* and EITARO WADA

1984

Geochimica et Cosmochimica Acta Vol. 48. pp. 1135-1140

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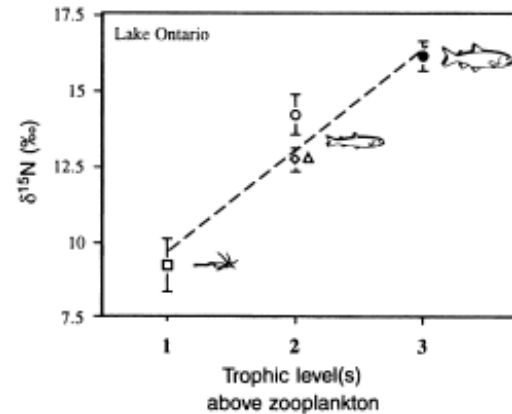
If you measure the $\delta^{15}\text{N}$ of a consumer and of the baseline item supporting it, you can infer the number of steps between the the consumer and the food web baseline, i.e. its trophic position

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



$$\text{TP} = \frac{\delta^{15}\text{N}_{\text{Cons}} - \delta^{15}\text{N}_{\text{Base}}}{3.4} + 1$$

With

$\delta^{15}\text{N}_{\text{Cons}}$ = $\delta^{15}\text{N}$ of consumer of interest (here, lake fish)

$\delta^{15}\text{N}_{\text{Base}}$ = $\delta^{15}\text{N}$ of baseline supporting this consumer (here, zooplankton)

3.4 = Mean trophic enrichment factor

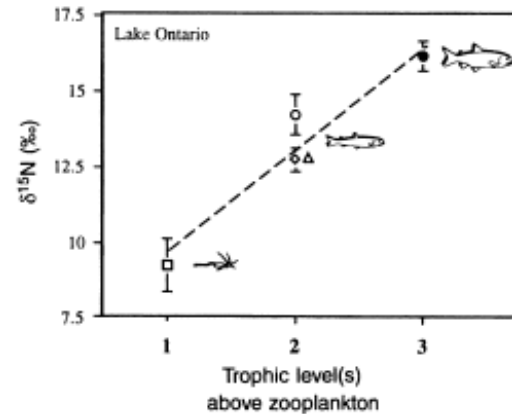
1 = Trophic position of the baseline

Stable isotopes and trophic position

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$$\text{TP} = \frac{\delta^{15}\text{N}_{\text{Cons}} - \delta^{15}\text{N}_{\text{Base}}}{3.4} + 1$$

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

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 10844–10847, October 1996

Comparison of aquatic food chains using nitrogen isotopes

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

GILBERT CABANA*† AND JOSEPH B. RASMUSSEN



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

With

$\delta^{15}N_{\text{Cons}}$ = $\delta^{15}N$ of consumer of interest

$\delta^{15}N_{\text{Prim}}$ = $\delta^{15}N$ of a primary consumer belonging to the same food web

3.4 = Mean trophic enrichment factor

2 = Trophic position of the primary consumer

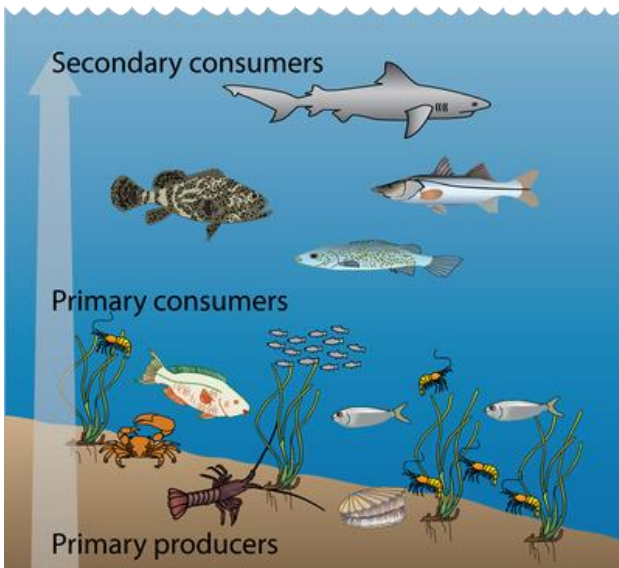


Unionidae mussels

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 ▼▶

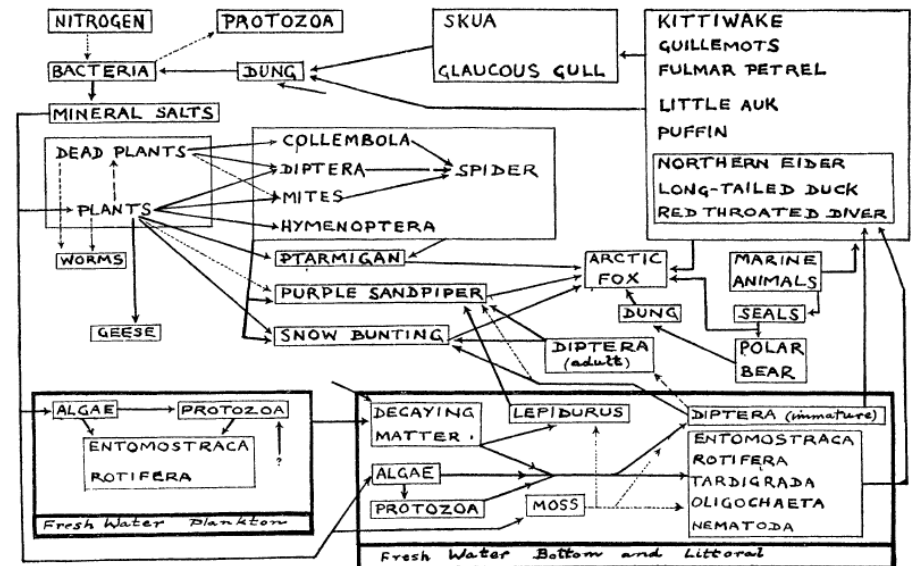
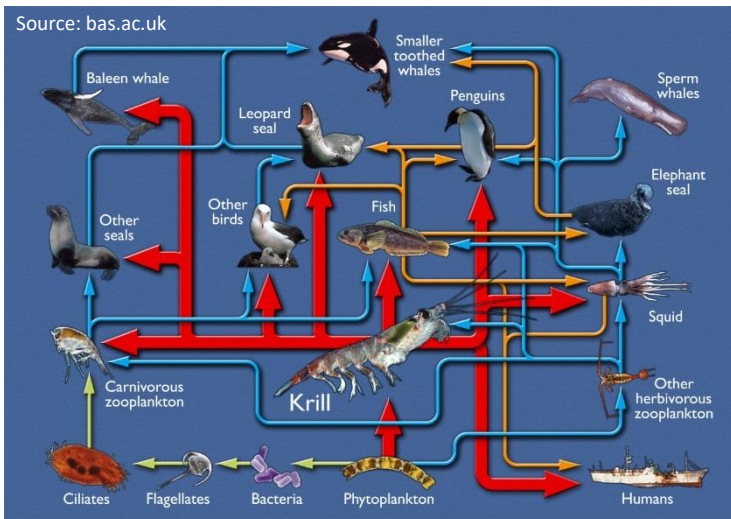


FIG. 2. Diagram of "Nitrogen Cycle" on Bear Island.

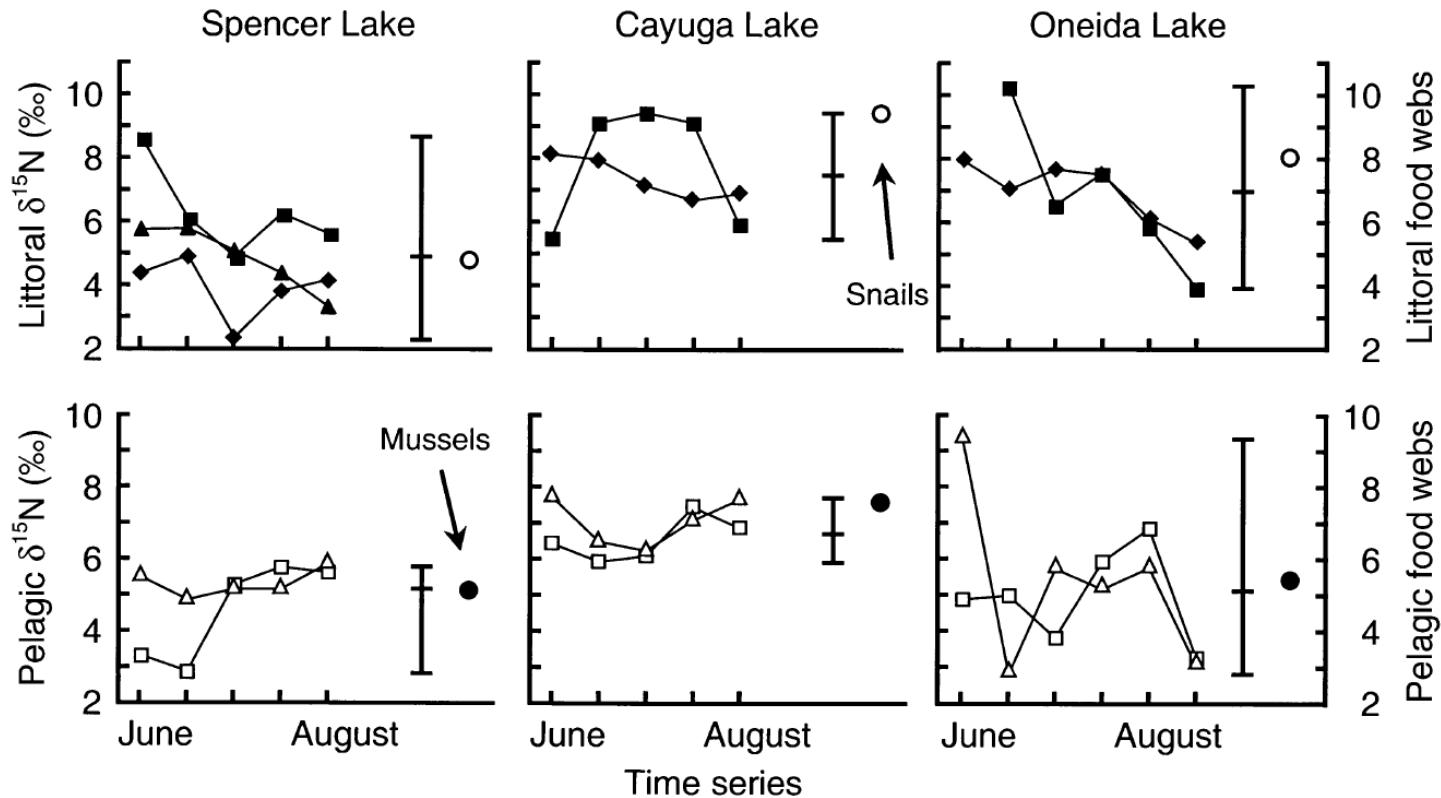
..... Probable, but no evidence from here.
 ----- Transformation.

Stable isotopes and trophic position

Ecology, 83(3), 2002, pp. 703–718
© 2002 by the Ecological Society of America

USING STABLE ISOTOPES TO ESTIMATE TROPHIC POSITION: MODELS, METHODS, AND ASSUMPTIONS

DAVID M. POST^{1,2,3}



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

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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

λ = Trophic position of "baseline"

Stable isotopes and trophic position

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Then, for two baselines

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

With

$\delta^{15}N_{\text{Cons}}$ = $\delta^{15}N$ of consumer

$\delta^{15}N_{B1}$ = $\delta^{15}N$ of "baseline" 1

$\delta^{15}N_{B2}$ = $\delta^{15}N$ of "baseline" 2

$\Delta^{15}N$ = Trophic enrichment factor

λ = Trophic position of "baselines"

α = Contribution of baseline 1 to the diet of the consumer

Stable isotopes and trophic position

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USING STABLE ISOTOPES TO ESTIMATE TROPHIC POSITION: MODELS, METHODS, AND ASSUMPTIONS

DAVID M. POST^{1,2,3}



How to estimate α ? With a (simple) mixing model based on carbon...

$$\alpha = \frac{\delta^{13}\text{C}_{\text{Cons}} - \delta^{13}\text{C}_{\text{B2}}}{\delta^{13}\text{C}_{\text{B1}} - \delta^{13}\text{C}_{\text{B2}}}$$

Considering no isotopic fractionation of carbon:

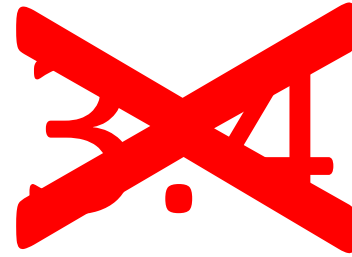
$$\delta^{13}\text{C}_{\text{Cons}} = \alpha \cdot \delta^{13}\text{C}_{\text{B1}} + (1 - \alpha) \cdot \delta^{13}\text{C}_{\text{B2}}$$

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



3.4

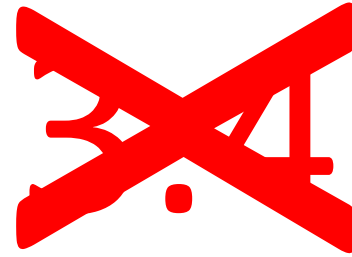
Using a suitable $\Delta^{15}\text{N}$ is important



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 $\Delta^{15}\text{N}$ is important



OIKOS 102: 378–390, 2003

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

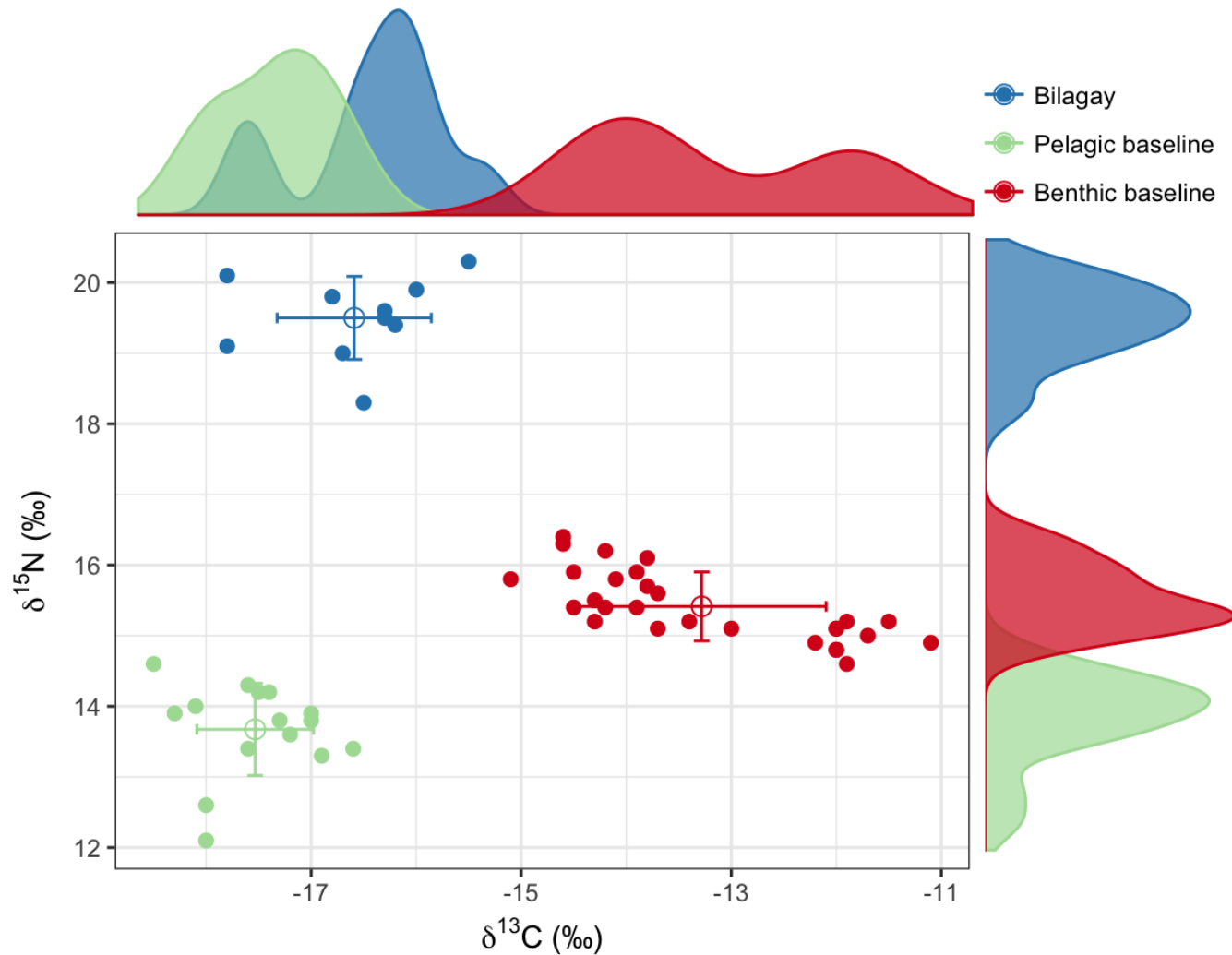
Using a suitable $\Delta^{15}\text{N}$ is important

Table 3. Mean (\pm 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.

Consumer	$\Delta\delta^{13}\text{C}$		$\Delta\delta^{15}\text{N}$		$\Delta\delta^{34}\text{S}$	
	Trophic shift	t-test	Trophic shift	t-test	Trophic shift	t-test
All animals	+0.5 \pm 0.13 (102)		+2.3 \pm 0.18 (73)		+0.5 \pm 0.56 (12)	
Diet type						
Vascular plants	+0.4 \pm 0.28 (34)	t = 0.39;	+2.4 \pm 0.42 (19)	t = 0.34;	-0.9 \pm 0.61 (6)	t = 3.83;
All other diets	+0.5 \pm 0.14 (68)	p = 0.70	+2.2 \pm 0.20 (54)	p = 0.73	+1.9 \pm 0.42 (6)	p = 0.003*
Protein content						
High	+0.6 \pm 0.16 (44)	t = 1.10;	+2.4 \pm 0.22 (38)	t = 0.61;	+1.9 \pm 0.51 (5)	t = 2.80;
Low	+0.5 \pm 0.19 (58)	p = 0.27	+2.2 \pm 0.30 (35)	p = 0.054	-0.5 \pm 0.65 (7)	p = 0.019*
Metabolism						
Poikilotherms	+0.4 \pm 0.14 (91)	t = 1.13;	+2.3 \pm 0.20 (65)	t = 0.45;	+0.5 \pm 0.56 (12)	-
Homeotherms	+0.9 \pm 0.37 (11)	p = 0.26	+2.0 \pm 0.38 (8)	p = 0.66	-	
Nitrogenous waste						
Ammonia	+0.4 \pm 0.18 (49)	t = 0.71;	+2.3 \pm 0.28 (32)	t = 0.14;	+1.9 \pm 0.51 (5)	t = 2.80;
Urea/uric acid	+0.5 \pm 0.19 (53)	p = 0.48	+2.3 \pm 0.24 (41)	p = 0.89	-0.5 \pm 0.65 (7)	p = 0.019*
Environment						
Aquatic	+0.4 \pm 0.17 (50)	t = 0.58;	+2.3 \pm 0.28 (33)	t = 0.12;	+1.9 \pm 0.51 (5)	t = 2.80;
Terrestrial	+0.5 \pm 0.19 (52)	p = 0.56	+2.3 \pm 0.24 (40)	p = 0.90	-0.5 \pm 0.65 (7)	p = 0.019*
Analysis						
Whole organism	+0.3 \pm 0.14 (84)	t = 2.93;	+2.1 \pm 0.21 (58)	t = 1.92;	-0.5 \pm 0.65 (7)	t = 2.80;
Muscle	+1.3 \pm 0.30 (18)	p = 0.004*	+2.9 \pm 0.32 (15)	p = 0.090	+1.9 \pm 0.51 (5)	p = 0.019*
Lipid removal (muscle)						
Lipid removed	+1.8 \pm 0.29 (5)	t = 1.17;	+3.2 \pm 0.43 (3)	t = 0.46;	-	-
No treatment	+1.1 \pm 0.35 (13)	p = 0.26	+2.8 \pm 0.40 (12)	p = 0.065	+1.9 \pm 0.51 (5)	
Acidification (whole)						
No treatment	+0.5 \pm 0.17 (62)	t = 2.11;	+2.4 \pm 0.24 (36)	t = 2.82;	-0.8 \pm 0.81 (5)	t = 0.64;
Acidified	-0.2 \pm 0.21 (22)	p = 0.038*	+1.1 \pm 0.29 (15)	p = 0.007*	+0.2 \pm 1.25 (2)	p = 0.55

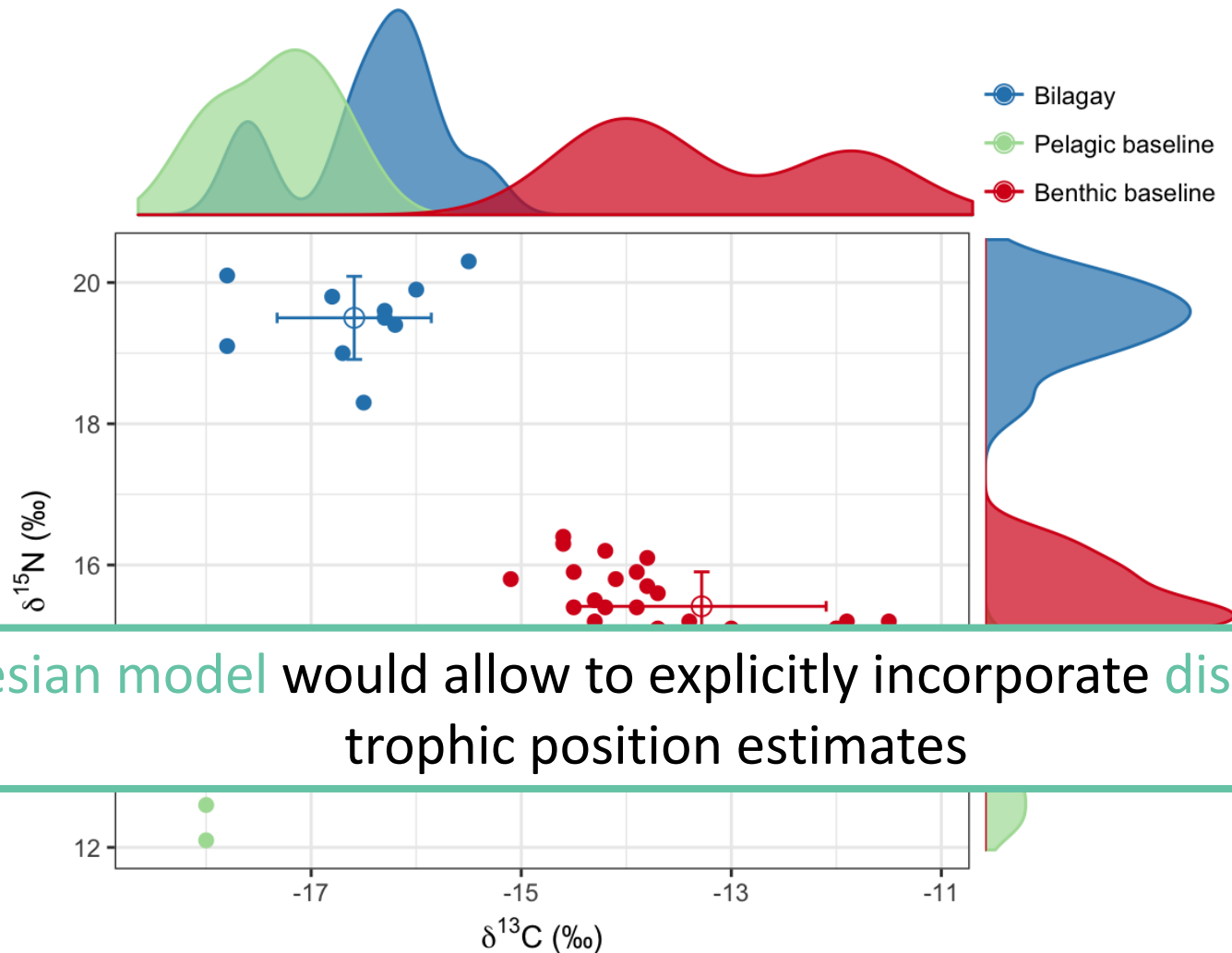
Fitting variability in the picture

Stable isotope ratios of consumers and baseline items, as well as trophic enrichment factors, are variable : natural variability + analytical error



Fitting variability in the picture

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

Received: 5 October 2017

Accepted: 13 March 2018







Methods Ecol Evol. 2018;9:1592–1599.

DOI: 10.1111/2041-210X.13009

APPLICATION

Methods in Ecology and Evolution 

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

Claudio Quezada-Romegialli^{1,2}  | Andrew L. Jackson³  | Brian Hayden^{4,5}  |
Kimmo K. Kahilainen^{5,6}  | Christelle Lopes⁷  | Chris Harrod^{1,2,8} 



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- Input data: $\delta^{15}\text{N}_{\text{Cons}}$, $\delta^{15}\text{N}_{\text{Base}}$, $\Delta^{15}\text{N}$ (optional: $\delta^{13}\text{C}_{\text{Cons}}$, $\delta^{13}\text{C}_{\text{Base}}$, $\Delta^{13}\text{C}$)
- 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 α (you need carbon data)
- Output: distribution of solutions (credibility intervals)
- Allows comparisons of distributions

Bayesian estimation of trophic position

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Practical course: get the files at
doi.org/10.5281/zenodo.3903263

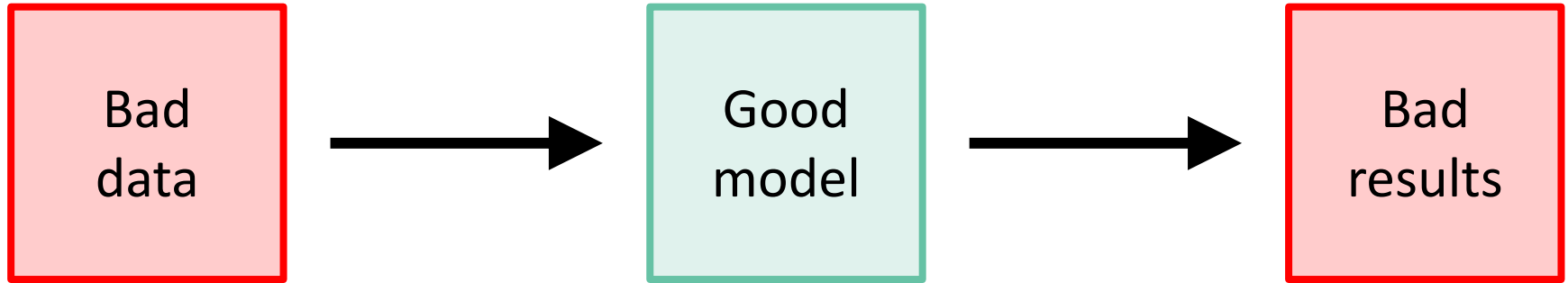
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Bayesian estimation of trophic position

"Junk in, junk out" paradigm

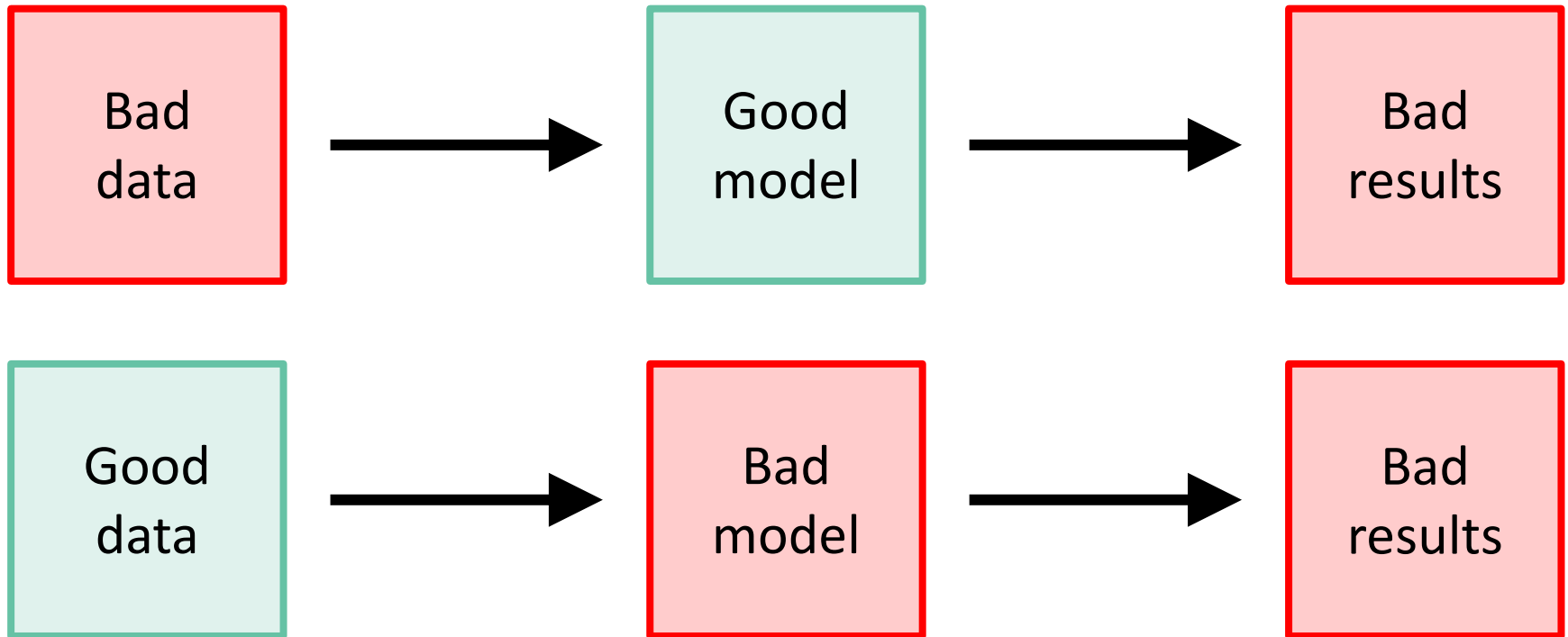
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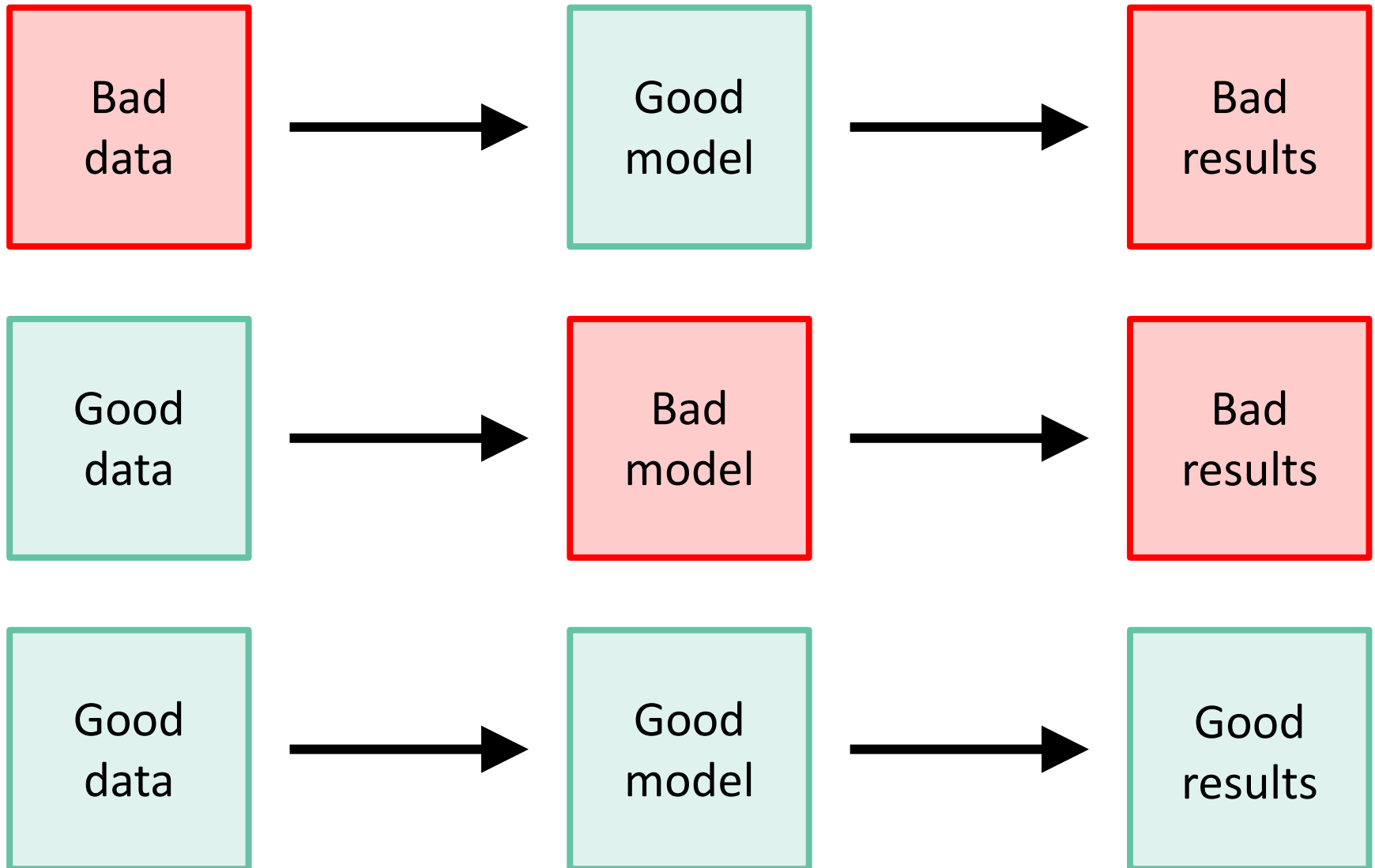
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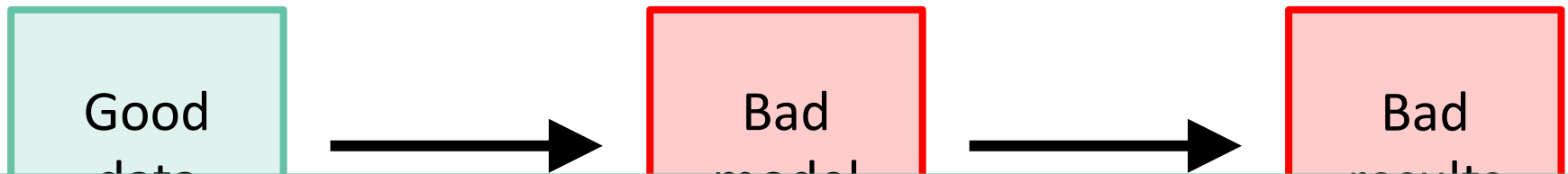
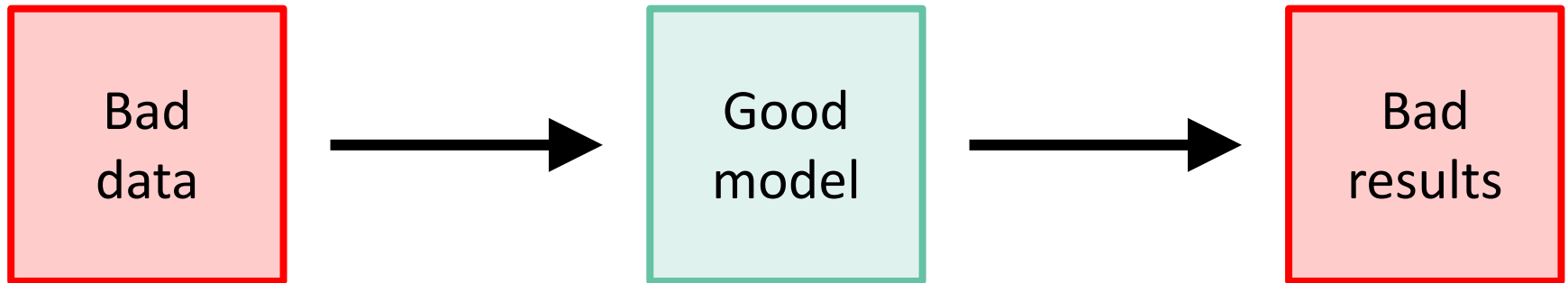
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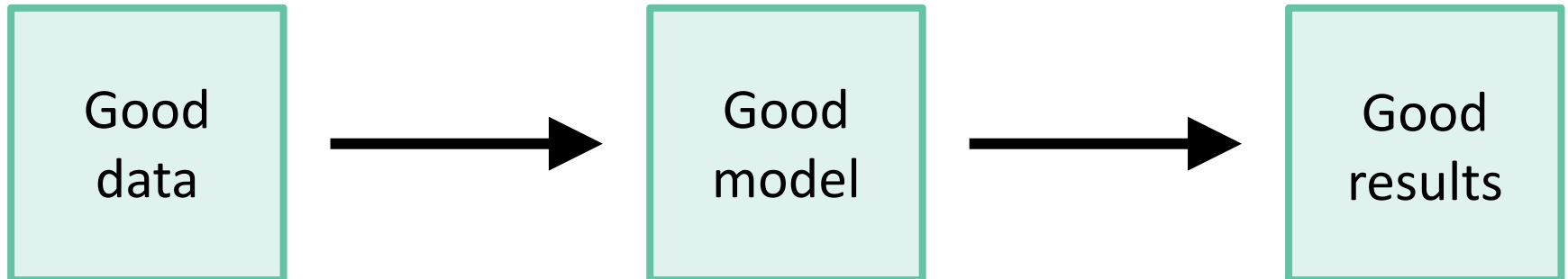


Bayesian estimation of trophic position

"Junk in, junk out" paradigm



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

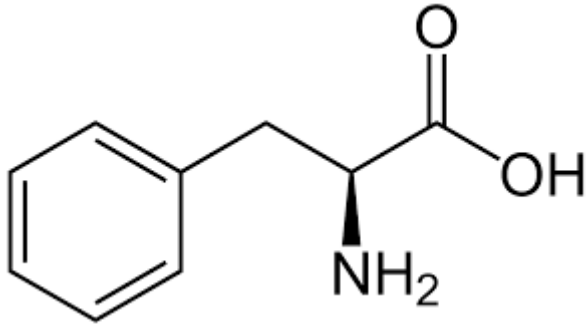


Using amino acid $\delta^{15}\text{N}$ to estimate TP

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

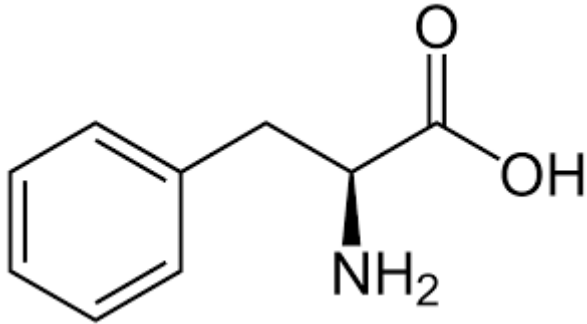
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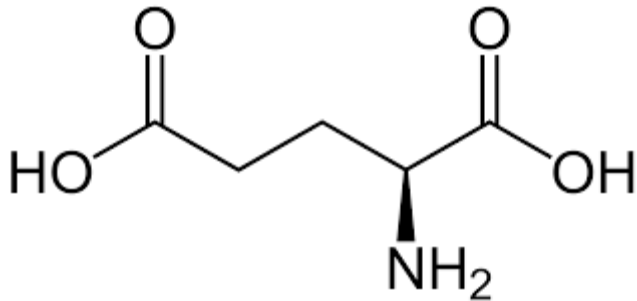
Essential amino acids (e.g. phenylalanine) cannot be synthesized by animals

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

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



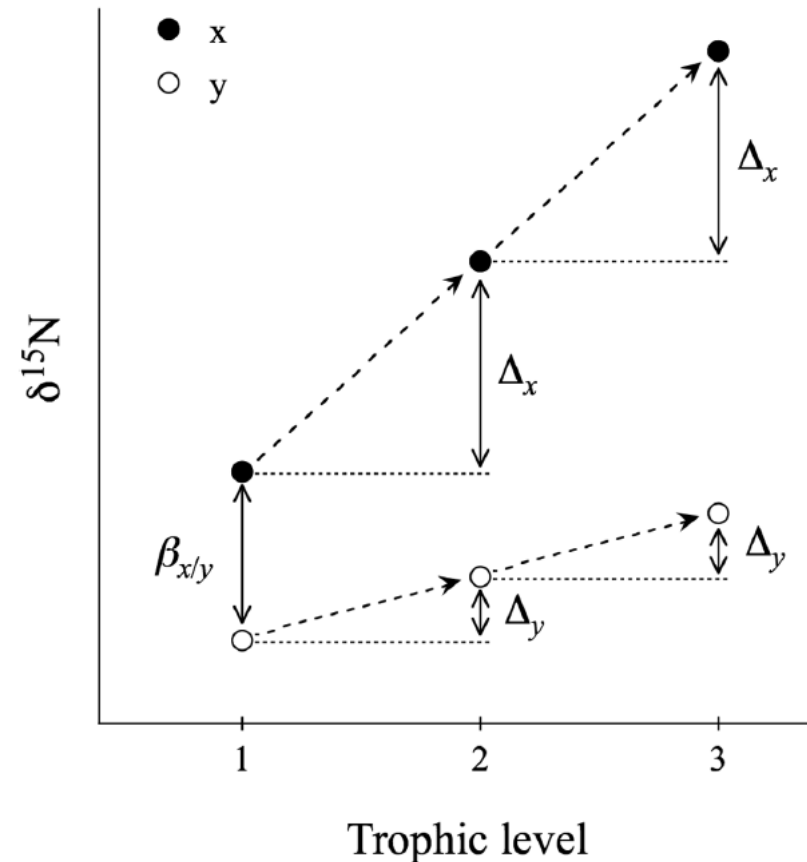
Non-essential amino acids (e.g. glutamic acid) can be synthesized by animals, and are involved in many metabolic reactions

Using amino acid $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ increase with each trophic level.

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



LIMNOLOGY and OCEANOGRAPHY: METHODS

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

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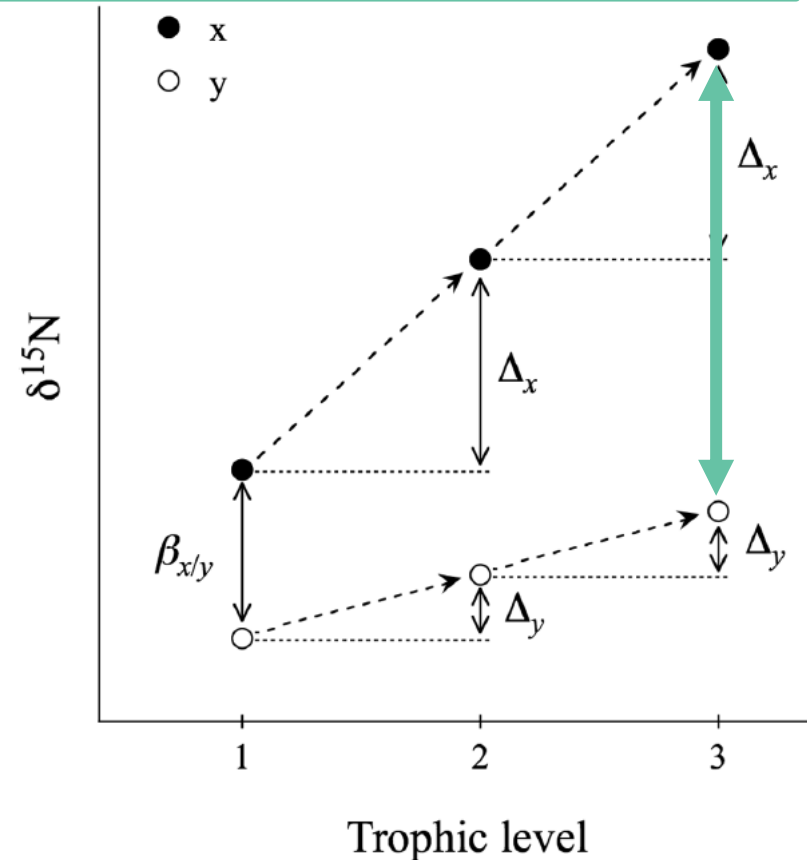
Limnol. Oceanogr.: Methods 7, 2009, 740-750
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Using amino acid $\delta^{15}\text{N}$ to estimate TP

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

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

$$\text{TP} = \frac{\delta^{15}\text{N}_x - \delta^{15}\text{N}_y - \beta_{x,y}}{\Delta_x - \Delta_y} + 1$$

With

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

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

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

Δ_x = Trophic enrichment factor for trophic amino acid(s)

Δ_y = Trophic enrichment factor for source amino acid(s)



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+ : No need to sample and analyse isotopic baseline, nor even to identify 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. Amino acid metabolism is complex, many open questions...



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Oecologia (2017) 184:317–326
DOI 10.1007/s00442-017-3881-9

CONCEPTS, REVIEWS AND SYNTHESSES

‘Trophic’ and ‘source’ amino acids in trophic estimation: a likely metabolic explanation

T. C. O’Connell¹

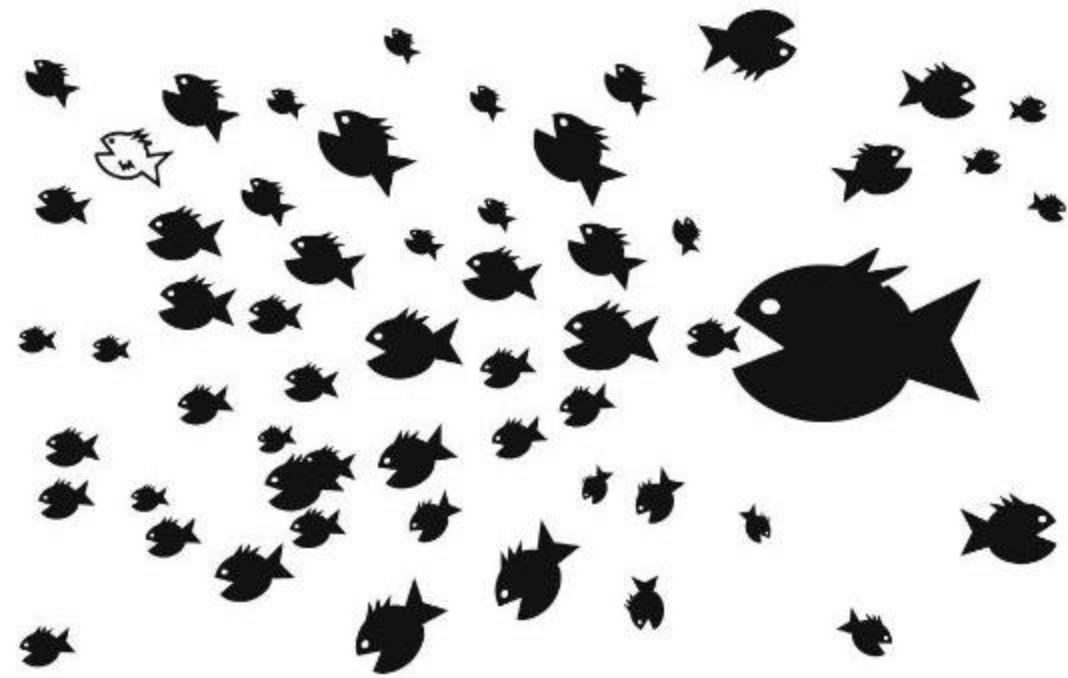
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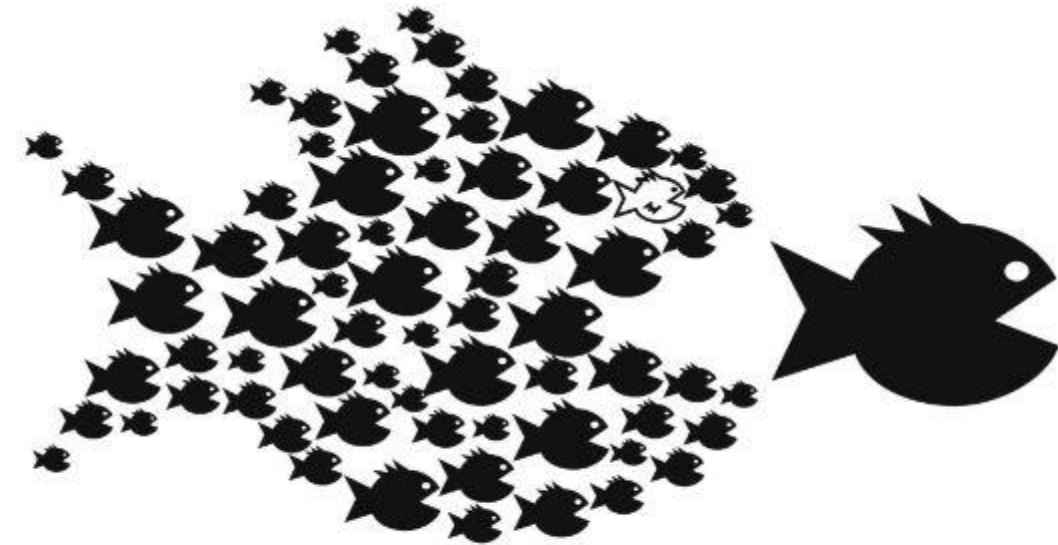


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Thanks for your
attention



ORGANIZE!

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