Science of the Total Environment 951 (2024) 175848

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Exploring the impact of high salinity and parasite infection on antioxidant and immune systems in *Coris julis* in the Pityusic Islands (Spain)

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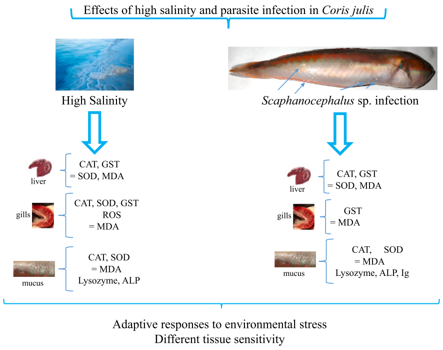
HIGHLIGHTS

- Salinity and parasites trigger antioxidant and immune defences in *Coris julis*.
- The effects of salinity were more evident in gills and the parasite in mucus.
- The response of *C. julis* to both stressors is enough to avoid oxidative damage.
- Analysing diverse tissues reveals early signs of stress impacts on marine fauna.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Damià Barceló

Keywords:
Coris julis
Parasite
Salinity
Pityusic islands
Oxidative stress

ABSTRACT

Climate change associated with human activities alters marine ecosystems and causes imbalances and abrupt changes in sea conditions. Scarce freshwater resources for human consumption often prompt the construction of desalination plants, which discharge significant amounts of brine into the sea, potentially elevating salinity levels. Furthermore, global trade together with higher temperature and pollution can facilitate the spread of parasites. The aim of this study was to assess the potential effects of salinity, an abiotic stressor, and *Scaphanocephalus* sp. parasitic infection responsible for black spot disease, a biotic stressor, on *Coris julis*, a common fish in the Balearic Islands (Spain). Fish were sampled from an area affected by a desalination plant, one with a high rate of parasite infection and a control area, and biomarkers were analysed in the liver, gills and epithelial

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https://doi.org/10.1016/j.scitotenv.2024.175848
Received 29 May 2024; Received in revised form 23 August 2024; Accepted 26 August 2024
Available online 27 August 2024

mucosa. Both salinity and the parasite induced increases in catalase (CAT) and glutathione s-transferase activities in the liver, while superoxide dismutase (SOD) did not show significant changes. The effects of salinity were evident to a greater extent in the gills with an increase in the activity of all enzymes, as well as in the production of reactive species. The effects of the parasite were mainly observed in the mucus with significant increases in CAT and SOD activities. Regarding immune response markers in the mucus, both stressors induced an increase in lysozyme and alkaline phosphatase activities, and in the case of the parasite, also an increase in immunoglobulins. Malondialdehyde, as an indicator of oxidative damage, remained unchanged. In conclusion, both abiotic and abiotic stress induce a stress situation in *C. julis* that responds by activating its antioxidant and immune defence mechanisms but does cause oxidative damage. The differential tissue response to different stressors highlights the value of analysing multiple tissues to detect early indicators of diverse impacts on marine fauna.

1. Introduction

Fluctuations in the ocean are rapidly increasing due to climate change and human impacts (Halpern et al., 2019; He and Silliman, 2019). These changes can lead to alterations in species, from genes to entire ecosystems, affecting many aspects of their life (Brierley and Kingsford, 2009). The Balearic Islands (Western Mediterranean, Spain), like most islands, are particularly vulnerable to direct human effects on the marine environment (Giorgi, 2006; Torres et al., 2021). Islands, with their limited resources due to restricted land area and fragile ecosystems, are particularly vulnerable to the impacts of climate change.

The high touristic affluence in the Balearic Islands, combined with the islands' limited freshwater resources, require the installation of desalinization plants (Torres et al., 2021). These plants render drinking water, thus alleviating the scarcity of freshwater sources in the islands. Conversely, the excess salts extracted from the water are discharged into the sea, creating areas with elevated salinity levels. Specifically, the island of Ibiza hosts a total of 3 desalinization plants, and one in Formentera based on reverse osmosis which together supplies 70 % of potable urban water in Ibiza and 100 % in Formentera (Vaquer-Sunyer et al., 2021). The impact of these plants has been examined in various environments revealing effects on sea grass beds (Capó et al., 2020; Fernández-Torquemada et al., 2005), soft-sediments (Riera et al., 2012), and temperate reefs (Clark et al., 2018). Discharges have also been found to influence fish behaviour and aggregation (Kelaher et al., 2020). Although direct effects are primarily observed in habitat-forming species such as the seagrasses, reefs and sediment environments, brine discharges result in significant increases in salinity and temperature, as well as the accumulation of metals, hydrocarbons and toxic anti-fouling compounds in receiving waters (Roberts et al., 2010). These latter pollutants can be accumulated in fish that inhabiting adjacent areas, potentially leading to adverse effects and oxidative stress.

Another consequence of both climate changes and anthropogenic pressures is the entry of new species. Such is the case with the parasite *Scaphanocephalus* sp. (Platyhelminthes, Trematoda, Heterophyidae), found around the island of Ibiza parasitizing *Xyrichtys novacula* (Cohen-Sánchez et al., 2023a, 2023b), and later observed spreading to other species of the Labridae family. This species is commonly found on the skin of many marine fish species (Dennis et al., 2019), where it becomes embedded in the skin and produces a cyst (metacercariae) around it. The affected fish typically develop a second outer dark tissue capsule for protection (Kohl et al., 2019) which gives this parasite infection the name of "black spot disease". The presence of this disease associated with *Scaphanocephalus* sp. has been evidenced in different areas including the Caribbean and the Gulf of Arabia, however, in the Mediterranean it was evidenced for the first time in 2015 in specimens of *X. novacula* (Cohen-Sánchez et al., 2023a, 2023b; Locke et al., 2024). This parasite is characterized by a complex life cycle, involving an initial mollusc host stage, followed by marine fish and finally fish-eating birds such as the osprey, *Pandion haliaetus*, as definitive hosts (Foronda et al., 2009; Galaktionov and Dobrovolskij, 2013). The presence of this parasite can induce physiological stress and alterations reproduction and behaviour (Cohen-Sánchez et al., 2023a, 2023b; Timi and Poulin, 2020), making hosts more vulnerable to other threats, such as fishing, diseases and predation (Lafferty, 2008).

Fish react to external factors in various ways in order to maintain physiological homeostasis (Banaee et al., 2024a; Faggio et al., 2014; Impellitteri et al., 2023). Externally, they can produce protective mucus, which serves as both a dynamic physical barrier and a biochemical barrier containing numerous immune molecules, such as lysozyme, alkaline phosphatase (ALP) and immunoglobulins in order to limit the growth of parasites (Reverter et al., 2018; Sridhar et al., 2021; Vallejo et al., 2009). Additionally, these environmental changes can induce stress in affected organisms, leading to increased production of reactive oxygen species (ROS) which, if excessive, can cause oxidative damage to biomolecules including lipids, proteins and DNA (Pinya et al., 2016). To mitigate oxidative damage and minimize the effects of ROS, organisms have developed antioxidant defence mechanisms that neutralize excess of ROS and maintains the redox balance while also limiting cell damage (Clarkson and Thompson, 2000; Matés et al., 1999). The main antioxidant enzymes are catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) which depends on glutathione reductase (GRd) to regenerate glutathione (Hoseinifar et al., 2020). In addition to antioxidant enzymes, another enzyme widely used as a biomarker is glutathione S-transferase (GST), a phase II enzyme in the detoxification process that conjugates glutathione with different xenobiotics, increases its solubility in water and facilitates its excretion (Allocati et al., 2018; Sureda et al., 2006). Malondialdehyde (MDA) is also used as a stress biomarker, as it is the end-product of lipid peroxidation process (Alomar et al., 2017; Banaee et al., 2024b).

Coris julis (Linnaeus, 1758), also known as rainbow wrasse, belongs to the family Labridae, and is one of the most common Mediterranean wrasses. It inhabits coastal waters, including rocky areas and sea grass beds, up to about 50 m depth (Lejeune, 1987). This species primarily feeds on zoobenthic species, such as molluscs and crustaceans (Pinnegar et al., 2000; Sureda et al., 2006). *C. julis* is a protogynous hermaphrodite as well as diandric and permanently dichromatic (Lejeune, 1987). Rainbow wrasses exhibit limited dispersal, displaying sedentary habits and with no described migration movements (Witkowski et al., 2016). This species is very common around the Balearic Islands (Riera et al., 1988). It is particularly suitable for study due to its abundance and adaptability, its sedentary lifestyle and feeding habits which facilitate bio-accumulation of xenobiotic compounds, as well as all this characteristics make them vulnerable to parasites (Fasulo et al., 2010).

This study aims to use *C. julis*, as a study case for the possible future conditions of the Mediterranean Sea. With increasing population, tourism, globalization, and global transport, impacts such as brine discharge from desalination plants and the spread of parasites are expected to escalate. Therefore, investigating the potential impacts of these changing environments on an adaptable species like *C. julis* can provide insights into the future trajectory of marine ecosystems.

2. Materials and methods

2.1. Sampling sites

For the present study, three sampling stations with differential characteristics have been selected: Talamanca (near a desalination plant), Cala Jondal (with a high prevalence of parasite infections) and Es Freus (control area) (Fig. 1). Specifically, the brine discharge area from the desalination plant is located to the east of the bay where Talamanca (38°54′49.8″N, 1°27′07.8″E) beach is situated, halfway to Sa Punta Grossa. Cala Jondal (38°52′05.4″N, 1°18′40.1″E) is a closed bay, with little water recirculation (Fig. 2). It is a popular site for boat anchoring, making it prone to black seawater and marine litter discharges, accumulating organic matter and pollutants in its sediments (Carreño and Lloret, 2021). This area is also considered a hotspot for *Scaphanocephalus* sp. parasite, with a high infection rate in different wrasse species such as *Thalassoma pavo* and *Symphodus tinca* (Cohen-Sánchez et al., 2023a, 2023b). The molecular identification of the parasite was carried out according to previously described procedure (Cohen-Sánchez et al., 2023a, 2023b). Es Freus (38°48′35.9″N 1°24′32.8″E), considered the control site, is part of a marine protected area where anchoring or recreational fishing is not allowed, and there is no evidence of parasite presence, maintaining normal salinity levels. In the three selected areas, rocky bottoms predominate with high coverage of *Posidonia oceanica*, where *C. julis* is abundant. In each area four different measures of temperature and salinity were carried out with a multiparameter probe Hanna HI9828.

2.2. Experimental procedure

A total of 27 fish ($N = 9$ per site) with similar size and weight were caught by line-fishing and worms were used as bait. The fish were then 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. Gills and liver samples were collected from all fish. Mucus samples were also taken from the epithelium of each of the sampled fish using a spatula, ensuring that the scales were not disturbed. After collection, the mucus was immediately placed in liquid nitrogen for preservation until transported to the laboratory, where it was stored at −80 °C until further use. For Cala Jondal, all captured fish presented evidence of parasite infection with at least

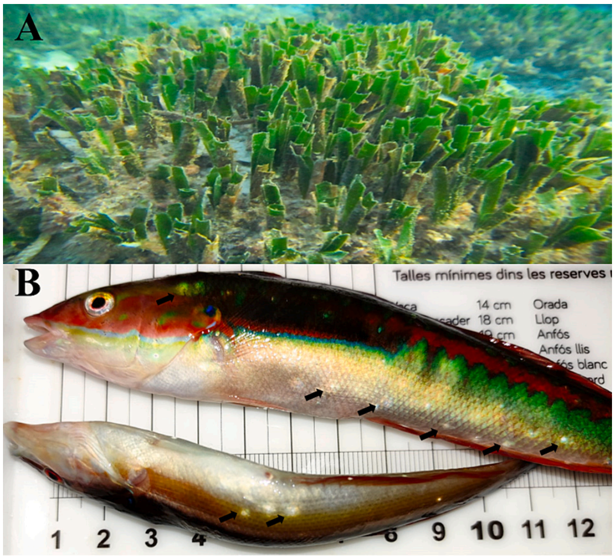

Fig. 2. Representative image of the effects of hypersaline water discharge in the Talamanca area on *Posidonia oceanica* leaves, which are notably reduced (A); and of a specimen of *Coris julis* from Cala Jondal infected by *Scaphanocephalus* sp. showing characteristic spots on its epithelium (black arrows).

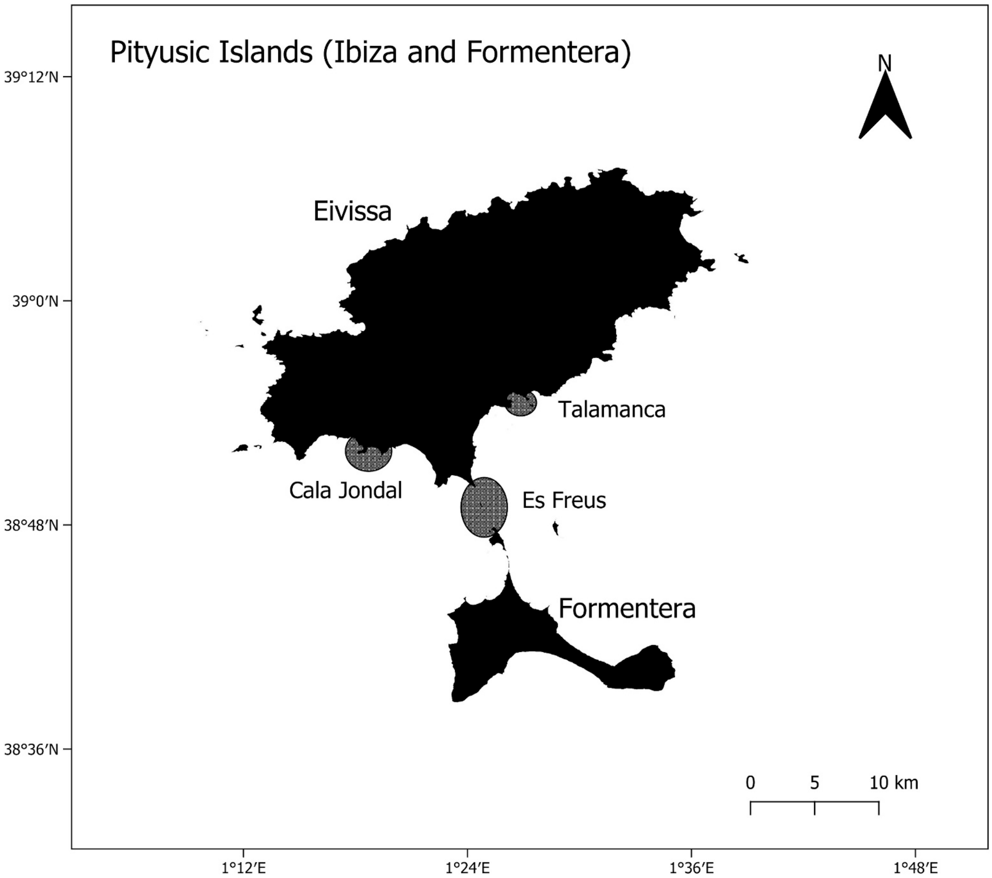

Fig. 1. Map of the sampling areas: Es Freus (Control), in a Marine Protected area (salinity 37.9 ± 0.1 PSU), Cala Jondal, area with high rates of parasite infection (salinity 38.0 ± 0.1 PSU) and, Talamanca, area directly affected by a desalination plant (salinity 40.1 ± 0.3 PSU). Sampling areas are highlighted with a shaded area.

counting 8 spots in each individual. Tissue samples were immediately placed in 1.5 mL tubes 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).

Prior to biochemical analysis, mucus samples were centrifuged (1500 ×*g* for 10 min at 4 °C, Sigma 3 K30) and the recovered supernatants used for the assays. The liver and gill tissues were homogenized using a TrisHCl buffer 100 mM, pH 7.5 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 (Merk S.L.U.), using bovine serum albumin as a standard to normalize all biochemical results.

2.3. Biomarker analysis

The activities of the antioxidant enzymes CAT and SOD were determined in liver, gills and mucus samples, whereas phase II detoxification enzyme GST was determined in liver and gill tissues. Also, the activity of the immune enzyme ALP and lysozyme were measured in mucus. Briefly, CAT activity (mK/mg prot) was determined following the method of Aebi based on the decomposition of H_2O_2 at 240 nm (Aebi, 1984). SOD activity (pKat/mg prot) was determined according to previously described method at 550 nm, using cytochrome as an indicator (Flohé and Ötting, 1984). GST activity (nKat/mg prot) was determined at 340 nm using GSH and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates (Habig et al., 1974). ALP activity in mucus was determined at 405 nm using *p*-nitrophenyl phosphate as a substrate. These enzymatic activities were monitored in a Shimadzu UV-2401 PC spectrophotometer at 25 °C. Lysozyme activity in 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).

ROS production was determined in liver and in gill homogenates using 2,7-dichlorofluorescin-diacetate (DCFH-DA) as indicator (Pinya et al., 2021). The fluorescence (Ex, 480 nm; Em 530 nm) was recorded at 25 °C for 1 h in FL 9800 Microplate Fluorescence Reader (Bio-Tek Instruments, Inc.). MDA levels, as a lipid peroxidation marker, were assayed in liver and gills by using a commercial colorimetric kit specific for MDA determination (Merk KGaA, Spain) following the manufacturer's instructions.

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

The effects of parasite presence and brine discharge outcome on oxidative stress biomarkers were assessed using a statistical analysis package (SPSS 27.0 for Windows®) (IBM® SPSS Inc., Chicago, IL, USA). The normality of the data was confirmed by the Shapiro–Wilk test and homogeneity of variance by the Levene's test. Statistical differences between the groups were carried out with one-way ANOVA followed by the Bonferroni post-hoc test. The results are presented as mean ± standard error of the mean (SEM) and $p < 0.05$ was considered statistically significant.

3. Results

A total of 27 fish, 9 per site and treatment, were caught in the three different sampling areas to proceed with the biomarker analysis of liver, gill and mucus. The mean total length (TL) of *C. julis* captured was 12.6 cm ± 0.3 cm ranging between 9.6 and 15.3 cm, and the mean weight was 18.3 g ± 1.1 cm ranging between 7.1 and 31.0 g. No differences in size and weight were observed in fish from the different sampling sites (Supplementary Material S1).

The salinity values of the different study areas presented the following values: the Talamanca area near the desalination plant 40.1 ± 0.3 PSU, while the areas of Cala Jondal (presence of the parasite) and Es Freus (control) presented normal values of 38.0 ± 0.1 and 37.9 ± 0.1 PSU, respectively. Temperature was similar in the three areas investigated: Talamanca 25.8 ± 0.1 °C, Cala Jondal 25.8 ± 0.1 °C and Es Freus 25.7 ± 0.1 °C.

Regarding the presence of parasites in the skin epithelium of the fish, no evidence was observed in the *C. julis* captured in the Talamanca and Es Freus areas, while in the Cala Jondal area an average of 14.3 ± 1.9 spots were reported per individual, with a range of between 8 and 23 parasites.

The activities of the antioxidant enzymes and GST in the liver of *C. julis* sampled in the three studied areas are presented in Fig. 3. Fish from the areas with high salinity and affected by the parasite exhibited greater activities of CAT (Bonferroni, $p = 0.039$ for salinity and $p = 0.022$ for parasite) and GST (Bonferroni, $p = 0.044$ for salinity and $p = 0.023$ for parasite), respect to the control area, whereas no statistical differences were observed in SOD activity (ANOVA, $p = 0.142$).

In the case of gill tissue, higher activity of GST was observed in the areas with high salinity and affected by the parasite when compared with the control group (Bonferroni, $p = 0.001$ for salinity and $p = 0.028$ for parasite), whilst for CAT and SOD, only salinity was found to be significantly increased respect to the control group (Bonferroni, $p = 0.011$ and $p = 0.012$, respectively) (Fig. 4).

For the collected mucus, significant statistical differences in CAT and SOD activities were also found between the three studied areas (Fig. 5). Specifically, CAT activity was higher in the salinity and parasitism groups compared to the control group (Bonferroni, $p = 0.024$ and $\text{p} = 0.001$, respectively). Similar results were observed for SOD with higher activities in the salinity and parasitism groups compared to the control group (Bonferroni, $p = 0.036$ and $p < 0.001$, respectively). For SOD, the enzyme activity was statistically higher in the parasitized group than in the high salinity one (Bonferroni, $p = 0.038$).

The levels of ROS production and MDA in liver and gills of *C. julis* are presented in Table 1. Although ROS production in the liver was higher in both salinity and parasite areas when compared to the control, the differences did not reach statistical significance (ANOVA, $p = 0.092$). However, for gills, statistical differences were only found for higher salinity respect to the parasite and control areas (Bonferroni, $\text{p} = 0.024$ and $p = 0.002$, respectively). No statistical differences were reported in MDA in any of the tissues analysed (ANOVA, $p = 0.981$ for liver and $p = 0.960$ for gills).

Lysozyme, ALP and Ig analysed in mucus of *C. julis* are shown in Table 2. Results evidenced that fish from both salinity and parasite areas presented significantly higher values of lysozyme ($\text{p} = 0.002$ and $\text{p} < 0.001$, respectively; Bonferroni) and ALP ($p = 0.016$ and $p = 0.004$ respectively, Bonferroni) when compared with the control group. However, for Ig, only the fish with parasite presence presented significant higher values respect to both the salinity and control groups (Bonferroni, $\text{p} < 0.001$ for both cases).

4. Discussion

The oceans are undergoing rapid changes driven by various external and internal factors (Irwin et al., 2015). These changes can significantly impact the behaviour, life cycles, and internal processes of the different

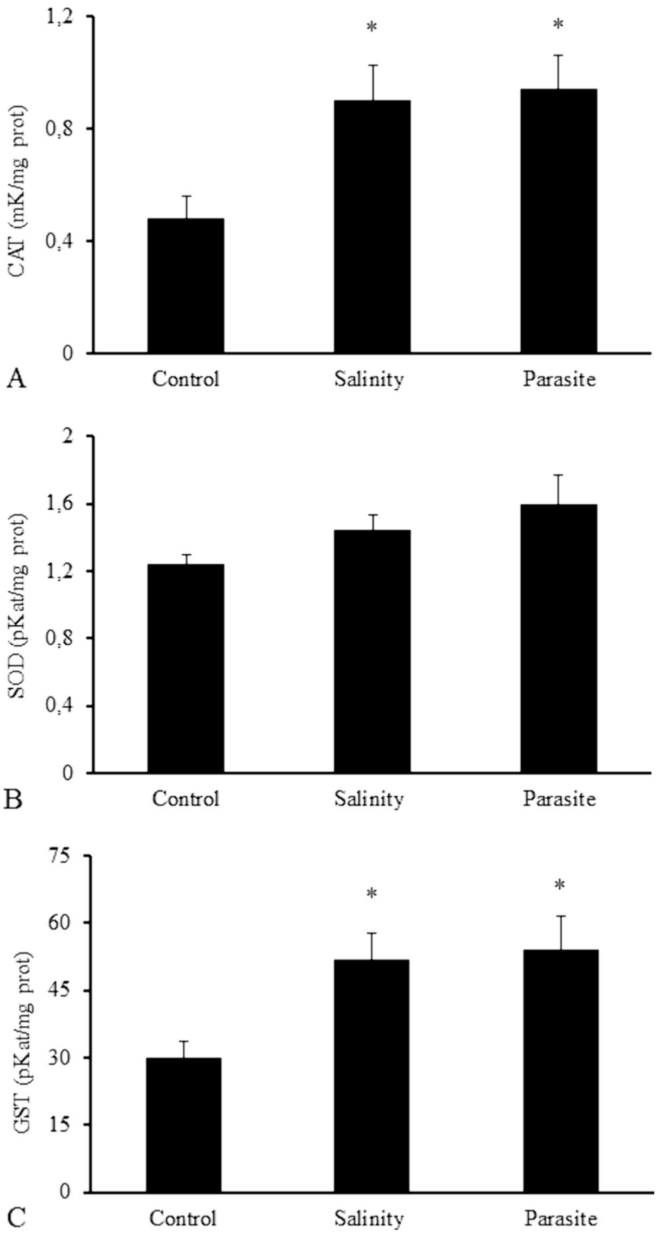

Fig. 3. Activities of (A) catalase (CAT), (B) superoxide dismutase (SOD) and (C) glutathione s-transferase (GST) in the liver of *C. julis* exposed to high salinity or parasite infection. * Indicates significant differences respect to control, $p < 0.05$.

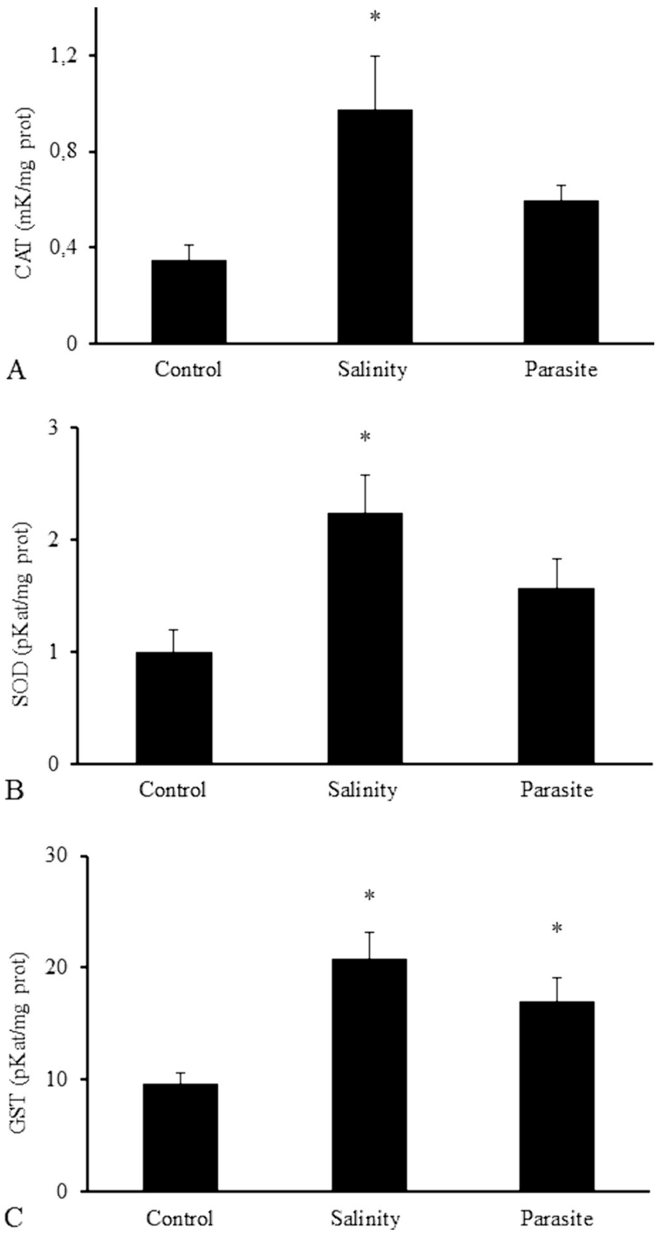

Fig. 4. Activities of (A) catalase (CAT), (B) superoxide dismutase (SOD) and (C) glutathione s-transferase (GST) in the gills of *C. julis* exposed to high salinity or parasite infection. * Indicates significant differences respect to control, $p < 0.05$.

species inhabiting them (Pörtner and Peck, 2010). One consequence of such changes is the production and accumulation of ROS in exposed organisms. Therefore, the analysis of antioxidant/prooxidant biomarkers can provide insights into whether these changes are causing oxidative stress. This study addressed two significant challenges affecting coastal species in the Pityusic islands: high salinity resulting from to brine discharges from desalinization plants, and parasite infection. The island of Ibiza, due to its reduced freshwater sources, depends mainly on desalinated water, leading to the discharge of substantial amounts of brine back into the sea, thereby altering local salinity levels (Vaquer-Sunyer et al., 2021; Torres et al., 2021). Specifically, this study focuses on the Talamanca area, where a desalination plant releases brine into its waters. Regarding parasites, *Scanocephalus* sp. was first identified in Ibiza in 2015 causing a skin disease in fish of the wrasse family known as black spot disease (Cohen-Sánchez et al., 2023a, 2023b). and has been seen to affect other species (personal observation). This parasite was initially described affecting *X. novacula*, but has subsequently been observed in other species such as *C. julis*, *T. pavo*, *Symphodus* sp. or *Labrus* sp. The main results of the present study revealed that both types of stressors induced a situation of oxidative stress, preferentially affecting the gills in the case of salinity and epithelial mucus in the case of the parasite, also accompanied by an immune response. Furthermore, the liver, as the body's main metabolic organ, also exhibited an increase in oxidative stress markers in both fish affected by salinity and the parasite.

Salinity stress can occur when there are rapid shifts in water salinity due to factors such as rainstorms, evaporation, tidal water flow, brine discharge or drought (Freitas et al., 2021). However, even smaller and gradual changes in salinity can also affect organisms, causing osmotic stress (Evans and Kültz, 2020). Such fluctuations in salinity have been found to impact fish species, inducing oxidative stress and impairing the immune response (Chowdhury and Saikia, 2020), and even interfering

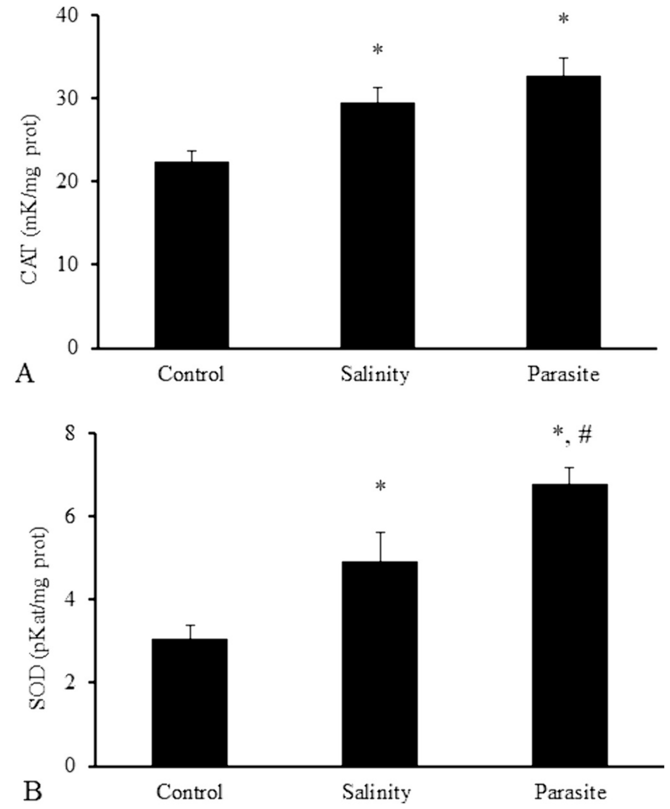

Fig. 5. Activities of (A) catalase (CAT) and, (B) superoxide dismutase (SOD) in the mucus of *C. julis* exposed to high salinity or parasite infection. * Indicates significant differences respect to control, # Indicates significant differences between salinity and parasite, $p < 0.05$.

Table 1
Reactive oxygen species production and malondialdehyde levels in liver and gills of *C. julis*.

	Control	Salinity	Parasite	*p*-Value
Liver				
ROS (% control)	100.0 ± 9.3	130.4 ± 6.9	110.9 ± 11.7	0.092
MDA (nM/mg prot)	5.59 ± 0.14	5.72 ± 0.64	5.64 ± 0.53	0.981
Gills				
ROS (% control)	100.0 ± 12.9	168.26 ± 15.3*	117.31 ± 7.8$^{\#}$	0.002
MDA (nM/mg prot)	8.37 ± 0.62	8.77 ± 1.02	8.73 ± 1.42	0.960

Abbreviations: ROS, reactive oxygen species; MDA, malondialdehyde. * differences respect the control group; # differences respect the salinity group, $p < 0.05$.

Table 2
Immune parameters in mucus of *C. julis*.

	Control	Salinity	Parasite	p-Value
Mucus				
Lysozyme (U/mg prot)	71.2 ± 3.8	92.0 ± 3.3*	96.0 ± 3.9*	<0.001
ALP (nKat/mg prot)	4.46 ± 0.39	6.29 ± 0.31*	6.66 ± 0.53*	0.003
Ig (ng/mL)	0.62 ± 0.03	0.65 ± 0.02	0.92 ± 0.04*,$^{\#}$	<0.001

Abbreviations: ALP, alkaline phosphatase; Ig, immunoglobulins. * differences respect the control group; # differences respect the salinity group, $p < 0.05$.

with their growth performance (Phuc et al., 2017). Most studies focus on freshwater species, particularly those used in aquaculture, given the susceptibility of fish farms to fluctuations in salinity levels. Fish farms predominantly rely on rivers and lakes for water sources. However, the rise in temperatures linked to climate change has resulted in escalated evaporation rates, consequently elevating salinity levels (Dawood et al., 2021, 2022; Phuc et al., 2017). Nonetheless, marine fish in particular tend to experience greater environmental variability due to the dynamic nature of the marine environment (Birnie-Gauvin et al., 2017). Changes in salt concentrations can trigger oxidative stress, as evidenced by the increased activities in antioxidant enzymes CAT and SOD observed in *Mytilus galloprovincialis* subjected to high salinity (Freitas et al., 2017). Our results align with these findings, as we observed higher activities of antioxidant enzymes and GST, mainly in gills because it is the tissue most directly exposed to salt stress. Also, an increase in the liver activities of CAT and GST has been observed, probably to cope with the demands that the increased salinity requires. In this sense, an increase in blood glucose levels, indicative of hepatic mobilization of energy resources has been observed in Asian seabass (*Lates calcarifer*) exposed to high salinity (Azodi et al., 2021). Similar to the present results, increased CAT and SOD activities were reported in the liver of sturgeon *Acipenser naccarii* exposed to high salinity levels, further highlighting the oxidative stress potential of salinity on fish (Martínez-Álvarez et al., 2002). Additionally, studies on olive flounder, *Paralichthys olivaceus*, have also reported an increase in the liver GST gene expression with the higher salinity exposure (Choi et al., 2008). Moreover, studies show that increased and continuous salinity stress can lead to a high accumulation of ROS, resulting in lipid peroxidation, as evidenced by the high MDA levels (Dragun et al., 2017; Liu et al., 2007). However, in the present study, an increase in ROS production has been observed in gills, but not in liver, which is not accompanied by higher MDA values as a marker of oxidative damage to lipids. The similar values in MDA in *C. julis* exposed to high salinity would indicate that although there is a stress situation with an increase in the production of ROS, the antioxidant response mechanisms are capable of avoiding oxidative damage.

Mucus is being more commonly used as a non-invasive method to study stress and immune response on fish. This component has been known to vary in composition under stressful conditions and therefore can be a good indicator of physiological stress (De Mercado et al., 2018; Rashidian et al., 2021; Vasto et al., 2010). In addition to the physical barrier with which mucus protects the fish, there are also components which act to prevent attacks such as parasite infestation. Previous studies evidenced that some components of exuded mucus including cortisol, glucose and lactate are modified in response to stressors and could be considered as feasible biomarkers that can be measured in non-invasive way (Fernández-Alacid et al., 2018, 2019). The exposure of European sea bass (*Dicentrarchus labrax*) to hyperosmotic (50 ‰) salinity conditions for 15 days increased the skin mucus osmolarity as well as the release of proteins, glucose and lactate (Ordóñez-Grande et al., 2021). Increased salinity has been also reported in *Cyprinus carpio* to increase secretory activity in intraepithelial and stromal telocytes and the contact with macrophages via telopodes (Emeish et al., 2023). However, there are no studies that analyse the effects of chronic exposure to high salinity in oxidative stress and immunological markers in marine fish. In the present study, the greater values of antioxidant enzymes, lysozyme and ALP in mucus of fish exposed to salinity is indicative of a stressful situation. The absence of changes in Ig levels, unlike the case of infection with the pathogen, would be indicative of a non-specific response to the salt stress situation.

Parasite infection has previously been found to cause oxidative stress in their hosts (Pawłowska et al., 2024). This particular parasite, *Scaphanocephalus* sp., found in *X. novacula* in the island of Ibiza for first time in 2015, also triggered an oxidative stress response in the infected fish in a direct relationship with the degree of infection (Cohen-Sánchez et al., 2023a, 2023b). Given the close taxonomic relationship between *C. julis* and *X. novacula* as members of the labrid family, this species of parasite is also capable to infect *C. julis* and induce the black spot disease. Biomarker analysis showed higher antioxidant activity in the liver and mucus of infected individuals, but not in gills suggesting that in the latter structure it is not significantly affected by the parasite. Antioxidant enzyme induction in liver indicates that the presence of the parasite infection leads to a situation of generalized oxidative stress (Bello et al., 2000; De Mercado et al., 2018; Marcogliese et al., 2005). In fact, the induction of antioxidant enzyme activities in the liver could arise from increased metabolic demands in response to parasitic infestation, leading to increased oxygen consumption and cellular stress. However, this increase in antioxidant activities allows the MDA values to be contained, avoiding oxidative damage. Similar enzyme induction has been observed in previous studies such as in *Pangasianodon hypophthalmus*, infected by the dactylogyrid monogenean *Thaparocleidus* sp. (Kumar et al., 2017) and *X. novacula* infected by the same *Scaphanocephalus* species (Cohen-Sánchez et al., 2023a, 2023b). Regarding the immune response in the mucus, the infection induced a significant increase in lysozyme and ALP, but also in Ig, indicating an innate and adaptive immune response. Similar responses were observed in various studies, where lysozyme and Ig levels were higher in the mucus of parasitized fish, such as in the large yellow croaker, *Pseudosciaena crocea*, infected by the ciliated protozoan parasite, *Cryptocaryon irritans* (Yin et al., 2015) or greater amberjack (*Seriola dumerili*) after infection with the monogenean parasite *Neobenedenia girellae* (Fernández-Montero et al., 2021). ALP has also been found to be elevated in parasitized fish, such as *Catla catla* infected with monogeneans (Kaur et al., 2018).

Thus, the results showed that the presence of parasites and high salinity can cause oxidative stress and immune response in different tissues (Fig. 6). Gill tissues exhibited higher antioxidant responses and ROS production in the groups exposed to salinity, likely due to direct seawater contact. Epithelial mucus, in direct contact with the parasite, showed elevated antioxidant enzymes and elements of the immune system in response to the infection. In contrast, the liver displayed increased antioxidant activity in response to both stressors, reflecting systemic effects on the organism as a whole. The increasing presence of parasites due to temperature changes and increased pollution could end up weakening the fish fitness, promoting secondary infections or making them more susceptible to predation (Marcogliese et al., 2005). However, further studies in more controlled conditions would be necessary to sustain that oxidative stress is solely due to the parasite as field-sampled animals can be influenced by other factors, such as organic and inorganic pollutants, which seem to be abundant in Cala Jondal.

5. Conclusion

Our comprehensive tissue analysis demonstrates the differential impact of stressors on the antioxidant and immune responses in *C. julis*. This approach allows for the examination of multiple threats and emphasizes the importance of selecting appropriate tissues based on the study's objectives. *C. julis* shows heightened oxidative stress, particularly in gill tissue under high salinity conditions and in epithelial mucus during parasite infections. These findings evidence the vulnerability of fish to environmental changes, especially those caused by human activities, which can exacerbate oxidative stress and increase susceptibility to pollutants and diseases. The present study highlights the adverse effects of two coastal threats, brine discharge and consequent salinity increase, and the presence of the parasite *Scaphanocephalus* sp. on *C. julis.* The observed antioxidant defence responses to these threats indicate their potential to induce significant stress. Additionally, the varying tissue responses underline the sensitivity of different tissues to environmental changes, showcasing the effectiveness of multi-tissue analysis in detecting early warning signs of diverse impacts on marine life.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.175848.

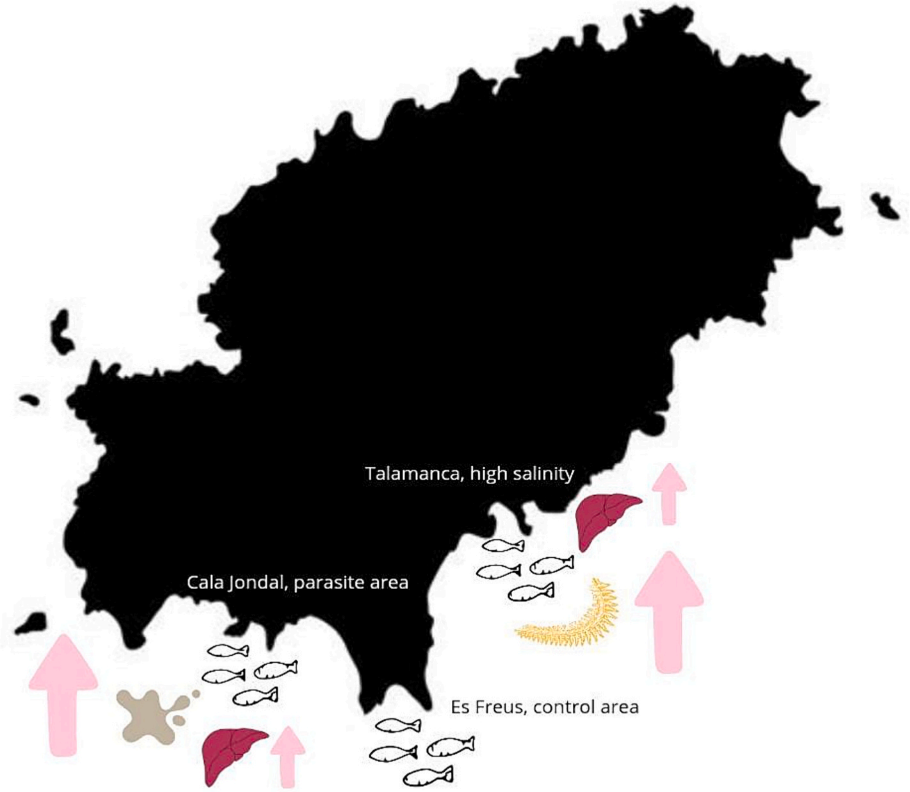

Fig. 6. Representative image of the different effects caused by high salinity and Scaphanocephalus sp. infection in *Coris julis*. Hypersaline water primarily affects the gills, while the infection mainly impacts the epithelial mucus. In both cases, effects on the liver are also observed, although they are less intense.

Funding sources

This work has been partially sponsored and promoted by the Comunitat Autònoma de les Illes Balears through the Direcció General de Recerca, Innovació I Transformació Digital and the Conselleria de Economia, Hisenda i Innovació via Plans complementaris del Pla de Recuperació, Transformació i Resiliència (PRTR-C17-I1) and by the European Union- Next Generation UE (BIO/006). Nevertheless, the views and opinions expressed are solely those of the author or authors, and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission are to be held responsible. This research was also funded by the Spanish Government, Institute of Health Carlos III (CIBEROBN CB12/03/30038). This work has also been financed by the Biodibal project, within the framework of the Collaboration Agreement between the University of the Balearic Islands and Red Eléctrica de España.

CRediT authorship contribution statement

Amanda Cohen-Sánchez: Writing – original draft, Methodology, Investigation, Formal analysis. **Antonio Box:** Writing – review & editing, Methodology, Investigation, Conceptualization. **José María Valencia:** Writing – review & editing, Methodology, Investigation. **Samuel Pinya:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Silvia Tejada:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Antoni Sureda:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

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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