Molecular characterization of the diet of the planktonic community in Málaga Bay (NW Alboran Sea)

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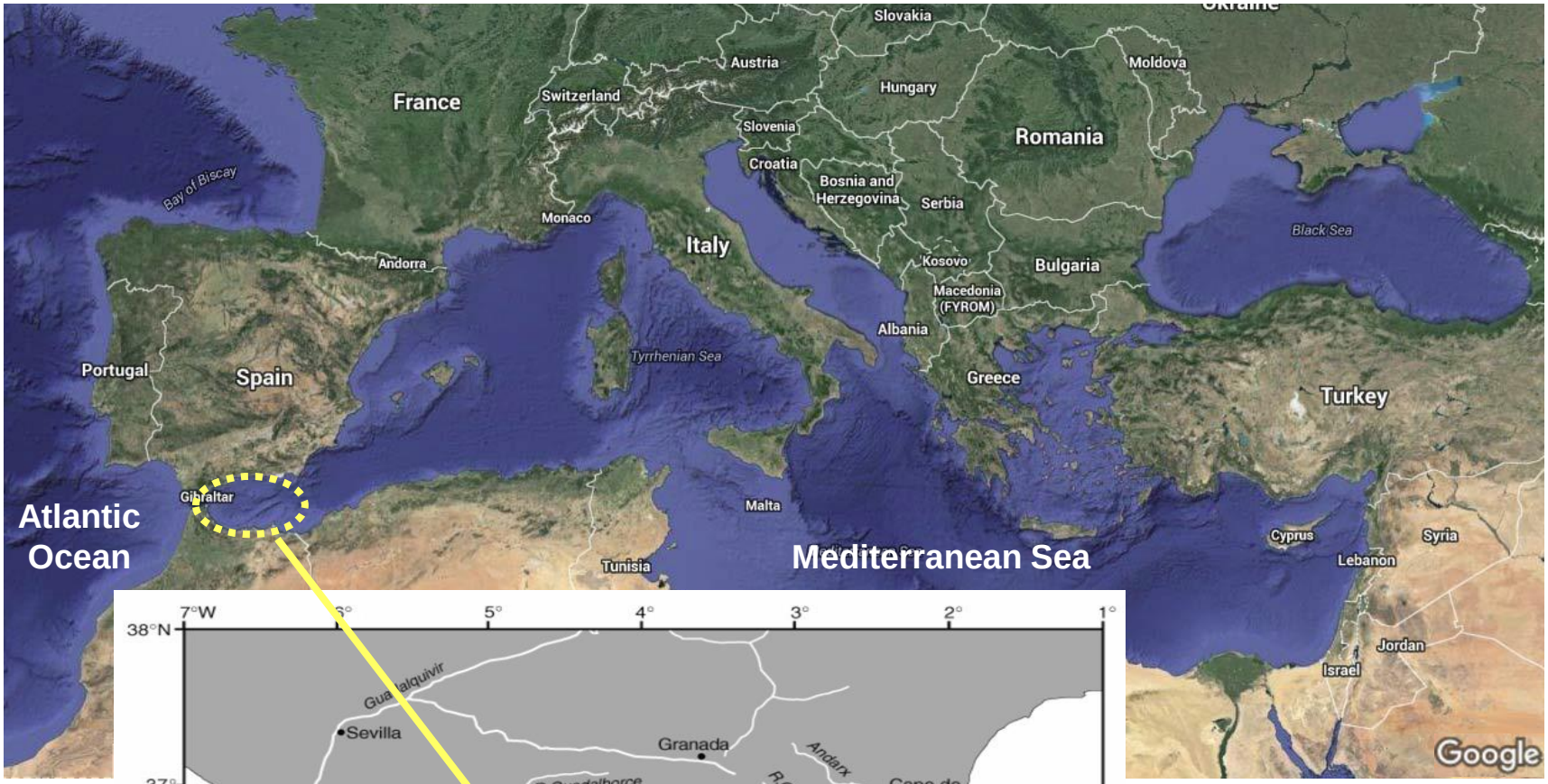
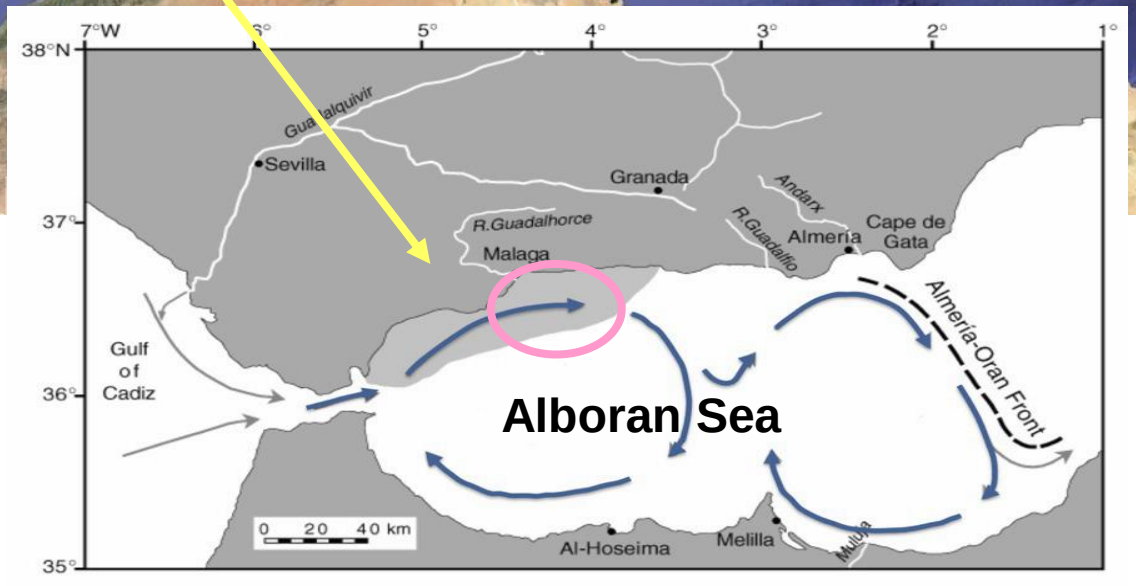
Invertimos en la pesca sostenible

Study area: Málaga Bay





Alboran Sea surface circulation

- **High plankton production**
- **Sardine and anchovy nursery**
- **Autumn season**

Objective

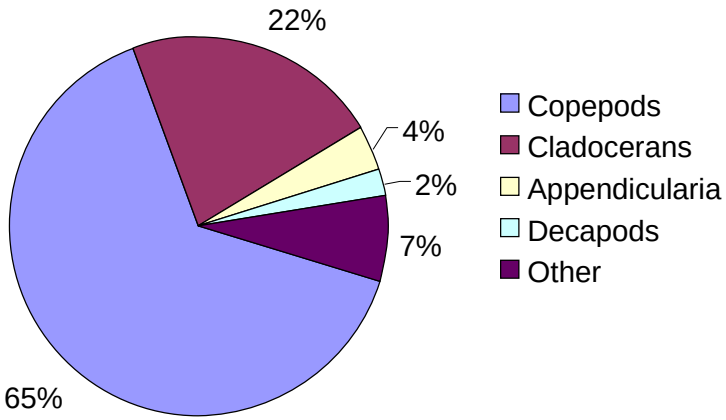
To characterise the diet of key plankton groups in the field

Specific questions

- Which are the most common coastal planktonic groups in autumn?
- What do they eat?

Most common planktonic taxa in autumn

Mesozooplankton



Group	Species	Relative abundance
Copepods	*Acartia clausi* *Paracalanus parvus/indicus* *Temora stylifera* *Oncaea spp./waldemari* *Clausocalanus parapergens/jobei* *Pseudocalanus elongatus*	up to 43% up to 18% up to 16% up to 40% up to 13% up to 8%
Cladocerans	*Penilia avirostris* *Podon sp.*	up to 33% up to 4%

Ichthyoplankton

Species	Relative abundance
Sardina pilchardus	48.9%

(García Jiménez *et al*. 2015)

Microplankton

Group	Taxa	Relative abundance
Diatoms	*Leptocylindrus danicus* *Leptocylindrus sp.* *Pseudonitzschia sp.*	4-65%
Dinoflagellates	Dinoflagellates <20 um *Gyrodinium sp.*	5-21%
Flagellates	Criptophytes Chlorophytes	62-90%
Microzooplankton	*Mesodinium rubrum* *Strombidium sp.*	<10%

(Yebra *et al*. **Poster ID-64, Session 4**)

Morphological/molecular identification of the community composition

What do they eat…

Difficulties:

- Microscopic analyses are not suitable for all taxa
- Most larvae vomit or defecate when collected
- High-throughput sequencing not always applicable

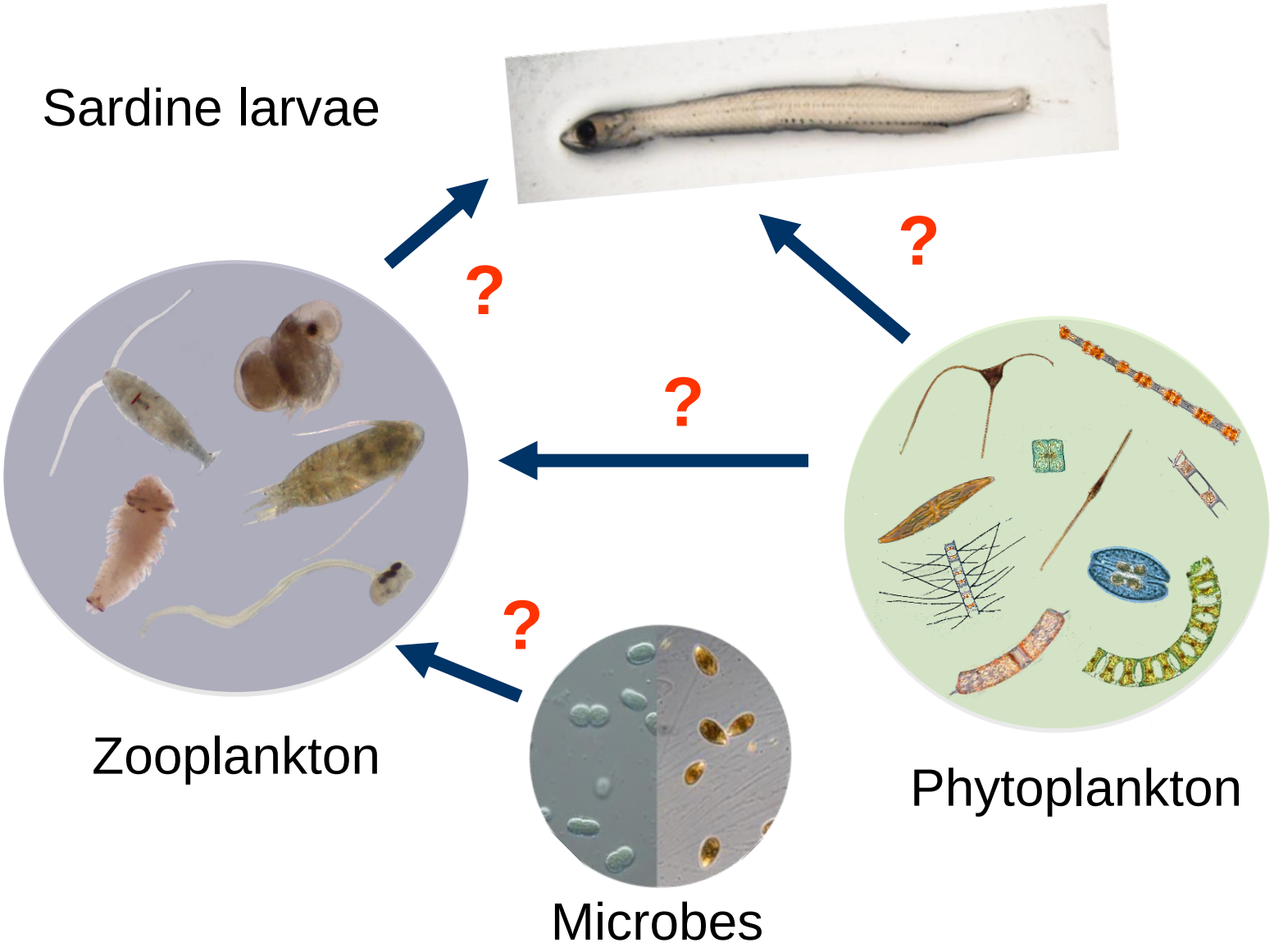
What do they eat…

Task 1- Identify potential preys

Task 2- Design multiplex PCR assays based on species specific custom design primers

Task 3- Run molecular analysis on predators to search for specific preys in their guts

Task 1- Potential preys identification



Task 1- Potential preys identification

Sardine larvae

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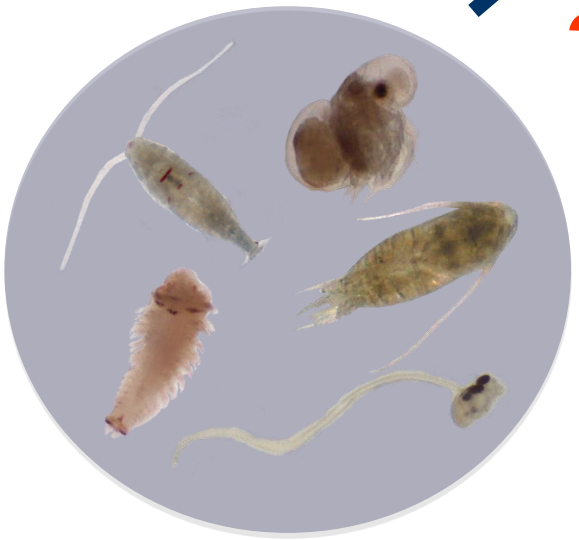


Zooplankton

Group	Species	Relative abundance
Copepods	***Acartia clausi***	up to 43%
	Paracalanus indicus	up to 18%
	Temora stylifera	up to 16%
	Oncaea waldemari	up to 40%
	Clausocalanus parapergens	up to 13%
	Pseudocalanus elongatus	up to 8%
Cladocerans	*Penilia avirostris*	up to 33%
	Podon sp.	up to 4%

Based on morphological and molecular identification

Task 2- Multiplex PCR design based on species specific custom designed primers

Species specific primers designed to run multiplex PCR analyses:

- Acartia & Temora: from published sequences (Genbank)
- Clausocalanus, Oncaea & Paracalanus: from local sequences obtained

To identify digested preys:

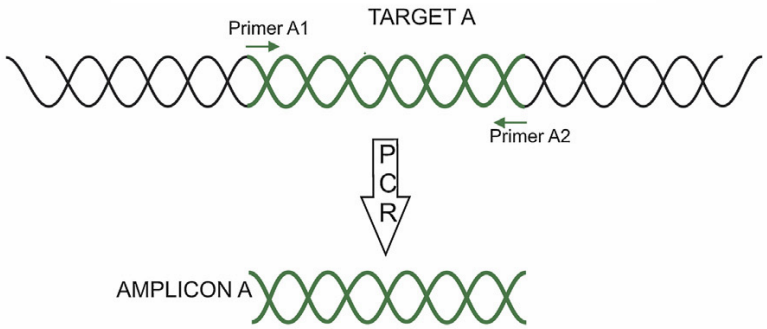
- Primers designed to amplify **short** specific regions of the COI mtDNA (between 100 and 200 bp)

To maximize sample size:

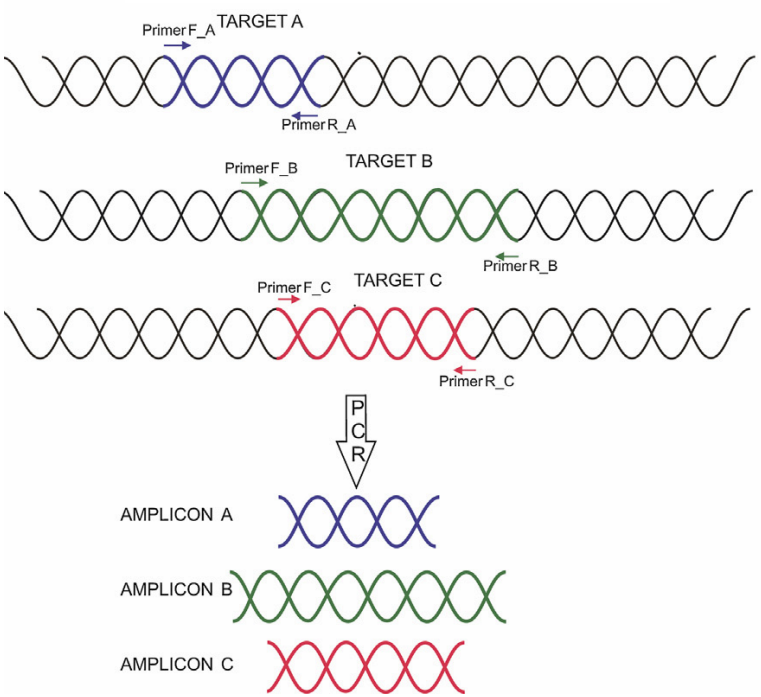
- Primers designed to produce amplicons of **different lengths**, so they will appear as different peaks in an electropherogram, and a **multiplex PCR assay** was designed
- Lab-on-a-chip (LOC) technology was applied: higher sensitivity (0.01 ng/µl) and resolution (± 5 bp) than agarose gels

Single versus Multiplex PCR assay

Single template PCR



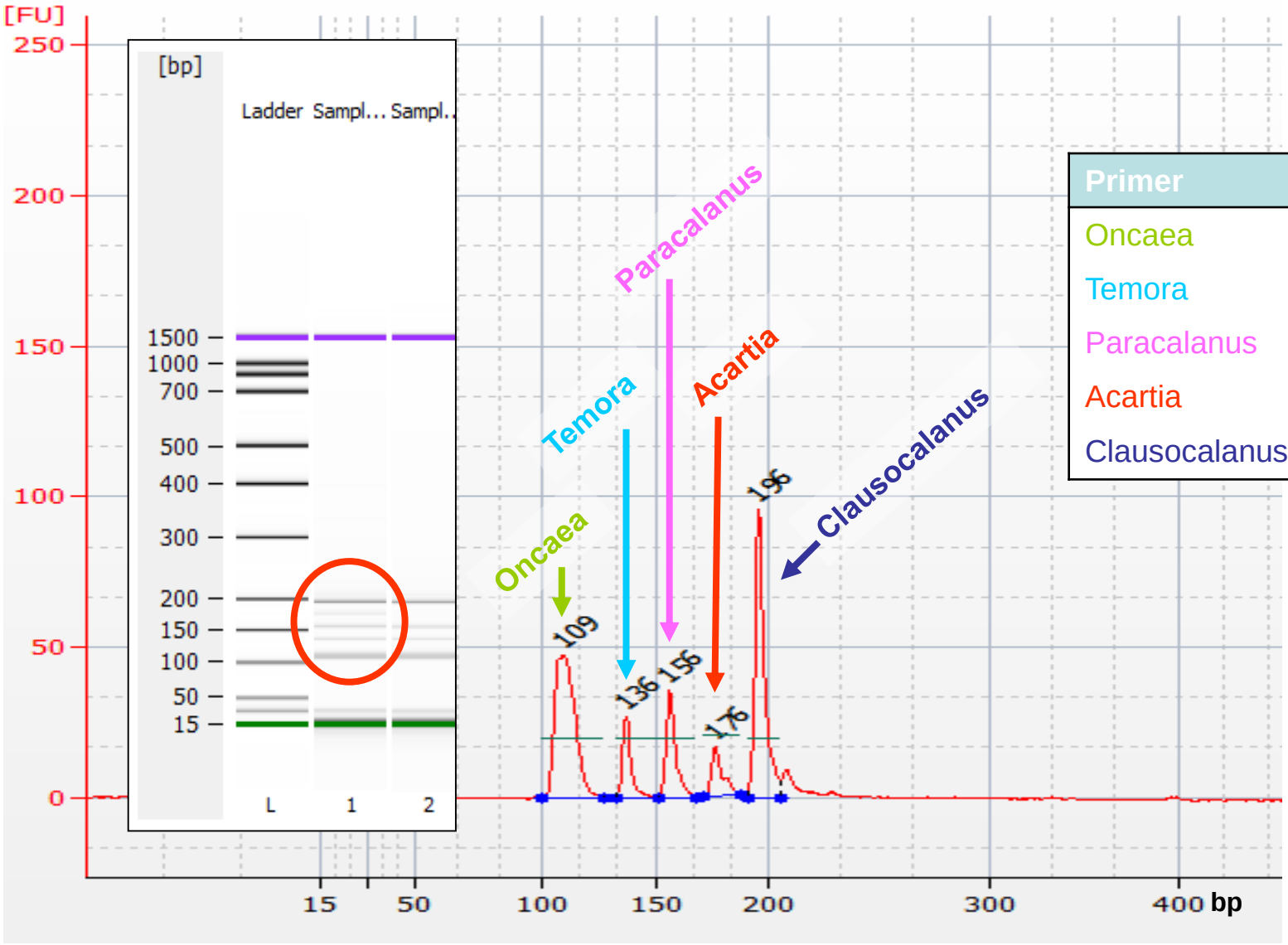
Multiplex PCR



Types of polymerase chain reactions

Kalle *et al*. (2014)

Copepod mix electropherogram

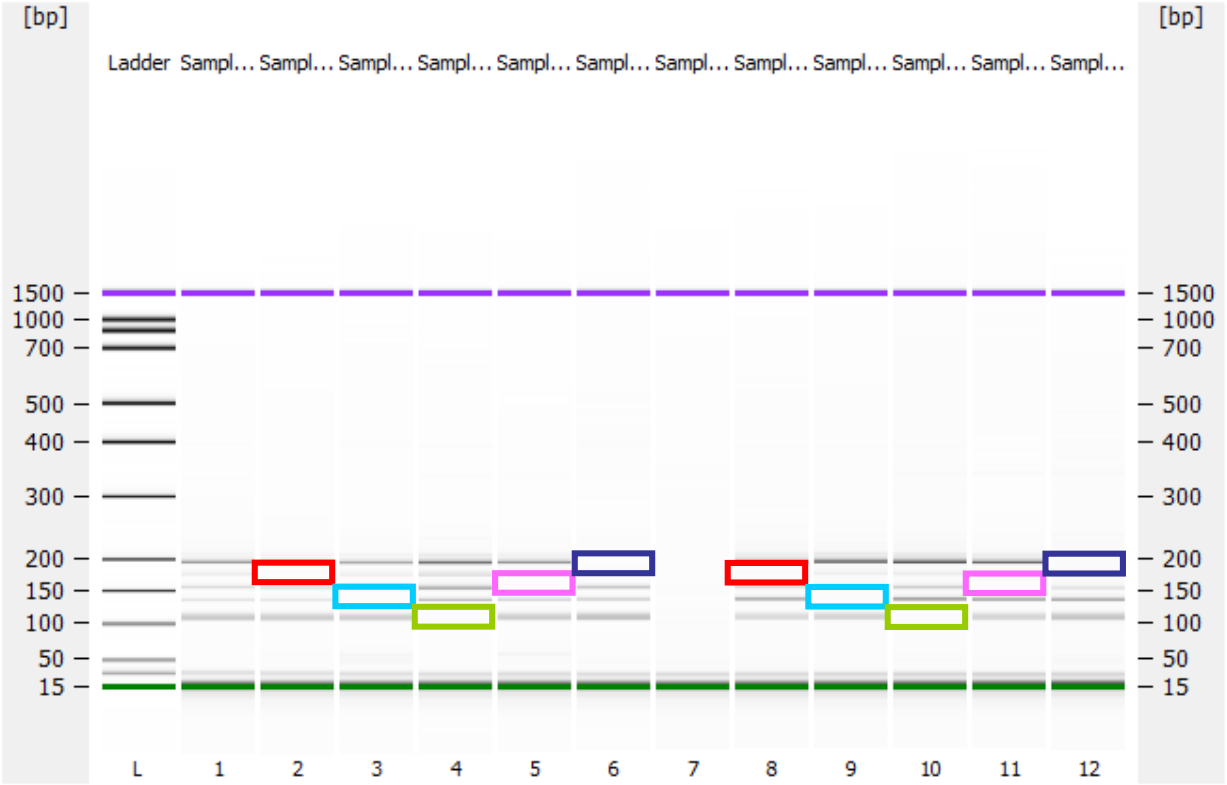


Primer	bp
Oncaea	108
Temora	136
Paracalanus	156
Acartia	175
Clausocalanus	196

Multiplex reaction mix optimisation

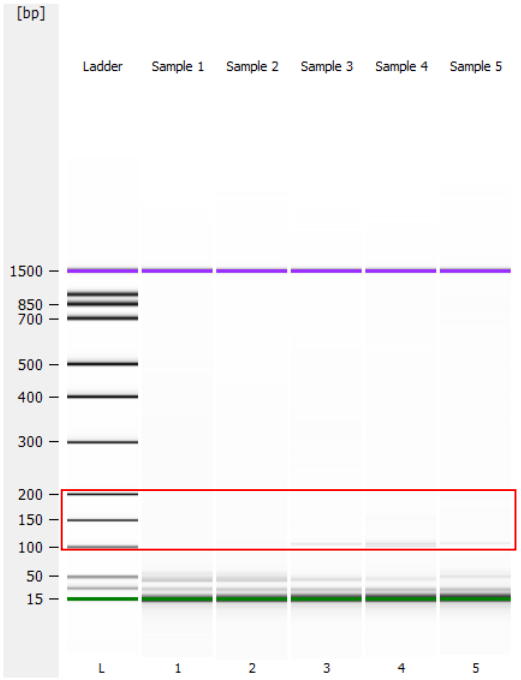
COP MIX	
Acartia	0.24 ng/µl
Temora	0.23 ng/µl
Oncaea	0.27 ng/µl
Paracalanus	0.27 ng/µl
Clausocalanus	0.20 ng/µl

PRIMER MIX	8 µl A, O, P / 1 µl T, C

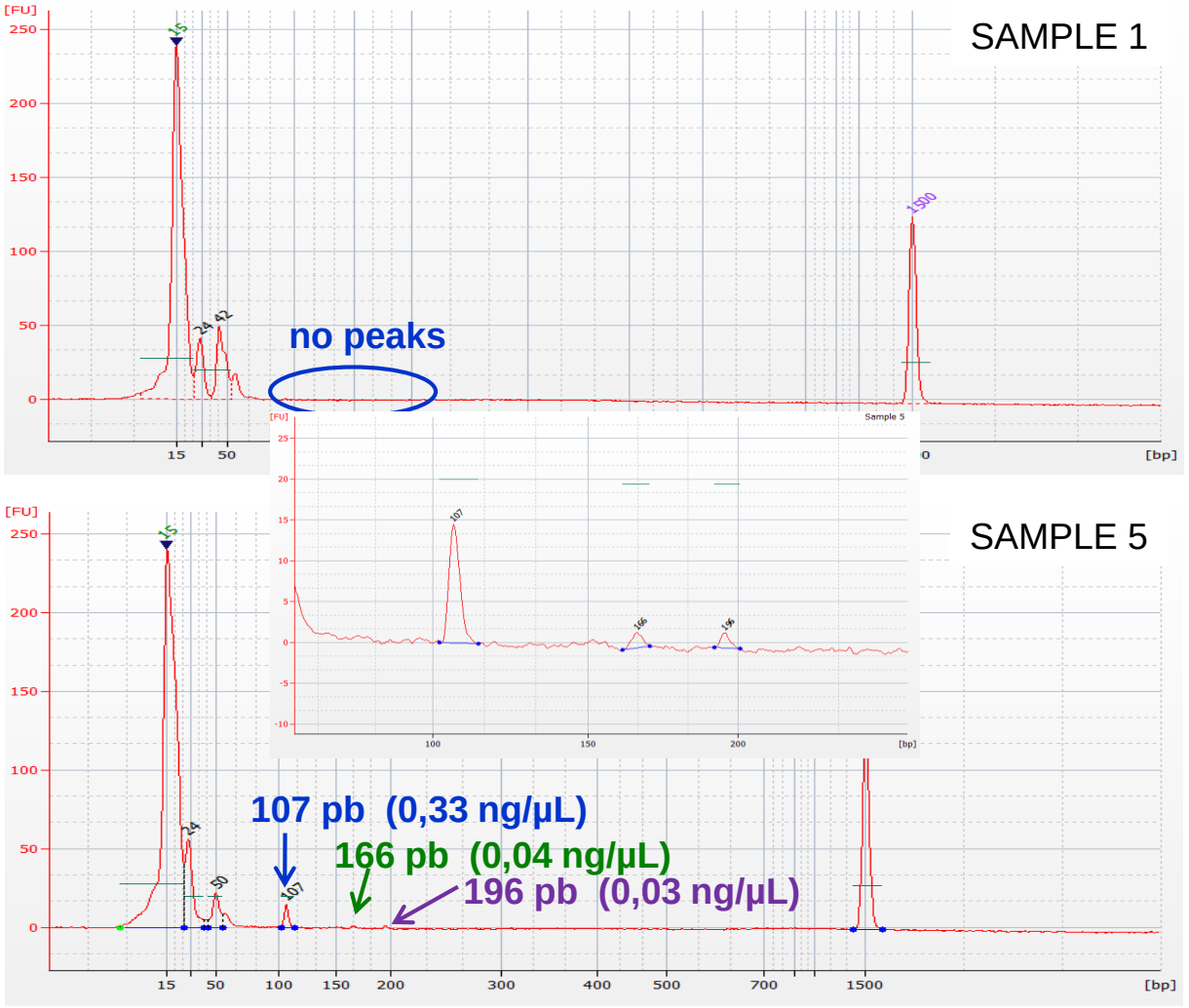


	DNA (0.5µl)
1	COP MIX
2	COP MIX - ACARTIA
3	COP MIX - TEMORA
4	COP MIX - ONCAEA
5	COP MIX - PARACALANUS
6	COP MIX - CLAUSOCALANUS
7	Water
8	COP MIX – ACARTIA + SARDINE 0.5µl
9	COP MIX - TEMORA+ SARDINE 0.5µl
10	COP MIX - ONCAEA+ SARDINE 0.5µl
11	COP MIX - PARACALANUS+ SARDINE 0.5µl
12	COP MIX - CLAUSOCALANUS+ SARDINE 0.5µl

Task 3- Multiplex PCR analysis of predators to search for specific preys in their guts

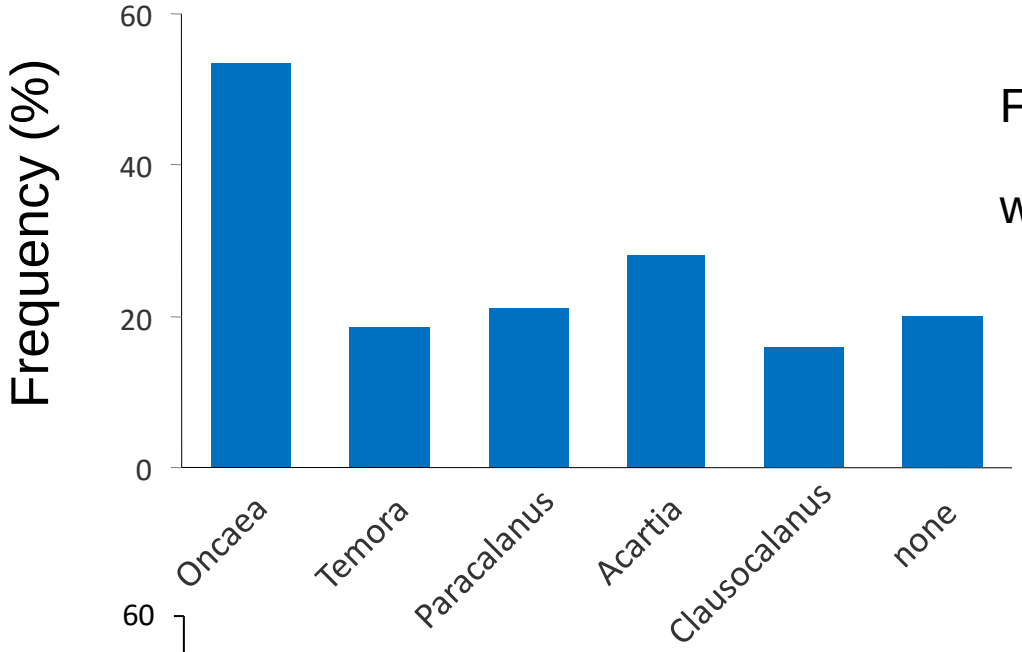


Lane	Sample	Total DNA
L	DNA marker	
1	Poolsard1	58,7 ng
2	Poolsard1	176,1 ng
3	Poolsard1	293,5 ng
4	Poolsard1	352,2 ng
5	Poolsard1	469,6 ng

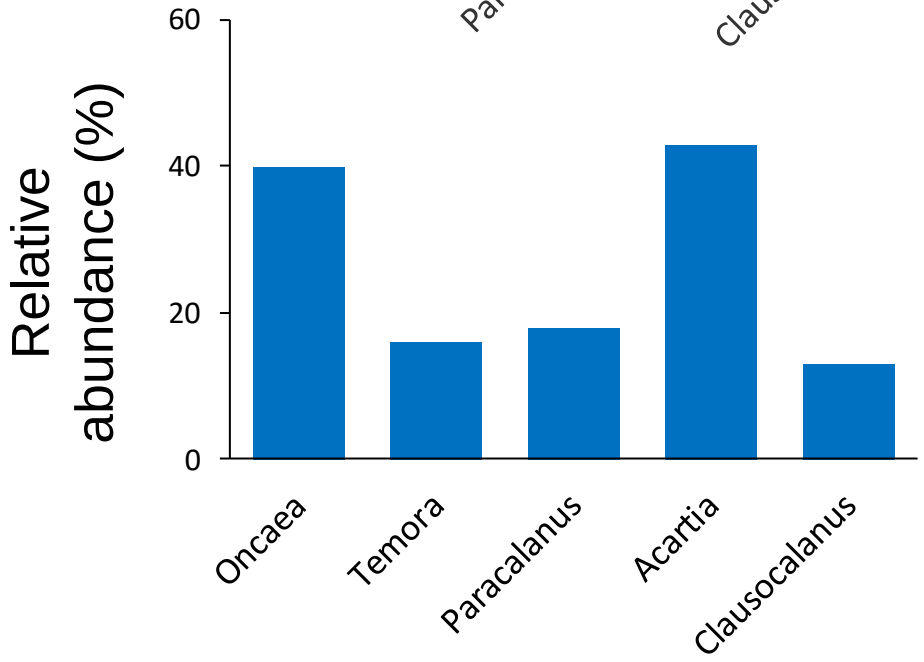


Electropherograms (DNA from *Sardina pilchardus* larvae guts)

Results



Frequency of prey DNA detected within sardine larvae guts



Autumn relative copepod abundance in Málaga Bay

Summary

- Most common plankton groups in autumn were sardine larvae, copepods and flagellates.
- We designed a multiplex PCR assay targeting potential sardine larvae preys.
- We successfully tested the presence/absence of some potential preys within sardine larvae guts.
- Sardine larvae preyed on the most abundant copepods present in the field.

Work in progress:

- Design of further assays to detect microplanktonic potential preys.

Acknowledgements

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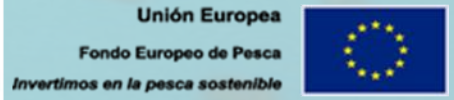

Thank you