Journal of
Marine Science and Engineering

MDPI

Article

Histopathologic Lesions in Bivalve Mollusks Found in Portugal: Etiology and Risk Factors

Daniel Pires [1], Ana Grade [2], Francisco Ruano [2] and Fernando Afonso [1,*]

[1] CIISA—Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Av. da Universidade Técnica, 1300-477 Lisbon, Portugal; dannigs10@hotmail.com
[2] IPMA—Instituto Português do Mar e da Atmosfera, Av. Alfredo Magalhães Ramalho, 1495-165 Lisboa, Portugal; agrade@ipma.pt (A.G.); fruano@ipma.pt (F.R.)
* Correspondence: fafonso@fmv.ulisboa.pt

Abstract: Bivalve mollusks are an important resource due to their socioeconomic value and to the historical and genetic value of some species. Two nationally important oyster species-Portuguese oyster (*Crassostrea angulata*) and Japanese oyster (*Crassostrea gigas*) from distinctive areas in Portugal were studied to evaluate their sanitary status. Oysters were sampled from four different sites in Portugal. Oysters collected from Japanese 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 was observed hemocytosis in the connective tissue, edema and metaplasia in the digestive gland and tissues necrosis. In wild populations from Sado and Mira estuaries the prevalence of mud blisters and gill lesions were higher than from populations produced on 0.50 m tables from mudflats. Biosecurity measures and diagnostic techniques are fundamental to control pathogenic agents, including the identification of pathogens at an early stage in their life cycles. This will prevent diseases and improve pathogen reduction on transport of animals from different countries and regions to new production areas to avoid the transmission of diseases.

Keywords: bivalve mollusks; oysters; histopathology; parasites

Citation: Pires, D.; Grade, A.; Ruano, F.; Afonso, F. Histopathologic Lesions in Bivalve Mollusks Found in Portugal: Etiology and Risk Factors. *J. Mar. Sci. Eng.* **2022**, *10*, 133. https://doi.org/10.3390/jmse10020133

Academic Editor: Snježana Zrnčić

Received: 1 October 2021
Accepted: 30 December 2021
Published: 20 January 2022

1. Introduction

In Portugal, in the late 20th century, the two most important commercial oysters were the flat Oyster (*Ostrea edulis*) and the Portuguese Oyster *(Crassostrea angulata)* [1]. Initially, flat oyster commerce was more important, but in the 20th century it was replaced by the Portuguese oyster. The culture of bivalve mollusks is an activity with high expression in Portuguese aquaculture. It represents 55% of the whole production, being the main species, clams, oysters and mussels [2].

There was a significant change in bivalve mollusks production. Initially, it was exclusively chosen for semi-intensive production in large areas. Nevertheless, at this moment there is an intensification of production in less area, increasing bivalve mollusks density. This option results in lower growth rates, lower product quality, lower fertility rates and increasing diseases [3].

Microbiological contamination of water where aquaculture production is present, in most cases, is the result of industries, urban activities and leisure activities, causing fecal contamination. [4–6]. The microbiological organisms present in bivalve mollusks are diverse, with different populations of bacteria (namely, *Escherichia coli*, *Clostridium perfringens*, *Vibrio parahaemolyticus*, *Salmonella* and *Listeria*) [7–9], enteric viruses (namely, norovirus, enteric calicvirus, hepatitis A virus and other enteroviruses) [10,11] and protozoa (namely, *Cryptosporidia* and *Giardia*) [12,13]. Generally, these microorganisms are not harmful to bivalve mollusks and do not cause lesions or disease.

Several diseases can affect different bivalve mollusks species and their production. Epizootics caused by fungi, viruses and protozoa can affect the entire bivalve production, as in Portugal and France in the 1970s where massive mortality caused by an iridovirus affected the Portuguese oyster production. Since 2009 a herpes virus (OsHV-1) has been the cause of massive mortalities in Japanese oyster (*Crassostrea gigas*), first in France and all over the most important European production countries of this species, including Portugal [3]. Japanese oyster aquaculture is a major primary production sector in many countries, but the industry has been threatened by mortality events for the last five decades [14]. In Portuguese and Japanese oyster, the main pathogens are virus, namely iridovirus, responsible for gill disease that affected Portuguese oyster production in the 1970's, and herpes viruses, namely Ostreid-herpesvirus 1 μvar (OsHV-1) that is related with summer oyster disease and affects the Japanese and Portuguese oyster populations [15]. From the first detection in the Eastern oysters (*Crassostrea virginica*) [14] in 1972, herpes virus has been identified in at least 20 bivalve species and has caused massive mortalities [14]. Herpes virus infecting bivalve mollusks are virulent pathogens of both larval and seed oysters. Mortalities of juvenile oysters associated with detection of herpes viruses have been reported from France, New Zealand, Spain and the USA [16–19].

In flat oyster, there are important diseases, including bonamiosis that is caused by *Bonamia ostrea* and *Marteilia refringens* that causes marteliosis and viruses such as *Ostreid-herpesvirus* 1 μvar [20,21].

Regarding mussels *Mytilus* sp., the main pathogens are protozoa, such as *Marteilia refringens* that is the causative agent of marteliosis and copepods, such as *Mytilicola intestinalis* which causes red worm disease [22,23].

Japanese clam, European clam and cockles have been mainly affected by protozoa, such as *Perkinsus olseni*, the causative agent of Perkinsiosis [24–27] and *Haplo*s*poridium tapetis* [26,28]. *Perkinsus olseni* is responsible for the high mortality of cockleshell populations. Perkinsiosis is caused by the protozoan *P. olseni*. This disease is a very common disease in clams and has caused massive mortalities in clam populations, contributing to the decrease in their production and consequently affecting their socioeconomic value [29]. Typical lesions that occur include cell disorganization, autolysis and necrosis of cells, both within and near the lesion [24].

The high prevalence of this pathogen in clams can be attributed to several factors, such as degraded sediments, high animal densities per unit area, lack of physical barriers between different culture beds, and transfer of animals carrying the disease [1].

Biotoxins can affect the nervous, the gastrointestinal and the respiratory systems, being fatal in some severe cases. They originate in various microalgae and dinoflagellates that produce these toxic elements in their metabolism. Mollusk bivalves are filter-feeder animals, and they can retain and accumulate those microalgae and the biotoxins, and as such, they can be a source of contamination for humans [30,31].

The accumulation of biotoxins in aquatic organisms depends on their feeding activity, on their metabolism, and on their elimination rate, modifying the transfer of toxins in the trophic chain [32].

Contaminant metals can cause several problems in humans, and the contamination of bivalve mollusks with different concentrations of heavy metals can be dangerous, namely lead, cadmium and mercury. They can accumulate high concentrations from water, and from the sediments and they have a wide bioaccumulation range of heavy metals depending on the species [33–35].

The aim of the present work was to study the sanitary condition in oyster production in four different populations of oysters. The objective was to describe the general health status, studying anatomo and histopathological lesions and parasites, in the Portuguese oyster (*Crassostrea angulata*) and Japanese oyster (*Crassostrea gigas*) from different production areas in Portugal, in order to evaluate their health condition.

2. Materials and Methods

The map (Figure 1) shows the four different areas where oysters were collected.

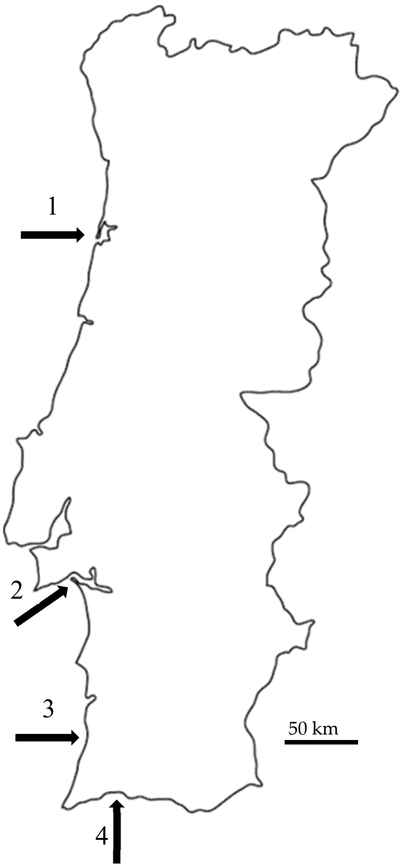


Figure 1. The map shows the areas were oysters were collected in mainland Portugal: Aveiro lagoon (1), Sado estuary (2), Mira estuary (3) and Alvor lagoon (4).

Oysters (9–11 cm, aged 24 months old) were sampled from four different sites of the Portuguese coast. *Crassostrea gigas* is an exotic species that was introduced in Portugal, and so, its presence is restricted to some production areas (1 and 4). In areas 2 and 4, *Crassostrea angulata* were collected from wild beds.

Portuguese oysters from Sado estuary (n = 30) and Mira estuary (n = 30) and Japanese oyster from Alvor lagoon (n = 30) and Aveiro lagoon (n = 30) were collected in May 2019. In May, in the four sites, oysters have already spawned and are more susceptible to environment stressors and increasing chances of histopathological lesions.

Individuals were randomized collected in production areas present in four mesotidal estuarine areas indicated in the map (Figure 1). The areas are located on the west Portuguese coast (1, 2 and 3) and in the south Portuguese coast (4). All the four production areas are under a strong oceanic influence because of the proximity to the coastal water and they have an influence of fresh water. For production, oysters are placed inside plastic bags that are put on tables with a distance of 0.50 m from the bottom in areas 1 and 4. In Sado and Mira estuaries, oysters were collected in intertidal mudflats where oyster beds are located. In Aveiro lagoon there is a lagoon with several channels, stretching for 45 Km. Sado stuary includes a section of river, marshlands and channels and the dynamic tide extends for 65 Km. Mira estuary is a narrow estuary of the "Ria" pattern that extends for 40 km. Alvor lagoon has a water body and a mesotidal shallow lagoon that extends for 15 Km.

Oyster were collected during low tide at a distance from the mouth of 1 Km (1), 17 Km (2), 15 Km (3) and 1 Km (4). Temperature and salinity of the water were, respectively: (1) 18.6 and 31.5; (2) 19.1 and 33.1; (3) 19.7 and 33.6; (4) 20.1 and 31.9.

After being collected, oysters were immediately transported on ice to the laboratory. To survey the presence of lesions, parasites and diseases anatomic and histopathological examinations were used as main diagnostic methods. Oysters were opened with a knife and the samples, including all organs and tissues, were collected with a tweezer and a scalpel, Tissue samples were prepared for histopathology processing, following the protocol used in Pathology laboratory of IPMA. Samples were fixed in Davidson's fixative for 48 h, dehydrated and embedded in paraffin. Sections with 5 μm thick were stained with Hematoxylin-Eosin and mounted on a microscopic glass slide.

Histological preparations were carefully examined under light microscopy (Motic BA-410), looking for the presence of lesions and parasites in oysters.

3. Results

In most of the samples no lesions were observed in organs (Figure 2A–C) The macroscopic lesions (Figure 3A–C), microscopic lesions (Figure 4A–F,I) and parasites (Figure 4G,H) were recorded.

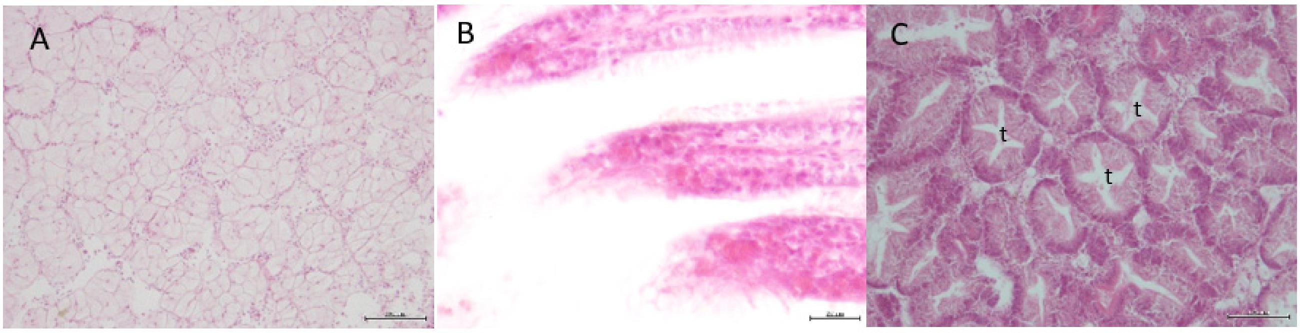
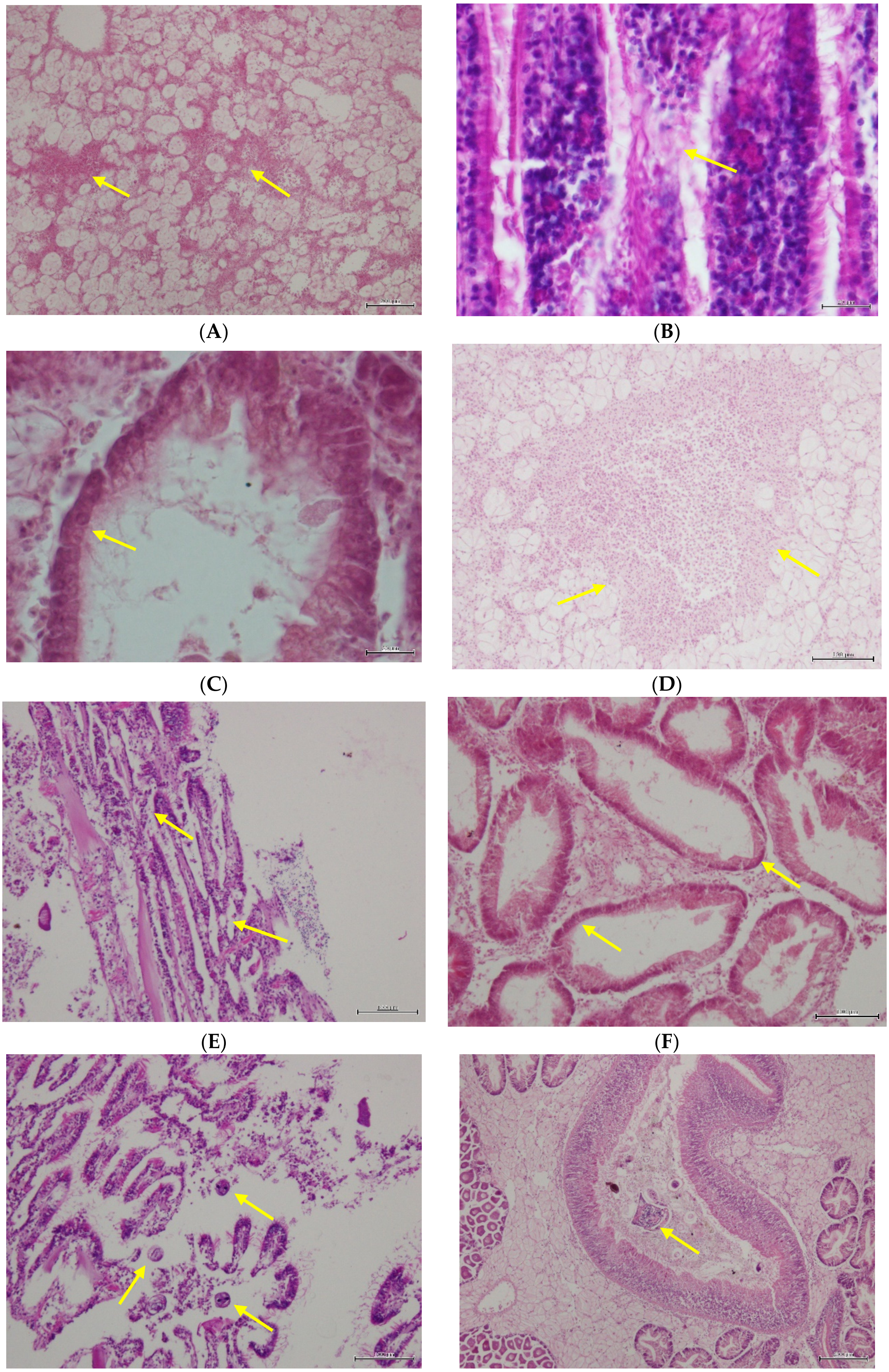


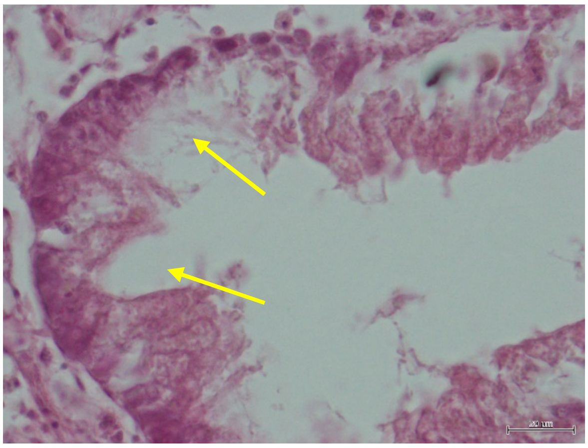
Figure 2. Histological sections stained with hematoxylin and eosin, where different tissues and organs are shown. (**A**) Connective tissue normal is present (Mag = 40×; bar = 200 μm) (*C. angulata* tissue). (**B**) The normal gill with filaments where a ciliary system is observed (*C. gigas* tissue) (Mag = 200×; bar = 20 μm). (**C**) The normal digestive gland presents a regular tubule (t) thickness, characterized by their dense areas and star shape (*C. gigas* tissue). (Mag = 40×; bar = 200 μm).

Figure 3. Macroscopical lesions observed in oysters. (**A**) Gills lesions (arrow) in *C. angulata* (bar = 3 cm). (**B**) Shell disease (arrow) in *C. angulata* (bar = 3 cm). (**C**) Mud blisters (arrows) in *C. gigas* (bar = 3 cm).



(**A**) (**B**) (**C**) (**D**) (**E**) (**F**) (**G**) (**H**)

Figure 4. *Cont*.



(**I**)

Figure 4. Microscopical lesions observed in oysters (tissue sections are stained with hematoxylin and eosin). (**A**,**D**) An inflammatory response is observed with the presence of hemocyte in the connective tissue (arrows) (*C. gigas* tissue). (Mag = 40×; bar = 200 µm). (**B**) Necrosis of gill tissue (arrow) (*C. angulata* tissue) (Mag = 200×; bar = 20 µm). (**C**) Metaplasia of digestive gland (arrows) (*C. angulata* tissue). (Mag = 200×; bar = 20 µm). (**E**) Necrosis of gill tissue (arrows) (*C. angulata* tissue). (Mag = 100×; bar = 100 µm). (**F**) Metaplasia of digestive gland (arrows) (*C. angulata* tissue). (Mag = 100×; bar = 100 µm). (**G**) *Trichodina* sp. (arrows) in gill tissue (*C. angulata* tissue). (Mag = 100×; bar = 100 µm). (**H**) *Mytilicola* sp. (arrow) in intestine (*C. gigas* tissue). (Mag = 40×; bar = 200 µm). (**I**) Loss of epithelium of the digestive gland (arrows) (*C. gigas* tissue) (Mag = 200×; bar = 20 µm).

In samples of *C. gigas* from Aveiro lagoon, it was observed only 3% of oysters with edema, 3% with shell disease and 10% with *M. ostrea*.

Finally, in samples from Aveiro lagoon, a prevalence of 13% *Trichodina* sp. infections, 13% *Ancistrocoma* sp. infections and 10% *Mytilicola* sp. infections. Concerning tissue lesions and morphological changes, 26% of wild oysters showed necrosis, 45% showed hemocytosis, indicating an inflammatory process, mainly in connective tissue. It was also observed a prevalence of 3% of ceroidosis in the connective tissue, edema in 15 % and 18% showed metaplasia in the digestive gland. Regarding farmed oysters, the following lesions and prevalence were found: necrosis (6%) in different tissues, hemocytosis (27%), ceroidosis (5%), edema (18%) and metaplasia (5%).

Samples of wild *C. angulata* from Sado estuary show the following results (Tables 1 and 2): 20% presented mud blisters, mainly caused by *Polydora* sp.; 33% with gills lesions; 10% had shell disease associated with the presence of the fungus *Ostracoblabe implexa*; and 17% showed *Myicola ostrea* copepods.

Table 1. Prevalence of macroscopic lesions and parasites in different populations.

Macroscopic Lesions and Parasites	Site 1	Site 2	Site 3	Site 4
Tricodina sp.	13%	0%	13%	23%
Ancistrocoma sp.	13%	0%	30%	13%
Mytilicola sp.	10%	0%	3%	10%
Myicola ostrea	10%	17%	0%	0%
Shell disease	0%	10%	10%	0%
Mud blisters	3%	20%	97%	10%
Gills lesions	0%	33%	50%	0%

Table 2. Prevalence of microscopic lesions in different populations.

Microscopic **Lesions**	**Site 1**	**Site 2**	**Site 3**	**Site 4**
Necrosis	13%	13%	40%	23%
Hemocytosis	36%	36%	53%	16%
Ceroidosis	10%	0%	6%	0%
Edema	36%	0%	30%	0%
Metaplasia	3%	26%	10%	6%

Portuguese oysters sampled in Sado estuary, it was found a prevalence of 30% infections with *Trichodina* sp., characterized by a shape similar to a disc, a circlet of eosinophilic denticles, a ciliary fringe and a horseshoe shaped nucleous, in gills and mantle epithelium. A prevalence of 20% infections with *Ancistrocoma* sp., characterized by a Spindle-shaped ciliates with large, granular and polymorphic nuclei, in the digestive gland tubules and connective tissue. Copepods (*Mytilicola* sp.), characterized by an elongated cylindrical shape and a redish colour were observed within the intestine of oysters with the prevalence of 17% [36].

Samples of wild *C. angulata* Mira estuary showed mud blisters (96%), 50% had gills lesions, 10% presented edema and 10% shell disease.

In samples from Mira estuary, it was found a prevalence of 13% infections with *Trichodina* sp., 30% infections with *Ancistrocoma* sp. and 3% infections with *Mytilicola* sp.

Finally, in samples of *C. gigas* from Alvor lagoon 10% presented mud blisters.

In farmed oysters sampled from Alvor lagoon, it was observed a prevalence of 23% *Trichodina* sp. infections and a prevalence of 27% *Ancistrocoma* sp. Copepod (*Mytilicola* sp.) were observed within the intestine of oysters at a prevalence of 3%.

4. Discussion

Cultivation and harvesting of bivalve mollusks are two important activities, and it is fundamental to preserve the natural ecosystems that support those activities and lead to increasing natural production and improving its quality. Bivalve mollusks production depends on the external factors [37], including water parameters, such as temperature variations, food change, biotoxins and pathogens (including parasites, bacteria, viruses) or anthropogenic, such as chemical contaminants (heavy metals, pesticides), organic contaminants (fecal contamination) and overexploitation of natural banks [1,38]. It is wildly known that the marine environment provides stress to oysters during their life cycle, namely changes in environmental parameters, changes in the availability of food and the display of various toxic pollutants [39–41]. Oysters are very resistant to changes of temperature and salinity that are present in the ecosystems where they live, namely estuaries and lagoons. As other euryhaline organisms, oysters can live in water where large variations of salinity occur. For short periods of time salinity values could range between 2% and 38% [1,42]. Similarly, oysters are resistant to temperature changes between 8 °C and 30 °C [1]. There are several factors that influence the growth of mollusk bivalves such as temperature, oxygen and the presence of different species of phytoplankton. Once they are filter-feeders, the quality and the quantity of filtered food define the outcome of their growth [43].

The regularly consumption of bivalve mollusks by different populations and the growth of shellfish aquaculture production show the importance of these animals and their monitoring must be undertaken to avoid diseases and mortality in oyster populations. Hereby we are presenting the results of the sanitary monitoring of oyster production in four different production areas. In general, it was observed that low numbers of parasites were present in oysters being *Trichodina* sp. and *Ancistrocoma* sp. infections the most common ones present in the areas that were studied. The presence of *Trichodina* sp., *Ancistrocoma* sp., *Mytilicola* sp. and *Myicola ostrea* are commonly observed in oyster populations and in massive infestations could lead to host weakness [36,44–46]. Lesions of the internal organs, such as metaplasia, may reflect physiological stress, contaminants or the presence of large parasitic loads [1,44]. In our study, hemocytosis, cerodosis and edema were mainly present in the interstitial connective tissue. These lesions and those observed in the epithelium of the diverticula of the digestive gland, including the hemocytic infiltration and necrosis were usually associated and could be related with the presence of the parasites. In wild populations from Sado and Mira estuaries the prevalence of mud blisters and gill lesions were higher than from populations produced on tables 0.50 m from mudflats.

The identification, characterization and registration of pathologic processes in oysters constitute a set of important measures for sanitary control. Based on the results of the sanitary control appropriate biosecurity tools could be developed and implemented leading to the control of diseases spread and transmission. Finally, knowledge and ability to manage risk of different pathogen will contribute to the sustainability of oyster production and protection of animal and human health.

Aquatic ecosystems can be modified due to human activities such as overharvesting and destruction of substrates [47]. In addition, transfers of oysters, between countries and regions can spread diseases and invasive species [47–49]. Concurrently with increase of the socio-economic importance of mollusk farming in Portugal all associated risk factors are affecting this production. It is essential to have stricter control at all stages of production of the different bivalve mollusk species. This is the only way to improve economic performance without jeopardizing animal welfare and at the same time to prevent diseases in oyster populations and their dissemination to different areas. In Portugal, IPMA (https://www.ipma.pt/en/) (accessed on 14 September 2021) undertakes regular analysis to the water, including in the areas where animal species that are produced to be consumed by populations. In the areas where animals were collected no contaminants were detected. Biotoxins are not harmful to bivalve mollusks but, when they are consumed by humans, they can cause several serious problems. As such, the control and monitoring of levels of contaminants are fundamental to prevent any risk to public health.

Diseases are important risk factors, specific or not, of different species of bivalve mollusks, 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 mollusks remain in these areas, and the introduction of seeds of unknown origin. Effective biosecurity measures and correct diagnostic techniques are essential to control pathogenic agents. The identification of pathogens should include those at an early stage in their life cycles, to control and to prevent their multiplication and proliferation. In the production areas from 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 mollusk populations, namely avoiding overcrowding and preventing diseases. In Sado and Mira estuaries, wild populations show that oysters are more susceptible to lesions than oysters produced on tables under the supervision of producers.

Research on genetic resistant bivalve populations to different pathogenic agents and the use of probiotic bacteria are important strategies to prevent diseases caused, namely by different *Vibrio* species [50,51] that can cause mortality outbreaks, that did not occur in the areas that were studied. Furthermore, restoration programs can be implemented in areas where ecosystems were negatively affected [49]. The success of bivalve mollusk production can only be achieved, if appropriate measures are applied, namely biosecurity, correct and efficient diagnostic methods, effective compliance with legislation and the dissemination of knowledge among populations.

Author Contributions: Methodology (F.A.; F.R.); Investigation (A.G.; D.P.); Writing—original draft preparation (D.P.; F.A.); Writing—review and editing (A.G.; F.R.). All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by MAR2020: MAR-02.05.01-FEAMP-0010.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ruano, F. Fisheries and farming of important marine bivalves in Portugal. *NOAA Tech. Rep. NMFS* **1997**, *3*, 191–200.
2. Government of Portugal. *Fisheries Statistics 2018*; Government of Portugal: Lisboa, Portugal, 2019.
3. Grizel, H.; Bachere, E.; Mialhe, E.; Tige, G. Solving parasite-related problems in cultured molluscs. *Int. J. Parasit.* **1987**, *17*, 301–308. [CrossRef]
4. McAllister, T.; Topp, E. Role of livestock in microbiological contamination of water: Commonly the blame, but not always the source. *Anim. Front.* **2021**, *2*, 17–27. [CrossRef]
5. D'Ugo, E.; Marcheggiani, S.; D'Angelo, A.; Caciolli, S.; Puccinelli, C.; Giuseppetti, R.; Marcoaldi, R.; Romanelli, C.; Mancini, L. Microbiological water quality in the medical device industry in Italy. *Microchem. J.* **2018**, *136*, 293–299. [CrossRef]
6. Maran, N.; Crispim, B.; Iahnn, S.; Araújo, R.; Grisolia, A.; Oliveira, K. Depth and Well Type Related to Groundwater Microbiological Contamination. *Int. J. Environ. Res. Public Health* **2016**, *13*, 1036. [CrossRef] [PubMed]
7. Pasquale, V.; Romano, V.; Rupnik, M.; Capuano Bove, D.; Aliberti, F.; Krovacek, K.; Dumontet, S. Occurrence of toxigenic Clostridium difficile in edible bivalve molluscs. *Food Microbiol.* **2012**, *31*, 309–312. [CrossRef] [PubMed]
8. Martinez, O.; Rodriguez-Calleja, J.; Santos, J.; Otero, A.; Garcia-Lopez, M.L. Foodborne and Indicator Bacteria in Farmed Molluscan Shellfish before and after Depuration. *J. Food Prot.* **2009**, *72*, 1443–1449. [CrossRef] [PubMed]
9. Ukwo, S.; Ezeama, C.; Obot, O. Microbiological safety and toxic element contaminants in bivalve shellfish from intertidal mudflats of IKO estuary, Niger delta, Nigeria. *South Asian J. Food Technol. Environ.* **2019**, *5*, 846–854. [CrossRef]
10. Gabrieli, R.; Macaluso, A.; Lanni, L.; Saccares, S.; Giamberardino, F.; Petrinca, B.; Divizia, M. Enteric viruses in molluscan shellfish. *New Microbiol.* **2007**, *30*, 471–475.
11. Iaconelli, M.; Purpari, G.; Della Libera, S.; Petricca, S.; Guercio, A.; Ciccaglione, A.; Bruni, R.; Taffon, S.; Equestre, M.; Fratini, M.; et al. Hepatitis A and E Viruses in Wastewaters, in River Waters and in Bivalve Molluscs in Italy. *Food Environ. Virol.* **2015**, *7*, 316–324. [CrossRef]
12. Schets, F.; Van Den Berg, H.; Husman, A. Determination of the Recovery Efficiency of Cryptosporidium Oocysts and Giardia Cysts from Seeded Bivalve Mollusks. *J. Food Prot.* **2013**, *76*, 93–98. [CrossRef] [PubMed]
13. Gomez-Couso, H.; Mendez-Hermida, F.; Castro-Hermida, J.; Ares-Mazás, E. Cryptosporidium Contamination in Harvesting Areas of Bivalve Molluscs. *J. Food Prot.* **2006**, *69*, 185–190. [CrossRef] [PubMed]
14. Alfaro, A.; Nguyen, T.; Merien, F. The complex interactions of Ostreid herpesvirus 1, Vibrio bacteria, environment and host factors in mass mortality outbreaks of Crassostrea gigas. *Aquaculture* **2019**, *11*, 1148–1168. [CrossRef]
15. Barbosa-Solomieu, V.; Renault, T.; Travers, M.-A. Mass mortality in bivalves and the intricate case of the Pacific oyster, Crassostrea gigas. *J. Invertebr. Pathol.* **2015**, *131*, 2–10. [CrossRef] [PubMed]
16. Burge, C.; Judah, L.R.; Conquest, L.; Griffin, F.; Cheney, D.; Suhrbier, A.; Vadopalas, B.; Olin, P.; Renault, T.; Friedman, C. Summer seed mortality of the Pacific oyster, Crassostrea gigas Thunberg grown in Tomales Bay, California, USA: The influence of oyster stock, planting time, pathogens, and environmental stressors. *J. Shellfish Res.* **2007**, *26*, 163–172. [CrossRef]
17. Hine, P.; Wesney, B.; Hay, B. Herpesviruses associated with mortalities among hatchery-reared Pacific oysters, Crassostrea gigas. *Dis. Aquat. Org.* **1992**, *12*, 135–142. [CrossRef]
18. Renault, T.; Le Deuff, R.; Lipart, C.; Delsert, C. Development of a PCR procedure for the detection of a herpes-like virus infecting oysters in France. *J. Virol. Meth.* **2000**, *88*, 41–50. [CrossRef]
19. Elandaloussi, L.; Carrasco, N.; Andree, K.; Furones, D.; Roque, A. Esdeveniments de mortalitat de lostró del Pacific (*Crassostrea gigas*) en el delta del Ebre. Estudi de cas. In Proceedings of the II Simposi d'aqüicultura de Catalunya, Sant Carles de la Rapita, Spain, 15–17 October 2009.
20. World Organisation for Animal Health (OIE). Infection with *Bonamia ostreae*. In *Manual of Diagnostic Tests for Aquatic Animals*; World Organisation for Animal Health: Paris, France, 2018; Chapter 2.4.3.
21. The Food and Agriculture Organization (FAO). Cultured Aquatic Species Information Programme. *Ostrea edulis* (Linnaeus, 1758). 2018. Available online: http://www.fao.org/fishery/culturedspecies/Ostrea_edulis/en (accessed on 15 May 2021).
22. The Food and Agriculture Organization (FAO). Cultured Aquatic Species Information Programme. *Mytilus galloprovincialis* (Lamarck, 1819). 2018. Available online: http://www.fao.org/fishery/culturedspecies/Mytilus_galloprovincialis/en (accessed on 15 May 2021).
23. The Food and Agriculture Organization (FAO). Cultured Aquatic Species Information Programme. *Mytilus edulis* (Linnaeus, 1758). Available online: http://www.fao.org/fishery/culturedspecies/Mytilus_edulis/en (accessed on 15 May 2021).
24. Azevedo, C. Fine structure of *Perkinsus atlanticus* sp. *(Apicomplexa, Perkinsea) parasite of the clam Ruditapes decussatus from Port. J. Parasitol.* **1989**, *75*, 627–635.
25. Azevedo, C. Virus-like particles in *Perkinsus atlanticus* (Apicomplexa, Perkinsidae). *Dis. Aquat. Org.* **1990**, *9*, 63–65. [CrossRef]
26. The Food and Agriculture Organization (FAO). Cultured Aquatic Species Information Programme. *Ruditapes decussatus* (Linnaeus, 1758). 2018. Available online: http://www.fao.org/fishery/culturedspecies/Ruditapes_decussatus/en (accessed on 15 May 2021).

27. The Food and Agriculture Organization (FAO). Cultured Aquatic Species Information Programme. *Ruditapes philippinarum* (Adams & Reeve, 1850). 2018. Available online: http://www.fao.org/fishery/culturedspecies/Ruditapes_philippinarum/en (accessed on 15 May 2021).
28. Vilela, H. Sporozoaires parasites de la palourde, *Tapes decussatus* (L.). In *Revista da Faculdade de Ciências, Lisboa*; University of Lisbon: Lisbon, Portugal, 1951; Volume 1, pp. 379–386.
29. Ruano, F.; Batista, F.; Arcangeli, G. Perkinsosis in the clams *Ruditapes decussatus* and *Ruditapes philippinarum* in the Northeastern Atlantic and Mediterranean Sea: A review. *J. Invertebr. Pathol.* **2015**, *131*, 58–67. [CrossRef]
30. Vale, P. *Marine Biotoxins*; Faculty of Veterinary Medicine, Technical University of Lisbon: Lisbon, Portugal, 2004; Volume 99, pp. 3–18.
31. Bricelj, V.; Shumway, S. Paralytic shellfish toxins in bivalve Molluscs: Occurence, transfer kinetics, and biotransformation. *Rev. Fish. Sci.* **1998**, *6*, 315–383. [CrossRef]
32. Costa, P.; Costa, S.; Braga, A.; Rodrigues, S.; Vale, P. Relevance and challenges in monitoring marine biotoxins in non-bivalve vectors. *Food Control* **2017**, *76*, 24–33. [CrossRef]
33. Gupta, S.; Singh, J. Evaluation of mollusc as sensitive indicatior of heavy metal pollution in aquatic system: A review. *IOAB J.* **2011**, *2*, 49–57.
34. Nour, H. Distribution and accumulation ability of heavy metals in bivalve shells and associated sediment from Red Sea coast, Egypt. *Environ. Monit. Assess* **2020**, *192*, 353. [CrossRef]
35. Souza, R.; Garbossa, L.; Campos, C.; Vianna, L.; Vanz, A.; Rupp, G. Metals and pesticides in commercial bivalve mollusc production areas in the North and South Bays, Santa Catarina (Brazil). *Mar. Pollut. Bull.* **2016**, *105*, 377–384. [CrossRef] [PubMed]
36. Bower, S.; Mcgladdery, S.; Price, I. Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish. *Annu. Rev. Fish Dis.* **1994**, *4*, 1–199. [CrossRef]
37. Gallardi, D.; Dörner, J.; Carbonell, P.; Pino, S.; Farías, A. Effects of Bivalve Aquaculture on the Environment and Their Possible Mitigation: A Review. *Fish. Aquac. J.* **2014**, *5*, 3. [CrossRef]
38. Wan, W.; Lu, G. Heavy Metals in Bivalve Mollusks. In *Chemical Contaminants and Residues in Food*, 2nd ed.; Schrenk, D., Cartus, A., Eds.; Woodhead Publishing Limited: Cambridge, UK, 2017; pp. 553–594.
39. Lacoste, A.; Jalabert, F.; Malham, S.K.; Cueff, A.; Poulet, S.A. Stress and Stress-Induced Neuroendocrine Changes Increase the Susceptibility of Juvenile Oysters (*Crassostrea gigas*) to Vibrio splendidus. *Appl. Environ. Microbiol.* **2001**, *67*, 2304–2309. [CrossRef]
40. Kennedy, V.; Newell, R.; Eble, F. *The Eastern Oyster Crassostrea virginica*; Maryland Sea Grant College: College Park, MD, USA, 1996.
41. Garnier, M.; Labreuche, Y.; Garcia, C.; Robert, M.; Nicolas, J.-L. Evidence for the Involvement of Pathogenic Bacteria in Summer Mortalities of the Pacific Oyster Crassostrea gigas. *Microb. Ecol.* **2007**, *53*, 187–196. [CrossRef] [PubMed]
42. Héral, M.; Deslous-Paoli, J. Oyster Culture in European Countries. In *Estuarine and Marine Bivalve Mollusk Culture*; Menzel, W., Ed.; CRC Press: Boca Raton, FL, USA, 1991; pp. 153–190.
43. Sarà, G.; Mazzola, A. Effects of trophic and environmental conditions on the growth of *Crassostrea gigas* in culture. *Aquaculture* **1997**, *153*, 81–91. [CrossRef]
44. Iglesias, D.; Rodriguez, L.; Gómez, L.; Azevedo, C.; Montes, J. Histological survey of Pacific oysters Crassostrea gigas (Thunberg) in Galicia (NW Spain). *J. Invertebr. Pathol.* **2012**, *111*, 244–251. [CrossRef]
45. Sabry, R.; Gesteira, T.; Magalhães, A.; Barracco, M.; Guertler, C.; Ferreira, L.; Vianna, R.; Silva, P. Parasitological survey of mangrove oyster, Crassostrea rhizophorae, in the Pacoti River Estuary, Ceará State, Brazil. *J. Invertebr. Pathol.* **2013**, *112*, 24–32. [CrossRef] [PubMed]
46. Aguirre-Macedo, M.; Kennedy, C. Diversity of metazoan parasites of the introduced oyster species Crassostrea gigas in the Exe Estuary. *J. Mar. Biol. Assoc. U.K.* **1999**, *79*, 57–63. [CrossRef]
47. Pogoda, B. Current Status of European Oyster Decline and Restoration in Germany. *Humanities* **2019**, *8*, 9. [CrossRef]
48. Bromley, C.; McGonigle, C.; Ashton, E.C.; Roberts, D. Bad moves: Pros and cons of moving oysters—A case study of global translocations of Ostrea edulis Linnaeus, 1758 (Mollusca: Bivalvia). *Ocean. Coast. Manag.* **2016**, *122*, 103–115. [CrossRef]
49. Ruesink, J.L.; Lenihan, H.S.; Trimble, A.C.; Heiman, K.W.; Micheli, F.; Byers, J.E.; Kay, M.C. Introduction of non-native oysters: Ecosystem effects and restoration implications. *Annu. Rev. Ecol. Syst.* **2005**, *36*, 643–689. [CrossRef]
50. Beaz-Hidalgo, R.; Romalde, S.; Figueras, M. Diversity and pathogenecity of Vibrio species in cultured bivalve molluscs. *Environ. Microbiol. Rep.* **2010**, *2*, 34–43. [CrossRef] [PubMed]
51. Azéma, P.; Lamy, J.-B.; Boudry, P.; Renault, T.; Travers, M.-A.; Dégremont, L. Genetic parameters of resistance to Vibrio aestuarianus, and OsHV-1 infections in the Pacific oyster, Crassostrea gigas, at three different life stages. *Genet. Sel. Evol.* **2017**, *49*, 23. [CrossRef] [PubMed]