FISH IMMUNE AND OXIDATIVE STRESS REACTION TO EMERGING PARASITE IN THE BALEARIC ISLANDS

Silvia Tejada, Amanda Cohen-Sánchez, José María Valencia, Antonio Box, Llorenç Gil, Samuel Pinya, Antoni Sureda

Abstract: Global change has a deep impact on the distribution and prevalence of diseases in fish, leading to the emergence of new pathogens. A recent observation on Ibiza Island in the Balearic Islands highlighted the presence of black spot disease linked to a digenean fluke belonging to the genus *Scaphanocephalus* in *Xyrichthys novacula* specimens.
The study aimed at assessing the antioxidant and immune responses in mucus and spleen of *X. novacula*, that are infected by *Scaphanocephalus* sp.
Two sites were selected, one devoid of the parasite and other with its presence. Fish were divided by severity of infection (low or high). Weight and size were measured, and mucus and spleen samples were obtained. As the infection was increased, the body index was worst and antioxidant enzymes activities and immunological parameters in mucus increased. However, the systems could not prevent lipid peroxidation. Also increases in immunological determinations were observed in the spleen.
In conclusion, *Scaphanocephalus* sp. in *X. novacula* triggers an immune response and oxidative stress in the mucus as the infection is more severe. These changes are accompanied by a decrease in their body condition.

Keywords: Ectoparasite; Immune system; Oxidative stress; Pearly razorfish

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Introduction

Global change, encompassing factors such as displacement from natural areas, habitat fragmentation, overexploitation, anthropogenic pressure, among others, has a profound and far-reaching impact on the distribution and prevalence of diseases in different animal species, including fish. This dynamic situation is leading to the emergence of new pathogens that can significantly affect the animal who is used as host [12].

A recent observation surrounding the Island of Ibiza in the Balearic Islands (Mediterranean Sea, Spain) has highlighted the presence of black spot disease in specimens of *Xyrichthys novacula* (Linnaeus, 1758), commonly known as pearly razorfish. This disease is linked to a digenean fluke belonging to the genus *Scaphanocephalus* [11]. The presence of the ectoparasite in this fish is particularly concerning given that the pearly razorfish is highly valued as a food source on the islands, suggesting that the presence of this parasite could have substantial impact in social and economic areas [6].

The black spot disease has been described as one of the most common characteristic manifestations of ectoparasite infections [5]. In addition, a rise of the environmental temperature could imply higher reproduction rates of parasites which could accelerate transmission within wild host–parasite systems and increase the overall abundance of the parasites.

In light of these concerns, this study aimed at assessing the antioxidant and immune responses in both mucus and spleen of *X. novacula*, with a specific focus on the extent of infection by *Scaphanocephalus* sp. Understanding these responses is crucial for developing strategies to mitigate the impacts of this emerging health threat on both the fish population and the local economy.

Materials and Methods

The study involved capturing fish in two distinct sandy bottom areas (19 meters depth) in waters of the Ibiza Island (Balearic Islands), the first one was devoid of the parasite and considered as a control site (Sa Mola, Formentera); and another one where the parasite was observed to be present, so as it was considered the infected area (Es Cubells, Ibiza southern) (Figure 1). The animals were captured by hook and line with worms as bait during October 2022.

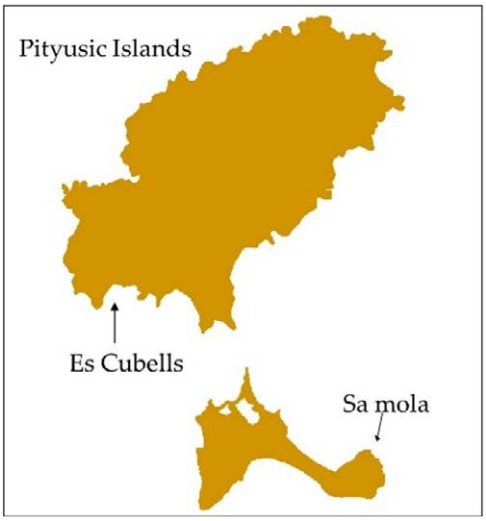

Figure 1 – Sa Mola (control) and Es Cubells (infected) areas where the study was performed.

The spots observed in the fish caught in the infected area were counted, so the animals were classified into two groups based on the severity of infection: low infection (from 1 to 15 spots) and high infection (>15 spots). When the sample size was achieved (10 for controls and 20 for infected, n=10/group), the other captures were returned to the sea.

Under anaesthesia (tricaine methane sulfonate), weight and size were measured for calculate the body index condition [8,9], and mucus and spleen samples were obtained and immediately frozen for posterior analysis. Oxidative stress was evaluated though the measurement of the activities of several enzymes (catalase, superoxide dismutase and glutathione peroxidase) following the procedures described in previous works [1,4,3]. To evaluate if the antioxidant systems protect the organism, the lipidic peroxidation was evaluated by means of measuring the malondialdehyde levels by a colorimetric assay kit following the manufacturer's instructions (Merk KGaA). The immunological parameters were evaluated by spectrophotometry (Shimadzu Corporation) (alkaline phosphatase, myeloperoxidase, lysozyme) or as previously described [7].

Data was analysed by one-way analysis of variance (ANOVA) followed by the Levene test to determine the statistical differences considering $p < 0.05$. Data was also checked for normality of the variance. The statistical package IBM SPSS Statistics software (SPSS) was used for the analysis of the data.

Results

A sample of a specimen of *Xyrichthys novacula* affected by the ectoparasite *Scaphanocephalus* sp is shown in the figure 2.

Figure 2 – Example of *Xyrichtys novacula* infected by *Scaphanocephalus* sp. (genetically identified).

The levels of the antioxidant enzymes in mucus of the *X. novacula* are represented in figure 3. A statistically significant increase was observed in the infected fish for catalase respect the control. For superoxide dismutase and glutathione peroxidase, the rise was significant in the animals with higher infection, when compared to the respective controls.

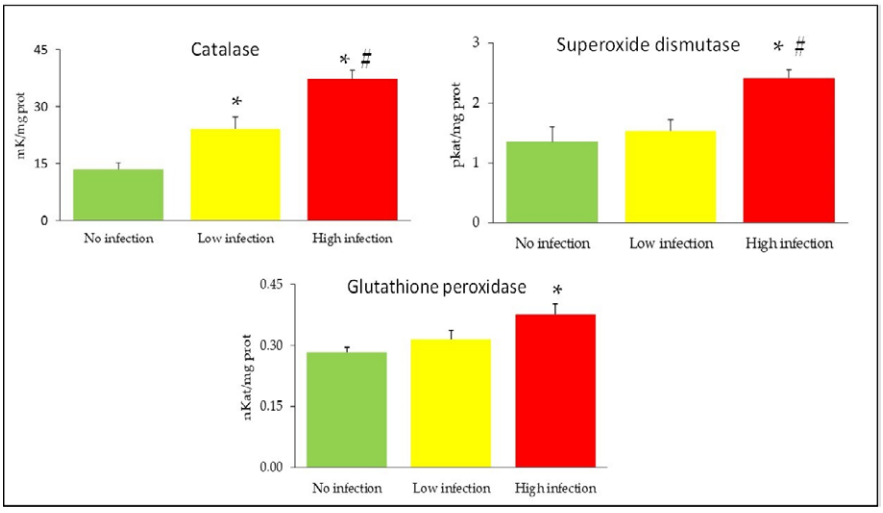

Figure 3 – Antioxidant enzyme response in mucus of razorfish in Sa Mola (control) and Es Cubells (infected). Bars represent mean ± SEM. * *vs* control, # *vs* low infection. One-way ANOVA, $p<0.05$.

The Malondialdehyde (MDA), as a marker of lipidic damage, values in mucus of the *X. novacula* specimens are included in table 1. Those animals with a higher infection (more than 15 spots) showed an elevated MDA values when compared with control and the fish lessen infected (1-15 spots).

Table 1 – Malondialdehyde (MDA) levels (mean ± SEM) in mucus of razorfish in Sa Mola (control) and Es Cubells (infected). * *vs* control, # *vs* low infection. One-way ANOVA, $p<0.05$.

	MDA (nmol/mg protein)
Control (no infection)	1.76±0.15
Low infection (1-15 spots)	1.82±0.29
High infection (>15 spots)	2.98±0.39$^{*,\#}$

Figure 4 includes the results observed in the immunological variables studied in the mucus and spleen of the razorfish. The rise in the values of the markers study was higher as the infection was more severe.

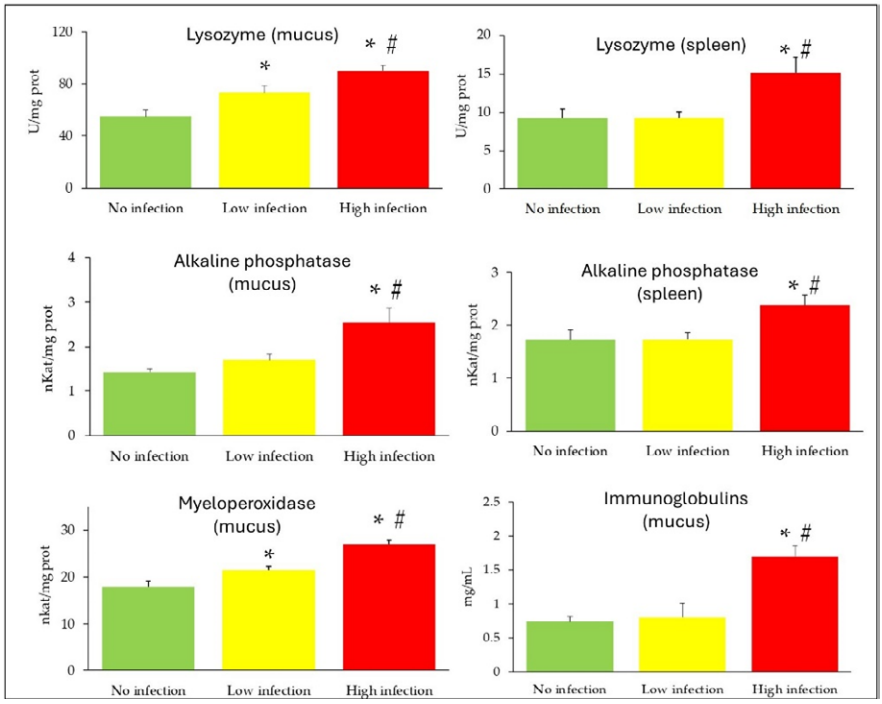

Figure 4 – Immunological parameters in mucus and spleen of razorfish in Sa Mola (control) and Es Cubells (infected). Bars represent mean±SEM. * *vs* control, # *vs* low infection. One-way ANOVA, $p<0.05$.

Discussion

As the degree of infection increased, a noticeable decline in the body condition index was observed, indicating the detrimental impact of the ectoparasite

Scaphanocephalus sp. One significant finding of this research was the increasing activity of the antioxidant enzymes in the mucus, including catalase, superoxide dismutase, and glutathione peroxidase, as the infection level increased. This increase in antioxidant defences aimed to counteract the oxidative stress imposed by the parasite. This defensive response seems to be partially effective in the fish with fewer spots, correlated with a fewer degree of infection of these animals. However, despite the activity of the antioxidant systems of the fish, the higher infection levels led to an elevation in malondialdehyde concentrations, which indicated lipidic oxidative damage. This rise highlights that the group with the most extensive infection exhibited an overwhelmed state of the antioxidant defenses. These results are consistent with previous research conducted with the same fish species [2,13].

In addition to the antioxidant response, the study also revealed a corresponding increase in various immunological parameters as the infection severity rose. These parameters included lysozyme, alkaline phosphatase, myeloperoxidase, and immunoglobulins. Also, lysozyme and ALP activities in the spleen of the higher infected specimens of *X. novacula* were observed although no differences were showed in the low infection. Similar results have been described in a previous study in which the damage in fish gills infected with an ectoparasite was more severe as the infection increased, affecting both the antioxidant system and the immunological parameters [13]. The study findings underscore the complexity of the immune and antioxidant responses in *X. novacula*, revealing both the capacity and the limits of the fish's defenses against parasitic infection.

Altogether, this immune response was indicative of the fish's attempt to combat the infection by *Scaphanocephalus* sp. This research contributes valuable insights into the broader understanding of how global change and emerging pathogens impact marine life, emphasizing the need for ongoing monitoring and adaptive management strategies to protect vulnerable species and ecosystems.

Conclusion

The presence of *Scaphanocephalus* sp. in *X. novacula* triggers both an immune response and a state of oxidative stress in the mucus as the infection becomes more severe. These changes in the physiology of the fish are accompanied by a decrease in their overall body condition. The potential effects that this ectoparasite can have on affected *X. novacula* populations highlight the need for continued with the studies, and more extensive research to monitor and understand the long-term impact of this infection.

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