EFFECTS OF ABIOTIC (SALINITY) AND BIOTIC (ECTOPARASITE) STRESSORS ON *CORIS JULIS* (LINNAEUS, 1758) IN IBIZA, BALEARIC ISLANDS: A BIOMARKER ANALYSIS

Antoni Sureda, Amanda Cohen-Sánchez, Guillem Mateu-Vicens, Pere Ferriol, LLorenç Gil, Antonio Box, Samuel Pinya, Silvia Tejada

Abstract: Stressful situations include abiotic factors such as exposure to hypersaline waters derived from desalination plants and biotic factors such as infection by new parasites introduced by human activities. The aim was to study the effects of an abiotic factor (salinity) and a biotic factor (ectoparasite) on the small coastal wrasse *Coris julis* (Linnaeus, 1758). Specimens were obtained in three areas of the Island of Ibiza (Balearic Islands): control area, area influenced by the desalination plant and area with high levels of parasite infection. Biomarkers of oxidative stress in the gills, liver and epithelial mucus were analysed, as well as immunological markers in the mucus. The two stress factors induced a differential response, with a greater effect of salinity on the gills and a greater effect of the parasite on the liver and mucus. Innate immunity increased in the mucus of fish under both stressors, and immunoglobulin levels increased only in the presence of parasite. In conclusion, *C. julis* specimens affected by salinity and an ectoparasite respond with an increase in antioxidant and immunological defence mechanisms.

Keywords: Biomarkers, Balearic Islands, Oxidative stress, Immune response

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Referee List (DOI 10.36253/fup_referee_list)
FUP Best Practice in Scholarly Publishing (DOI 10.36253/fup_best_practice)

Antoni Sureda, Amanda Cohen-Sánchez, Guillem Mateu-Vicens, Pere Ferriol, LLorenç Gil, Antonio Box, Samuel Pinya, Silvia Tejada, *Effects of Abiotic (Salinity) and Biotic (Ectoparasite) Stressors on* Coris julis *(Linnaeus, 1758) in Ibiza, Balearic Islands: A Biomarker Analysis*, pp. 307-312, © 2024 Author(s), CC BY-NC-SA 4.0, DOI: 10.36253/979-12-215-0556-6.28

Introduction

Anthropic activity represents a continuous challenge for species that are constantly subjected to different stress situations [4]. Stressful situations include abiotic factors such as exposure to hypersaline waters derived from desalination plants and biotic factors such as infection by new parasites introduced by human action or an increase in the infectious load favoured by pollution.

In the Balearic Islands, high human pressure associated with tourism, especially in summer, causes an increase in water demand that cannot be met by the natural water reserves and, it must therefore be met through the osmosis industry [9]. Recently, the presence of an ectoparasitic trematode of the genus *Scaphanocephalus* that affects the skin of fish from the wrasse family causing 'black spot disease' has been observed in Ibiza [2]. Any factor that causes stress to organisms induces an activation of the metabolism to face this new situation. This fact translates into an increase in the production of reactive species (ROS) which, in turn, induces the activation of antioxidant defence mechanisms [8].In this sense, to avoid 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. Furthermore, any element exogenous to the body induces an activation of immune defences to protect the body. Therefore, the analysis of biomarkers allows to evaluate the potential effects of different stressors on organisms by determining the same parameters.

Coris julis (Linnaeus, 1758), commonly known as the rainbow wrasse, is part of the Labridae family and is one of the most frequently found wrasses in the Mediterranean Sea. It lives in coastal regions, such as rocky areas and seagrass beds, at depths of up to 50 meters [5]. This species is ideal for research due to its high population and adaptability, its sedentary nature, and feeding habits that allow it to accumulate environmental pollutants.

Altogether, the objective of the present work was to study the effects of an abiotic factor (salinity) and a biotic factor (ectoparasite) on the small coastal wrasse *C. julis*. Specimens were obtained in three areas of the Island of Ibiza (Balearic Islands): control area, area influenced by the desalination plant and area with high levels of parasite infection. Biomarkers of oxidative stress in the gills, liver and epithelial mucus were analyzed, as well as immunological markers in the mucus.

Materials and Methods

A total of 27 fish (N=9 per site) of similar size and weight were caught using line-fishing. The abundance of parasites was visually assessed by counting the observable spots on each fish. Gills and liver samples were collected from all fish, and mucus samples were taken from the epithelium of each fish. The experimental procedures were approved by the Ethics Committee for Animal Experimentation of the University of the Balearic Islands (Ref. 020/06/AEXP).

The activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) were measured in liver, gill, and mucus samples, while the phase II detoxification enzyme glutathione S-transferase (GST) was assessed in liver and

gill tissues following established methods [8]. Additionally, the activity of the immune enzymes alkaline phosphatase (ALP) and lysozyme, as well as the levels of immunoglobulins, were measured in mucus [2]. Reactive oxygen species (ROS) production was determined in liver and gill homogenates using 2,7-dichlorofluorescin diacetate as an indicator, and malondialdehyde levels were also measured in both tissues using a commercial colorimetric kit [1].

Statistical analyses were evaluated using the statistical software package SPSS 27.0 for Windows® (IBM® SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to confirm the normality of the data, and the Levene's test was used to verify homogeneity of variance. Statistical differences between groups were analysed using one-way ANOVA followed by the Bonferroni post-hoc test.

Results

A total of 27 fish, 9 from each site and treatment were caught. The average total length of *C. julis* was 12.6 cm ± 0.3 cm, and the average weight was 18.3 g ± 1.1 g. There were no statistically significant differences in size or weight among fish from the different sites. Salinity values were as follows: near the desalination plant had 40.1 ± 0.3 PSU, while the area affected by the parasite and Es Freus (control) had normal values of 38.0 ± 0.1 and 37.9 ± 0.1 PSU, respectively. Regarding skin parasites, none were found in fish from the control and salinity sites, whereas in the area affected by the parasite fish had an average of 14.3 ± 1.9 spots per fish.

The two stress factors induced a differential response, with a greater effect of salinity on the gills and a greater effect of the parasite on the liver and mucus (Figures 1 and 2). In general, an increase in the activities of the antioxidant enzymes, catalase and superoxide dismutase (SOD), and in the production of ROS was observed in the gills of fish affected by salinity, while this increase was greater in the liver in fish affected by the parasite. The activity of the detoxification enzyme, glutathione s-transferase (GST), was increased in both tissues and under the two stressors, while no changes were observed in malondialdehyde (MDA) levels as an indicator of oxidative damage.

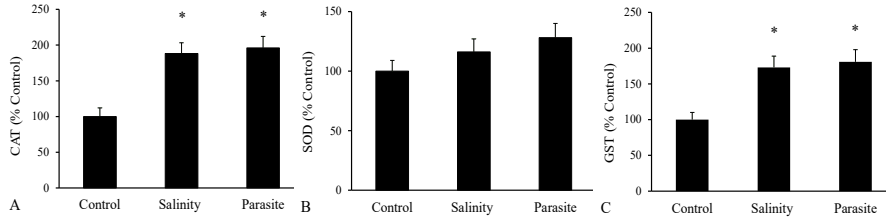

Figure 1 – Activities of (A) catalase (CAT), (B) superoxide dismutase (SOD) and (C) glutathione s-transferase (GST) in the liver of *C. julis*. * Indicates significant differences respect to control, $p<0.05$.

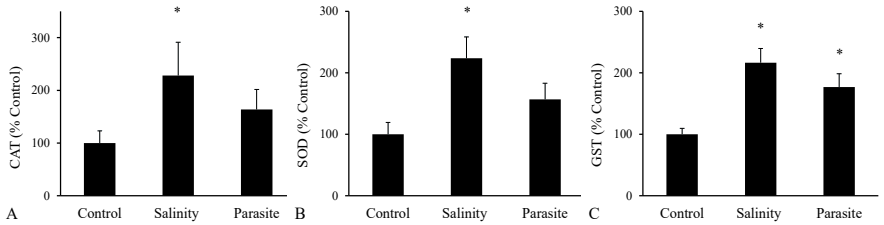

Figure 2 – Activities of (A) catalase (CAT), (B) superoxide dismutase (SOD) and (C) glutathione s-transferase (GST) in the gills of *C. julis*. * Indicates significant differences respect to control, $p<0.05$.

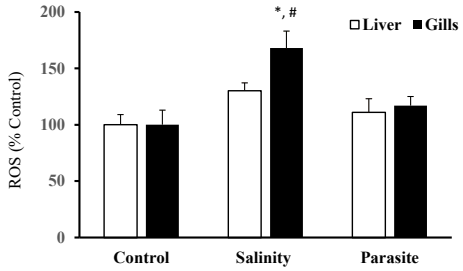

Figure 3 – Reactive oxygen species production in the liver and gills of *C. julis*. * Indicates significant differences respect to control, # Indicates significant differences respect to parasite, $p<0.05$.

Lysozyme and alkaline phosphatase activity increased in the mucus of fish under the influence of salinity and parasitism, and immunoglobulin levels increased only in the presence of parasite.

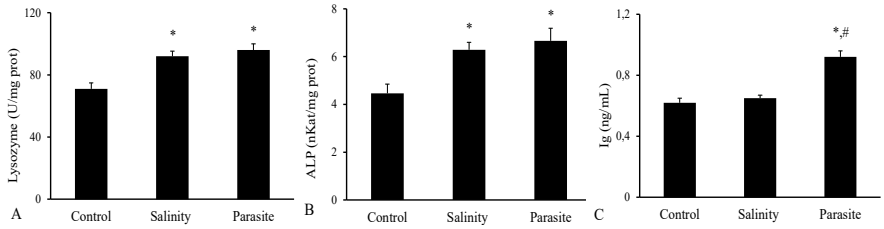

Figure 4 – Activities of (A) Lysozyme, (B) Alkaline phosphatase (ALP) and (C) Immunoglobulin (Ig) levels in the mucus of *C. julis*. * Indicates significant differences respect to control, # Indicates significant differences respect to parasite, $p<0.05$.

Discussion

The oceans are rapidly changing due to various external and internal factors, impacting the behaviour, life cycles, and internal processes of marine species [7]. One effect of these changes is the production and accumulation of reactive oxygen species (ROS) in exposed organisms. Analysing antioxidant/prooxidant biomarkers can indicate if these changes are causing oxidative stress. This study focused on two major challenges for coastal species in Ibiza Island: high salinity from desalination plant brine discharges and parasite infections.

Ibiza relies heavily on desalinated water due to limited freshwater sources, leading to significant brine discharge into the sea and altering local salinity [9]. Regarding parasites, *Scanocephalus* sp., first identified in Ibiza in 2015, causes black spot disease in wrasse family fish [2]. Initially found in *X. novacula*, it has also been observed in other species such as *C. julis* and *Thalassoma pavo*. The main findings of this study revealed that both stressors induced oxidative stress, primarily affecting the gills in the case of salinity and epithelial mucus with the parasite, along with an immune response. Additionally, the liver, as the main metabolic organ, showed increased oxidative stress markers in fish affected by both salinity and parasites.

Changes in salt concentrations can trigger oxidative stress, as shown by the increased activities of antioxidant enzymes CAT and SOD in *Mytilus galloprovincialis* exposed to high salinity [3]. Our results support these findings, revealing higher activities of antioxidant enzymes and GST, primarily in the gills, which are the tissues most directly exposed to salt stress. Additionally, an increase in liver activities of CAT and GST was observed, likely to meet the demands imposed by the elevated salinity. The increased values of antioxidant enzymes, lysozyme, and ALP in the mucus of fish exposed to salinity indicate a stressful situation. The absence of changes in Ig levels, unlike the response to pathogen infection, suggests a non-specific response to salt stress.

Parasite infection has been found to cause oxidative stress in hosts [6]. The induction of antioxidant enzyme activities in the liver could result from increased metabolic demands due to parasitic infestation, leading to higher oxygen consumption and cellular stress. However, this rise in antioxidant activities helps contain MDA values, preventing oxidative damage. The infection caused a significant increase in lysozyme, ALP, and Ig in the mucus, indicating both an innate and adaptive immune response.

Conclusion

This study highlights the adverse effects of two coastal threats —brine discharge increasing salinity and the presence of the parasite *Scaphanocephalus* sp.— on *C. julis*. The observed antioxidant defence responses to these threats suggest a significant potential risk and stress induction. Additionally, the varying responses across different tissues underscore their sensitivity to environmental changes, demonstrating the value of multi-tissue analysis in identifying early warning signs of various impacts on marine life.

Acknowledgements

This work has been partially sponsored and promoted by the Comunitat Autònoma de les Illes Balears through the Servei de Recerca i Desenvolupament, Direcció General d'Universitats, Recerca i Estudis Artístics and the Conselleria d'Educació i Universitats 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.

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