SUPPLEMENT

REVISTA DE Biología Tropical

https://doi.org/10.15517/rev.biol.trop..v72iS1.58993

Study of histopathology on *Arbacia lixula* (Arbaciidae: Arbacioida) and *Paracentrotus lividus* (Parechinidae: Camarodonta) with bald sea urchin disease symptoms in Gran Canaria Island, Spain

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Received 27-VI-2023. Corrected 06-XII-2023. Accepted 03-I-2024.

ABSTRACT

Introduction: Sea urchin diseases have been documented in several locations worldwide, with reported occurrences of bacterial, protozoan, fungal, and algal infections.

Objective: This study aimed to investigate pathogen agents in populations of *Arbacia lixula* and *Paracentrotus lividus* along the coast of Gran Canaria Island (Central-East Atlantic, Spain).

Methods: Sampling was conducted at San Cristobal beach, on the Northeast side of the island, where sea urchins were manually collected from depths of 1-3 m during June, July, and October 2022. Swab samples were taken from the external and internal areas of the lesions and cultured on various media plates.

Results: Eight different pathogen agents, including bacteria and fungi, were identified, with *Vibrio alginolyticus* being the most frequently observed bacteria in all diseased sea urchin samples. Additionally, ciliated protozoans were found within the tests, potentially acting as opportunistic parasites.

Conclusions: This research provides a unique perspective on bald sea urchin disease by identifying a significant number of associated pathogens, including *Candida*, previously unreported in diseased organisms. Furthermore, the study highlights the presence of an inflammatory response in tissues with bacterial colonies, offering crucial insights into understanding this sea urchin disease.

Key words: rocky shore; *Vibrio*; sea urchin mortality; Canary Islands; Webbnesia.

RESUMEN

Estudio de histopatología en *Arbacia lixula* (Arbaciidae: Arbacioida) y *Paracentrotus lividus* (Parachinidae: Camarodonta) con síntomas de la enfermedad del erizo desnudo en la Isla de Gran Canaria, España

Introducción: Las enfermedades en los erizos de mar han sido descritas en muchas localidades alrededor del mundo, y se han asociado con la presencia de infecciones por bacterias, protozoarios, hongos y algas.

Objetivo: Este estudio tuvo como finalidad investigar sobre los agentes patógenos que afectan a las poblaciones de *Arbacia lixula* y *Paracentrotus lividus* a lo largo de la costa de la Isla de Gran Canaria (Atlántico Centro-Oriental, España).
Métodos: El muestreo fue llevado a cabo en la playa de San Cristóbal, al noreste de la isla, dónde los organismos fueron capturados entre 1-3 metros de profundidad, durante junio, julio y octubre del año 2022. Se tomaron muestras en la zona interna y externa de la lesión en cada organismo, y se cultivaron en varios medios de cultivo.
Resultados: Fueron identificados ocho agentes patógenos diferentes, incluyendo bacterias y hongos, y siendo *Vibrio alginolyticus* la bacteria más frecuentemente observada en todas las muestras de erizos enfermos. Además, se observaron protozoarios ciliados dentro de los caparazones, actuando potencialmente como parásitos oportunistas.
Conclusiones: Esta investigación proporciona una perspectiva única sobre la enfermedad del erizo desnudo al identificar un número significativo de patógenos asociados, incluida *Candida*, que no se había reportado previamente en organismos enfermos. Además, el estudio destaca la presencia de una respuesta inflamatoria en tejidos con colonias bacterianas, lo que ofrece información crucial para comprender esta enfermedad de los erizos de mar.

Palabras clave: costas rocosas; *Vibrio*; mortalidad de erizos de mar; Islas Canarias; Webbnesia.

INTRODUCTION

Sea urchin diseases have been described around the globe, particularly those that caused several mass mortalities, including those that occurred in the 80´ with *Diadema antillarum* (Philippi, 1845) in the Caribbean Sea (Lessios, 1988), and *Strongylocentrotus droebachiensis* (O.F. Müller, 1776) in Nova Scotia, Canada (Jones et al., 1985). In both cases, the infection agents were not deeply described. In addition, sea urchin diseases have been reported between 2001 and 2020 in some regions of Webbnesia: Madeira (Portugal), and Tenerife and La Palma in Canary Islands (Spain), mainly in *Diadema africanum* (Rodríguez, Hernández, Clemente & Coppard, 2013), but in other species as *Paracentrotus lividus* (Lamarck, 1816), *Arbacia lixula* (Linnaeus, 1758), and *Sphaerechinus granularis* (Lamarck, 1816) (Clemente et al., 2014; Dyková et al., 2011; Girard et al., 2011; Gizzi et al., 2020; Hernández et al., 2020; Salazar-Forero et al., 2022), and it is important to highlight that there are no reports of mass mortalities or diseases in Gran Canaria Island.

In recent times, some authors have reported the pathogenic action of different infectious agents in sea urchins, such as bacteria, protozoan, fungi, and algae, being bacteria the most common agent appearing in all diseases (Dyková et al., 2011; Gizzi et al., 2020; Grech et al., 2019; Grech et al., 2022; Hernández et al., 2020; Hewson et al., 2023; Jangoux, 1987; Salazar-Forero et al., 2022; Shaw et al., 2024; Shimizu et al., 1995; Wang et al., 2013b; Wang et al., 2023). In the case of bacteria, one of the groups most frequently described in marine habitats is that of the *Vibrio* genus, which increases its concentration during the temperature rise and produces blooms (Mira-Gutiérrez & García-Martos, 1998). Within the *Vibrio* genus, *Vibrio alginolyticus* (Miyamoto et al., 1961) is the most abundant and halotolerant species in temperate marine ecosystems (Mira-Gutiérrez & García-Martos, 1998).

Several authors around the globe have reported *Vibrio* spp. in diseased tissues of some sea urchin's species, such as *D. africanum, Strongylocentrotus intermedius* (A. Agassiz, 1864), and *P. lividus* (Becker et al., 2008; Clemente et al., 2014; Gizzi et al., 2020; Grech et al., 2022; Salazar-Forero et al., 2022; Shimizu et al., 1995; Wang, Chang et al., 2013; Wang, Feng et al., 2013; Wang et al., 2023). However, there is some controversy because the same bacteria are isolated from healthy sea urchins, leading to their classification as opportunistic rather than primary pathogens. For this reason, it is essential to resort to histopathological studies to visualize tissue alterations and to complement the diagnosis of this disease (Virwani et al., 2021).

Different protozoan species have been described in some echinoderms as infection

agents. For example, Hernández et al. (2020) reported that the mass mortality events of *D. africanum* in Madeira and the Canary Islands were associated with anomalous southwest storms that resuspended bottom sediment and favored the appearance of paramoebas promoting paramoebiasis. Also, Scheibling and Lauzon-Guay (2010) described how the amoebic disease has been producing sea urchin massive mortalities in the North Atlantic Ocean, because of intense tropical cyclones near warm coastlines, which has facilitated the spread of the disease. Furthermore, Jangoux (1987) in his echinoderm diseases review described the presence of the ciliate *Orchitophrya stellarum* (Cépède, 1907) which parasites the gonads of *Asterias rubens* (Linnaeus, 1758). Finally, Virwani et al. (2021) reported the presence of ciliates and encysted metazoan parasites in diseased test tissues on *Tripneustes ventricosus* (Lamarck, 1816) from St. Kitts, Caribbean Sea. It is crucial to emphasize that certain authors have associated the increase of pathogen bacteria with the rising seawater temperature. Indeed, the collaborative work of Garrabou et al. (2022) showed that the increase in seawater temperature in the Mediterranean Sea between 2015–2019 is the cause behind the mass mortality of 23 taxa and seven phyla, with the shallow water echinoderms, distributed between 0 and 10 m depth, included in this list.

In this context, this work aimed to characterize the infection agents and describe the histopathology and disease observed in *Arbacia lixula* and *Paracentrotus lividus* populations on the coast of Gran Canaria Island (Northeast Atlantic, Spain).

MATERIALS AND METHODS

Study area: This study was conducted on San Cristobal beach, on the northeast coast of Gran Canaria Island (Canary Islands, Spain; 27°45´N 15°45´W) (Fig. 1). This urban beach of the capital city of Las Palmas of Gran Canaria, the most populated area of the island, is a highly anthropogenic coast close to the La Luz harbor, one of the most important ports in

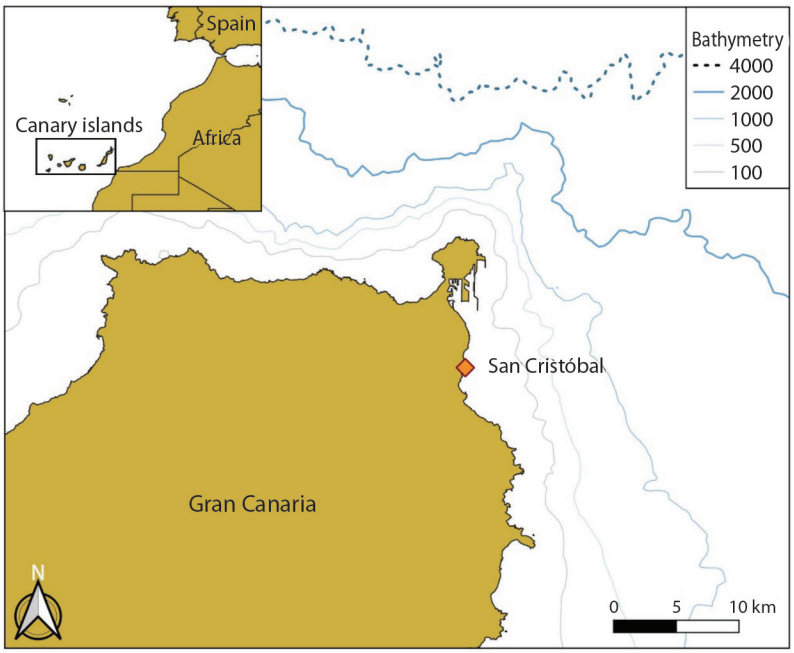

Fig. 1. San Cristobal beach location, where sea urchins (*Paracentrotus lividus* and *Arbacia lixula*) were collected (Gran Canaria Island, Spain).

the northwest Africa area because of its great influence on fish products and goods traffic of the region.

Field survey: Diseased and apparently healthy individuals of the sea urchins of *Arbacia lixula* and *Paracentrotus lividus* were manually collected between 1 and 3 m depth, with temperatures over 23 °C, according to data collected by the State Ports Buoy Network, Government of Spain, for the eastern area of Gran Canaria (Ministerio de Transporte, Movilidad y Agenda Urbana- Puertos del Estado, 2022). All individuals were larger than 4 cm in test diameter and were collected with basic apnea equipment in June, July, and October 2022. After being collected, the live sea urchins were transported to the laboratory in a small box with seawater.

Additionally, one visual census was carried out to count the number of complete sea urchin tests in an area of 30 m^2 (two parallel transects of 15 m^2) to estimate test density (Fig. 2A, Fig. 2B); some sea urchins with the presence of illness marks, and tests suspected that they could have been broken due to fishing activity or predation were discarded.

Laboratory: Tissue samples were taken on the external and internal face of lesions, scraping the test surface with a sterilized swab, and in the external and internal face of the healthy sea urchin. The samples were then placed on plates with different culture media (Blood agar, MacConkey agar, Baird Parker and Sabouraud agar (Pronadisa, Laboratorios Conda, Madrid, Spain)). The culture plates were incubated at 25 ºC during 7 days of aerobiosis. Strains obtained

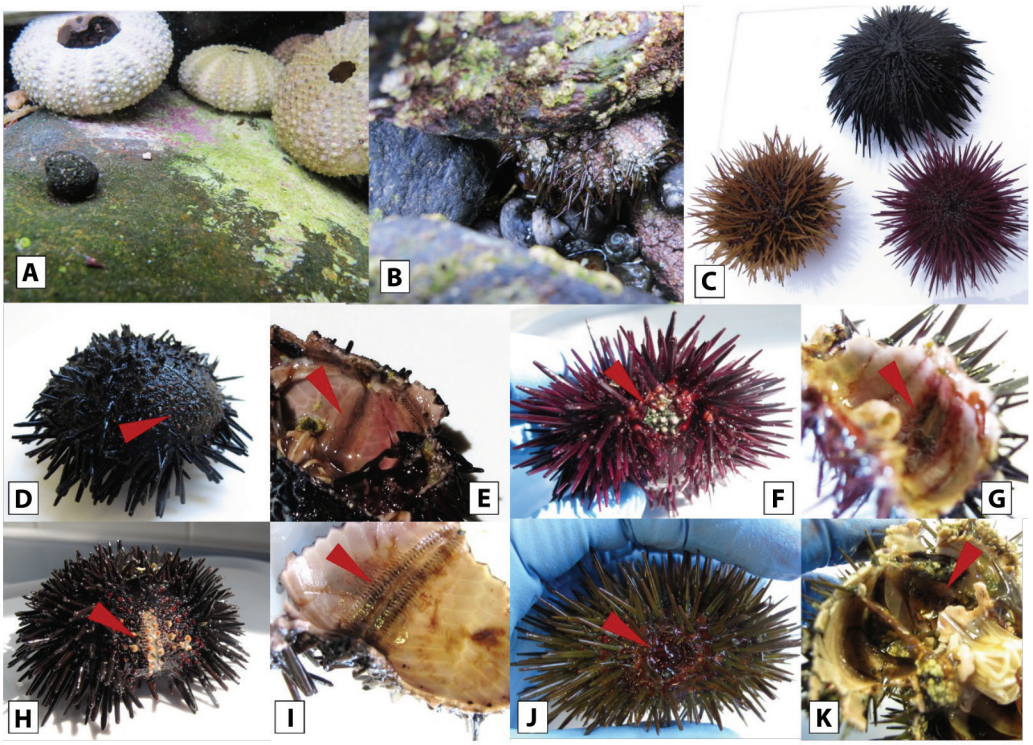

Fig. 2. A. Sea urchin tests around the sampling area; **B.** test of a recently dead sea urchin with areas of the test showing the presence of disease; **C.** healthy sea urchins: black sea urchin in the upper part of the image is *Arbacia lixula* and the two in the lower area (greenish and purple) are *Paracentrotus lividus*; **D.** and **H.** *A. lixula* individuals with the presence of external test damage caused by disease; **E.** and **I.** *A. lixula* organism with the presence of internal test damage caused by the disease; **F.** and **J.** *P. lividus* individuals with the presence of external test damage caused by the disease; **G.** and **K.** *P. lividus* specimens with the presence of internal test damage caused by the disease.

from culture were identified through mass spectrometry, employing MALDI-TOF technique (Autoflex III, Bruker Daltonics GmbH). This method is a standard tool in clinical microbiology due to its rapid and dependable microorganism identification capabilities (Siller-Ruiz et al., 2017), creating a spectrum based on the protein profile using a complete database of more than 16 000 strains covering more than 5 000 species and more than 1 000 genera of marine microorganisms (Bizzini & Greub, 2010; Croxatto et al., 2012).

Samples of the test, gonad, and tube feet from diseased and healthy sea urchin were fixed in formaldehyde (4 %) for 48 hours. Test samples were decalcified for 5 minutes. After that, all samples were dehydrated with graded ethanol and xylene series, and embedded in paraffin using a routine histological process. Serial cross sections (4 µm) were made and stained with Hematoxylin and Eosin (H&E) and Gram stains. The slides were mounted and examined with a light microscope (Olympus BX51TF, Japan).

Analysis: The frequency of appearance of the infection agent in the organisms was represented using ggplot2 package (Wickham, 2016) in R v4.2.3 (R Core Team, 2023), and the map was graphed using QGIS software (QGIS.org, 2023).

RESULTS

Description of samples: The counting of 192 complete sea urchin tests in 30 m^2, resulted in a density of 3.84 dead sea urchins/m^2. In general, all the complete tests showed marks associated with diseases (Fig 2A, Fig. 2B).

Overall, the signals of disease in both sea urchin species were bare test surfaces, without spines or pedicellaria, and discoloration of the test in comparison with the healthy calcarean tissue (Fig. 2D). Additionally, it was observed that when the disease was advanced, the test in the affected area was weakened. In the case of *A. lixula*, the affected area was in cream color with brown-reddish zones (Fig. 2H, Fig. 2I) compared to the blackish color of the healthy test. For *P. lividus*, the affected area was in a greenish color (Fig. 2F, Fig. 2G) compared to the dark red color of the healthy test. In the last phases of the disease, lesions resulted in the perforation of the test (Fig. 2J, Fig. 2K), exposing the coelomic cavity. In addition, the inside of the injured area was darkened to different degrees (Fig. 2E, Fig. 2G, Fig. 2I, Fig. 2K).

Microscopically, an inflammatory reaction composed mainly of coelomocytes and pigment cells, was observed at the edge of the injured areas from diseased sea urchins (Fig. 3, inset I). In the affected spines, groups of gram-negative bacterial colonies (Fig. 4A, Fig. 4B), were observed. Moreover, ciliate protozoan (Fig. 4C) inside the sea urchin test and metazoans inside the pedicellaria were found in affected sea urchins (Fig. 4D). Neither bacterial colonies nor the presence of parasitic forms was observed in the apparently healthy sea urchins collected.

Analysis of infection agents: A total of 17 sea urchins, 13 sick (7 of *Arbacia lixula* and 6 of *Paracentrotus lividus*), and four apparently healthy individuals of both species were sampled. The infection agents vary between species and health conditions, but *Vibrio alginolyticus* was the most frequent bacteria in sick specimens of both species on the internal or external side of the tests. The other microorganism which appeared in both species was *Candida* sp. Variations among species considering other infection agents were found: in case of sick *A. lixula*, *Acinetobacter johnsonni*, *Pseudomonas frederiksbergensis* and *Vibrio mytili* were found. However, on *P. lividus* we found *Vibrio harveyi* and *Pseudomonas rhodesiae* (Fig. 5). On the other hand, no infection agents in healthy *A. lixula* internal tissues and *P. lividus* external tissues were found, even though *V. alginolyticus* was detected in external tissues of *A. lixula*.

Comparing the infection agents on sea urchins reported in the bibliography in different areas of the globe, some authors describe the presence of *Vibrio* in samples of *P. lividus, S. intermedius, D. africanum* and

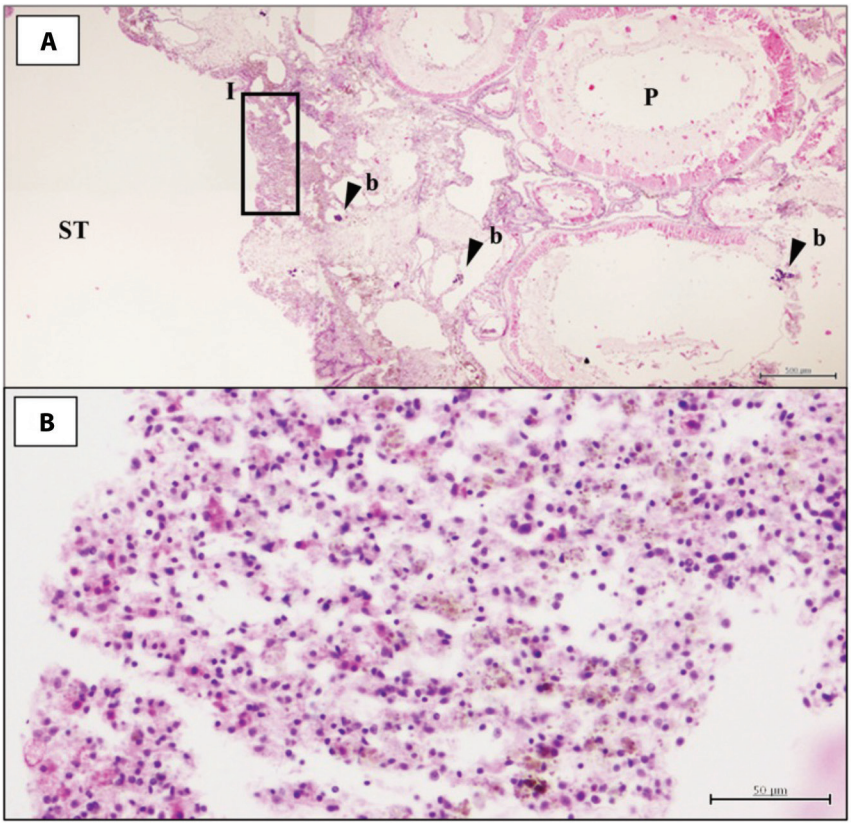

Fig. 3. Histological test preparation with the general view of the injured area. **A.** ST: sick tissue, b: bacteria colony, P: non-affected spine base, I: inflammation area with coelomocytes and pigment cell in the edge of lesion (scale bar = 500 µm); **B.** close-up of the area in the box of Fig. 3A, showing inflamed areas with a high concentration of coelomocytes (dark purple cells) (scale bar = 50 µm).

Strongylocentrotus purpuratus, but *V. alginolyticus* was only reported in an association with *D. africanum* and *S. purpuratus* (Table 1).

DISCUSSION

The present study provides fundamental pathology data for two species of common sea urchins in Gran Canaria, *P. lividus* and *A. lixula*, with a particular focus on visually infected individuals. Test lesions were found to be a prevalent feature among reports of urchin diseases, establishing a description of the morphological representation of this condition. Diseased sea urchins present bare surfaces in the test associated with tissue necrosis, loss of spines and tube feet, and some greenish or brownish areas in the most compromised tissue, features that have been associated with bald sea urchin disease (Bauer & Young, 2000; Becker et al., 2008; Clemente et al., 2014; Dumont et al., 2004; Hernández et al., 2020; Hughes et al., 1985; Virwani et al., 2021).

The samples of sick sea urchins collected in San Cristobal beach were consistent with the description of bald sea urchin disease. All the affected organisms exhibited varying degrees of infection, manifesting the described characteristics associated with this disease. In the case of *P. lividus*, the bald surface had a greenish color and in *A. lixula*, it had a brown-reddish color; in both cases the test presented loss of spines and tube feet, and in advanced disease condition, it also included epidermal tissue loss and perforation of the test.

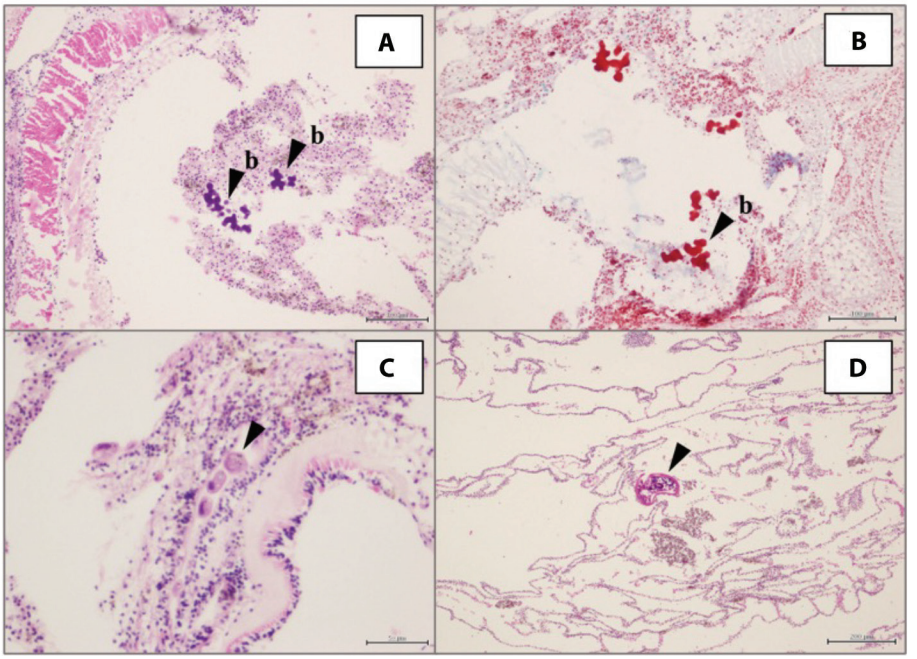

Fig. 4. Histological test preparation with the presence of pathogen organisms: **(A)** bacteria colony in the injured spine base, cross-sections stained with H&E (scale bar = 100 µm); **(B)** bacteria colony stained with Gram stain, denoting their gram-negative characterization (red color) (scale bar = 100 µm); **(C)** Ciliate protozoa inside the test (scale bar = 50 µm); **(D)** Metazoan inside the pedicellaria (scale bar = 200 µm).

The presence of protozoans and metazoans in different tissues has been reported in previous works (Francis-Floyd, 2020; Grech et al., 2022), and specifically the presence of amoebas has been described in several works as an infection agent in sea urchin diseases (Dyková et al., 2011; Hernández et al., 2020; Jones et al., 1985; Salazar-Forero et al., 2022; Scheibling & Lauzon-Guay, 2010). In our study, we found different protozoa and metazoan species in diseased sea urchin tests, spines, and tube feet, while other authors described them just in the rigid structures (spines and test). According to Jones et al. (1985), infiltration of protozoan is frequently observed in the organic matrices of spines but the notable impacts of the disease are primarily evident in the spine bases. The presence of these microorganisms associated with echinoderms, has been extensively documented by multiple authors (Jangoux, 1987), and in some cases, they have been considered commensal in the gut of sea urchins because they inhabit the digestive systems of certain echinoids without causing disease (Grech et al., 2022). In our samples, we found that these organisms were proximate to diseased tissues, suggesting they might function as opportunistic pathogens rather than the primary cause of the disease.

Considering the presence of bacteria within the lesions, numerous studies have reported several bacterial species consistent with bald sea urchin disease; in the Pacific Ocean, *Vibrio* genus was reported in *S. intermedius* sick sea urchin in Japan and China (Wang, Feng et al., 2013; Wang et al., 2023). In the Mediterranean Sea, bacteria were present in lesions of *P. lividus* and *A. lixula* (Maes & Jangoux, 1984), and *Vibrio* genus in *P. lividus* (Becker et al., 2008; Grech et al., 2022). In the Canary and Madeira archipelagos, *V. alginolyticus* was reported in affected tissues of *D. africanum, A. lixula,* and

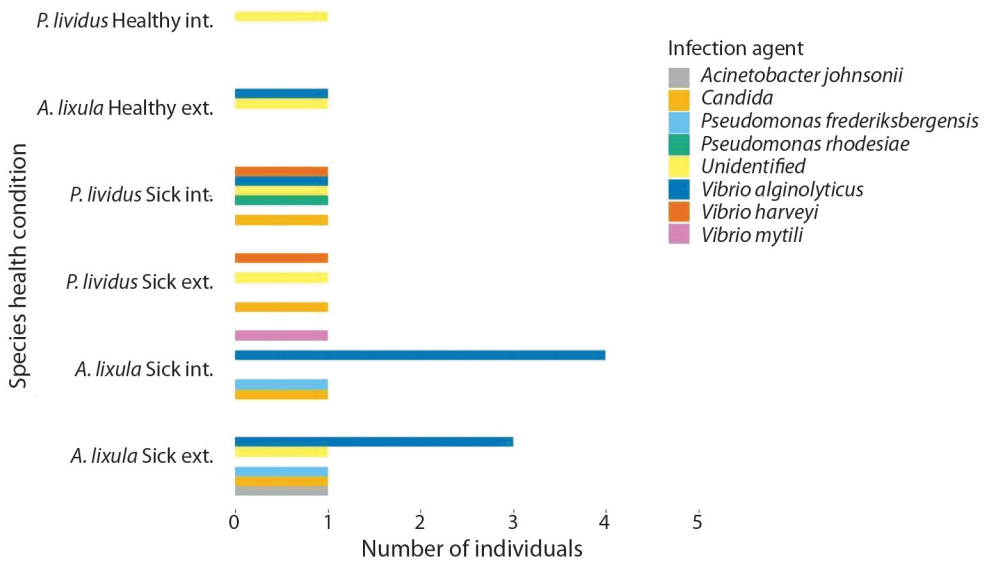

Fig. 5. Frequency of appearance of the infection agent in *Arbacia lixula* and *Paracentrotus lividus* organisms, both healthy and sick.

P. lividus (Clemente et al., 2014; Gizzi et al., 2020; Salazar-Forero et al., 2022), and in *S. purpuratus* grown in aquariums (Shaw et al., 2024); this species was frequent in almost all the samples of healthy and sick individuals in our study, inside and outside the diseased tissue. Additionally, the second most abundant bacterial species found both in our study and in other studies was *V. harveyi* in *P. lividus* samples, which coincides with the finding of Wang, Feng et al. (2013) in hatcheries in China. Furthermore, Shaw et al. (2024) reported the presence of *Acinetobacter* sp. in *S. purpuratus* hatcheries consistent with our findings in diseased tissues of *A. lixula*. We also found other pathogen agents in diseased tissues of both species (Table 1) that together could be producing the signal of the disease.

Importantly, while it is true that many of these bacteria, such as *V. alginolyticus* and *V. harveyi*, can be found in the marine environment, we can assert that, when present within the internal region of the diseased tissue, they serve as contributors to the development of the disease. Moreover, Shaw et al. (2024) highlighted that diseased sea urchins could be exposed to great abundance, concentration, or diversity of opportunistic microorganisms, potentially intensifying the pathogenic effects.

Our research has effectively established a histopathology association between the presence of bacterial colonies in the injured areas and an accompanying inflammatory response in the affected tissue. This evidence strongly suggests that these bacterial colonies could be the causative agents of the disease.

The presence of *V. alginolyticus* on the external tissues of healthy *A. lixula* is expected because this is one of the most frequent bacteria in marine ecosystems. Similarly, the presence of other unidentified bacteria within the tests of both sea urchin species is an expected result as it is widely recognized that the coelomic fluid of sea urchins harbors a diverse bacterial community. Indeed, Clemente et al. (2014) reported that in some instances, individuals harbor pathogens in their coelomic fluid without exhibiting any outward signs of disease. Even upon experimental injection with a pathogen-containing suspension, the individuals did not contract the disease throughout the duration of the infection experiments, indicating that the

Table 1

Comparison between the infectious agents reported in different species of sea urchin.

Author	Sampling year	Locality	Sea urchin species	Pathogen agent
Jones et al., (1985)	1982	Nova Scotia	*Strongylocentrotus droebachiensi*	*Paramoeba*
Maes and Jangoux (1984)	1984	Mediterranean Sea and North Atlantic, France	*Echinus esculentus, Sphaerechinus granularis, Psammechinus miliaris Arbacia lixula* and *Paracentrotus lividus*	Bacterial infection
Shimizu et al., (1995)	1990-1992	Fisheries Breeding Center, Japan	*Strongylocentrotus intermedius*	*Vibrio* and *Aeromonas*
Becker et al., (2008)	2006	Atlantic coast, Brittany, France	*Paracentrotus lividus*	*5 Alphaproctobacteria, 8 Gammaprotobacteria* and *1 Fusobacteria.* Some were identified as *Vibrio* sp., *Colwellia* sp., *Stappia* sp., *Bacteroidetes* sp. and *Cytophagales* sp.
Girard et. al., (2011)	2003	Tenerife, Spain	*Paracentrotus lividus*	Bald sea urchin disease related with global climate warming
Dyková et al., (2011)	2010	Tenerife, Spain	*Diadema africanum*	*Neoparamoeba branchiphila*
Wang, Feng et al. (2013)	2009-2010	Heishijiao Hatchery, China	*Strongylocentrotus intermedius*	*Vibrio splendidus, V. shilonii, V. harveyi, Pseudoalteromonas tetraodonis, Shewanella aquimarina*
Clemente et al, (2014)	2009-2010	Madeira Island (Portugal) to the Canary Islands (Spain)	*Diadema africanum*	*Vibrio alginolyticus.*
Virwani et al. (2021)	2017-2018	St. Kitts, Caribbean Sea	*Tripneustes ventricosus*	Ciliates and encysted metazoan parasites
Hernández et al., (2020)	Since 2001	Tenerife and La Palma, Spain	*Diadema africanum*	*Paramoeba branquiphila*
Gizzi et al., (2020)	2018	Madeira, Portugal	*Diadema africanum*	In the coelomic fluid: *Aeromonas salmonicida* and non-identified β-hemolítica (gram-negative) bacteria species (as *V. alginolyticus)*
Grech et al., (2022)	2019	Sardinia (Italy)	*Paracentrotus lividus*	*Vibrio splendidus,* protozoa and metazoan observed too
Salazar-Forero et al., (2022)	2020	After Filomena Storm. Tenerife, Spain	*Diadema africanum*	*Neoparamoeba brachiphula, Vexillifera minutissima, Vibrio alginolyticus*
			Sphaerechinus granularis	*Neoparamoeba brachiphila, Vexillifera minutissima*
			Paracentrotus lividus	
			Arbacia lixula	
Federico et al., (2023)	2019-2020	Napoli (Italy)	*Paracentrotus lividus*	*Vibrio anguillarum, Aeromonas salmonicida, Tenacibaculum* spp.
Wang et al. (2023)	2020	Sea urchin farm in Weihai, Shandong Province, China	*Strongylocentrotus intermedius*	*Vibrio coralliilyticus*
Hewson et al., (2023)	2022	Saba (Caribbean Netherlands) and St. John (USVI)	*Diadema antillarum*	*Philaster* spp (ciliate)
Zirler et al., (2023)	2022	Levantine coast of Greece and Turkey	*Diadema setosum*	Infection agents were not described
Shaw et al., (2024)	–	Southern California Sea Urchin Company (Corona del Mar, CA)	*Strongylocentrotus purpuratus*	Many different pathogenic bacteria, including *V. alginolyticus, V. coralliilyticus*, *Flexibacter* sp., *Acinetobacter* sp., *Tenacibaculum* sp., *Colwellia* sp., Flexibacteraceae, Rhodobacterales, *Cohaesibacter gelatinilyticus*, *Stappia*, Psychrobacter, Staphylococcus, and Saprospiraceae
Our study	2022	Gran Canaria, Spain	*Arbacia lixula*	*Acinetobacter johnsonii* *Pseudomonas frederiksbergensis* *Vibrio alginolyticus* *Vibrio mytili* *Candida*
			Paracentrotus lividus	*Pseudomonas rhodesiae* *Vibrio alginolyticus* *Vibrio harveyi* *Candida*

presence of this diversity of bacteria is not necessarily related to the appearance of the disease.

Furthermore, it is important to mention that in our samples the detected pathogen agent varied according to the individual despite the lesions showed having the same characteristics. This result coincides with that reported by Becker et al. (2008). The diverse symptoms associated with this disease suggest that pathology can vary significantly between individuals, because of the presence of different opportunistic infection agents, producing different levels of the bald sea urchin disease (Shaw et al., 2023). Finally, the histological work of Becker et al. (2008) revealed that bacteria infecting *Paracentrotus lividus* test were confined to the central zone, whereas the peripheral zone exhibited a high concentration of cells that function as a barrier and the surrounding tissues remained unaffected, impeding the spread of the infection.

The presence of *Candida* in *A. lixula* and *P. lividus* affected surfaces represents a new record in sea urchins' infection agents. There is only one previous work that reports other species of fungi in sea urchin spines in the Pacific Ocean, but those fungi were not considered as pathogens (Dabrowa et al., 1964). This information shows a novel perspective on the bald sea urchin disease, adding new possible pathogens that cause this disease.

Even with all the infectious agents described above, it is important to highlight that these diseases seem to have a multifactorial origin, influenced by physiological and environmental factors (temperature variation, adverse factors, and plankton blooms), and life cycles in trophic networks (Federico et al., 2023). These adverse characteristics in the conditions agree with the season of our study, with the highest temperatures reported in Gran Canaria Island during 2022, and with the reproductive season of the sea urchin species in the area (Núñez-González et al., unpublished data). According to what was described above, the presence of these pathogenic agents could be inducing the weakening of the organism. First for the immune system, but then because of diminished adhesion to the substrate due to the external lesions and loss of the tube feet that affect the normal behavior of the sea urchin (Girard et al., 2011), and make them prone to being attacked by predators (Hughes et al., 1985). In addition to these physiological effects, our study reveals the presence of an inflammatory response within the affected tissues where bacterial colonies are present, providing crucial insight into the understanding of this sea urchin disease.

In this sense, we highlight the importance of histopathology as the primary tool for comprehending the impact of pathogens, not only on tissues but also on the overall development of sea urchins in their natural habitat. The utilization of histopathology enables a comprehensive assessment of the effects caused by these pathogens.

Finally, the exhaustive review of Feehan & Scheibling (2014) and Sweet (2020) indicated that research about disease patterns in marine key species, such as sea urchins, could be affected by climate change and other human-induced pressures, such as pollution and fishing activities which affect the top-down control exerted by predators. While the species of the study are not frequently consumed by the inhabitants of Gran Canaria Island, they hold significant value as bait for fishing some species of fish. It is crucial to enhance our understanding of disease dynamics in marine ecosystems and recognize the role that disease plays in driving alterations within these ecosystems.

The effect of the disappearance of coastal sea urchins can produce important changes in ecosystem dynamics, as the case reported by Sangil & Hernández (2022), where the decrease in *D. africanum* population implied a rapid recovery of non-crustose macroalgae in the Canary Archipelago. However, the impact of species' disappearance in our study remains unexplored and should be worthy of further studies. We emphasize the need to continue monitoring marine species and to gain insight into the development of these diseases within marine ecosystems, to provide new methods for the efficient management and governance of coastal resources. By doing so, we can

implement conservation measures to safeguard these species for future generations.

Ethical statement: the authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

ACKNOWLEDGMENTS

The authors gratefully thank the Government of the Canary Islands, for providing the scientific permit Nº: 168/2020 - Tomo: 1, Libro: 401, Date: 10/06/2020, to collect all the samples for this study. We also thank Sarah Piaugeard for helping during the sampling campaigns, Eleinis Ávila-Lovera for helping us to improve the manuscript, and Sam C. for helping us correct English grammar.

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