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Black spot disease related to a trematode ectoparasite causes oxidative stress in *Xyrichtys novacula*

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ABSTRACT

Xyricthys novacula is very popular fish species in the Balearic Islands and a main target for recreational fishing. Over the last years, spots were observed on many individuals, which seems to be progressively more common. The aim of the present study was to identify the parasite, determine its abundance in a marine protected area (MPA) and in a non-protected one with more anthropic influence, and to evaluate the antioxidant and immune responses to this parasite presence by studying biomarkers in both liver and epithelial mucus. Analysis of genetic sequences established with 98.6–98.4% certainty that the parasite is a digenean fluke *Scaphanocephalus* sp. 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. The activities of lysozyme and superoxide dismutase and total immunoglobulin concentration were significantly higher in mucus of *X. novacula* with more parasites. Similarly, in liver, a higher parasite load is related to higher activities of catalase, glutathione peroxidase and glutathione *S*-transferase, whereas malondialdehyde remained similar. In conclusion, *Scaphanocephalus* sp. affects *X. novacula*, inducing an immune and antioxidant response in epithelial mucus and in liver. The potential influence of the environment on parasite transmission, prevalence and abundance require further research to determine whether it makes fish more susceptible to infections.

1. Introduction

Xyrichtys novacula (Linnaeus, 1758) or pearly razorfish is a small wrasse found in warm latitudes of the Atlantic Ocean and the Mediterranean Sea (Castriota et al., 2005; Schneider, 1992). It is highly benthic, associated to shallow sandy or muddy bottoms (Cardinale et al., 1997; Katsanevakis, 2005), sometimes in *Zostera* meadows. It buries its head into the sediment for protection and it has rarely been seen deeper than 150 m (Alós et al., 2012; Fischer et al., 1981). This fish is a highly appreciated target for recreational fishers in the Balearic Islands (Box et al., 2009). In 2015, a fisherman reported spots on the skin of the pearly razorfish caught in certain areas of the island of Eivissa. These spots were identified as the trematode ectoparasite *Scaphanocephalus* sp., and it appears to be spreading their range to different areas around the Island (Fig. 1).

The metacercaria of the digenean fluke *Scaphanocephalus* sp. is commonly found in the skin of many wild and net-pen reared marine fish species. When present in large numbers, the parasite causes 'black spot disease' (Dennis et al., 2019). The metacercariae lodge in the fish skin where they produce tiny clear cysts around themselves, in turn, this induces the fish to produce a second outer dark connective tissue capsule (Kohl et al., 2019). The Digenea are characterized by complex life cycles

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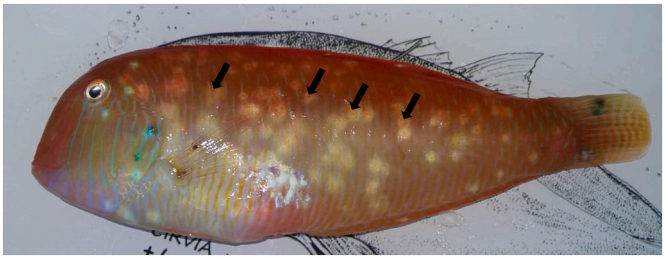

Fig. 1. Representative image of pearly razorfish (*Xyrichtys novacula*) sampled in Eivissa and Formentera (Balearic Islands) significantly affected by *Scaphanocephalus* sp.

involving several hosts and developmental stages. *Scaphanocephalus* sp.'s life cycle probably includes coastal molluscs as the first intermediate host, marine fish as the second, and fish predatory birds such as osprey, *Pandion haliaetus*, as definitive hosts, although this is not established conclusively (Foronda et al., 2009; Galaktionov and Dobrovolskij, 2003). Parasite classification based on morphology alone remains a source of many scientific controversies. As an alternative to classical methods, molecular tools are used to identify digenean life stages (Anderson, 1999; Athokpam et al., 2016; Cribb et al., 1998; Le et al., 2017). DNA sequence data facilitate detection of variation between closely related species that may be morphologically similar or indistinguishable (McManus and Bowles, 1996). LSU rDNA gene regions are successfully used to analyse genetic differentiation within digenean families (Dennis et al., 2019; Kaukas and Rollinson, 1997).

Parasites can affect the physiology, morphology, reproduction, and behaviour of the hosts (Timi and Poulin, 2020) and make them more susceptible to predation, fishing or diseases (Lafferty, 2008). To deal with ectoparasites, fish produce external mucus that acts as a dynamic physical and biochemical barrier and contains numerous immune molecules, such as lysozyme and immunoglobulins that contribute to limiting the development of certain parasites (Reverter et al., 2018; Sridhar et al., 2021; Vallejo et al., 2009). The presence of a pathogenic agent induces stress in the affected organisms and leads to an increased production of reactive oxygen species (ROS) that, if excessive, can cause oxidative damage (Pinya et al., 2016). Organisms have therefore developed an antioxidant defence system that neutralizes excess ROS molecules, maintaining an equilibrium and limiting cell damage (Clarkson and Thompson, 2000; Matés et al., 1999). Among the main antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) which depends on glutathione reductase (GRd) to regenerate glutathione (Hoseinifar et al., 2020) are useful tools. Moreover, glutathione *S*-transferase (GST) has a fundamental role as a phase II enzyme in the detoxification process, exhibits glutathione peroxidase activity and catalyzes the reduction of organic hydroperoxides to their corresponding alcohols (Allocati et al., 2018; Sureda et al., 2006). It is interesting to mention the use as stress biomarkers of some of the oxidation products such as malondialdehyde (MDA), an end-product of the lipid peroxidation (Alomar et al., 2017). Previous studies have shown that the presence of any factor involving metabolic stress induces an antioxidant and inflammatory response in the liver as a protective mechanism against its metabolic activation. In fact, histological alterations in the liver, an increase in the activity of antioxidant enzymes and in the expression of cytokines have been observed after infection with ectoparasites (Tu et al., 2019; Wang et al., 2022). Therefore, although the relationship between digenean fluke infection and oxidative stress on tissues is largely unknown, alterations on physiological responses of liver and mucus may be promising biomarkers of parasite infection.

X. novacula is very important for the local and family economy of Ibiza and Formentera Islands, and from a cultural point of view. A parasite such as this one, which manifests itself visibly on the skin of its fish host, can have a negative impact on the fishery and the islanders. In addition, this parasite has been found increasingly throughout the years, not only in *X. novacula,* but also in other wrasse species. Both the local importance of this species and the progressive expansion of this parasite make this topic worth studying.

The objective of this study was to identify the trematode ectoparasite detected in *X. novacula* and to investigate its effects on the immune and antioxidant defence systems in the epithelial mucus, as the structure directly affected by the parasite, and liver, as the main metabolic organ, of its host. The influence of the environment is also considered, as parasite abundance is compared between fish caught in a marine protected area (MPA) and a non-protected area with greater human pressure.

2. Methods

2.1. Sampling

A total of 48 razorfish were fished around the island of Ibiza. 26 were fished from the MPA area named Es Freus and 22 from the non-protected area Cala Jondal by line fishing, using worms as bait (Fig. 2). Cala Jondal area is a sheltered bay with calm waters and protected from the prevailing easterly winds in summer, which facilitates that numerous boats anchor in this area. The anchoring of leisure boats entails damage to *Posidonia oceanica*, engine noise, antifouling toxicity, discharge of black seawater and marine litter, artificial lights, and animal feeding, among other effects, requiring urgent regulations on these activities (Carreño and Lloret, 2021). Es Freus Marine Reserve is a protected area in which the anchoring of boats is prohibited as well as recreational fishing. In this area only fishing with traditional minor gear or with scientific purposes are allowed under strict permission of the pertinent authority. This area also has a surveillance service that ensures compliance with current regulations. Es Freus was considered the control group as little to no parasites were reported in that area, whilst Cala Jondal was the parasitized area. The animals were captured during October 2020 to avoid the ban period, which protects the reproductive season of the fish. The two sampling areas were established (Fig. 1) and random sampling points at a distance of 100 m from each other were chosen to perform the experimental fishing. The fish were anesthetized with tricaine methane sulfonate (MS-222) (1 g/10 L water) to minimize stress. The parasite abundance was determined visually by counting the observable spots on the fish. For those fish that presented visible spots, and therefore were considered parasitized, a flesh piece containing skin papules was collected, kept in 100% ethanol and sent to the *Laboratorio de Investigaciones Marinas y Acuicultura* (LIMIA), for the species to be genetically identified. In addition, mucus and liver samples were collected on board from 32 specimens: 16 with 0–1 parasites from Es Freus (classified as low parasite abundance) and 16 with $\geq$7 parasites from Cala Jondal (classified as high parasite abundance) for biochemical analysis. To avoid overlapping in the determinations, those fish with an intermediate number of parasites were discarded. Tissue samples were placed in 1.5 mL tubes and immediately introduced in a liquid nitrogen container. The samples were kept in liquid nitrogen until they reached the laboratory, where they were stored individually at −80 °C until they were used for biochemical determinations. 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).

2.2. Molecular identification

Metacercaries from 14 flesh pieces containing skin papules were excysted from these papules using dissecting needles (Waikagul and Thaenkham, 2014) and the DNA extraction was initiated immediately. DNA extraction was performed using the Macherey-Nagel XS DNA Tissue extraction kit following the manufacturer's instructions.

Polymerase chain reactions (PCR) were used to amplify the 28S

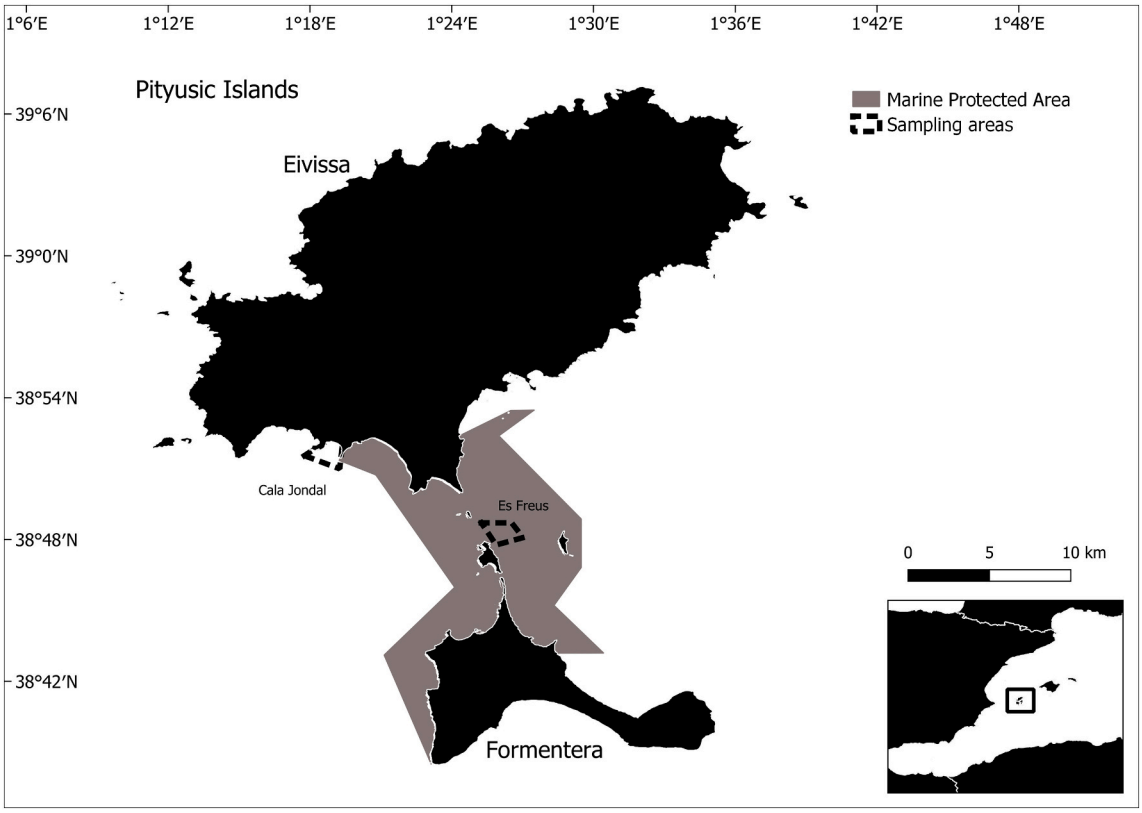

Fig. 2. Location of the different sampling sites in the two selected areas in the Pityusic islands (Ibiza and Formentera), with the marine protected area delimited in grey.

rDNA partially, including variable expansion regions D1-D3 of the of the parasites using the primers pairs LSU5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC-3′) and 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3′) (Littlewood et al., 2000; Olson et al., 2003) and SCAPH28F (5'-CCATGTCAACATTACCGGACTCATTGCTG-3′) and SCAPH28R (5'-TATCCCGGGTACGACTGATAATACATCGTT-3′). PCR reactions were performed in a total volume of 20 μL containing 1 μL of genomic DNA, 10 μL of KAPA Taq Ready Mix DNA Polymerase (KapaBiosystems), 0.8 μL (20 mM) of each primer, and water to make up the final volume. After a pre-denaturation period for 5 min at 95 °C the amplification protocol consisted of 40 cycles of 95 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min followed by 72 °C for 10 min.

PCR products were separated in 1% agarose in TAE 1 × buffer gels (*w/v*), stained with GelRed® Nucleic Acid Gel Stain (Biotium, CA, USA) including a High-Ranger 1000-bp DNA ladder size standard (Norgen-Biotek, ON, Canada) and visualized on a UV transilluminator. Amplicons were purified using mi-PCR purification Kit (Metabion International, Germany) following the manufacturer's instructions and sequenced in a 3130xl DNA automated sequencer (Applied Biosystems, CA, USA). The sequences were aligned and edited using the BioEdit 7.1.3.0 software package (Hall, 1999) and compared using the basic local alignment search tool (BLAST) of the National Center for Biotechnology Information (NCBI). GenBank accession numbers for the obtained sequences were from OK045681 to OK045688.

2.3. Antioxidant and immune response analysis

Once at the laboratory, mucus samples were centrifuged (1500 ×*g* for 10 min at 4 °C) and the recovered supernatants were stored at −80 °C until biochemical analysis. The liver tissues were homogenized using a TrisHCl buffer at a 7.5 pH using a small sample dispersing system (Ultra-Turrax® Disperser, IKA) and then centrifuged (9000 ×*g*, for 10 min, 4 °C; Sigma 3 K30) (Solomando et al., 2020). The supernatants were collected and kept in the freezer at −80 °C until analysis. For all samples, total protein content was determined in a microplate reader (BioTek®, PowerWaveXS) by a colorimetric method (Biorad Protein Assay), using bovine serum albumin (BSA) as a standard to normalize all biochemical results.

The activities of the antioxidant enzymes activities of CAT (Aebi, 1984) and SOD (Flohé and Ötting, 1984) were determined in liver and mucus samples whereas GPx (Flohé and Günzler, 1984), GR (Goldberg, 1984) and GST (Habig et al., 1974) were determined only in liver tissues. All enzymatic activities were monitored with a Shimadzu UV-2401 PC spectrophotometer at 25 °C. MDA levels, as a lipid peroxidation marker, were assayed in liver by using a commercial colorimetric kit specific for MDA determination (Merk, Spain). Lysozyme activity in fish mucus was measured using a bacterial suspension of *Micrococcus lysodeikticus* cells (Lee and Yang, 2002). The activity was monitored at 450 nm in a microplate reader (BioTek®, PowerWaveXS). Total immunoglobulin (Ig) concentration was determined after precipitation with 12% 10,000 kDa polyethylene glycol for 2 h (Milla et al., 2010). After centrifugation (1000 ×*g* for 10 min), the supernatants were collected and the protein levels determined. The total Ig concentration was calculated by subtracting this value from the total protein concentration in the mucus before precipitation.

2.4. Statistical analysis of data

For the statistical analysis, two groups were defined depending on the degree of infection by *Scaphanocephalus* sp.: low infection, including individuals from Es Freus with 0–1 parasites per fish, and high infection, fish from Cala Jondal with over 7 parasites per fish. Due to the lack of normality of the data, a non-parametrical Kruskall-Wallis test was performed to assess the statistical differences between groups. Results were expressed as mean ± standard error of the mean (S.E.M.) and all differences were considered significant at $p < 0.05$. Statistical analyses

were carried out using R version 3.5.3 (R Core Team 2019).

3. Results

3.1. Parasite abundance

A total of 48 fish were caught (22 in Cala Jondal and 26 in Es Freus), with an average size of 14.7 ± 2.6 cm and weight of 38.9 ± 25.1 g. Fish specimens affected by the parasite presented a pigmented dermatopathy that reflected a focal dermatitis caused by the encysted digenean metacercaria, compatible with a diagnosis of black spot disease.

The number of parasites was found to be significantly different in the two locations, with a higher number of parasites found in Cala Jondal (12.3 ± 11.4 parasites/individual) when compared to Es Freus (1.3 ± 1.3 parasites/individual) ($p < 0.05$). Parasite abundance ranged from 0 parasites in Es Freus to an individual with 51 parasites in Cala Jondal (Table 1).

3.2. Parasite identification

Amplification was obtained by PCR in 8 out of 14 analysed metacercaries. A sequence with 1283 base pairs of the LSU rDNA gene of the parasite was obtained by aligning the sequences of the different PCR amplicons. The excysted metacercaries were identified as *Scaphanocephalus* sp. based on molecular parameters. The sequences obtained in this study ($N = 8$) showed 98.6–98.4% identity to the 4 *Scaphanocephalus* sp. sequences available in GenBank (MN160569, MN160569, MT461356, MK680936). LSU rDNA obtained sequences were deposited in GenBank under the accession numbers OK045681 to OK045688.

3.3. Antioxidant and immune response

The immune and antioxidant biomarker results from mucus are presented in Figs. 3 and 4. No significant differences were observed in CAT activity in the mucus when comparing fish with low and high parasite abundance ($p = 0.14$) (Fig. 3A). Significant differences were found for SOD activity between locations ($p = 0.02$), being higher in those *X. novacula* with more parasites (Fig. 3B). Also, immune parameters, lysozyme activity (Fig. 4A) and total Ig concentration (Fig. 4B) in mucus were significantly higher ($p = 0.02$ and $p = 0.03$, respectively) in the specimens of *X. novacula* affected by the parasite.

The biomarkers of oxidative stress determined in liver are presented in Table 2. The CAT, GPx and GST activities were significantly higher in the fish that presented a higher parasitic infection ($p = 0.04$, $p < 0.05$ and $p = 0.01$), while SOD, GRd and MDA showed a similar trend without statistical differences ($p = 0.19$, $p = 0.89$, $p = 0.79$).

4. Discussion

The present results have shown that black spot disease induces an immune response and causes oxidative stress in *X. novacula*. All parasites recovered that yielded molecular data, were consistent with *Scaphanocephalus* sp. This kind of parasites is frequently found on the pectoral fins and skin of fish reef species such as parrotfishes (Shimose et al., 2020). Furthermore, diet studies of *X. novacula* have showed that this fish feeds mainly on molluscs, but also on shrimps, crabs, mysids, polychaetas, sipunculans, echinoderms and teleosts (Beltrano et al., 2006; Cardinale et al., 1997; Castriota et al., 2005). This feeding pattern is compatible with the putative life cycle of *Scaphanocephalus* sp. whose first intermediate hosts are molluscs (Galaktionov and Dobrovolskij, 2003; Kohl et al., 2019). However, whether the fish are infected by feeding on molluscs or by being in contact with free swimming cercariae near molluscs remains unclear and needs additional research.

The presence in the environment of natural and anthropic stressors including pollutants or excess organic matter can lead to an increase in ROS production (Centeno, 2008). Interactions with other species have been also reported to induce oxidative stress, such as the exposure of *Oreochromis* sp. to cyanobacteria (Jos et al., 2005) or the arrival of invasive species on native species (Jos et al., 2005; Sureda et al., 2006). In accordance with this previous data, the interaction of *X. novacula* with *Scaphanocephalus* sp. affects the host and appears to cause oxidative stress.

The increasing concern for animal welfare leads scientists to search for new non-invasive ways to study organisms. As a non-invasive way to obtain information related to the immune and oxidative stress levels, mucus from the fish skin has been used. Fish mucus has been shown to vary and increase in quantity and cell size under stressful conditions, such as handling and disease (Vatsos et al., 2010), so that it is considered a good indicator of physiological stress (De Mercado et al., 2018). Mucus acts not only as a physical barrier but also has a series of components to try preventing parasitic infection. Increased antioxidant enzymes have been observed in fish mucus following exposure to pollutants (Dzul-Caamal et al., 2016). In the current study, lysozyme and total Ig concentrations were significantly higher in mucus in severely parasitized fish, indicating that these fish generate an immune response against the infection (Saurabh and Sahoo, 2008). In this sense, it has been previously described that mucus is capable to limit parasite load for ectoparasitic monogeneans (Buchmann and Lindenstrøm, 2002). In accordance, increased lysozyme activity has been observed in mucus of greater amberjack (*Seriola dumerili*) after infection with the monogenean parasite *Neobenedenia girellae* (see Fernández-Montero Torrecillas et al., 2021). An increase in lysozyme activity and Ig levels was also observed in mucus of large yellow croaker, *Pseudosciaena crocea*, infected by the ciliated protozoan parasite, *Cryptocaryon irritans* (see Yin et al., 2015). In addition to these immunological parameters, the activities of the antioxidant enzymes CAT and SOD have been analysed in the same mucus, observing a significant higher SOD activity in fish presenting a higher parasite load. These results indicate that the parasite induces a stressful situation associated with the fish immune response to the pathogen.

In the liver, significant higher CAT, GPx and GST activities were detected in the most parasitized *X. novacula* group. This antioxidant enzyme induction in liver is indicative of generalised oxidative stress related to the parasite infection. In fact, increased oxidative stress in liver caused by parasites has also been reported in previous studies (Bello et al., 2000; De Mercado et al., 2018; Marcogliese et al., 2005). An increased oxidative burst and the activities of CAT, SOD and GST were also observed in liver of the pangasius catfish, *Pangasianodon hypophthalmus*, infected by the dactylogyrid monogenean *Thaparocleidus* sp. (Kumar et al., 2017). Parasite infections can lead to elevated oxidative stress and comprised metabolic demands, meaning that, in addition to direct effects, parasites might make fish more vulnerable to other pollutants, to excess of organic matter or to additional pathologies (Marcogliese et al., 2005).

Although most of the reported studies on oxidative stress caused by parasites in fish were focused on lipid peroxidation (MDA) (Bello et al., 2000; Mozhdeganloo and Heidarpour, 2014; Stumbo et al., 2012), no significant differences were observed in *X. novacula* liver MDA levels in the present study, suggesting that the antioxidant response was enough

Table 1
Abundance of *Scaphanocephalus* sp. in the skin of pearly razorfish (*Xyrichtys novacula*) sampled at Cala Jondal and Es Freus in Eivissa and Formentera (Balearic Islands).

N° *Scaphanocephalus* sp.	0–1	2–5	5–10	>10	Min	Max	Total
Cala Jondal	0 (0%)	2 (9.1%)	12 (54.5%)	8 (36.4%)	3	51	22 (100%)
Es Freus	16 (61.5%)	10 (38.5%)	0 (0%)	0 (0%)	0	5	26 (100%)

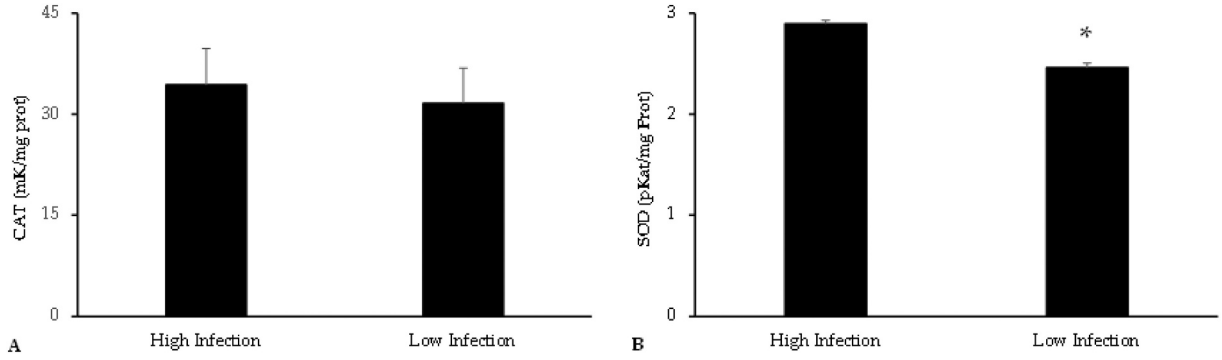

Fig. 3. Activities of catalase (CAT) (A) and superoxide dismutase (SOD) (B) in mucus of pearly razorfish (*Xyrichtys novacula*) sampled in Eivissa and Formentera (Balearic Islands) depending on the degree of infection by *Scaphanocephalus* sp. Data are presented as mean ± S.E.M. * indicates significant differences between groups (Kruskall-wallis test, $p < 0.05$).

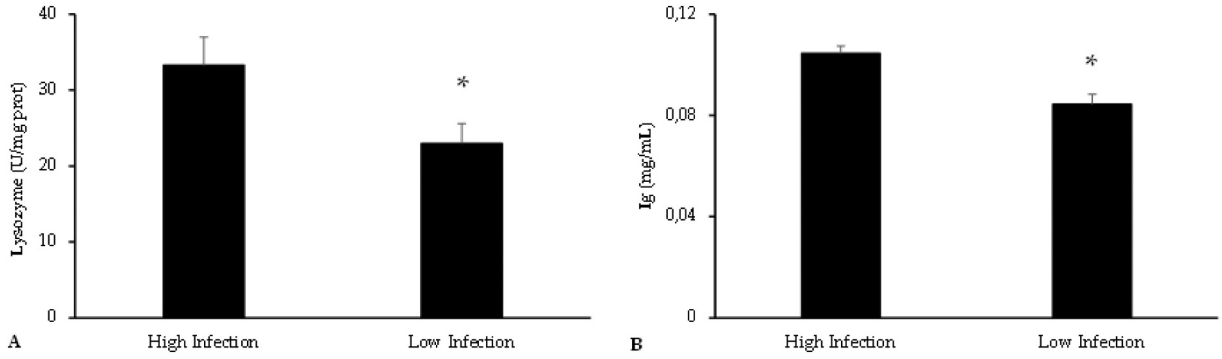

Fig. 4. Activity of lysozyme (A) and total immunoglobulin (Ig) concentration (B) in mucus of pearly razorfish (*Xyrichtys novacula*) sampled in Eivissa and Formentera (Balearic Islands) depending on the degree of infection by *Scaphanocephalus* sp. Data are presented as mean ± S.E.M. * indicates significant differences between groups (Kruskall-wallis test, $p < 0.05$).

Table 2
Oxidative stress biomarkers determined in liver homogenates of pearly razorfish (*Xyrichtys novacula*) sampled in Eivissa and Formentera (Balearic Islands) depending on the degree of infection by *Scaphanocephalus* sp.

Infection degree	CAT	SOD	GPx	GRd	GST	MDA
High	67.7 ± 6.8	0.3 ± 0.03	1.2 ± 0.1	0.5 ± 0.06	2.4 ± 0.4	0.6 ± 0.08
Low	50.1 ± 3.5*	0.2 ± 0.02	0.6 ± 0.05*	0.4 ± 0.04	1.5 ± 0.2*	0.6 ± 0.06

Catalase (CAT); Superoxide dismutase (SOD); Glutathione peroxidase (GPx), Glutathione reductase (GRd); Glutathione S-Transferase (GST); Malondialdehyde (MDA). Data are presented as mean ± S.E.M. * indicates significant differences between groups (Kruskall-wallis test, $p < 0.05$).

to prevent oxidative damage to lipids in the liver. Thus, parasitized fish seem to respond to the infection by activating their antioxidant systems. Parasitism can cause changes in the metabolic pathways of its host to counteract the infective process. Therefore, an increase in antioxidant mechanisms in parasitized fish could be related to the increased metabolic activity induced by the parasites (Kumar et al., 2017; Radovanović et al., 2015).

Parasite abundance seems to be related to favourability of the environment for the parasite. Katahira et al. (2021) suggested that infectious parasites hot spots are found in nearshore sandy areas and fish which inhabit deep areas would not be affected as much, due to spatial mismatch. These results match those of the current study. Despite fish being caught at similar depths, Cala Jondal is generally shallower, which could contribute to higher parasite abundance. However, at the moment, the first intermediate host is a unidentified mollusc (Kohl et al., 2019). This association is known to be much more host specific and, therefore, the presence and abundance of this host greatly conditions the existence of *Scaphanocephalus* sp. (Dennis et al., 2019). Thus, it is possible that Cala Jondal is a more favourable environment of this host, and, consequently, enables a higher quantity of parasites. Moreover, Cala Jondal is a fished area, whilst Es Freus is an MPA, where recreational fishing is prohibited. In this sense, it is possible that fishing has removed predators of intermediate hosts, making them more numerous, as seen in various studies (Baum and Worm, 2009; Soler et al., 2015; Walters et al., 1999).

In addition, fish vulnerability to infection cannot be overlooked. Cala Jondal is a semi-closed, heavily used area, particularly by recreational seasonal yachts, which are suspected of emptying sewage in this area in addition to releasing potentially polluting waste products. This, coupled with fishing pressure, can make fish more vulnerable to parasite infection. In short, these and other possible factors can also contribute to generating oxidative stress. In this sense, it would be advisable to carry out studies based on artificial infection with parasite in healthy fish, and check the response in the mucus and liver but also in tissues with an immunological role such as the spleen and kidney to evaluate the effects of the infection, avoiding environmental interference. Also, further studies are needed to rule out the possibility that pollutants or other environmental conditions create increased vulnerability to the parasite.

5. Conclusions

In conclusion, *Scaphanocephalus* sp. infection affects the antioxidant and immune response of *X. novacula*, and these changes were observed in liver and epithelial mucus. The increase in the secretion of lysozyme and total Ig, and the activation of antioxidant defences in the mucus of

X. novacula indicated that a metabolic and immune response was generated against infection by *Scaphanocephalus* sp. There is a geographical relationship between parasite distribution and the environmental and human pressure, which appears to be associated with higher abundance of parasitism. However, more studies are necessary to assess whether the increased presence of contaminants may make *X. novacula* more vulnerable to the parasite or if other factors related to the environment would increase the load of the parasite. In addition, long-term studies are required to assess the progression of the infection to currently unaffected areas and its possible effects on the fitness of their hosts, both at individual and population levels.

Institutional review board statement

The study was conducted in accordance with the EU Directive 2010/63/EU for animal experiments and has been approved by the Ethics Committee for Animal Experimentation of the University of the Balearic Islands (Ref. 020/06/AEXP).

Ethics approval statement

The study was approved by the Ethics Committee for Animal Experimentation of the University of the Balearic Islands (Ref. 020/06/AEXP).

CRediT authorship contribution statement

Amanda Cohen-Sánchez: Investigation, Formal analysis, Writing – original draft. **José María Valencia:** Methodology, Investigation. **Antonio Box:** Conceptualization, Investigation, Resources. **Antònia Solomando:** Investigation, Formal analysis. **Silvia Tejada:** Investigation, Funding acquisition, Visualization. **Samuel Pinya:** Conceptualization, Methodology, Investigation, Funding acquisition. **Gaetano Catanese:** Methodology, Investigation. **Antoni Sureda:** Conceptualization, Methodology, Investigation, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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