

Is the parasitisation of the eggs an additional threat for the Mediterranean sardine?

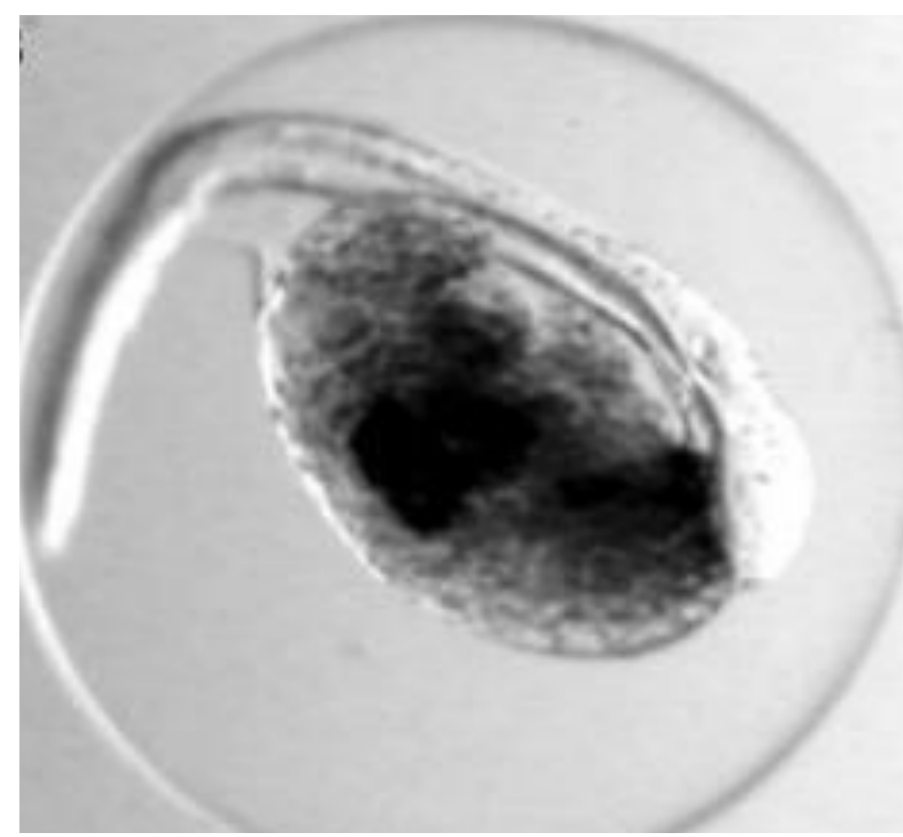
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INTRODUCTION

Ichthyodinium chabelardi is a marine dinoflagellate (Syndiniales) which acts as an endobiotic parasite in the embryos and yolk sac larvae of several fish species in marine ecosystems and aquaculture tanks. It has been reported in commercially important species like *Sardina pilchardus* (Hollande & Cachon, 1952), *Sparus aurata* (Statoudakis et al., 2000), *Merluccius merluccius* (Meneses et al., 2003) and *Gadus morhua* (Pedersen et al., 1993).



Infected egg by *Ichthyodinium chabelardi* Skovgaard et al. (2009).

As *I. chabelardi* has been reported to induce death in sardine yolk sac larvae (Gestal et al., 2006), this species is in the spotlight of the present study. Sardine is a capital breeder (Ganias et al., 2004) since it feeds intensively on plankton during the maximum primary production in spring-summer and accumulates energetic reserves to assume the cost of reproduction in winter (Albo-Puigserver et al., 2017). Especially in the northwestern Mediterranean Sea, overfishing, seawater temperature, and **parasite infection** become critical threats (Frigola-Tepe et al., 2022).

The **main goal** of this research was to develop and validate a genetic method based on DNA barcoding to record the presence of *I. chabelardi* in European sardine eggs and apply it to samples from the Catalan Coast.

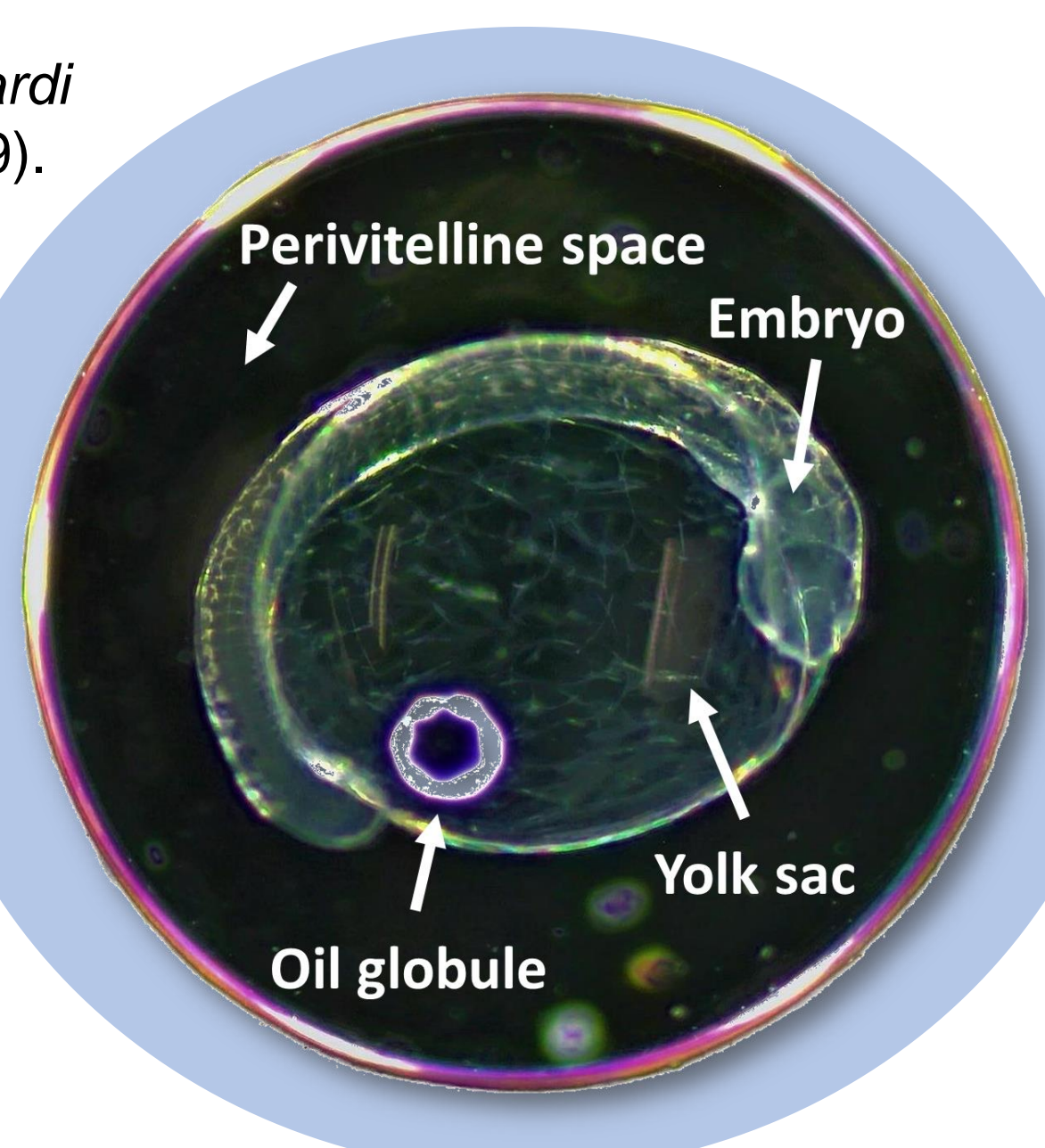
METHODOLOGY

Sardine eggs were collected with a **bongo net** (500 µm) along the coastline of Sant Feliu de Guíxols (Northwestern Mediterranean Sea). The collection of eggs was carried out during the spawning season in winter. Bongo net was pulled horizontally for **45 minutes** at depth of **5-10 m**. Plankton attached to the net was preserved in marine water.

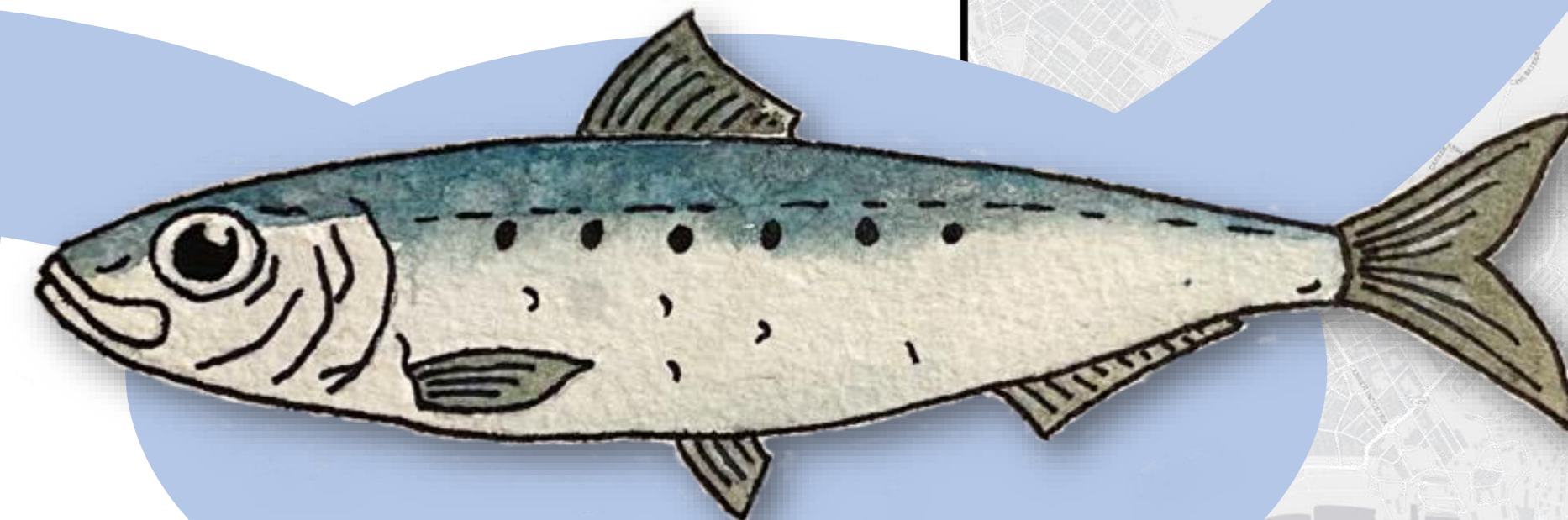
Genetic marker

Nuclear Small subunit (SSU) ribosomal RNA (aprox 300bp).

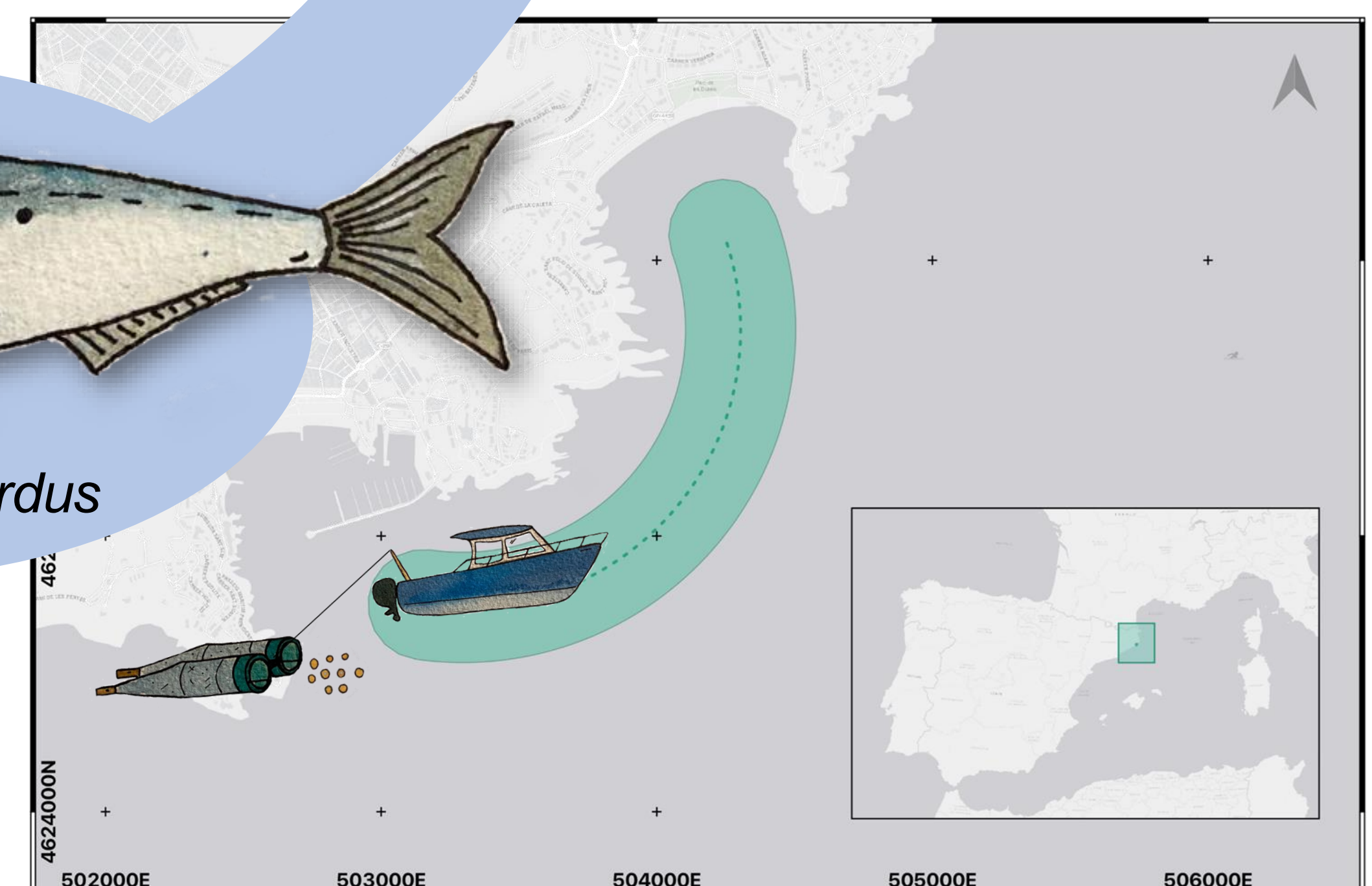
Synthetic positive control (**G-Block sequence**).



Visual identification of eggs



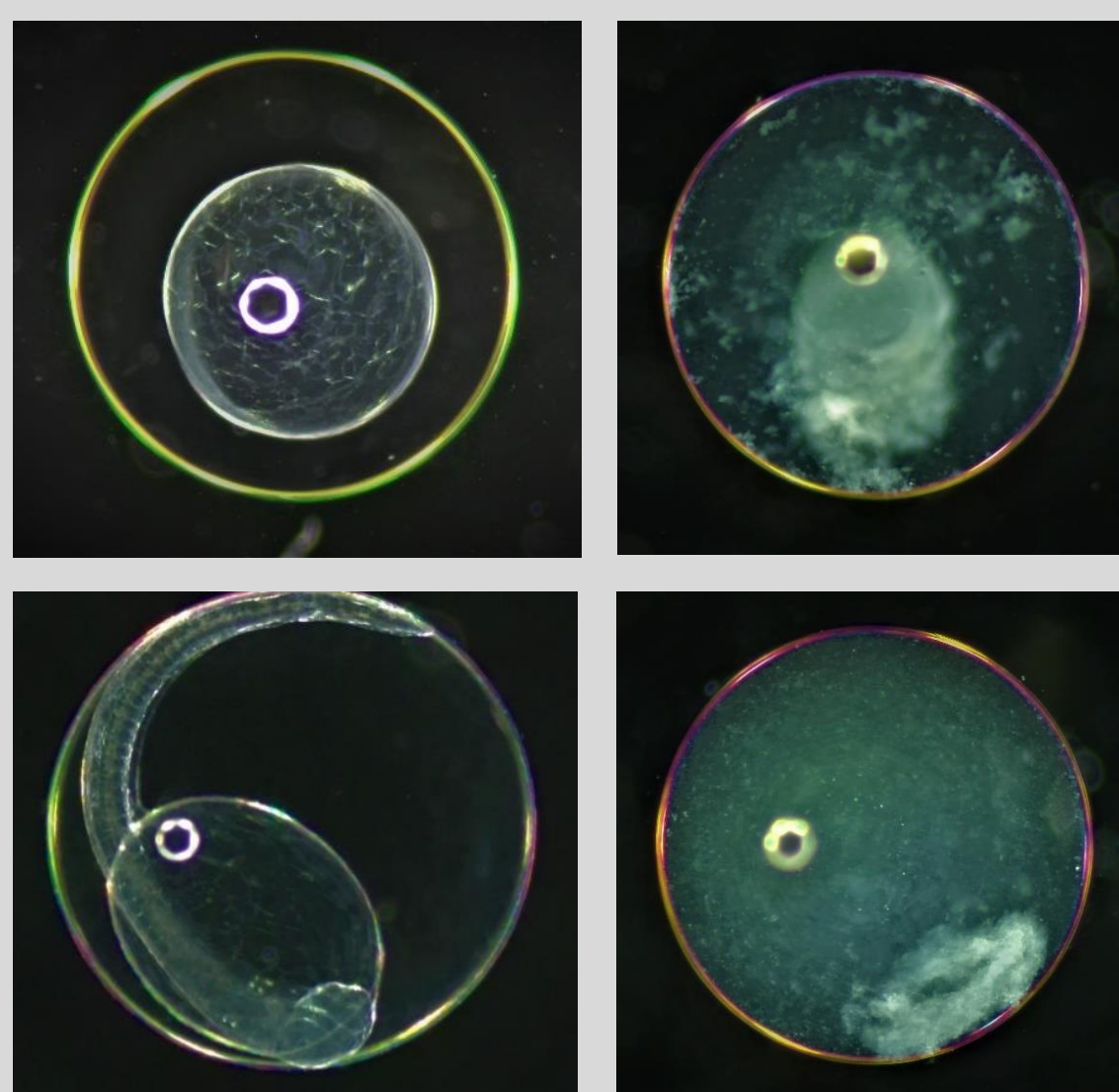
Sardina pilchardus



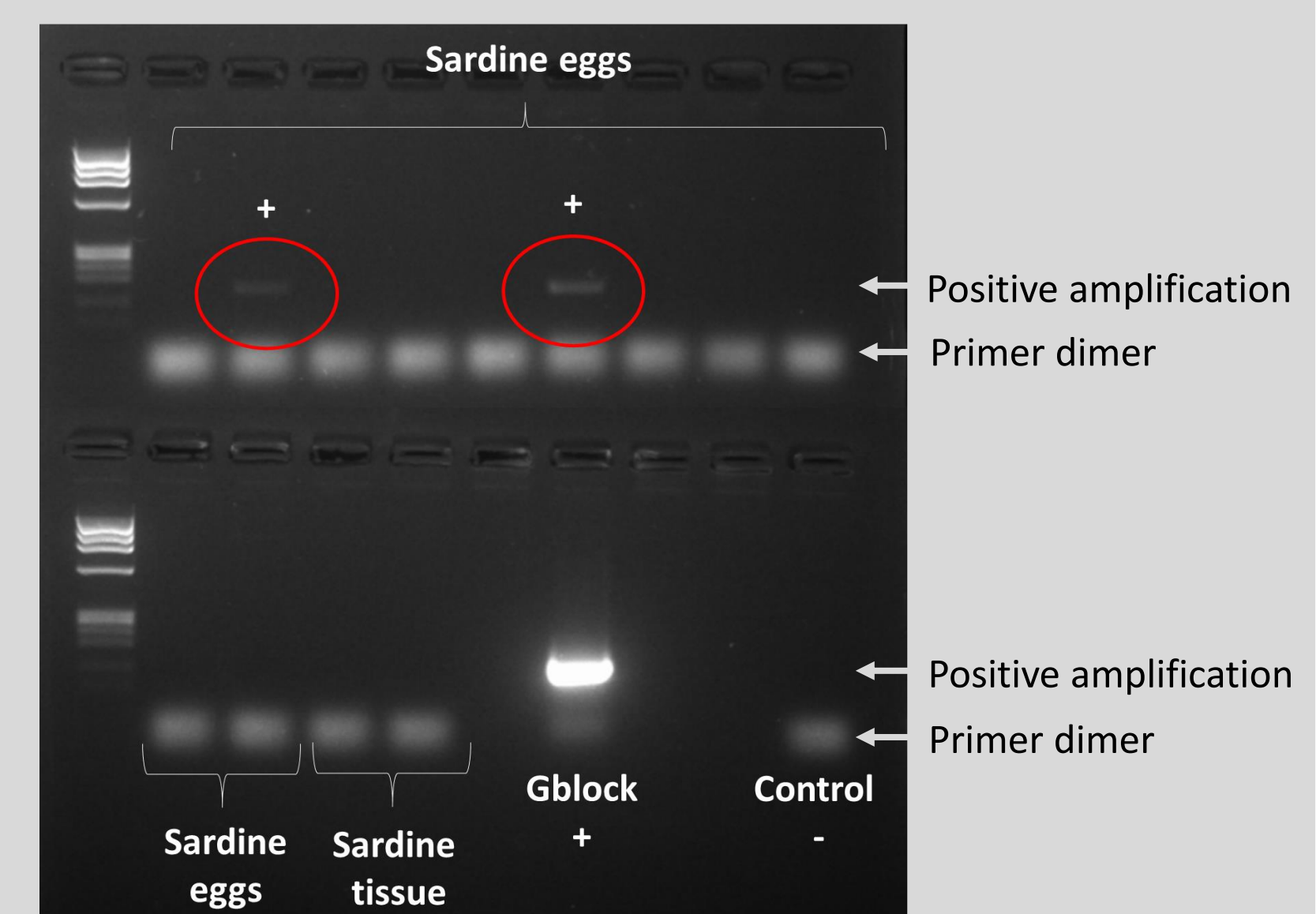
RESULTS

- PCR Amplification found 36 parasitized eggs of a total of 426. *I. chabelardi* appeared with a prevalence of 8.10%.
- Nested PCR, which increases specificity of the PCR and confirmed the infection of the eggs, raised the prevalence to 10.56%.

426 *Sardina pilchardus* eggs identified by visual keys and confirmed by genetic methods.



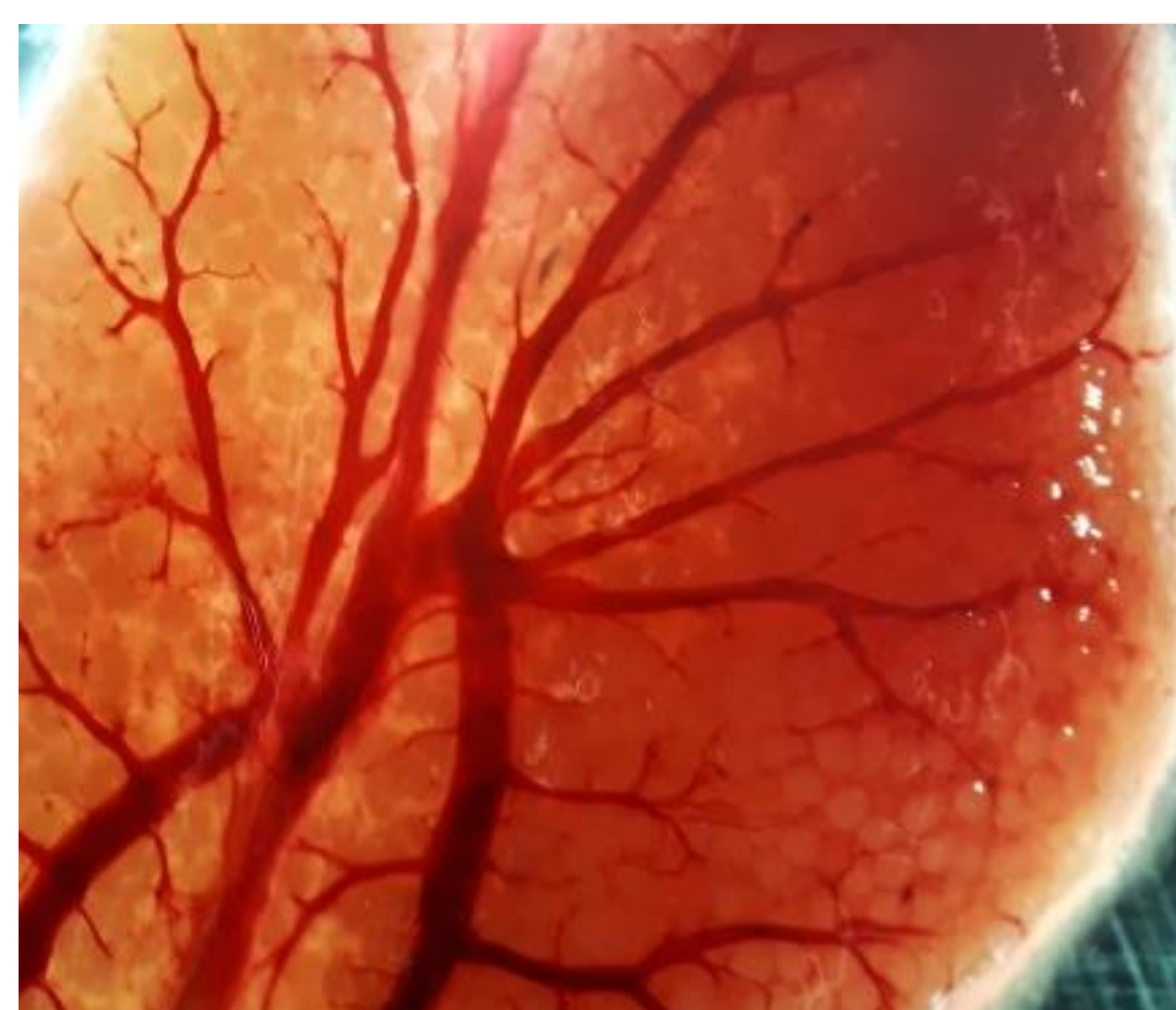
The functionality of **Gblock** was confirmed with parasitized eggs of *Scomber scombrus*.



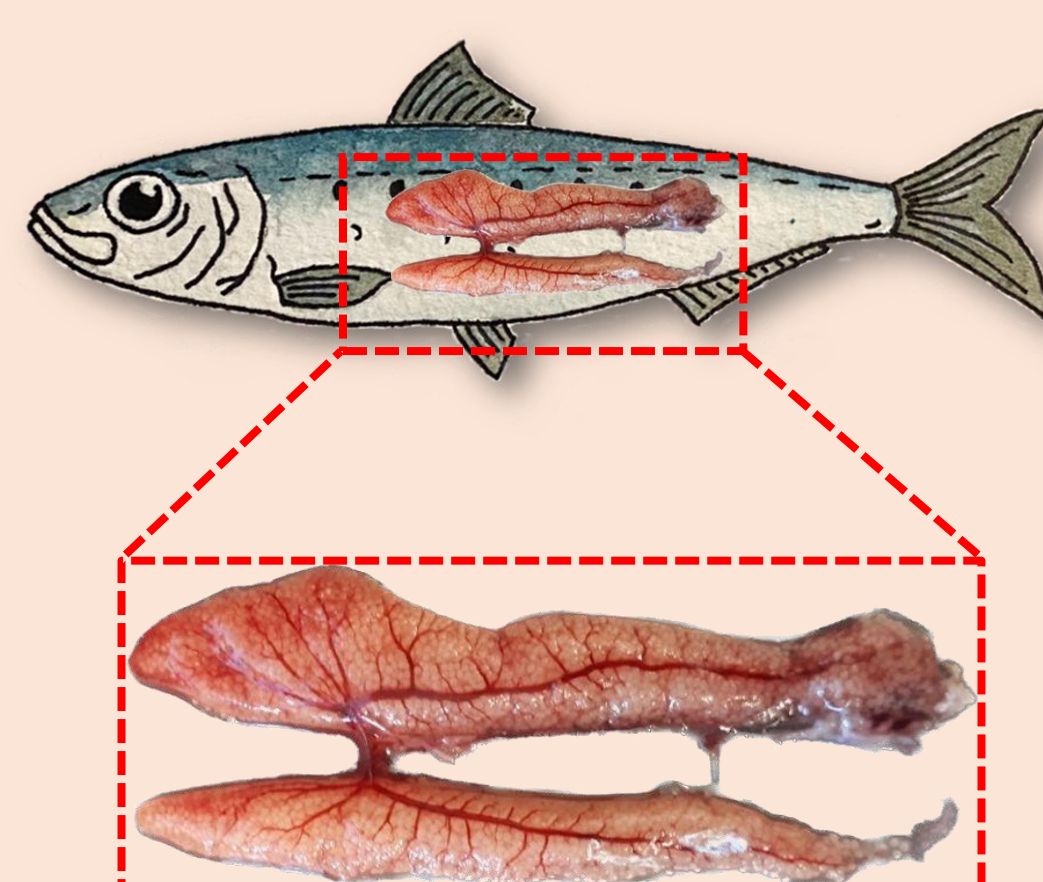
Agarose Gel electrophoresis shows the infection of the eggs by *Ichthyodinium chabelardi*.

CONCLUSIONS

Although fish spawn many eggs, only a few will become fertilized and develop into juvenile fish. Fish eggs can be subjected to predation and diseases (Wootton, 1991). Thus if we add a 10% of parasite infection of planktonic stages with a 100% of mortality, this could directly affect the recruitment success of the Mediterranean sardines.



NEXT STEP...



We are currently determining if sardine eggs are infected by vertical or horizontal transmission. With the analysis of sardine gonads, we want to determine the infection origin.

