

Haplosporidium pinnae as a Molecular Marker of Disease Progression in *Pinna nobilis*

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INTRODUCTION

Pinna nobilis is the largest endemic bivalve mollusc in the Mediterranean Sea. Since 2016, it has been experiencing mass mortality events caused by a multifactorial disease that is driving the species toward extinction. These events have been associated to the combined action of several etiological agents (e.g., bacteria, viruses, and especially the protozoan *Haplosporidium pinnae*) and to changes in environmental factors, such as temperature and salinity, which may favour the propagation of *Haplosporidium pinnae*. To counteract this dramatic situation, conservation efforts, including the EU-funded LIFE PINNA project, have been implemented. As part of this project, we monitored the levels of microorganism infection in 30 *Pinna nobilis* specimens belonging to the Venetian Lagoon and kept in the University of Genoa aquaria.



Figure 1. Deceased *Pinna nobilis* specimens

AIMS

This study aimed to standardize a procedure for identifying the stages of disease progression based on *Haplosporidium pinnae* infection levels.

MATERIALS AND METHODS

To achieve this goal, mantle fragments were collected monthly from live individuals using non-lethal and non-invasive techniques to monitor infection levels over time. For deceased specimens, tissue samples were taken from four anatomical districts (mantle, adductor muscle, gills, and digestive gland) to study infection dynamics. Quantitative PCR (qPCR) was then performed on DNA extracted from these samples to assess infection levels through CT values (i.e., the number of amplification cycles required for the detection and amplification of pathogenic DNA).

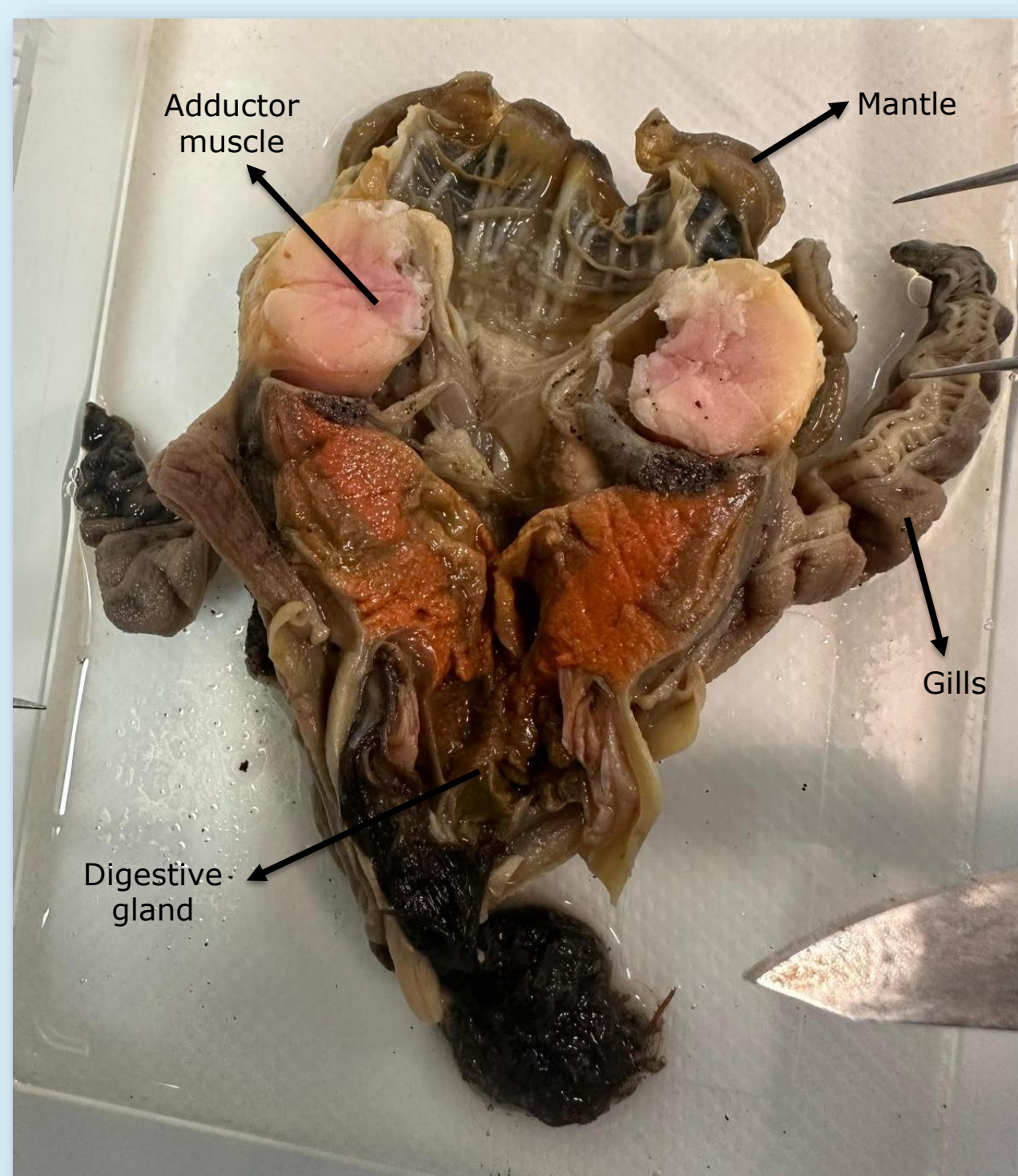


Figure 2. Deceased *Pinna nobilis* specimen

RESULTS AND DISCUSSION

Based on the results obtained, we identified three cycle threshold (CT) ranges that reflected the health status of the individuals:

1. **CT \geq 32** → **Absence of infection**
2. **24 \leq CT \leq 32** → **Low infection level** associated with the early stages of the disease.
3. **CT \leq 24** → **High infection levels** corresponding to advanced or terminal disease stages. Individuals exhibiting such CT values typically died within approximately 30 days.



Based on the infection patterns and *Haplosporidium pinnae* responses observed in the fan mussels, three main categories of individuals were distinguished:

1. **Resistant to infection** → Individuals showing no significant infection.
2. **Highly sensitive** → Individuals that died even with mild infections confined to the mantle.
3. **Less sensitive** → Individuals able to survive with moderate mantle infections, succumbing only when all organs were severely affected.

CONCLUSION

In conclusion, these analyses support the use of *Haplosporidium pinnae* infection levels in the tissues of fan mussels as a molecular markers for monitoring of diseases' progression in individuals kept in captivity.