

NON-LETHAL SAMPLING METHOD FOR THE
ANALYSIS OF FATTY ACID PROFILES IN
EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Mickaël PERON, Fabienne LE GRAND, Victor SIMON, Jean-Baptiste
QUEMENEUR, David MAZURAIS, Philippe SOUDANT, Marie VAGNER

Lipids

Source & **reserve** of energy



Triglycerides



Neutral lipids (**NL**) / **Reserve** lipids

Strong dietary influence

Structure & function of **membranes**



Phospholipids



Polar lipids (**PL**) / **Membrane** lipids

Lipids

Source & **reserve** of energy



Triglycerides



Neutral lipids (**NL**) / **Reserve** lipids

Strong dietary influence

Structure & function of **membranes**

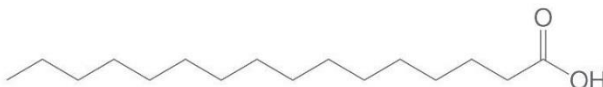


Phospholipids

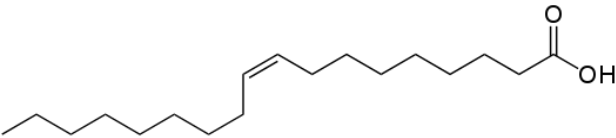


Polar lipids (**PL**) / **Membrane** lipids

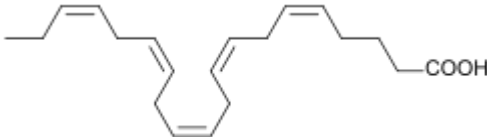
Fatty acids



Saturated
(*e.g* Palmitic acid)

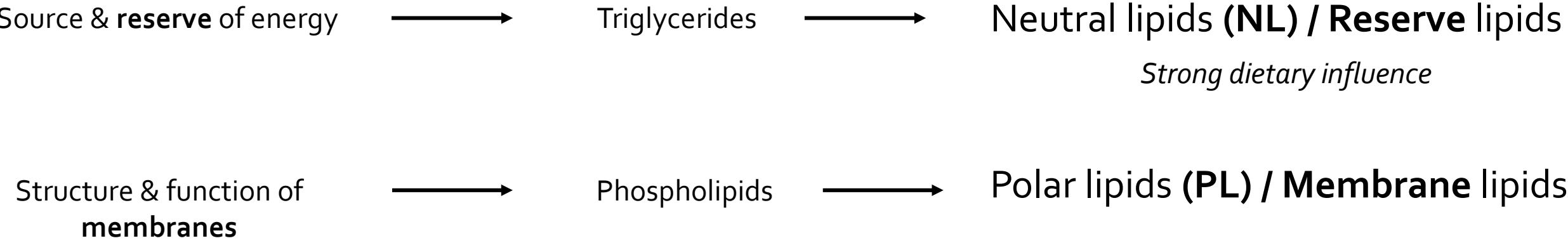


Mono-unsaturated
(*e.g* Oleic acid)

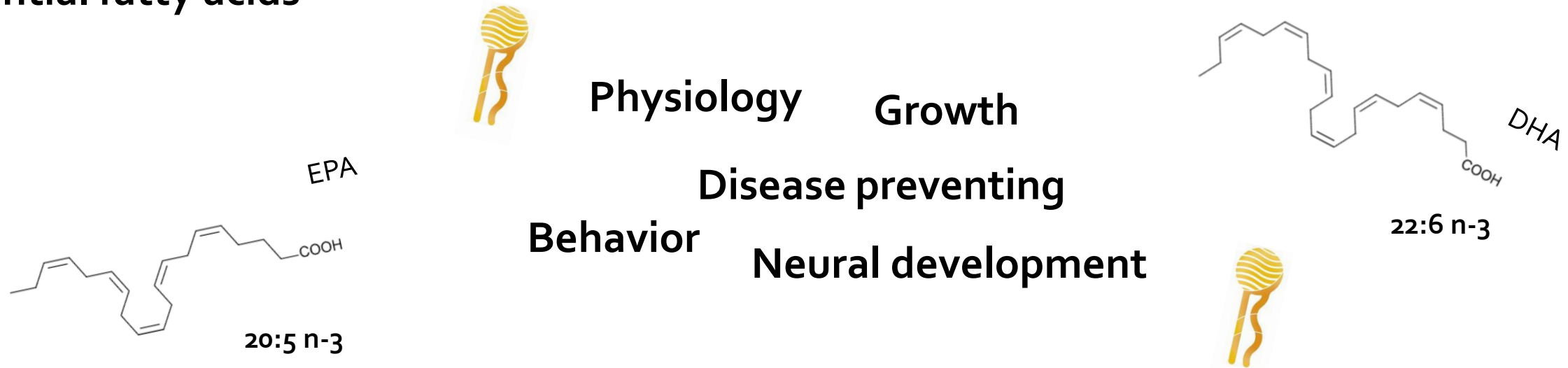


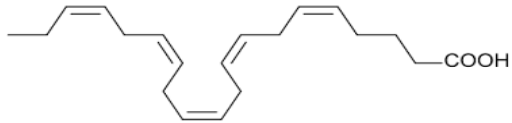
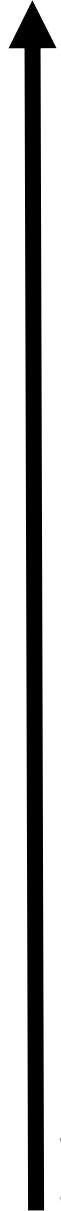
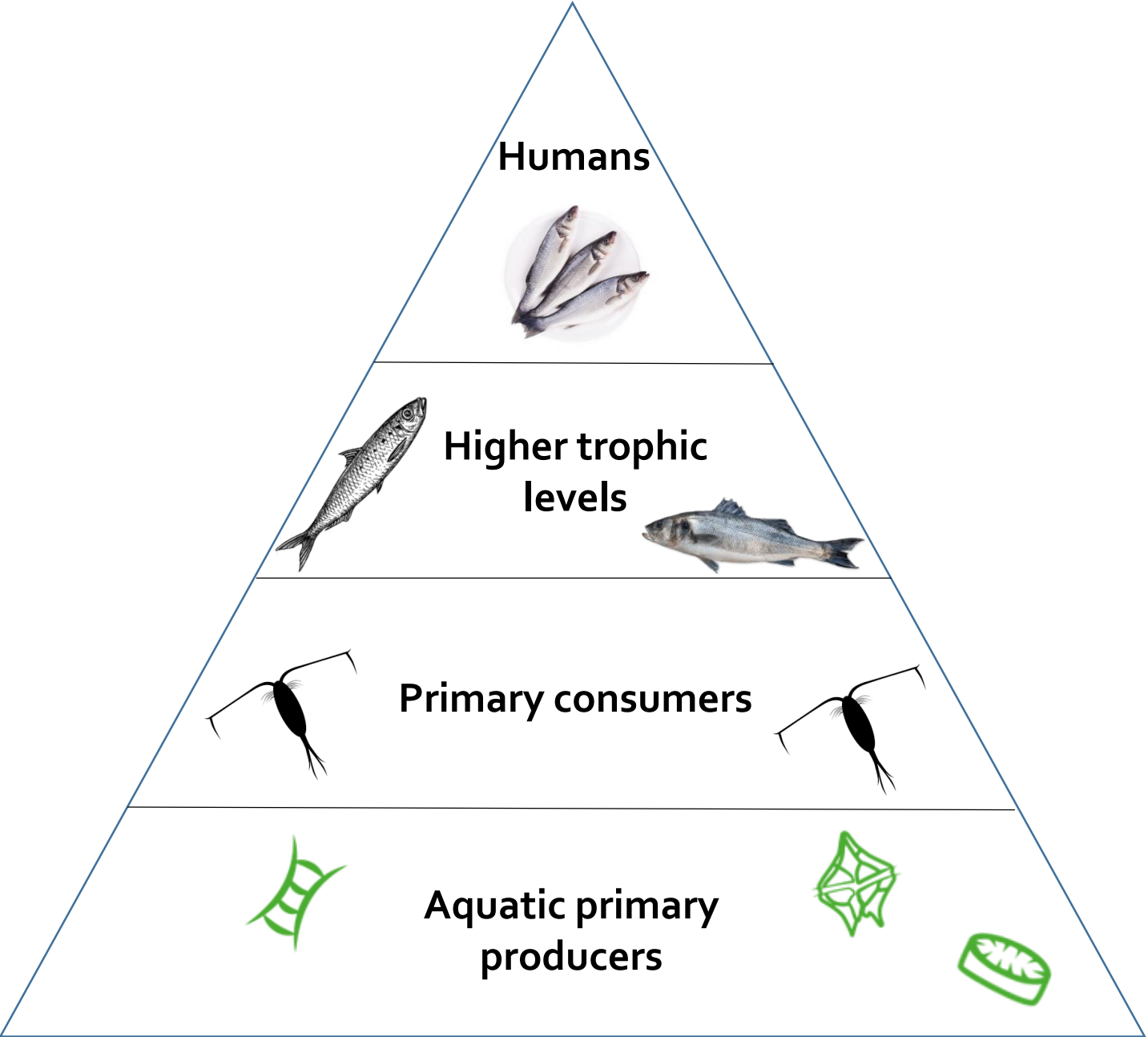
Poly-unsaturated
(*e.g* Eicosapentaenoic acid)

Lipids

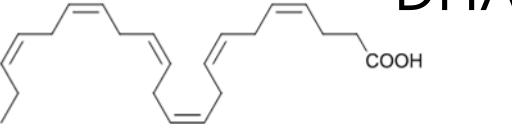


Essential fatty acids





EPA

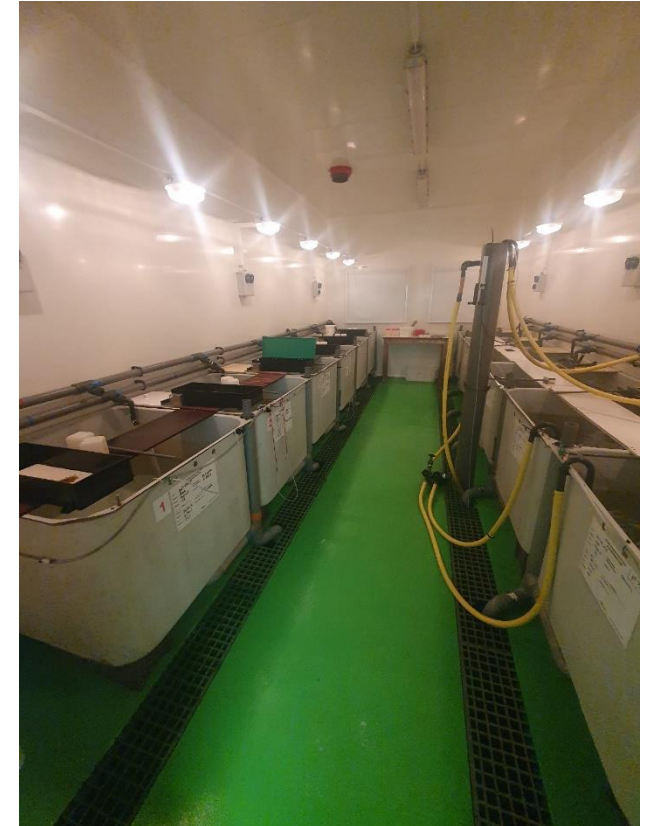


DHA

FA Analysis in Fish

Applications ?

- Experimental biology → Nutritional conditioning experiments, FA of physiological relevance



FA Analysis in Fish

Applications ?

- Experimental biology → Nutritional conditioning experiments, FA of physiological relevance
- Ecological studies → Trophic information through FA biomarkers



FA Analysis in Fish

Applications ?

- Experimental biology → Nutritional conditioning experiments, FA of physiological relevance
- Ecological studies → Trophic information through FA biomarkers
- Aquaculture → Feeding trials (monitoring FA composition over time)



Tissue sampling for FA analysis



Tissue sampling for FA analysis

Muscle



Brain



Tissue sampling for FA analysis

Muscle



Brain



Liver



Tissue sampling for FA analysis



Classic samples :

- Lethal, most of the time (Brain, liver...)
- One sample at one time (+ inter-individual variability)



**No idea of the evolution
of the FA in tissues**

Why following FA composition over time ?

- Experimental studies -> Testing the effects of various parameters affecting FA composition (T°, diet..)

Why following FA composition over time ?

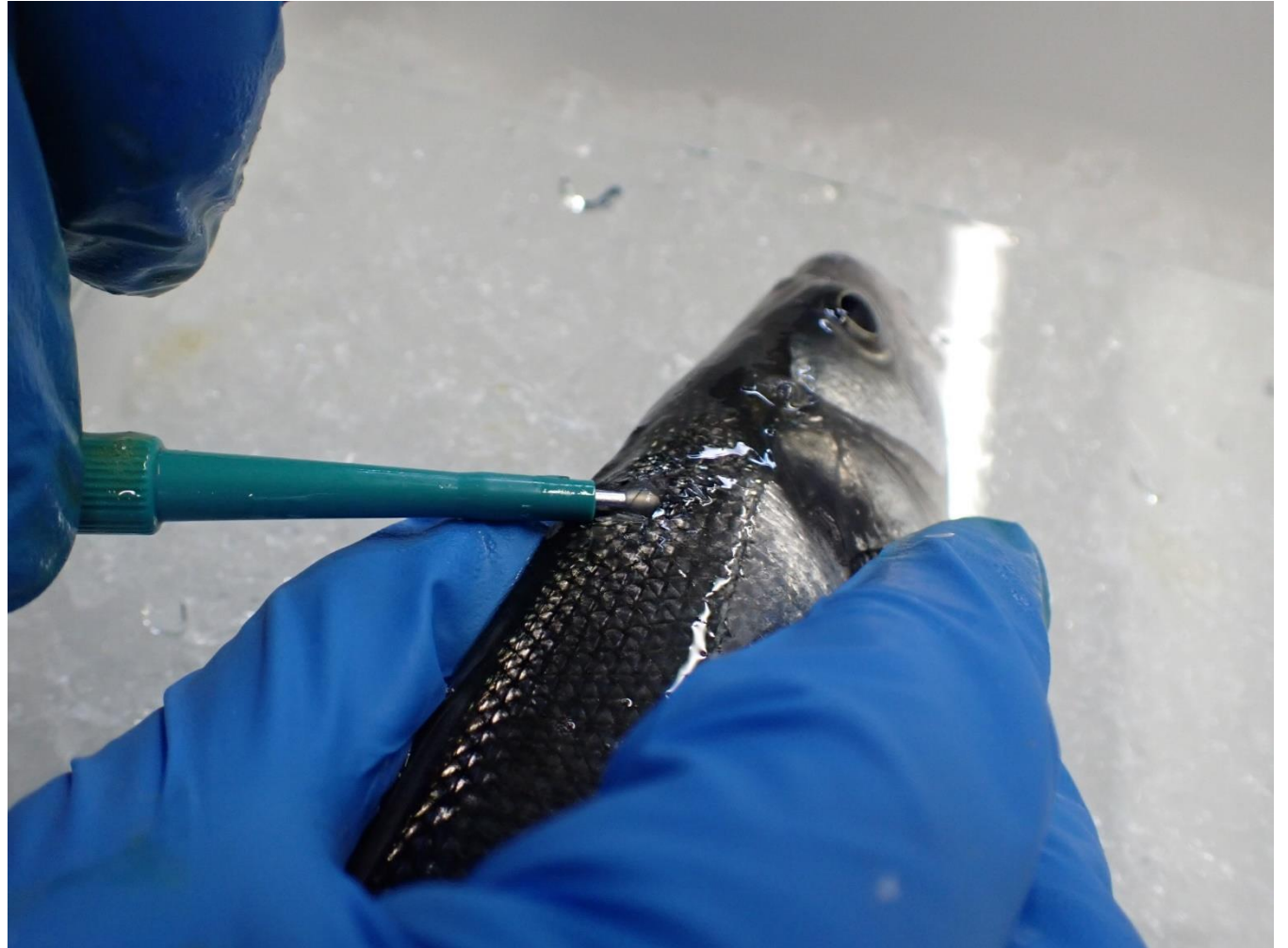
- Experimental studies -> Testing the effects of various parameters affecting FA composition (T°, diet..)
- Acquiring information about the behavior of FA in tissue : Turn-over of FA, incorporation...

Why following FA composition over time ?

- Experimental studies -> Testing the effects of various parameters affecting FA composition (T°, diet..)
- Acquiring information about the behavior of FA in tissue : Turn-over of FA, incorporation...

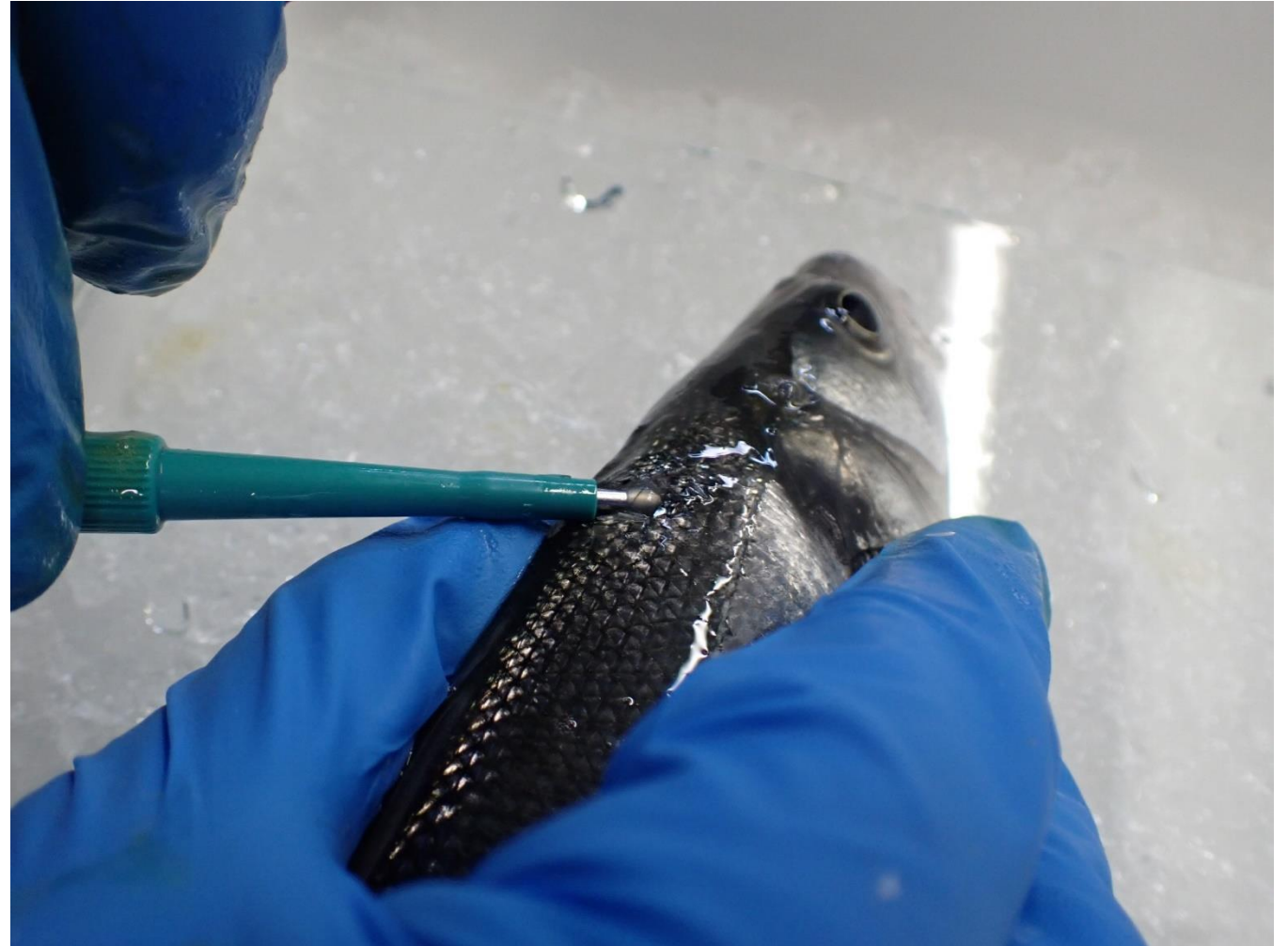
Need of non-lethal sampling method to study FA composition overtime (and having individual monitoring)

The subcutaneous biopsy



The subcutaneous biopsy

- Non-lethal method
- Sampling of white muscle
- Feasible on various fish size
- Showed no impact of growth if done 1 month apart



Major questionings :

- Is the quantity of biopsied tissue sufficient for routine analysis (usually 50mg) ?



Major questionnings :

- Is the quantity of biopsied tissue sufficient for routine analysis (usually 50mg) ?
- How to process the sample ?



Major questionnings :

- Is the quantity of biopsied tissue sufficient for routine analysis (usually 50mg) ?
- How to process the sample ?
- Is the quantity of extracted FA similar to a classic sample ?



Major questionnings :

- Is the quantity of biopsied tissue sufficient for routine analysis (usually 50mg) ?
- How to process the sample ?
- Is the quantity of extracted FA similar to a classic sample ?
- Is the FA profile similar between method and replicates ?



Major questionnings :

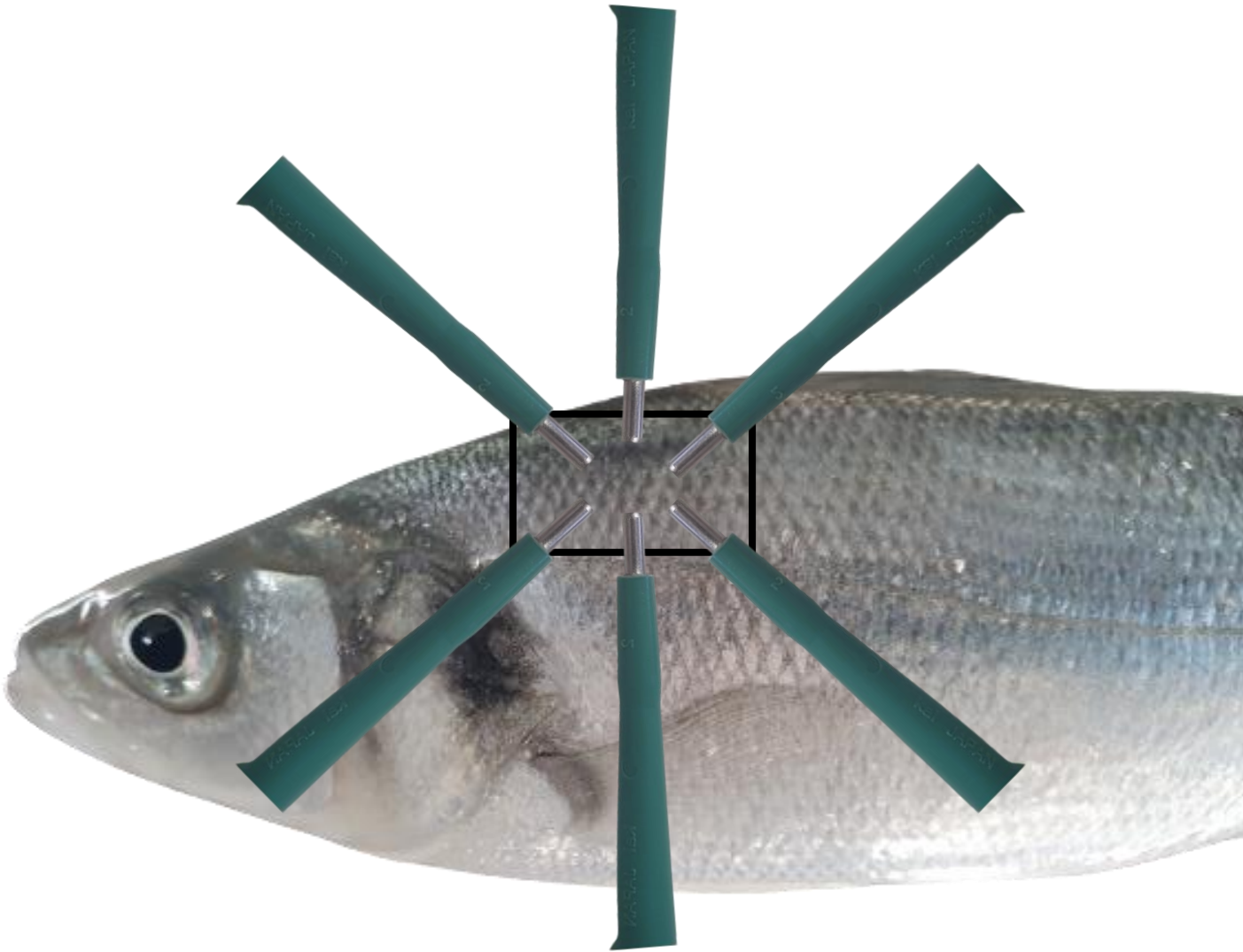
- Is the quantity of biopsied tissue sufficient for routine analysis (usually 50mg) ?
- How to process the sample ?
- Is the quantity of extracted FA similar to a classic sample ?
- Is the FA profile similar between method and replicates ?

**Is subcutaneous biopsy a viable method for
FA analysis in fish ?**

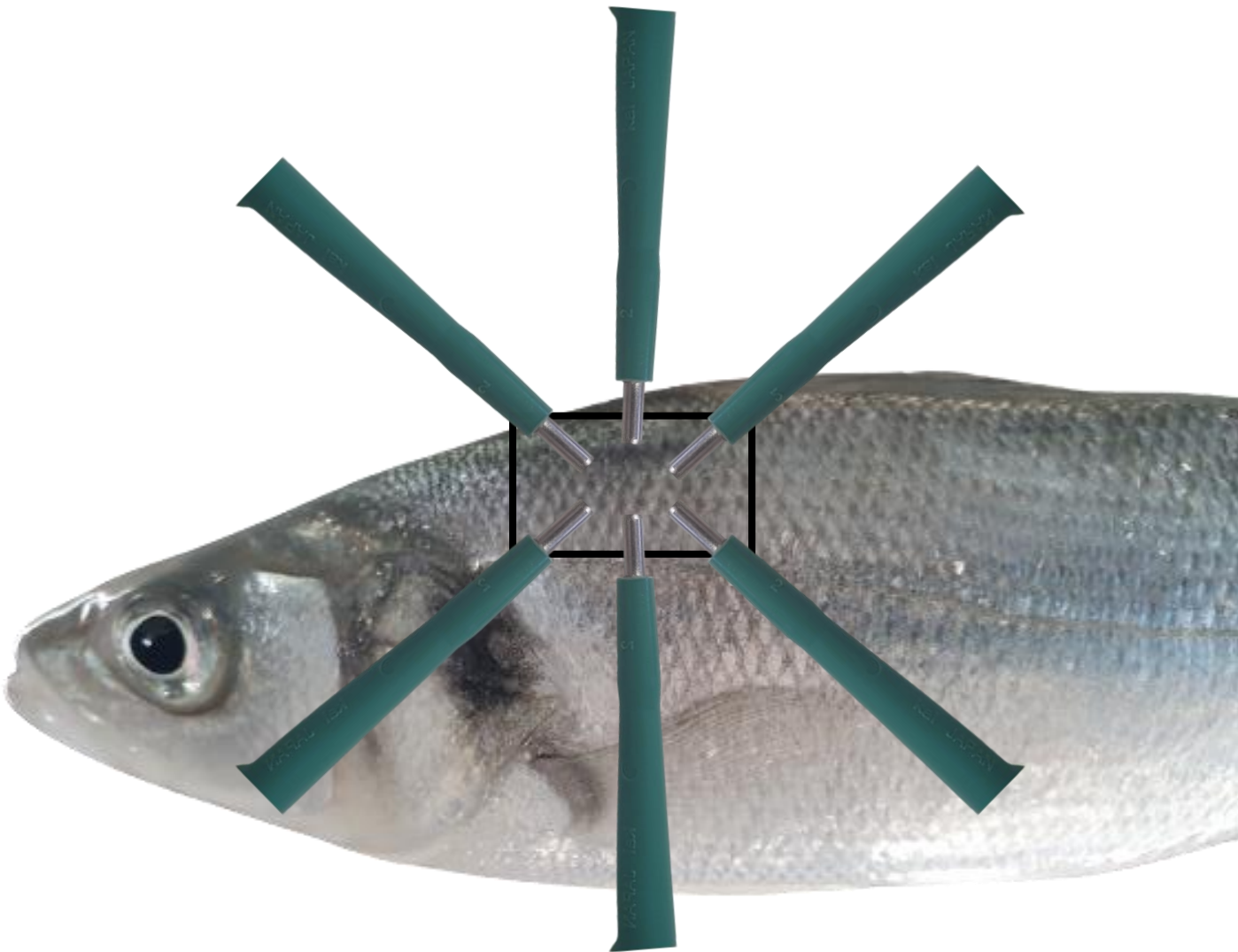


Material and Methods

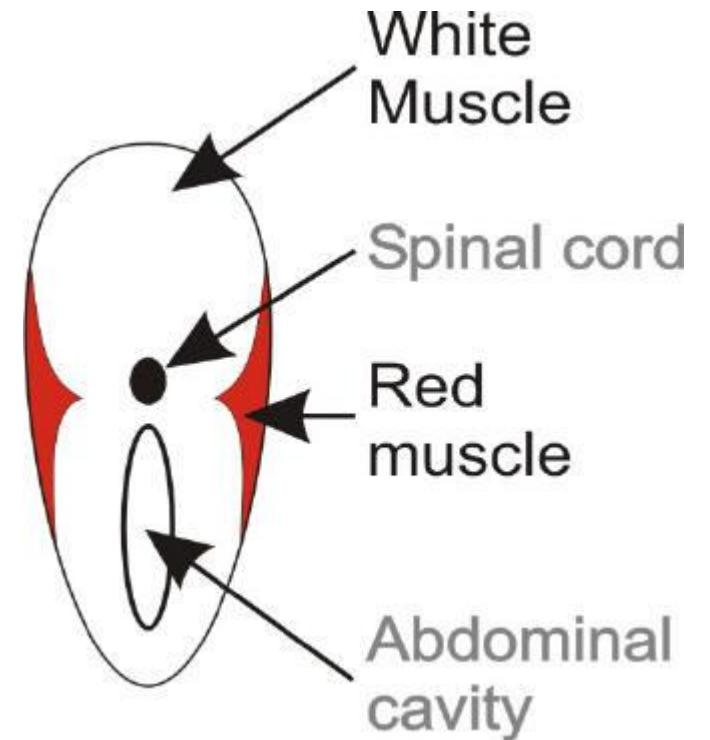
- 6 biopsy on the same space on each fish – White muscle
- White muscle sampling of the whole space



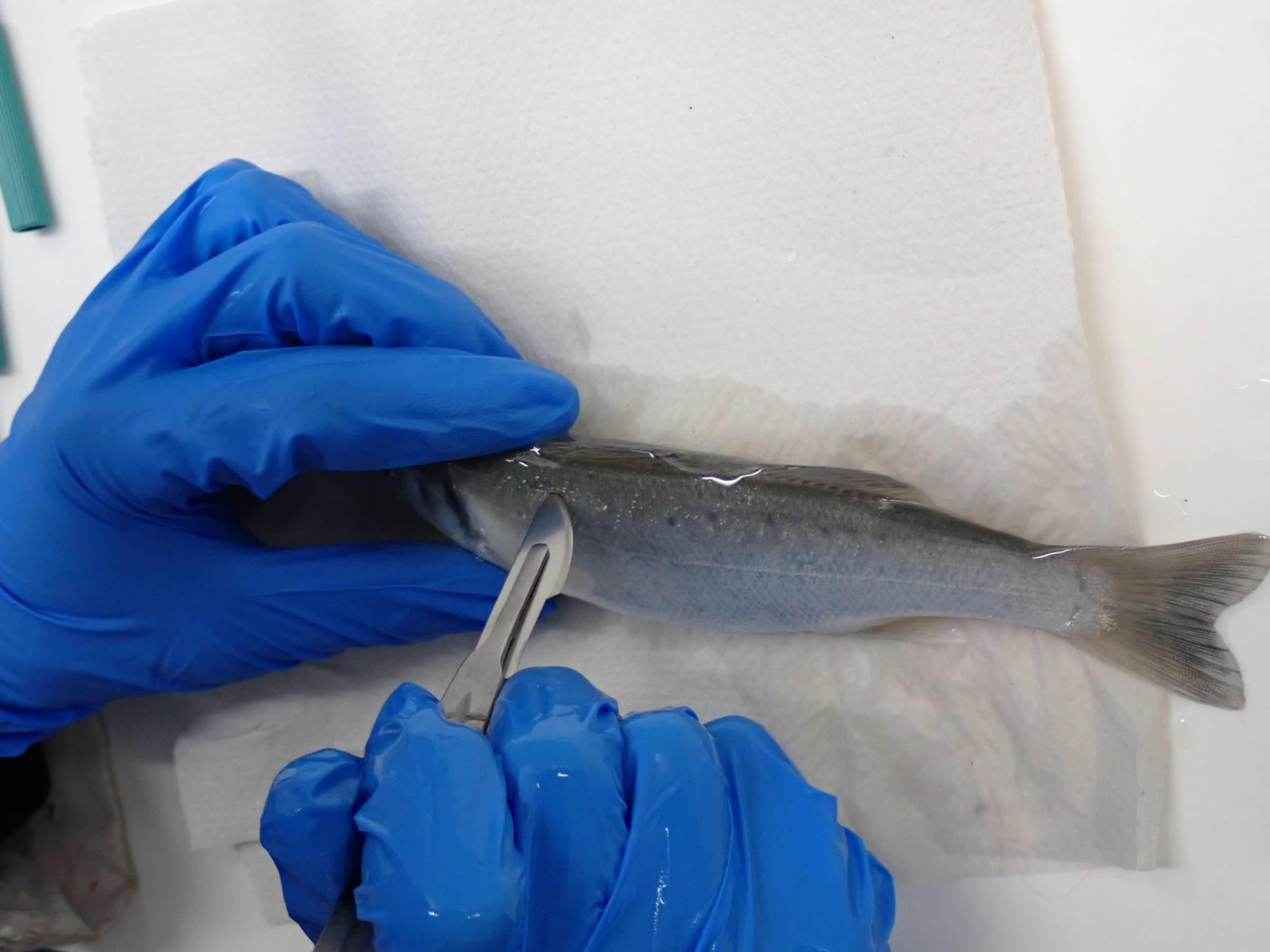
- 6 biopsy on the same space on each fish – White muscle
- White muscle sampling of the whole space



- 6 biopsy on the same space on each fish – White muscle
- White muscle sampling of the whole space



Step 1 : Small cut on the skin





Step 1 : Small cut on the skin

Step 2 : Inserting biopsy punch



Ø 2 mm

Used for human medicine (sterile)



Step 1 : Small cut on the skin

Step 2 : Inserting biopsy punch

Step 3 : Pushing in and turning the punch

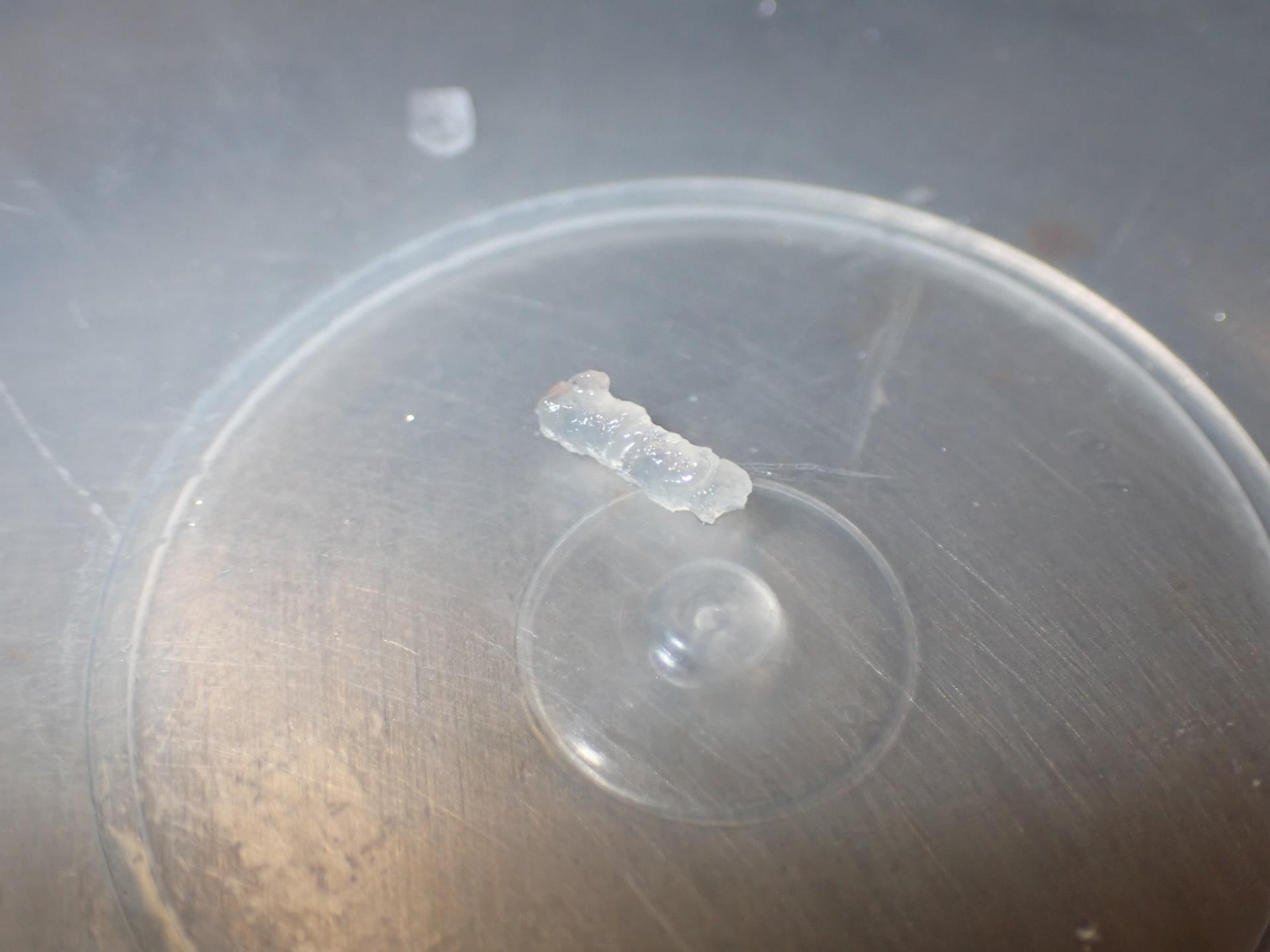


Step 1 : Small cut on the skin

Step 2 : Inserting biopsy punch

Step 3 : Pushing in and turning the punch

Step 4 : Lifting and removing the punch



Step 1 : Small cut on the skin

Step 2 : Inserting biopsy punch

Step 3 : Pushing in and turning the punch

Step 4 : Lifting and removing the punch

Step 5 : Extracting tissue



Step 1 : Small cut on the skin

Step 2 : Inserting biopsy punch

Step 3 : Pushing in and turning the punch

Step 4 : Lifting and removing the punch

Step 5 : Extracting tissue

Step 6 : Applying antiseptic



Step 1 : Small cut on the skin

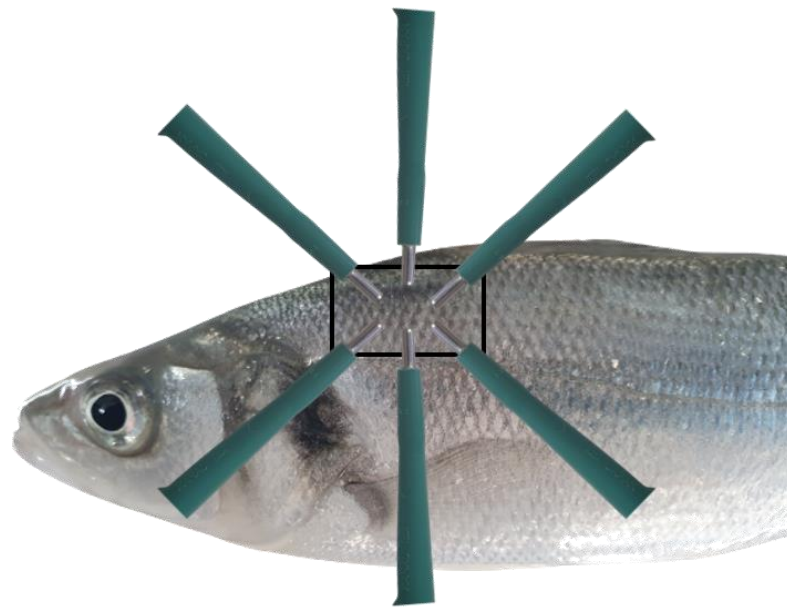
Step 2 : Inserting biopsy punch

Step 3 : Pushing in and turning the punch

Step 4 : Lifting and removing the punch

Step 5 : Extracting tissue

Step 6 : Applying antiseptic & surgical glue



N= 6 fish
Juvenile European
seabass
~15 cm, ~50g



N= 6/fish



N= 1/fish **Whole Muscle**



Whole Muscle



Grinding 50mg of tissue



6mL Solvent (CHCl_3 -MeOH)

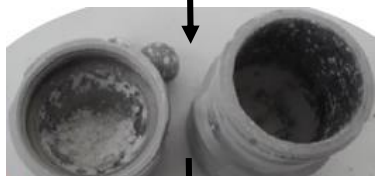


1mL for lipid extraction and separation



Fatty acid profiles
with GC-FID





6mL Solvent (CHCl_3 -MeOH)



1mL



Fresh

6mL Solvent



6mL



Freeze-Dried

6mL Solvent



6mL

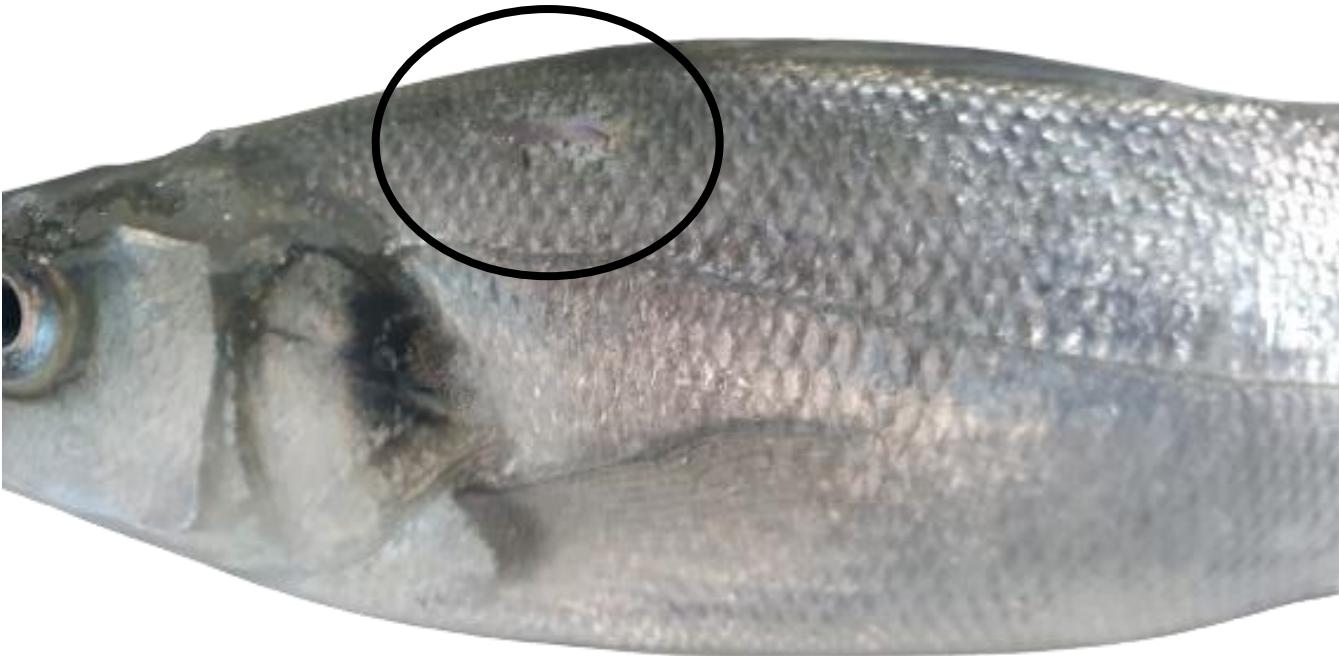


Results & Discussion



Recovery of the fish :

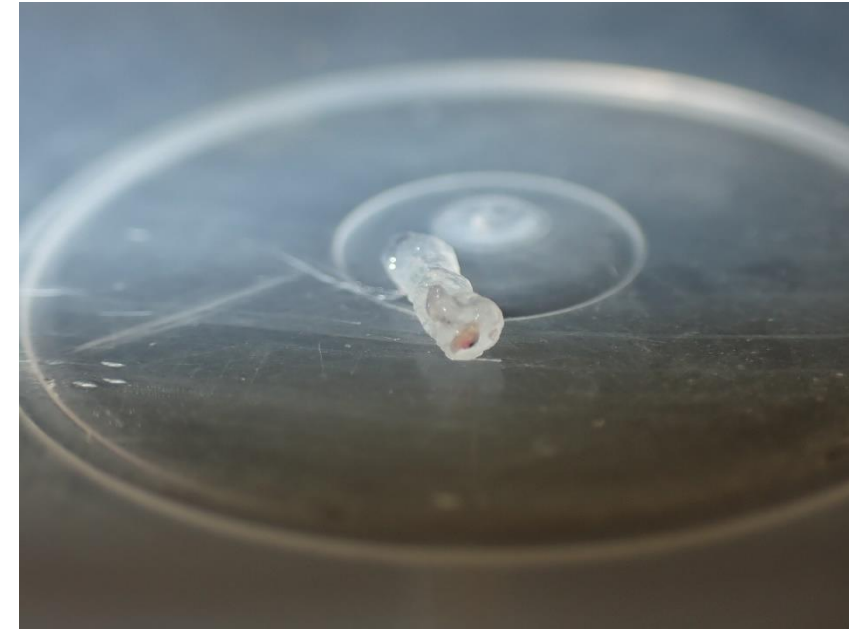
- No observed mortality
- Small scar after 1 month



- Quantity of tissue :

Mean sample weight with biopsy was **8,8 (\pm 3,5) mg.**

Minimum weight was **1,4 mg** and maximum was **14,4 mg.**

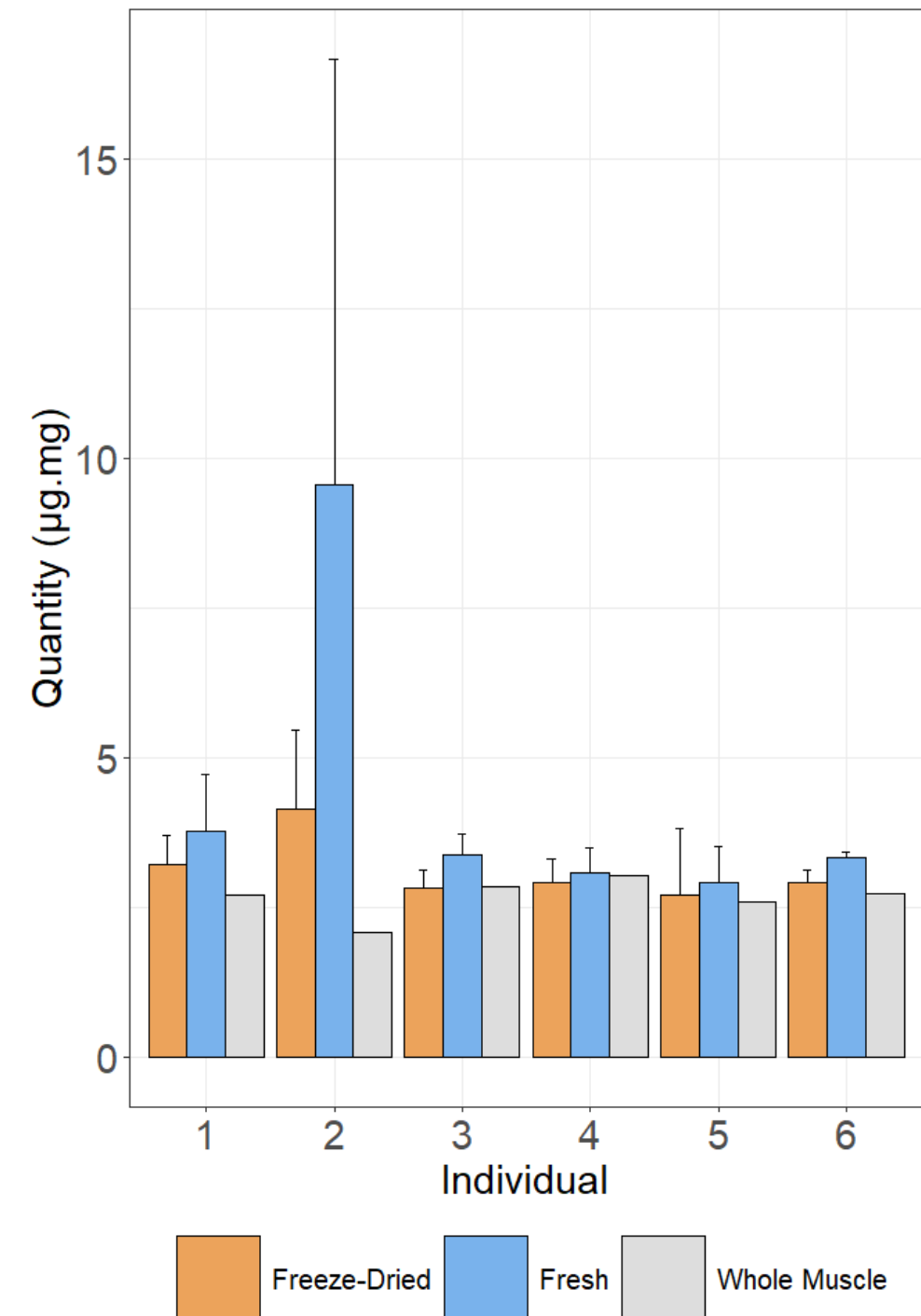


- Number of identified FA :

	NL	PL
F	55	63
FD	61	59
WM	60	67

Quantity of extracted FA in **PL** :

- Lower variability
- One highly variable sample (manipulation bias ?)
- Overall approximative quantities of FA



Need to strengthen method
for quantity analysis

Quantity of extracted FA in **NL** :

- Very high variability

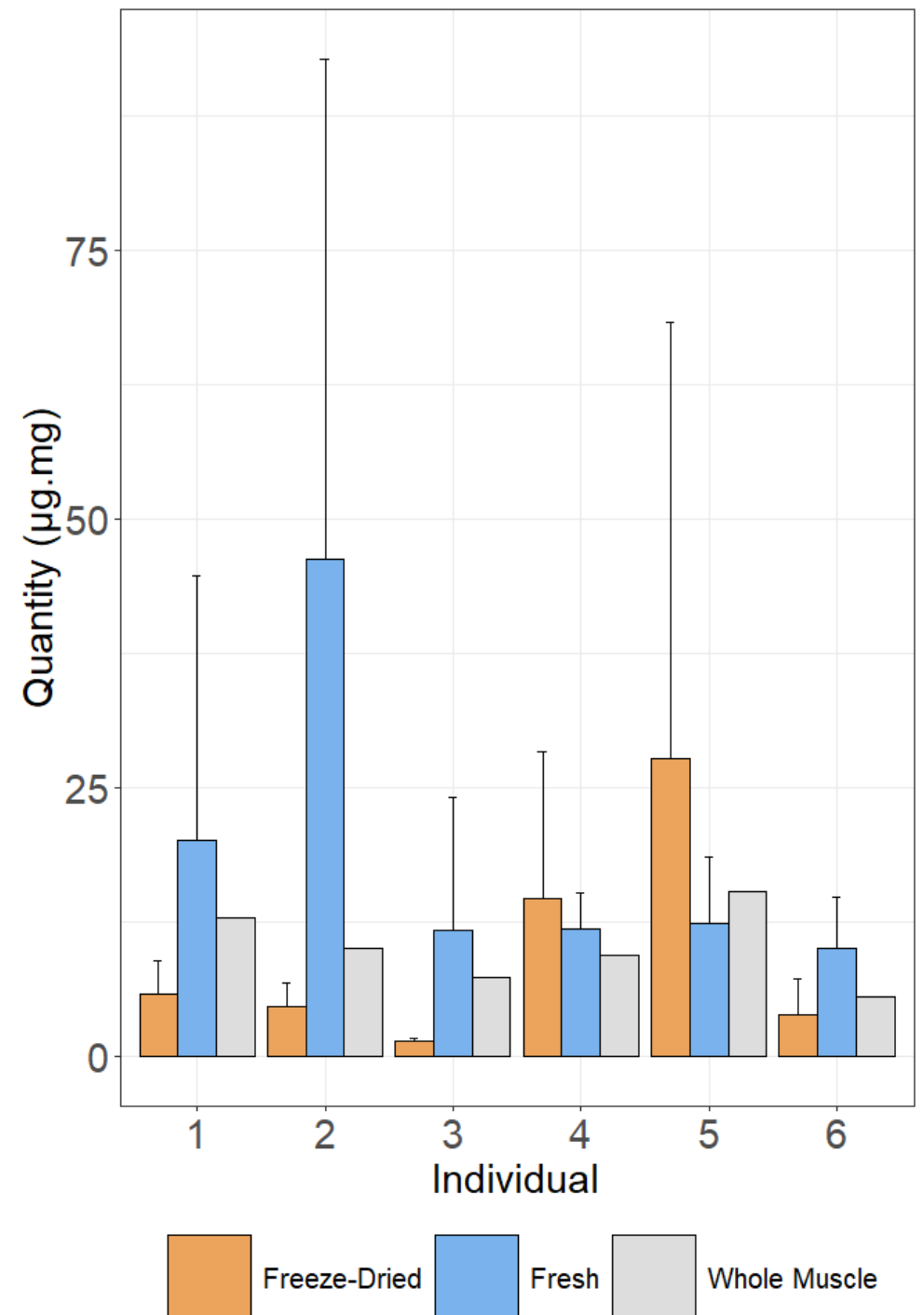
Among replicates & individuals

Lipid droplets under the skin ?

Sampling of skin ?



Questionable method for
quantity analysis



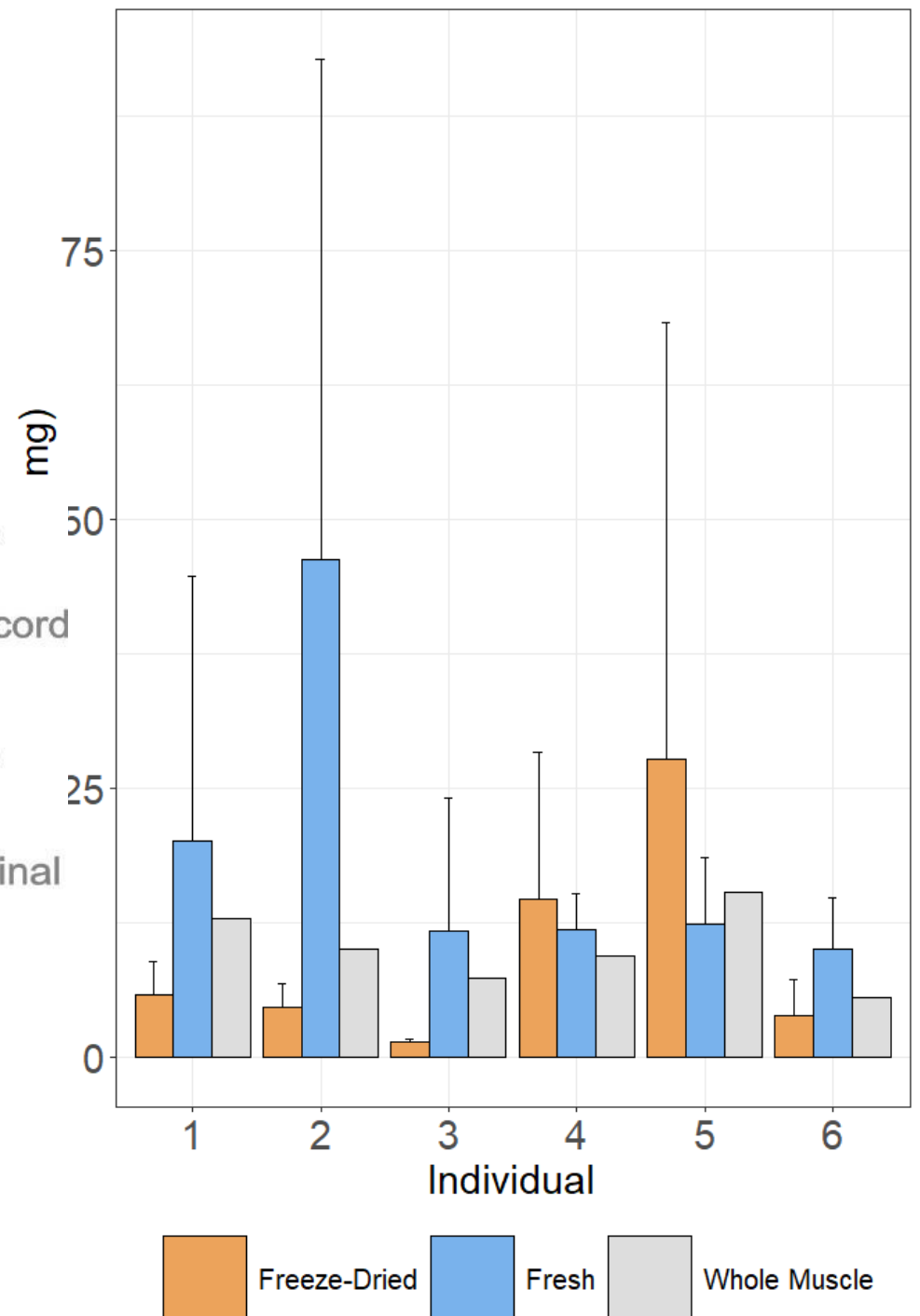
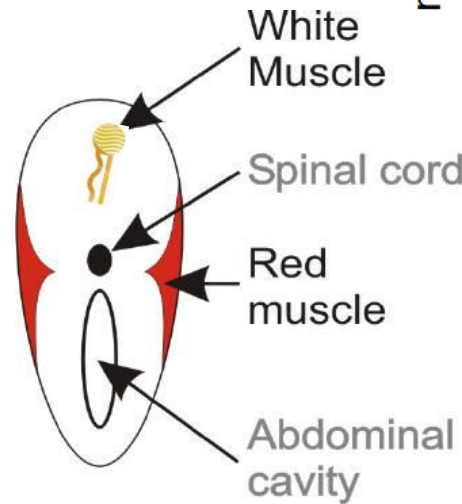
Quantity of extracted FA in **NL** :

- Very high variability

Among replicates & individuals

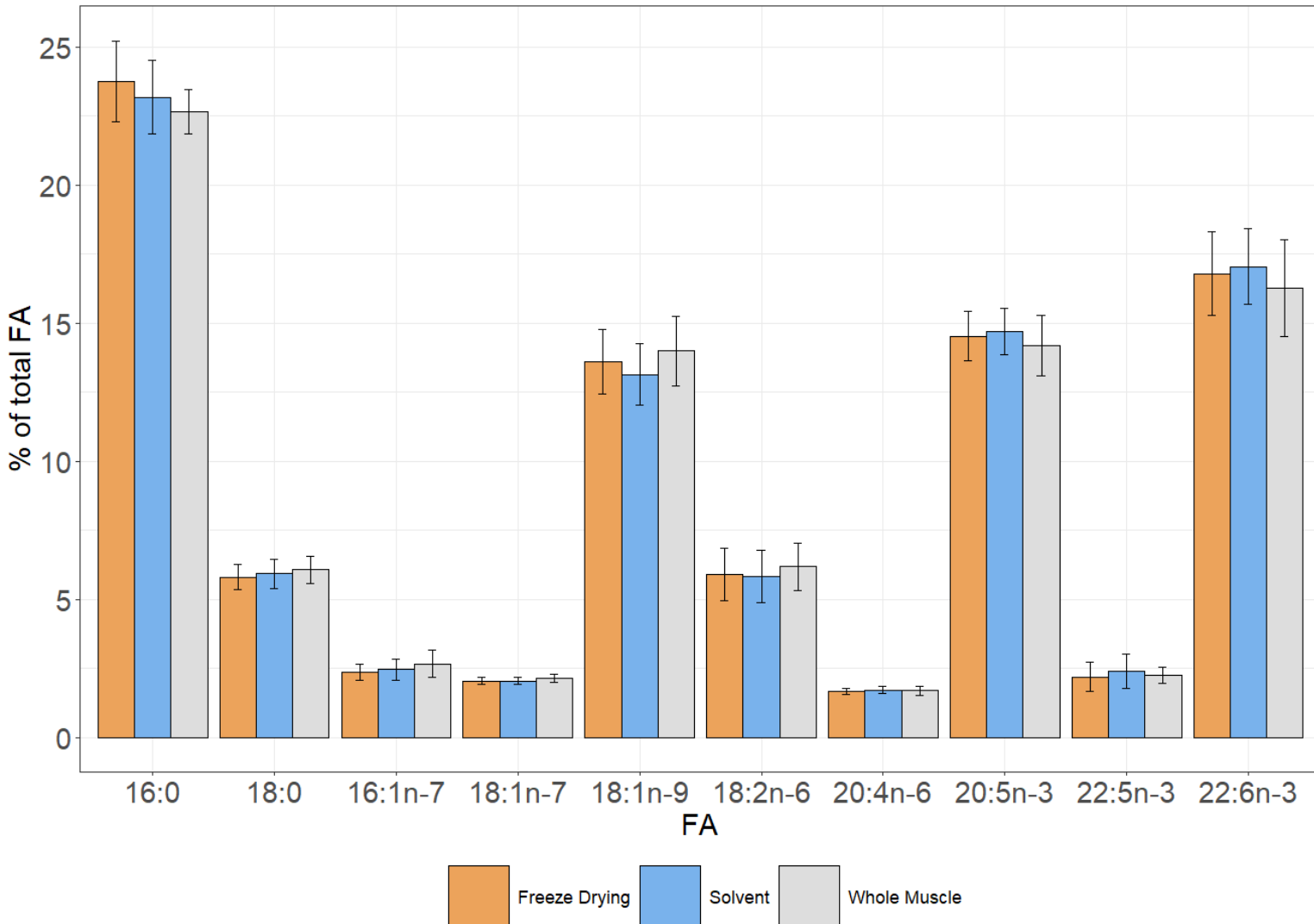
Lipid droplets under the skin ?

Sampling of skin ?



Questionable method for
quantity analysis

Proportion of 10 major FA (% of total FA) in PL (all individuals)

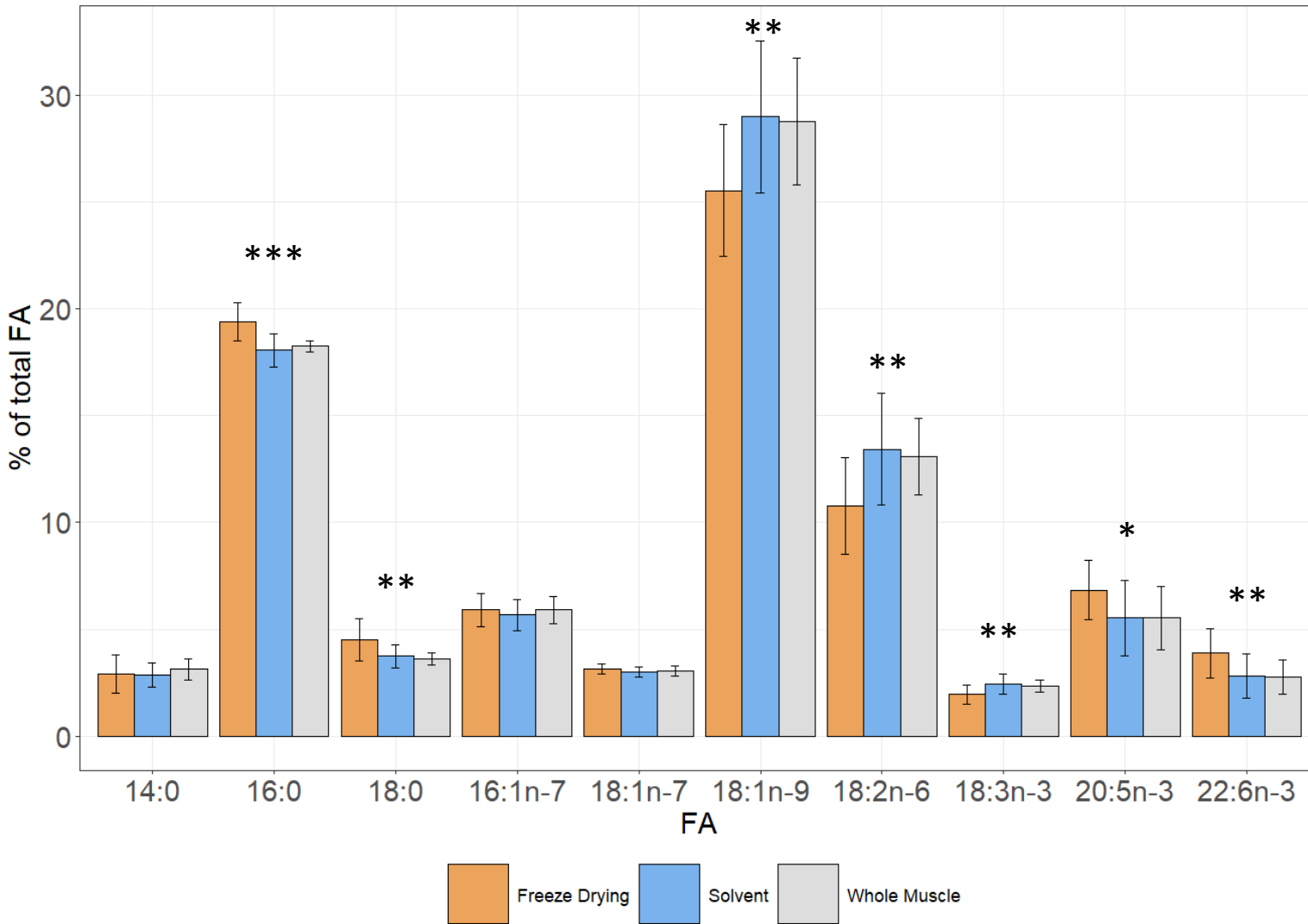


FA profiles in **PL** :

No significant differences in FA profiles depending on method = no aberrant values in profiles

-> **Need to check among the same individual**

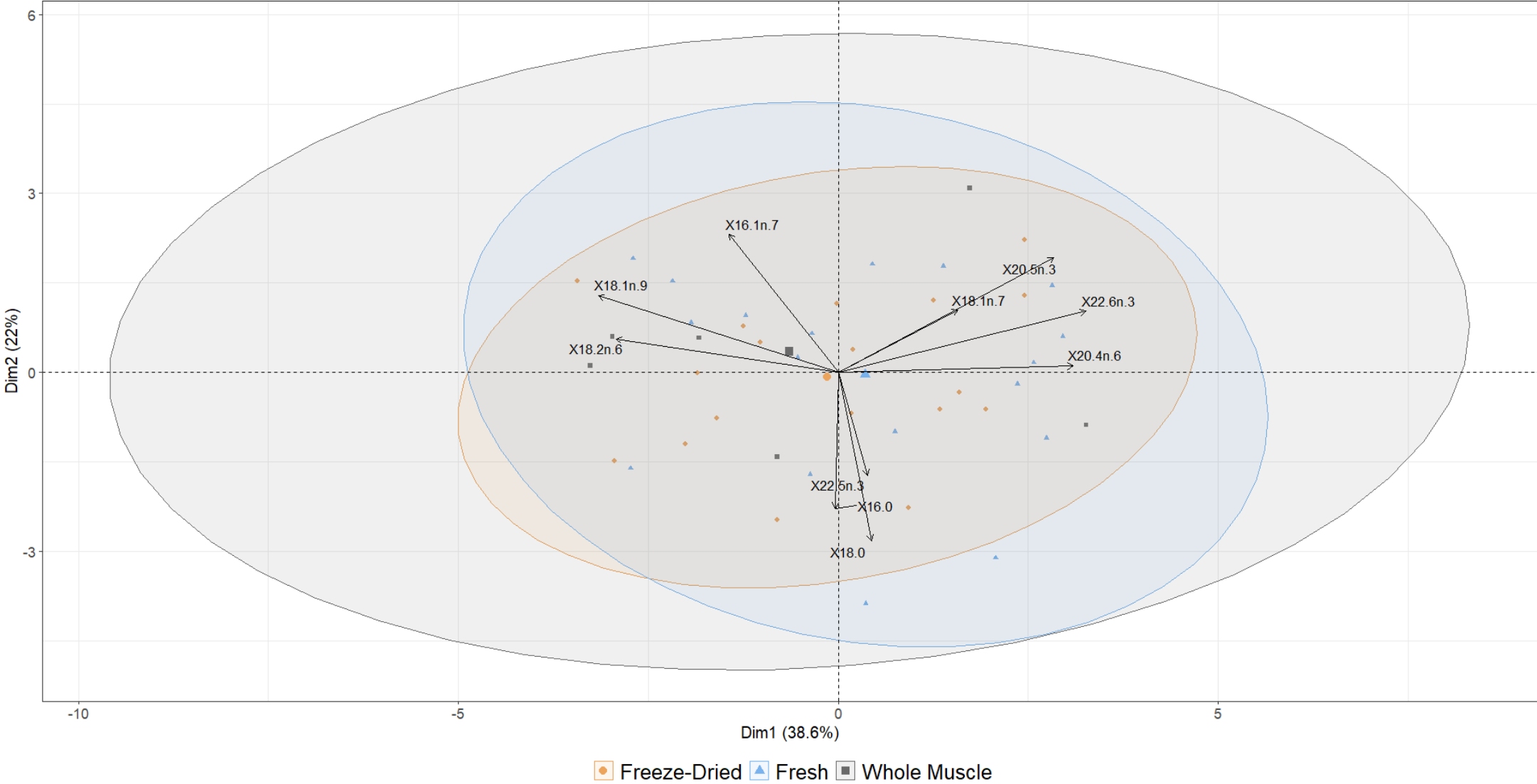
Proportion of 10 major FA (% of total FA) in NL (all individuals)



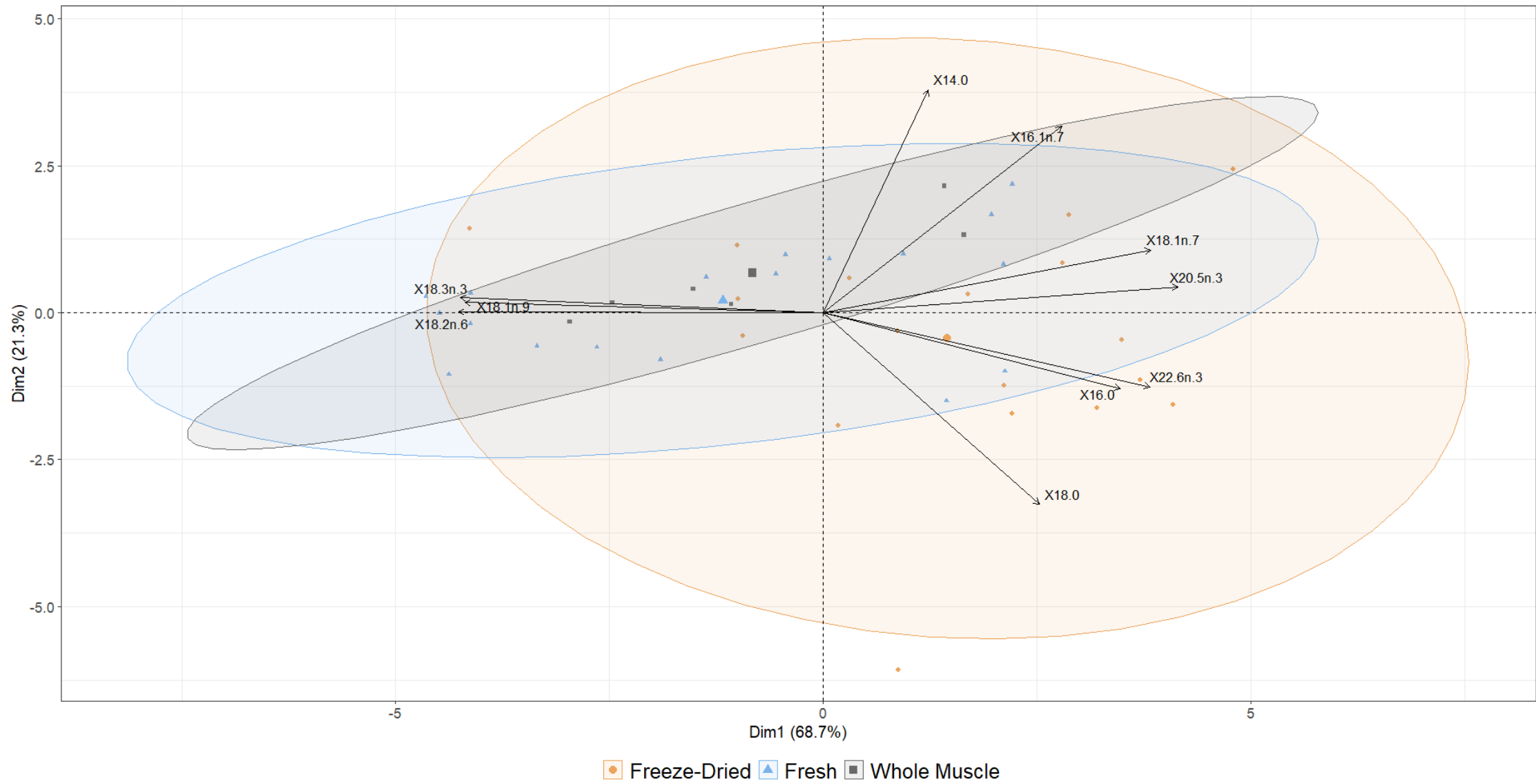
FA profiles in **NL** :

- Significant differences between methods in 7/10 major FA
- Freeze-Dried distinguish from 2 others
- Variability related to NL (reserve lipids)

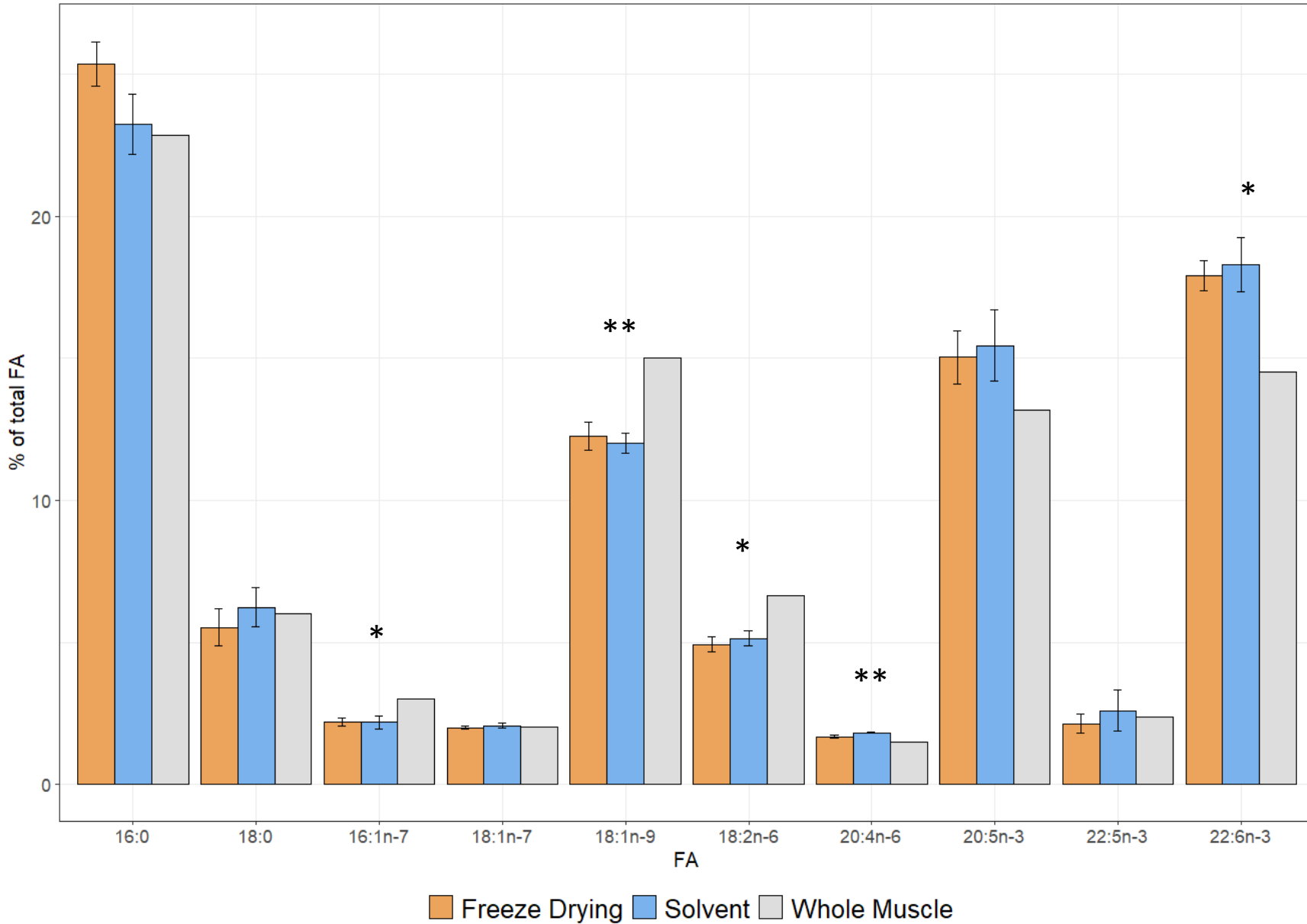
PCA – 10 major FA - PL



PCA – 10 major FA - NL



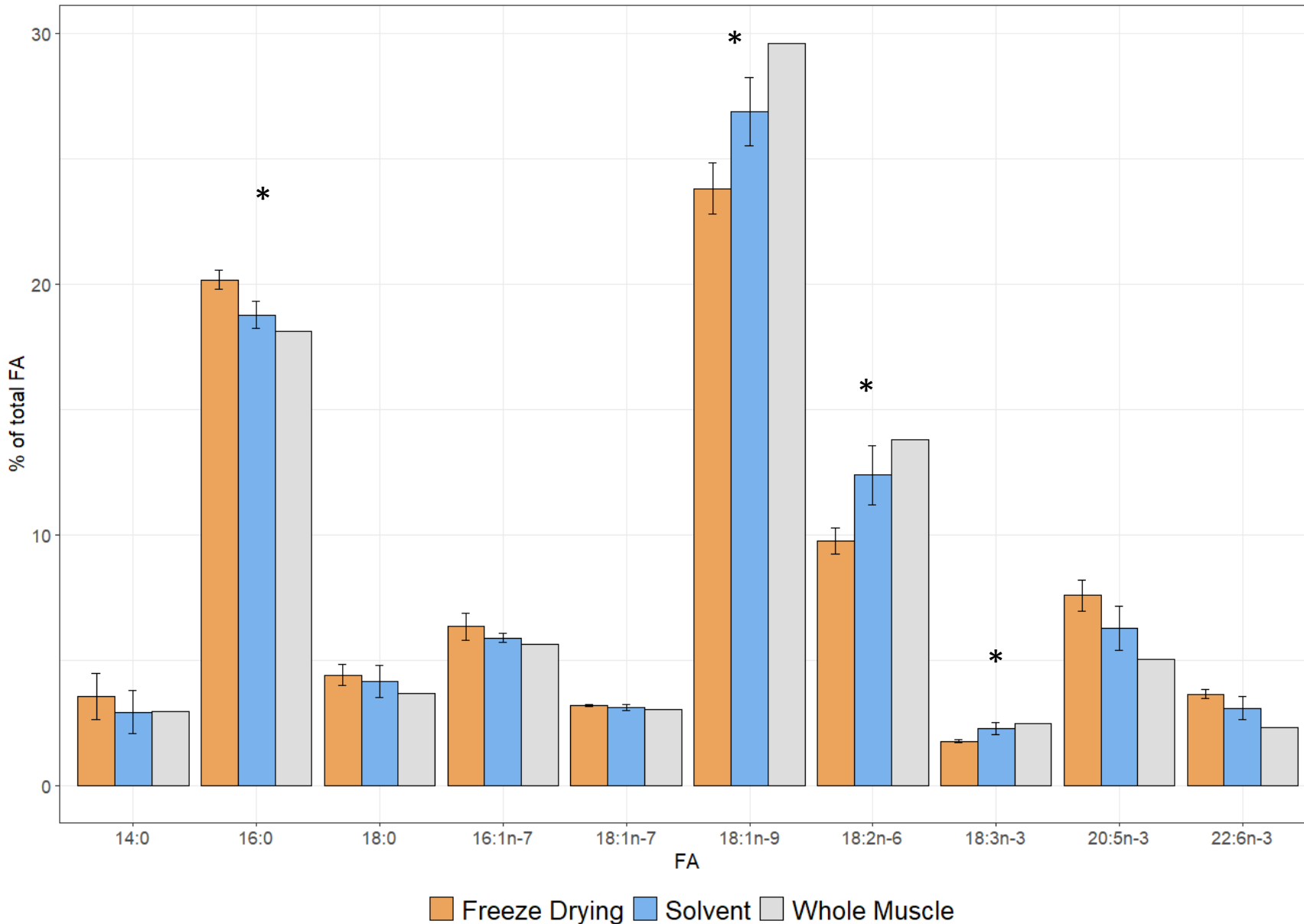
Proportion of 10 major FA in PL (% of total FA) - Fish 1



PL FA profile – Fish n°1 :

- 5/10 FA different between methods
- Low intra-individual variability
- Differences on major FA

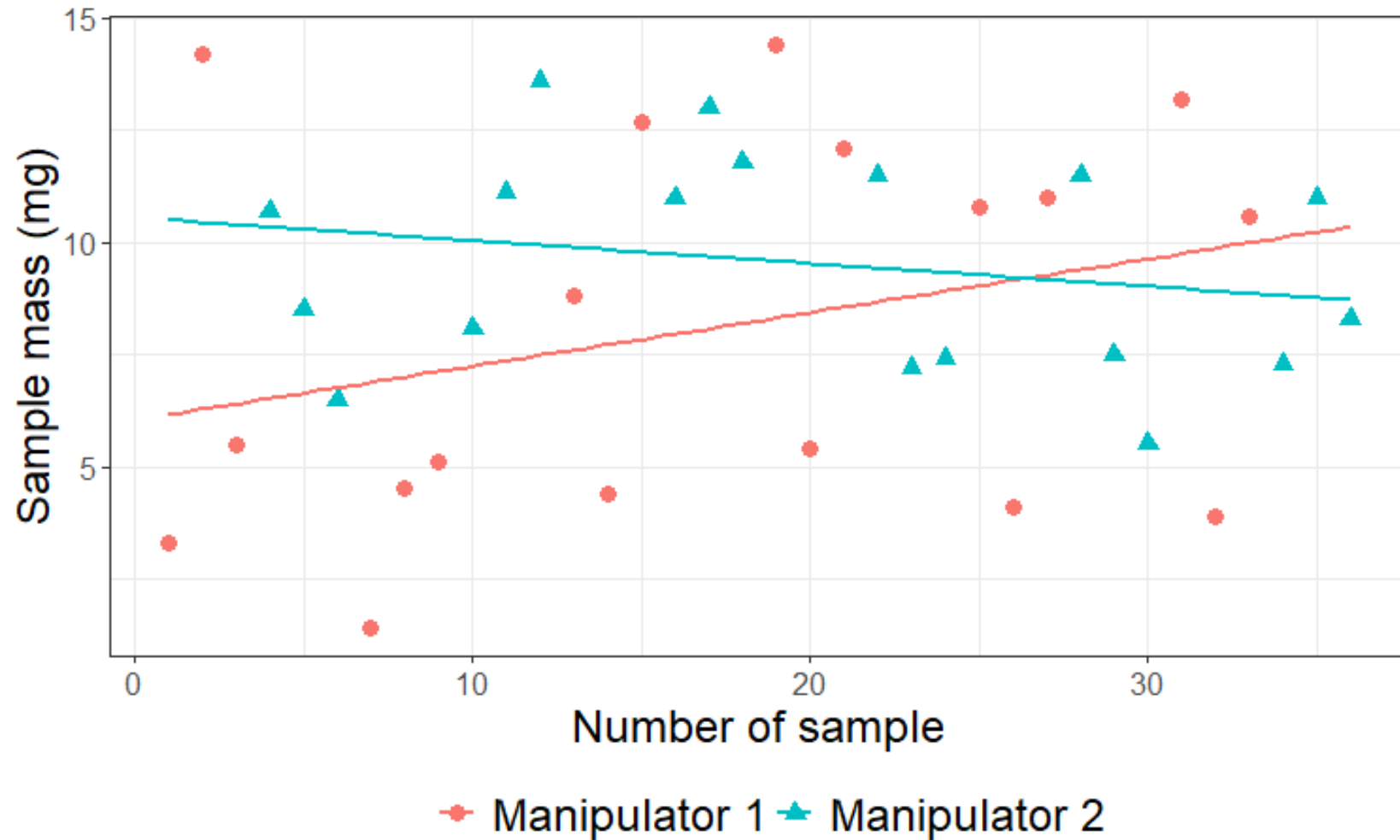
Proportion of 10 major FA in NL (% of total FA) - Fish 1



NL FA profile – Fish n°1 :

- 4/10 FA different between methods
- Low intra-individual variability
- Quantity variability does not affect proportion

Mass of biopsied tissue over time



- Mass doesn't seem to stabilize over time
- Very variable even for the same manipulator
- No pattern of improvement over time

General conclusion on the subcutaneous biopsy for FA analysis :

- The sample mass is always enough to obtain major FA profiles with a routine analysis but the mass is very hard to stabilize

General conclusion on the subcutaneous biopsy for FA analysis :

- The sample mass is always enough to obtain major FA profiles with a routine analysis but the mass is very hard to stabilize
- Need further investigation to evaluate the best processing method (but both work)

General conclusion on the subcutaneous biopsy for FA analysis :

- The sample mass is always enough to obtain major FA profiles with a routine analysis but the mass is very hard to stabilize
- Need further investigation to evaluate the best processing method (but both work)
- Quantity of extracted FA is **VERY** variable (especially NL) : Maybe because of lipid droplets in the sample, need to take a precious care while sampling.

General conclusion on the subcutaneous biopsy for FA analysis :

- The sample mass is always enough to obtain major FA profiles with a routine analysis but the mass is very hard to stabilize
- Need further investigation to evaluate the best processing method (but both work)
- Quantity of extracted FA is **VERY** variable (especially NL) : Maybe because of lipid droplets in the sample, need to take a precious care while sampling.
- FA profiles in proportion are less variable than quantity

General conclusion on the subcutaneous biopsy for FA analysis :

- The sample mass is always enough to obtain major FA profiles with a routine analysis but the mass is very hard to stabilize
- Need further investigation to evaluate the best processing method (but both work)
- Quantity of extracted FA is **VERY** variable (especially NL) : Maybe because of lipid droplets in the sample, need to take a precious care while sampling.
- FA profiles in proportion are less variable than quantity
- Real need to asses the effect of the biopsy on fish fitness and on **different species**

General conclusion on the subcutaneous biopsy for FA analysis :

- The sample mass is always enough to obtain major FA profiles with a routine analysis but the mass is very hard to stabilize
- Need further investigation to evaluate the best processing method (but both work)
- Quantity of extracted FA is **VERY** variable (especially NL) : Maybe because of lipid droplets in the sample, need to take a precious care while sampling.
- FA profiles in proportion are less variable than quantity
- Real need to asses the effect of the biopsy on fish fitness and on **different species**

It's a promising method for further application of studying FA profiles over time

