MULTIPLE APPLICATIONS OF CARBON AND NITROGEN ISOTOPE MEASUREMENTS: (1) TROPHIC NICHES AND (2) LINKING ECOTOXICOLOGY AND TROPHIC ECOLOGY

Gilles Lepoint
Laboratory of Oceanology – University of Liège

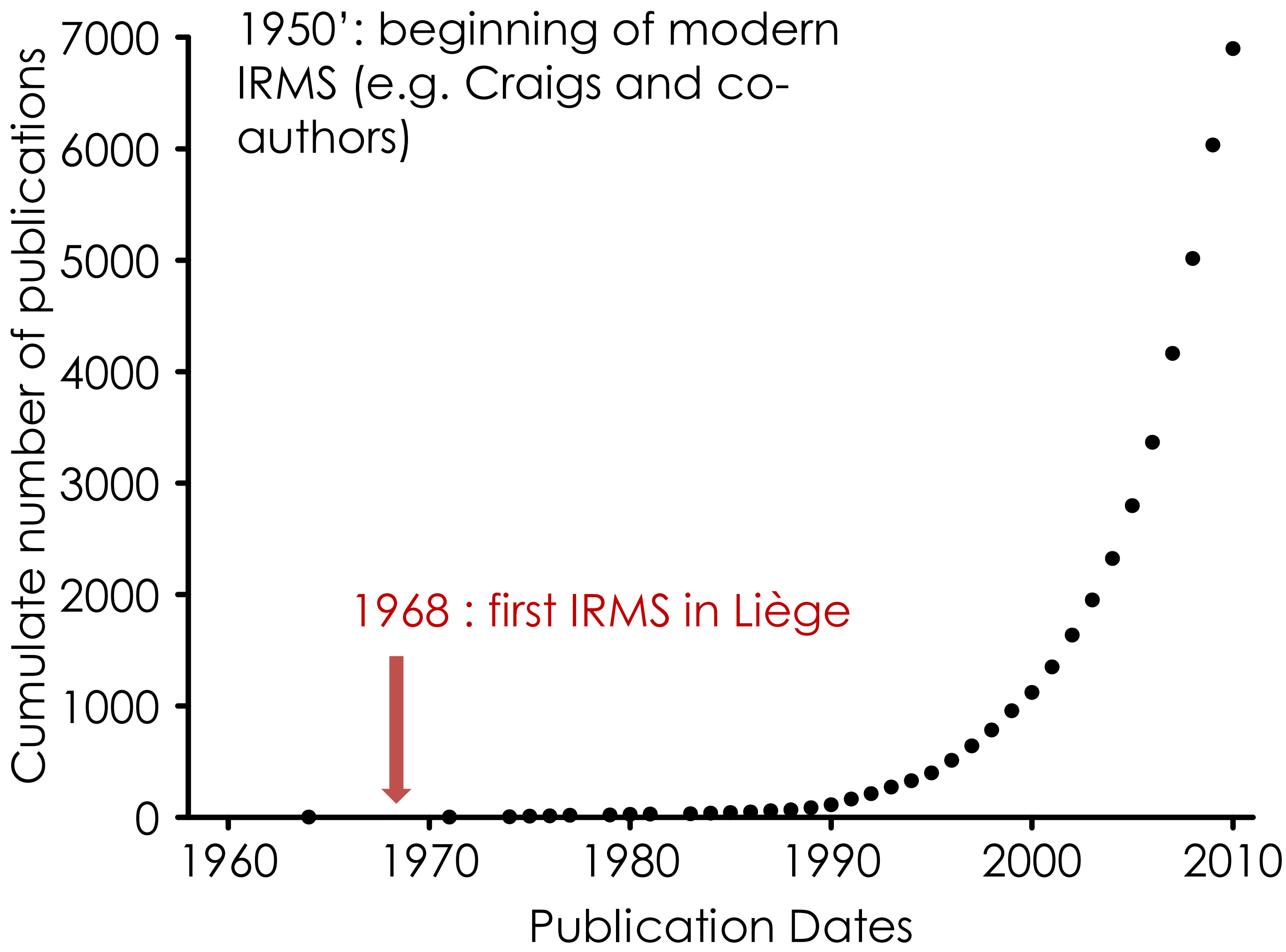
Partim of the Specialist course: Applications of Biomarkers in Aquatic food web studies (28th January -01st February)

-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

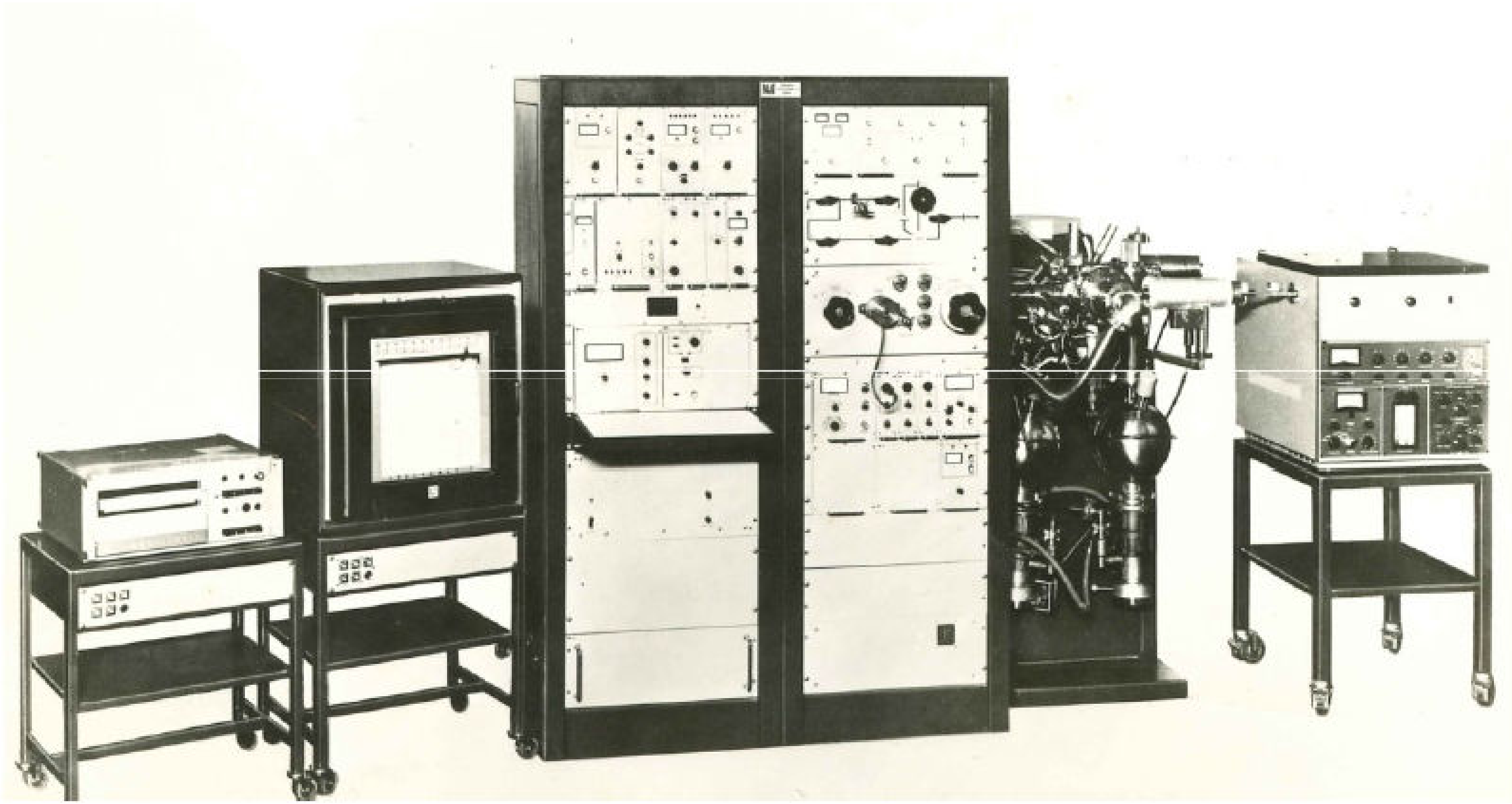
- Welcome
- Irms in Liège: few words about a long history
- IRMS instrumentation
- Applications 1: To assess trophic niches and their changes
- Applications 2: to link Ecotoxicology and Trophic Ecology
- Take home message

IRMS in Liège: few words about a long history

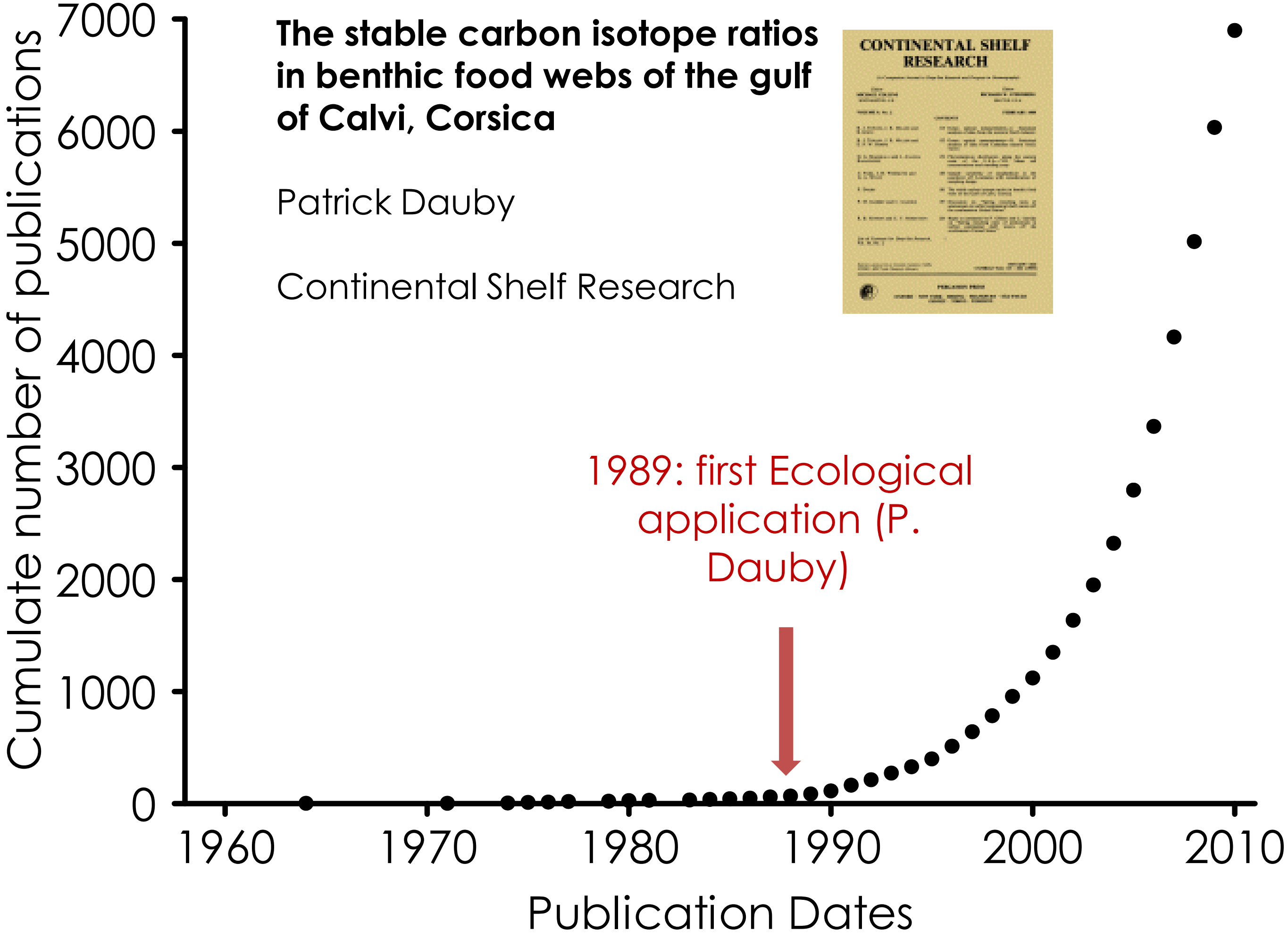


Data: IsiWeb of Knowledge, key words: (stable isotope) and (Nitrogen or Carbon) (Environmental Sciences and Agriculture)

Varian-MAT CH5 (later Finnigan, now ThermoFisher)



IRMS IN LIÈGE: FEW WORDS ABOUT A LONG HISTORY



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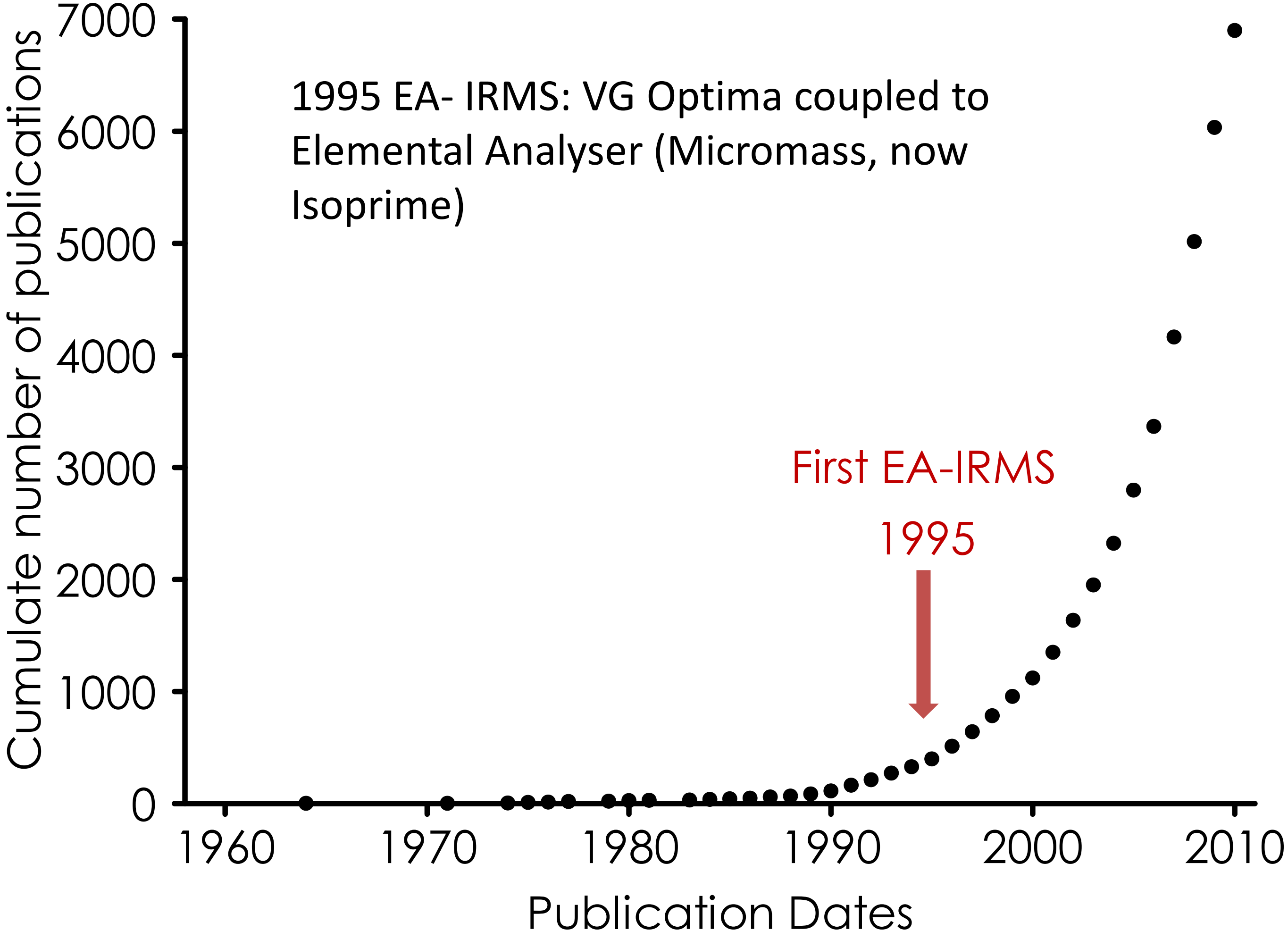
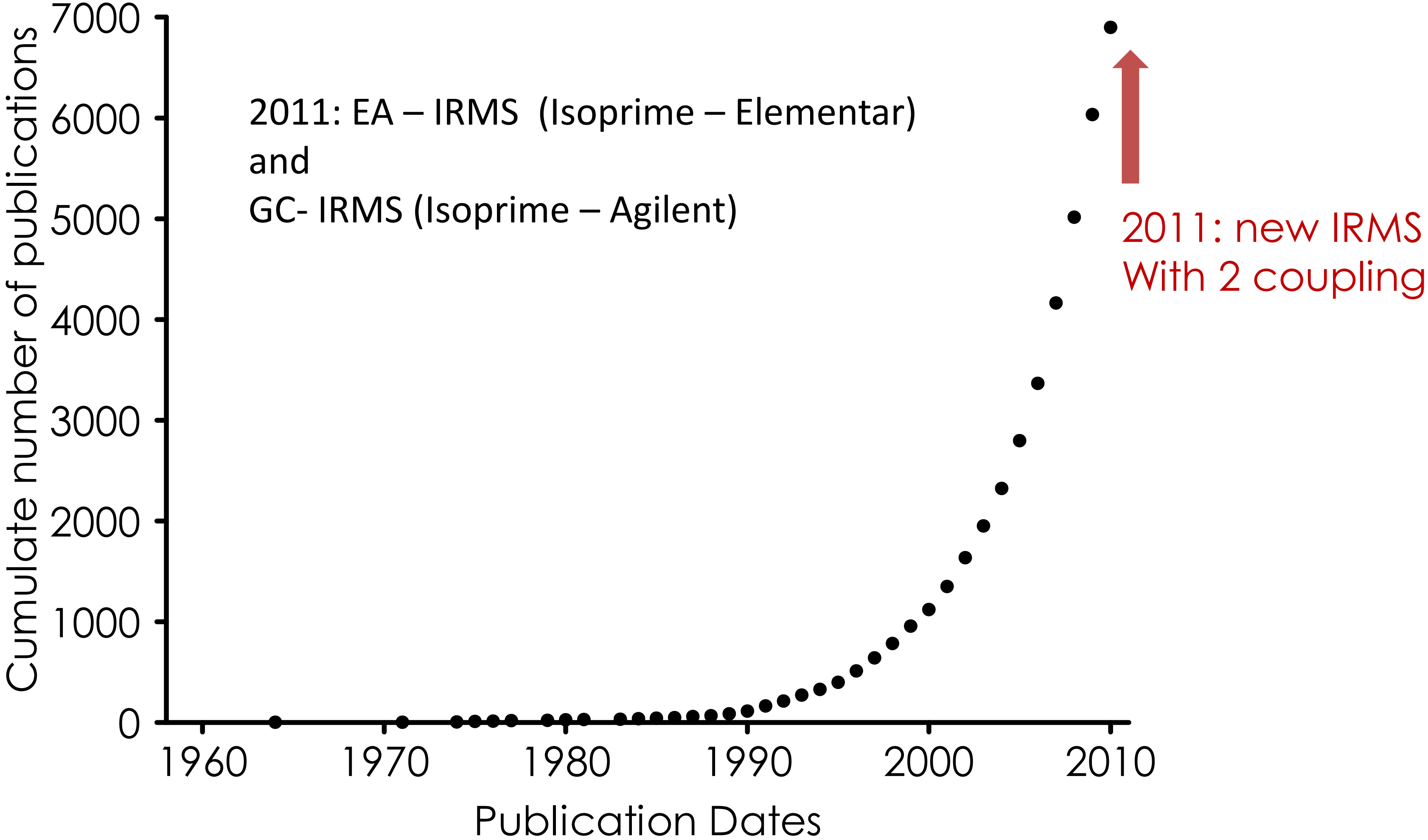
IRMS in Liège: few words about a long history



IRMS in Liège: few words about a long history



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de Liège
ULg

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***

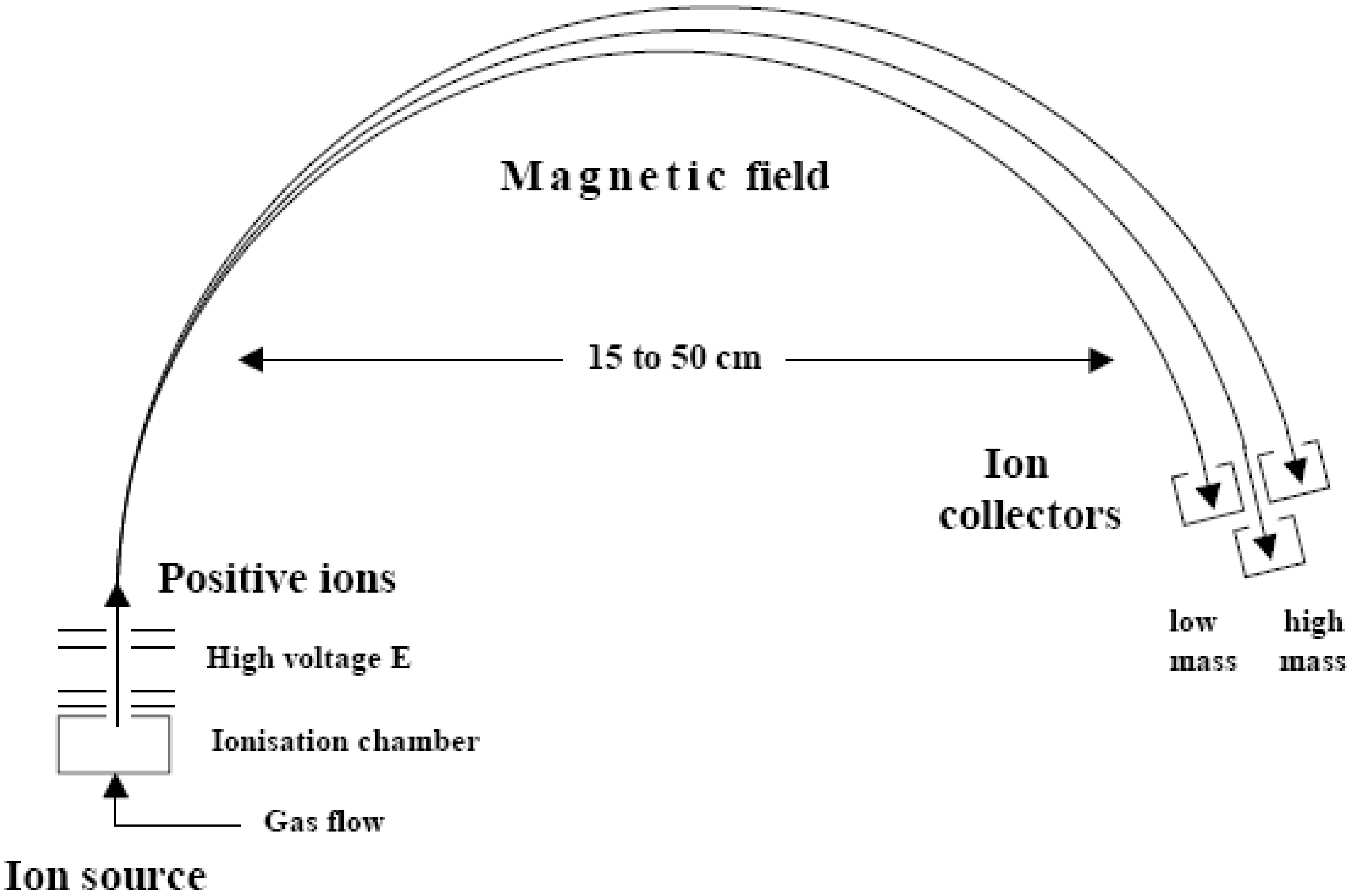
INSTRUMENTATION: IRMS

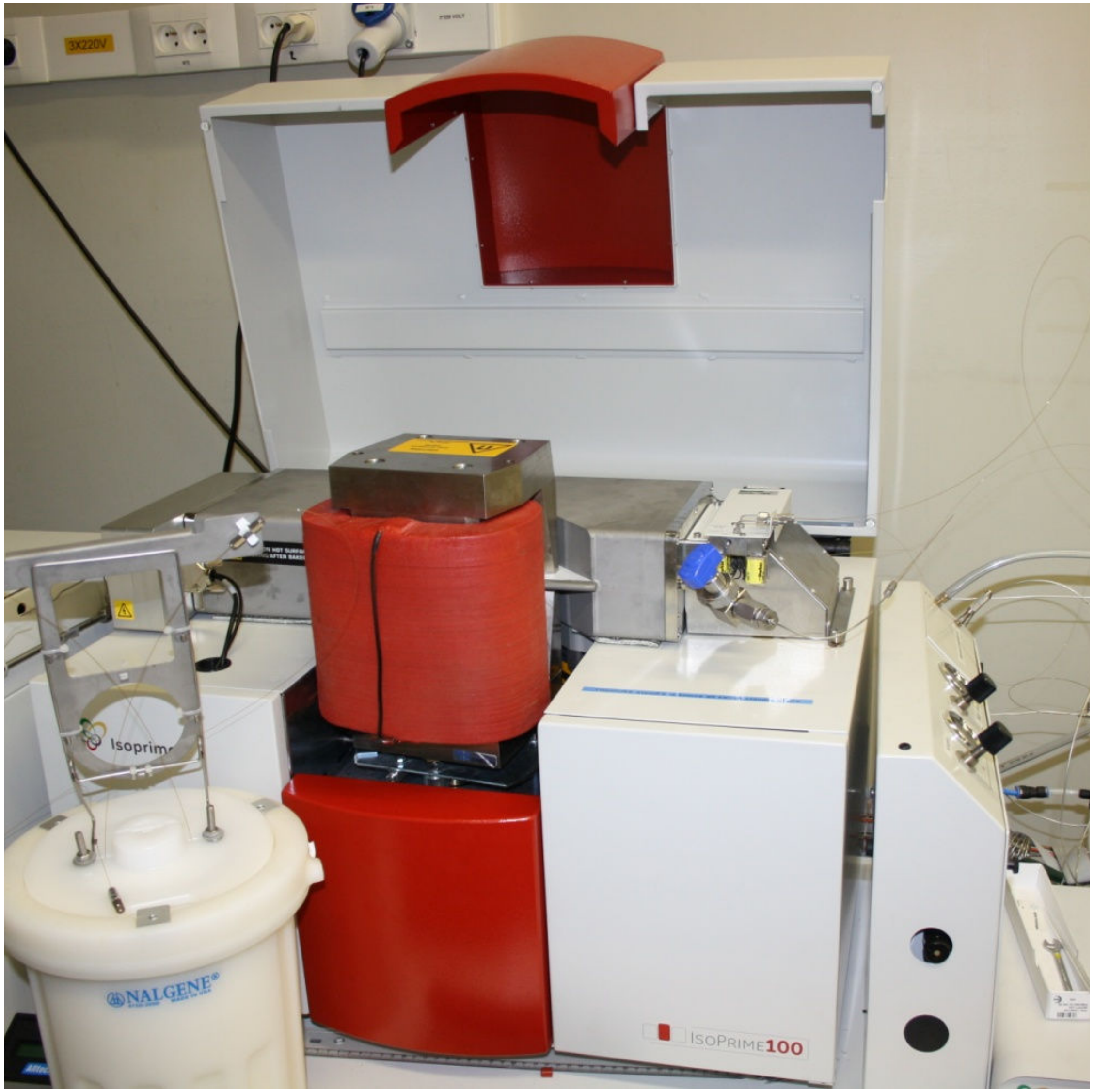
- How we measure?

Mass spectrometer equation

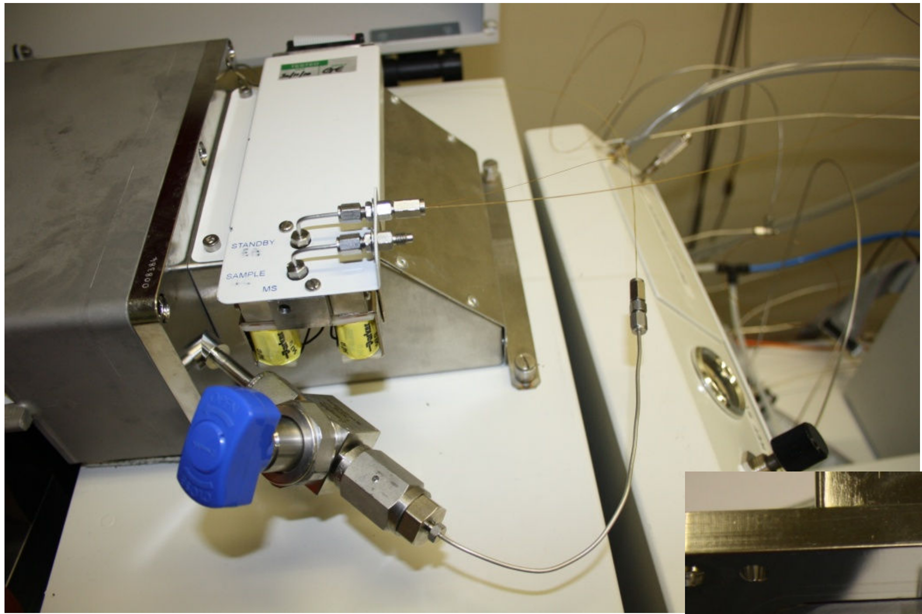
$$\frac{M}{Z} = \frac{B^2 R^2}{2V}$$

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.

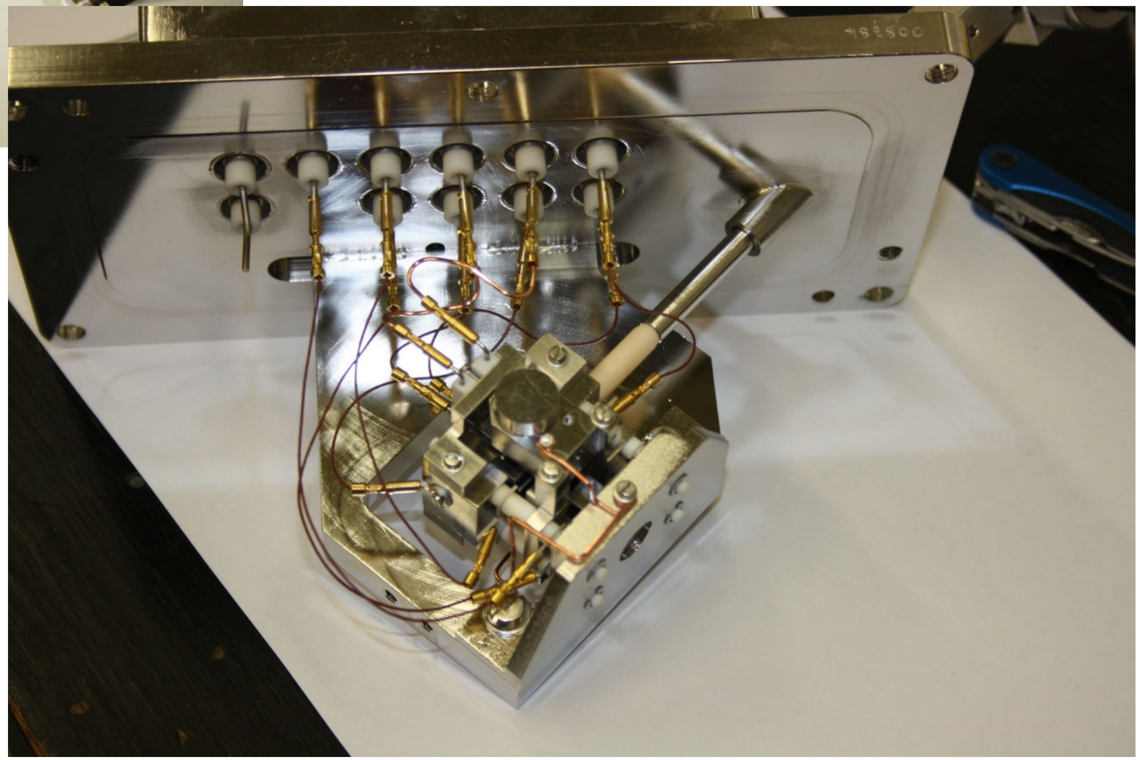

Magnetic field
15 to 50 cm
Ion collectors
Positive ions
High voltage E
Ionisation chamber
Gas flow
Ion source
low mass
high mass



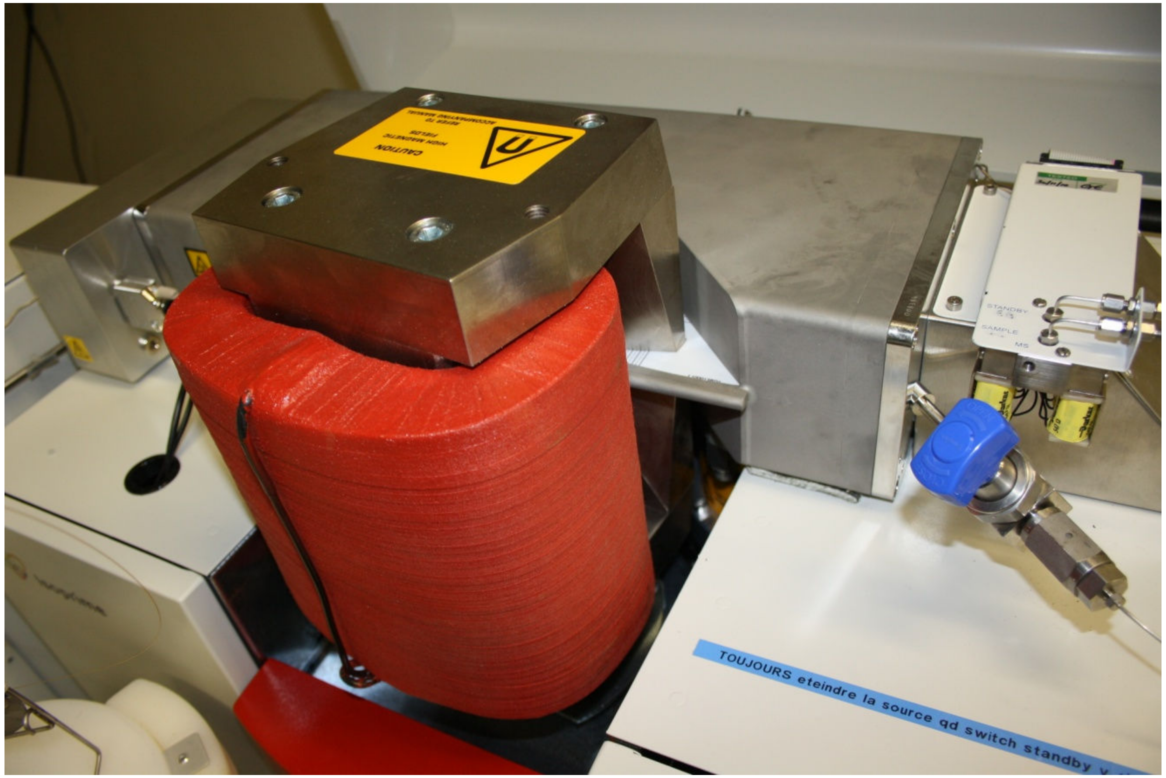
IRMS Isoprime 100 (Isoprime, UK)



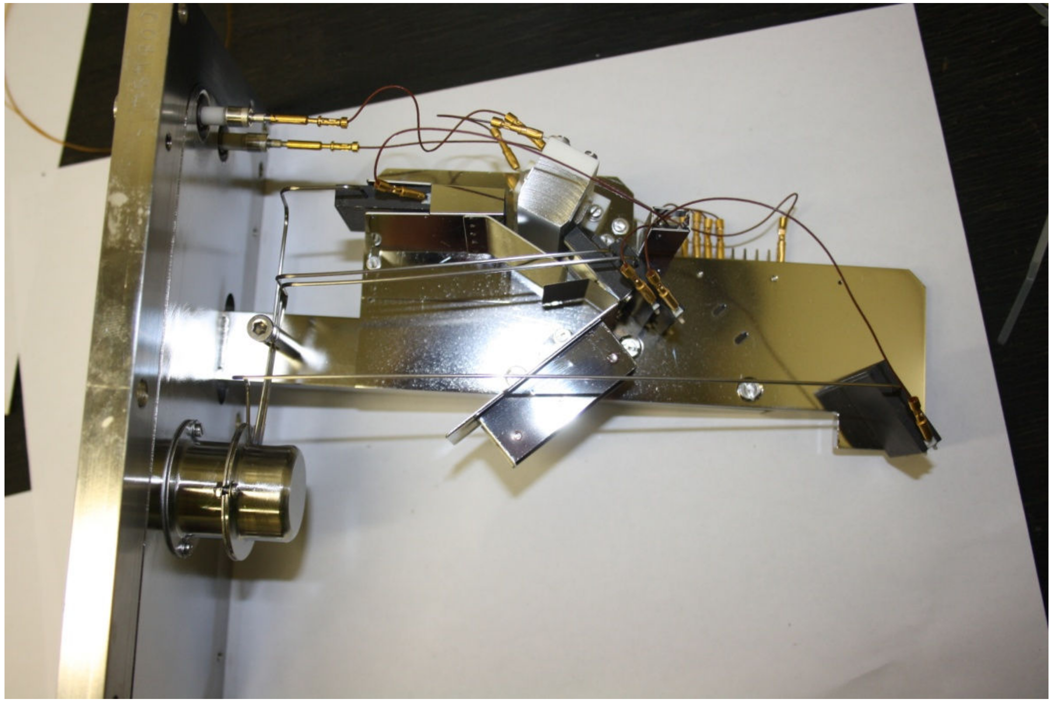
Sample inlet

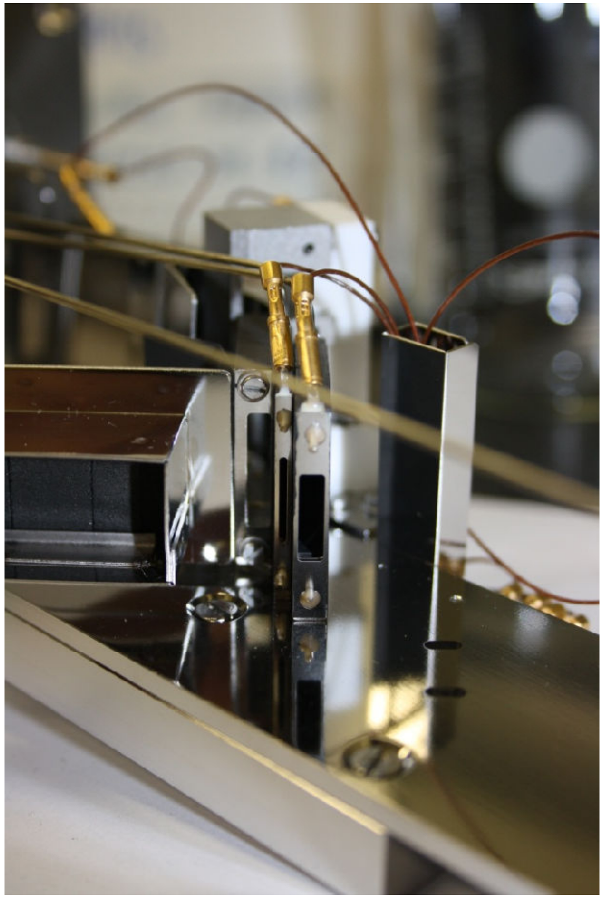


Source



Magnet and Flying tube



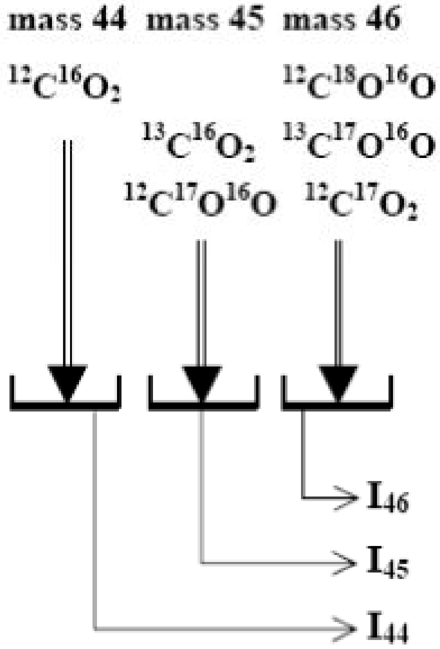


Collectors and Detection

Detection/amplification (Faraday cup)

Calculation

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



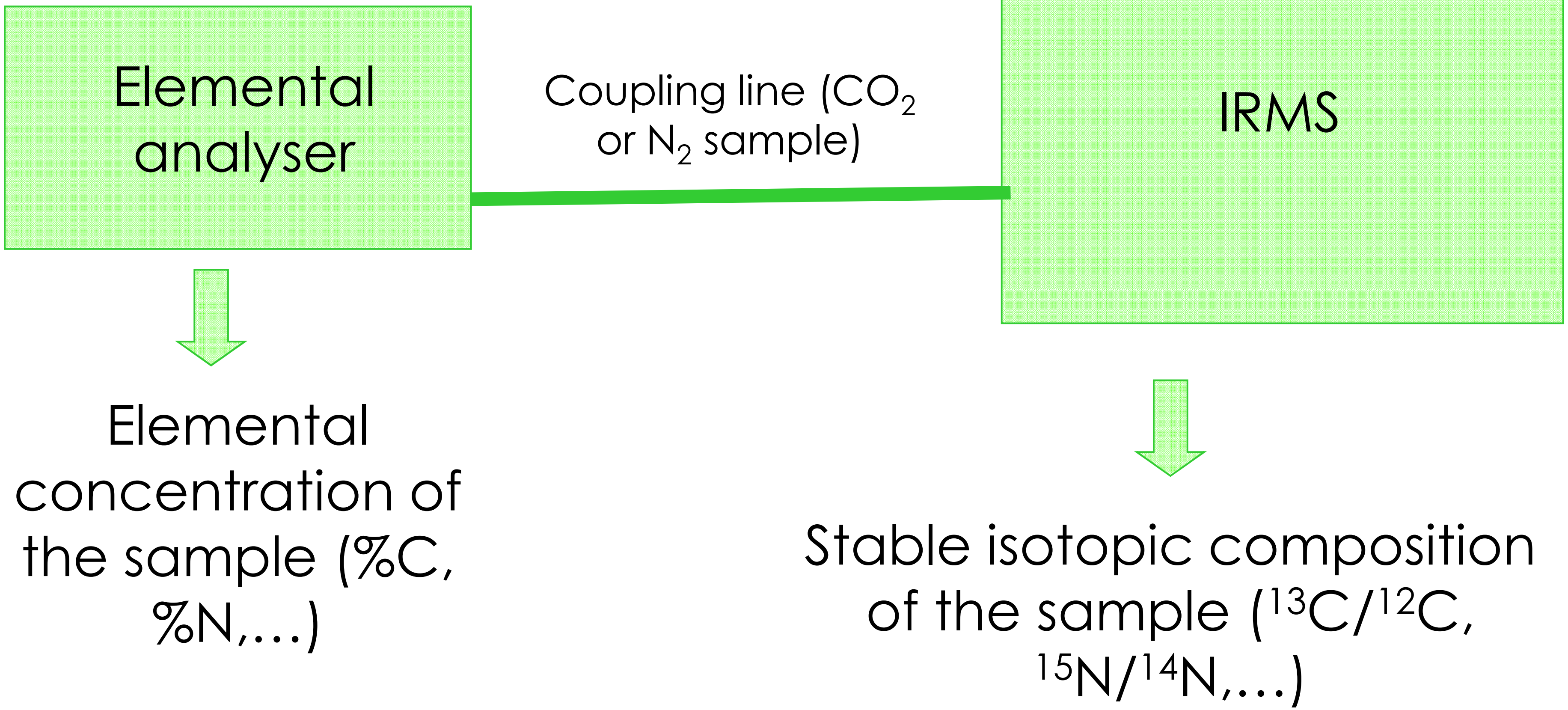
Collectors schema (for CO_2)

•Need to convert sample into simple gaz

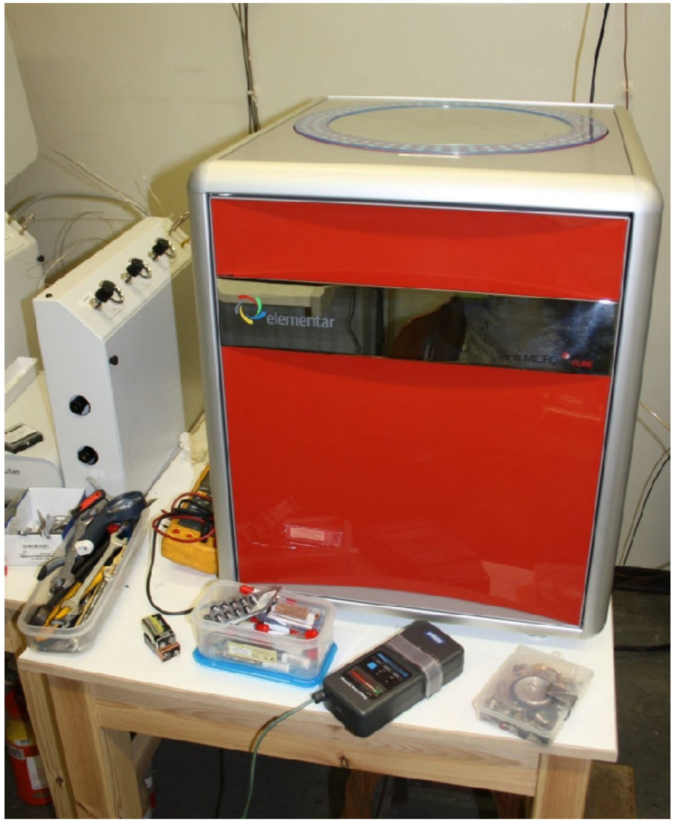
⇒ Preparation off line (till 1990') or on line = coupling of 2 instruments

Example 1: Coupling EA -IRMS

Solid or liquid samples
⇒ Combustion and conversion in CO_2, N_2



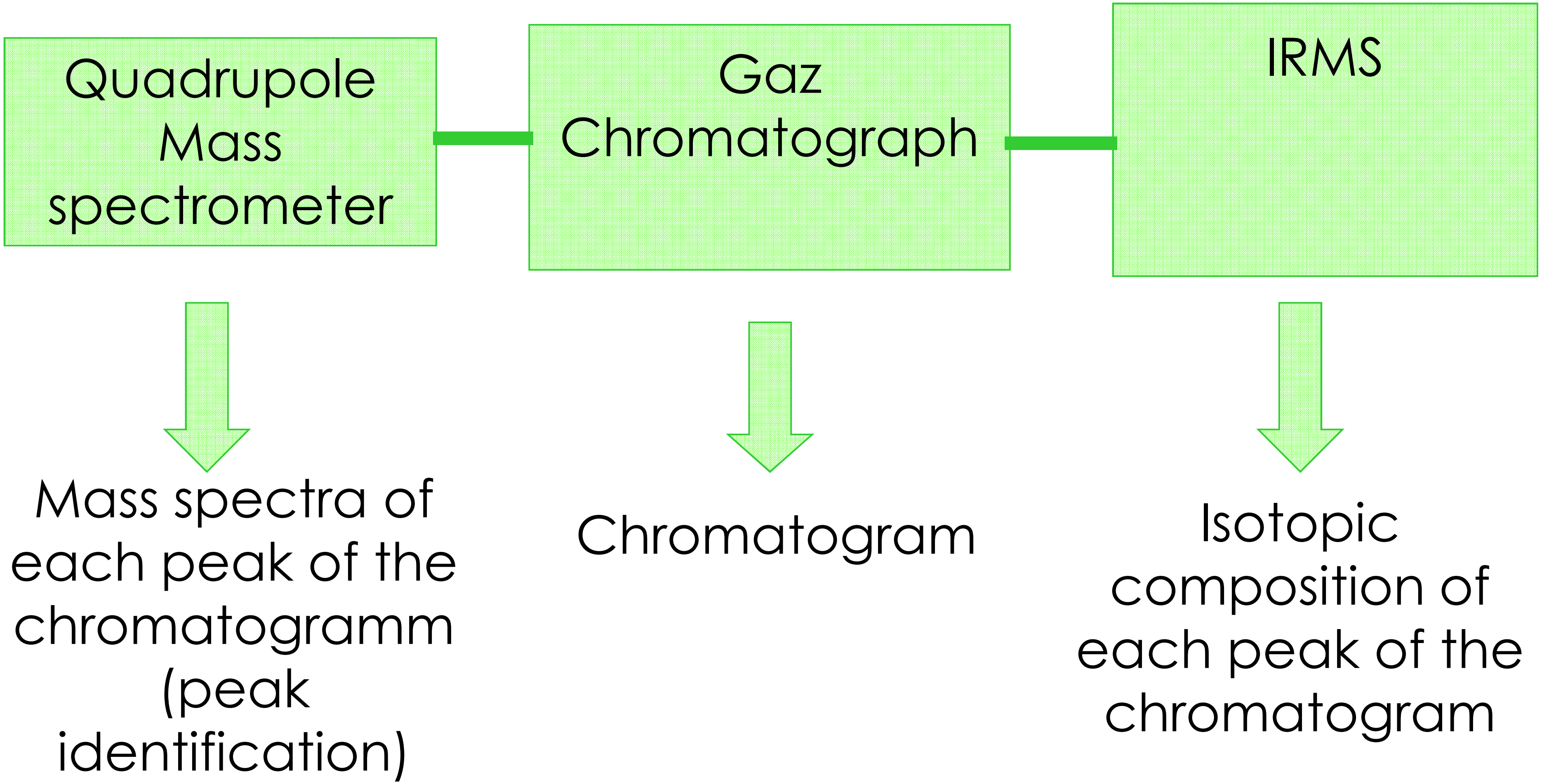
⇒ BULK stable isotopes composition



Elemental Analyser
(VarioMicro cube,
Elementar, Germany)

He: carrier gaz (<u>CONTINUOUS FLOW</u>)
O_2: sample combustion
N_2 et CO_2: isotopic reference gaz

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



⇒ Compound specific stable isotope analysis (CSIA)

MS-GC-IRMS

Delta notation : practical and international

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

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

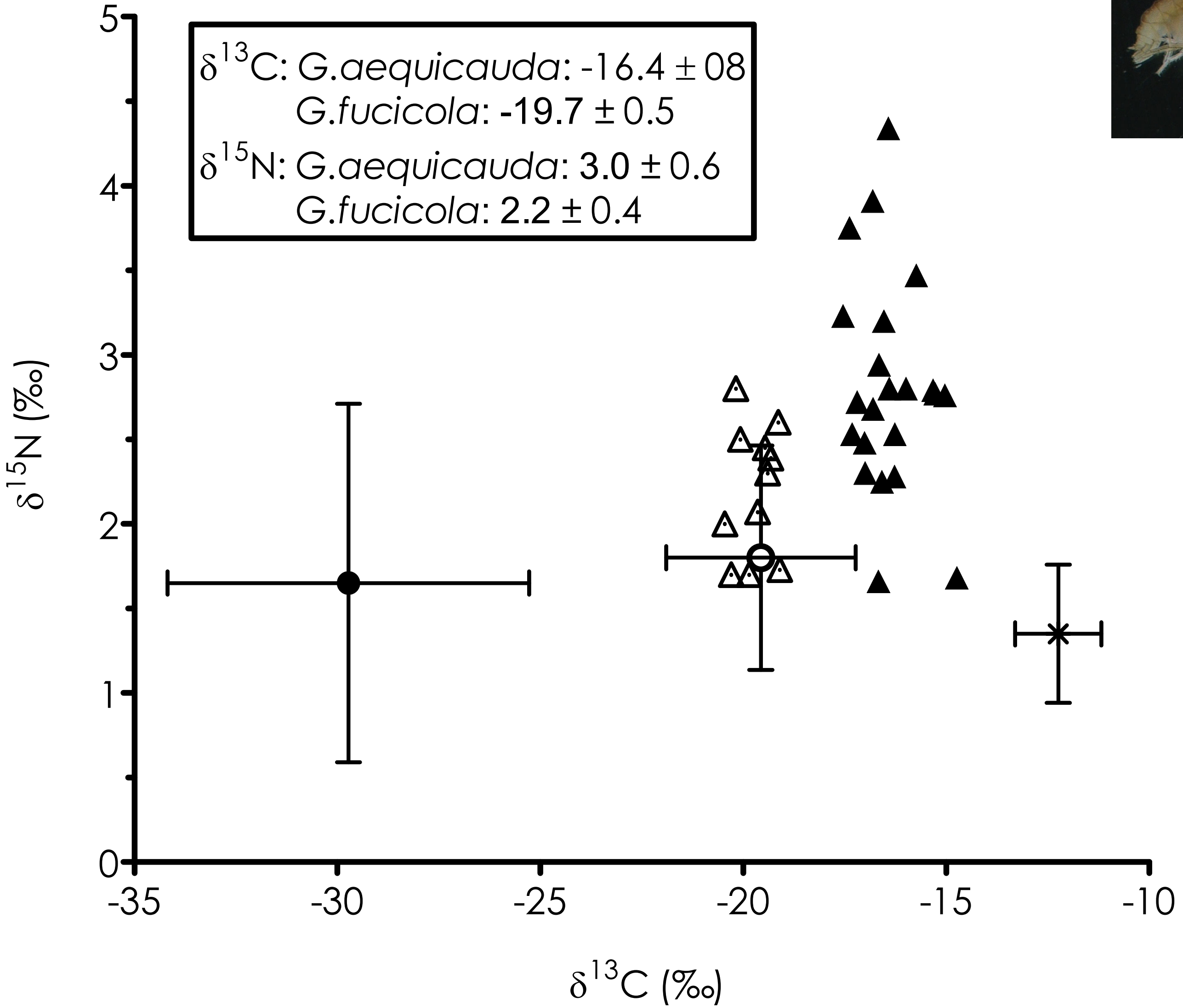
II. APPLICATIONS 1: TO ASSESS TROPHIC NICHES AND THEIR CHANGES

- Generalities
- Trophic diversity of Pomacentrid fishes
- Intra-specific trophic diversity in a Pomacentrid species
- Ontogenic trophic shift in an Acanthurid fish

Generalities

- Trophic niche = one aspect of ecological niche (sensu Hutchinson)
- Why to study trophic niche?
- How stable isotopes may be use for trophic niche studies?

⇒ To use isotopic variability and to calculate associated metrics



Source: Lepoint et al., Mar Biol Research 2006

CAUSES OF ISOTOPIC VARIABILITY IN CONSUMER

1. If diet constant between individual:

- Isotopic variability of the diet
- V

2. I

-

AN

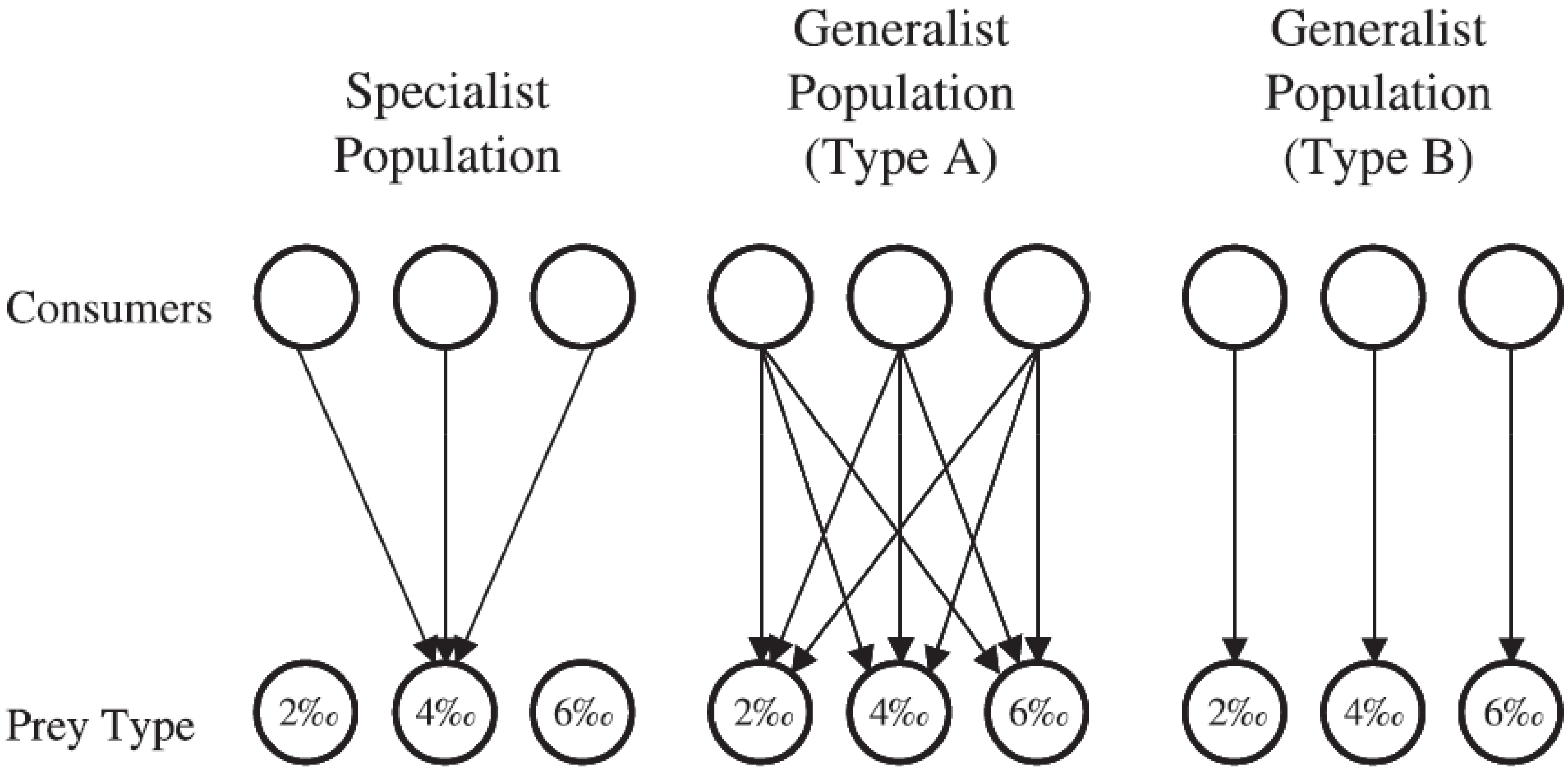
Then Isotopic niche never equal Trophic niche

Because:

*** Isotopic variability of sources**
*** Limited by possibilities to discriminate isotopically the different food sources**

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

Level of diet specialization in a population



Source: **Bearhop** et al., J Anim Ecol 2004: 73, 1007-1012

If we assume that diet/tissue fractionation is constant (4‰), prey isotope ratios remain constant over time and that Type A individuals consume all prey types in equal amounts then:

(A) Sampling a tissue that integrated dietary information over long temporal scales would likely give consumer population values (mean ± s^2) of

Specialist	Generalist (Type A)	Generalist (Type B)
8‰ ± 0	**8‰ ± 0**	**8‰ ± 4**

(B) Sampling a tissue that integrated dietary information over short temporal scales (with a large sample size) would likely give consumer population values (mean ± s^2) of

Specialist	Generalist (Type A)	Generalist (Type B)
8‰ ± 0	**8‰ ± 4**	**8‰ ± 4**

(C) Assuming that the tissue being sampled integrates dietary information over a shorter period than the diet varies over, serial sampling the same tissue (integrating very short-term dietary information, such as blood plasma samples, or short sections from feathers, hair or possibly whiskers) from the same individual over time would likely give individual values (mean ± s^2) of

Specialist	Generalist (Type A)	Generalist (Type B)
8‰ ± 0	**8‰ ± 4**	**6, 8 or 10‰ ± 0**

Source: **Bearhop** et al., J Anim Ecol 2004: 73, 1007-1012

A STEP FURTHER: DEVELOPMENT OF ISOTOPIC NICHE METRICS

•We need objective measurements of isotopic niche to assess trophic niches

$\Rightarrow$ Isotopic niches metrics

•Convex hull approach (e.g. Layman and co-authors),

•Bayesian statistics approach (e.g. Jackson, Parnell, Inger and co-authors) (module SIBER in SIAR mixing model)

Journal of Animal Ecology

Journal of Animal Ecology 2011, **80**, 595–602 doi: 10.1111/j.1365-2656.2011.01806.x

Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R

Andrew L. Jackson[1]*, Richard Inger[2], Andrew C. Parnell[3] and Stuart Bearhop[2]

Ecology, 88(1), 2007, pp. 42–48

CAN STABLE ISOTOPE RATIOS PROVIDE FOR COMMUNITY-WIDE MEASURES OF TROPHIC STRUCTURE?

Craig A. Layman,[1,5] D. Albrey Arrington,[2] Carmen G. Montaña,[3] and David M. Post[4]

[1]*Marine Science Program, Department of Biological Sciences, 3000 NE 151st Street, North Miami, Florida 33181 USA*
[2]*Loxahatchee River District, 2500 Jupiter Park Drive, Jupiter, Florida 33458-8964 USA*
[3]*Universidad de Los Llanos Ezequiel Zamora, UNELLEZ, Guanare, Apartado Postal 3310 Venezuela*
[4]*Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut 06520-8106 USA*

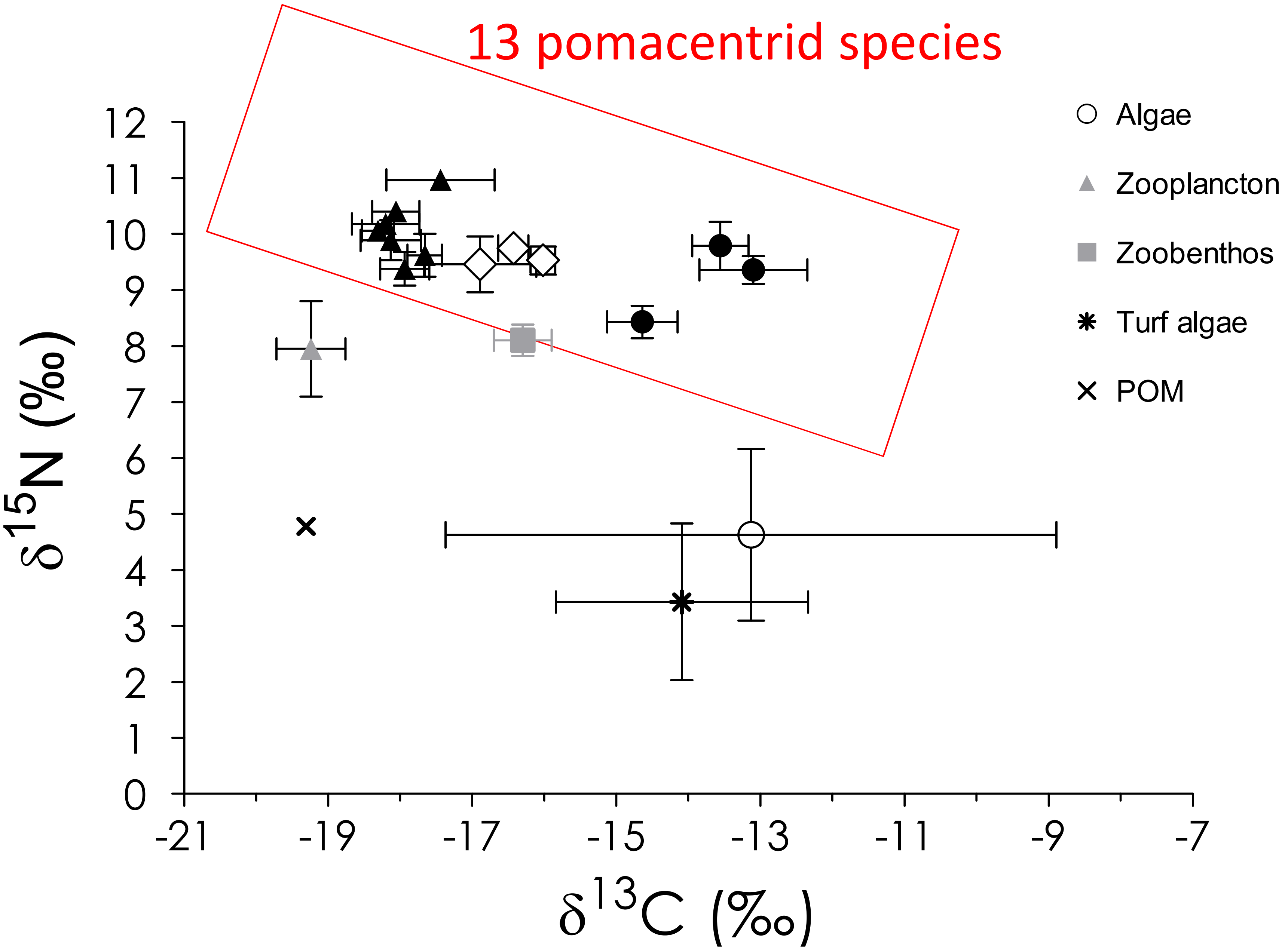
Last philosophical comment

•A model is a model, not the reality

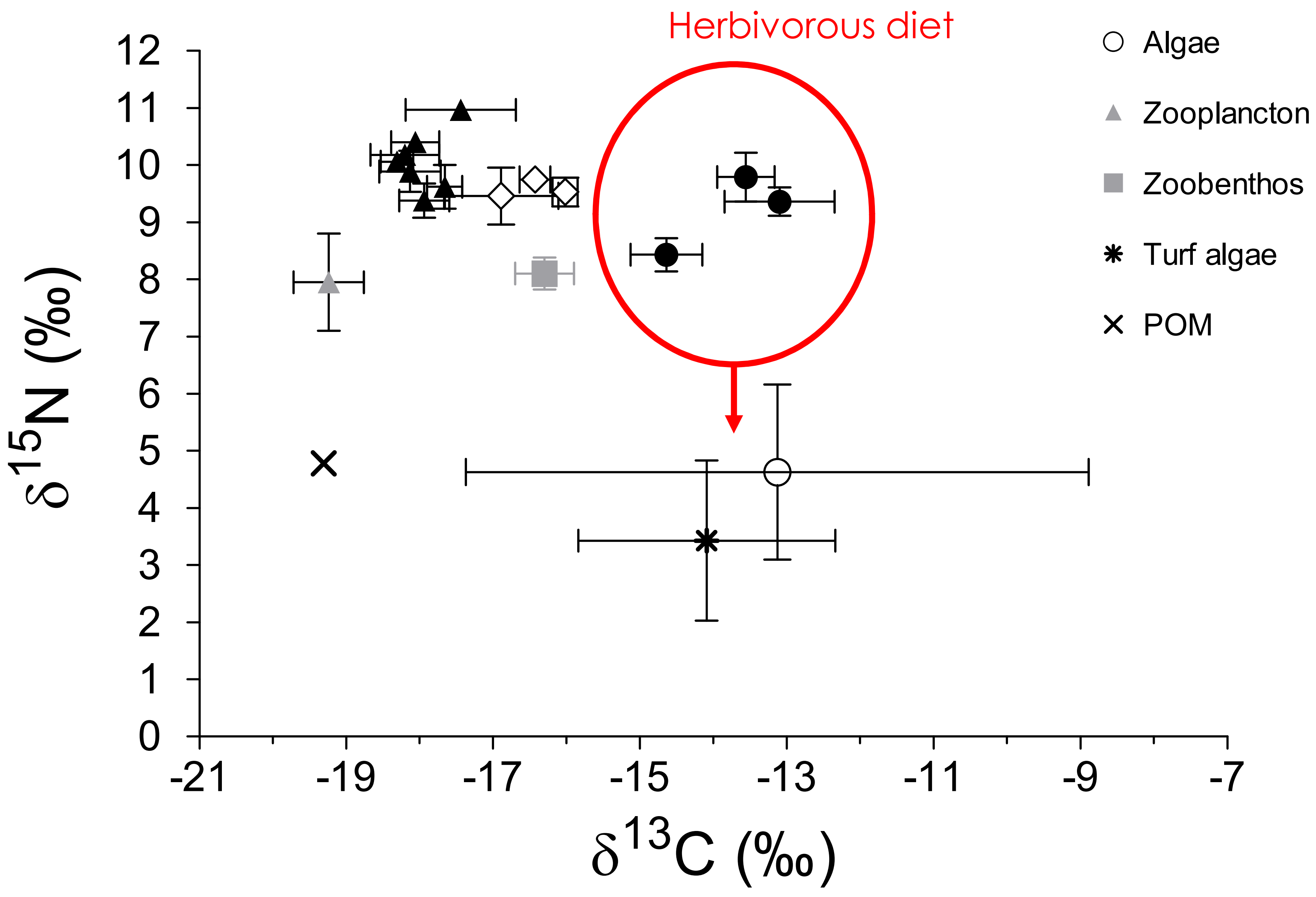
•The question strongly determine the sampling design for stable isotope purpose

•Complex problem needs multidisciplinary approach : stomach contents are not always obsolete

Trophic diversity of Pomacentrid fishes



Source: Frédérich et al. 2008, Ichtyological Research



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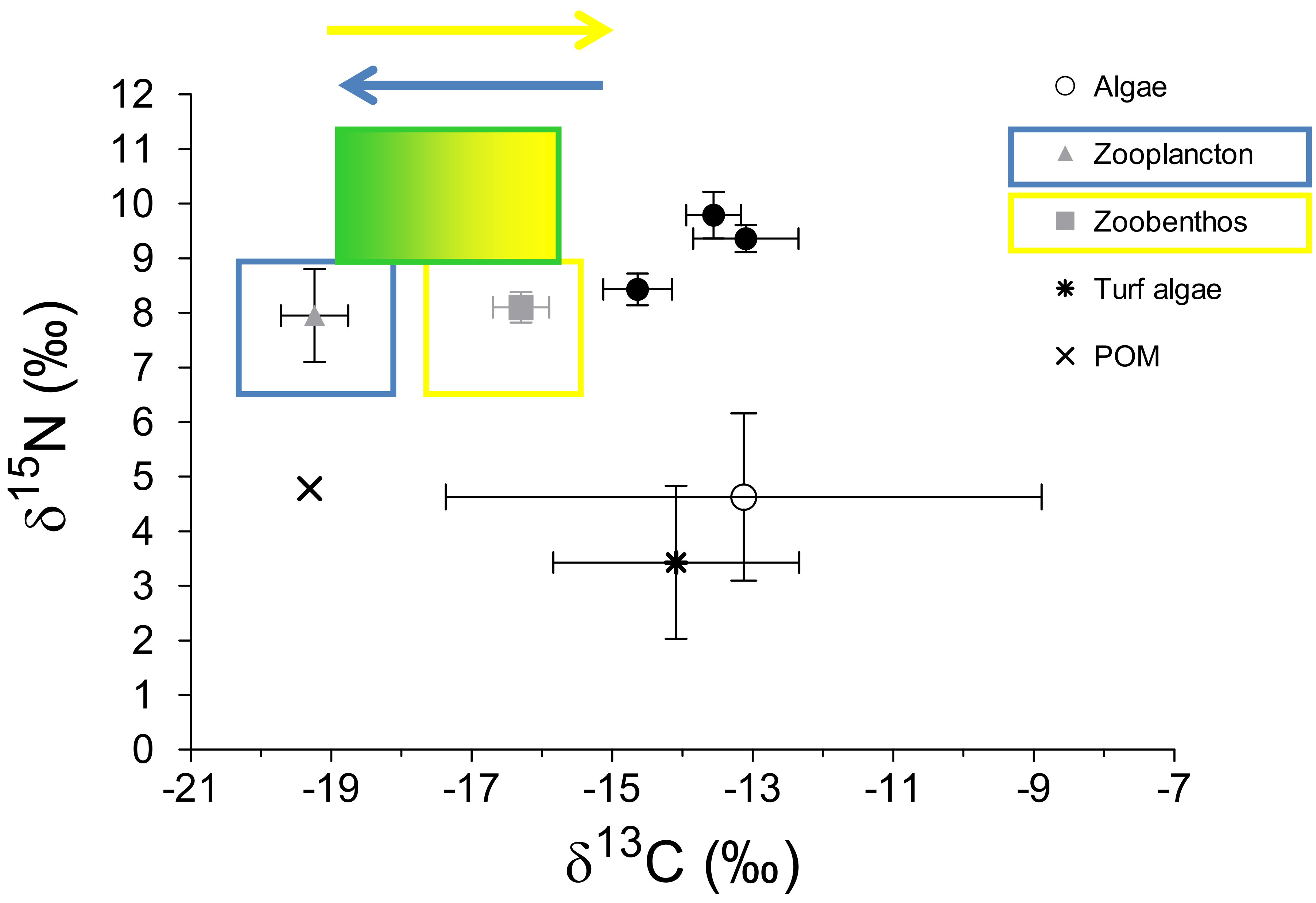
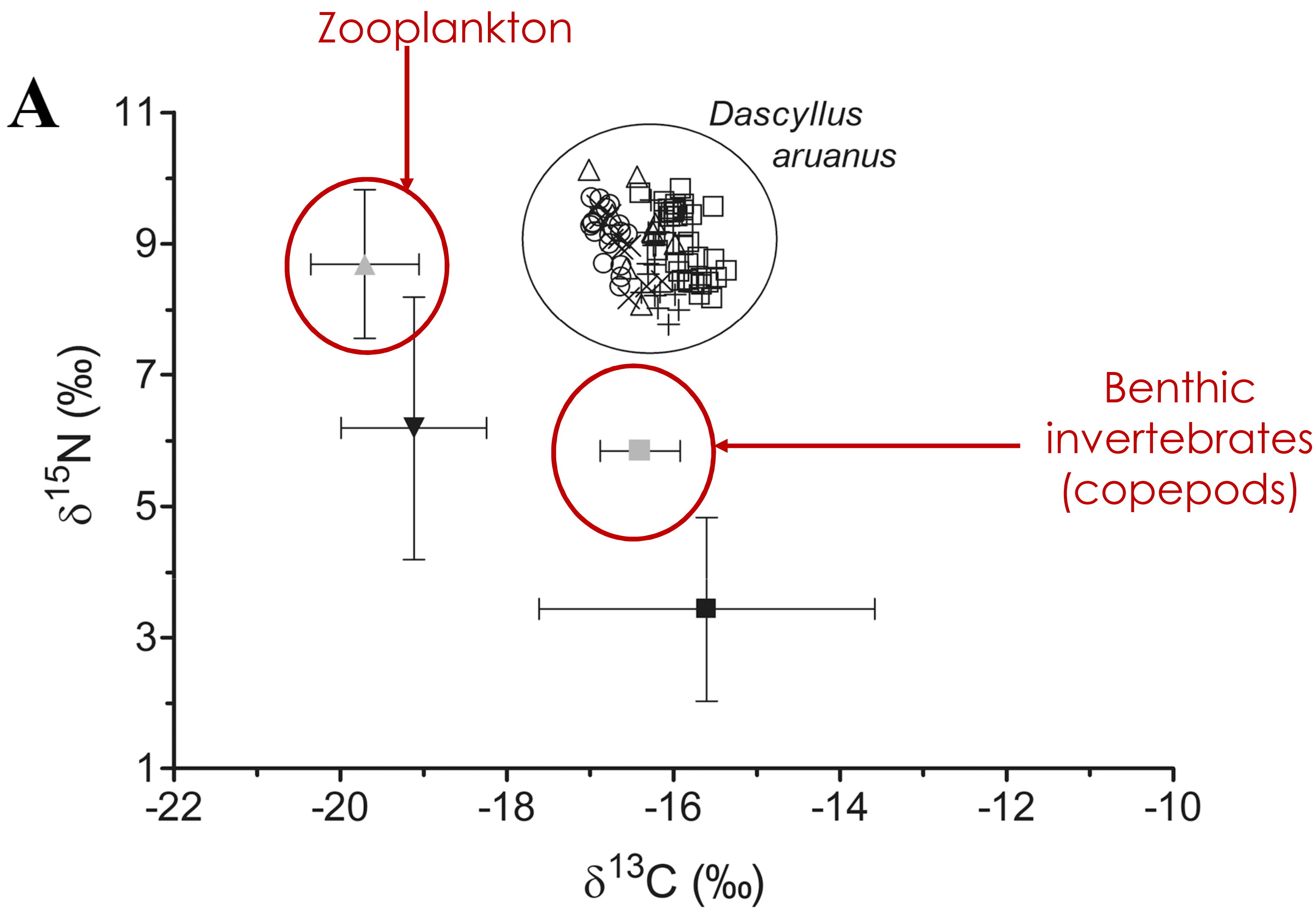


Source: Frédérich et al. 2008, Ichtyological Research

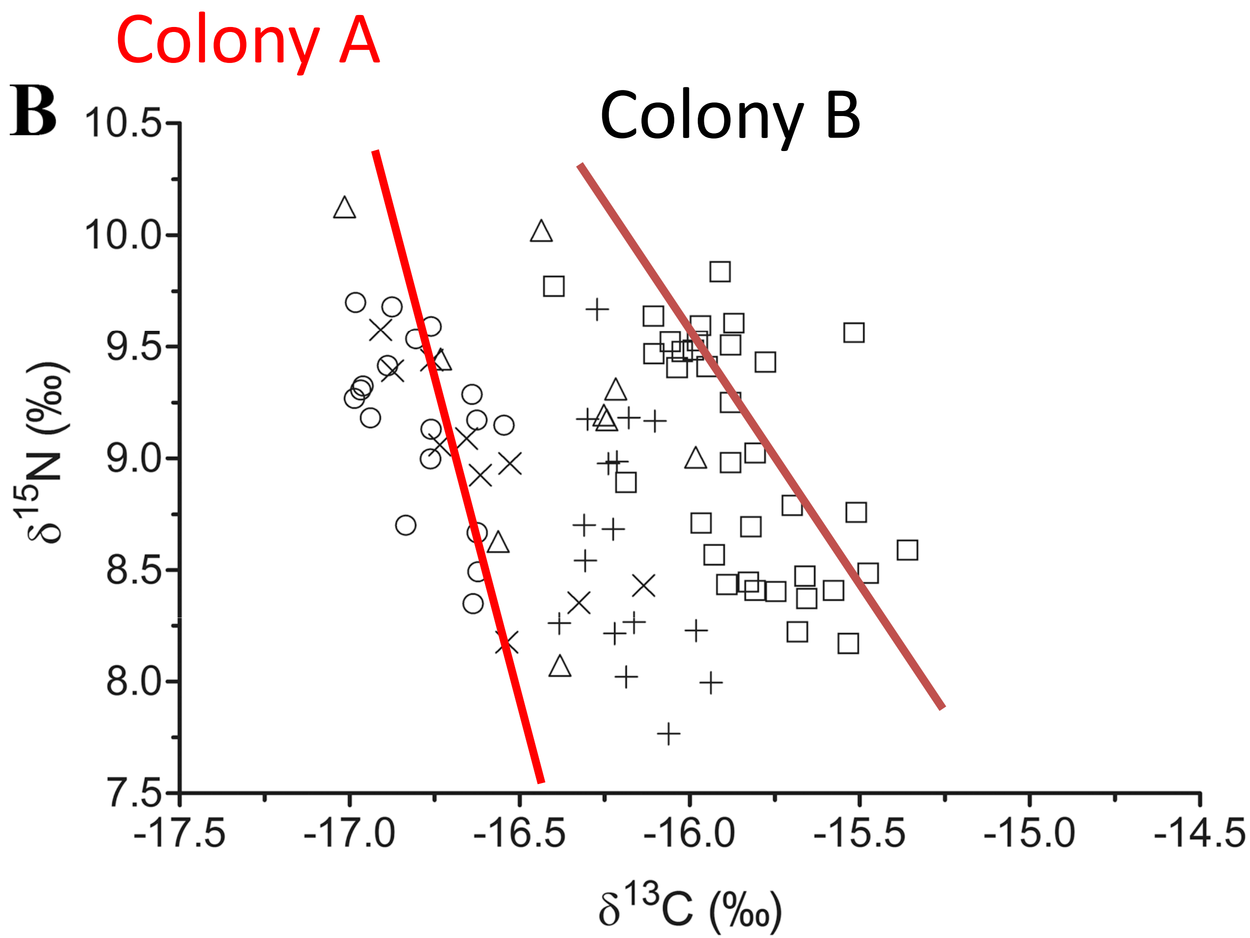
INTRA-SPECIFIC TROPHIC DIVERSITY IN A POMACENTRID SPECIES

Dascyllus aruanus



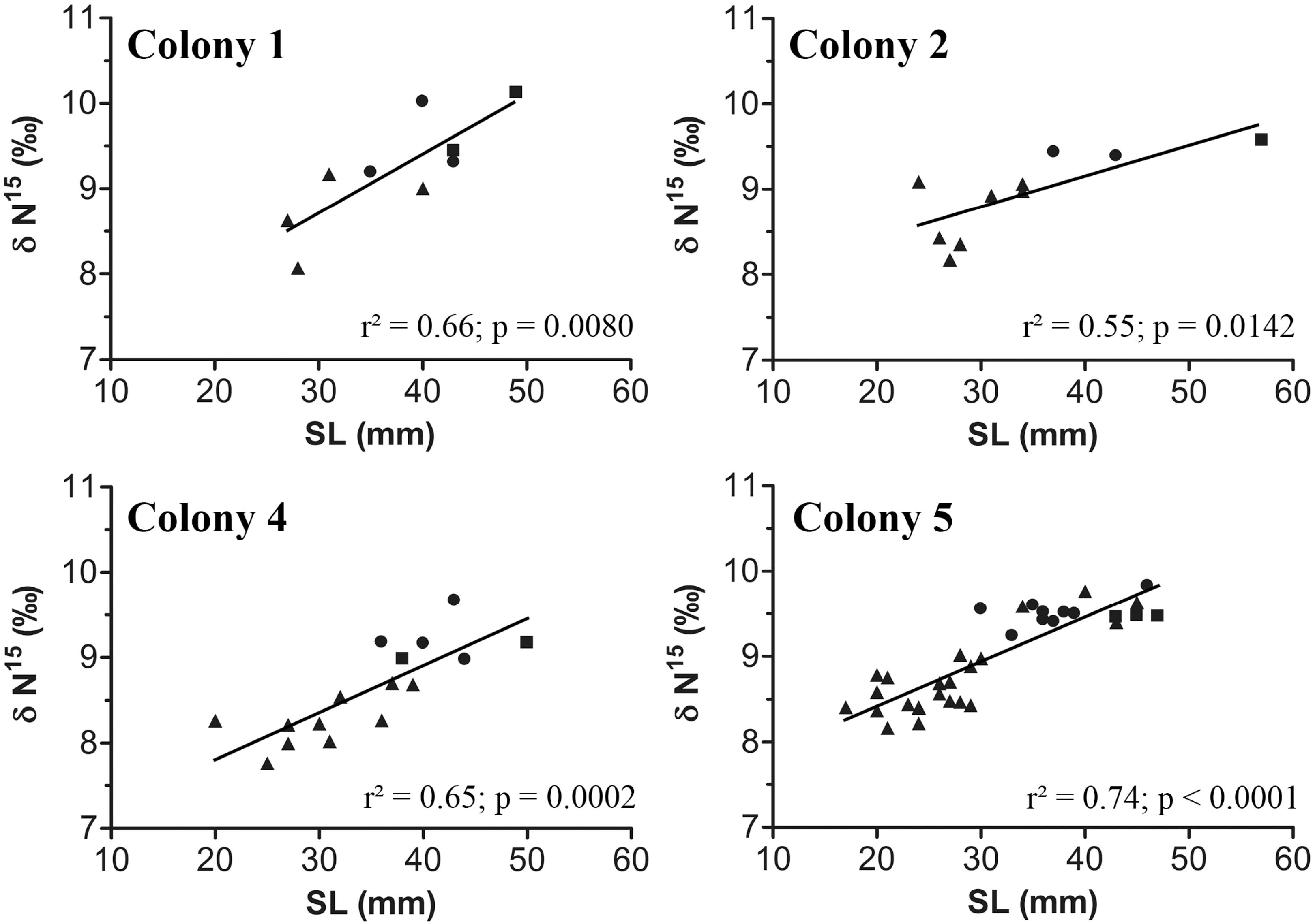
- Inter-individual variability: ≈ 2‰

Source: Frédérich et al. 2010, Copeia

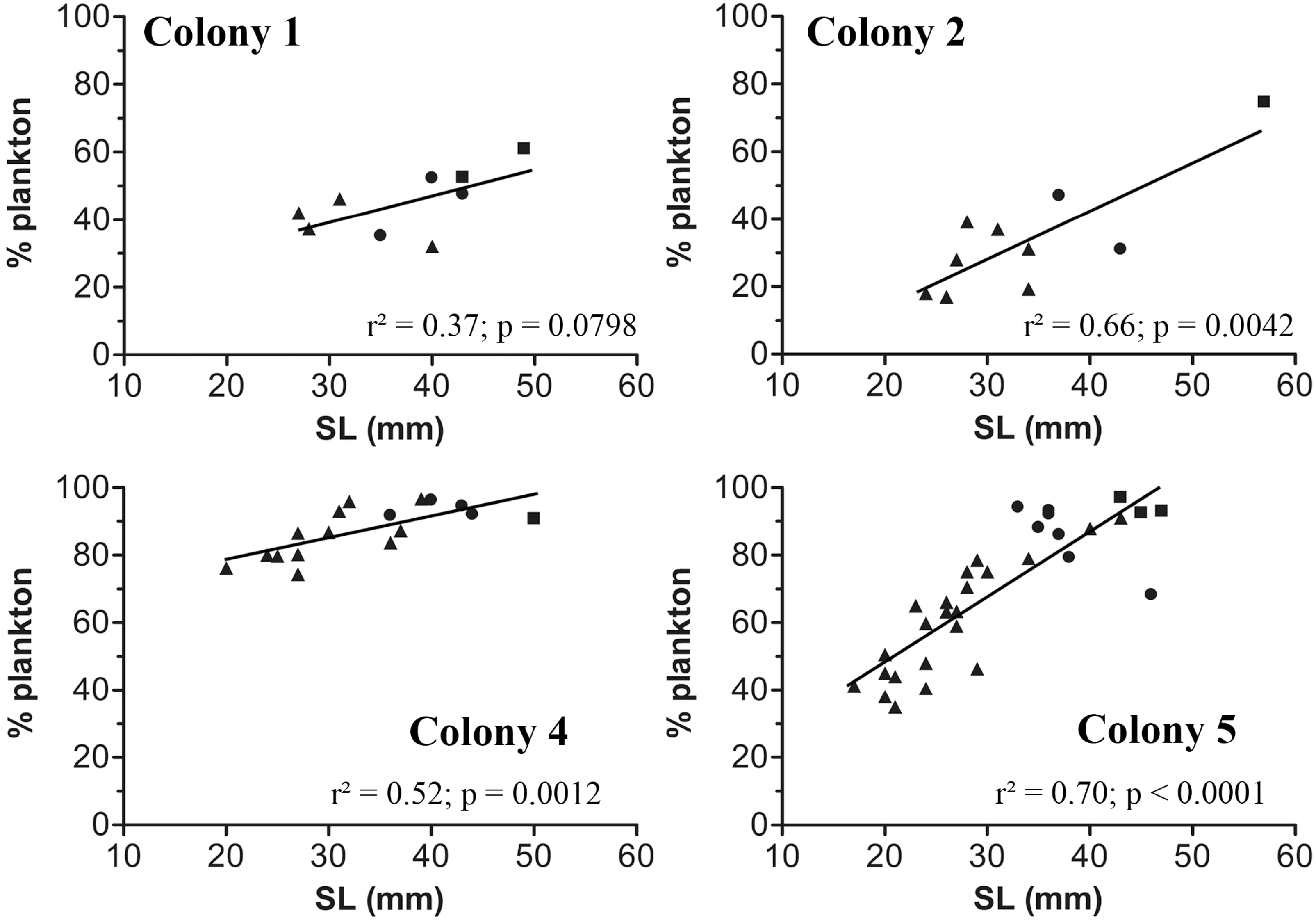


- Co-variations $\delta^{13}C$ et $\delta^{15}N$, but variable between colonies

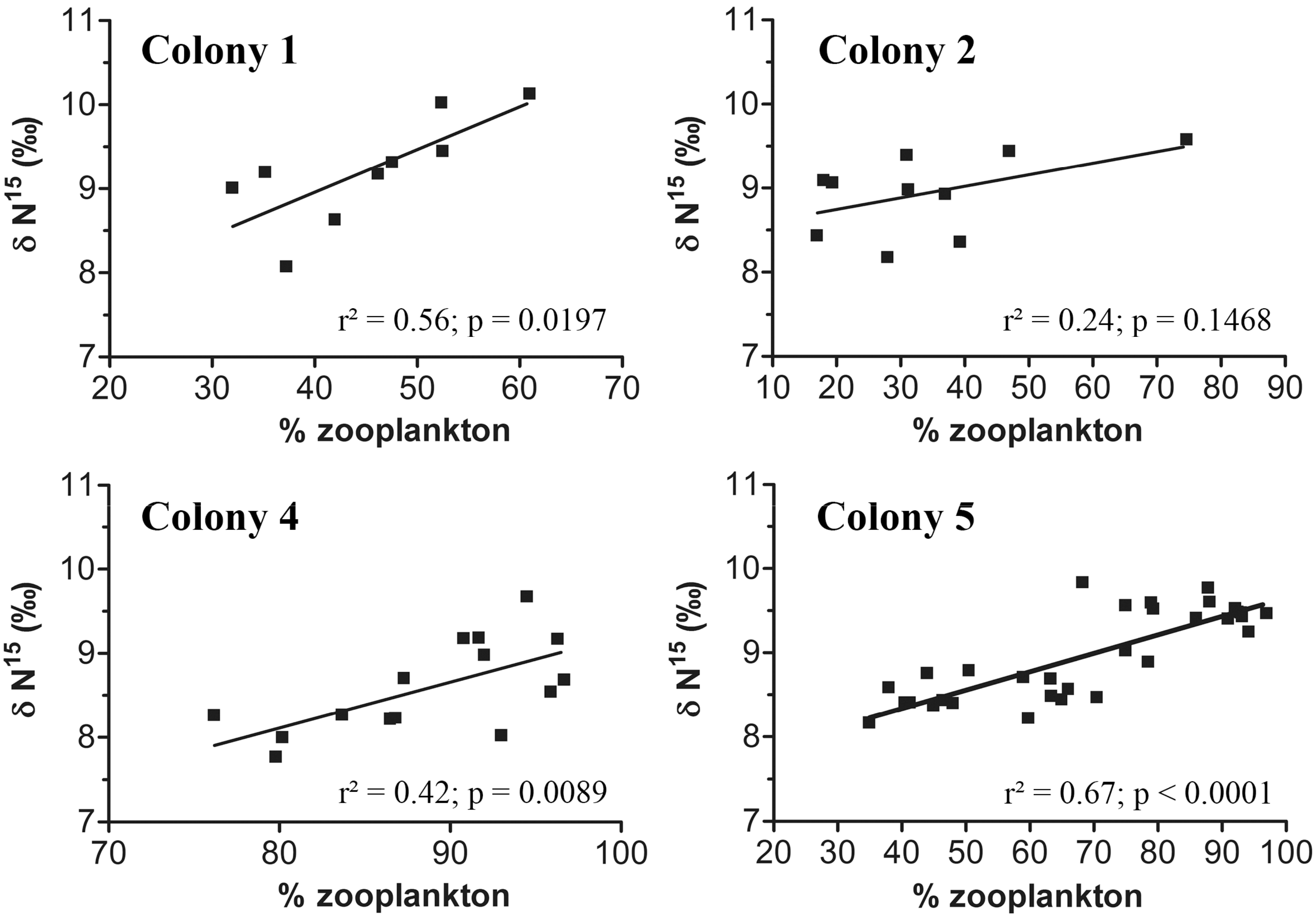
Source: Frédérich et al. 2010, Copeia



Source: Frédérich et al. 2010, Copeia



Source: Frédérich et al. 2010, Copeia



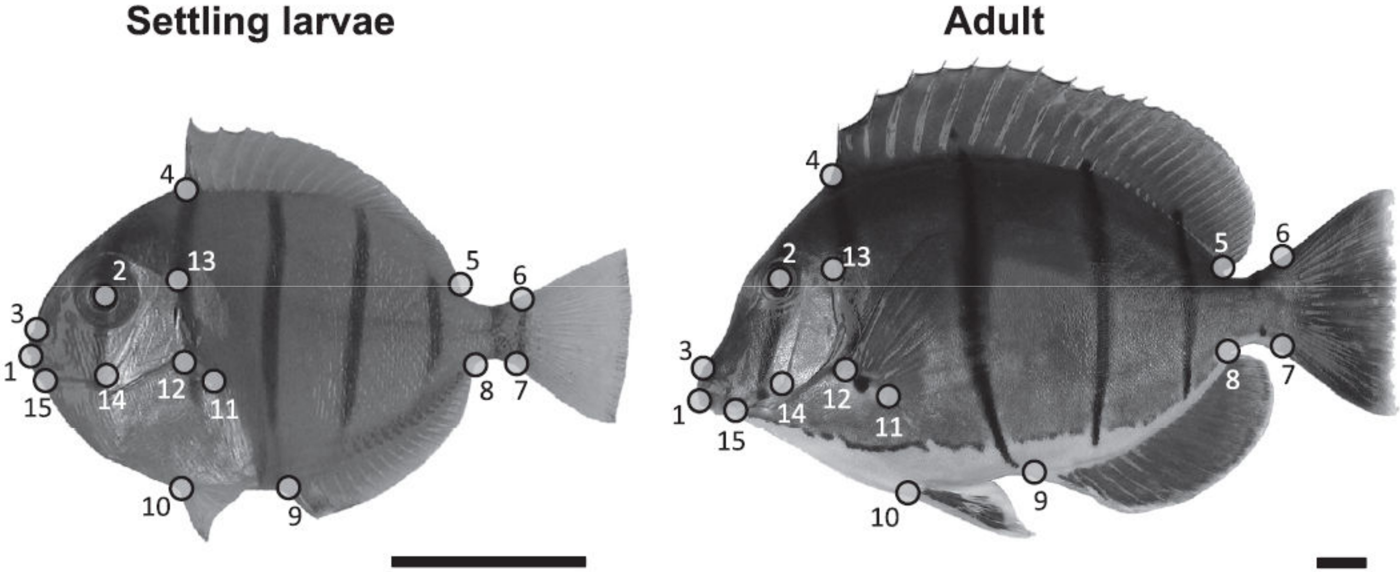
Source: Frédérich et al. 2010, Copeia

- 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

ONTOGENIC TROPHIC SHIFT IN AN ACANTHURID FISH



Acanthurus triostegus

Source: Frédérich et al. Frontiers in Zoology 2012, 9:8

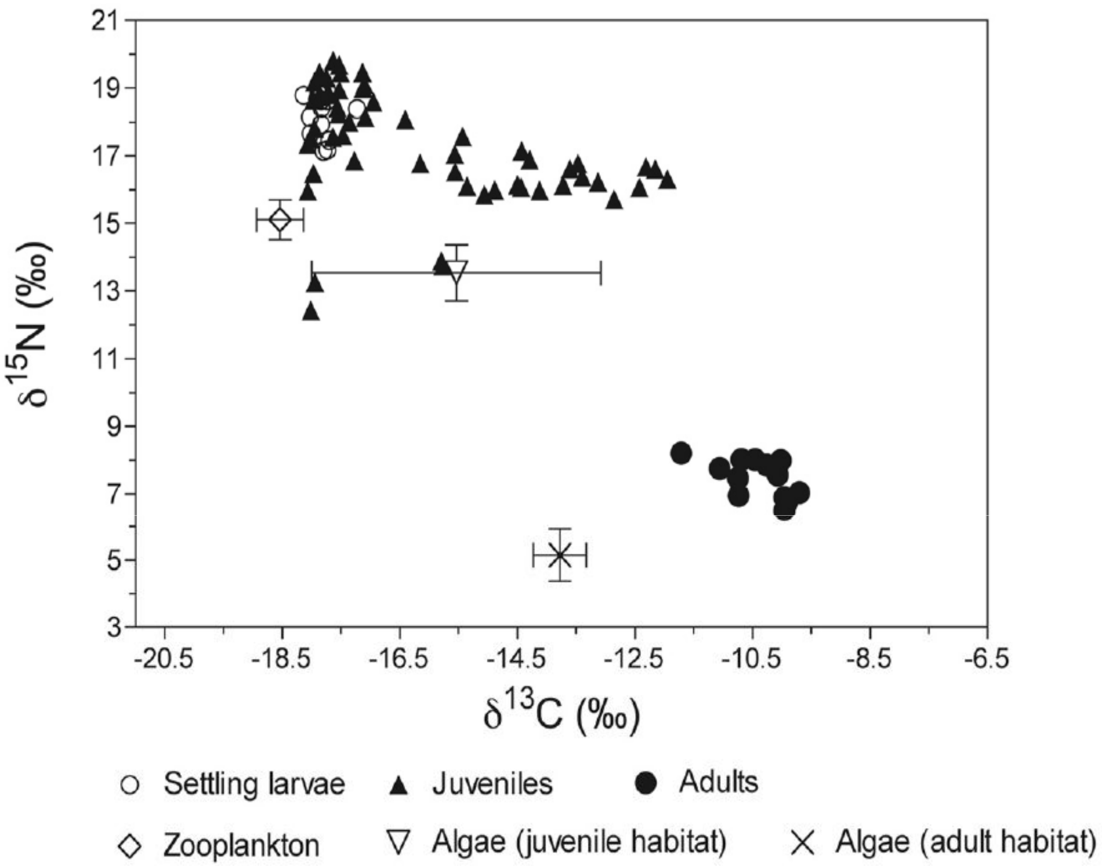


Figure 5 Isotopic map of sample populations of *Acanthurus triostegus* and their food sources. Mean (± SD) d15N and d13C ratios of food items (zooplankton and algae from both juvenile and adult habits) collected from the lagoon and isotope values of all *Acanthurus triostegus* (settling larvae, juveniles, adults).

Source: Frédérich et al. Frontiers in Zoology 2012, 9:8

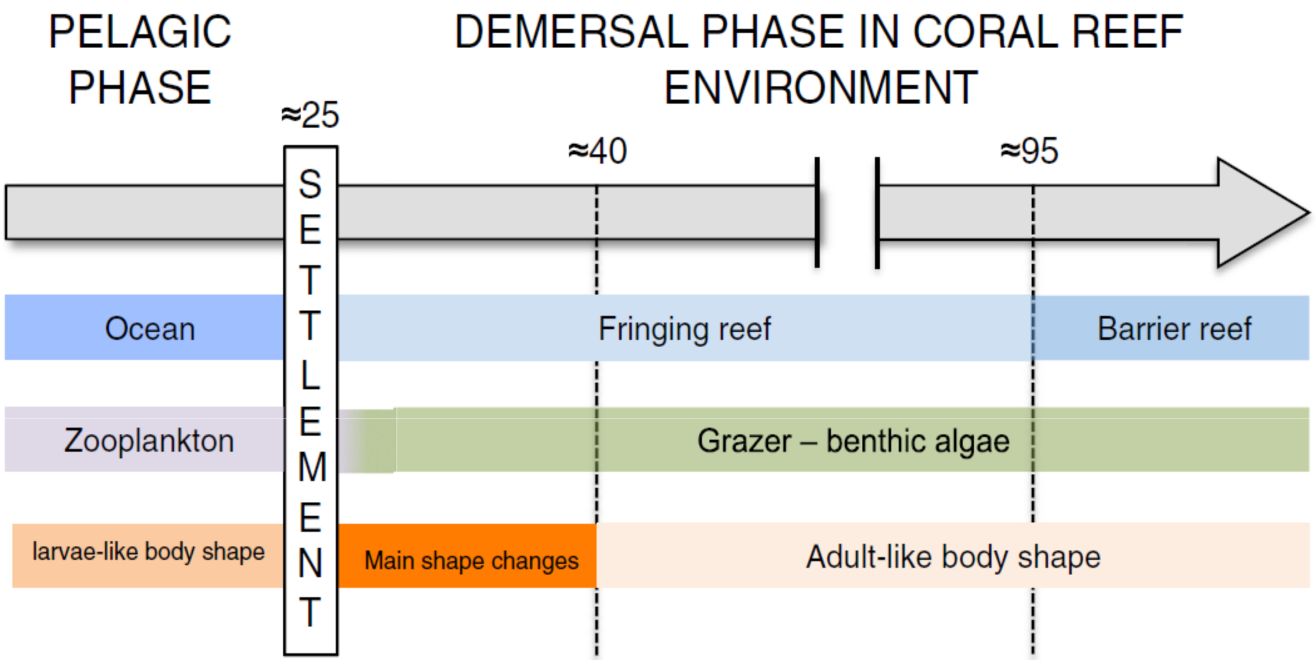


Figure 6 Schematic representation of the ecological and shape changes observed during the ontogeny of *Acanthurus triostegus*. See the text for details. The grey arrow represents the ontogenetic scale. The numbers above the scale refer to the approximate fish size (standard length in mm) when the changes occur. Variations in habitat, diet and morphology are illustrated by a gradient of blue, green and orange, respectively.

Source: Frédérich et al. Frontiers in Zoology 2012, 9:8

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 organochlorate pesticide

Generalities

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

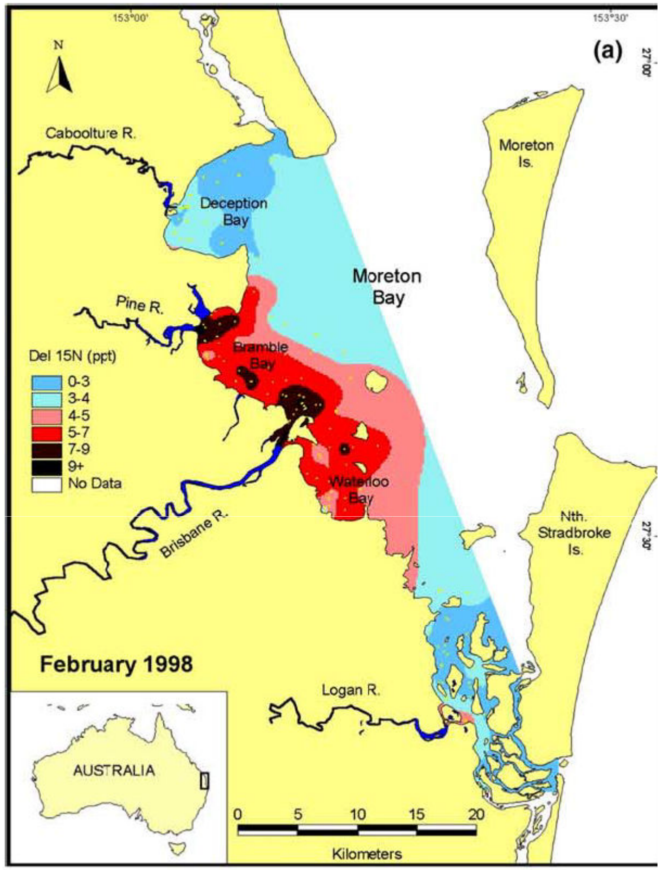


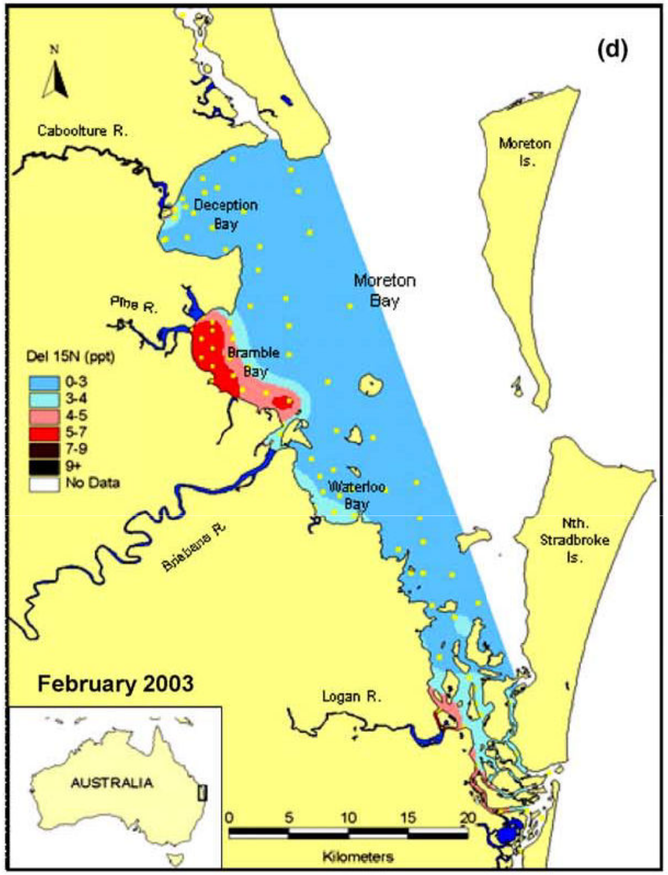

Generalities

•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

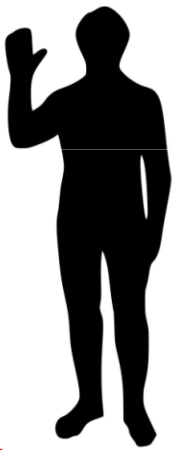
Spatial and temporal responses of marine gastropods and biofilms to urban wastewater pollution in a Mediterranean coastal area

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

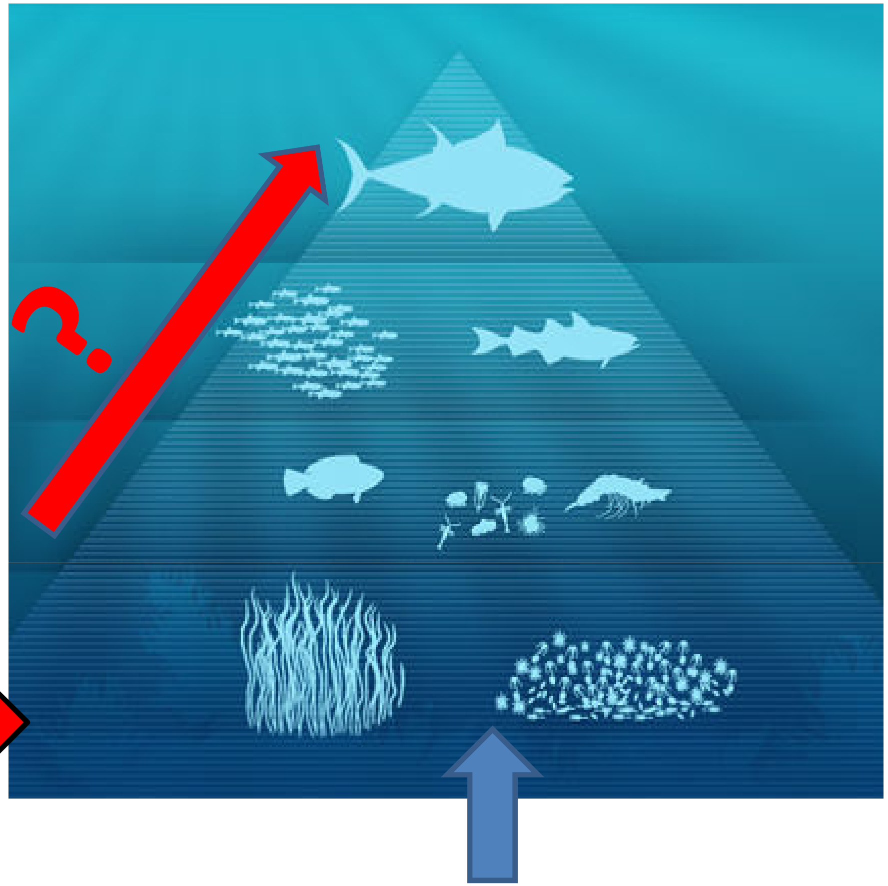
- Main objective: to assess the efficiency of a new set of potential early bioindicators of urban wastewater pollution for Mediterranean coastal areas in a environmental monitoring context.

- Potential bioindicators: epilithic biofilm and two of their consumers

PRINCIPLE TO USE DELTA ^{15}N AS A TRACER OF WASTEWATER NITROGEN



wastewater
Nitrogen =
high $\delta^{15}N$ values



Ambient nutrients

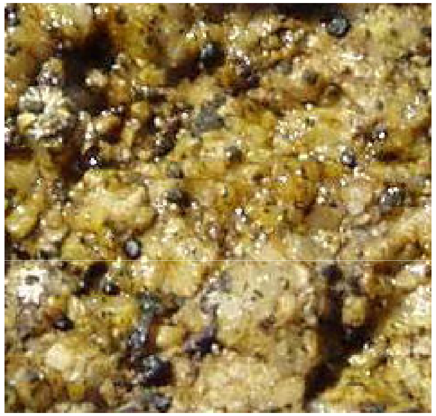
Causes:

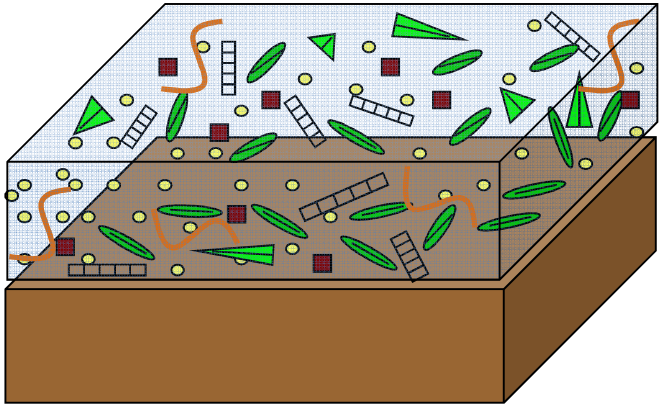
- Human = high trophic level
- Fractionation during mineralisation process
- Freshwater and terrestrial nutrient

Sources: Vermeulen S. (phD thesis, 2012)

Epilithic biofilms

- ubiquitous communities
- 1st colonization step





macroalgal sporeling

diatom

cyanobacteria

protozoa

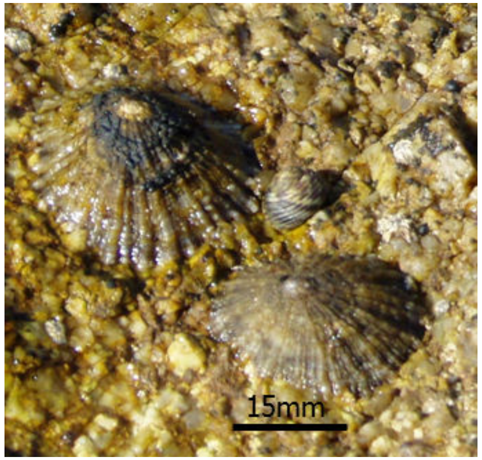
bacteria

animal

Extracellular Polymeric Substances (EPS)

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

BIOFILM CONSUMERS

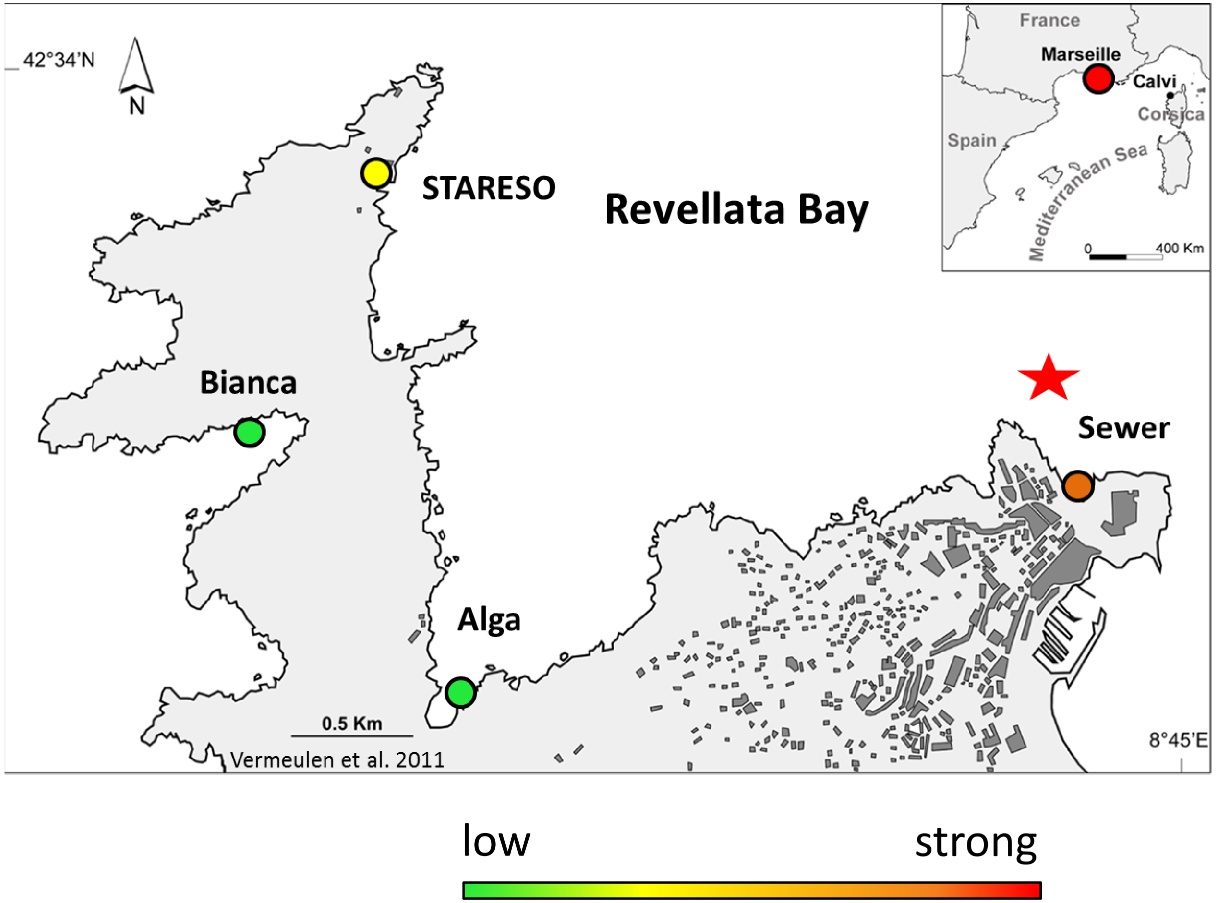


Patella caerulea

Monodonta turbinata

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

SAMPLING DESIGN



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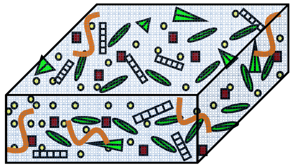
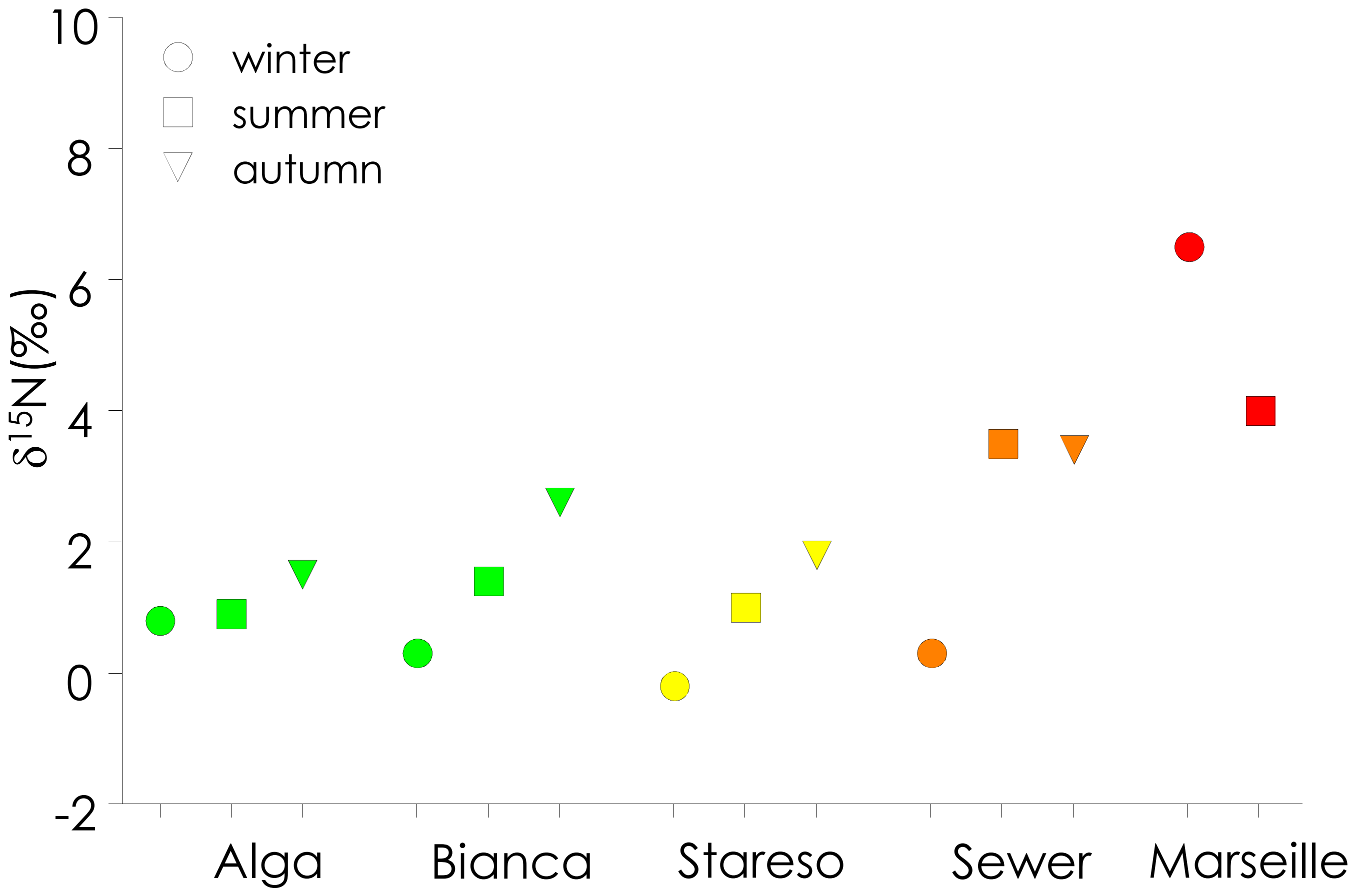
Epilithic biofilms

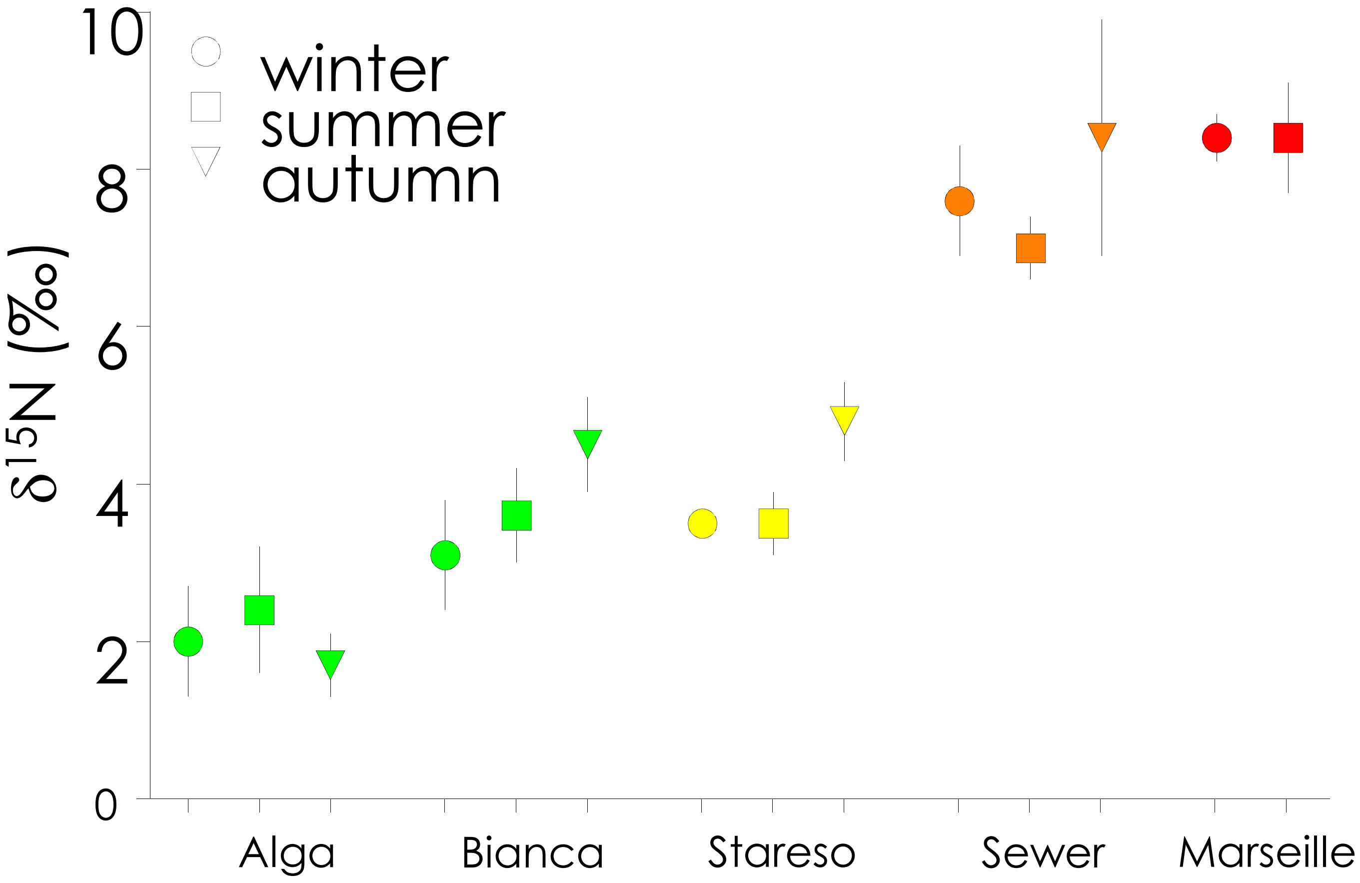




Sources: Vermeulen S. (phD thesis, 2012) and Vermeulen et al., (2011) Mar Ecol Prog Ser 422: 9-22

- ^{15}N increase = algal incorporation of wastewater nitrogen and / or shift in biofilm composition
- seasonal response to summer pulses (fast turnover rates)

Limpets (*Patella caerulea*)

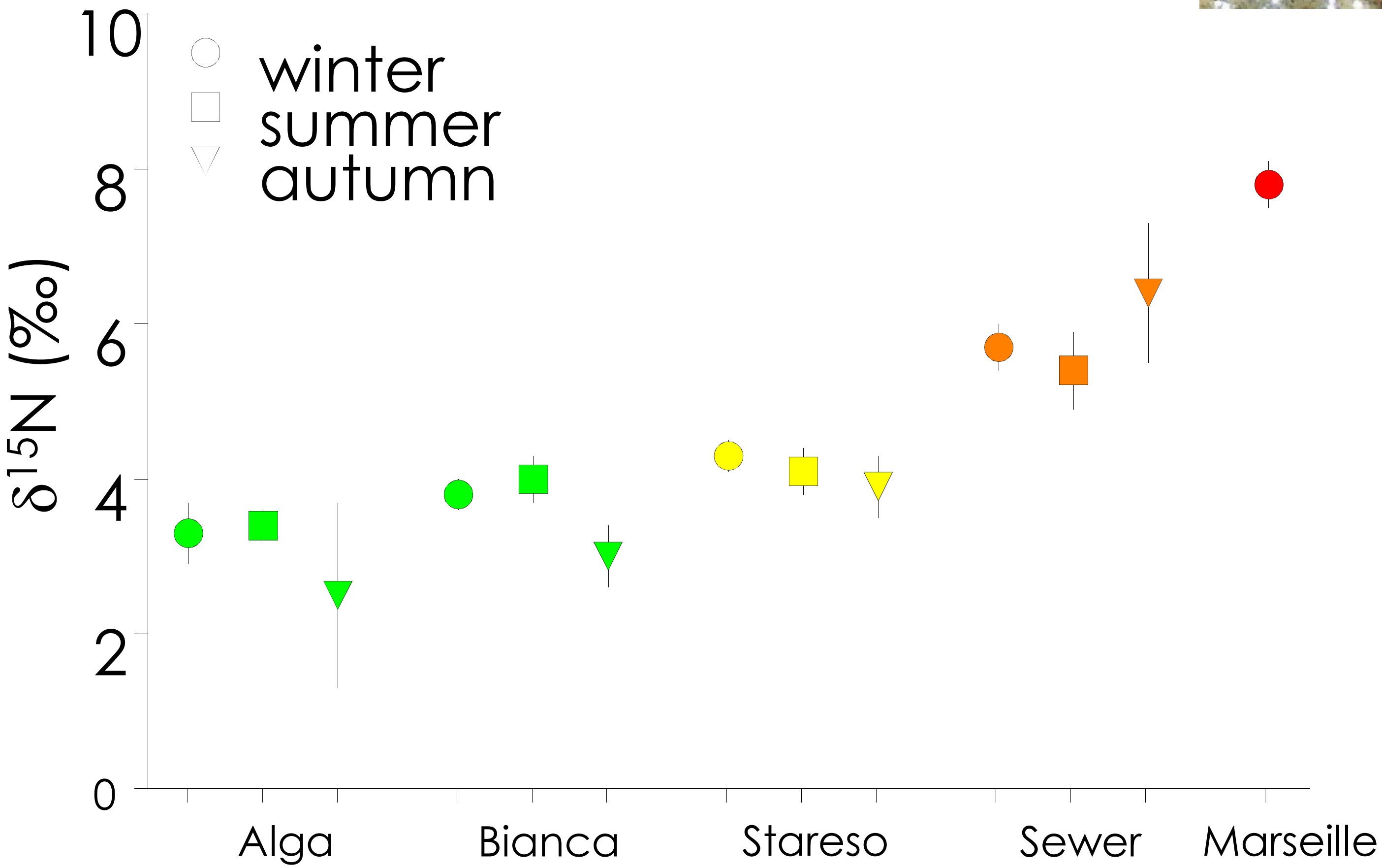


Sources: Vermeulen S. (phD thesis, 2012) and Vermeulen et al., (2011) Mar Ecol Prog Ser 422: 9-22

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

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

Snails (*Monodonta turbinata*)



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

•More expected signal of gradual exposure to wastewater

•Lower isotopic variances than limpets

$\Rightarrow$ Response differ form one compartment/species to another

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

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)

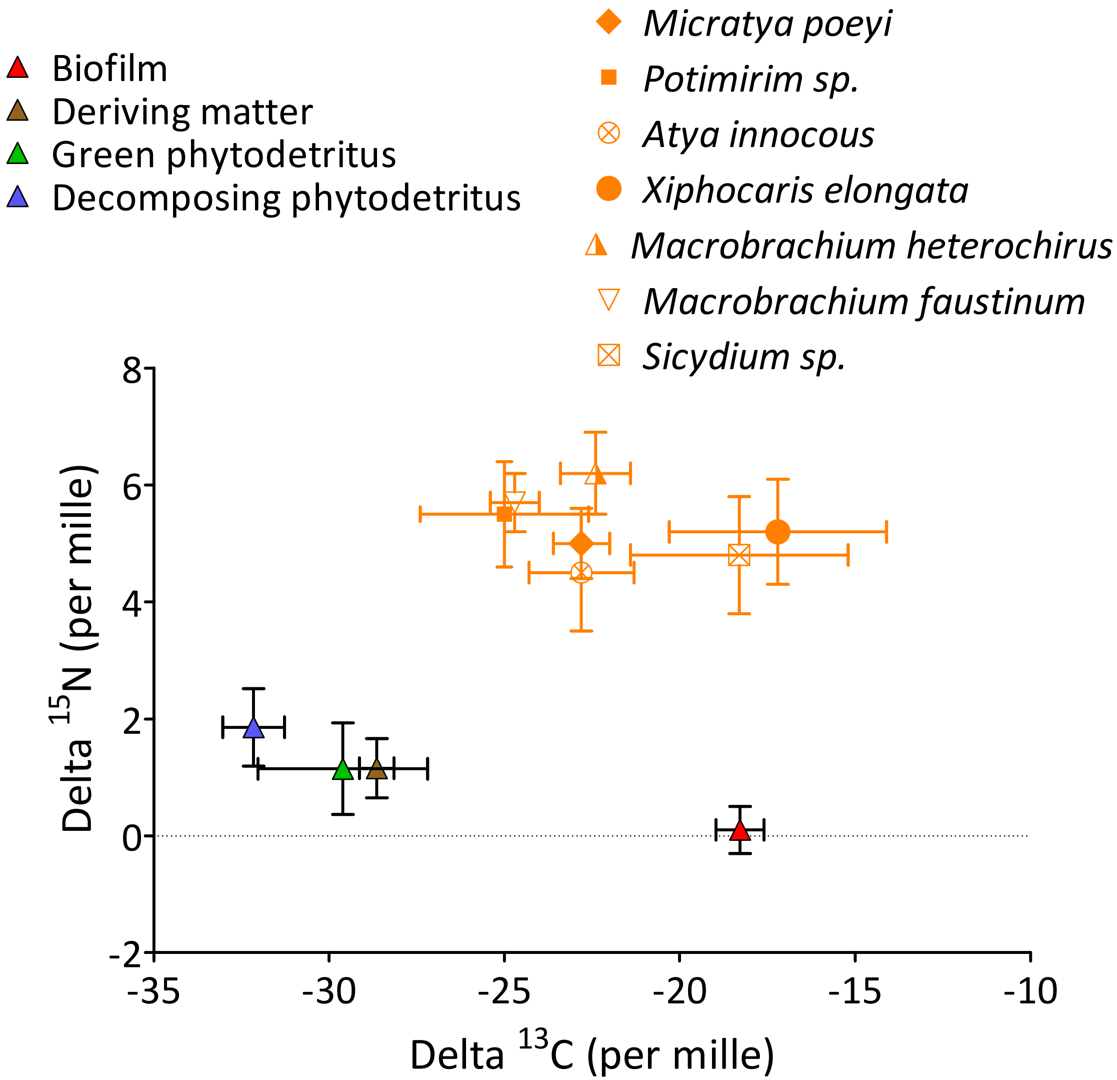
PROBLEMATICS

- Heavy contamination by organochlorine pesticides (Banana culture)

- What is the general structure of the trophic web?

- Is there a relation between trophic level and pollutant contamination

Stable isotopic compositions of potential food sources and consumers sampled in the River Pérou (Guadeloupe)



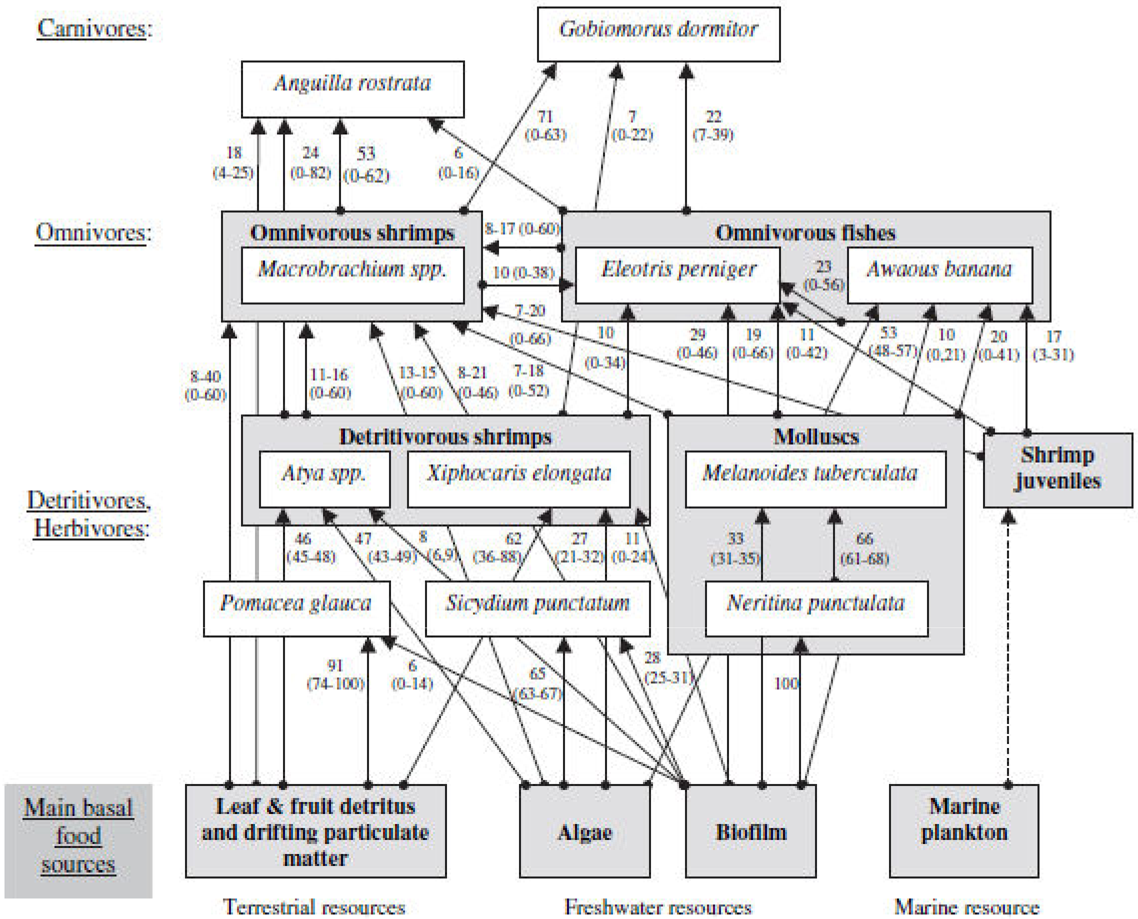


Fig. 2 Food web of a Caribbean stream (Grande-Anse, Guadeloupe), based on stable isotope analysis (grey units show elements with similar carbon isotopic signatures and constitute the dominant potential food sources of this aquatic ecosystem during the dry season. Mean percent contributions of sources to adult consumers are indicated below the arrows when ≥5%. They are followed by the minimum and the maximum in parentheses (the presence of two means indicates a range of contributions for the different species of a genus). Arrows start with a black dot and link species and/or units). 208 × 179 mm (600 × 600 DPI).

Source: Coat et al. (2009) Freshwater Biology 54, pp. 1028-1041 ,

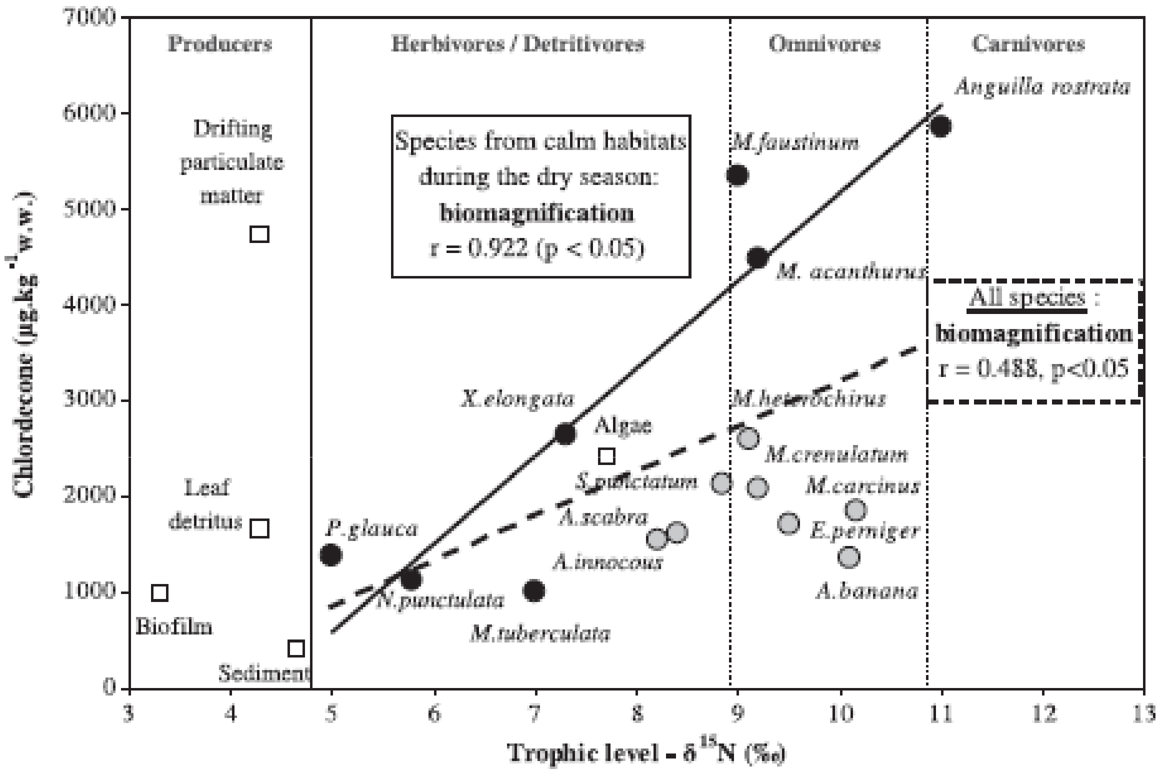


Fig. 3. Chlordecone concentrations versus trophic level measured in river samples during the dry season (the hatched regression line represents the statistically significant relationship in biota (all circles), the complete regression line only takes into account the species living in calm habitats (black 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

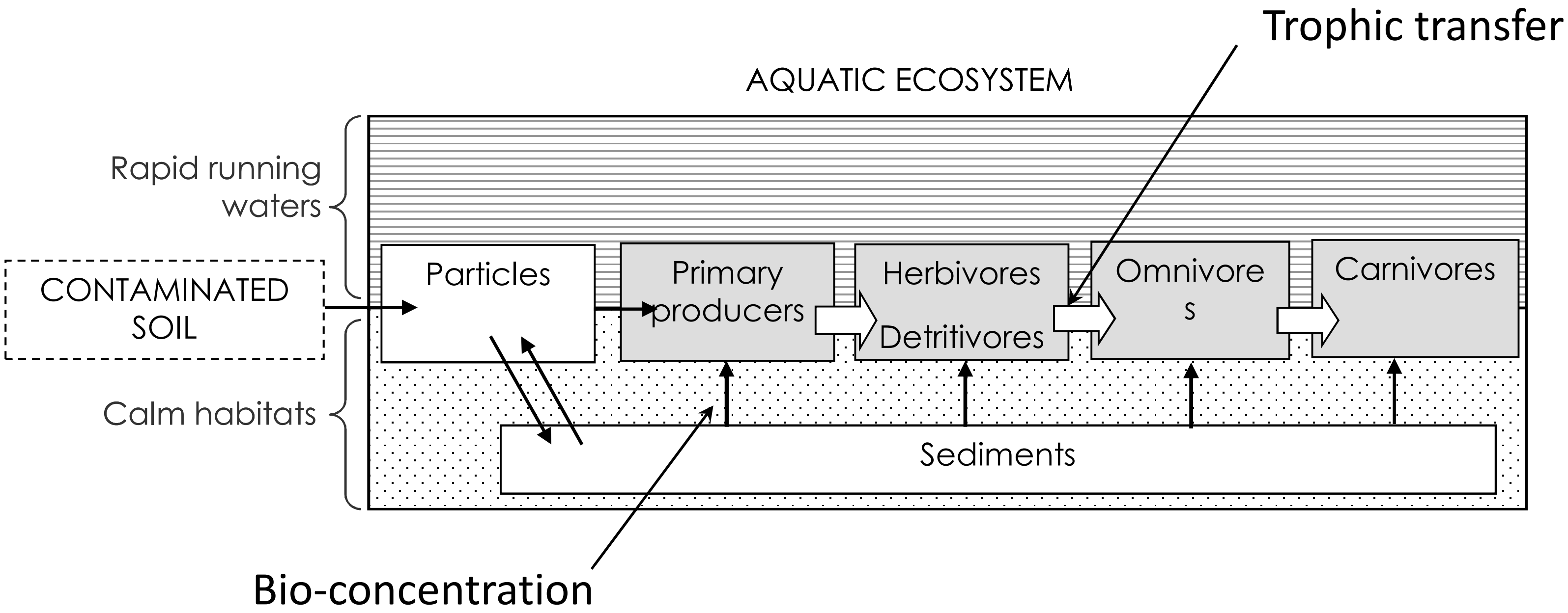


Figure 10. Model of chlordecone transfer in the aquatic ecosystem, separated into calm and turbulent habitats (the transfer of chlordecone between abiotic, white, and biotic, grey, compartments is symbolised by black arrows for direct contamination (bioconcentration) or white arrows for contamination by food (trophic transfer))

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

IV. TAKE HOME MESSAGE

- EA-IRMS = bulk isotopic composition by combustion of a sample
- GC-IRMS = stable isotopic composition of "small" specific compounds (AA, Fatty acids in trophic ecology)
- Isotopic approach is a powerful technique (particularly when associated with other approaches)
- but numerous limitations and assumptions
- First the question, then the sampling design

Thank you for your attention

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