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Influence of Winter Storms on the Sea Urchin Pathogen Assemblages

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In recent years, recurrent sea urchin mass mortalities in the Canary Islands have been registered. These mortality-related events have decimated 93% of the eastern Atlantic populations of the barren-forming sea urchin *Diadema africanum*. Two severe episodes of rough southeastern seas led to winter storms in February 2010 (Xynthia) and February 2018 (Emma) and preceded the last mass mortality event. We hypothesized that these events are related to the mass mortalities registered during the February in those years. Previous studies identified *Neoparamoeba branchiphila* as the causal agent of the disease, possibly acting in synergy with *Vibrio alginolyticus* and/or other pathogens. To determine the link between winter storms and the sea urchin pathogen community, we monitored the marine pathogen assemblage before and after the winter storm Filomena (February 2020) on Tenerife Island, on different habitats (sea water, sediment and algae) and in four species of sea urchin hosts (*D. africanum*, *Arbacia lixula*, *Paracentrotus lividus* and *Sphaerechinus granularis*). A total of six pathogens, including *N. branchiphila*, *Vexillifera minutissima*, *Acanthamoeba* sp., *Vahlkampfia* sp., *V. alginolyticus* and green colonies of *Vibrio* spp., were identified. Only small amoebas were found in sea urchins, while *Vibrio* species were more common in seawater, sediment and algae substrates. *V. alginolyticus* was occasionally detected in three sea urchins specimens, while *N. branchiphila* was found in the coelom of all four sea urchin studied. As previously hypothesized, a significant pathogen increment in seawater and in the sea urchin species *D. africanum* and *P. lividus*, was found after Filomena. Our results confirmed the relationship between the winter storms and marine pathogen dynamics. However, further studies are needed to demonstrate the direct relationship between these pathogen increases and the sea urchin mass mortalities.

Keywords: Killer Storm, pathogens, sea urchin, mass mortalities, communities

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INTRODUCTION

Over the last several years, the frequency and severity of disease spread in marine animals is increasing (Harvell et al., 1999; Lafferty et al., 2004; Feehan et al., 2012; Nowak and Archibald, 2018), a process that is related to an unbalanced ecosystem caused by human activities. It is anticipated that this growth will continue in the currently climate change context (Harvell et al., 2002). The echinoderms are one of the taxa having several reports of spread diseases that have been associated

with massive mortality events (Lessios et al., 1984; Tajimaa et al., 2007; Hernández et al., 2020; Sweet, 2020). Since 1970, extensive mortality events involving sea urchins caused by pathogenic agents has been reported in almost 30 genera of sea urchins from different parts of the world (Maes and Jangoux, 1984; Tajimaa et al., 2007; Feehan et al., 2012; Wang et al., 2013). However, the most studied cases are the green sea urchin *Strongylocentrotus droebachiensis* off the coast of Nova Scotia (Scheibling and Stephenson, 1984; Scheibling et al., 2010; Feehan et al., 2012) and *D. antillarum* in the Caribbean Sea (Lessios et al., 1984); the latest because of the strong effects on the structure and dynamics of the coral reef communities of the Caribbean, which have had a very slow recovery after 30 years and are still far from their initial states (Lessios, 2016). In Nova Scotia, the massive mortalities events are cyclicals and are associated with storm-induced paramoebiasis, better known as the "Killer Storm" (Scheibling and Lauzon-Guay, 2010; Scheibling et al., 2010; Feehan et al., 2012).

The spread of these diseases has been associated with extremes in environmental conditions, such as storms and sea temperature increases, which synergistically facilitate the dispersion of pathogenic agents thus causing an increase in their presence in the ecosystems over the last several years because of the global warming (Tajimaa et al., 2007; Scheibling and Lauzon-Guay, 2010; Scheibling et al., 2010; Feehan et al., 2012). Because sea urchins are key herbivores in the benthic communities, any density-related changes could introduce a risk for the stability and the structure of the benthic ecosystems and generate an impact throughout trophic cascade processes (Fernandez et al., 2006; Clemente et al., 2014; Trowbridge et al., 2019). In the East Atlantic Ocean, two mass mortality events have been reported, one after the storms Xynthia in February 2010 and one after Emma in February 2018 (Hernández et al., 2020) that affected the sea urchin *Diadema africanum*. The first mortality event was observed to last from October 2009 to April 2010 during which the sea urchin population was reduced by 65%; however, this event occurred without compromising the reproductive success of the species (Clemente et al., 2014). During the second event, the population was reduced by 93% around Tenerife and La Palma Islands (Hernández et al., 2020) and promoted a stable shift toward a macroalgae-dominated system (Sangil and Hernández, 2021). These mortality events also affected the Madeira Archipelago; but in contrast to what happened around La Palma and Tenerife Islands, some recovery of the sea urchin populations after the second mortality event did occur (Gizzi et al., 2020). All mortality cases were attributed to sea urchin bald disease because of the similarities in the lesions observed in the epidermis of echinoids. In the first set of studies, it was suggested that *Vibrio algynoliticus* was the principal pathogenic agent and during the second event, only *Neoparamoeba branchiphila* was found in moribund individuals. Nevertheless, in the first event the sea urchin specimens under study were collected in an advanced state of disease or were even dead. Therefore, the possibility of a synergy with other causative pathogens, such as the amoeba *N. branchiphila*, was not ruled out (Dyková et al., 2011; Clemente et al., 2014). It is relevant to highlight at this point that a similar amoeba (*Paramoeba invadens*) was identified as the causative agent of the disease in the sea urchins *Strongylocentrotus droebachiensis* in the west Atlantic. Both periods of mortality were associated with unusually rough seas due to southwest storms in February 2010 and 2018, suggesting that this kind of storm also induces paramoebiasis in the East Atlantic, supporting the hypothesis "Killer Storm" that has been suggested for the West Atlantic shores (Scheibling and Lauzon-Guay, 2010; Scheibling et al., 2010; Feehan et al., 2012). This hypothesis supposes a positive correlation between the intensity of the storm and the propagation of paramoebiasis (Scheibling and Lauzon-Guay, 2010). The East Atlantic mortality events presented completely different average temperatures; hence, no clear relationship with sea surface temperatures can be found (Hernández et al., 2020).

Despite the number of extensive reported mortality events, in many cases, the causal agents had not been studied, and in most of these cases, it was not identified because of the difficulties related to the microbiological studies (Trowbridge et al., 2019; Sweet, 2020). Nonetheless, bacteria, fungus, protozoa and even algae have been considered as infectious agents (Wang et al., 2013), probably potentiated by the interaction of multiple stress drivers such as temperature changes, storms and environmental degradation (Trowbridge et al., 2019). The identification of pathogens in the marine environment is difficult, but this type of study can be harder and bring more challenges when free-living amoebae (FLA) are studied. In the first place, amoebae are diverse, they have a cosmopolitan distribution, and they can be isolated from most environments and infect many different animals. Because they have a two-stage life cycle, morphological studies are based on the identification of trophozoites and cysts (Walochnik, 2018). The observation of FLA requires many hours in front of a microscope, and sometimes it is difficult to focus on an agar medium. The observation of cysts is widely used in amoebae identification; yet, many times, this finding is not supported with molecular data (Walochnik, 2018). In this situation, we found another drawback because DNA extraction of marine amoebae requires high quantity of cells; also, the procurement and maintenance of axenic cultures is complicated because the amoebae usually are in a mixed culture with bacteria (Nowak and Archibald, 2018). In some cases, we must consider a symbiotic relation between the amoeba and bacteria, because intracellular presence of *Vibrio* sp. has been detected in amoebae (Mac Phail et al., 2020). In addition, the availability of molecular marker data and the isolated strains of invertebrates in collections are low (Dyková et al., 2007, 2011). This drawback makes molecular studies even more difficult.

On January 6–8, the storm, Filomena, arrived at the Canaries after originating inside the United States (US) and moved through the Atlantic until it was positioned in the north of the Canary Islands. This storm produced intense rains and generalized winds of 70 to 80 km/h from the SW and NW, which generated waves of 2 to 3 m (AEMET, 2021). These conditions seemed appropriate for obtaining information on the dynamics of the pathogen community after the passage of the storm and its possible relationship with sea urchin mortalities. Therefore, the presence of this pathogen before and after Filomena was examined in different type of substrates (water, sediment and algae) and sea urchin species (*Diadema africanum*,

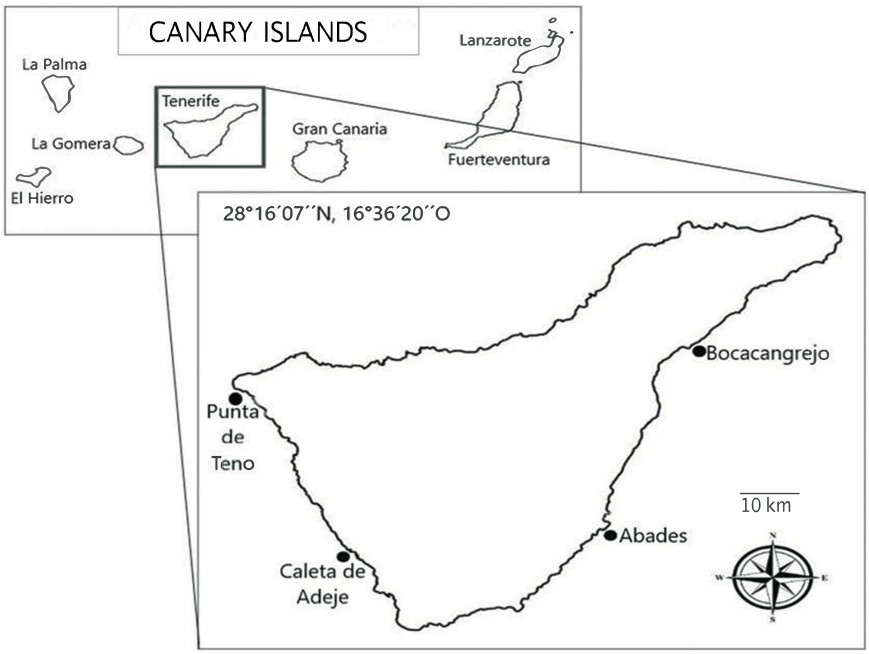

FIGURE 1 | Map showing the sampling localities visited in the present work off Tenerife Island (Canary Archipelago, Spain).

Paracentrotus lividus, *Sphaerechinus granularis* and *Arbacia lixula*) to understand the mechanisms of the introduction of pathogens and its dynamics to determine how the sea urchin mass extensive mortality events could occur.

MATERIALS AND METHODS

Sampling from four locations on the island of Tenerife was conducted (28°16′07″North, 16°36′20″West): Boca Cangrejo, Abades, La Caleta de Adeje and Punta de Teno (**Figure 1**) before the storm season in August 2020 and during the month of January 2021, some days after the Filomena was finished. The storm produced waves of 2 to 3 m from the S and SW (**Figure 2**). Generally, Tenerife Island is affected by trade winds that generate waves of moderate energy with an annual medium height of 1.4 m. Between October and March, the sea swell intensifies and winds from the North Atlantic approach the island from the north/northwest (NNW) and northwest (NW), producing waves of 2 to 3 m. Due to the orientation of coasts, the eastern and southern shores of the island are more protected from the NW/NNW swells, and the average wave is less than in other orientations (Yanes et al., 2006).

To locate and characterize the presence of pathogens, we selected three types of substrates: (1) sea water, (2) sediment and (3) algae; we also evaluated the possibility that some of the common sea urchin species (*D. africanum*, *A. lixula*, *P. lividus*, and *S. granularis*) could act as pathogen hosts. For each locality, we collected by free diving three replicates of sediment using 50 ml cores, 25 cm^2 of macroalgae community and 1.5 L of water; each replicate was collected at about 1–2 m depth and spaced about 3 m apart. Additionally, depending on the availability, three individuals for each sea urchins were collected by visual searching between 1 and 3 m depth for 45 min (**Table 1**). All samples were transported in the refrigerator to the laboratory facilities.

Sample Process and Experimental Design

Each sample was grown in two different media: (1) petri plate (60 mm × 15 mm) of thiosulphate-citrate-bile-sucrose Agar (TCBS) Merck™, a selective medium for *Vibrio* isolation, composed of bile salts, thiosulphate, citrate and alkaline pH of 8.6. These components inhibit the growth of other bacteria and the saline characteristics of the medium promote the growth of *Vibrio* spp. The differentiation process is based on fermentation of as sucrose detected by indicators (thymol blue and bromothymol blue), which change color to yellow *via* a sucrose reaction. Strains undergoing sucrose fermentation produce yellow colonies, while the others show green colonies. The sizes of the colonies can be different, but generally, they have a diameter of 0.5 mm minimum (Corry et al., 1995) and (2) the other medium used was a petri plate (100 mm × 20 mm) of nutritious granulated agar (ANN) DIFCO™ 2%, a solid medium for amoebae isolation but does not guarantee exclusive growth of amoebae. Both culture mediums were prepared following the instructions of the manufacturer using Milli-Q® water.

Sea water samples were prepared using a vacuum ramp PALL No 1540 and nitrocellulose membranes with pores of 0.45 μm diameter. The first 100 ml from each bottle was filtered. Using a Bunsen burner and tongs, the membrane was separated from the filter and was grown on the TCBS

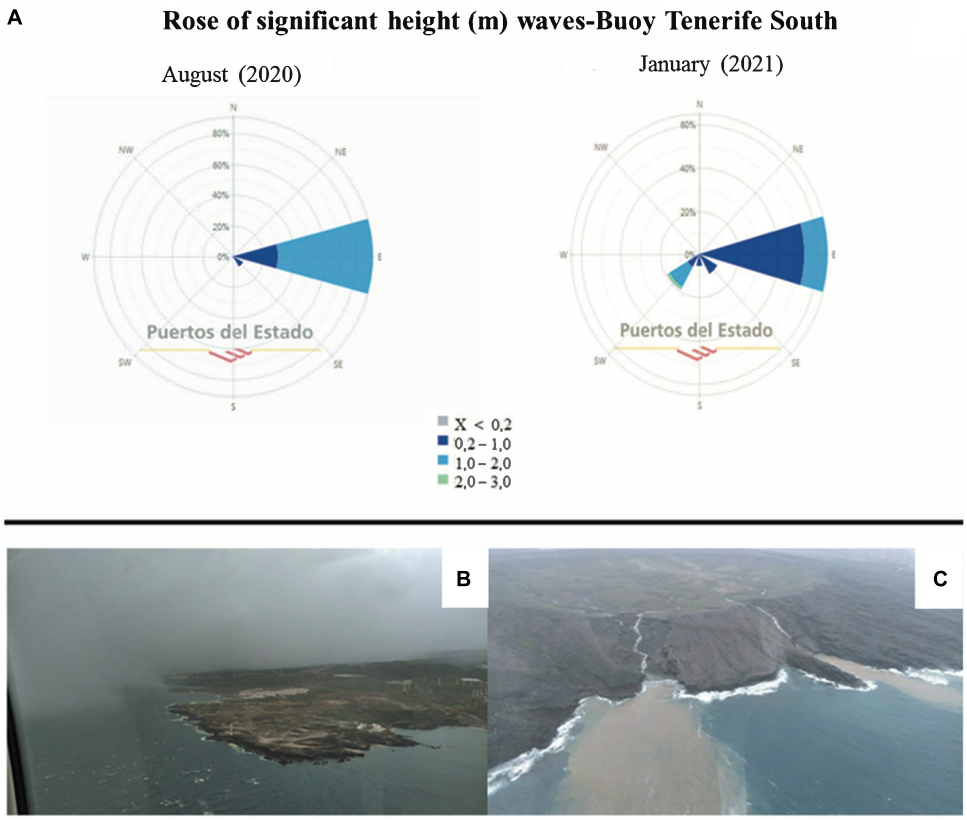

FIGURE 2 | (A) Significant wave height Rose (m) for August 2020 (pre-storm) and January 2021 (post-storm) from the buoy of Tenerife South (del Estado, 2021). **(B)** Image of Pta. Rasca (south tip of Tenerife), where the westerly weather is clearly visible during the period of Filomena storm; **(C)** Image of Montaña Pelada (Tenerife), where the flow of the ravines in the post-storm period is clearly visible. Aerial photographs by Ignacio Herranz Duarte.

plate after which the remaining water was filtered, and the membrane was placed face down in the Petri plates with ANN to facilitate transfer from the membrane to the culture medium.

For the algae assemblages samples, with the help of tweezers, a small piece of algae turf of 2 cm^3 were seeded directly onto both growing media. An individual of each algae species that composed the turf was left for later taxonomical identification (**Table 2**).

For the sediment samples, a cotton swab was introduced for the obtaining a small sample, which was later added directly to both growing media.

For the sea urchin coelom samples, the sea urchin was positioned on its back and using a 29 Gx 1.27 cm insulin syringe, 1 ml of coelom from each sea urchin was extracted. The syringe was introduced in the peristomal membrane at an angle that avoided contact with the Aristotle's lantern (Dyková et al., 2011), and the coelomic fluid was cultured in each selective medium.

Vibrio sp. Isolation

A total of 76 samples from pre-storm and 78 from post-storm were cultured and incubated at 37°C for 24 h in a temperature controlled room. After which time the presence of *Vibrio* spp. colonies was identified using API (Analytical Profile Index) 20E test.

Amoebae Isolation

A total of 76 cultures from pre-storm and 78 from post-storm were incubated at 20°C in the laboratory and observed weekly using an inverted microscope Leica DMIL at 10 and 20 X to identify the presence of amoebae (Pussard and Pons, 1977). We took pictures using the microscope EVOS Floid Imaging System. Additionally, 30 replications were made to isolate amoebas and keep the cultures free of bacteria and fungi.

Identification of the Amoeba Species Present in the Cultures

Identification was made using morphological characters such as the shape of trophozoites, the pseudocysts for the genus Neoparamoeba (Lima et al., 2017) and the double-walled cysts shape unique in Acanthoamoeba. For the DNA extraction, two procedures were used. For the first procedure, 2 ml of the cold Page's amoeba saline solution (PAS) was used in the Petri plate and amoebae were separated with a staff and the liquid was pipetted into an Eppendorf tube for centrifugation for 20 min at 1500 rpm. The supernatant

TABLE 1 | Number of sea urchin specimens of each species collected before and after the storm Filomena.

		A. lixula	*D. africanum*	*P. lividus*	*S. granularis*
Pre-storm	Abades	3	3	0	3
	Boca Cangrejo	3	2	3	3
	Caleta	3	3	3	0
	Teno	3	3	3	2
Post-storm	Abades	3	3	3	3
	Boca Cangrejo	3	1	3	3
	Caleta	3	3	3	0
	Teno	3	3	3	2

TABLE 2 | Algae species community that composed the turfs used for cultivation on growing media at each locality.

Locality	Algae species
Abades	*Dictyota dichotoma*
	Dictyota sp.
	Lobophora schneideri
	Rhodophyta
Boca Cangrejo	*Asparagopsis taxiformis*
	Jania sp.
	Lobophora schneideri
La caleta de adeje	*Corallina ferreyrae*
	Codium sp.
	Dictyota dichotoma
	Lobophora schneideri
Punta de teno	*Asparagopsis taxiformis*
	Lobophora schneideri
	Rhodophyta

was collected and the DNA was extracted in an automatized system Maxwell 16®. In the case that the amoebae were found in just one area of the plate and in a very small proportion, that particular piece of agar was cut and put directly into a cartridge for the extraction of DNA in the Maxwell 16®. In both cases, after 40 min, the DNA was collected from the elution buffer cell and stored at −20°C until PCR was performed.

The amplification of DNA was carry out with a Termocycler Arktik™ (Thermo Scientific™), using 18S rRNA specific primers for free living amoebae (FLA) and for Valkampfidae Amoebae (VAHL), following the protocol described in Reyes-Batlle et al. (2021). The primers were P-FLA F 5′-CGCGGTAATTCCAGCTCCAATAGC-3′/P-FLA R 5′-CAGGTTAAGGTCTCGTTCGTTAAC-3′ (Tsvetkova et al., 2004) and VAHL-1 5′-GTCTTCGTAGGTGAACCTGC-3′/VAHL-2 3′-CCGCTTACTGATATGCTTAA-5′ (De Jonckheere and Brown, 2005). The expected amplicon was between 500 and 550 pb long. For the mixture, 5 μl buffer, 1 μl dNTPs, 0.5 μl forward and 0.5 μl reverse primers, 0.25 μl Taq, and 32.75 μl water were used. For each sample, two sets were prepared, the first one with 10 μl of DNA, and the second one with 4 μl of DNA and 6 μl of water. For the positive control of FLA 6 μl Acanthoamoeba DNA + 4 μl MIC, for the positive control of VAHL 6 μl *Naegleria fowleri* DNA + 4 μl MIC and for the negative control in both cases 10 μl MIC were used. Once the samples were amplified, the electrophoresis was performed using a 2% agarose gel with 1% TAE buffer. A charged buffer was used to weight the fragments and the marker PCRBIO Ladder IV, which works from 100 to 1500 pb, was used to calculate the molecular weights of the DNA samples. *P. brachiphila* sequence have been uploaded to ZENODO and are free accessible (Lorenzo-Morales and Hernández, 2020).

Statistical Analyses

We used the SPSS 19 (IBM) and PRIMER7 + PERMANOVA (Anderson et al., 2008; Clarke et al., 2014) statistical packages to perform all analyses. Chi2 test was applied specifically for the most frequently detected pathogen species (*Vibrio algynoliticus*, *Neoparamoeba branchiphila* and *Vexillifera minutissima*) (**Figure 3**) to determine significant variations on their presence/absence proportion after the passage of the storm. For the pathogen assemblage analysis the presence/absence of pathogen data was used for calculating the similarity matrix using Bray–Curtis index. The triangular matrix of similarities was then used to obtain a cluster grouping to determine the principal groups of pathogens. Using the SIMPROF procedure, significant differences between groups were determined. A non-metric MDS was used for better visualize the obtained results. The effects of time (pre-/post-storm periods) and the substrate/host (sea water, sediment, algae and sea urchin species) on the pathogen community were tested using a two-way factor permutational multivariate analysis of variance (PERMANOVA) in which both factors were treated as fixed factors with two and seven levels, respectively.

RESULTS

A total of 156 cultures from the August 2020 sampling were examined, 78 in ANN culture media and 78 in TCBS culture media. In January 2021, 152 cultures were examined, 76 in ANN and 76 in TCBS. Six different pathogens were found:

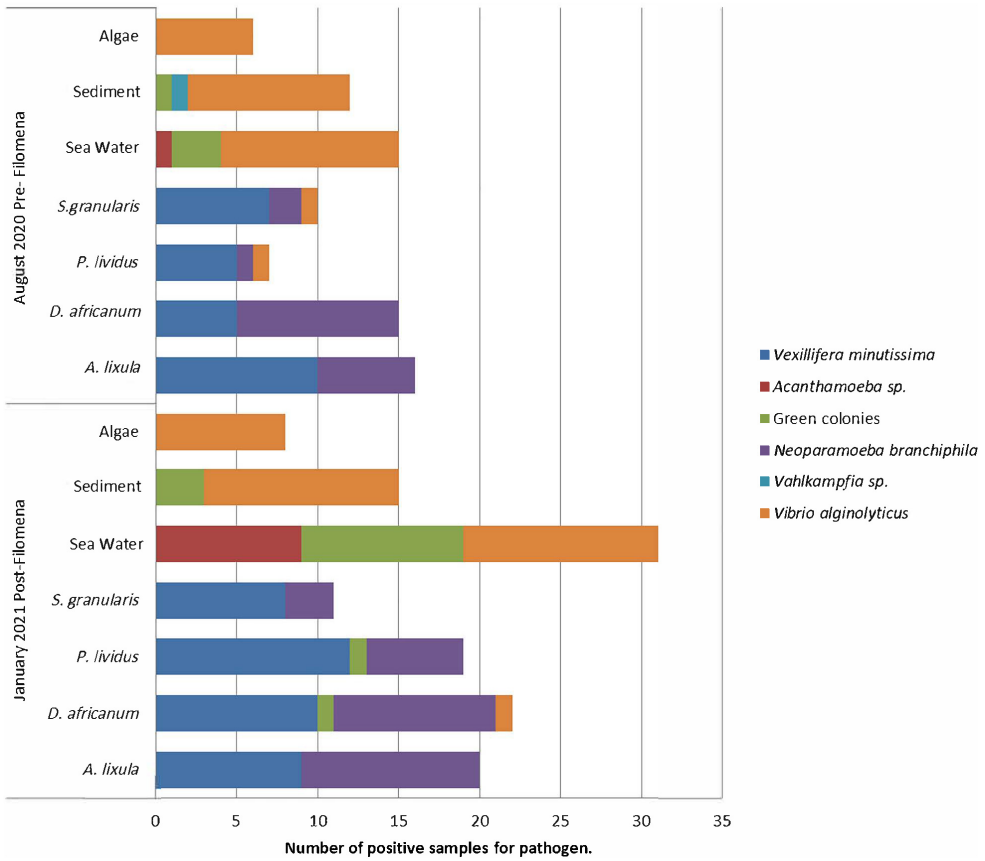

FIGURE 3 | Bar chart of the number of positive samples for the different pathogens grown on the different substrates/host collected before (pre-) and after (post-) the Filomena storm.

(1) yellow colonies of the bacteria *Vibrio alginolyticus*, (2) green colonies of bacteria *Vibrio* sp., and (3) the amoeba *Acanthamoeba* sp., (4) the amoeba *Vahlkampfia* sp., (5) the amoeba *Neoparamoeba branchiphila* and (6) the amoeba *Vexillifera minutissima*. In January 2021 (post-Filomena storm) a higher number of positive per sample were obtained for all pathogens. Particularly, in the seawater samples, highlighted by the large number of positive for three pathogens: (1) green colonies of *Vibrio* sp., (2) *V. alginolyticus* and (3) *Acanthamoeba* sp. (**Figure 3**).

Vibrio spp. Isolation

In 62 analyzed samples, 29 before the storm and 33 after, yellow colonies identified as *V. alginolyticus* were observed on the three substrates (**Figures 4A–C,E,F**) and in three sea urchin's species, before storm in the case of *P. lividus* and *S. granularis*; and after in the case of *D. africanum* (**Figures 4A,D**). Additionally, in the sea water sample from Teno, collected in August 2020 (pre-storm), it was found *Vibrio harveyi* (**Figure 4G**). Also, the growth of green colonies, which increased by 13%, was observed, specifically in sea water samples, sediment and two species of sea urchins (**Figures 3**, **4D**).

Amoebas Isolation

A total of 156 ANN plates were followed. A cyst and trophozoites of *Acanthamoeba* sp. in a sample of sea water from La Caleta de Adeje (**Figure 5A**) and a cyst of *Vahlkampfia* sp. (**Figure 5B**) in a sediment sample from Abades were found in samples taken before the storm. *N. branchiphila* was observed on 19 plates containing sea urchin coelomic fluid (**Figure 5C**) and *V. minutissima* was found in 27 plates of sea urchins (**Figure 5F**). Most of the post-Filomena substrate samples presented *Acanthamoeba* sp. The sequencing of one of the strains allowed the identification of genotype T4. Even though the positive control did not run adequately, the molecular marker allowed us to deduce that the amplified fragment was around 500 pb, and it was *Acanthamoeba*. The other amoeba found in January 2021 was *N. branchiphila*, identified by the trophozoites and the pseudocyst (**Figures 5D,F**). When compared with samples from August 2020, this amoeba showed a 13% increase in amount (**Figure 5A**) while *V. minutissima* had an increase of 15% (**Figure 5F**).

Statistical Analysis

The Chi2 test found no differences on the presence/absence of *V. alginolyticus* on samples proportion after the passage of the

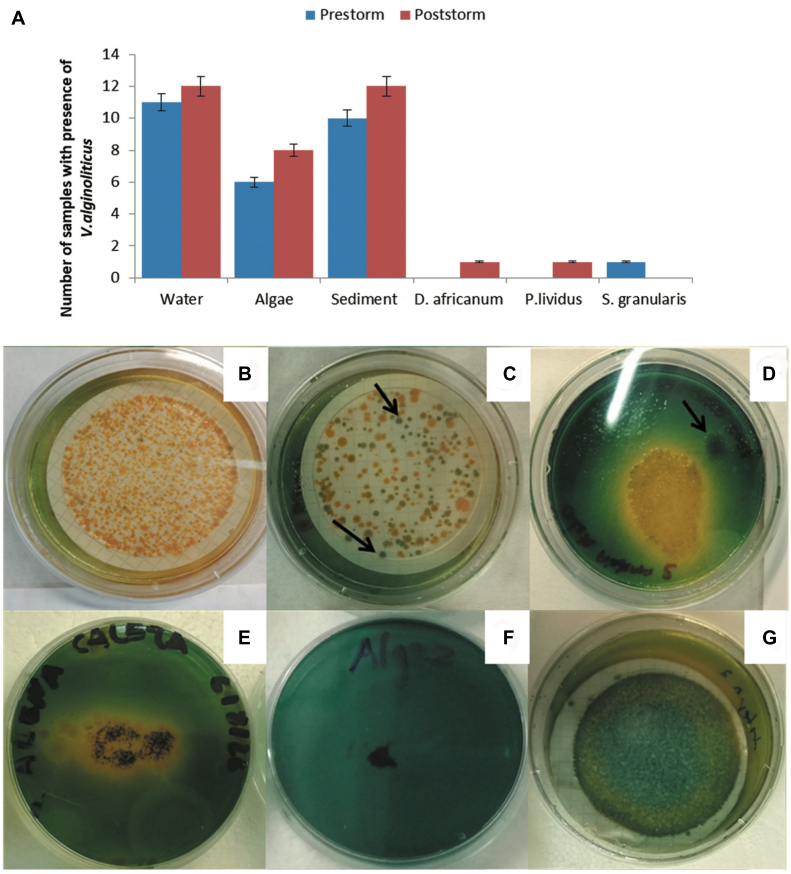

FIGURE 4 | Isolation of *Vibrio* species: **(A)** the bar graph shows the presence of *V. alginolyticus* on substrates/hosts before and after of Filomena's storm (5% error bars). Cultures photographs: **(B)** positive growth of yellow colonies of *V. alginolyticus* post-storm in water sample from Teno; **(C)** positive growth of *V. alginolyticus* post-storm in water sample from Abades; **(D)** Positive growth of *V. alginolyticus* and green colonies (see arrow) post-storm in coelomic liquid of *Diadema africanum* from La Caleta de Adeje; **(E)** positive growth of *V. alginolyticus* pre-storm in sediment collected in La Caleta de Adeje; **(F)** negative growth of algae sample from Boca Cangrejo; **(G)** positive growth of *V. harveyi* in water sample from Teno.

Filomena storm (**Figure 4A**). However, a significant increase of samples with *N. brachiphila* was found for the sea urchins *A. lixula* and *P. lividus* (**Figure 5C**); parallel to an increase of samples with *V. minutissima* presence in the case of *D. africanum* and *P. lividus* (**Figure 5F**).

The groups obtained by SIMPROF (**Figure 6**), better visualized with the MDS plot (**Figure 7**) were classified by different pathogen communities. In general, we just observed *V. minutissima* and *N. branchiphila* in the sea urchin species, while in the sea water, sediment, and algae turf samples, the most observed colonies were *Vibrio algynoliticus*. Additionally, we found atypical observations that included *Acanthamoeba* in sea water samples, *Vahlkampfia* in sediment samples, and *Vibrio* spp. (green colonies) in four sea urchin samples. The PERMANOVA analysis (**Table 3**) showed a significant interaction between the factor "pre- and post-storm" and the factor "substrate/hosts" on the presence of pathogens. In other words, Filomena produced a significant change in the presence of pathogens that depend on the substrate/hosts studied. A significant increase in the presence of pathogens was found in sea water samples and the hosts *A. lixula*, *D. africanum* and *P. lividus*. No effect was found in the other substrates/hosts (**Figure 3**).

DISCUSSION

We found six different pathogens in the studied samples: (1) *Vibrio alginolyticus*, (2) green colonies of *Vibrio* sp., (3) *Acanthamoeba* sp., (4) *Vahlkampfia* sp., (5) *Neoparamoeba branchiphila* and (6) *V. minutissima*. The pathogen community depended on the studied substrate/host, for example *N. branchiphila* and *V. minutissima* were found only in sea urchins, while *Vibrio* sp. was present in sea water, sediment and algae samples. The presence of *N. branchiphila* was exclusively on the coelom of the four studied species of sea urchin. Filomena storm produced significant changes in the pathogen communities of seawater and the hosts, *A. lixula*, *D. africanum* and *P. lividus*. The main effect was an increase in their presence in that substrate and hosts. However, no extensive sea urchin mortality event was reported that year, perhaps because Filomena was a mild storm especially with respect to the intensity of winds and waves from

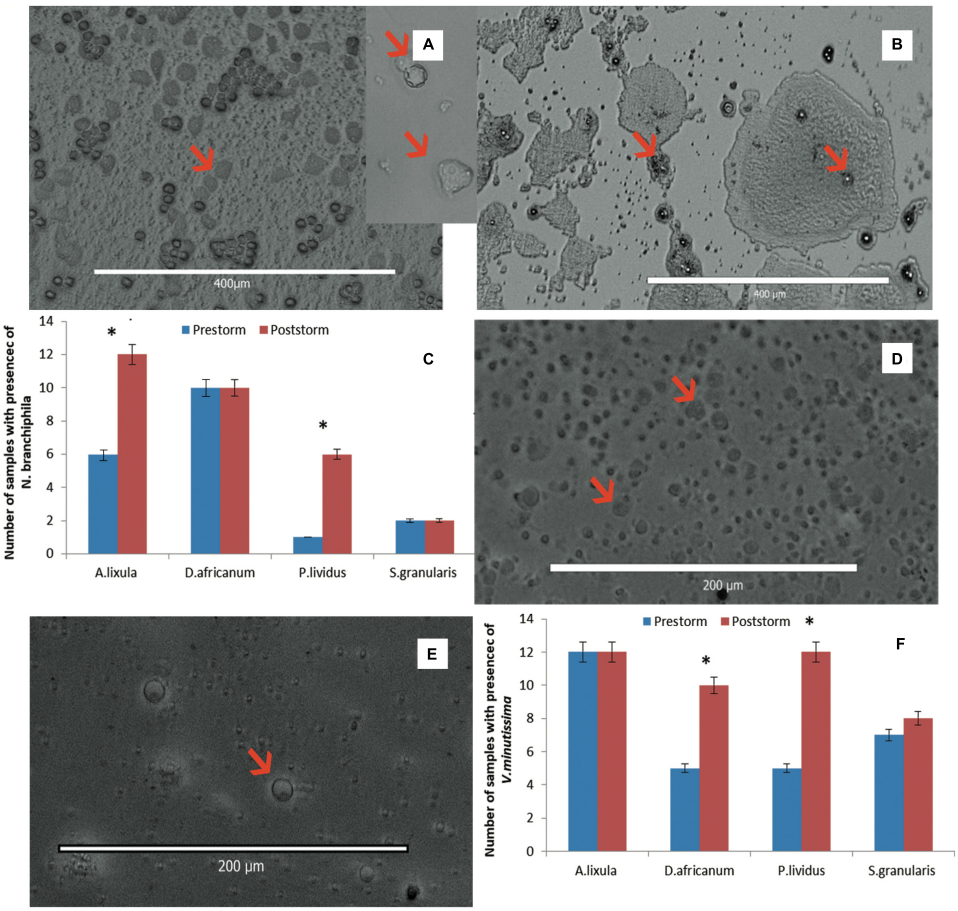

FIGURE 5 | (A) Trophozoites and cyst of Acanthamoeba genotype T4, the arrows shows the double-walled cysts and the shape of trophozoite; **(B)** cysts of *Vahlkampfia* sp. in a sediment sample from Abades; **(C)** bar graph that shows the presence of *N. branchiphila* in sea urchins before (pre-) and (post-) Filomena's storm (5% error bars), asterisks shows significant differences of $P < 0{,}005$; **(D)** pseudocysts of *N. branchiphila* **(E)** typical pseudocysts of *N. branchiphila*; **(F)** bar graph that shows the presence of *Vexillifera minutissima* in sea urchins before (pre-) and (post-) Filomena's storm (5% error bars), asterisks shows significant differences of $P < 0{,}005$.

the S and SW since the values did not reach the intensity during Emma and Xynthia as reported by Hernández et al. (2020).

Vibrio sp. was always found in sea urchins and *V. alginolyticus* in most of the seawater, sediment and algae samples. This result can be interpreted as a stable pathogen community of the sea urchins. In this sense bacteria of the genus *Vibrio* sp. have been previously associated with the development of skin lesions in different species of echinoids (Becker et al., 2008; Sweet, 2020). Specifically, *V. alginolyticus* was previously isolated from moribund and dead individuals during the sea urchin mortality event of 2009–2010 that occurred in the East Atlantic (Clemente et al., 2014). Also, it is important to mention that *V. harveyi*, a marine bacterium characterized by bioluminescence that is associated with vertebrate and invertebrate diseases (Austin and Zhang, 2006), was found in one of the seawater samples. *Vibrio* has also been associated with poor water quality of the environment (Martony et al., 2018). In relation to the algae substrate, we detected *Vibrio* sp. but no amoebae were found; this finding could be a result of the secondary metabolites of the selected algae substrate, which can have antimicrobial, antioxidant and cytotoxic activities (Gamal, 2010; García-Davis et al., 2018; Carroll et al., 2019).

We only found *V. minutissima*, which is a marine amoeba previously isolated from sediments in the Baltic Sea (Garstecki and Arndt, 2000), and *N. branchiphila*, which has previously been related to the extensive mortality of *D. africanum* in the East Atlantic (Dyková et al., 2011; Hernández et al., 2020), in the sea urchin samples. Nonetheless, *N. branchiphila* has only been observed in moribund individuals of *D. africanum* (Dyková et al., 2011) and in healthy individuals of *P. lividus* and *Heliocidaris erythrogramma* (Dyková et al., 2007). In this case, any of the studied sea urchins presented symptoms of the disease. The genus *Neoparamoeba* has also been observed in conjunction with *Vexillifera* sp. confirming a pathogen community; which is also present in the gills of the Atlantic salmon (Bermingham and Mulcahy, 2007). *N. branchiphila* was not observed in any of the seawater, sediment or algae samples in our study, suggesting that the amoeba does not have a stable population in these

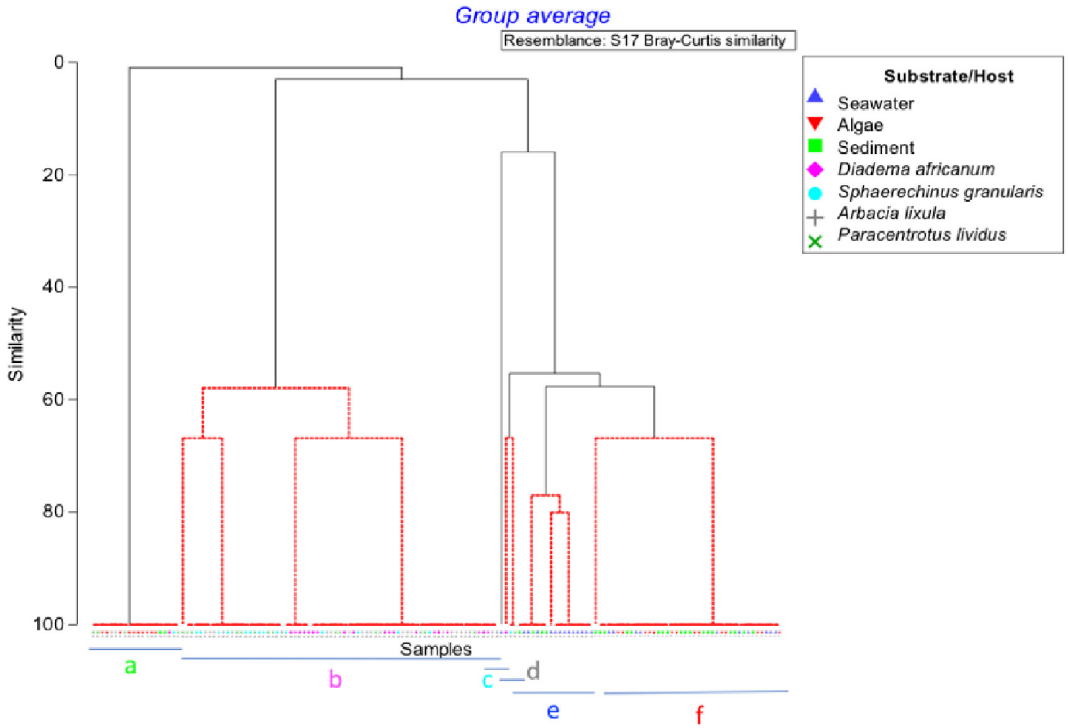

FIGURE 6 | SIMPROF dendrogram showing the significant six clusters of samples formed in red. Each group has a letter from a to f.

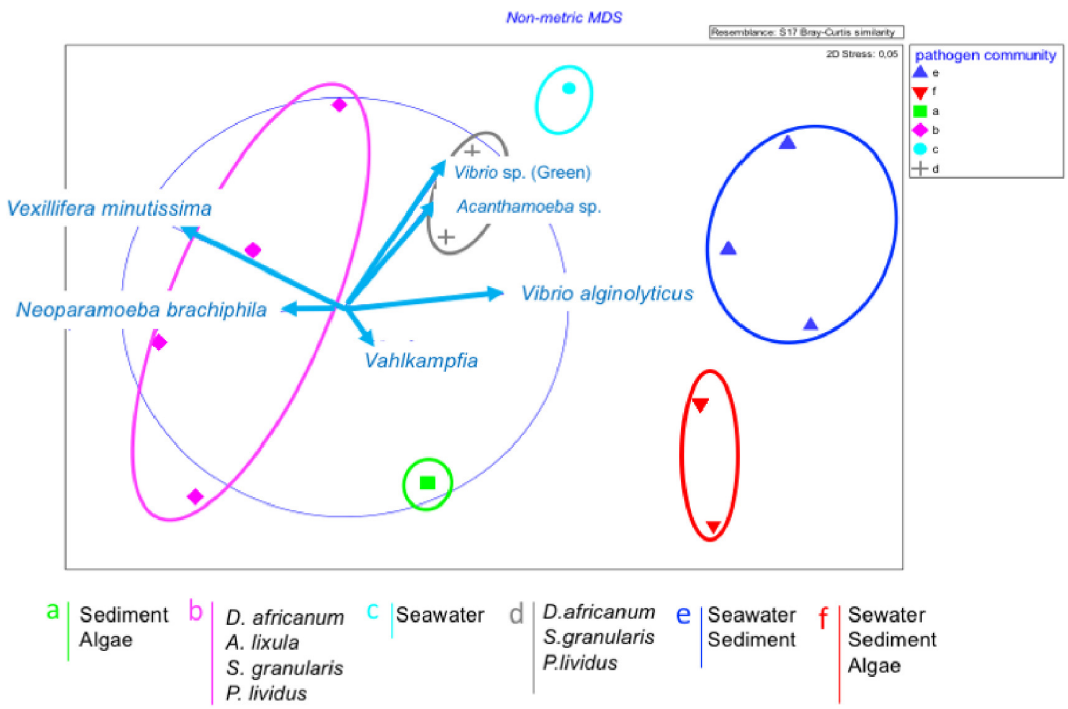

FIGURE 7 | Non-metric multidimensional scaling (nMDS) plots of the dominant pathogen clusters and assemblages. Dominant pathogens which highlight the differences are shown as vector.

TABLE 3 | (A) PERMANOVA analysis of the pathogen assemblages for the factors "Season" (Pre-/Post- storm), "Substrate/Host" (Sea Water, Sediment, Algae, *Diadema africanum, Sphaerechinus granularis, Arbacia lixula, Paracentrotus lividus*) and their interactions. **(B)** PAIWISE analysis of the interaction term. Season (Pre-/Post- storm) is compared across the levels of "Substrate/Host" term.

(A) PERMANOVA	***df***	**SS**	**MS**	**Pseudo-F**	**P(perm)**
Season	1	3.397	3.397	10.784	0.001
Substrate/Host	6	80.304	13.384	42.486	0.001
Season X Substrate/Host	6	7.066	1.178	3.739	**0.001**
Residual	140	44.103	0.315		
Total	153	135.42			
(B) PAIRWISE	***t***	**P(perm)**			
Sea Water	3.614	**0.001**			
Sediment	1.198	0.332			
Algae	0.804	0.687			
Diadema africanum	2.442	**0.012**			
Sphaerechinus granularis	0.956	0.631			
Arbacia lixula	2.182	0.051			
Paracentrotus lividus	2.131	**0.017**			

df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; Pseudo-F: F value by permutation and P(perm): P-value base on permutations. t: t-test values and P(perm): P-value base on permutations. Significant relevant factors are mark with a p-value in bold.

substrates or that the storms did not have the magnitude to promote vertical mixing in the studied localities. *Acanthamoeba* sp. was only found in the seawater samples; this amoeba is an opportunistic free-living parasite widely distribute worldwide and can be found in several environments, such as sweet water bodies, seawater, air and different kinds of soil (Lorenzo-Morales et al., 2005a). On the Canary Islands, this amoeba has been previously isolated from many seawater samples (Lorenzo-Morales et al., 2005b). Is interesting to highlight here that one of the isolated strains of *Acanthamoeba* sp., which was identified by the irregular shape of the trophozoites and cysts with double wall with hexagonal or star shape (Lorenzo-Morales et al., 2005b), was sequenced and identified as genotype T4. This genotype is widely distributed in environments shared with humans and is also associated with some human pathologies even in some animals (Martinez, 1991; Macivera et al., 2013). Even though no studies associating *Acanthamoeba* sp. as a sea urchin pathogen (Tajimaa et al., 2007; Becker et al., 2008; Wang et al., 2013), *Acanthamoeba* sp. is part of the pathogen community of seawater with *Vibrio* sp. and both presented increases with the passage of the storm. This result shows that the storm had an influence on the pathogen's propagation (Gizzi et al., 2020). We also found cysts of *Vahlkampfia* genus in the sediments; this species has been associated with human infections and can be localized to several aquatic and terrestrial environments (De Jonckheere, 2006; Reyes-Batlle et al., 2021).

Significant changes in the pathogen community were found after the storm in the sea urchin species *A. lixula*, *P. lividus* and *D. africanum* and in seawater samples, which had an increase of 51% after the storm, maybe because of the vertical mixing of sediments caused by the waves in the case of *Vibrio* species. The continuous presence of *N. branchiphilain* in the sea urchin species during both seasons may indicate that these sea urchins may acts as a reservoir for the amoeba. In Nova Scotia, for example, it was also observed that *Paramoeba invadens* remained in the sea urchin *S. droebachiensis* that had recovered from the disease. In that case, it was also observed that those sea urchins only developed the diseases when the temperature increased above the infection threshold (Scheibling and Stephenson, 1984; Feehan et al., 2012). This finding could mean that the amoebae remained in the sea urchins for some time after the infection occurred (Jones, 1985; Jones and Scheibling, 1985; Feehan et al., 2013). The presence of *N. branchiphila* in the sea urchins also could be related to recurrent episodes that had introduced the pathogen to Tenerife's coast, in a manner like that described by Feehan et al. (2016) for the coasts of Nova Scotia. It appears that the individuals that survived were resistant to the infection (Scheibling et al., 2010). This idea has also been hypothesized for the survivors *D. antillarum* in the Caribbean Sea (Beck et al., 2014; Lessios, 2016). It may be due to loss of virulence that can happen in the species of genus *Neoparamoeba* sp. (Dyková et al., 2000), which are maintained in sea urchins but do not induce the symptoms of the disease. In our case, lack of a clear correlation with the sea surface temperature and the mortality events was found (Hernández et al., 2020).

The study of causative agents of diseases that are spread in the marine environment is often complicated due to difficulties in detecting potential pathogens in host tissues that are surrounded by water (Feehan et al., 2013); the presence of just one pathogen does not imply disease development or the identification; therefore, histological studies are needed and also the verification of Koch's postulates (Martony et al., 2018). Perhaps viewing diseases in the marine environment as the interaction of various agents allows a better understanding of the disease (Sweet, 2020). With respect to the four samples of sea urchin coelom, two in August and two in January, we observed the growing of *V. alginolyticus* colonies and in only one sample the amoeba was present; however, individuals presenting symptoms of the disease at the moment of capture were seen in some cases (Clemente et al., 2014). Assessing the influence of pathogens on the disease is a difficult task. The presence of bacteria in the coelomic fluid could be the result of a poor-quality environment (Martony et al., 2018), such as observed in the water samples when the pathogen community showed a significant increase after Filomena. Similarly, the presence of *N. branchiphila* in healthy individuals has been observed and could means that *N. branchiphila* is not the only trigger of the disease as other agents may be involved in the development of this (Dyková et al., 2011; Clemente et al., 2014; Hernández et al., 2020). The next studies must also verify the pathogenic activity of *V. minutissima* because the genus has been found in some fish and has shown the capability of colonizing gills in addition to pathogenic activity (Bermingham and Mulcahy, 2007). It is also necessary to evaluate the disease using the Koch's ecological

postulates (Sweet, 2020) for evaluating the dysbiosis and existence of an intermediate host.

Finally, the fact that the winter storms, including Filomena, are related to an increase on the presence of pathogens proves that the environmental changes associated with global change could benefit the presence of pathogens of sea urchins, which are key species in structuring the biodiversity composition of the rocky bottoms in the Atlantic (Hernández et al., 2008). Faced with this situation, continuous monitoring of pathogens in the this region is necessary and must be done in parallel with the monitoring of climatic events as stormy periods, temperature increases, and others that could benefit the presence of the pathogens. In the climate change context, it is important to understand the disease dynamics in marine organisms and the role as inducers of ecosystems have on development management policies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JCH and JL-M conceived of the presented idea. JCH, CES-F, and SG-D contributed the field work. CES-F and MR-B contributed the lab work. JL-M verified the analytical methods. JCH supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.