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Editorial

Microbial Diseases of Marine Organisms

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Healthy oceans and marine environments provide critical life support functions upon which human health and well-being depend [1]. Multiple benefits are derived from marine and coastal ecosystem at local, regional and global scales, ranging from pollution control, storm protection, shoreline stabilization and habitats for species to climate mitigation and food provisioning.

At present, we are facing increasing threats to the sustainability of the marine environment caused by industrialization, tourism, marine traffic and global warming. Marine organisms, whether they are prey or predators in the food chain, are very important members of the marine ecosystem. They form associations with microorganisms, including protists, bacteria, fungi and viruses, and their relationships are mostly mutually beneficial symbiont systems. However, environmental changes induced by anthropogenic impact and climate changes may alter the symbiont relationship, and microorganisms could influence the health, physiology, behaviour and ecology of marine animals [2]. Over time, many different pathogenic microorganisms have been reported as causes of mortality of fish, molluscs, crustaceans and other marine organisms. These disease outbreaks may lead to a large decline in the host population, resulting in the endangerment of the affected species and causing an imbalance in the marine environment.

This Special Issue, titled "Microbial Diseases of Marine Organisms", is conceived as a contribution to the knowledge of the deleterious impacts of microorganisms on marine fish, molluscs, crustaceans, cetaceans or other organisms. Data presented in this Special Issue provide valuable information which could be used for establishing strategies for disease mitigation and control and consequently contribute to preserving the sustainability of the marine ecosystem. A variety of hosts such as corals [3]; different species of endemic, farmed or commercial bivalve mollusc species [4–6]; decapods [7]; and wild fish in public aquaria [8] and aquaculture facilities [9] endangered by different microorganisms is presented in this issue.

We have learnt that coral reefs are among the most biodiverse biological systems on Earth [3]. They are classified as marine invertebrates and filter the surrounding food and other particles in seawater, including pathogens such as viruses. Viruses act as both pathogens and symbionts for metazoans. Marine viruses, which are abundant in the ocean, are mostly single- or double-stranded DNA and single- or double-stranded RNA viruses. These findings were obtained using advanced identification methodologies to detect the presence of viruses in coral reefs; PCR analyses, metagenomic analyses, transcriptomic analyses; and electron microscopy. The review paper in this issue discusses and presents the discovery of different viruses in the marine environment and their hosts, the viral diversity in coral and also the presence of viruses in corallivorous fish communities in reef ecosystems. The detection methods were described, as well as the occurrence of marine viral communities in marine sponges. It was concluded that marine viral communities play a crucial role in biogeochemical cycles in the ocean, indirectly and directly. These viral communities induce mortalities and diseases in the reef ecosystem through abiotic and biotic factors, which cause disturbances in the symbiotic relationship between the coral and their surrounding hosts. The review emphasizes marine viruses from the ocean, coral-associated viruses and marine sponge and coral fish viruses in reef ecosystems, examining

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previous research via traditional methods to modern advanced approaches. The findings of this review have enhanced our understanding of coral–virus interactions and enriched our understanding of reef-associated virus interactions and the diversity of viral communities in marine environments.

Other studied species were red claw crayfish (*Cherax quadricarinatus*) and red swamp crayfish (*Procambarus clarkia*), important for food and ornamental purposes, imported to South Korea from China and Indonesia [7]. The research comprised PCR testing to detect infectious hypodermal and hematopoietic necrosis virus (IHHNV or Decapod penstylhama-parvovirus 1). IHHNV was detected in tissue samples pooled from nine out of ten batches of red claw crayfish imported from Indonesia. Phylogenetic analysis of PCR amplicons from representative pools clustered the IHHNV strain with infectious-type II sequences commonly detected in Southeast Asian countries, rather than with type III strains detected previously in white leg shrimp (*Penaeus vannamei*) cultured in South Korea. IHHNV DNA was detected most frequently in the muscle, followed by hepatopancreas and gill tissues, suggesting that red claw crayfish could be a potential carrier of the virus. It was concluded that transboundary movements can cause significant environmental disturbance and provide opportunities for the inadvertent translocation of disease-causing pathogens to new locations. The detection of IHHNV infection in *C. quadricarinatus* by identifying the viral DNA in tissue samples of the commodity imported into South Korea as the IHHNV type II strain indicates that red claw crayfish could be a potential carrier of the infectious IHHNV, posing a threat to the cultured and wild population of crustaceans in South Korea.

The most represented marine species of this issue were bivalve molluscs: from farmed European flat oysters (*Ostrea edulis*) [5], followed by farmed and free-living Portuguese oysters (*Crassostrea angulata*) and Pacific oysters (*Crassostrea gigas*) [6], to the critically endangered noble pen shell (*Pinna nobilis*) endemic to the Mediterranean area [4]. Research on flat oysters describes the first occurrence and molecular identification and epidemiology of parasites from the genera *Bonamia*, phylogenetically positioned into a clade microcell within the genus *Haplosporidia* in a farming area along the Croatian Adriatic Coast [5]. PCR analysis and sequencing for SSU rDNA gene and BLAST analysis confirmed infection with *Bonamia exitiosa*. Although prevalence in a five-year period ranged from 3.3 to 20% at the different sites, there were no mortalities reported from the infected sites, and it seemed that infection of flat oysters with *B. exitiosa* did not affect their health. Attempt to prove the Pacific oyster as a putative vector of the parasite failed. The phylogenetic analysis did not disclose any information on the source of *B. exitiosa* origin. Since the Croatian isolate showed 100% similarity to previously sequenced isolates from Chile or Australia based on the SSU rDNA gene, the sequencing of additional genes or the whole genome should be carried out to provide us with more details on the phylogeny of the Croatian isolates. More comprehensive molecular studies of the *B. exitiosa*, together with an investigation of the natural population of *O. stentina*, which are susceptible species from production areas and natural beds along the Eastern Adriatic coast, could confirm the natural-historical origin of the parasite *B. exitiosa.* Other studied bivalve molluscs species are species nationally important for Portugal; Portuguese oyster (*Crassostrea angulata*) and Pacific oyster (*Crassostrea gigas*) from four distinctive areas in Portugal were studied to evaluate their sanitary status [6]. Collected Pacific oyster populations were cultivated in a strong ocean-influenced environment, and Portuguese oyster populations were cultivated in wild beds. The histopathological examination of both oyster species revealed the presence of parasites in gills, mantle epithelium, digestive gland tubules and connective tissue, with a moderate prevalence. In both populations, hemocytosis was observed in the connective tissue, oedema and metaplasia in the digestive gland and necrosis in the tissues. In wild populations from the Sado and Mira estuaries, the prevalence of mud blisters and gill lesions was higher than from populations produced on 0.50 m tables from mudflats. It was concluded that diseases are important risk factors which are caused, in many cases, by non-compliance with basic management rules, namely, the level of animal load in production areas, the length of time the bivalve molluscs remain in these areas, and

the introduction of seeds of unknown origin. Effective biosecurity measures and correct and early diagnostic techniques are essential to control pathogenic agents. In the production areas of the Aveiro and Alvor lagoons, these measures are implemented by producers and by the authorities. Producers understand that it is essential to prevent mortalities in bivalve mollusc populations, namely, avoiding overcrowding and preventing diseases while in Sado and Mira estuaries, wild populations were proven to be more susceptible to lesions than oysters produced on tables under the supervision of producers. Differently from previous research, another study is dedicated to the noble pen shell (*Pinna nobilis*), the largest bivalve (60–120 cm), endemic to the Mediterranean Sea [4]. It is an inhabitant of shallow waters along the Croatian Adriatic coastline and suffered high mortalities similar to other parts of the Mediterranean. The results of the study presented in this Special Issue contribute a description of the diagnostics of causative agents of mortalities and epidemiology in Mljet National Park and the Northern Adriatic. It seems that mortalities were caused by infection with the haplosporidian parasite *Haplosporidium pinnae* and bacterium *Mycobacterium* sp. The spreading pattern of the mortalities based on a pilot study undertaken in Mljet National Park, an area with a dense population of noble pen shells, was evaluated. The results of the study support the hypothesis that the increase in mortalities was influenced by high temperatures, as peak mortalities in the studied area occurred in August with a sea temperature of more than 26 °C. In addition, multifactorial causality was proven, as the presence of *Mycobacterium* sp. alone was detected a long time before mortalities occurred, but coinfection of *Haplosporidium pinnae* was also detected after mortality. Still, a multidisciplinary experts' approach is needed to explain the phenomenon and to set up an efficient program for the protection of noble pen shell from extinction.

The focus of the next two papers are marine fish: one studied lesser-spotted dogfish (*Scyliorhinus canicula*) juveniles reared in public aquaria which suffered from infection with two different species from the genus *Vibrio* [8], and the other studied the skin microbiota of farmed fish correlated to the use of antibiotics [9]. Although elasmobranchs are endangered species in the Mediterranean Sea, classified as on the decline due to habitat degradation and consequent to the direct impacts of fishing, lesser-spotted dogfish, a small demersal shark, is classified as being of least concern (LC) by the IUCN. Its diet and habitat requirements, as well as easy reproduction in captivity, make them favourable species in public aquaria. Reports on infectious diseases affecting sharks are scarce, and therefore, research on their susceptibility to different infectious agents may contribute to the mitigation of elasmobranchs' decline. This research proved susceptibility to two *Vibrio* species: *V. crassostreae*, previously described as a pathogen of molluscs and fish, and *V. cyclotrophicus*, reported in molluscs and as a member of the microbiome of marine copepods [8]. This study described suitable diagnostic methodology to identify specifically *V. crassostreae* and *V. cyclitrophicus*, underlining the criticalities in the identification process concerning the techniques adopted (API® 20E, MALDI-TOF MS, molecular biology) and the need for developing specific guidelines for the identification of non-major pathogenic *Vibrio* species. In addition, it highlighted the need for in-depth studies of the pathogenic mechanism of these bacteria in sharks. Bacteria from the genus *Vibrio*, together with those from the genus *Pseudomonas*, were prevalent in the study of skin microbiota of farmed European seabass (*Dicentrarchus labrax*) [9]. Some of the microbiota that were identified are known to be pathogenic to fish: *V.alginolyticus*, *V. anguillarum* and *V. harveyi*. *Vibrio* strains showed higher resistance to studied antibiotics compared to previous studies. This study provides, for the first time, information on the cultivable skin bacteria that were associated with healthy European seabass under culture conditions with and without the use of antibiotics. The obtained information will be useful in assessing how changes in cultivable microbiota may affect the health of farmed European seabass, indicating a potential problem for fish health management during disease outbreaks. Interestingly, some resistant bacteria were detected among isolated bacteria, a component of skin microbiota from the farm without the use of antibiotics that raised many different questions on the influence of the fish farms on the environment but also the influence of the environment and anthropogenic activity

on fish farming. The presence of resistant microorganisms could potentially endanger consumers' health and contribute to horizontal gene transmission. However, there is little data on the antimicrobial resistance gene transmission (AGR) in the marine environment and future studies should put a lot of effort into elucidating pathways and possibilities of AGR spreading.

Each of the published articles in this Special Issue tackled one of the niches within the marine environment and fulfilled a strong need for further and more detailed research on different marine organisms and their interactions with different microbes and the outcomes of these interactions.

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