

Insights into Tuna Trophic Level and Physiology from Compound-Specific Nitrogen Isotope Analysis of Proteinaceous Amino Acids

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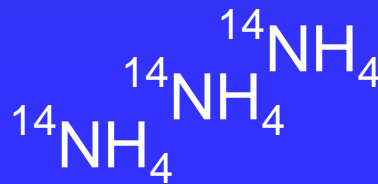
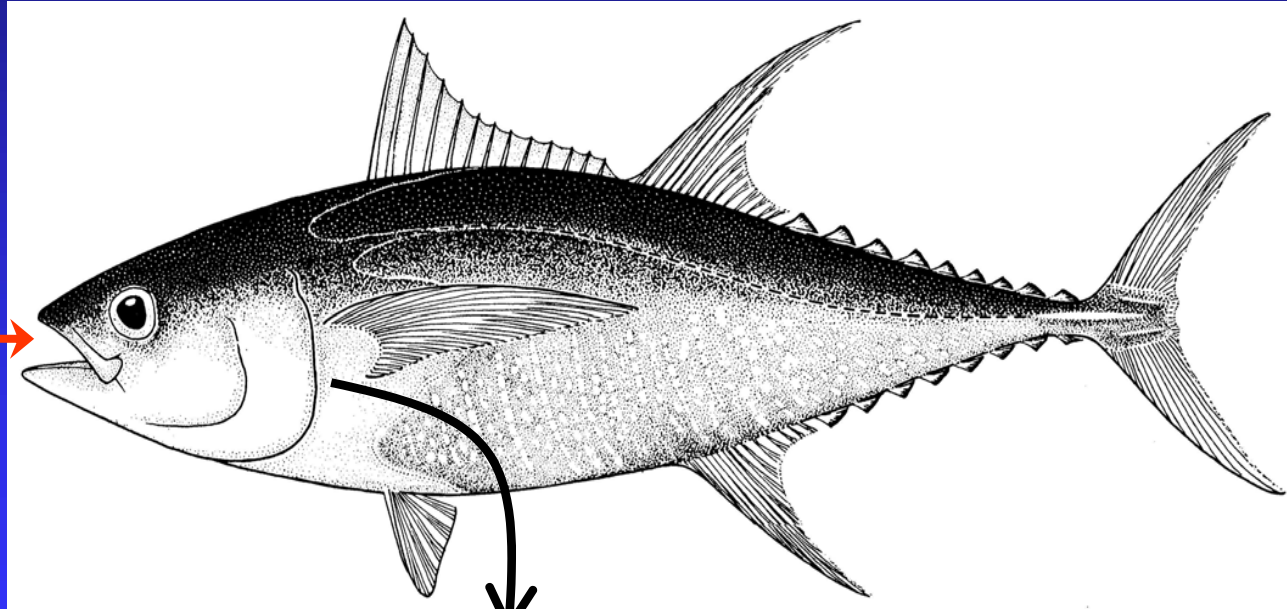
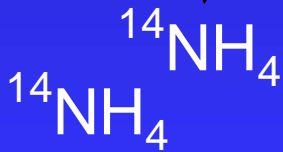
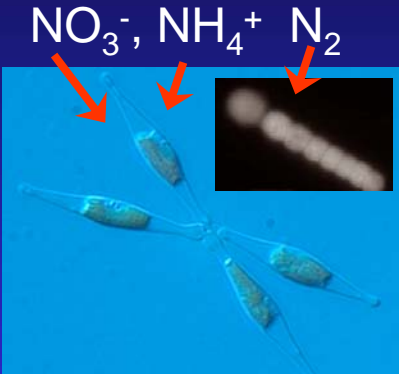
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Brief Outline

- Introduction and inspiration
- Analytical methods
- Sample analysis: proof-of-concept example
- An intriguing example
- Conclusions and implications

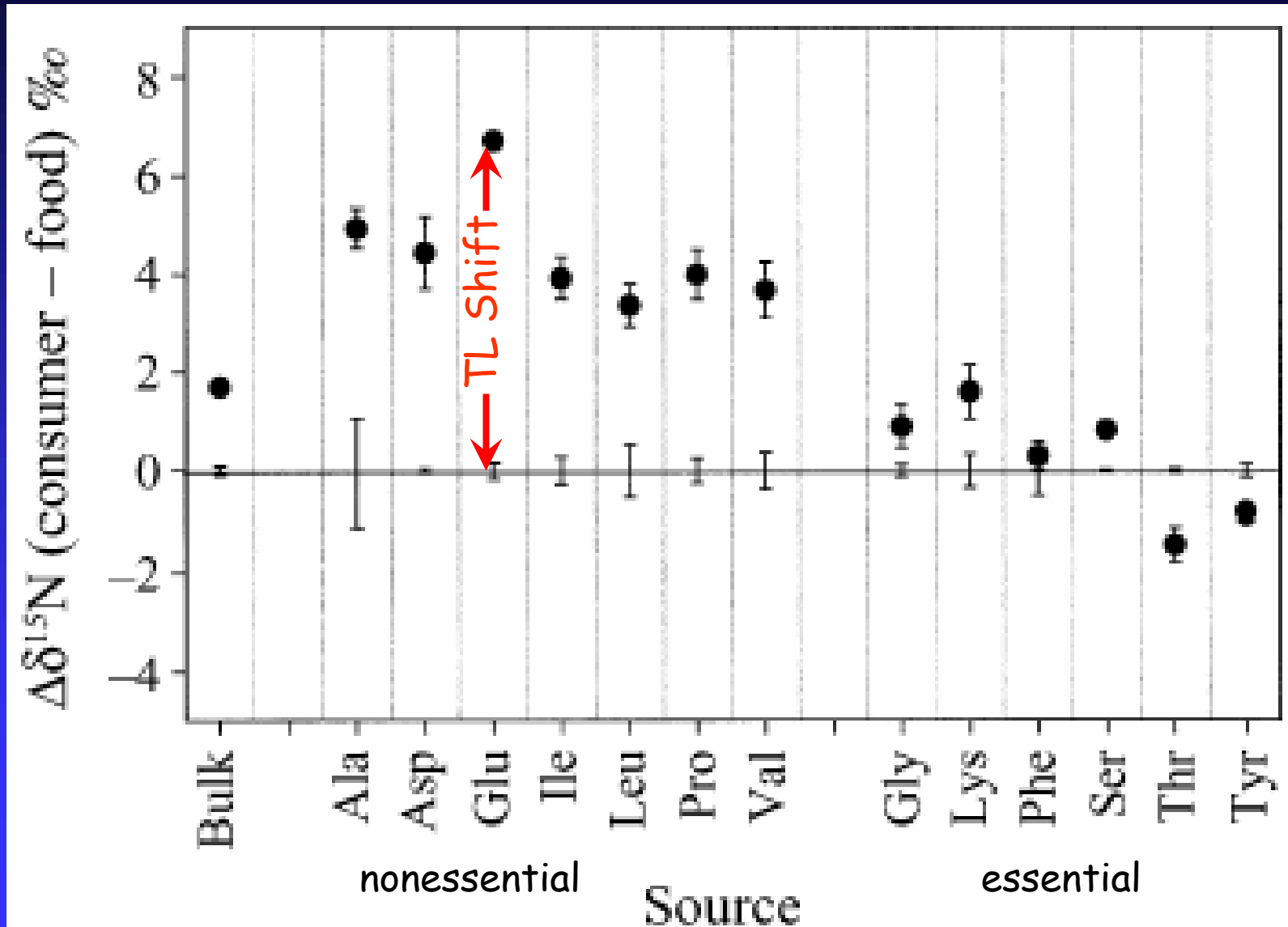
Nitrogen Isotope Fractionation

$$\delta^{15}\text{N}_{\text{predator}} = 3.0 + \delta^{15}\text{N}_{\text{prey}} (\text{‰})$$



More of the lighter isotope (^{14}N) is excreted than the heavier isotope (^{15}N) during metabolism

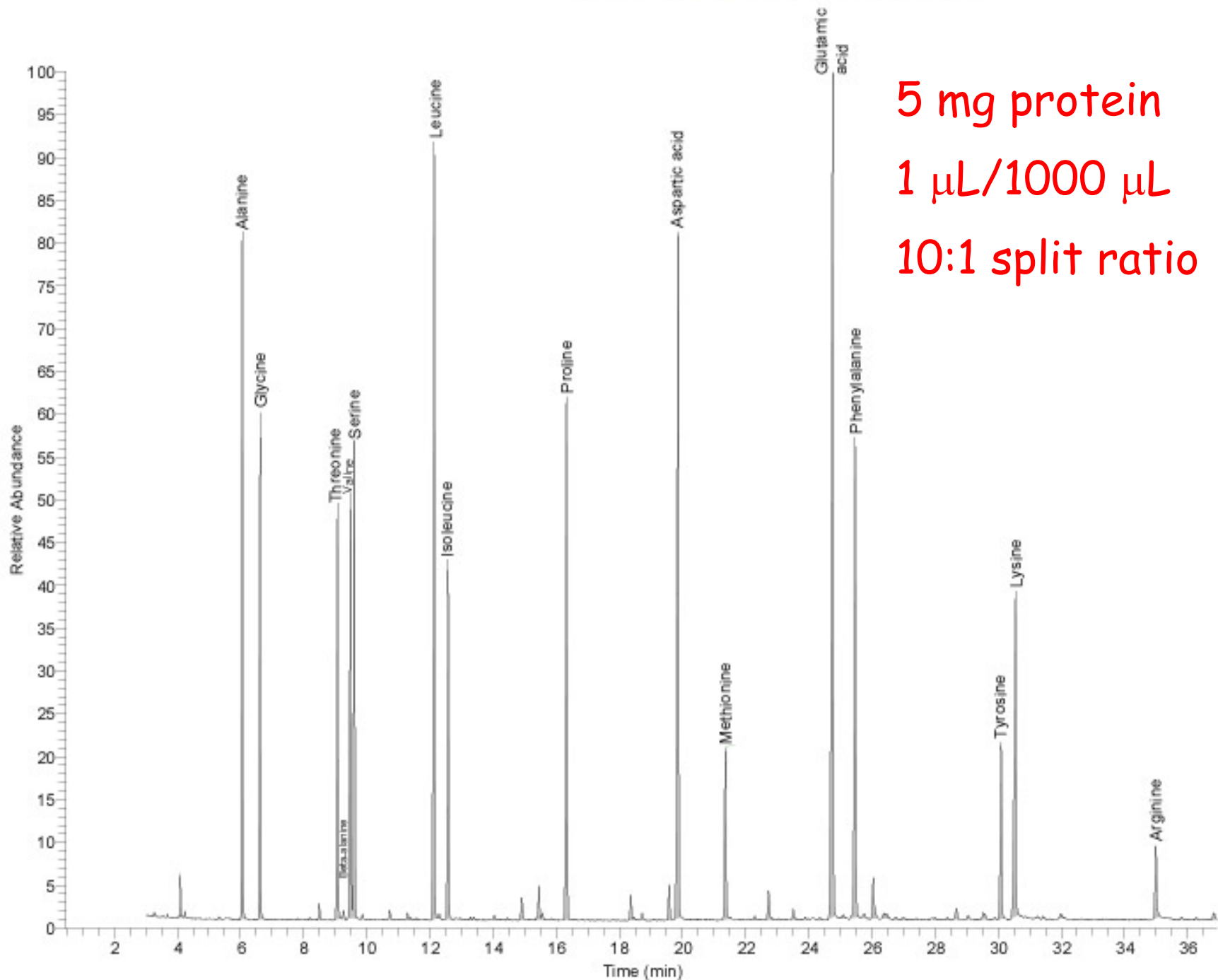
Inspiration



McClelland and Montoya (2002, Ecology, 83:2173)

Method Overview

- Hydrolysis (Cowie and Hedges 1991, *L&O* 37:703)
 - ◆ Boil in 6N HCl, ~1 h (150°C)
 - ◆ Clean up
 - ◆ Filtration
 - ◆ Ion exchange
- Esterification (Hofmann *et al.* 2003, *Isotopes Environ. Health Studies*, 3:233)
 - ◆ Heat at 110°C, 1 h acetyl chloride:isopropanol (1:4)
- Acylation
 - ◆ Heat at 100°C, 15 min methylene chloride:trifluoroacetic anhydride (3:1)

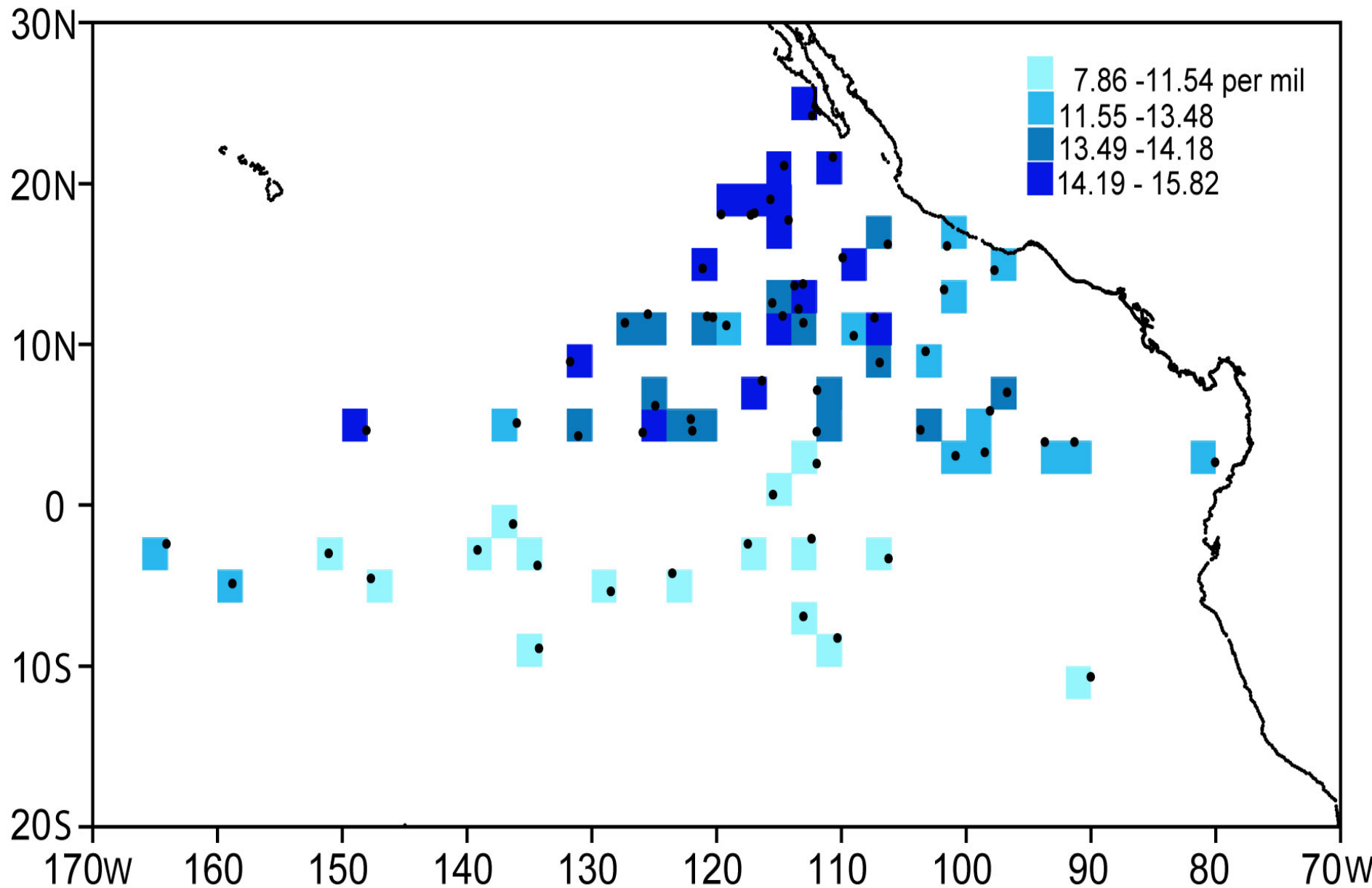


5 mg protein
1 μ L/1000 μ L
10:1 split ratio

Stable Isotope Analysis

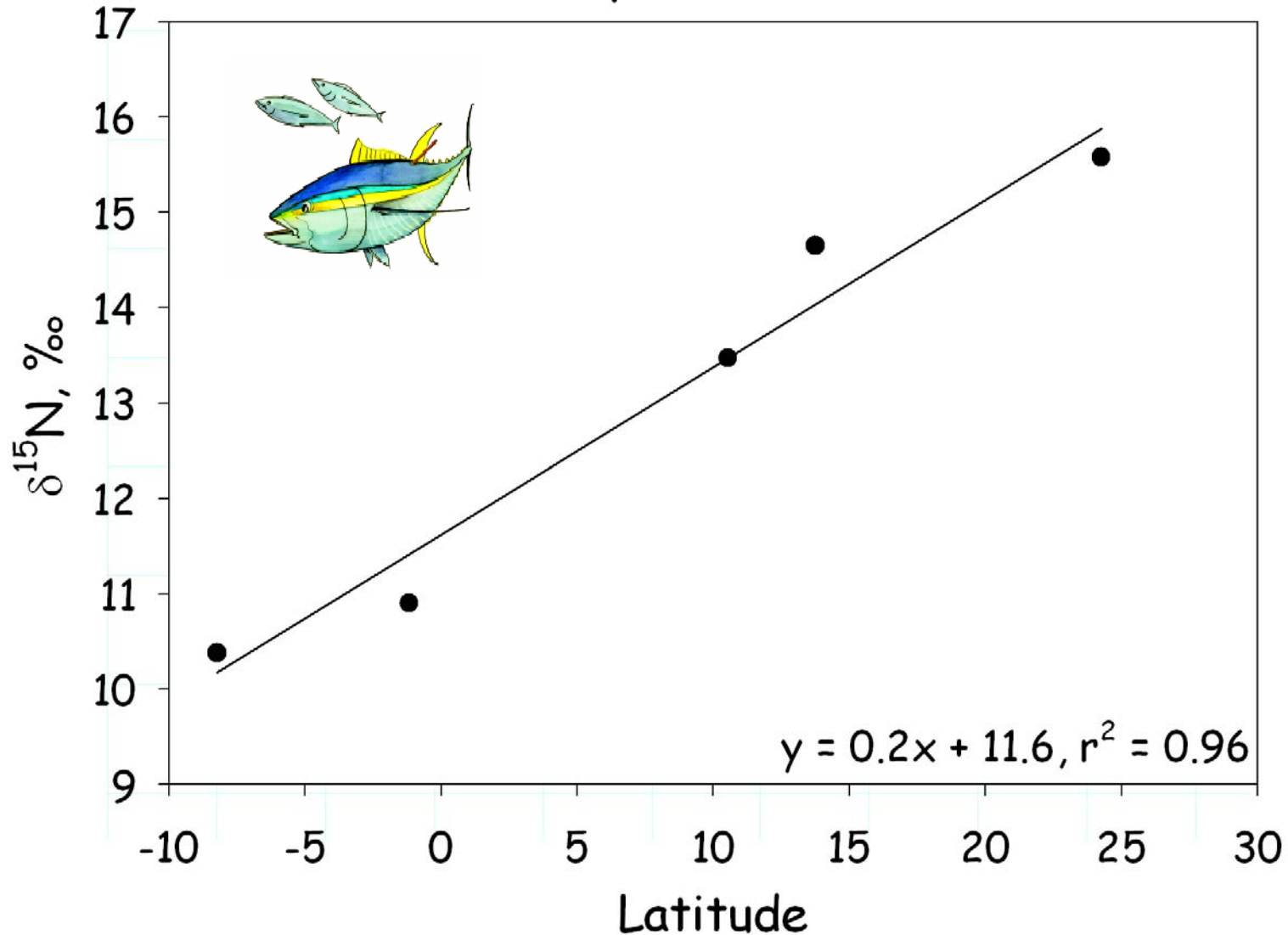
- Analysis time
 - ◆ 35-40 minutes; ~12 amino acids
- Sensitivity
 - ◆ 5 mg protein (0.5 mg)
 - ◆ 0.5 μL /100 μL (0.5 μL /10 μL)
- Accuracy and Precision
 - ◆ Accuracy (standards) $\sim \pm 0.5\text{‰}$ (0.2-0.8‰)
 - ◆ Precision (standards) $\sim \pm 0.2\text{‰}$ (0.1-0.4‰)
 - ◆ Precision (samples) $\sim \pm 0.3-1.0\text{‰}$

Yellowfin Tuna $\delta^{15}\text{N}$



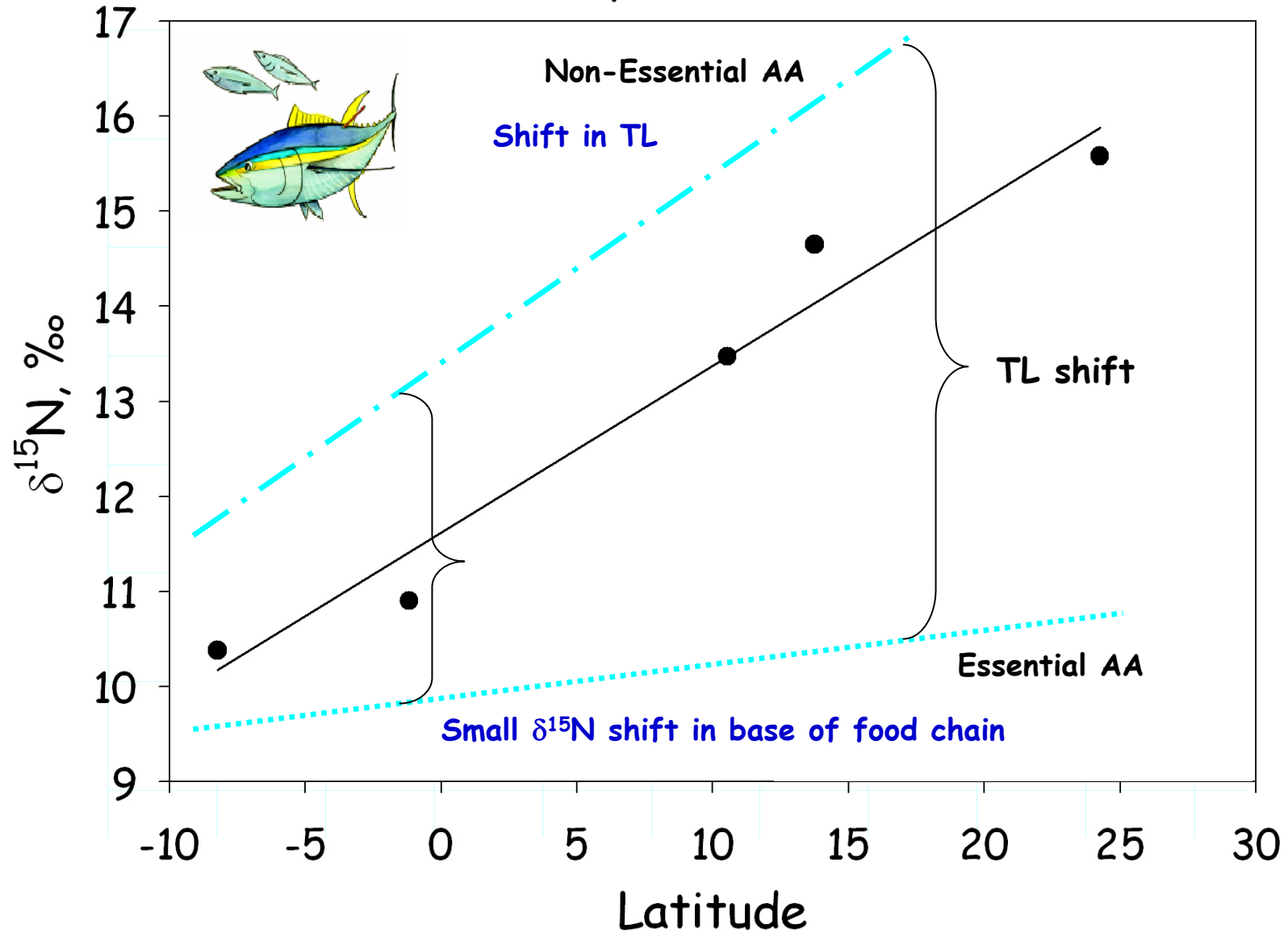
$\delta^{15}\text{N}$ of Bulk White Muscle Tissue

Eastern Tropical Pacific Sashimi



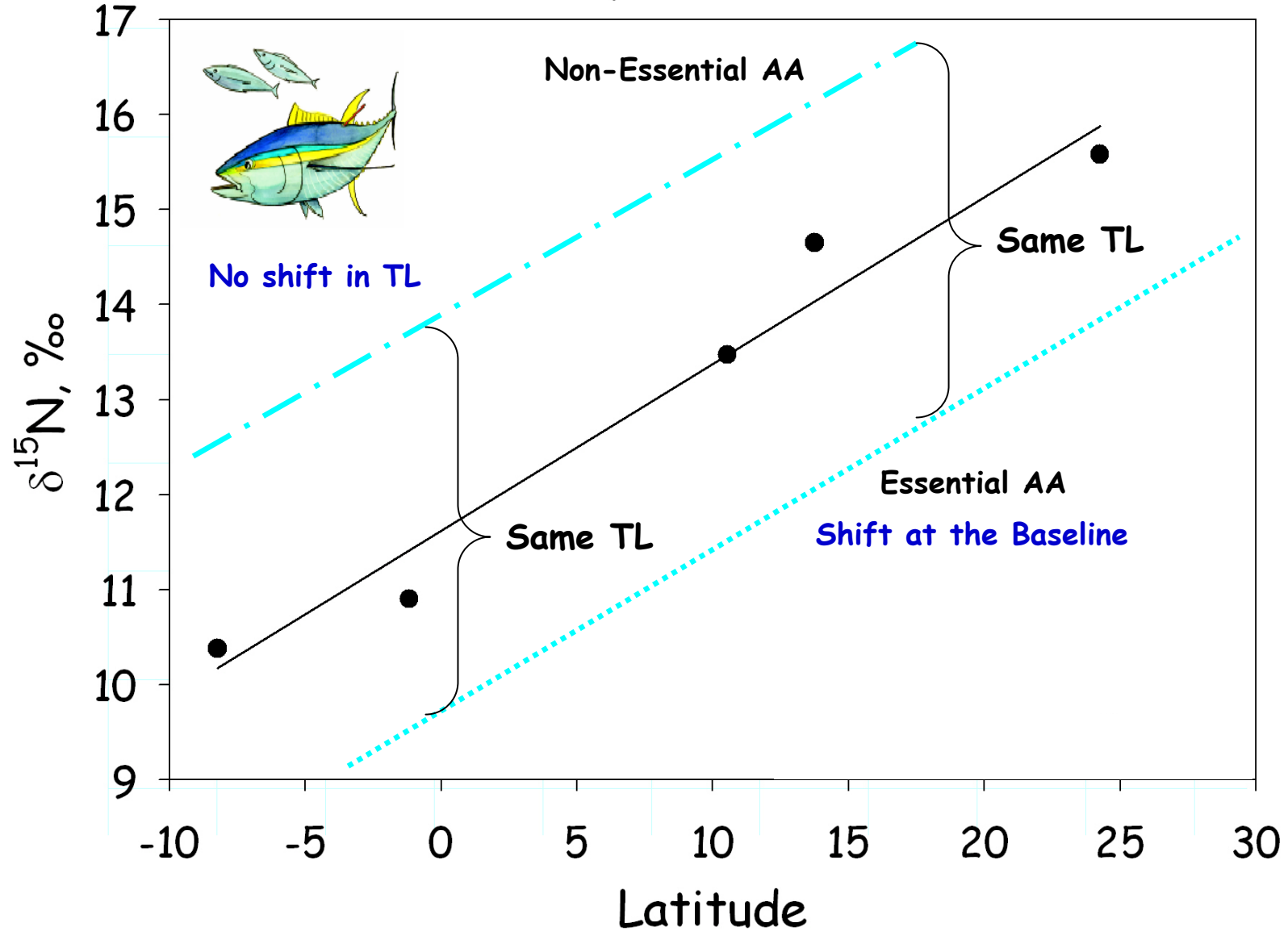
$\delta^{15}\text{N}$ of Amino Acids?

Eastern Tropical Pacific Sashimi



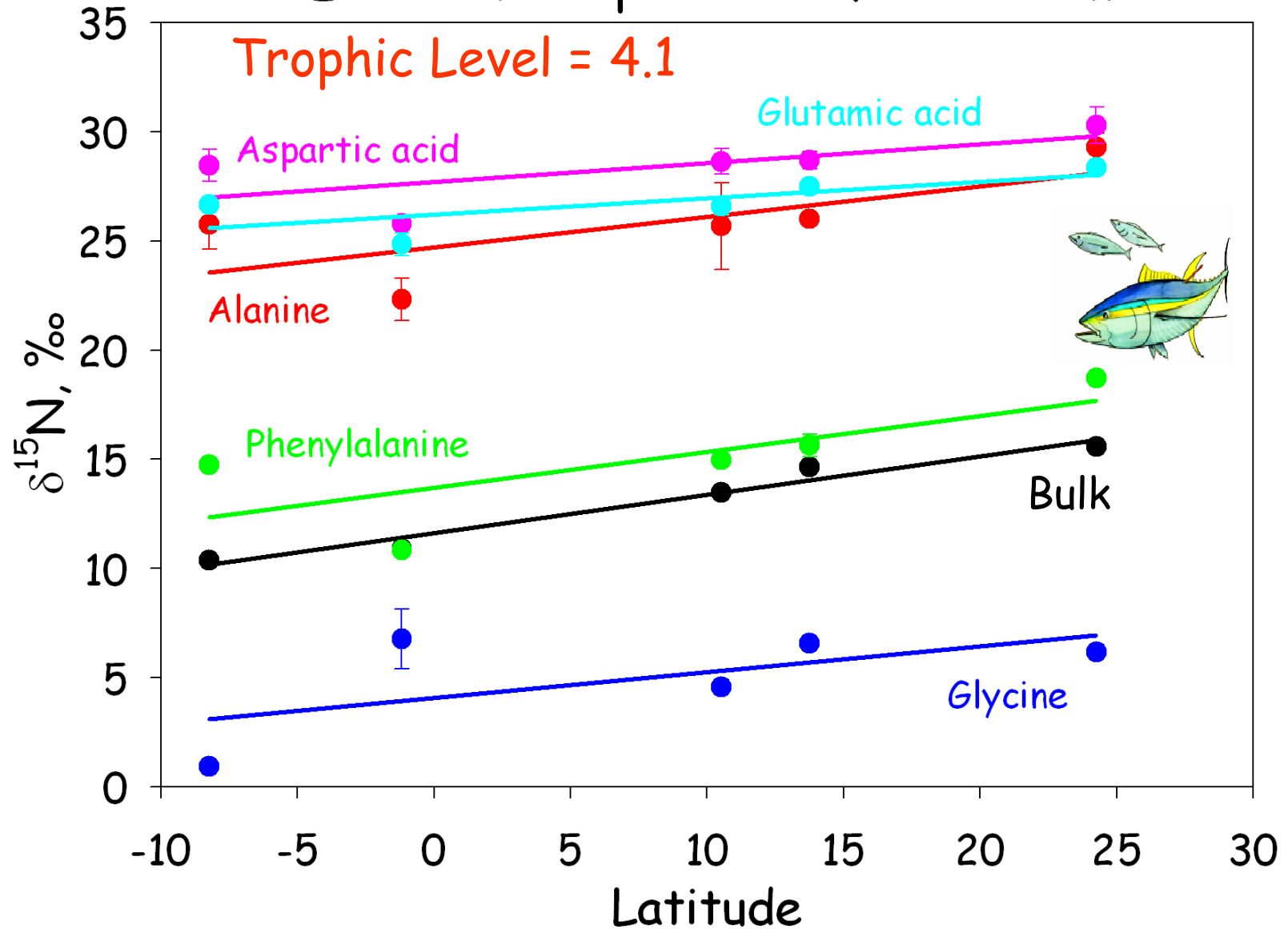
$\delta^{15}\text{N}$ of Amino Acids?

Eastern Tropical Pacific Sashimi

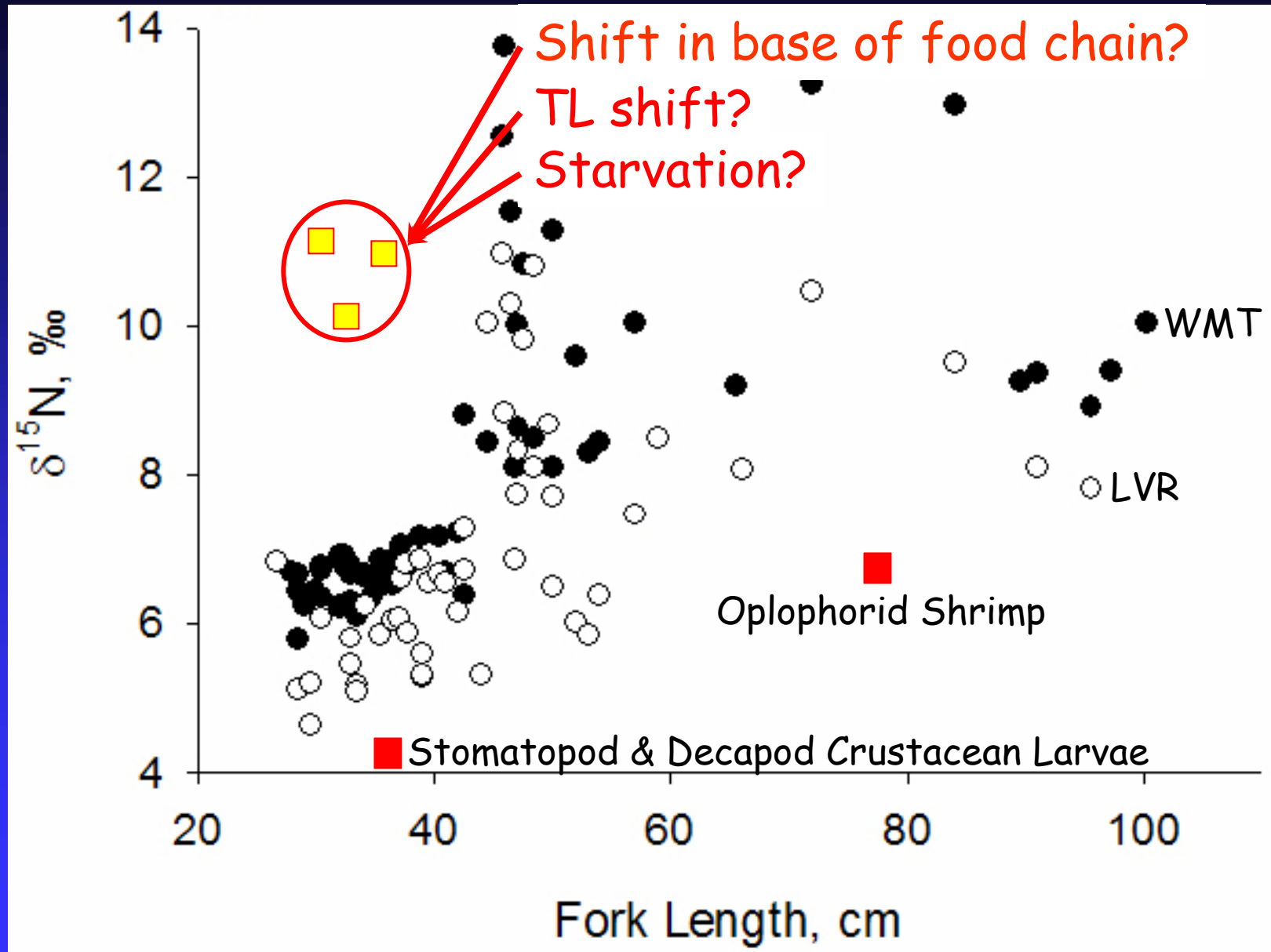


$\delta^{15}\text{N}$ of Amino Acids in WMT

Eastern Tropical Pacific Sashimi

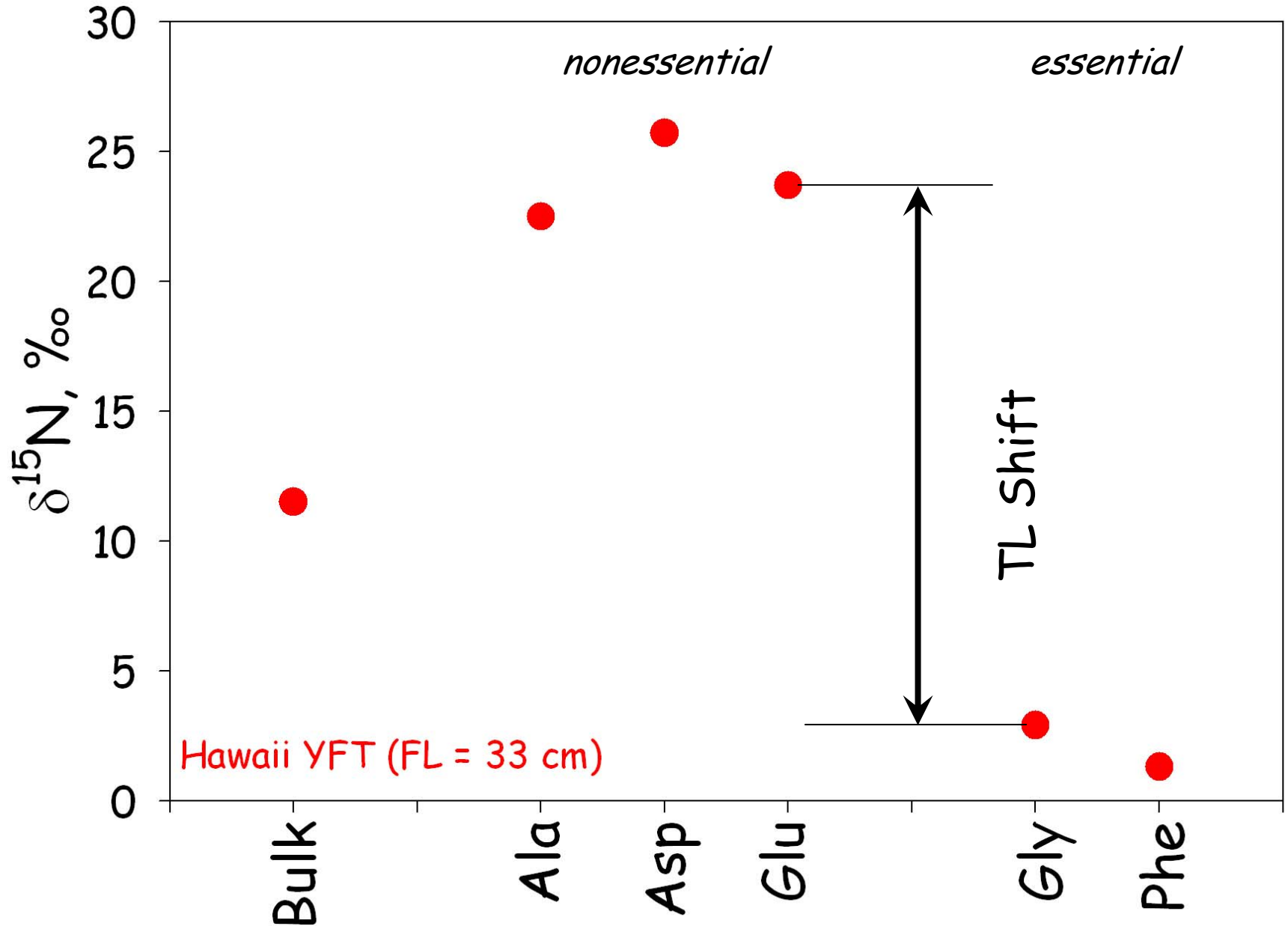


Ontogenetic Shift in Yellowfin Tuna from Oahu



(Graham *et al.* 2005 submitted)

Starving Tuna?



Conclusions and Implications

- $\delta^{15}\text{N}$ of amino acids in sashimi
 - ◆ Essential - track $\delta^{15}\text{N}$ of base of the food chain
 - ◆ Nonessential - track trophic level and physiology
 - ◆ Characterize $\delta^{15}\text{N}$ of base of food web and trophic level in same tuna sample
- Implications
 - ◆ Test if tuna trophic level changed with time
 - ◆ Allows migration reconstruction for comparison with archival tags
 - ◆ Monitor tuna health - recognize starvation and natural mortality?

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