An attempt to use stable isotopes in clam spoilage assessment

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3 topics

Clams

Spoilage

Stable isotopes

topic #1

Clams…

…why did I chose clams to study spoilage?

One of the most popular and profitable molluscs exploited in rearing plots in the Mediterranean

Used as a food source for centuries

Let's make a **poll**:

Consumption of clams during a meal?

Let's make **another** poll:

Sick after a meal of clams?

Usually seafood and clam **intoxications** can have 3 origins:

Toxins DSP, PSP, NSP, ASP

Microorganisms *Clostridium, Listeria, Vibrio, Escherichia, Salmonella…*

Spoiled food…

Goal of this study…

…assess the potential use of carbon and nitrogen **stable isotopes** as spoilage indicators for clams (*Ruditapes decussatus*)

…in order to better understand and assess the progressive loss of freshness that occurs in all fresh (sea)foodstuffs

topic #2

Spoilage…

…how does seafood becomes spoiled?

Seafood tissue

rich in protein and non-protein N (e.g. amino acids, TMAO, creatinine)

low in carbohydrate resulting in a high post mortem pH (>6.0)

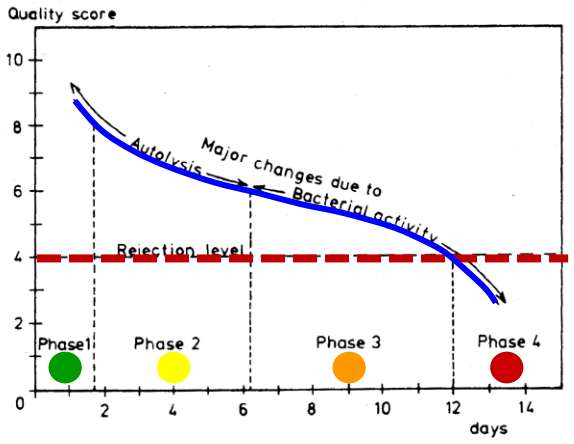
The condition named "**spoilage**" is not clearly defined in objective terms

…but is related with ***post-mortem*** conditions

Obvious signs of **spoilage** are:

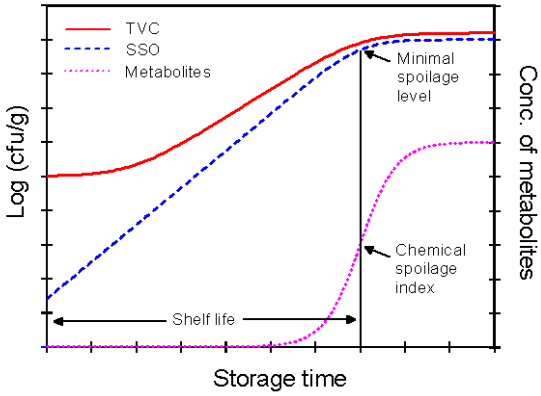
- detection of off-odours and off-flavours
- slime formation
- gas production
- discoloration
- changes in texture

Spoilage is a very **complex** process
[1] autolysis; [2] microbiota metabolism; [3] oxidative reactions



Huss, 1994

SSO (specific spoilage organisms) are the main responsible for spoilage characteristics



http://sssp.dtuaqua.dk/HTML_Pages/Help/English/MSM/MSM-intro/msm.htm

Besides **sensory analysis**… spoilage can be assessed through:

chemical analysis of specific compounds (*e.g.* TVB-N)

determination of **microorganisms** (*e.g.* SSO)

Although these indicators support

public health decisions

regarding the consumption of seafood…

…they usually **lack precision** in defining spoilage pathways

Critical issues on seafood spoilage:

chemical indicators of spoilage can be originated by autolytic processes or by microbiota metabolism

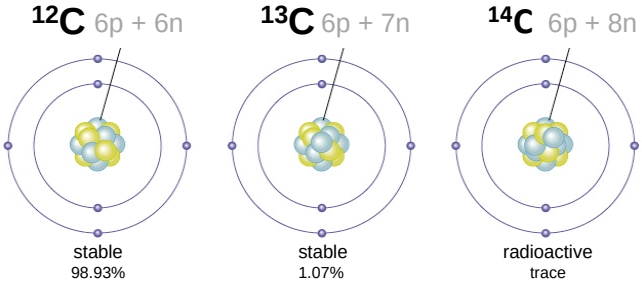
microbiota presence doesn't always mean that spoilage is occurring

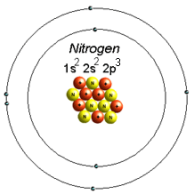
topic #3

Stable isotopes…

…what is the relation between spoilage and stable isotopes?

Stable isotopes are…





7p + 7n 7p + 8n

^{14}N	^{15}N
14.00307	15.0001
99.63%	0.37%
Stable	Stable

Stable isotopes are **traditionally** used in…

Geochemistry

chemical processes controlling the lithosphere, hydrosphere and atmosphere

Ecology and environmental sciences

flow of organic matter in food webs

Recent approaches use stable isotope analysis in…

Archaeology

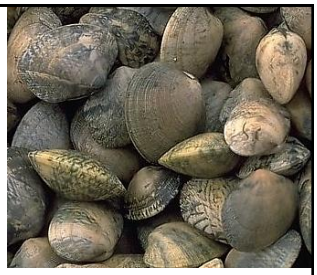
human dietary preferences in the past

Food sciences

food products authentication, traceability and geographic origin…

…never used as a seafood spoilage tracer

Harvested clams (n=136)
were conserved at 5 ºC



Sampling: d0, d8, d10 and d12
(34 clams/sampling moment)

Parameters:

- moisture
- condition index + percentage edibility
- TVB-N + pH
- nitrogen & carbon percentage
- nitrogen & carbon stable isotopes

Nitrogen and carbon isotopes were determine by an **thermal mass spectrometer**

Stable isotopes abundance is expressed in **‰**

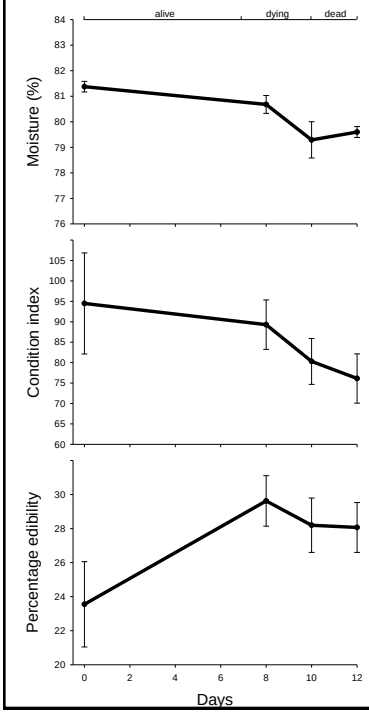
$$\delta^{n}X = ([R_{sample}/R_{standard}] - 1) \times 10^{3}$$

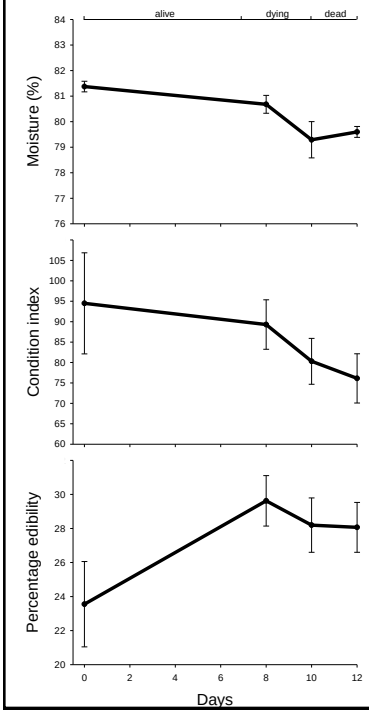
X = N or C
n = atomic mass of heaviest isotope
$R = {}^{15}N:{}^{14}N$ or $R = {}^{13}C:{}^{12}C$

$\delta^{15}N$ standard = N_2 atm
$\delta^{13}C$ standard = Belemnitella Americana (V-PDB)

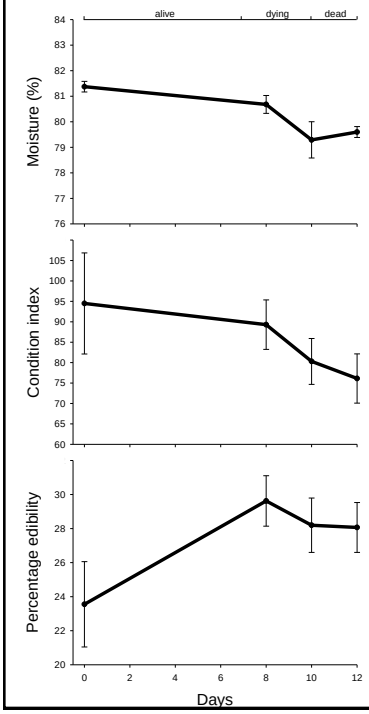
Stable isotope analysis main assumption:

clam tissues (edible part) are composed by different isotopes from the same element, which are **differentially discriminated** during chemical reactions



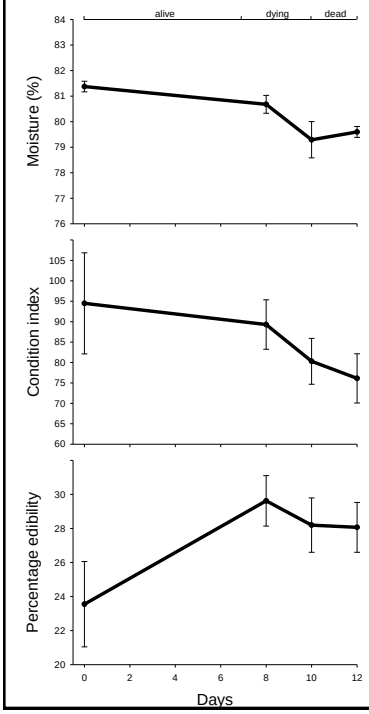


Moisture ↓ caused by dehydration



Moisture ↓ caused by dehydration

Condition index ↓ caused by dehydration, starvation and spoilage



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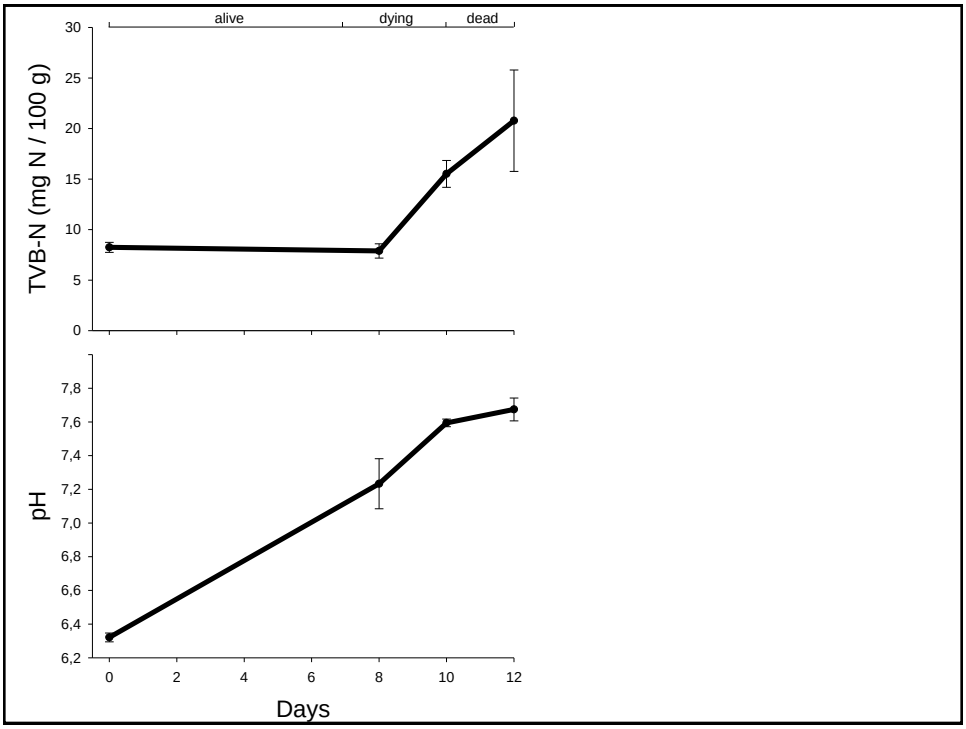
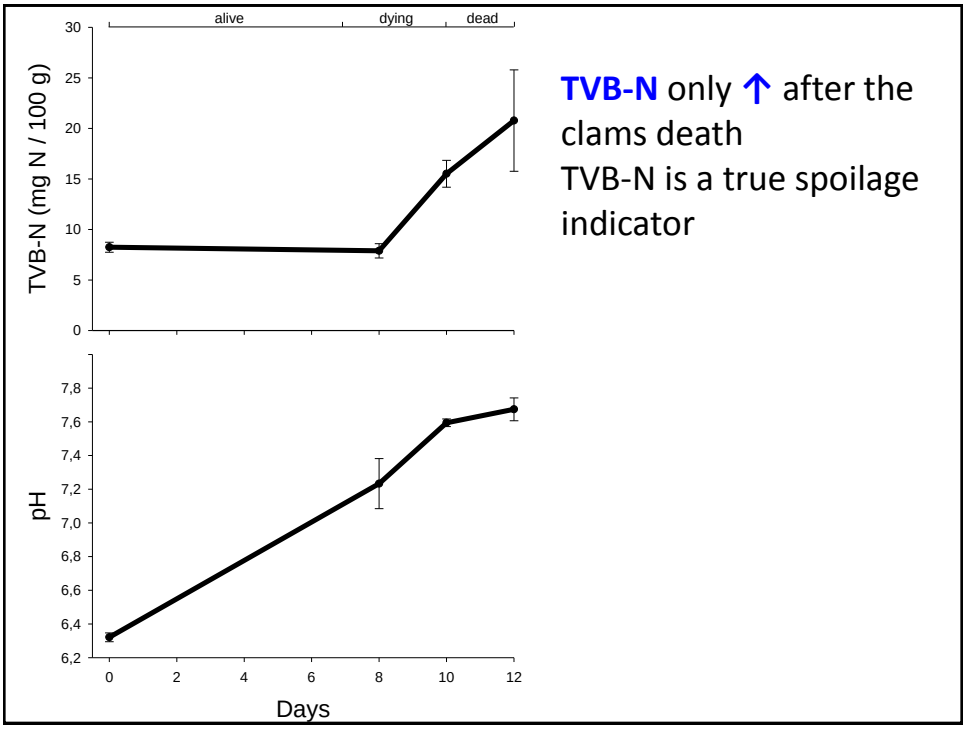
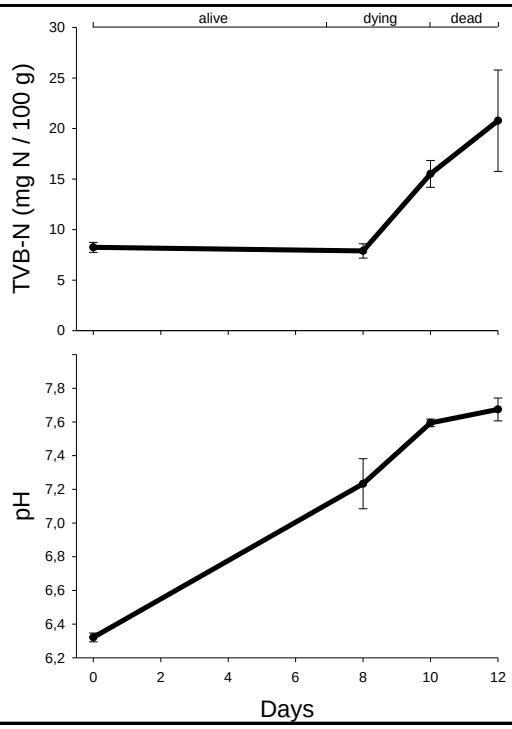
Percentage edibility is a **bad index** because is influenced by the loss of intravalvar water





TVB-N only ↑ after the clams death

TVB-N is a true spoilage indicator



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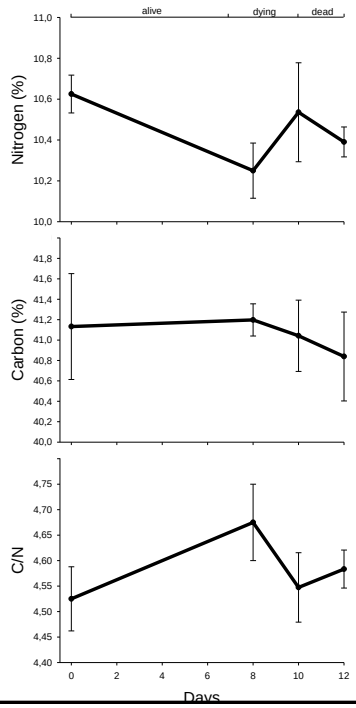
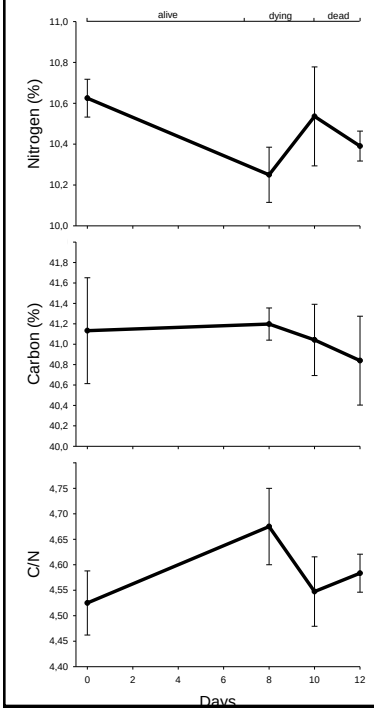
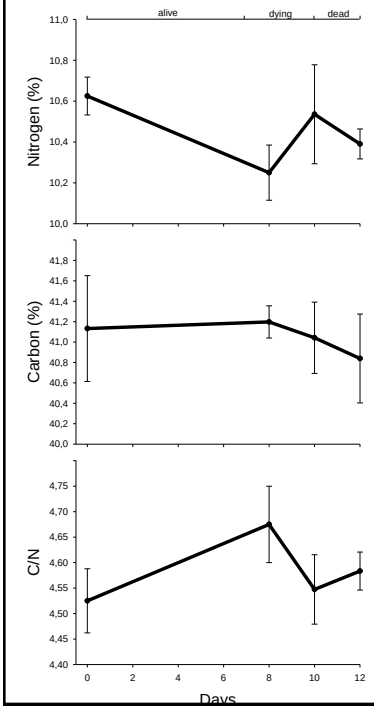
pH ↑ is caused by the formation of basic compounds (TVB-N) related to starvation and spoilage





Nitrogen ↓ d0 to d8 caused by starvation; **Nitrogen** ↑ d8 to d10 caused by microbiota colonization
Nitrogen minor ↓ d10 to d12 caused by equilibrium between microbiota and TVB-N



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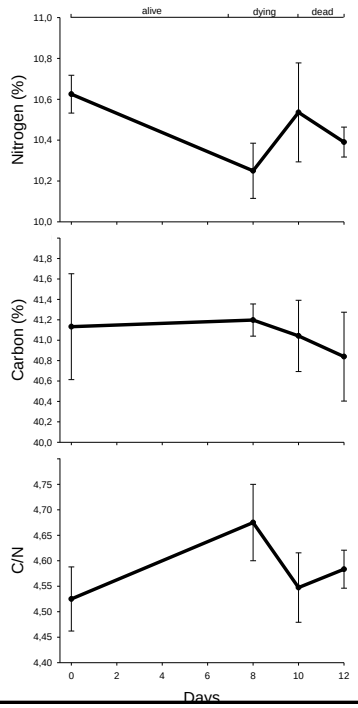
Carbon only ↓ slightly after clams death caused probably by decarboxylation reactions



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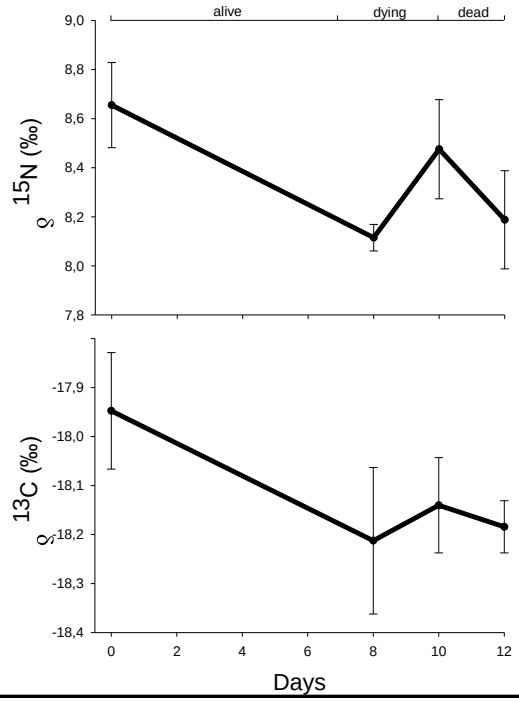
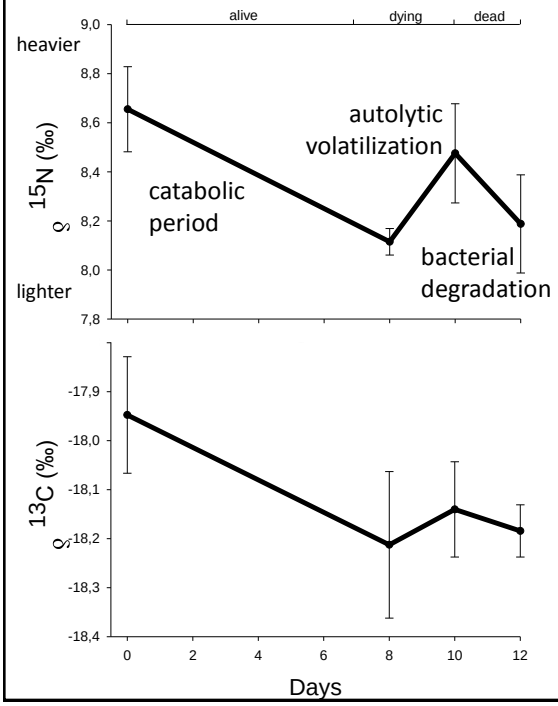
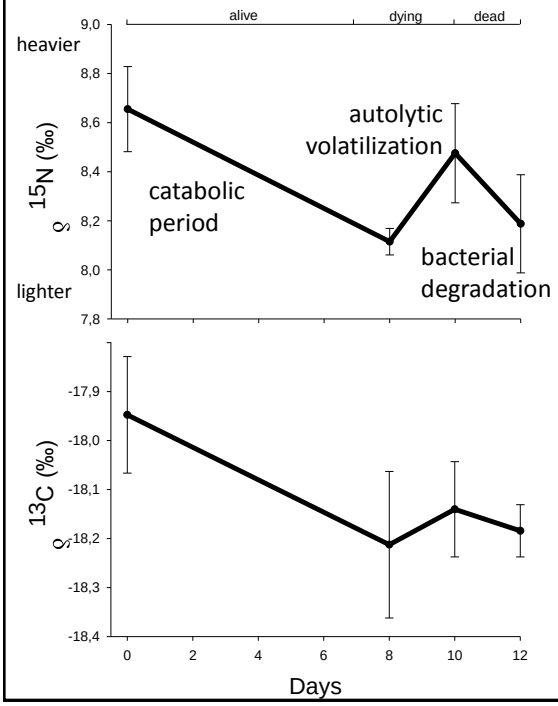
C/N reflects nitrogen variations





$\delta^{15}N$ similar to **nitrogen**



$\delta^{15}N$ similar to **nitrogen**

$\delta^{13}C$ no significant changes during the experiment

Final considerations…

…clams did not dye all in the same day, introducing a temporal error in all parameters…

…TVB-N, pH and condition index reflect spoilage conditions

Final considerations…

…only nitrogen stable isotopes show relation to spoilage phases…

…carbon is not conclusive…

Final considerations…

…sulfur stable isotopes might be interesting regarding spoilage cause by bacteria that use sulfur and sulfate as electron acceptors

Thank YOU!

… and I would like to **acknowledge**! *funding and support from:*

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