Consumer Amino Acid Nitrogen Isotopes
First Feeding Experiment: Amino Acid $\delta^{15}$N

“Trophic” Amino Acids: Ala/Asp/Glu/Pro/Ileu/Leu/Val

“Source” Amino Acids: Gly/Ser/Tyr/Lys/Phe/Thr

McClelland and Montoya 2002
Mechanisms: Trophic versus Source

Metabolic Nitrogen Pool

Amino Acid Group
- Asp#
- Pro#
- Val# Leu# Ile#
- Gln#
- Glu#
- free NH₃
- Ala#
- Lys†
- Met†
- Hist†
- Thr†
- Gly† Ser†
- Phe†
- Tyr†

# Trophic
† Source
‡ Metabolic

O’Connell 2017
Trophic Amino Acids: Glu/Ala/Asp/Pro/Ileu/Leu/Val

Source Amino Acids: Lys/Phe

Aquatic Food Web

\[ TP_{\text{Glu-Phe}} = \frac{\delta^{15}N_{\text{Glu}} - \delta^{15}N_{\text{Phe}} + 3.4}{7.6} + 1 \]

\[ \beta_{x/y} = 3.4 \pm 0.9\% \]
\[ \Delta_x = 8.0 \pm 1.2\% \]
\[ \Delta_y = 0.4 \pm 0.5\% \]
\[ TDF_{\text{Glu-Phe}} = \Delta_x - \Delta_y = 7.6 \pm 1.0\% \]

Chikaraishi et al. 2009, 2014
Sampling Approach:
13 Pacific Nations
Target & Bycatch Species (3 species)
~$4000 = 1 Satellite Tag
$8/day = Data
~$5000 for 3-month Deployment (1 Individual)
111 Elephant Seals = $555,000*
*does not include cost of deployment or data analysis
Tuna Bulk Tissue $\delta^{15}$N IsoScapes

*Thunnus albacares*

*Katsuwonus pelamis*

*Thunnus obesus*
A Common Problem with Mobile Marine Animals

**BASELINE?**

**TROPHIC LEVEL?**

OR

+1.5 ($\delta^{13}C$) +3.0 ($\delta^{15}N$)

+1.5 ($\delta^{13}C$) +3.0 ($\delta^{15}N$)

+1.5 ($\delta^{13}C$) +3.0 ($\delta^{15}N$)
Tuna Amino Acid $\delta^{15}$N: Baseline Shift

$$TF_{T-S} = 7\% \text{ per TL}$$

$$TL = 1 + \left(\frac{T_{AA} - S_{AA}}{7}\right)$$
Tuna Amino Acid $\delta^{15}$N: Trophic Level Shift

$$TF_{T-S} = 7\% \text{ per TL}$$

$$TL = 1 + \left(\frac{T_{AA} - S_{AA}}{7}\right)$$

Source AA

Trophic AA

Bulk Muscle

Popp et al. 2007
Tuna $\delta^{15}$N Variation is Driven by Baseline Shifts

$$TP_{\text{Trophic-Source}} = \frac{\delta^{15}\text{N}_{\text{Trophic}} - \delta^{15}\text{N}_{\text{Source}} + \beta_{\text{Trophic-Source}}}{TDF_{\text{Trophic-Source}}} + 1$$

Amino acid $\delta^{15}$N-based trophic level estimates ($4.3 \pm 0.5$) are similar to those based on stomach content analysis.
Defining Tuna Population Stocks in the Eastern Tropical Pacific

Geographical patterns in tuna $\delta^{15}N$ values reflect fluctuations in nitrogen isotopes at the base of the food web, not trophic level.

Can we estimate north-south movements?
And what can this tell us about tuna population stock structure?
Thunnus albacares

Move North-South?

Katsuwonus pelamis
Conclusions

Observed shifts in tuna bulk tissue $\delta^{15}N$ values reflect shifts in the nitrogen isotope baseline.

Isotope data suggest differences in net movements among species.

Yellowfin and skipjack tuna exhibit smaller-scale random movements with little net north-south directional movements (spatial population structure).

Bigeye tuna move quickly across large distances.
Influence of Physiology on Beta (β)?

\[
TP_{Trophic-Source} = \frac{\delta^{15}N_{Trophic} - \delta^{15}N_{Source} + \beta_{Trophic-Source}}{TDF_{Trophic-Source}} + 1
\]

Ramirez et al. 2021

Mostly Aquatic

Mostly Terrestrial

Conventional β_{aquatic}

Conventional β_{Terrestrial C4}

Eukaryotic microalgae
Cyanobacteria
Macrophytes
POM
Moss
Seagrass
Macrophyte
Cactus
Fern
Foliar
Grass
Vine
Shrub
Tree
Leaf litter
Some trophic-source pairs (Pro-Lys) show little change with dietary protein, while others (Glu-Phe) show a significant decrease.

Whiteman et al. 2021
Consumer–Diet $\Delta^{15}$N: Primary Consumers

Source

Aquatic, ammonia
Aquatic, urea/uric acid
Terrestrial, ammonia
Terrestrial, urea/uric acid

McMahon and McCarthy 2016
Consumer–Diet $\Delta^{15}$N: Tertiary Consumers

![Graph showing $\Delta^{15}$N values for different amino acids. The graph includes symbols representing different sources: Aquatic, ammonia (purple diamonds), Aquatic, urea/uric acid (blue diamonds), Terrestrial, ammonia (green squares), and Terrestrial, urea/uric acid (green squares). The graph is labeled with amino acids: Glu, Asp, Ala, Ile, Leu, Pro, Val, Gly, Ser, Phe, Lys, Met, and Thr. The x-axis represents the amino acids, and the y-axis represents $\Delta^{15}$N values in percent (‰).]
Diet Quality and Excretion Mode Matter

\[ f(x) = -2.3x + 5.1 \]
\[ r^2 = 0.61, P < 0.01 \]

Amino acid imbalance (Diet-consumer mol %)

\[ \Delta^{15}N_{C-D} (\%o) \]

1-2° consumer, ammonia
1-2° consumer, urea/uric acid
3+° consumer, ammonia
3+° consumer, urea/uric acid
Patterns in Amino Acid $\delta^{15}N$: Proxy for Nitrogen Balance?

Marion Island, South Africa

Mirounga leonina

Dr. Nico Lubcker

Dr. John Whiteman

Lubcker et al. 2016, 2017
\( \delta^{15}N \) Values of Many Amino Acids are Higher During Fasting

Fasting (endogenous)

Independent (exogenous)

Lubcker et al. 2020
Exogenous (dietary protein)

Endogenous (skeletal muscle)

\[ \Delta ^{15}N \text{N-AAs} \]

\[ ^{14}N\text{-Urea} \]

Gluconeogenesis: A Critical Pathway

Glucose

\[ \Phi \text{-enolpyruvate} \]

Pyruvate

\[ \text{Oxaloacetate} \]

\[ \alpha \text{-Ketoglutarate} \]

Fumarate

\[ \text{Succinyl-CoA} \]

Ala, Ser, Gly, Glu, Pro, Asp Phe, Tyr (glucogenic)

\[ 15^N\text{-AAs} \]

(plasma)

\[ ^{14}N\text{-Urea} \]

(pee)
Alanine $\delta^{15}N$ is Lower During Fasting

Lubcker et al. 2020
Interorgan cycle that transports nitrogen from skeletal muscle to the liver using alanine as a carrier during nutritional stress (i.e., fasting).
No Change in Branch-Chained AAs and Glutamic Acid $\delta^{15}N$

![Graph showing amino acids and $\delta^{15}N$ values](image)

- **[N]**
- **Branch-Chained BCAT Enzymes**
- **Fasting (endogenous)**
- **Independent (exogenous)**

Lubcker et al. 2020

Amino Acid:
- Ala
- Asp
- Glu
- Ile
- Leu
- Val
- Pro
- Gly
- Ser
- Phe
- Lys
- Tyr

$\delta^{15}N$ values for each amino acid are plotted on the graph.
Significant (3-5‰) decreases in $\delta^{15}N$ from early (yolk) to late (placenta) term pregnancy in nearly all AAs (except Phe).

Preliminary results suggest either (1) protein sparing (decrease in $\Delta^{15}N_{\text{consumer-diet}}$), and/or (2) nitrogen ($^{14}N$ urea) recycled for reproduction.
Reproduction and Nitrogen Balance?
Balaenoptera musculus

Positive $\delta^{15}$N anomalies in females versus males.
Anomalies are associated with breeding grounds (Gulf of California).
At present, too few exist data to robustly assess taxon- and ecosystem-related variation in primary producer $\beta$ and AA-specific trophic discrimination factors* (especially true for terrestrial and freshwater aquatic ecosystems).

Comparison of trophic and source amino acids can provide an estimate of trophic level that only requires a single consumer tissue sample.

Amino acid $\delta^{15}N$ is also a promising tool to study animal eco-physiology, specifically processes that impact nitrogen balance (reproduction).

For $\delta^{15}N$, amino acids are classified as source and trophic depending on their involvement in the central metabolic nitrogen pool.

$\delta^{15}N$ analysis of source amino acids (Phe/Lys) provides a way of assessing baseline (primary producer) $\delta^{15}N$ composition that is sensitive to environmental conditions by analyzing consumer tissues.

*IMO