



Tuna tagging: an unique opportunity to investigate further research on trophic ecology, energy allocation and chemical bioaccumulation

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Main scientific and management results expected from tagging programs... and more...

Tagging programs widely recognized as important tools for understanding population dynamics of fisheries resources:

- Stock structure and migrations;
- Growth, natural mortality as a function of age and sex;
- Fish behavior as a function of their environment (e.g., impact of FADs)
- Fishing mortality, interactions between fisheries

➡ *No information on the physiological, chemical or energetic causes, consequences, costs or constraints associated with patterns of behavior or distribution*

➡ *To collect a multitude of interdisciplinary data both at tagging and recapture would allow to test hypotheses about mechanisms affecting fish life history*

➡ *Improvement of the fish stock assessment methods*

Fish tagging combined with non-invasive sampling method: past experiences

Fish	Tracking method	Non-invasive sampling method	References
Salmon	<ul style="list-style-type: none"> •Conventional tags & tattoos 	<ul style="list-style-type: none"> •caudal fin clip 	Cunjak et al 2005
Salmon & sculpin	<ul style="list-style-type: none"> •Passive integrated transponder tags 	<ul style="list-style-type: none"> •caudal fin clip 	Gray et al 2004 ; Cunjak et al 2005
Tuna, shark & marlin	<ul style="list-style-type: none"> •Conventional tags •Telemetric tags 	<ul style="list-style-type: none"> •blood from heart (5mL) 	Skomal & Chase 2002
Salmon	<ul style="list-style-type: none"> •Radio transmitter (gastric implants) 	<ul style="list-style-type: none"> •muscle / biopsy punch (0.1g) •blood from caudal vein (3mL) •gills / surgical tools (0.03g) 	Cooke et al 2005
Barracuda	<ul style="list-style-type: none"> •Telemetric tags •Biologging 	<ul style="list-style-type: none"> •muscle / biopsy punch (0.1g) •blood from caudal vein (3mL) 	O'Toole et al 2012

Insights from non-invasive sampling methods

MUSCLE

Genetic/genomic
(DNA Barcodes,
microarray)

Stable isotopes
(N, C, S)

Contaminants
(Trace metals,
organohalogenated,
endocrine disrupter)

Fatty Acids

Lipids

Population structure
& connectivity

Migration patterns

Foraging behaviour
Trophic ecology

Sex determination
Reproductive success

Growth / Ageing

Energetic / Condition
Contaminant exposure

BLOOD

Genetic/genomic
(DNA barcodes &
adducts, microarray)

Stable isotopes
(N, C, S)

Hormones &
reproductive
proteins (Vtg, Zrp)

Contaminants
(Endocrine disrupter,
metabolites)

Non-esterified
free fatty acids

Non-lethal sampling on a practical point of view

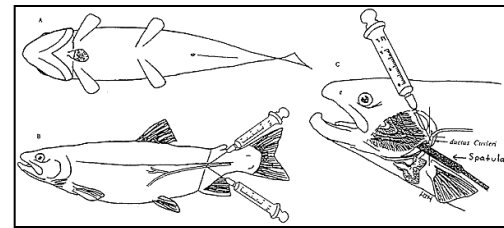
	Muscle Biopsy needle or punch	Blood Caudal vein or heart puncture with a needle on a heparinized syringe
Genetic/genomic	200-500mg (Kocan et al 2003)	1-5mL (SPC 1980; IATTC 1983; Wessel et al 2012)
Stable isotopes	1mg (Cunjak et al 2005)	1mL (Kline et al 2000)
Contaminants	200-800mg (Baker et al 2004; Schmitt & Brumbaugh 2007)	1-3mL (Schmitt & Brumbaugh 2007)
Lipids, fatty acids, reproductive proteins, hormones	200mg (Reprodott, 2002)	3mL (Skomal and Chase 1997 & 2002; Corriero et al 2004; Scoot et al 2006)



needle



punch



Are tunas at recapture still good for biological and chemical analyses ?

- Rapid freezing of fish followed by frozen storage prevent from cell damage and allow to retain fish sensory and nutritional properties
- Salt treatment such as brine immersion followed by cooling treatment provides a rapid convection freezing:

Advantages

(Toledo-Flores & Zall 1992)

- Decrease in water activity
- Less availability to microbial attack
- Enhancement of functional properties
- Increase of the shelf-life time

Inconvenients

(Aubourg & Ugliano 2002)

- Oxidation process leading to a modification of amino and fatty acid composition in muscle tissue, especially for fat fish

Reminder: Tuna recapture mainly depends on purse-seining that use brine freezing preservation to keep fish at -20 C or even lower for long trips.....

Objectives of the pilot study

1- To investigate the effects of brine freezing on four specific tracers:

- Fatty acids
- Lipids
- Stable isotopes
- Lipophilic chemicals

2- To examine the influence of the biopsy position on these tracers

The fatty acids

Fatty acids act as building blocks of lipids:

➤ **Saturated fatty acids (SFAs)**

➤ **Monounsaturated fatty acids (MUFAs)**

➤ **Polyunsaturated fatty acids (PUFAs) = Essential fatty acids (EFAs)**

Cannot be synthesized in animal tissues and must be taken through the diet

Omega 6

Omega 3

EFA deficiency leads to nutrition deficiency disease :

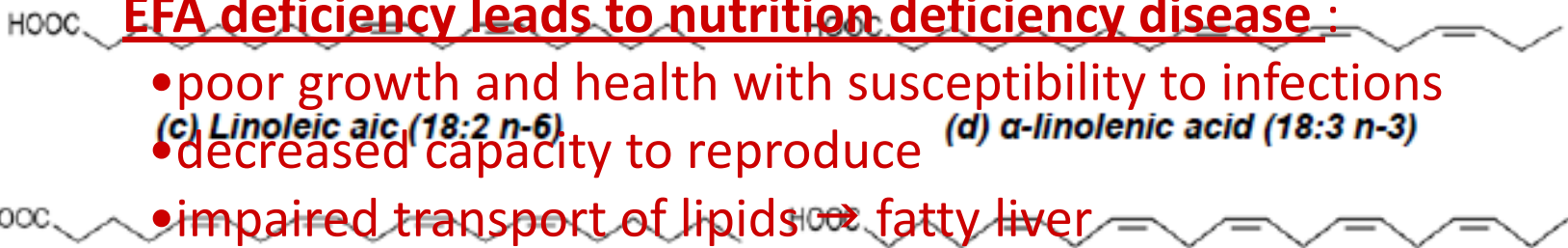
- poor growth and health with susceptibility to infections
- decreased capacity to reproduce

• impaired transport of lipids → fatty liver

• lowered resistance to stress

(e) Arachidonic acid (20:4 n-6)

(f) EPA (20:5 n-3)



Lipids as energetic tracers

Lipids are biological molecules that are insoluble in water but soluble in organic solvents

➤ **Structure lipids (SL)**: Mainly phospholipids and sterols

- Essential constituents of **cell membrane** and **nervous system**
- Components of **steroid hormones** that are critical intercellular messengers, and precursors for prostaglandin synthesis

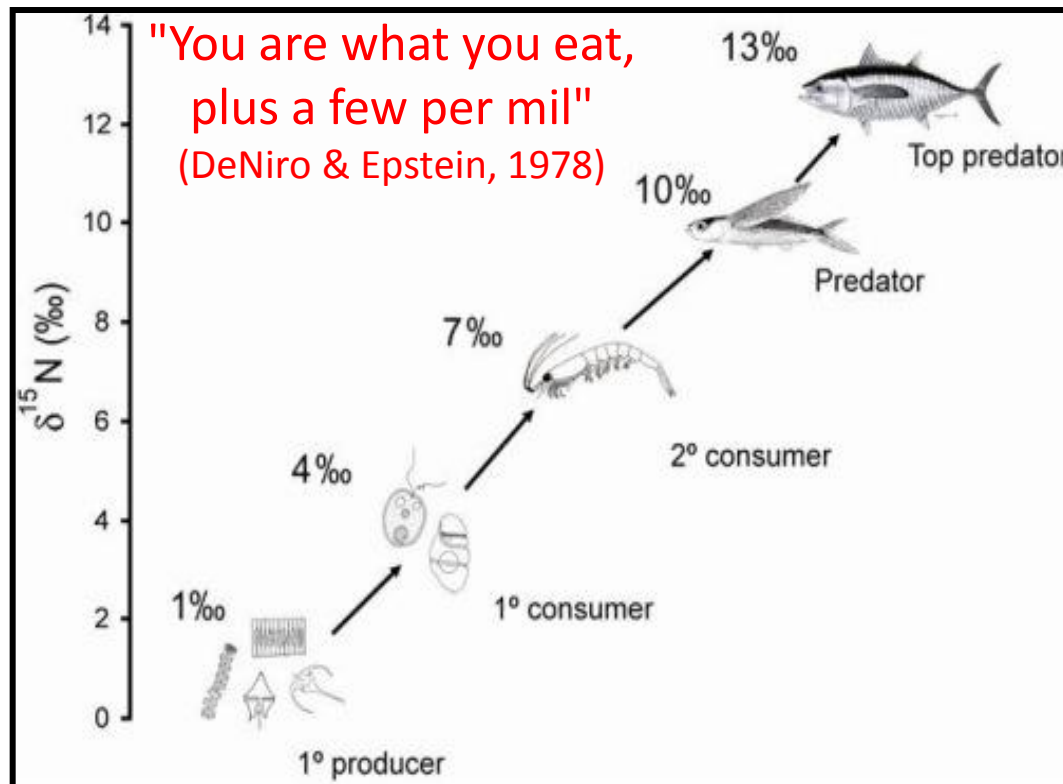
➤ **Reserve lipids (RL)**: mainly triacylglycerols and waxes

- As an **energy source** → high-energy value providing more energy per gram than carbohydrates and proteins (9 kcal.g^{-1})
- Supply the **fat-soluble vitamins** (A, D, E and K)
- Supply the **essential fatty acids** that cannot be synthesized

Trophic ecology and stable isotopes

➤ Stable isotopes determined on predators and preys to improve our understanding of trophic structure:

- Carbon stable isotopes ($\delta^{13}\text{C}$, $^{13}\text{C}/^{12}\text{C}$): identification of diet sources
- Nitrogen stable isotopes ($\delta^{15}\text{N}$, $^{15}\text{N}/^{14}\text{N}$): determination of trophic level



Stable isotopes =
an integration of the past
food habits depending
of the tissue turnover
(muscle → month / blood → days)

≠

Stomach contents =
a snapshot of the last meal

Basic information on lipophilic chemicals: not only indicators of contamination

➤ Chemicals of interest :

- Lipophilic or “fat-soluble” compounds with low volatility, high chemical stability
- Mainly from anthropogenic sources (industrial, urban and agricultural), the marine environment being the ultimate receptacle!

→ Omnipresent and persistent in marine ecosystems

→ Bioaccumulation in marine organisms: uptake through diet and storage in lipid-rich tissues

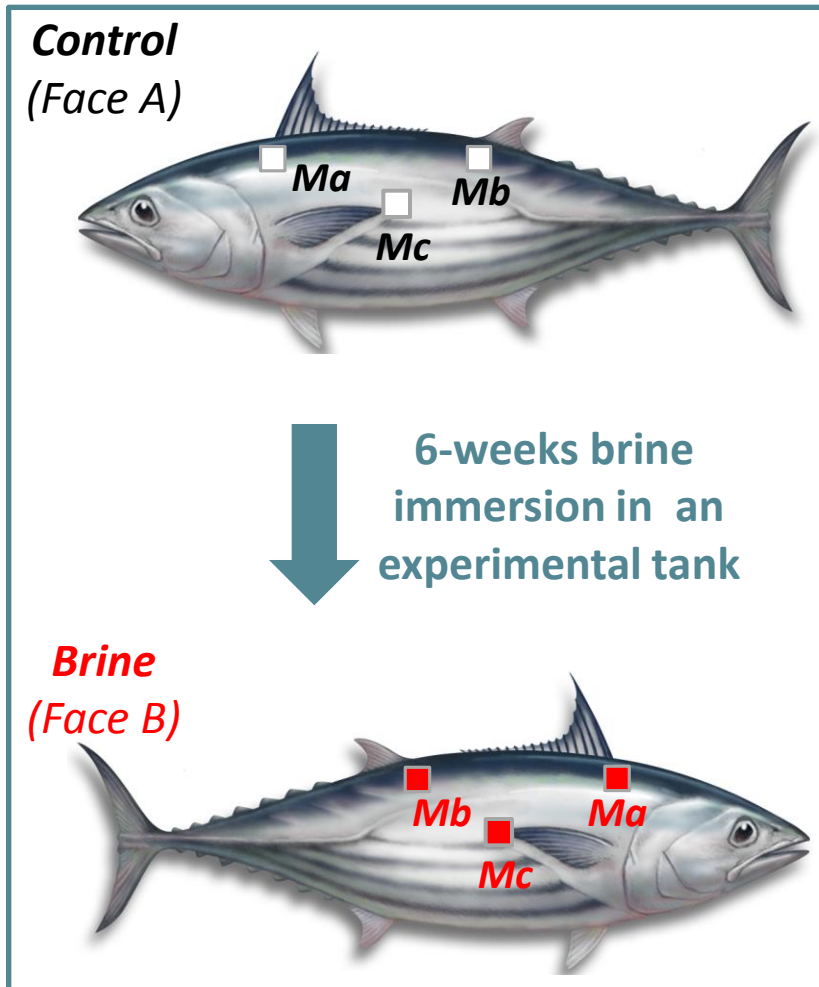
→ Biomagnification in marine foodwebs leading to high levels in top predators

➤ Lipophilic chemicals as novel and promising tools for research in fish biology and ecology:

- Trophic tracers to understand foodweb structure (Borga et al 2004)
- Population structure and migration biology (Dickut et al 2010)
- Growth and sexual dimorphism (Bodiguel et al 2010)
- Reproductive success (Wells et al 2005) → endocrine disrupting chemicals

Experimental design

➤ **Brine freezing on tuna purse-seiners** = Saturated sodium chloride (~300g/L) brine immersion tank with a propeller maintained at -20 °C for 6-weeks max.

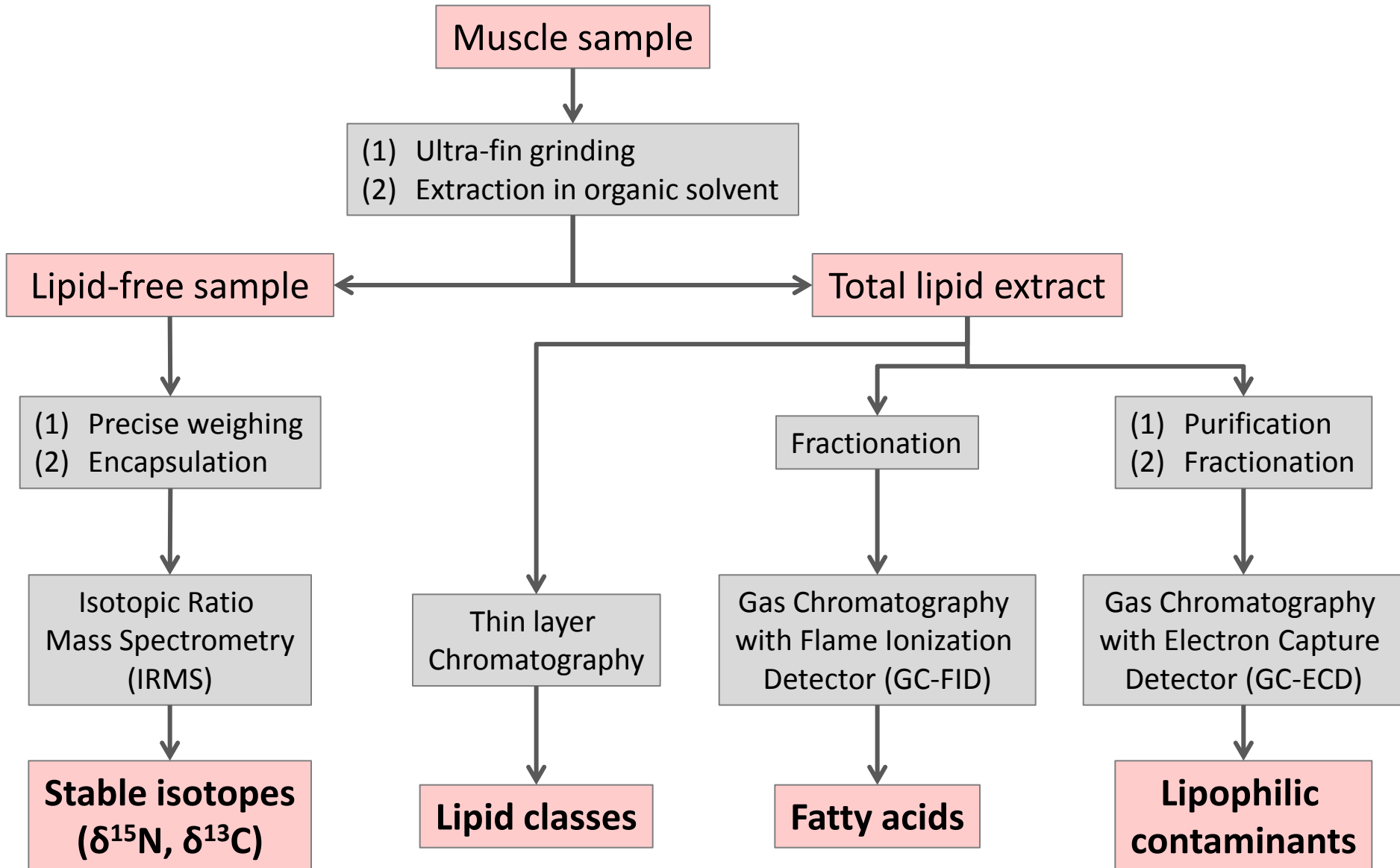


- Targeted fish = small tunas with thin skin

Species	Sp Code	<i>n</i>	RWt (kg)	FL (cm)
Skipjack	SKJ	6	2.3±1.3	46±8
Yellowfin	YFT	6	2.2±0.3	49±3

- *Ma* → samples from the dorsal musculature adjacent to the first dorsal fin
- *Mb* → samples from the dorsal musculature adjacent to the second dorsal fin
- *Mc* → samples from the ventral musculature

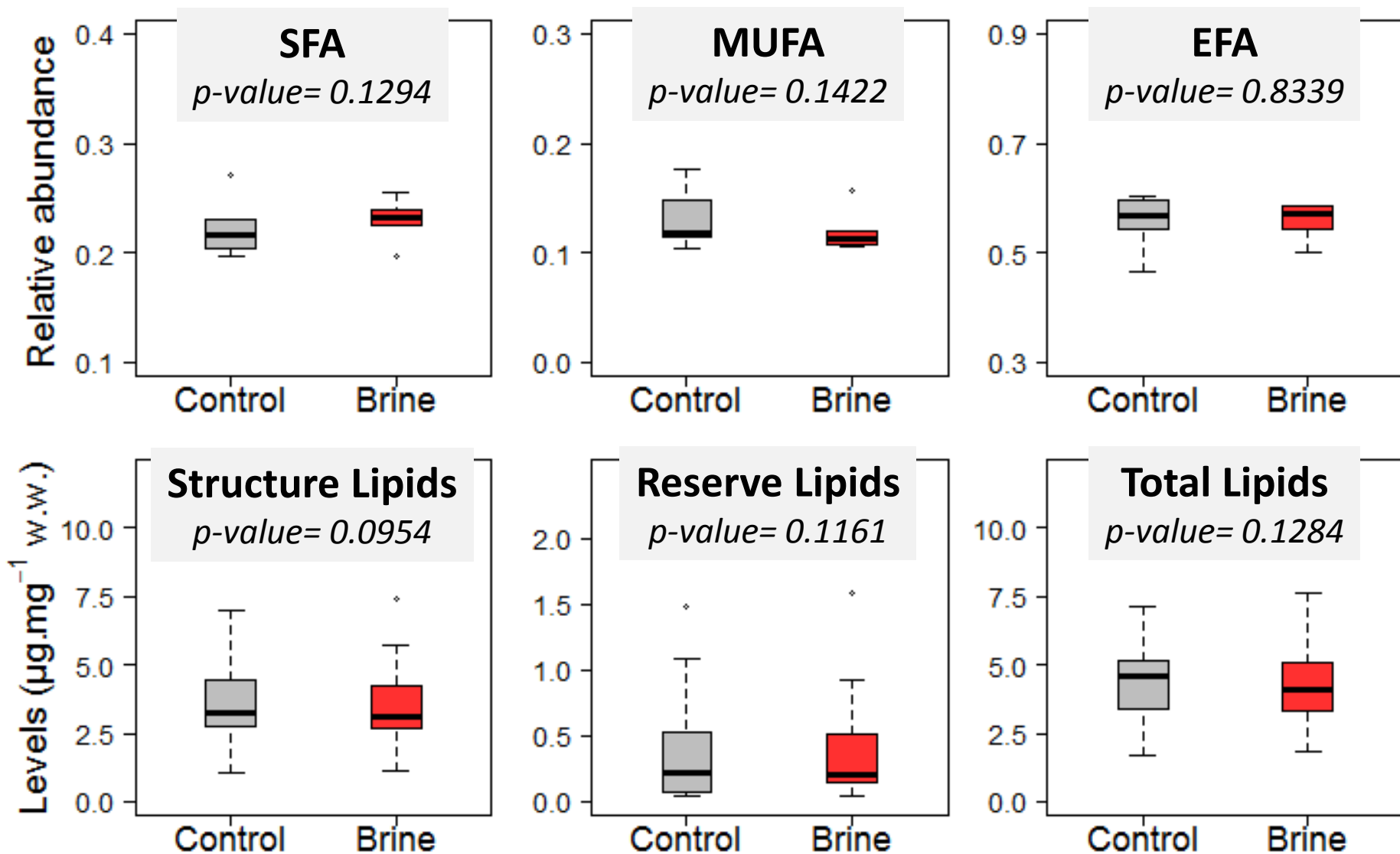
Analytical methodology



Objective 1

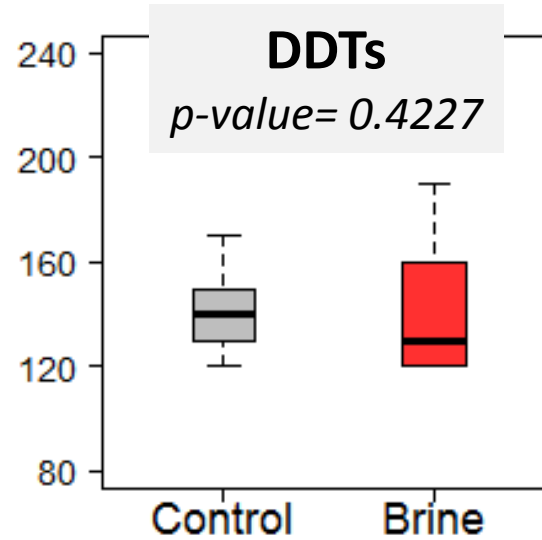
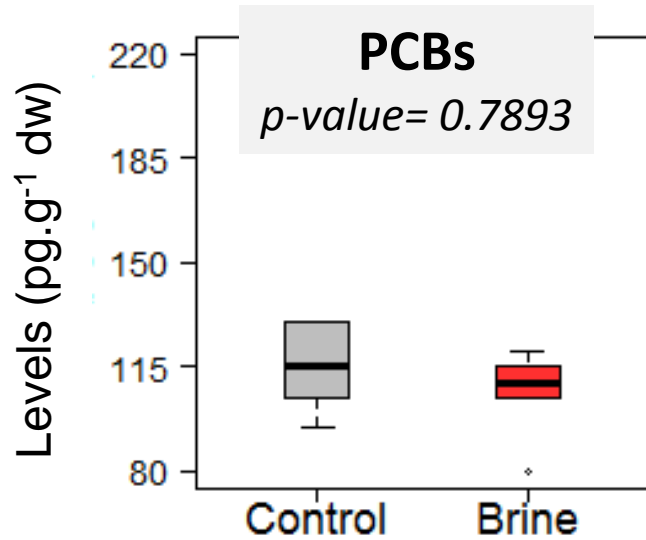
Is there any influence of brine storage on trophic, bioenergetic and chemical tracers determined in small tuna muscles?

Brine effect on tracers: (1) fatty acid & lipid patterns



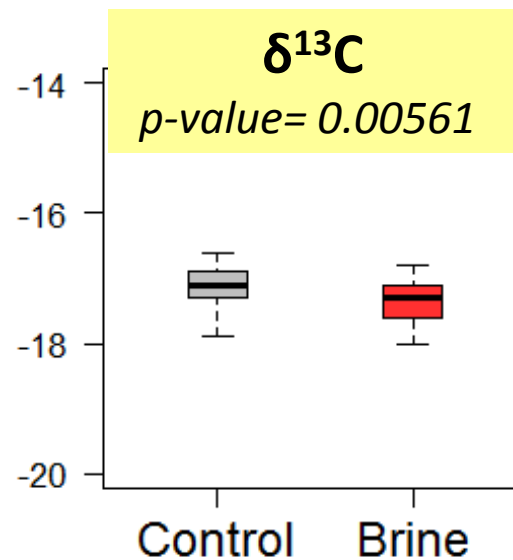
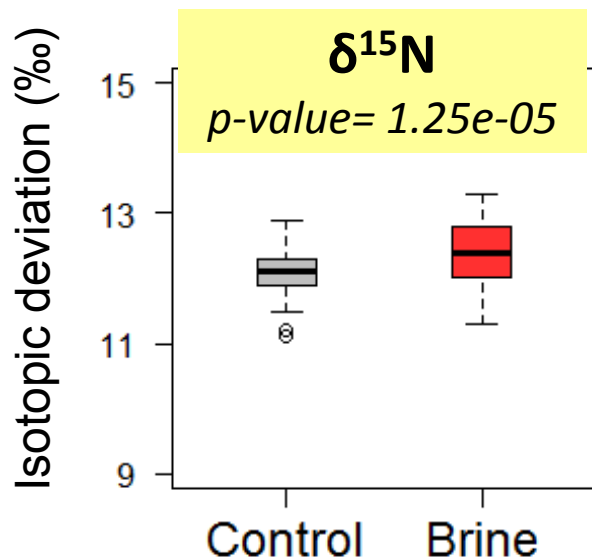
Brine effect on tracers:

(2) lipophilic chemicals & stable isotopes



PCBs: Polychlorinated biphenyls (industrial compounds)

DDTs: Organochlorine pesticides used against malaria



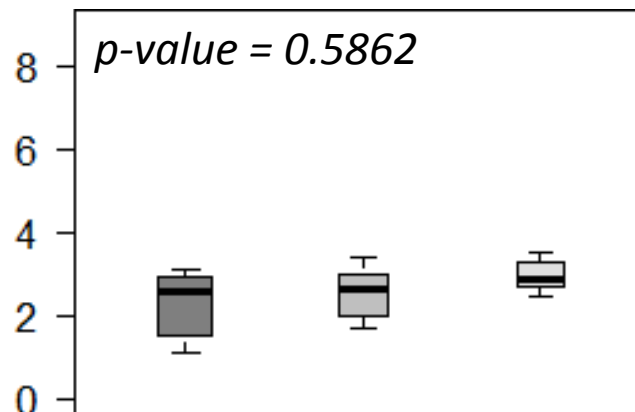
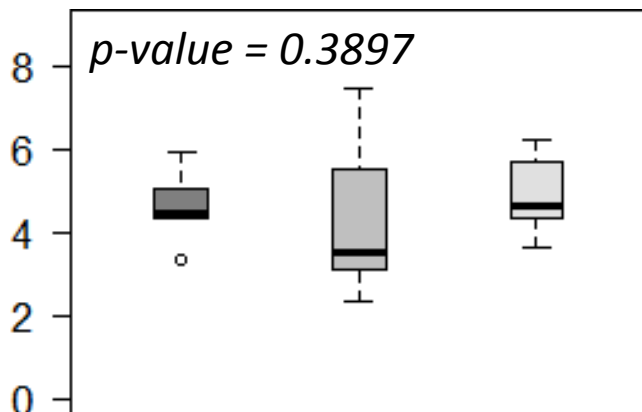
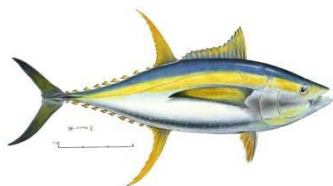
δ¹⁵N: Nitrogen isotopes

δ¹³C: Carbon isotopes

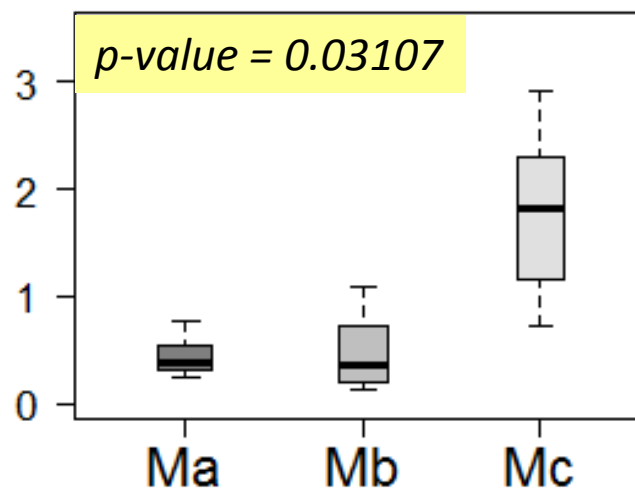
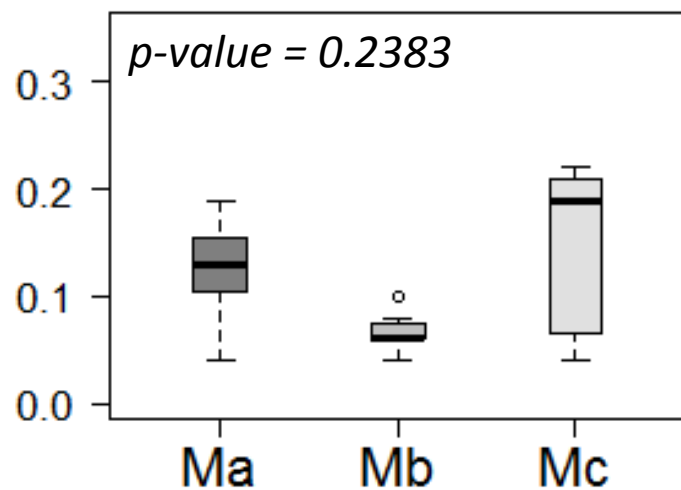
Objective 2

Is there any influence of muscle sample position on tracer's determination?

Effect of muscle position on lipid distribution

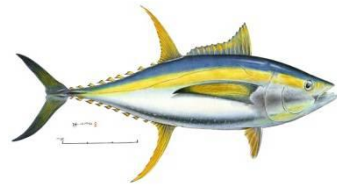


Structure lipids

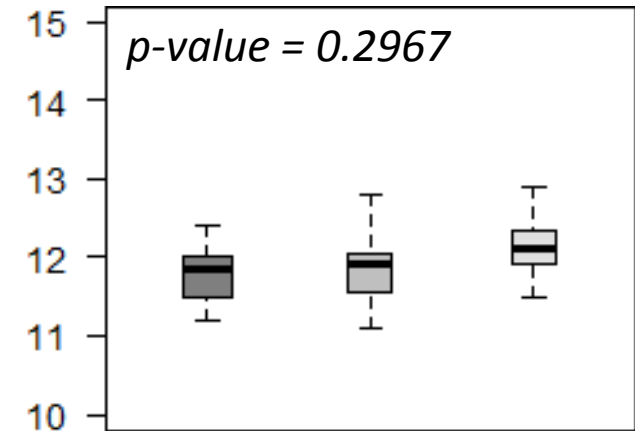
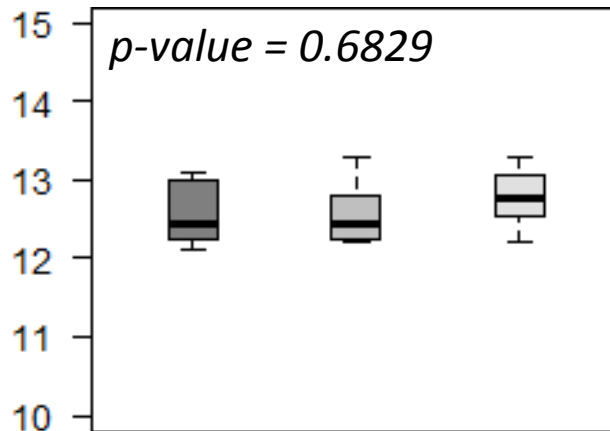


Reserve lipids

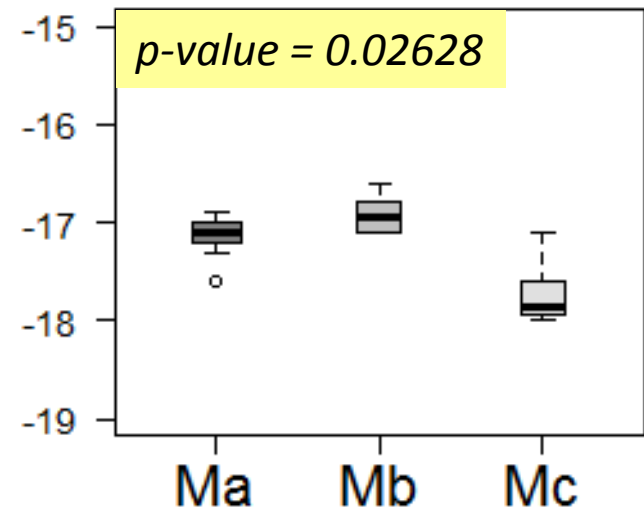
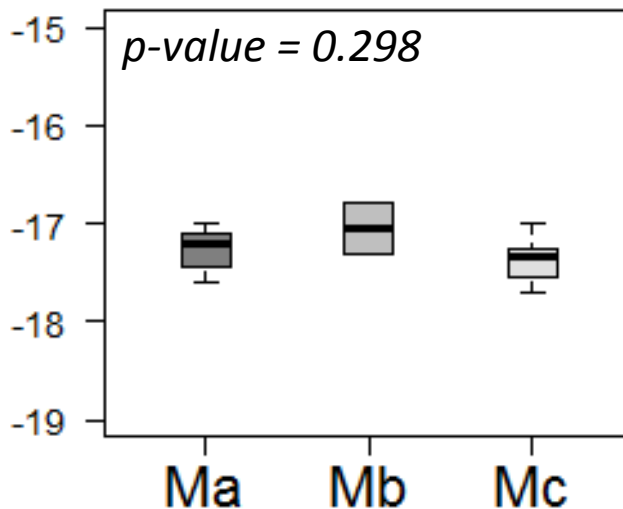
Effect of muscle position on isotopic signatures



Nitrogen isotopes ($\delta^{15}\text{N}\text{‰}$)



Carbon isotopes ($\delta^{13}\text{C}\text{‰}$)



Conclusions

1- Non-invasive tissue biopsies and blood sampling have been successfully realized on various tagged fish, including tunas and billfishes

2- The studied tracers (fatty acids, lipids and lipophilic chemicals) can be determined on the muscle of tagged fish whereas stable isotopes should be analysed on blood samples

3- Efforts must be done to establish a standardised protocol to collect muscle samples in tunas due to its heterogeneous biochemical composition

Next step... Waiting for the future tagging program to conduct such researches and improve knowledge on the biology, ecology and contamination of tunas