

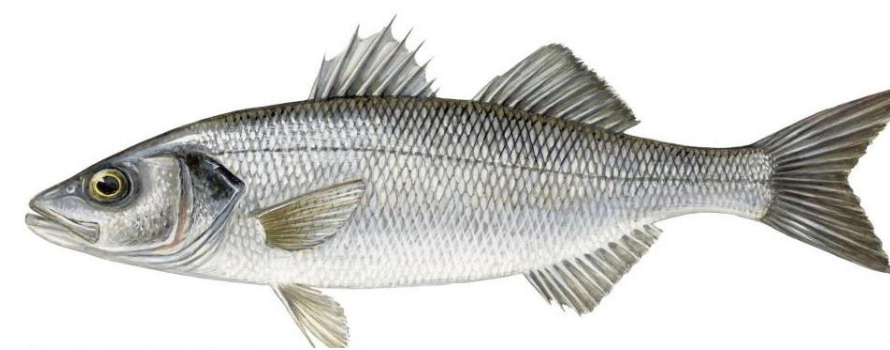
INTRODUCTION

Infectious diseases in Aquaculture

(caused by bacteria, viruses, fungi, and parasites)

leads to

Fish health ↓
 Growth rate ↓
 Reproduction ↓
 Fish mortalities ↑
 Economic losses ↑



Dicentrarchus labrax (European seabass)

- ✓ Widely distributed
- ✓ Great importance for Mediterranean aquaculture
- ✓ Production tripled between 2005 and 2019 [1]

INTERNSHIP GOAL

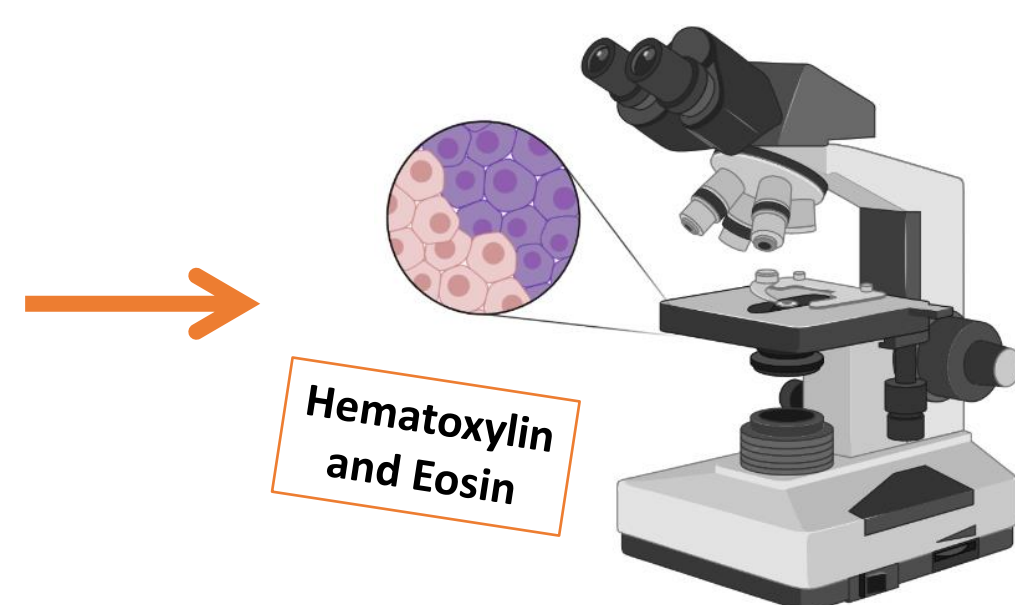
- Obtain skills related with **the diagnosis of unknown pathologies** in marine fish
- Acquire expertise in **histological and molecular biology techniques** (2 different approaches)

METHODOLOGY

- ✓ Samples from European seabass, with an **unknown pathology**, were collected for **analysis**

Histological approach

- Samples fixed in **buffered formalin** (24h) and stored in **ethanol 70%**
- **Standard histological tissue processing and staining**



Molecular approach

- To discriminate the possible **presence of several infectious organisms**, a DGGE (Denaturing gradient gel electrophoresis) [4] approach was applied:

- 1) DNA was extracted from samples, with a commercial kit
- 2) **Touchdown PCR** (bacterial 16S rRNA and eucaryotic 18S rRNA gene fragments amplified)
- 3) PCR products were resolved by **DGGE electrophoresis**
- 4) **Selected bands were sequenced and phylogenetically analysed** to identify the **closest known species**

RESULTS & DISCUSSION

- ✓ **Histological analyses** revealed the presence of **granulomas** in the **digestive tract** and **liver**

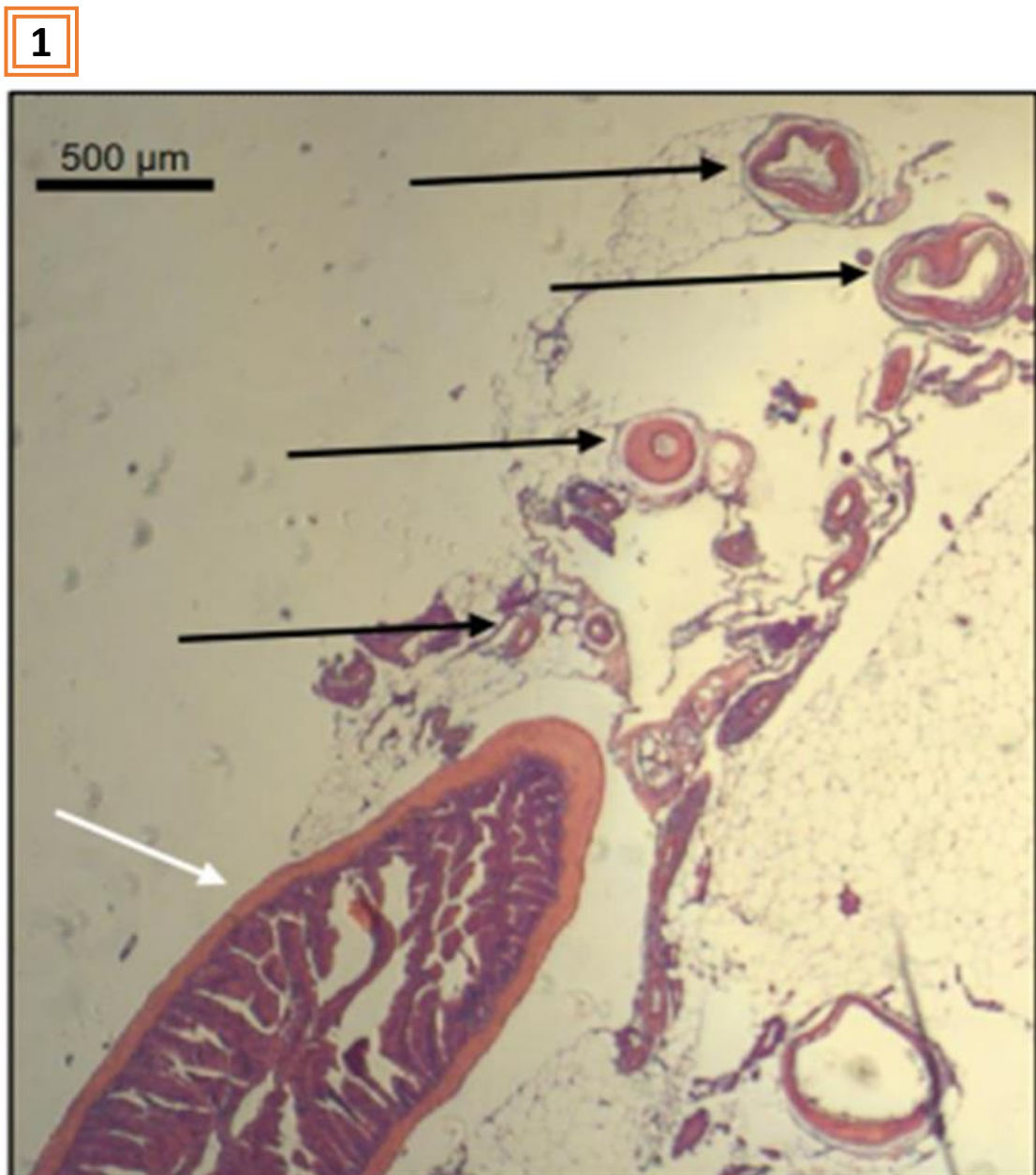


Fig. 1 - **Pyloric caeca** (white arrow) surrounded by a dense **granuloma formation** (black arrows) in the adipose tissue



Fig. 2 - **Granulomas** (arrow) surrounding the **liver**

- ✓ Closest relatives (BLAST) to the **sequenced PCR-DGGE gel bands** of the DNA extracted from the collected organs of European seabass.

Nearest neighbour	Similarity to nearest neighbour (%)	Accession number of nearest neighbour
Uncultured <i>Pseudomonas</i> sp.	87	MF034595.1
<i>Pseudomonas azotoformans</i>	92	MN733445.1
<i>Pseudomonas</i> sp.	87	MH236690.1
<i>Vibrio</i> sp.	98	DQ659046.1

- ***Pseudomonas* genus:** *Pseudomonas* sp. (can be an opportunistic pathogen of fish, including European seabass) associated with **granulomatous lesions** in the **liver** and **kidney** [3]
- ***Vibrio* genus:** associated with **Vibriosis** (a devastating disease affecting farmed fish species worldwide, including European seabass) associated with **granulomas**, in a **chronic phase** [5]

Various biotic agents can cause **granulomatous lesions** in European seabass

The **most common diseases** associated with **granulomas** are fish mycobacteriosis (caused by bacteria of the genus *Mycobacterium*) or pasteurellosis (causative agent is the *Photobacterium damsela* subsp. *piscicida*) [2]

However, these bacterial pathogens **were not found in our molecular analysis**

CONCLUSION

- ✓ **Molecular analyses** points to the **genus *Vibrio*** and ***Pseudomonas***
- ✓ Main bacterial pathogens related with fish granulomas **were not detected** in this study
- ✓ The exact aetiology behind the diseased European seabass **remains to be clarified**

In the future: conduct a more **thorough molecular analysis**, with the goal of **unveiling the particular species** within the bacterial genera found in this study

REFERENCES

- 1) **FishStat F** (2021) Global Aquaculture Production 1950-2019, <http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en>, Visited in 13/08/2021; 2) **Martínez-Lara P, et al. (2021)** Granulomatosis in fish aquaculture: a mini review. *Reviews in Aquaculture*; 3) **Roberts R (2012)** *Fish Pathology*, Fourth Edition, Wiley-Blackwell; 4) **Serra C. et al. (2018)** Gut microbiota and gut morphology of gilthead sea bream (*Sparus aurata*) juveniles are not affected by chronic oxide as digestibility marker. *Aquaculture Research*; 5) **Urku C (2021)** Isolation and characterization of *Pseudomonas putida* caused granulomas in cultured sea bass (*Dicentrarchus labrax*) in Turkey. *Journal of the Hellenic Veterinary Medical Society*