

Ectoparasite causes oxidative stress in the liver of the razorfish *Xyrichtys novacula*

*Ectoparàsits causen estrès oxidatiu al raor *Xyrichtys novacula**

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Resum: *Xyrichtys novacula* is very popular fish species in the Balearic Islands and a main target for recreational fishing. Spots on many individuals progressively are more common. This study aimed to identify and determine the abundance of the parasite in a marine protected area (MPA) and in a non-protected area with more anthropic influence evaluating the antioxidant and immune responses to this parasite by biomarkers in liver. The digenean fluke *Scaphanocephalus* sp. was identified as the ectoparasite. An average abundance of 12.3 ± 11.3 and 1.3 ± 1.3 parasites per individual was found for the non-protected area and the MPA, respectively. In liver, higher parasite load was related to higher activities of catalase, glutathione peroxidase and glutathione S-transferase, while similar lipidic damage in both groups was observed. In conclusion, *Scaphanocephalus* sp. affects *X. novacula*, inducing an immune and an antioxidant response. The potential influence of the environment on parasite transmission, prevalence and abundance requires further research to determine whether it makes fish more susceptible to infection.

Paraules clau: Antioxidants, Balearic Islands, activity, Pearly razorfish.

Abstract: *Xyrichtys novacula* és una espècie de peix molt popular a les Illes Balears i un objectiu principal per a la pesca recreativa. Les taques en molts dels individus són progressivament més freqüents. Aquest estudi volia identificar i determinar l'abundància del paràsit en una àrea marina protegida (MPA) i en una àrea no protegida amb més influència antròpica avaluant les respostes antioxidants i immunes a aquest paràsit mitjançant biomarcadors al fetge. El trematode digeni *Scaphanocephalus* sp. va ser identificat com l'ectoparàsit. Es va trobar una abundància mitjana de $12,3 \pm 11,3$ i $1,3 \pm 1,3$ paràsits per individu per a l'àrea no protegida i l'AMP, respectivament. Al fetge, la càrrega de paràsits més alta es va relacionar amb activitats més elevades de la catalasa, glutatió peroxidasa i glutatió S-transferasa, mentre que es va observar danyos lipídics similars als dos grups. En conclusió, *Scaphanocephalus* sp. afecta *X. novacula*, induint una resposta immune i antioxidant. La influència potencial del medi ambient en la transmissió, la prevalença i l'abundància del paràsit requereix més investigacions per determinar si fa que els peixos siguin més susceptibles a la infecció.

Key words: Antioxidants, Illes Balears, Raor.

INTRODUCCIÓ

Xyrichtys novacula (Linnaeus, 1758) or pearly razorfish is a small wrasse, highly benthic, associated to shallow sandy or muddy bottoms, and found in warm latitudes of the Atlantic Ocean and the Mediterranean Sea. It is highly appreciated for recreational fishers in the Balearic Islands (Box *et al.*, 2009). Spots, identified as the trematode ectoparasite *Scaphanocephalus* sp., on the skin of the *X. novacula* was reported in certain areas of Eivissa island in 2015.

The 'black spot disease' consist of the presence in the skin of fish of great numbers of the metacercaria of the digenean fluke *Scaphanocephalus* sp. (Dennis *et al.*, 2019) with complex life cycles involving several hosts (molluscs, fish and finally birds) and developmental stages (Foronda *et al.*, 2009). Nevertheless, parasite classification based on morphology is controversial, so DNA sequence data (LSU rDNA gene regions) facilitate it (Dennis *et al.*, 2019). The parasitized species can be more vulnerable since their conditions and behaviour can be altered. In addition, animals

have developed an antioxidant defence system to avoid an excess of a production of reactive oxygen species (ROS) that could induce oxidative damage because of a pathogenic agent (Pinya *et al.*, 2016), so that maintaining an equilibrium and limiting cell damage. Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) are some of the main antioxidant enzymes. The last one depends on glutathione reductase (GRd) to regenerate glutathione (Hoseinifar *et al.*, 2020). Glutathione S-transferase (GST) has a fundamental role as a phase II enzyme in the detoxification process and exhibits glutathione peroxidase activity and catalyses the reduction of organic hydroperoxides to their corresponding alcohols (Allocati *et al.*, 2018). Moreover, the end-product of the lipid peroxidation malondialdehyde (MDA) is used as a stress biomarker (Alomar *et al.*, 2017). Oxidative stress on tissues related to the ectoparasite is unknown, but changes of the physiological responses in the liver of the fish could be promising biomarkers of the parasite infection.

This study aimed to identify the trematode ectoparasite detected in the pearly razorfish and to study its effects on the antioxidant defence systems in the liver of the fish. It is also evaluated if the environment influenced infections by the parasite, comparing parasite abundance in fish caught in a marine protected area (MPA) and a non-protected area with greater human activity.

MATERIAL AND METHODS

Forty-eight razorfish were fished around the island of Ibiza. 26 were fished from the MPA area Es Freus and 22 from the non-protected area Cala Jondal by line fishing in October 2020 avoiding the reproductive season of the fish. Animals in Es Freus presented few or no parasites so they were considered as the control group; while fish from Cala Jondal were the parasitized group. Tricaine methane sulfonate (MS-222) (1g/10L water) was used to anesthetized the fish. The observable spots on the fish were counted to assess the parasite abundance. In order to genetically identified the ectoparasite, a flesh piece containing visible skin papules was collected, kept in 100% ethanol, and sent to the Laboratorio de Investigaciones Marinas y Acuicultura (LIMIA) for molecular identification. Liver samples were collected in 1.5 mL tubes from 32 specimens: 16 with 0-1 parasites from Es Freus and 16 with ≥ 7 parasites from Cala Jondal. Samples were stored at -80°C for later biochemical analysis. Experimental procedures with fish followed EU Directive 2010/63/EU for animal experiments and were approved by the Ethics Committee for Animal Experimentation of the University of the Balearic Islands (Ref. 020/06/AEXP). Once at the laboratory, the livers were homogenized and supernatants were collected. The total protein content and CAT, SOD, GR, GPx, and GST, and MDA levels were determined following the methodology used in the group (Sureda *et al.*, 2006). R version 3.5.3 (R Core Team 2019) was used for the statistical analysis, and two groups were defined depending on the degree of infection by *Scaphanocephalus* sp. Kruskal-Wallis test assessed the statistical differences between groups (differences at $p < 0.05$). Statistical analyses were carried out using.

RESULTS

A higher number of the ectoparasites *Scaphanocephalus* sp., genetically identified, were found in the *Xyrichtys novacula* specimens (Fig. 1) caught in the non-protected area of Cala Jondal when compared with the ones fished in the control site (Es Freus). In the Cala Jondal area the average number of spots in the fish was greater than 10 per individual, while in Es Freus it was practically undetectable. The enzymatic activities of CAT, GPx and GST were significantly higher in the liver of the fish with higher parasitic infection ($p < 0.05$). In addition, the activities of SOD and GRd, and the levels of the MDA showed a similar trend, although without statistical differences.



FIGURE 1. Example of *Xyrichtys novacula* infected by *Scaphanocephalus* sp.

DISCUSSION

Xyrichtys novacula feeds molluscs, shrimps, crabs, among others, being those the intermediate hosts of *Scaphanocephalus* sp. life cycle. The higher number of infected fish was found in Cala Jondal, that is generally shallower which a great number of intermediate hosts and more human impact; these could be factors that influenced the appearance of more ectoparasite abundance (Kohl *et al.*, 2019). It is known that interactions with other species can induce oxidative stress. In this work, statistical differences were observed in the activities of the antioxidant enzymes CAT, GPx and GST in the liver of the razorfish which showed a great number of ectoparasites when compared to the fish caught in the control site. This results could be related to the infection that was observed in the animals, indicating an activation of the antioxidant systems to avoid the induced oxidative stress. This result agrees previous works in which parasitism induced an increase of the antioxidant activities of CAT, SOD and GST (Kumar *et al.*, 2017). In addition, lipid damage was not observed in the present work, indicating that the antioxidant systems could prevent the damage in the infected fish.

CONCLUSION

In conclusion, the antioxidant system response of the razorfish *X. novacula* due to the infection by the ectoparasite *Scaphanocephalus* sp. is increased in the liver of the fish. However, more studies are necessary to know how the infection can affect the physiology and vulnerability of the species.

AGRAÏMENTS

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