

# Tracing Nitrogen in Food Webs using Compound Specific Isotope Ratio Mass Spectrometry

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# Biomarker      Signature for;

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- ⇒ Sterols      Faecal contamination, algae, higher plants, high-organic anaerobic environments
- ⇒ Bile Acid      Human and animal faecal matter
- ⇒ Ketones      Algal and paleoclimatic markers
- ⇒ Long-chain diols      Algal markers (e.g. Eustigmatophytes)
- ⇒ Fatty Acids      Algal and bacterial markers
- ⇒ Polar Lipids      Algal and bacterial biomass, community structure and nutritional status (from cell membrane)
- ⇒ Hydrocarbons      Oil spills / reserves, algal groups (diatoms, blue green algae)
- ⇒ C & N Isotopes      Biogeochemical studies of primary productivity and foodwebs

# Fitzroy River – macrotidal, subtropical estuary

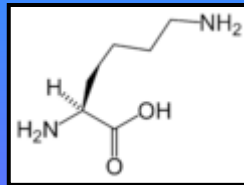






# New Markers for New Applications

## Amino Acids



Tracing the flow of nitrogen through biogeochemical systems

How much re-working is carried out by heterotrophic bacteria?

How important is N<sub>2</sub> fixation in this system? Cyanobacteria?

Measuring changes in trophic structures in pelagic ecosystems

★ Distinguishing between nutrient and trophic dynamics in ecosystems

Evaluating fishery impacts on ecosystems

# New Markers for New Applications

## Amino Acids

Essential versus non-essential amino acids describe carbon flow through Biochemical systems; e.g synthesis of specific carbon skeletons



# Amino Acids

## Essential amino acids

Valine  
Leucine  
Aspartate  
Glutamate  
Methionine  
Phenylalanine  
Alanine  
Proline  
Tryptophan  
\*Histidine  
\*Arginine

## Non-Essential amino acids

Glycine  
Serine  
Proline  
Aspartate  
Tyrosine  
Serine  
Glutamate  
Phenylalanine  
Tyrosine  
Cysteine  
Glutamine  
Asparagine

The difference in  $\delta^{15}\text{N}$  values of these two groups reflect the degree of transamination experienced during biosynthesis.



The bulk  $\delta^{15}\text{N}$  values in white muscle tissue of eastern pacific tuna ranged from 10 to 15‰ depending on latitude.

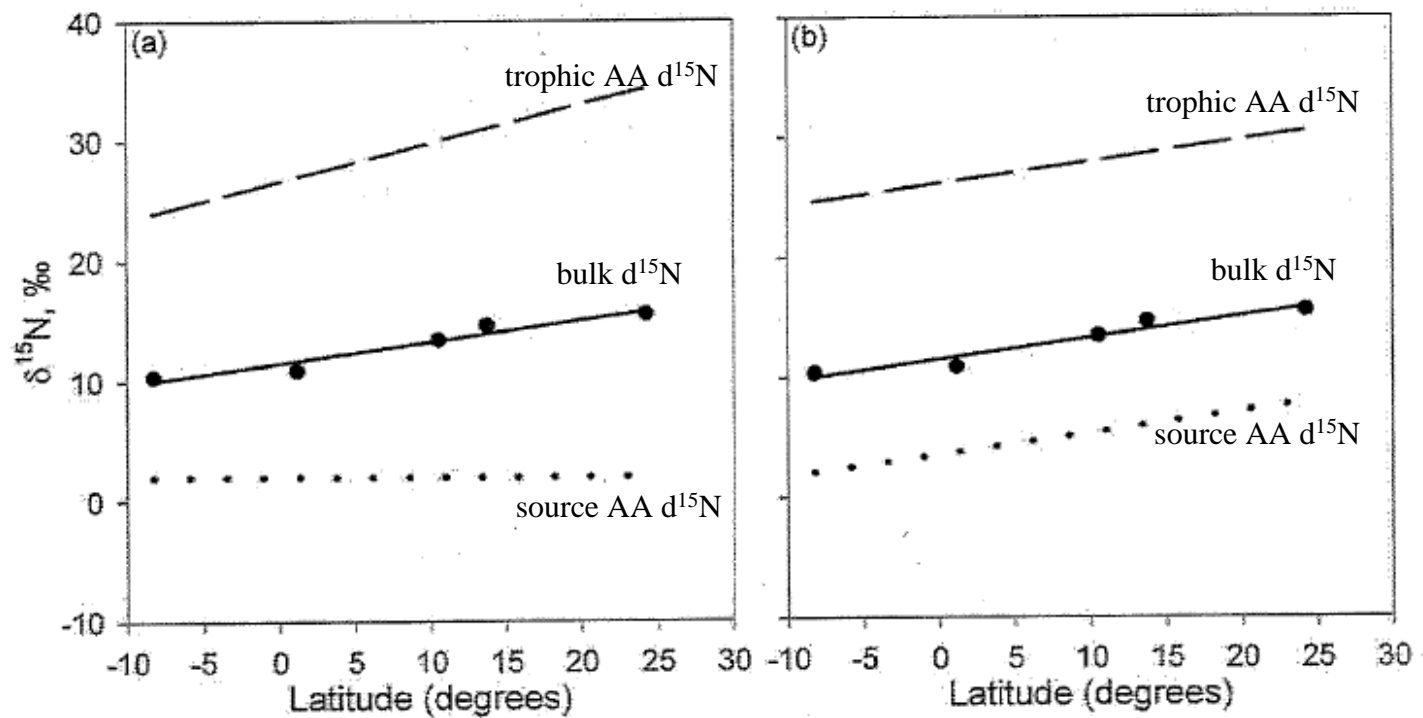
The amino acid  $\delta^{15}\text{N}$  values in white muscle tissue of eastern pacific tuna range from;

Source amino acids 2.2 to 7.5‰ depending on latitude

Trophic amino acids 27.5 to 30.8 ‰ depending on latitude

From Popp *et. al.*, 2008





a) No change in  $\delta^{15}\text{N}$  at the base of the food chain, but a trophic shift with latitude

b) No change in  $\delta^{15}\text{N}$  trophic level, but a shift in the base of the food chain with latitude

From Popp *et. al.*, 2008

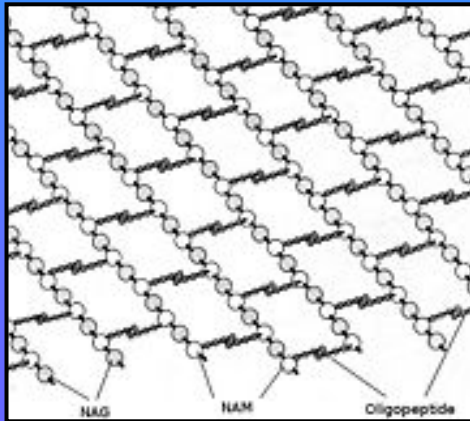
## Utilizing effluents in the production of shrimp / prawns

Benefits of the right microbial community structure to shrimp culture

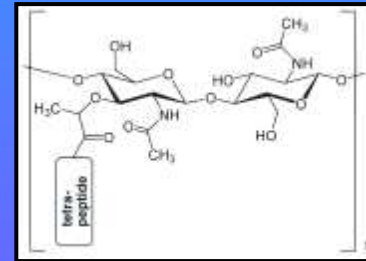
- Enhanced animal growth
- Improved animal product quality such as colour
- Improved water quality through reduction of ammonia and nitrite
- Enhanced animal health through stimulation of immune system

*Ju et. al. 2008*

# New Markers for New Applications

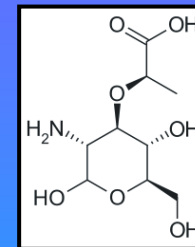


Peptidoglycan (or murein) forms  
The membrane of gram-positive  
And gram-negative bacteria



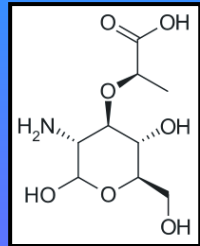
Peptidoglycan consists of alternating  
ether linked N-acetylglucosamine  
and N-acetylmuramic acid monomers

Muramic Acid



# New Markers for New Applications

## Muramic Acid



Measures how much bacteria is present in complex samples



Also contains nitrogen thus will compliment biogeochemical studies as both a source biomarker and an isotopic biomarker

In conjunction with pigment analyses can determine the community structure of floc cultures.

**Table 3** Profiles of phytoplankton community structure (%) of shrimp floc samples collected from outdoor raceways and cylindrical culture tanks

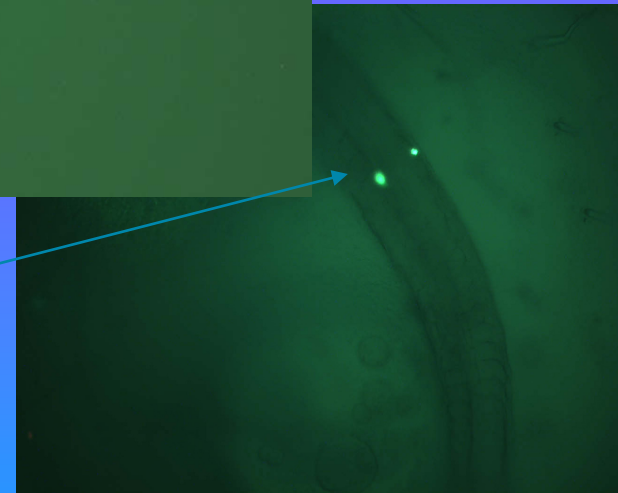
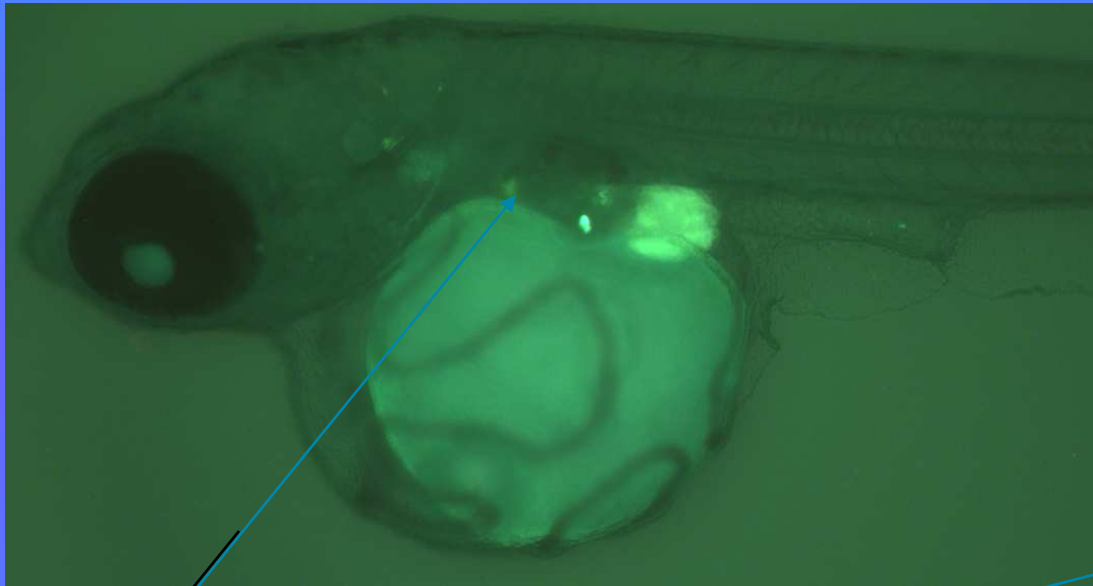
Algal class	Phytoplankton community composition (%)			
	Floc-A	Floc-B	Floc-C	Floc-D
Diatoms	4.3	8.2	84.4	81.7
Chlorophytes	82.3	75.4	5.5	9.8
Cyanobacteria	5.9	0.6	0.1	0.1
Dinoflagellates	2.6	6.9	7.3	7.5
Cryptophytes	5.0	8.9	2.7	0.9

**Table 4** Algae and bacteria biomass ( $\text{g kg}^{-1}$ ) and their ratios (dried sample basis) of floc materials collected from outdoor shrimp raceways and tanks

Biomass/floc type	Floc-A	Floc-B	Floc-C	Floc-D
Algae biomass	244.1	43.7	252.3	239.9
Bacteria biomass	12.8	78.3	25.6	34.2
Total	256.9	122.0	277.9	274.1
Algae:Bacteria (ratio)	19.1:1	0.5:1	9.9:1	7.0:1

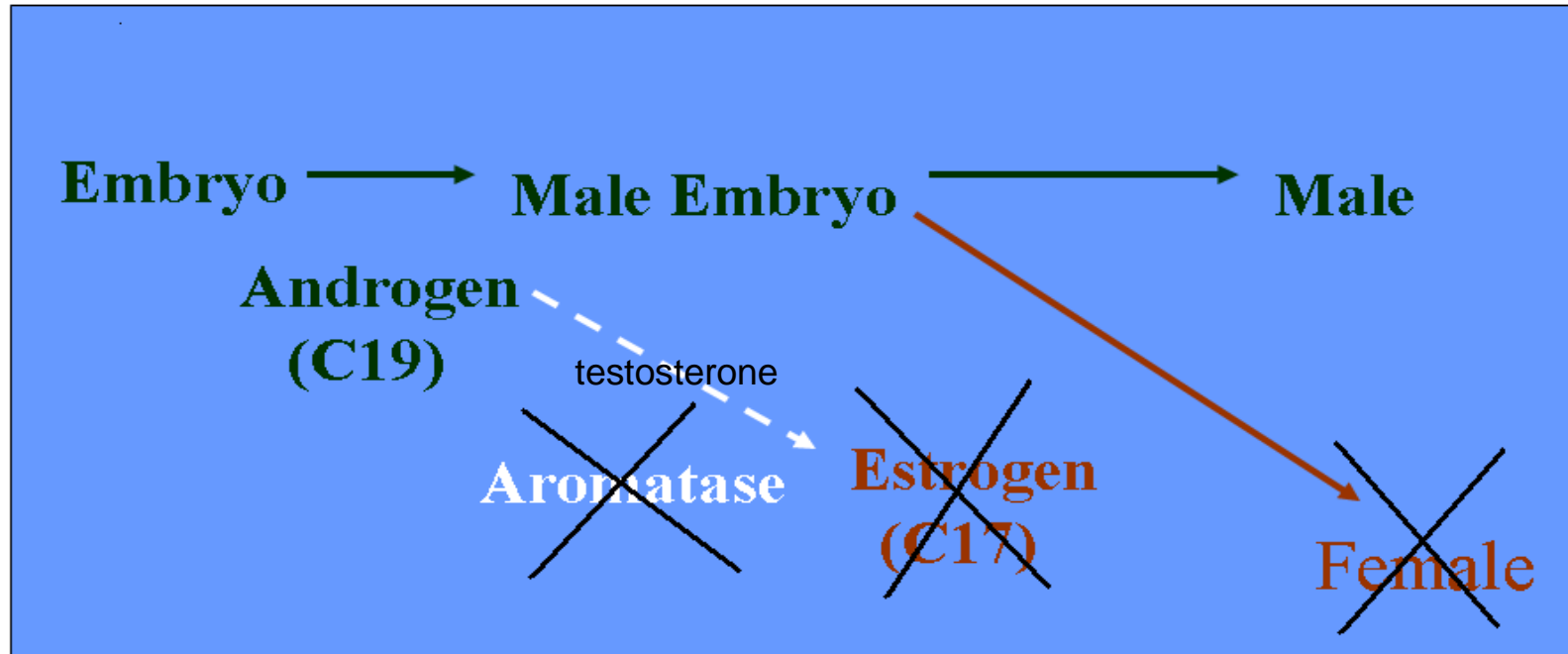


# Larval fish with genetic construct to switch off aromatase



Aromatase  
blocker ON

# Fish Sexual Development





# New Markers for New Applications

## Estradiol

Measurement of phenotypic expression of genetically modified organisms

↳ Eradication of pest species e.g. carp

Manipulated aquaculture species; e.g. breeding, triploid

## Environmental issues

↳ Imposex; e.g. tri-butyl tin exposure

Endocrine disruption studies



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