“The main use of stable isotopes involves magic. We cannot see, feel, touch, hear, smell, or taste stable isotopes with our normal senses, yet there they are, magical scraps of information fluttering gently all around us”

Brian Fry, 2006

Multiple applications of carbon and nitrogen isotope measurements: (1) Trophic ecology (generalities), (2) linking ecotoxicology and trophic ecology (3) Trophic niches

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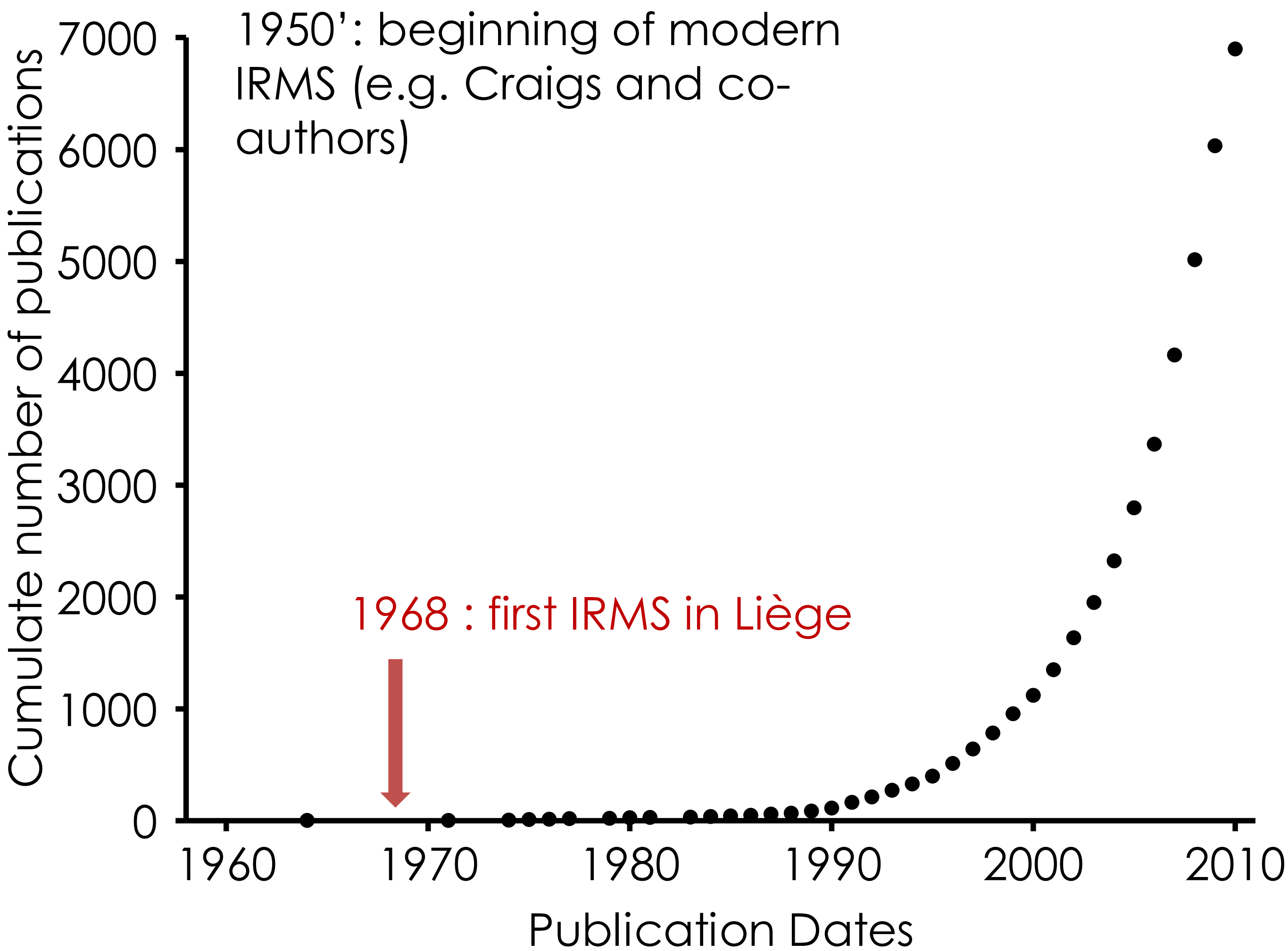
Partim of the Specialist course: Applications of Biomarkers in Aquatic food web studies (24-26 March 2014)

-sponsored by the Doctoral School of Natural Sciences (Ghent University),
-part of the Doctoral Programme on Marine Ecosystem Health and Conservation (MARES),
-organised by Marleen De Troch and Tom Moens (Marine Biology, UGent)

I. INTRODUCTION

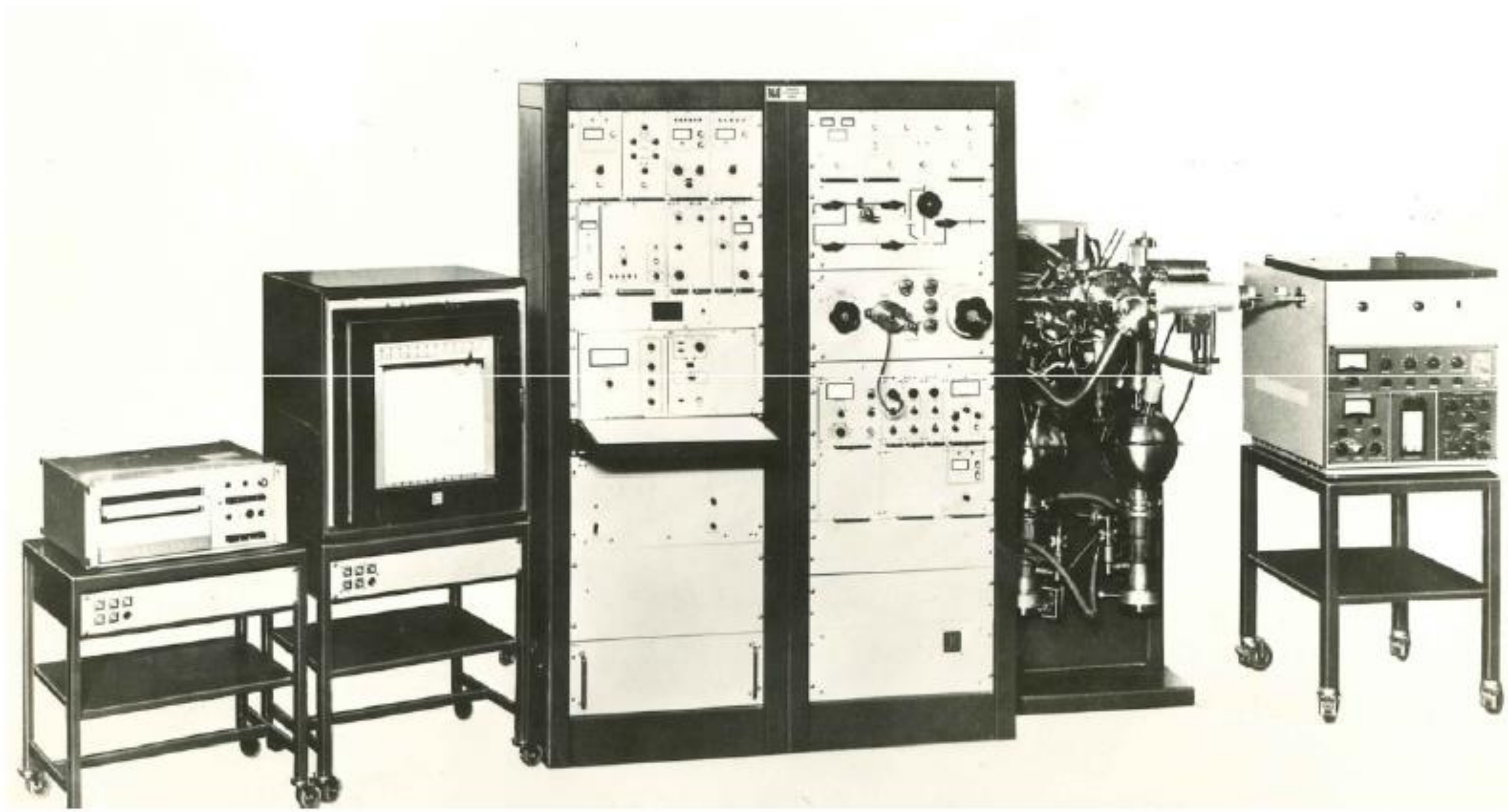
- IRMS IN LIÈGE: FEW WORDS ABOUT A LONG HISTORY
- IRMS INSTRUMENTATION
- GENERALITIES AND BASICS
- APPLICATIONS 1: TO ASSESS TROPHIC ECOLOGY (FOOD WEB STUDIES AND ANIMAL DIET VARIABILITY)
- APPLICATIONS 2: TO LINK ECOTOXICOLOGY AND TROPHIC ECOLOGY (EUTROPHISATION AND POP CONTAMINATIONS)
- APPLICATIONS 3: TO ASSESS TROPHIC ECOLOGY (TROPHIC NICHES) (DR LOÏC MICHEL, ULG)

IRMS in Liège: few words about a long history

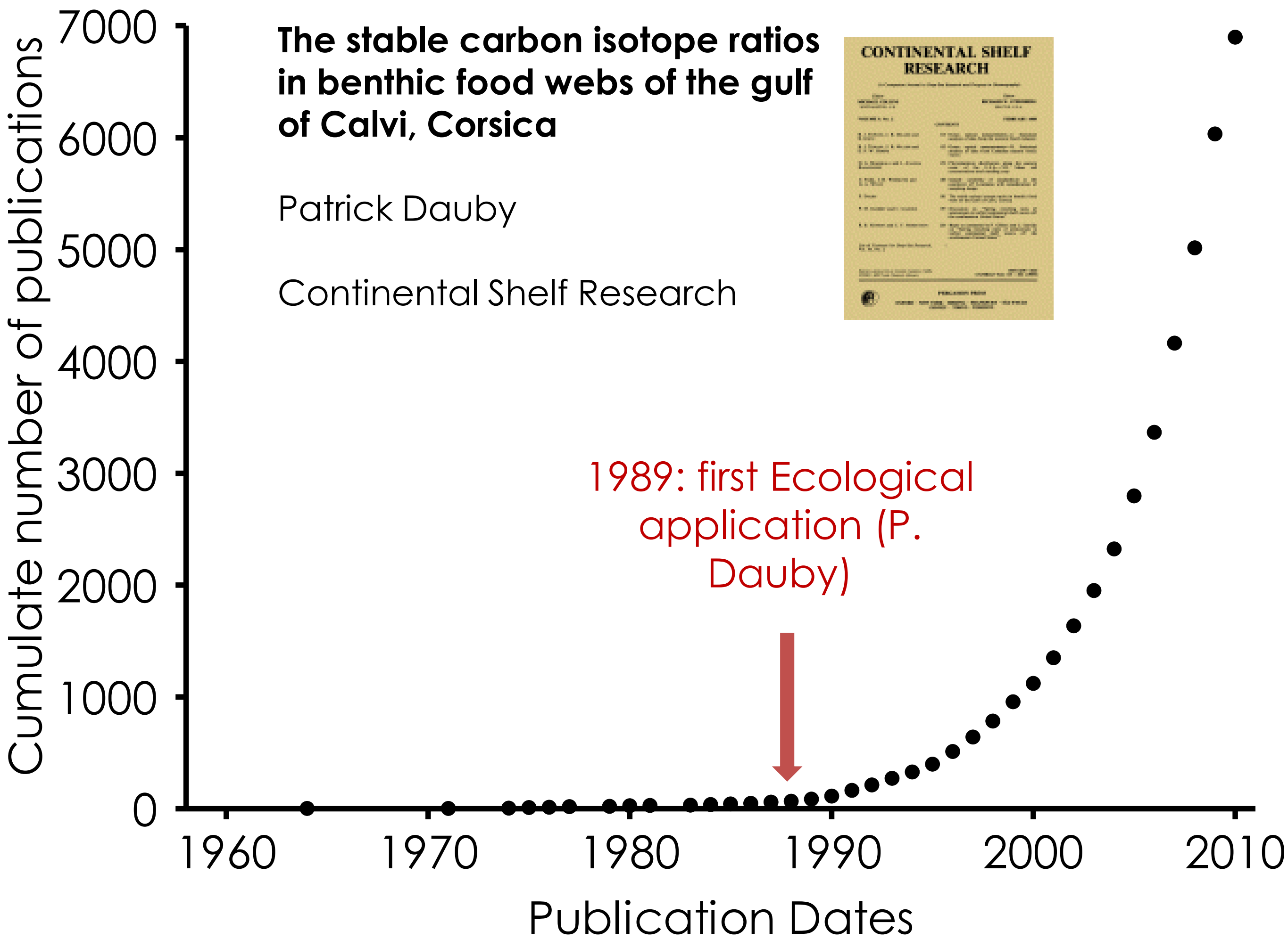


Data: IsiWeb of Knowledge, key words: (stable isotope) and (Nitrogen or Carbon) (Environmental Sciences and Agriculture)

Varian-MAT CH5 (later Finnigan, now ThermoFisher)



IRMS IN LIÈGE: FEW WORDS ABOUT A LONG HISTORY



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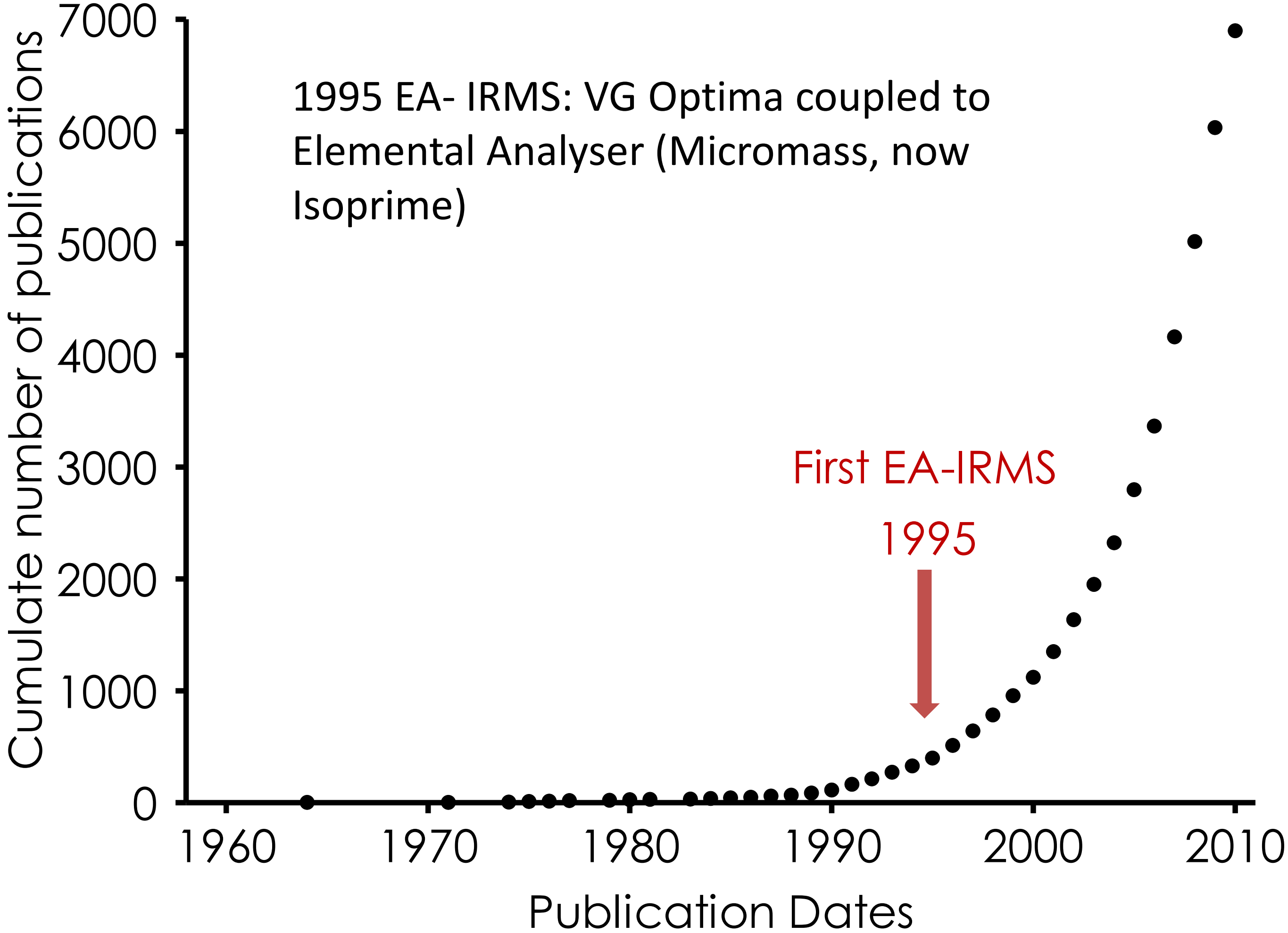
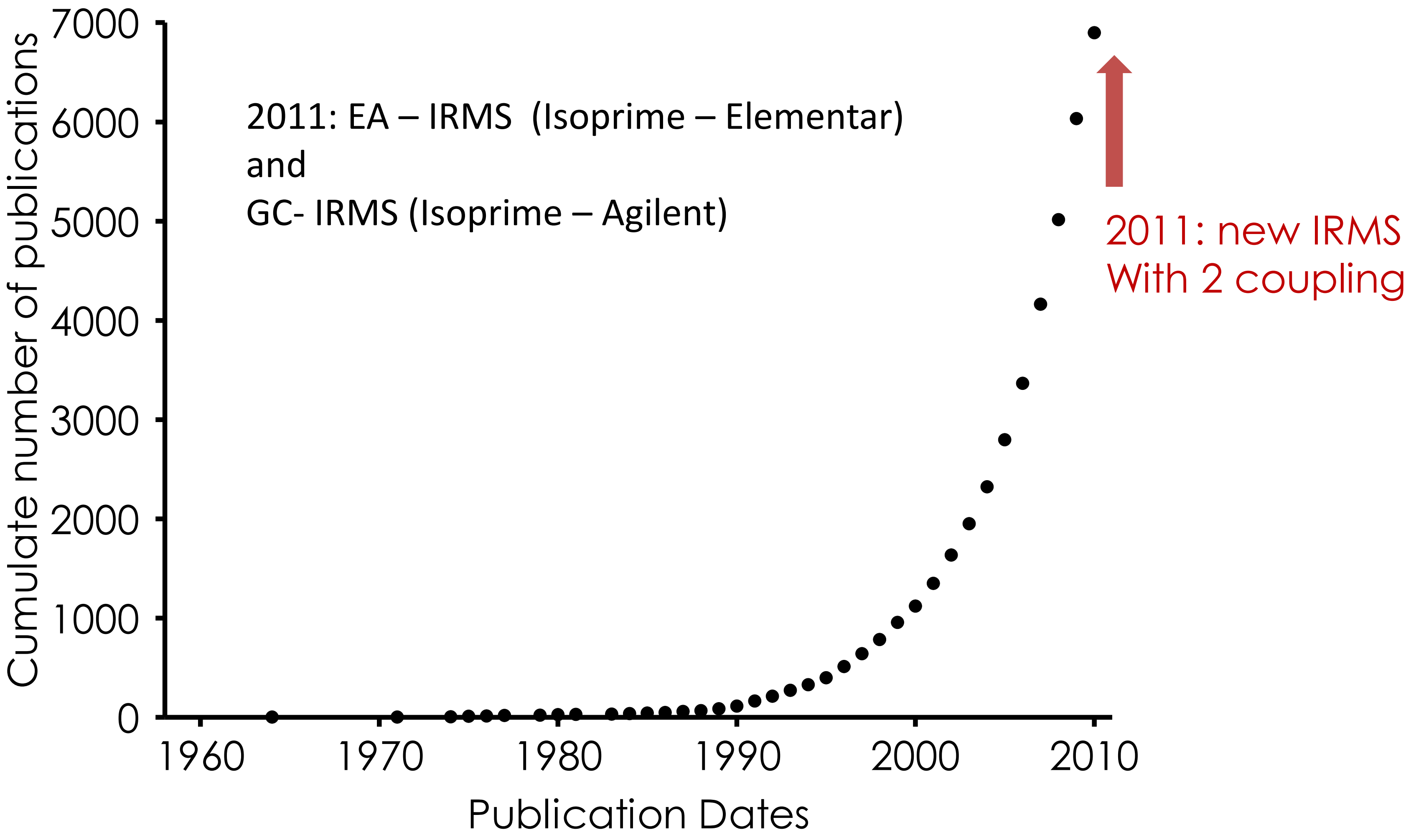
IRMS in Liège: few words about a long history



IRMS in Liège: few words about a long history



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de Liège

INSTRUMENTATION: IRMS

- What we measure?

$$^{X}R = \frac{AbondanceX}{AbondanceY}$$

With X and Y = 2 stable isotopes of an element

$\Rightarrow$ *Isotopic ratios* = RELATIVE MEASUREMENT

$\Rightarrow$ *"Isotope Ratio Mass Spectrometry"* = ***IRMS***

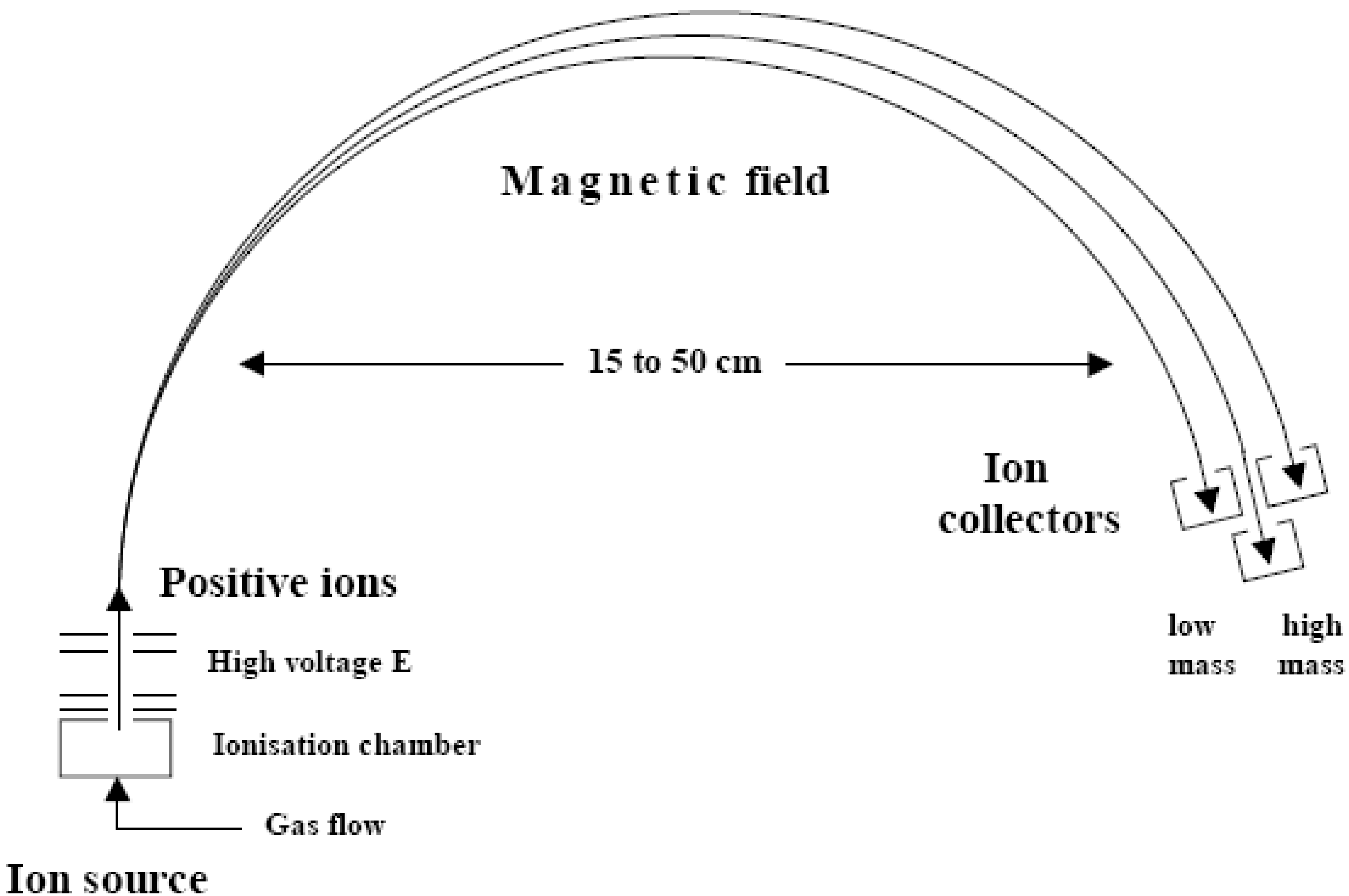
INSTRUMENTATION: IRMS

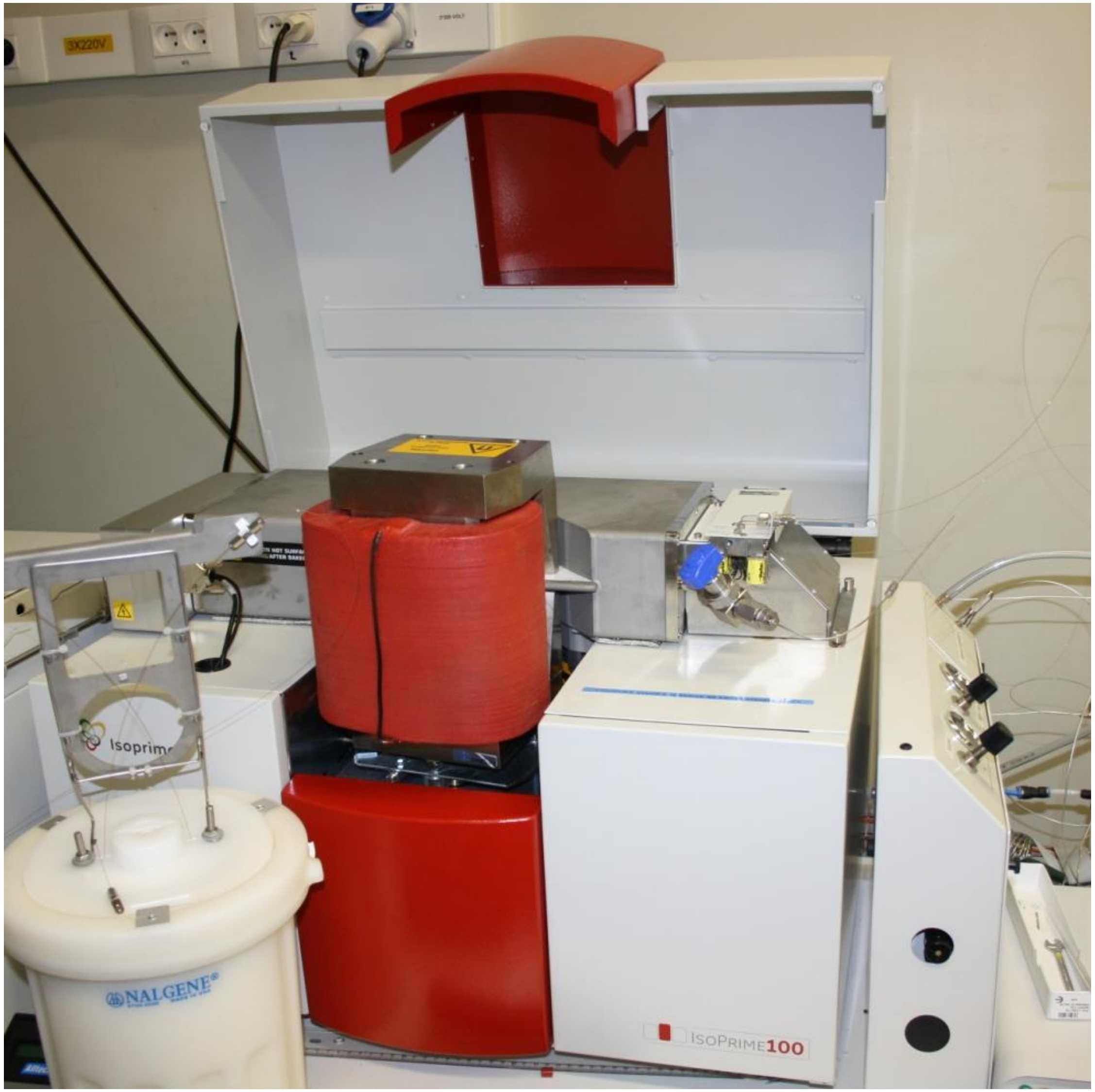
- How we measure?

Mass spectrometer equation

$$\frac{M}{Z} = \frac{B^2 R^2}{2V}$$

or: When an ion with a mass M and an electric charge Z is accelerated by a potential difference V through a magnetic field B, this ion move according to a circular orbite with a radius equal to R.

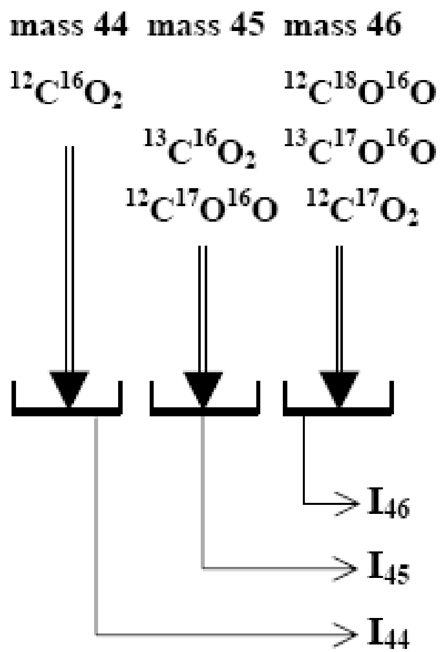

Magnetic field
15 to 50 cm
Ion
collectors
Positive ions
High voltage E
Ionisation chamber
Gas flow
Ion source
low
mass
high
mass



IRMS Isoprime 100 (Isoprime, UK)

•Measurements are done on simple gaz (CO_2, N_2, SO_2, H_2) NOT on atoms of C, N, O, H, S

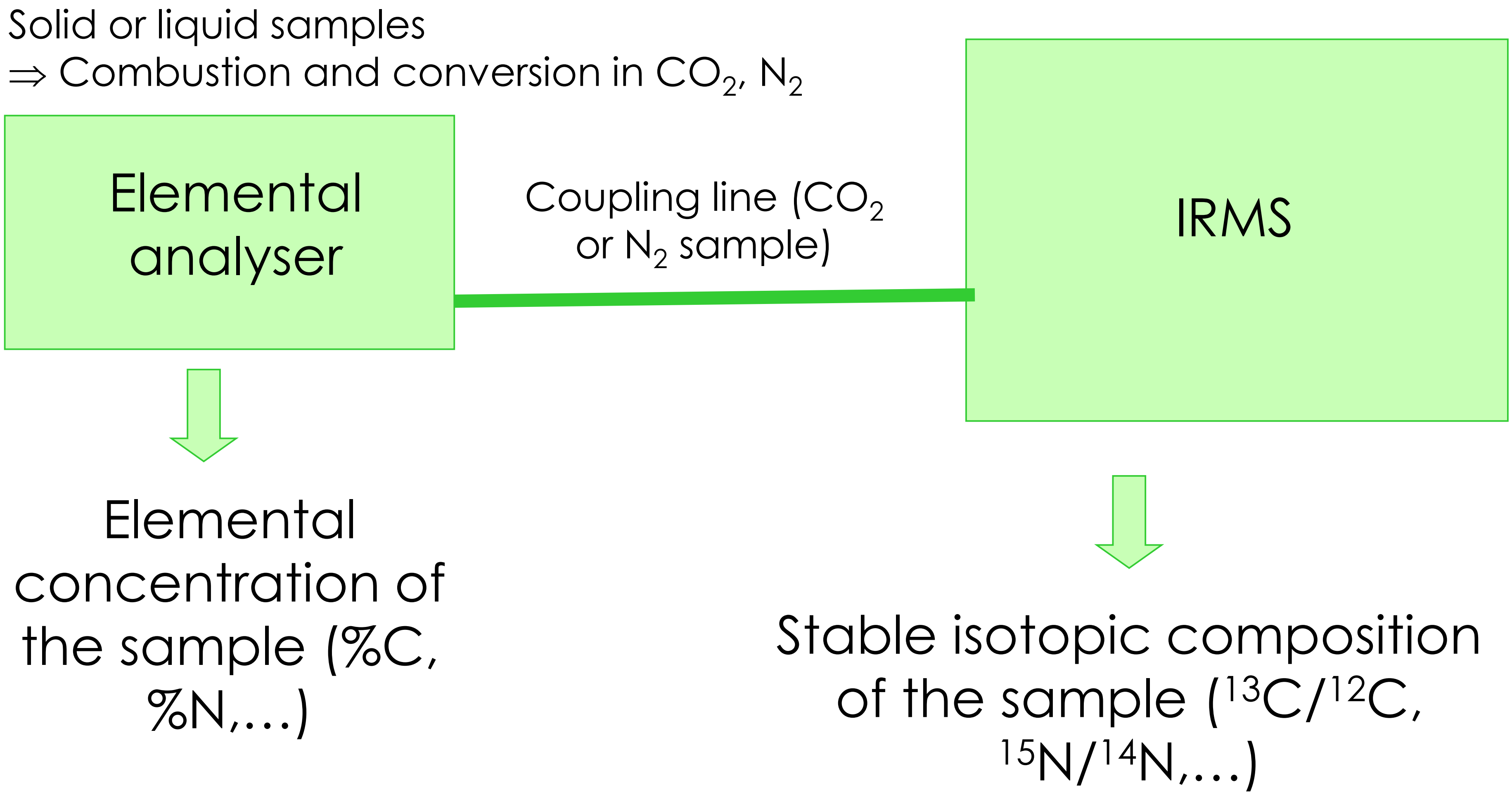
Collectors schema (for CO_2)



•Need to convert sample into simple gaz

⇒ Preparation off line (till 1990') or on line = coupling of 2 instruments

Example 1: Coupling EA -IRMS

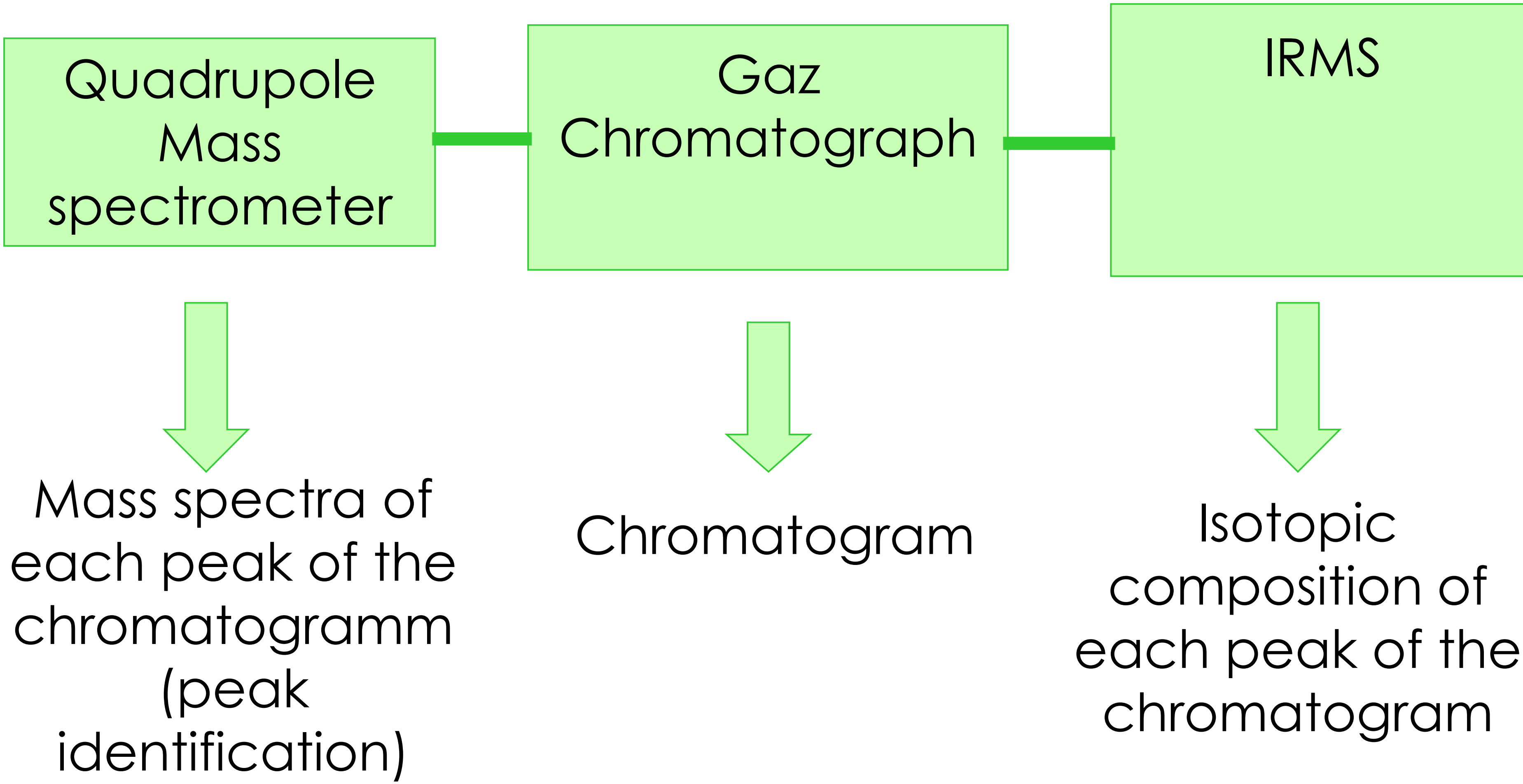


⇒ BULK stable isotopes composition

He: carrier gaz (CONTINUOUS FLOW)

Elemental Analyser (VarioMicro cube, Elementar, Germany)

Example 2: GC-IRMS and even more MS-GC-IRMS



⇒ Compound specific stable isotope analysis (CSIA)

MS-GC-IRMS

Delta notation : practical and international

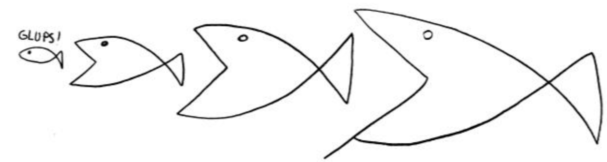
$$\delta X = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \times 1000$$

δ = deviation (in per mille) between the isotopic ratio of a sample and of an INTERNATIONAL standard

- Delta ^{13}C is NOT the quantity of ^{13}C in a sample but the deviation in per mille between the ratio $^{13}C/^{12}C$ of a sample and the ratio $^{13}C/^{12}C$ of a standard

Value	Signification
$\delta = 0$	Isotopic ratio of SAMPLE = Isotopic ratio of REFERENCE
$\delta > 0$	Isotopic ratio of SAMPLE higher than Isotopic ratio of REFERENCE $\Rightarrow$ heavy isotope more abundant in SAMPLE
$\delta < 0$	Isotopic ratio of SAMPLE lower than Isotopic ratio of REFERENCE $\Rightarrow$ heavy isotope less abundant in SAMPLE

II. APPLICATIONS 1: TO ASSESS TROPHIC ECOLOGY



Nyssen F.

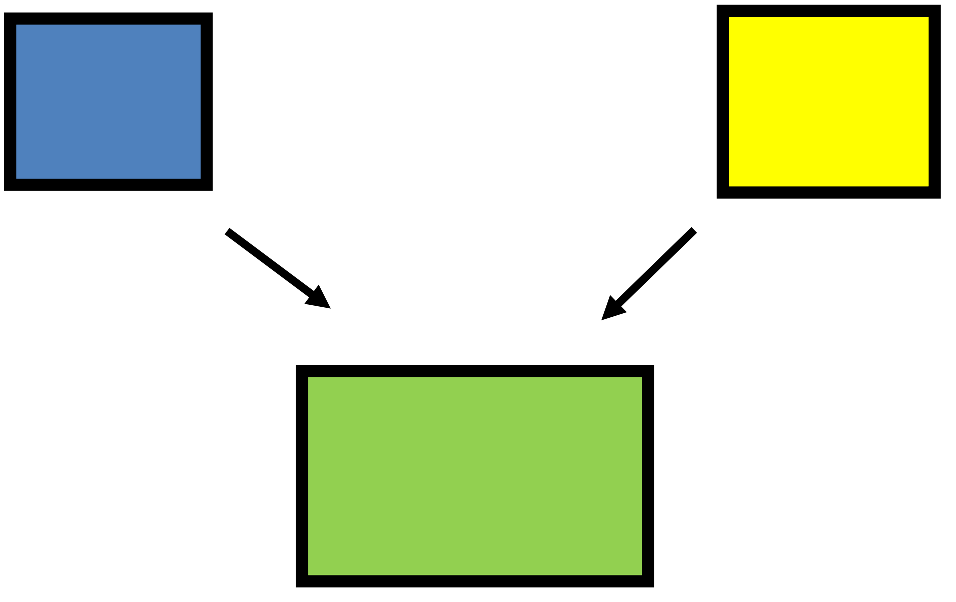
- Generalities and basics

- Trophic web associated to seagrass *Posidonia oceanica* meadows

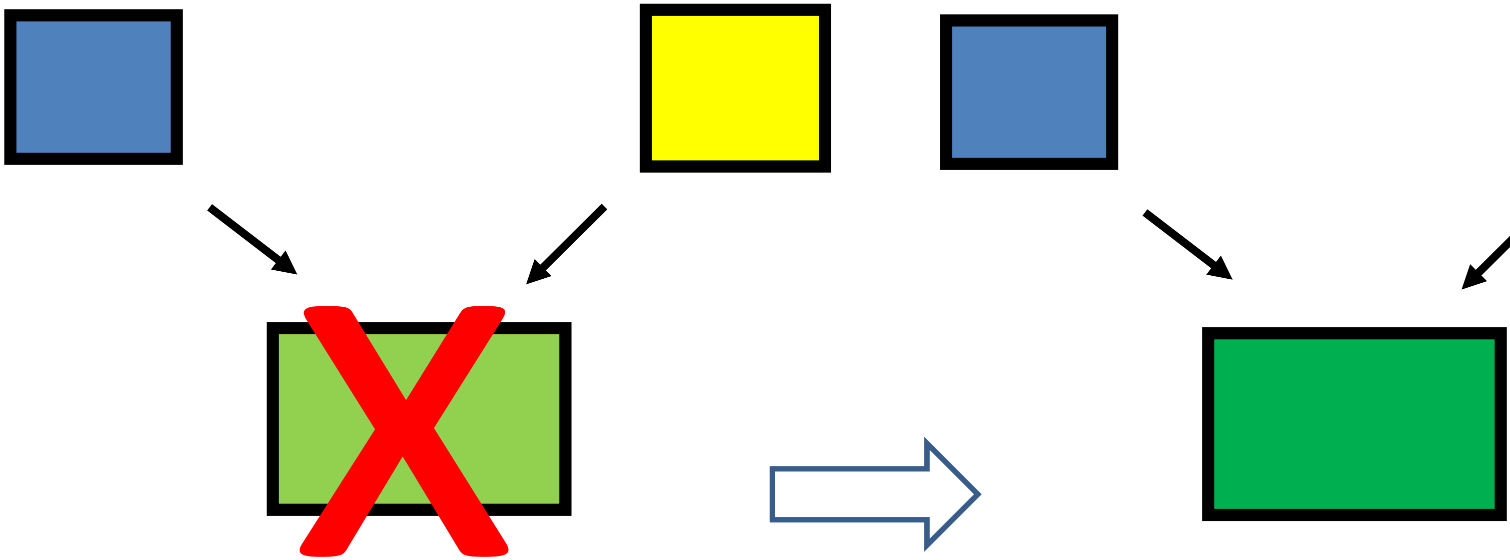
- Intra-specific trophic diversity in a Pomacentrid species

I. Generalities and Basics

"*You are what you eat…plus a few per mille*"
DeNiro & Epstein, 1978



MIXING Law:
"YOU ARE WHAT YOU EAT"



« Plus few per mille »

Plus few per mille = trophic enrichment factor (TEF)
= trophic fractionation factor

- Trophic enrichment factor (TEF) = difference between isotopic compositon of an organism and of its food

- = NET RESULTS OF ALL ISOTOPIC FRACTIONATIONS OCCURING DURING METABOLISM

- VARIABLE – NOT ALWAYS AN « ENRICHMENT » (i.e. an increase of the heaviest isotope abundance)

- Causes of variability (among other): phylogeny/diet/individual variability/tissues type

In summary:

The isotopic composition of an organism is the weighted mixing of the isotopic compositions of its food sources, modified by the isotopic fractionation**s**

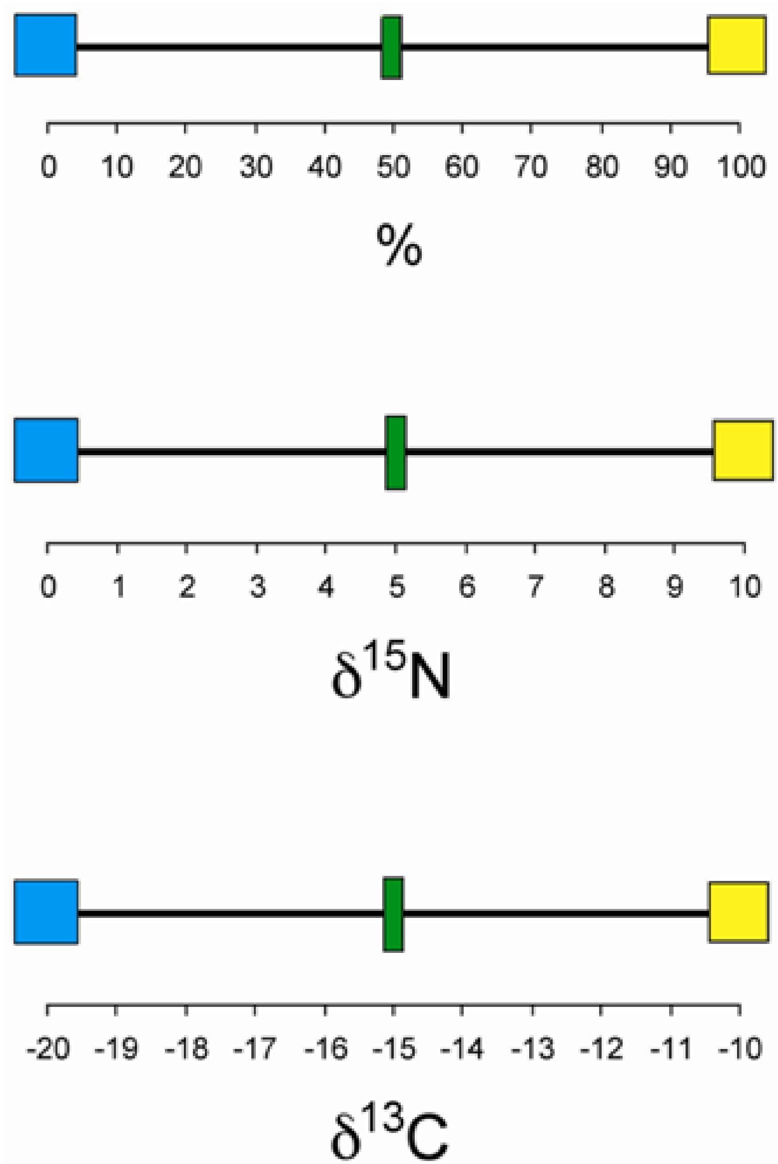
Mixing equation for 2 sources:

$$f_1 + f_2 = 1$$

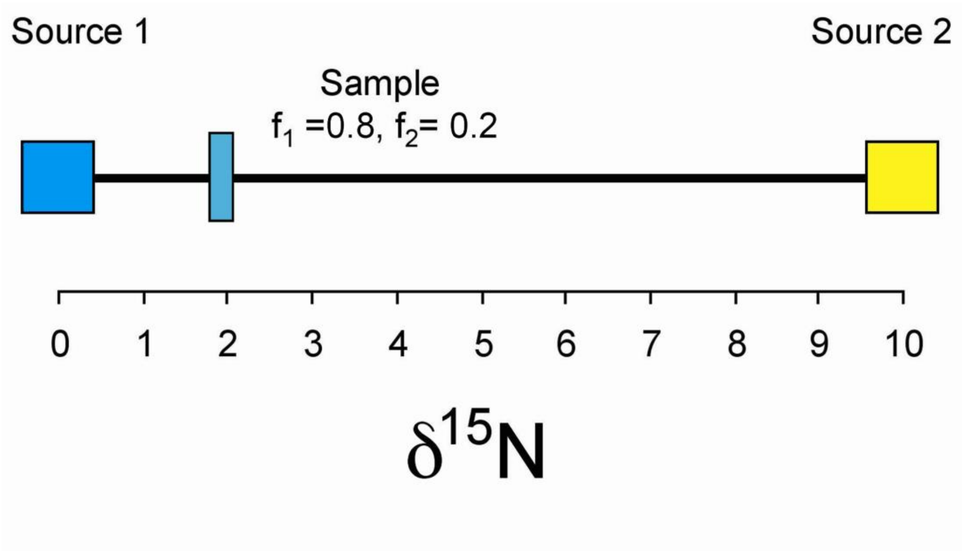
$$\delta m = (\delta_{source1} \times f_1) + (\delta_{source2} \times f2)$$

$$\delta m = \delta_{organism} - \delta_{fractionation}$$

Source A Source B



Source: Fry 2006



Source: Fry 2006

Mixing equation for n sources:

$$\delta_m = (f_a\delta_a + f_b\delta_b + f_c\delta_c + \ldots)$$

⇒ Complex mixing modelling

Examples:

A. Isosource (Philips & Gregg 2001) (www.epa.gov/wed/pages/models/isotopes/isosource.htm)

B. SIAR (Parnell et al. 2010) (cran.r-project.org/web/packages/siar)

Or MixSir

Causes of isotopic variability in consumer

1. If diet constant between individuals/populations/species:

- Isotopic variability of the diet
- Variability of isotopic fractionation between diet and consumer tissues

2. If not:

- Diet variability between individuals/populations/species

AND

- Isotopic variability of the diet
- Variability of isotopic fractionation between diet and consumer tissues

- Numerous applications in trophic ecology: trophic web delineation, diet variability, trophic niches
- Objectives and application types determine sampling design

<u>Few examples:</u>

- Trophic web delineation : * sampling of consumers <u>and</u> of food sources

* min number of samples: ± 6 (individuals or pool)

- Animal diet: * sampling of consumer (individual measurements if possible) and of sources

* as much as possible (depend upon ethics, cost, field constrainst) to assess diet variability

- Trophic niches (Loïc Michel): * only consumers must be sampled – but knowing general isotopic environment is better

* as much as possible (ALWAYS INDIVIDUAL measurements) (min 10 for me) because application based on isotopic variability

Last philosophical comments

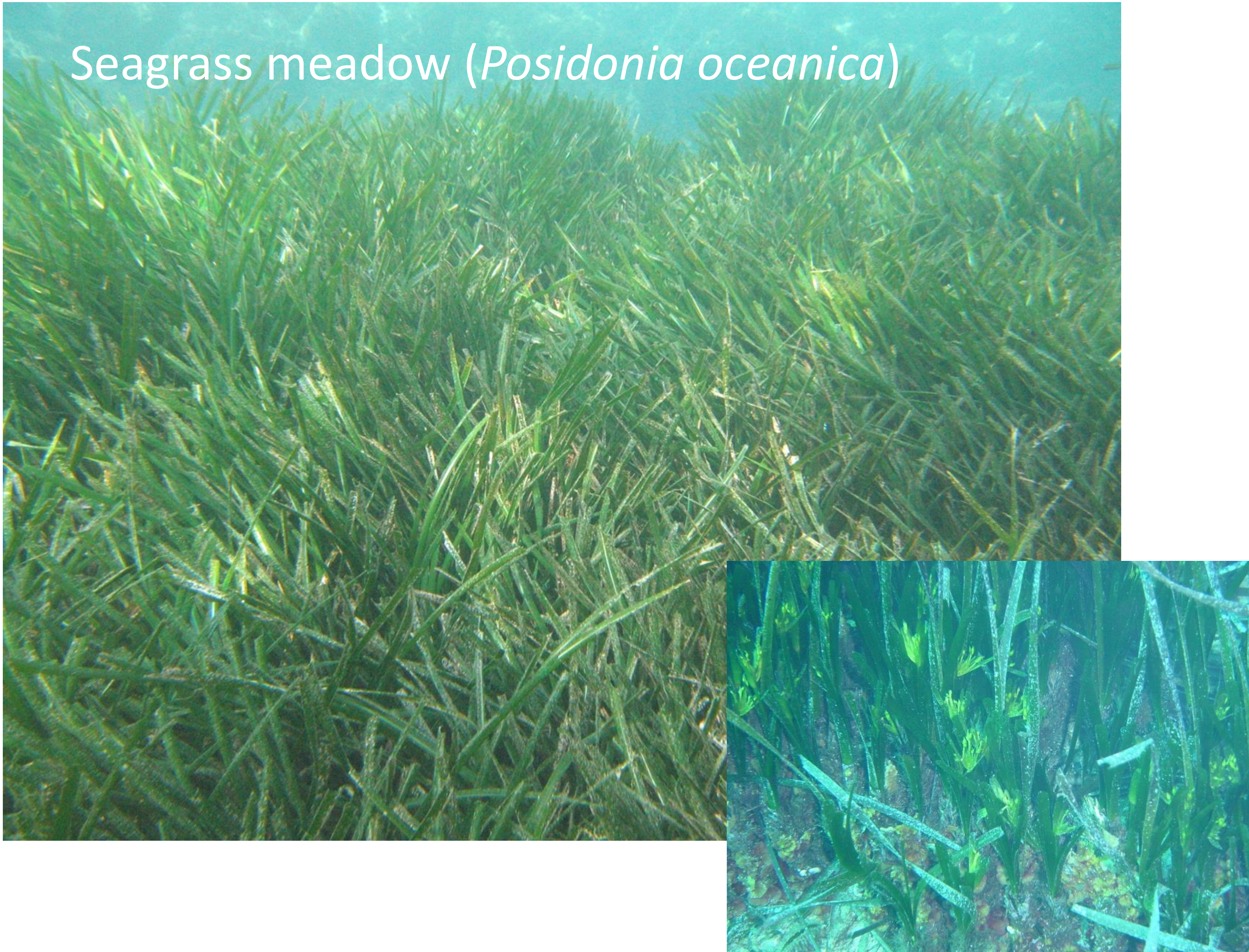
•A model is a model, not the reality

•The question strongly determine the sampling design for stable isotope purpose

•Complex problem needs multidisciplinary approach :
* Stomach contents are not obsolete
* Other tracers (FA, etc.) bring other (and complementary) information

II. APPLICATIONS: Delineation of a trophic web

Seagrass meadow (*Posidonia oceanica*)

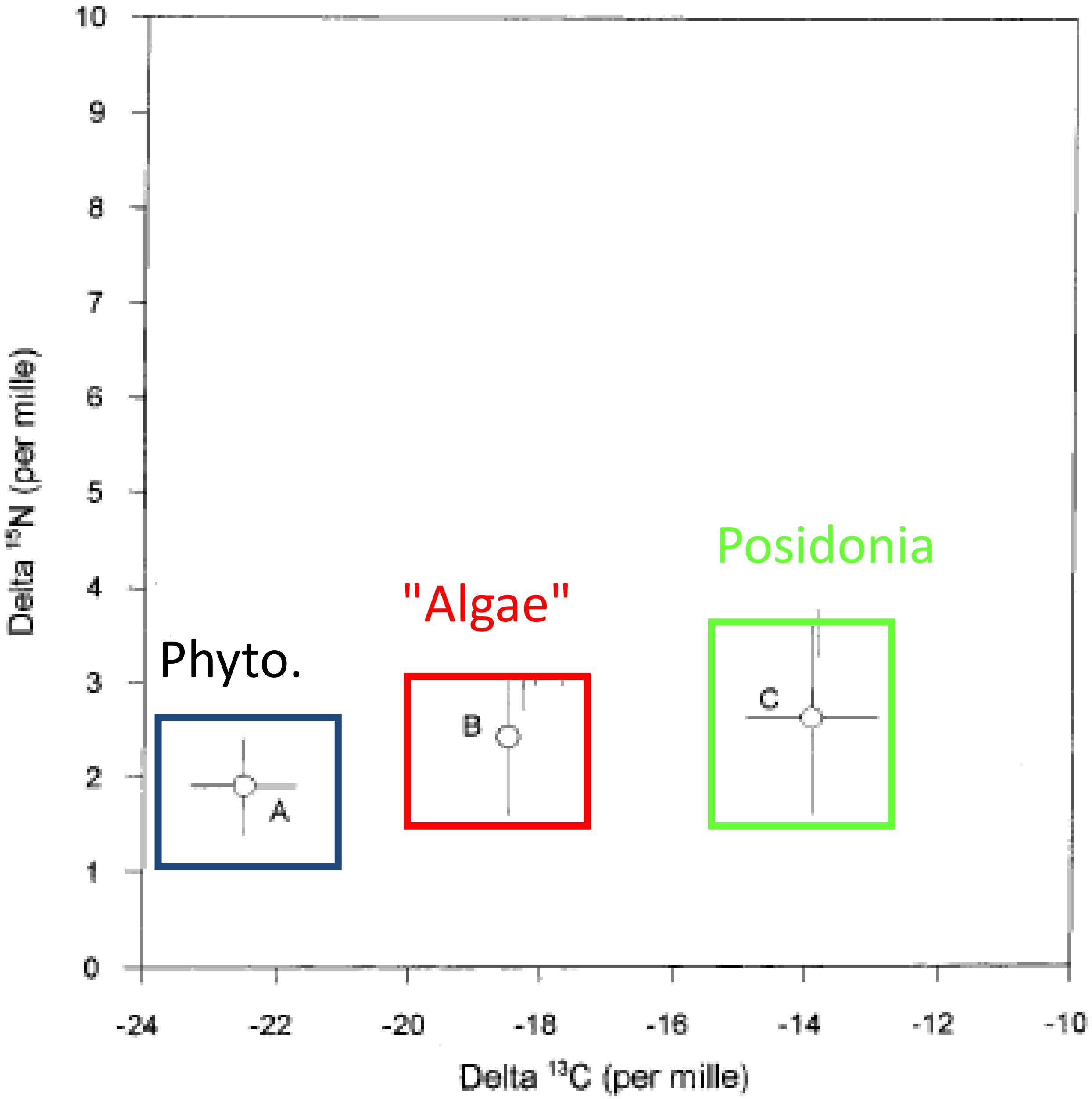


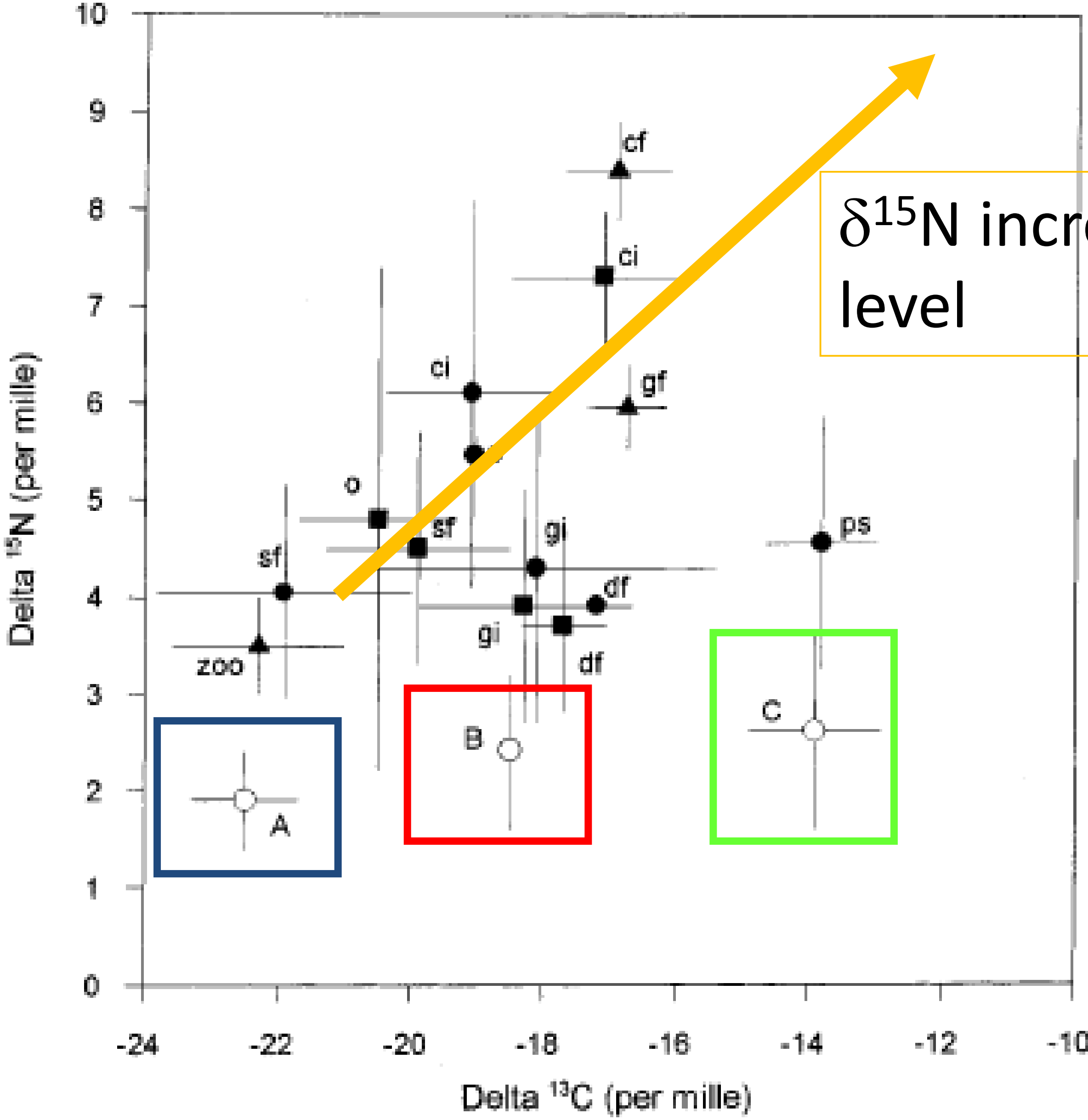
Epiphytic organisms

Leaf litter

+ sestonic material

Primary food sources



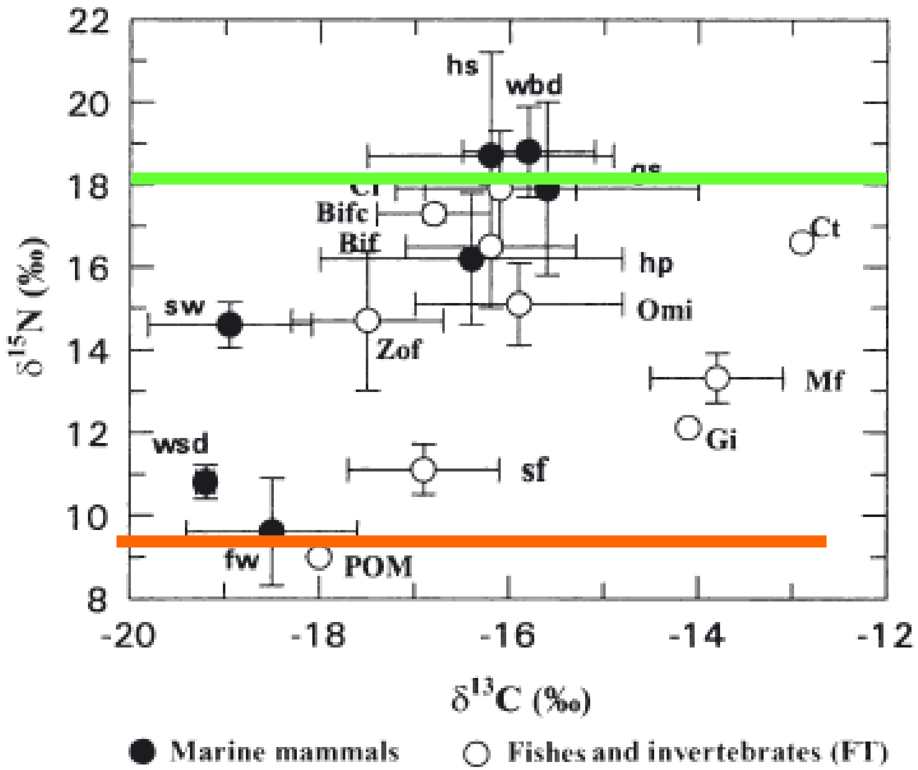


δ^{15}N increases with trophic level

Source: Lepoint et al. 2000

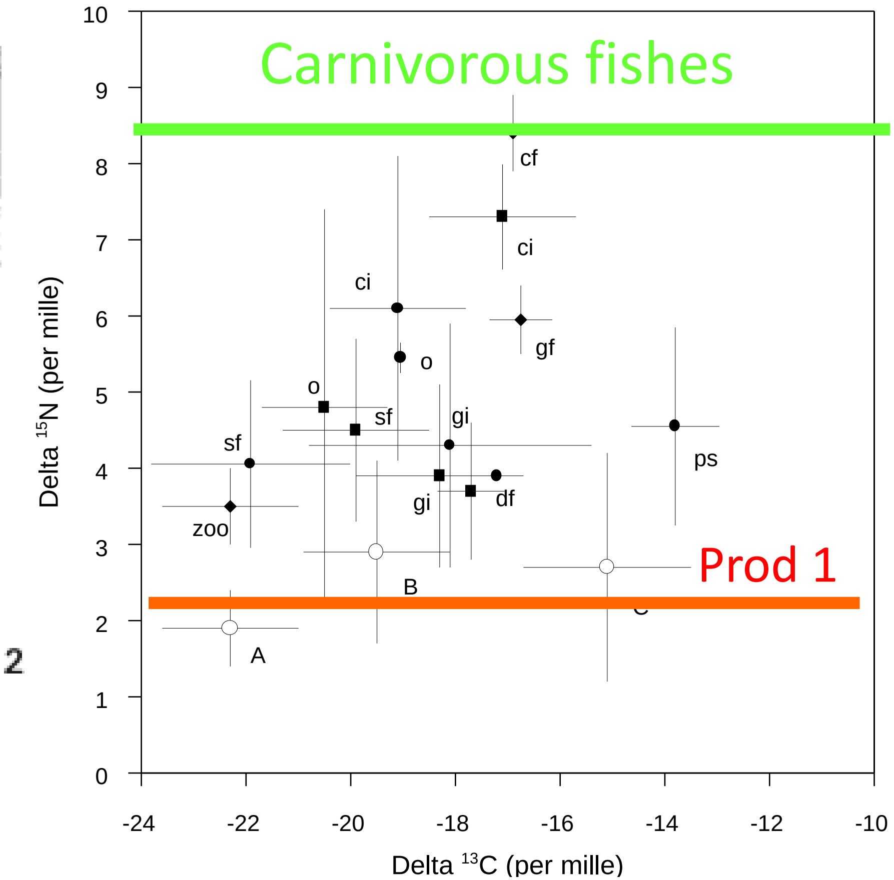
Base line variability

North Sea



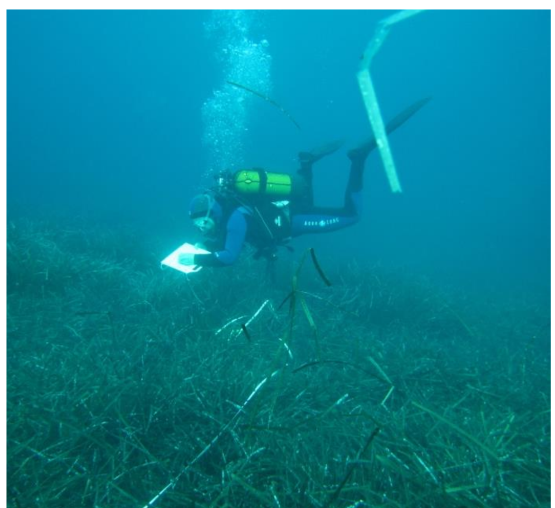
Das et al. MEPS 2003

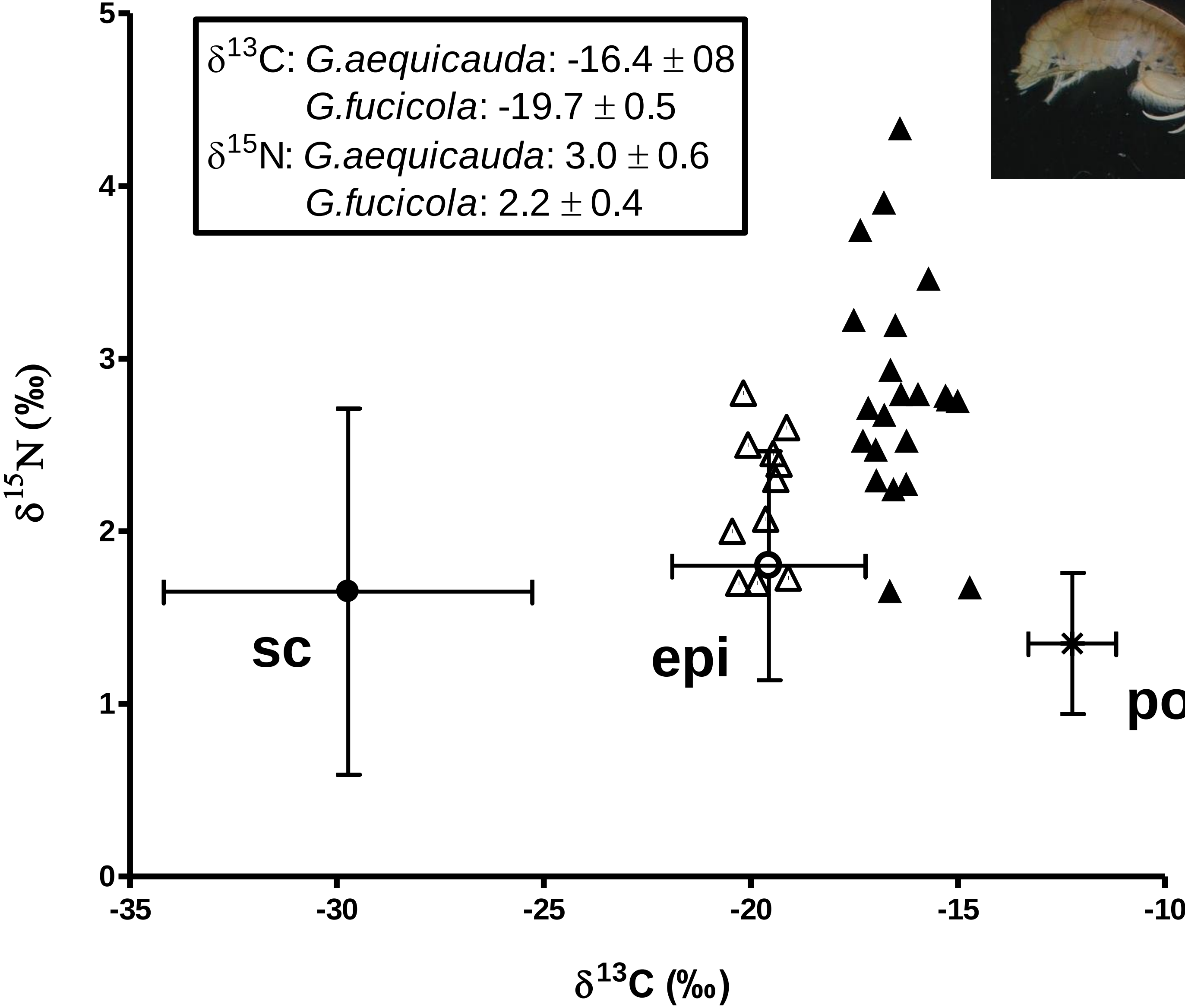
Mediterranean Sea



Lepoint et al. Mar Biol 2000

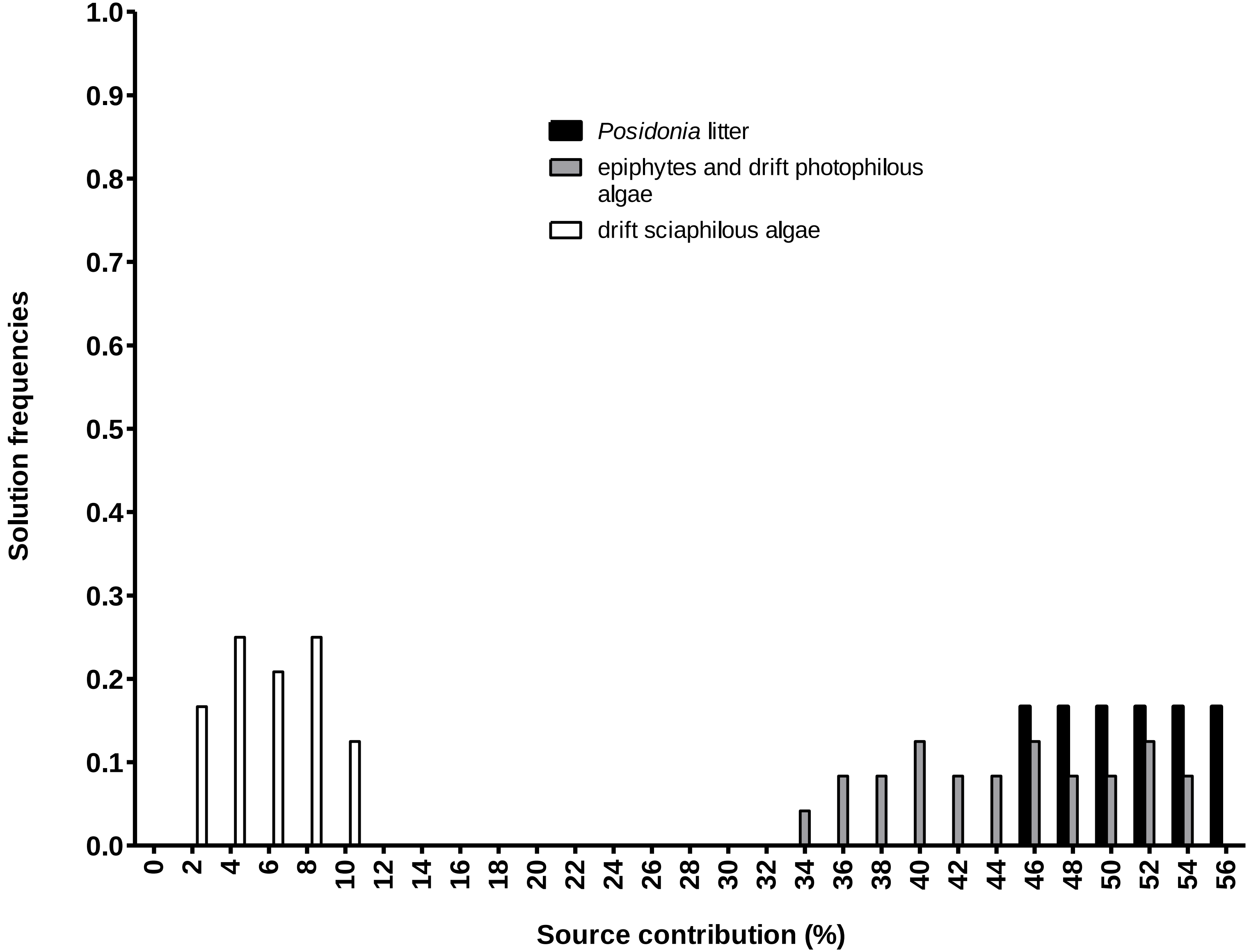
Exported macrophytodetritus





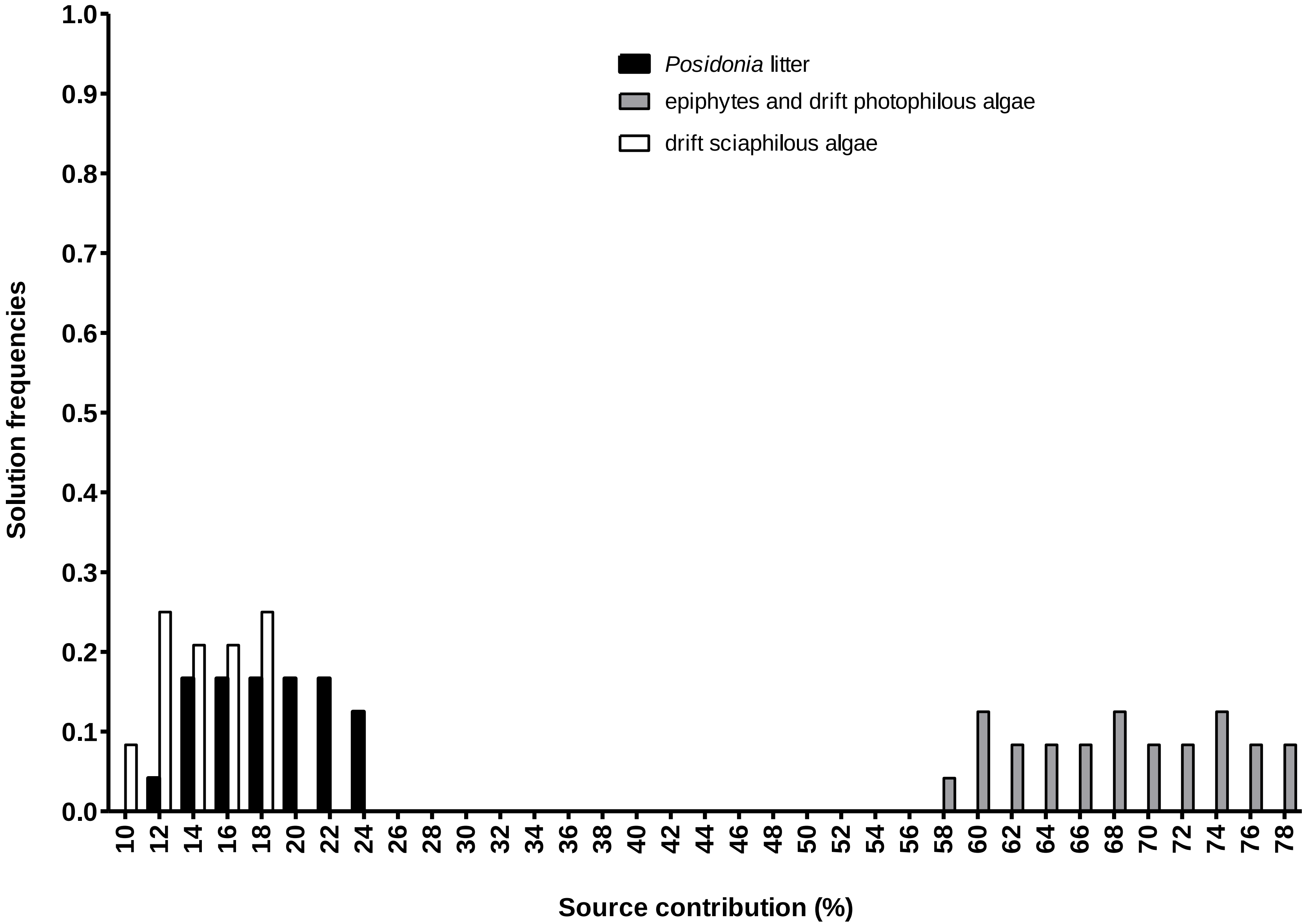
Source Lepoint et al. 2006

Gammarus aequicauda



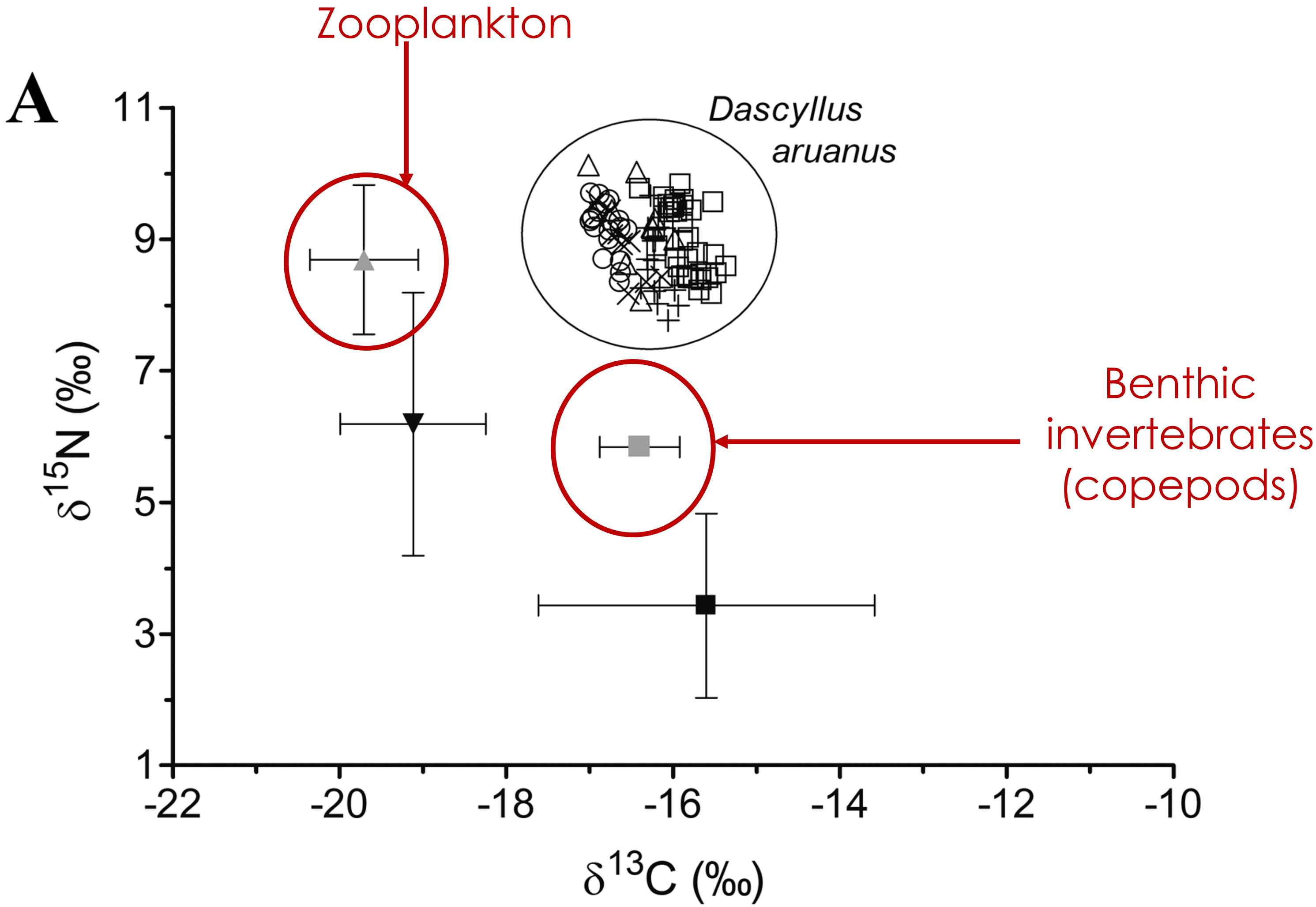
Source Lepoint et al. 2006

Gammarella fucicola



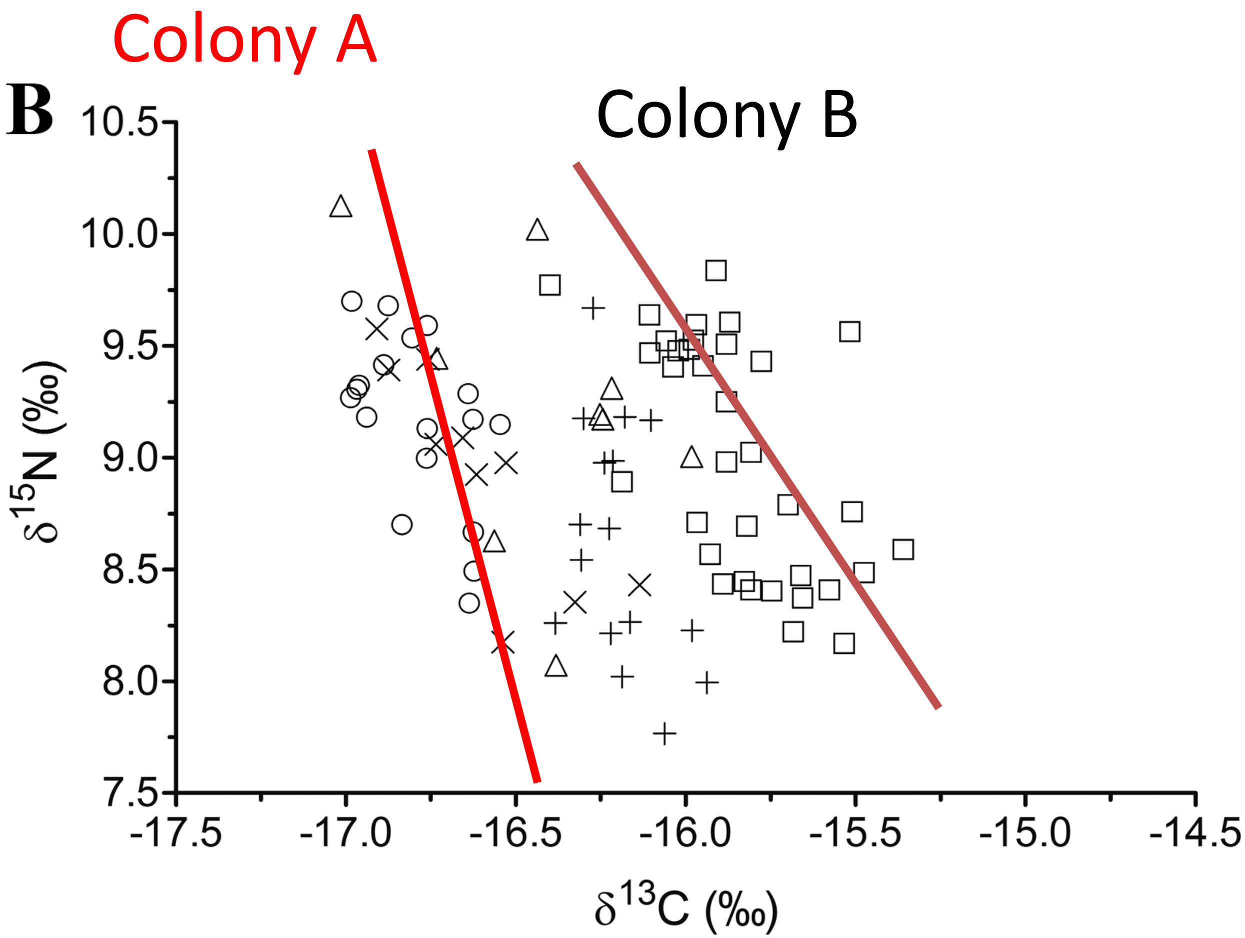
Intra-specific trophic diversity in a Pomacentrid species

Dascyllus aruanus



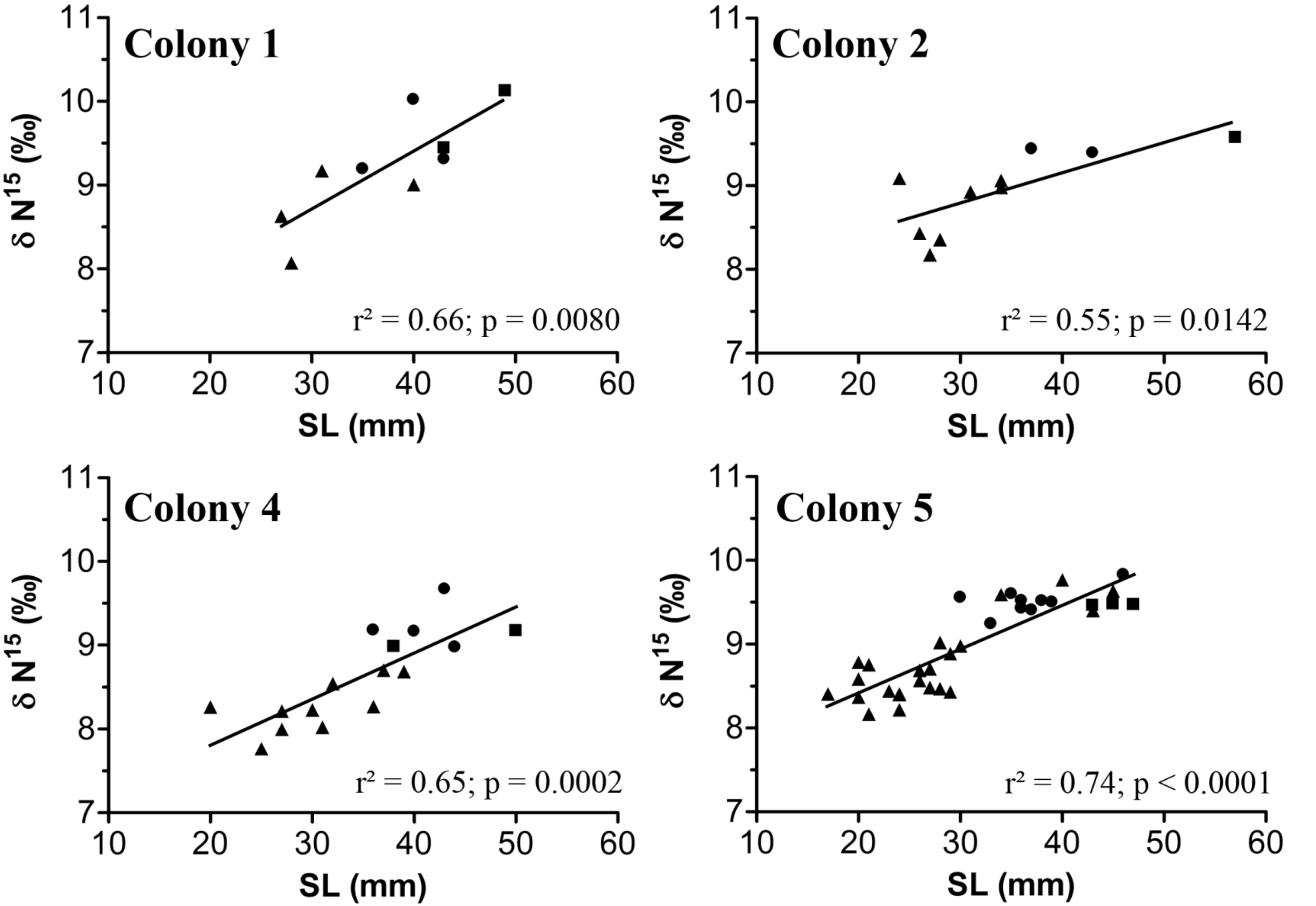
- Inter-individual variability: ≈ 2‰

Source: Frédérich et al. 2010, Copeia

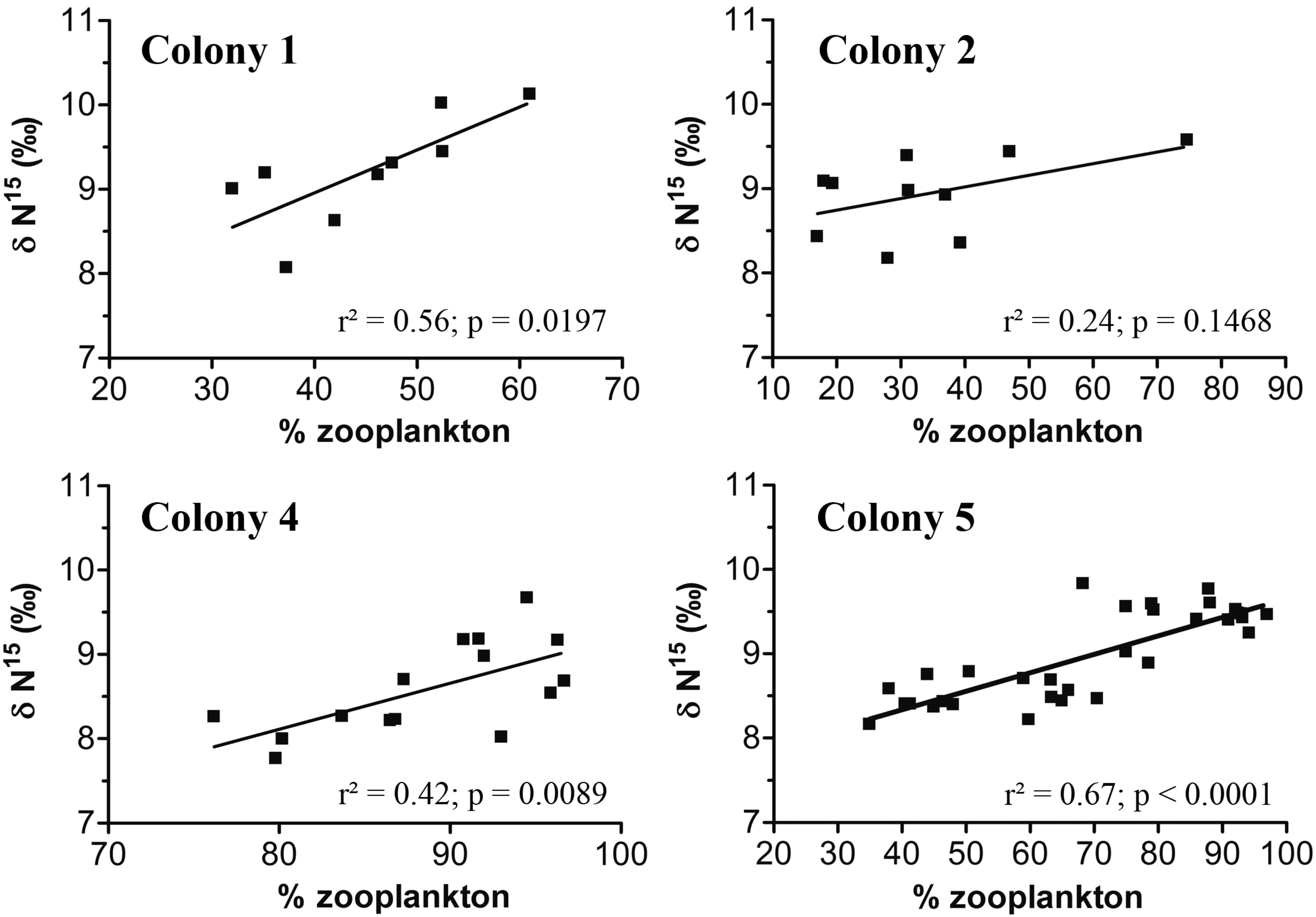


- Co-variations δ^{13}C et δ^{15}N, but variable between colonies

Source: Frédérich et al. 2010, Copeia



Source: Frédérich et al. 2010, Copeia



Source: Frédérich et al. 2010, Copeia

- Progressive trophic shift according to size (i.e. age, status in the colony)

- From zoobenthos to zooplankton

- Variable according to colony size = intra-population variability

III. APPLICATIONS 2: TO LINK ECOTOXICOLOGY AND TROPHIC ECOLOGY

- Generalities

- Case study 1 : to detect eutrophication in a marine coastal environment

- Case study 2: to elucidate contamination pathway of an organochlorine pesticide

GENERALITIES

- Why to use stable isotopes to detect or to understand pollution effect?




Generalities

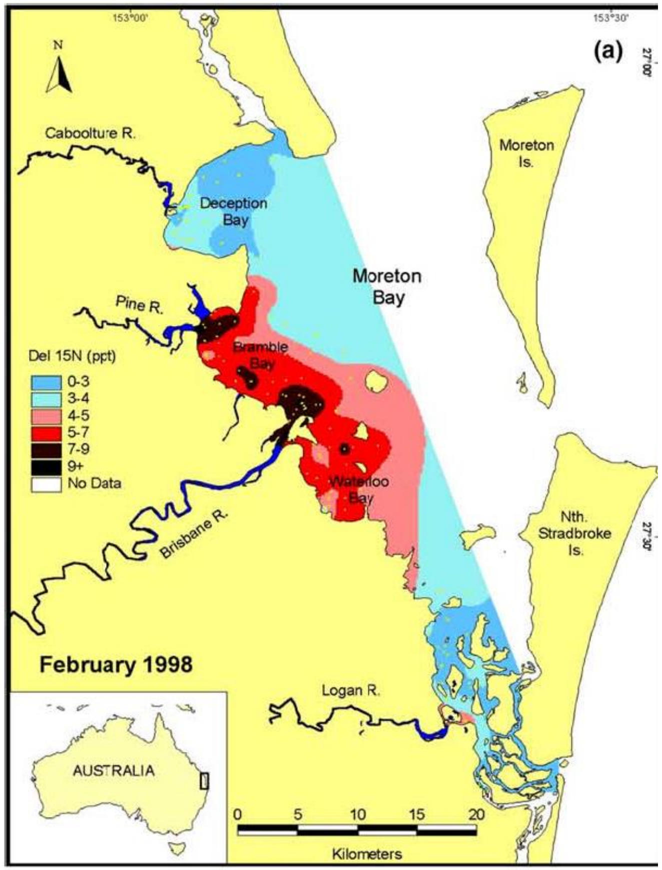
- Why to use stable isotopes to detect or to understand pollution effect?

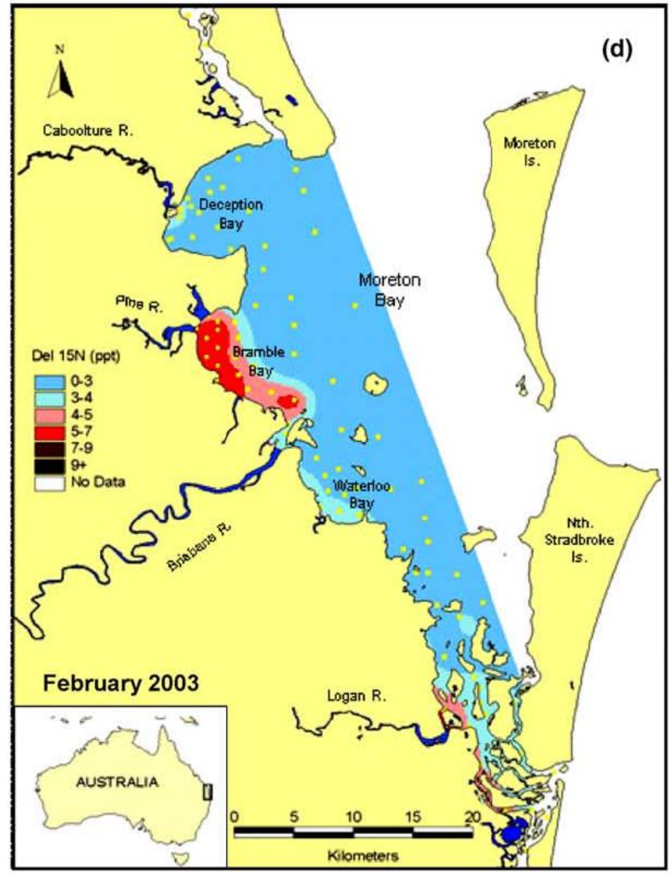
  - Pollution mapping

  - Problematic of diffuse/ponctual pollution

  - Understanding of pollution effect on trophic web

EXAMPLE OF ISOTOPIC MAPPING: MORETON BAY, AUSTRALIA





⇒ Isotopic mapping (IsoMap) or isototopic landscape/seascape (IsoScape) (www.isoscape.org)

Source: Costanzo et al. (2005), Mar Pollut Bull 54: 212-217

CASE STUDY 1 : TO DETECT EUTROPHICATION IN A MARINE COASTAL ENVIRONMENT

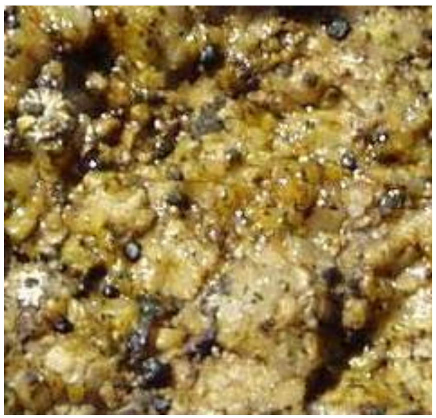
Spatial and temporal responses of marine gastropods and biofilms to urban wastewater pollution in a Mediterranean coastal area

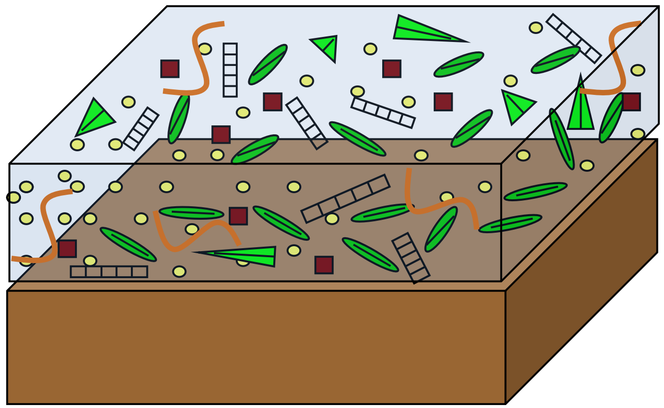
Sources: Vermeulen S. (phD thesis, 2012) and Vermeulen et al., (2011) Mar Ecol Prog Ser 422: 9-22

- Main objective: to assess the efficiency of a new set of potential early bioindicators of urban wastewater pollution for Mediterranean coastal areas in a environmental monitoring context.
- Potential bioindicators: epilithic biofilm and two of their consumers

Epilithic biofilms

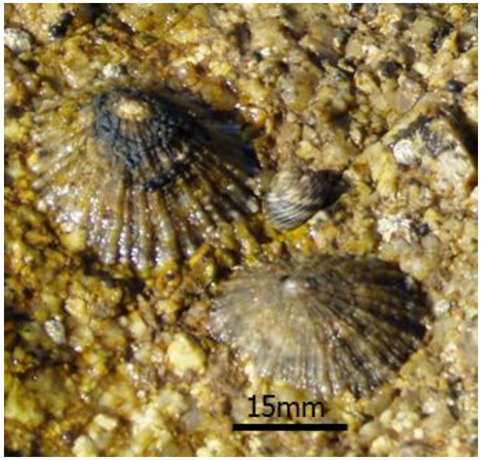
- ubiquitous communities
- 1st colonization step





Sources: Vermeulen S. (phD thesis, 2012) and Vermeulen et al., (2011) Mar Ecol Prog Ser 422: 9-22

Biofilm consumers

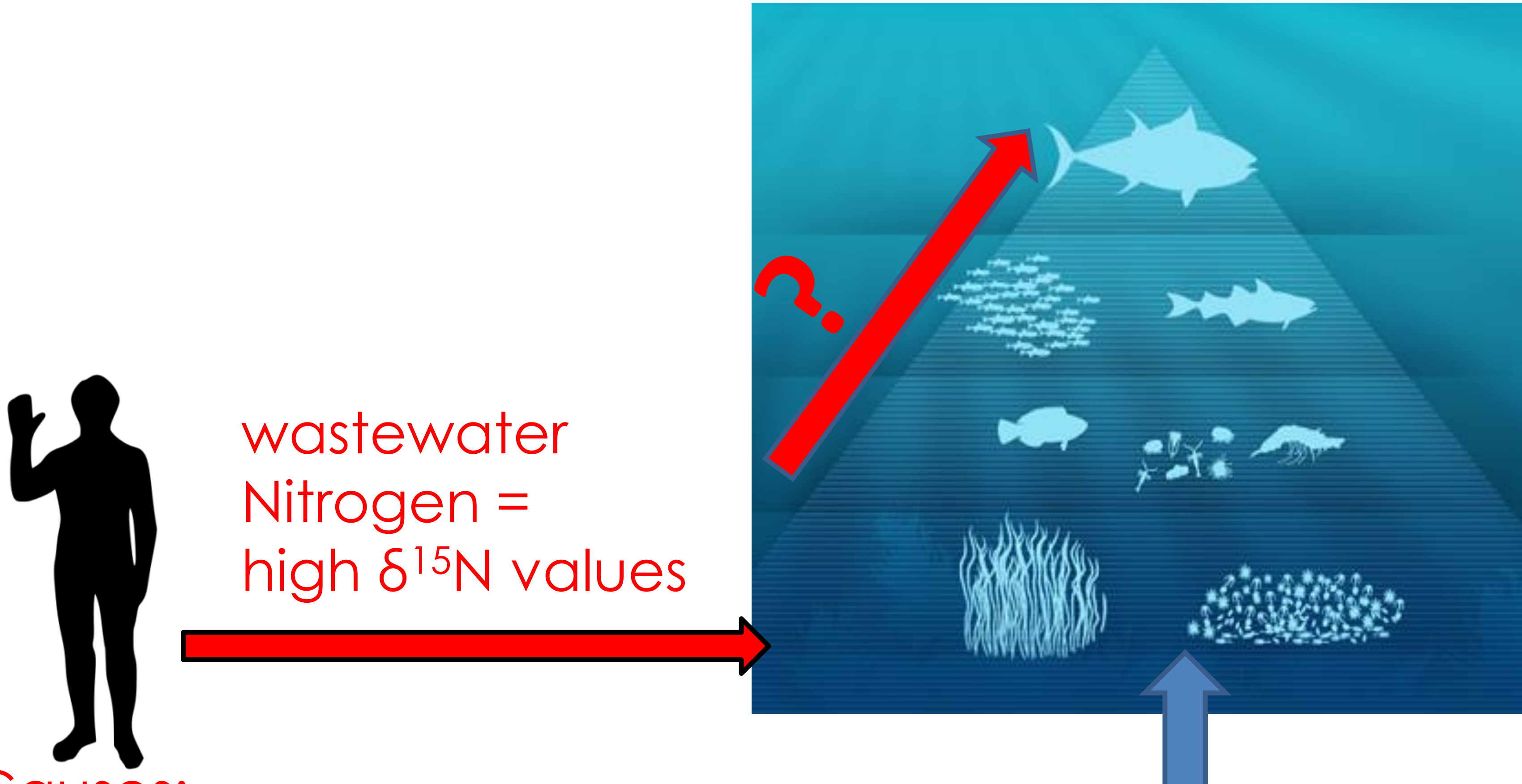


Patella caerulea

Monodonta turbinata

- most frequent organisms of the Mediterranean midlittoral zone
- easily accessible
- available all year long
- low mobility
- primary consumers (biofilms)

Principle to use delta ^{15}N as a tracer of wastewater nitrogen

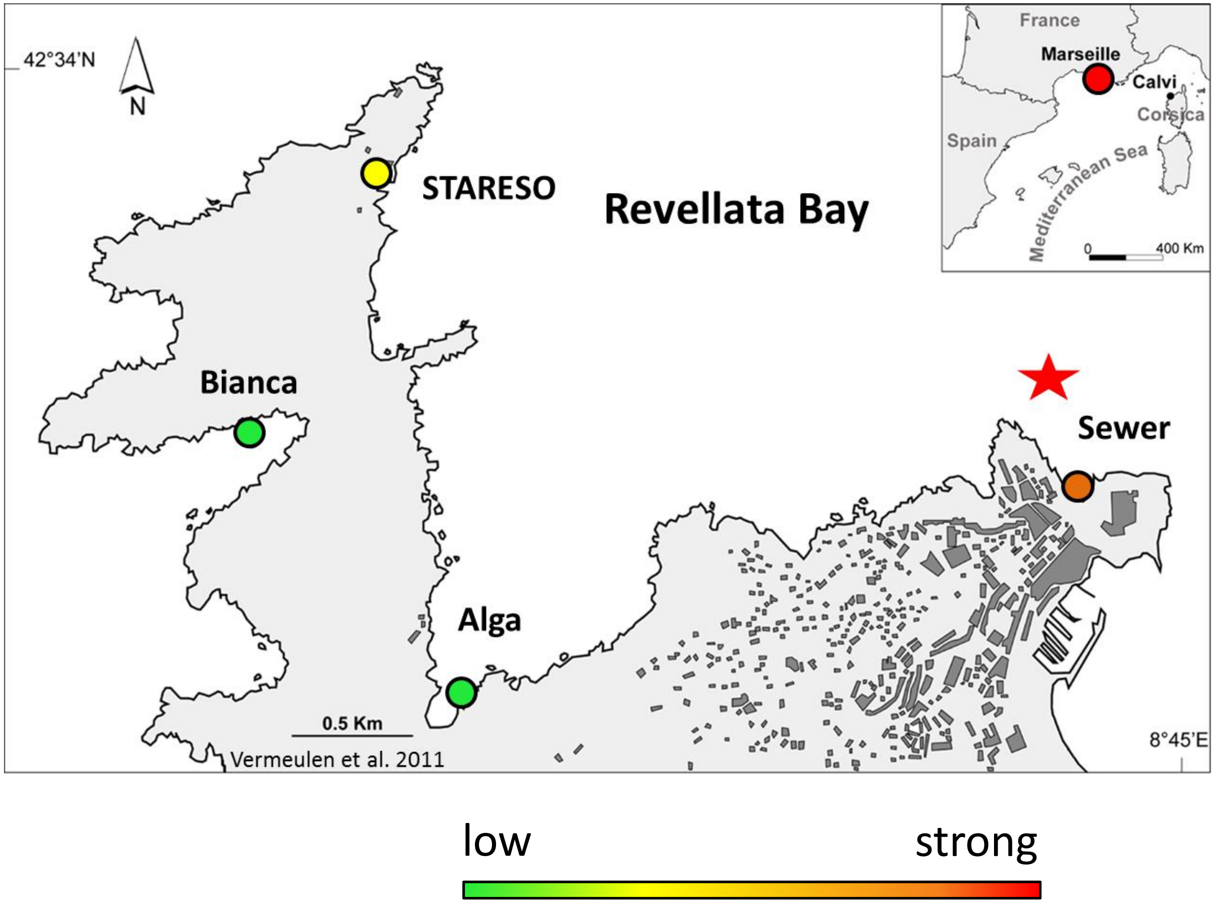


Ambient nutrients

Causes:
- Human = high trophic level
-Fractionation during mineralisation process
-Freshwater and terrestrial nutrient

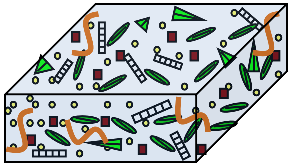
Sources: Vermeulen S. (phD thesis, 2012)

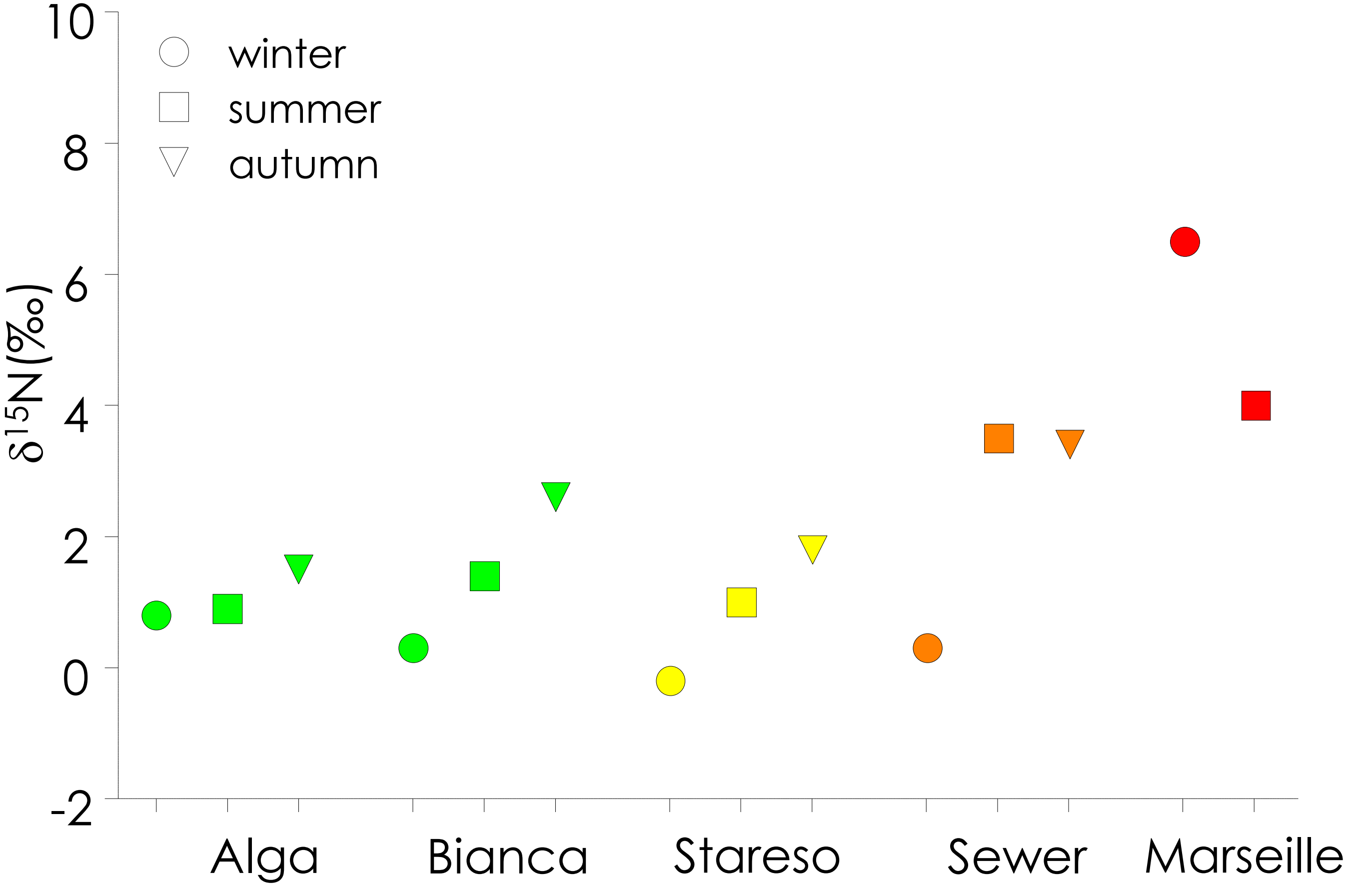
SAMPLING DESIGN



Sources: Vermeulen S. (phD thesis, 2012) and Vermeulen et al., (2011) Mar Ecol Prog Ser 422: 9-22

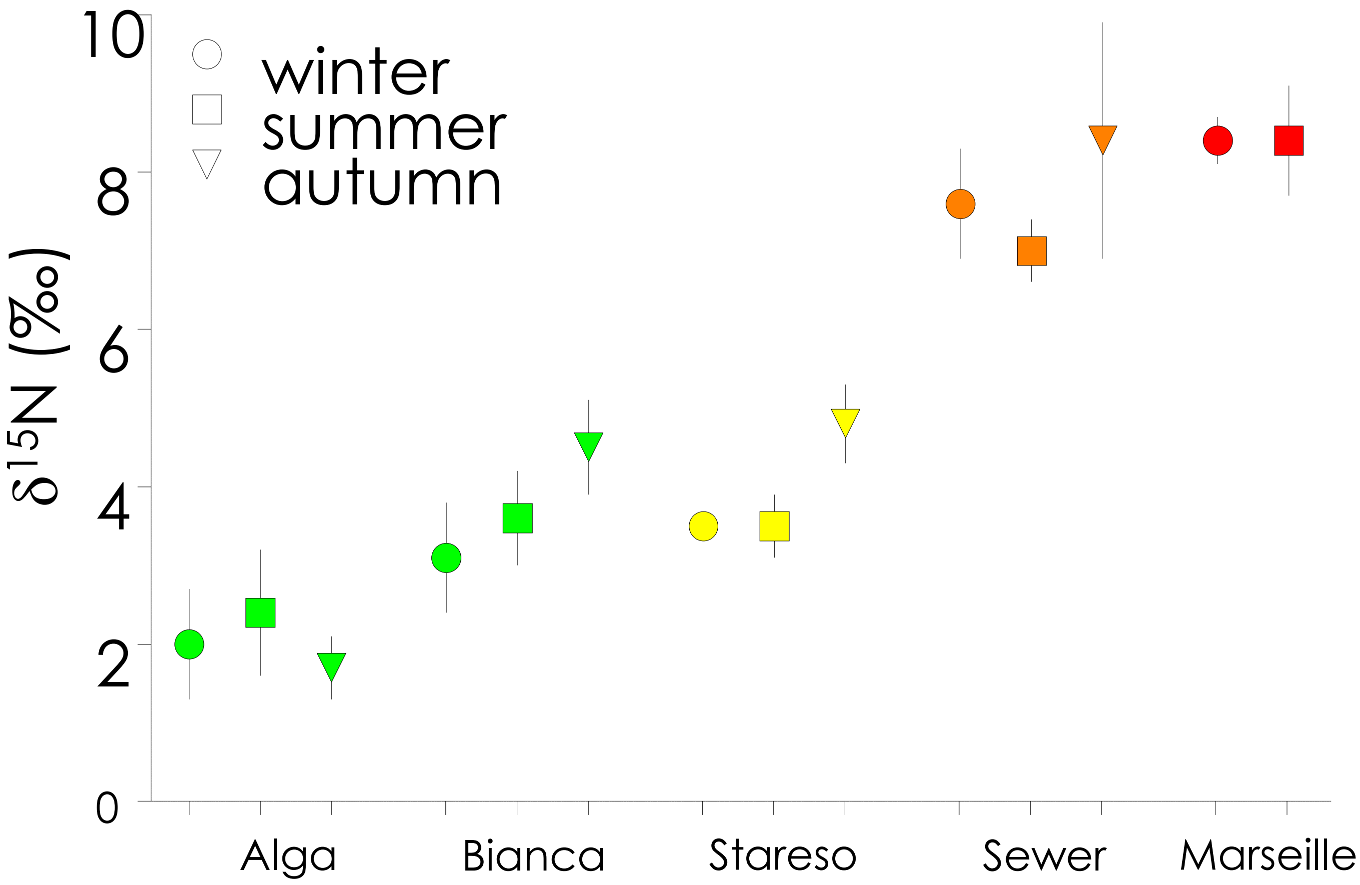
Epilithic biofilms





Sources: Vermeulen S. (phD thesis, 2012) and Vermeulen et al., (2011) Mar Ecol Prog Ser 422: 9-22

Limpets (*Patella caerulea*)



Sources: Vermeulen S. (phD thesis, 2012) and Vermeulen et al., (2011) Mar Ecol Prog Ser 422: 9-22

•Limpets fed from algae that incorporated wastewater nitrogen and / or from spatially differing community types

•Steady values across seasons (low turnover rate of muscles)

CASE STUDY 2: TO ELUCIDATE CONTAMINATION PATHWAY OF AN ORGANOCHLORINE PESTICIDE

Organochlorine pollution in tropical rivers (Guadeloupe): Role of ecological factors in food web bioaccumulation

Source: Coat 2010 (phD thesis), Coat et al. (2009) Freshwater Biology 54, pp. 1028-1041 , Coat et al. (2011), Environmental Pollution 159: 1692-1701

PROBLEMATICS

- Heavy contamination by organochlorine pesticides (Banana culture)

- Is there a relation between trophic level and pollutant contamination

Figure 1: Pérou River sampling site (Guadeloupe)

Figure 2: Example of crustacean species found the river Pérou fauna: *Atya innocous* (Atyidae) (a) ; *Macrobrachium heterochirus* (Palaemonidae) (b) ; *Xiphocaris elongata* (Xiphocarididae) (c) (photos: Nicolas Marichal)

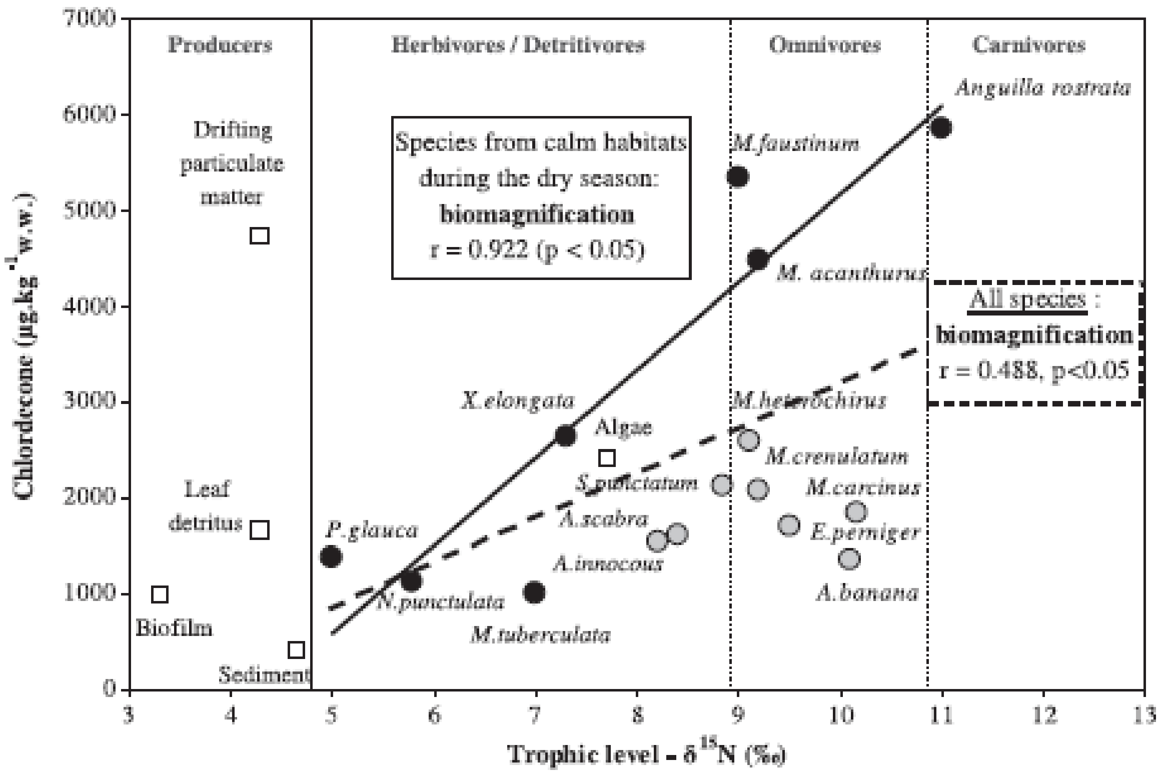


Fig. 3. Chlordecone concentrations versus trophic level measured in river samples during the dry season (the hatched regression line represents the statistically significant relationship in biota (all circles), the complete regression line only takes into account the species living in calm habitats (black circles), no relationship is observed for the species living in rapid running waters (grey circles)).

Source: Coat et al. (2011), Environmental Pollution 159: 1692-1701

IV. TAKE HOME MESSAGE

- Isotopic approach is a powerful technique (particularly when associated with other approaches)

- but numerous limitations and assumptions

- First the question, then the sampling design and methodological choice

Thank you for your attention

Acknowledgements

Firstly, I am sincerely grateful to Marleen De Troch who gives me the opportunity to participate in this course. I wish also to thank the numerous collaborators and co-authors who have contributed to this isotopic journey. I am Associated-Researcher at FRS-FNRS.

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