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Note

Temporal distribution of potentially pathogenic agents detected on carpet-shell clam, *Ruditapes decussatus* cultured in Galicia (NW Spain)

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Abstract – An histology and pathological survey on carpet-shell clam cultured in the south of Galicia (NW Spain) *(Ruditapes decussatus)* was carried out for a two years period (2000-2001). A temporal distribution of the potentially pathogenic agents were determined by histological analysis. In addition, stocks of molluscs reported to suffer mortalities during the studied period were also analyzed and the pathogenic burden determined. Pathogens do not follow a temporal pattern over studied period and no significant differences were detected nor on the presence nor in prevalence of these pathogens among all the analysed stocks. Therefore, no direct relationship can be established between infection by any pathogen and mortality episodes.

Key words: Pathology / Parasites / Histology / Clams / *Ruditapes decussatus* / Atlantic Ocean

1 Introduction

Shellfish aquaculture is a traditional activity of high economical importance in the west coast of Galicia (NW Spain). The natural bays or "*Rías"* provide shelter from the Atlantic Ocean, thus allowing cultivation of several bivalve mollusc species in natural beds. Although it is very difficult to estimate the mortality rate in these natural bays, in recent years shellfish fishermen have reported heavy mortalities in natural populations based on their extractive operating procedures of carpet-shell clam, *Ruditapes decussatus*, Manila clam, *Ruditapes philippinarum*, and pullet carpet-shell clam, *Venerupis pullastra*.

Mortalities have been traditionally attributed to the presence of pathogens when they showed high prevalence, and therefore, monitoring programmes of cultured populations have been suggested as tools for solving bivalve molluscs mortality problems (Bower and Figueras 1989).

Several pathogenic agents have been described in *R. decussatus*, *R. philippinarum* and *V. pullastra* from the Spanish coast (Figueras et al. 1992; Navas et al. 1992; Rodriguez et al. 1994; Villalba et al. 1993; Figueras et al. 1996; Ordás et al. 2001). In particular, symptoms of Brown Ring Disease (BRD), described in *R. philippinarum* and *V. pullastra* from France (Paillard et al. 1989), have also been detected in stocks of both *R. decussatus*, and *R. phillipinarum* cultivated in Galicia (Figueras et al. 1996). Haplosporidian (phylum Haplosporidia) and *Perkinsus* sp. (phylum Perkinsozoa) species are also present in *R. decussatus* from Galicia (Figueras et al. 1992; Novoa et al. 2004; Villalba et al. 2005). Related parasites have been responsible for high mortalities of oysters in the North American Atlantic coast since the late 1950s (Lauckner 1983). Moreover, they have also been associated with mortalities of *R. decussatus* from a depuration plant in Meira (SW Galicia) (Figueras et al. 1992). Recently, the presence of virus like particles has also been associated with mass mortalities in Galicia (Novoa and Figueras 2000).

The aim of this study was to determine the temporal distribution over an studied period of two years (2000-2001) of the potentially pathogenic agents presented on stocks of *R. decussatus* cultivated in the main areas of aquaculture in Galicia (NW Spain) and compare it to the pathogenic burden of mortality suffering reported stocks of *R. decussatus* all over the same period.

2 Materials and methods

2.1 Sampling methods

Samples of 60 adult clams were collected and processed for histopathological analysis on spring, summer, autumn and

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Table 1. Prevalence (%) of the potentially pathogenic agents detected on stocks of *Ruditapes decussatus* on year 2000 and on year 2001.

2000				
Pathogen/Season	Spring	Summer	Autumn	Winter
Brown Ring Disease	0	0	0	0
Rickettsiae	33.3	28.3	13.3	16.7
Perkinsus sp.	16.7	20	6.7	0
Nematopsis sp.	0	1.7	0	0
Other Gregarines	6.7	11.7	5	13.3
Haplosporidian organisms	20	5	0	0
Ciliates	50	13.3	13.3	30
Turbelarian organisms	0	8.3	3.3	10
Trematodes	10	3.3	5	0
Copepods	3.3	0	0	0

2001			
Pathogen/Season	Spring	Summer	Autumn
Brown Ring Disease	3.3	0	0
Rickettsiae	8.3	36.7	21.7
Perkinsus sp.	0	28.3	8.3
Nematopsis sp.	0	3.3	6.7
Other Gregarines	0	8.3	0
Haplosporidian organisms	0	5	6.7
Ciliates	3.3	13.3	18.3
Turbelarian organisms	11.7	5	0
Trematodes	23.3	1.7	5.0
Copepods	0	3.3	3.3

winter time on year 2000 and on spring, summer and autumn time on the year 2001. Additionally, samples of 60 adult clams were also analysed on every mortality episode reported by shellfish fishermen over this two years period. Samples were taken from the southern Rías of Galicia (Ría of Arosa, Ría of Pontevedra and Ría of Vigo) where the production achieved is higher due to upwelling processes.

2.2 Gross observation and histology

Clams were externally examined for symptoms of Brown Ring Disease (BRD). Body clams were fixed in Davidson's solution (Shaw and Battle 1957) for 24 hours. Oblique transverse sections, approximately of 5 mm thick, were taken from each specimen including mantle, gonad, digestive gland, gills, kidney and foot. Tissue samples were processed in an automatic tissue processor (Reichert-Jung Histokinette 2000), embedded in paraffin wax blocks and cut in a microtome. Sections of 5 μm were first deparaffinized, rehydrated and finally stained with haematoxylin- eosin. Clam histological sections were examined for the presence of parasites and pathological alterations with a microscope Nikon Optiphot.

3 Results

3.1 Mortality episodes

Four mortality episodes were reported by fishers all over the studied period. Two of theses episodes were reported in autumn and winter time of the year 2000 and during spring and summer time of the year 2001.

Table 2. Prevalence (%) of the potentially pathogenic agents detected on stocks of *Ruditapes decussatus*, reported to suffering mortalities on year 2000 and on year 2001.

2000		
Pathogen/Season	Autumn	Winter
Brown Ring Disease	1.7	0
Rickettsiae	20	15
Perkinsus sp.	6.7	30
Nematopsis sp.	0	1.7
Other gregarines	13.3	0
Haplosporidian organisms	0	6.7
Ciliates	6.7	0
Turbelarian organisms	0	0
Trematodes	0	0
Copepods	0	0

2001		
Pathogen/Season	Spring	Summer
Brown Ring Disease	1.7	3.3
Rickettsiae	55	36.7
Perkinsus sp.	15	10
Nematopsis sp.	1.7	10
Other gregarines	20	6.7
Haplosporidian organisms	15	0
Ciliates	20	6.7
Turbelarian organisms	0	0
Trematodes	1.7	0
Copepods	0	3.3

3.2 Gross observation and histology

Signs of BRD were detected in the 3.3% of the analyzed clams during the monitoring programme carried out during the spring of the year 2001 (Table 1).

The pathogenic burden of the analysed clams all over the sampling time is summarised (Table 1). Rickettsia-like organisms, protistan and metazoan parasites were detected (Figs. 1 to 3). No temporal distribution can be elucidate for any of the potentially pathogenic agents detected except for *Perkinsus* sp., which showed an slight increase on its prevalence on summer time of both years (Fig. 4).

The pathogenic burden of stocks of *R. decussatus* suffering mortalities is also presented (Table 2). During the two mortality episodes reported in autumn and winter time of the year 2000, Rickettsiae and *Perkinsus* sp., were detected with the higher prevalences (20% and 30% of prevalence respectively). Another two mortality episodes were reported in spring and summer time of the year 2001. In this case, Rickettsiae were also detected with the higher prevalence on both reported mortality episodes (55% and 37% respectively) (Table 2).

4 Discussion

The histopathological examination of all the stocks of *R. decussatus* revealed the occurrence of Rickettsia-like organisms, protistans and metazoan parasites (Table 1). No temporal distribution pattern could be determined for any of the potentially pathogenic agents during the two years sampling time, although in the case of *Perkinsus* sp. its prevalence was slightly

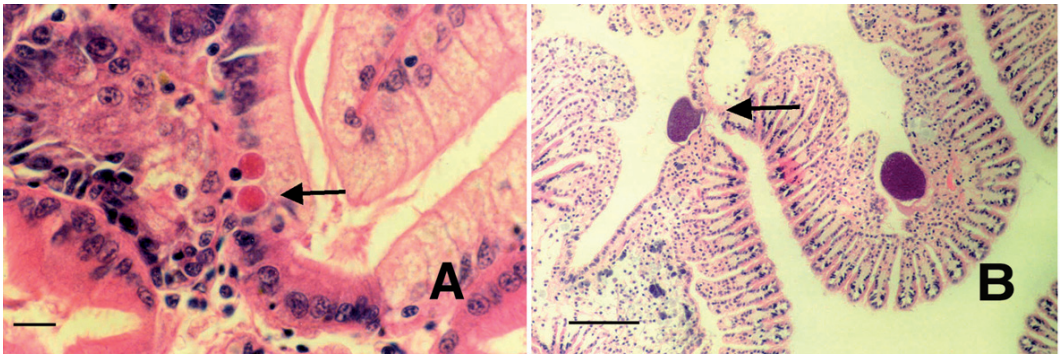

Fig. 1. Rickettsia-like organisms in the digestive tubules (A) and gills (B) of carpet-shell clams (*Ruditapes decussatus*). H & E stain. Scales bars: 50 μm.

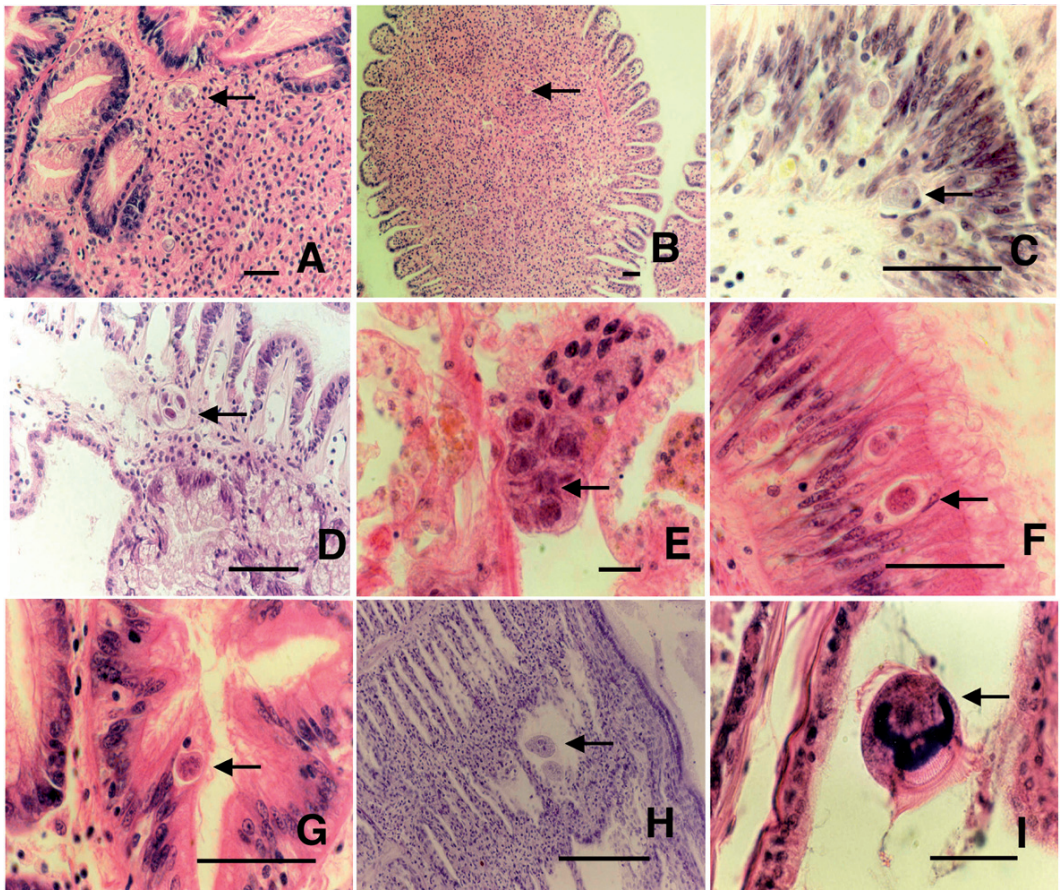

Fig. 2. Photomicrography of protistan infecting the 3 species of clams. Mature *Perkinsus* trophozoites associated with strong haemocytic infiltration in the digestive gland (arrow) (A) and gills (arrow) (B). Gregarines in width surrounded the intestinal epithelia. (C). *Nematopsys* sp. oocysts in gills (D). Coccidian-like organisms macrogamonts (arrow) (E). Multinucleate plasmodium (2-8 nuclei) of haplosporidian organisms in digestive epithelium (arrow) (F) and digestive gland (arrow) (G). Ciliates (arrow) (H) and *Trichodina* sp. (arrow) surrounded by a ciliary girdle (arrow head) in the gills (I). H & E stain. A, B, D, E, H scales bars: 50 μm. C, F, G and I scales bars: 20 μm.

higher in summer time both years due to the increase of the water temperature.

During the studied period, four mortality episodes were reported by fishermen. However, mortality rate was very difficult to determine in populations collected from natural bays since mortality episodes were only known when fishers estimate them. There were also difficulties when determining the time and place where the highest mortalities were taking place.

The histopathological examination of all the analysed stocks of clams reported to suffering mortalities revealed the occurrence of BRD with a very low prevalence, smaller than those reported by Drummond et al. (2007) for *R. phillipinum* in the same location. However, the same authors indicated that even clam mortalities were registered in natural conditions in this area, the experimental infection carried out with the pathogen agent of the BRD, *Vibrio tapetis*, did not result in

Fig. 3. Turbellarian *Paravortex*-like, in the intestinal lumen (arrow) (A) Trematodes cercariaes located in the intestine (arrow) (B). Copepods detected in the intestine (arrow) (C). H & E stain. Scale bars: 25 μm.

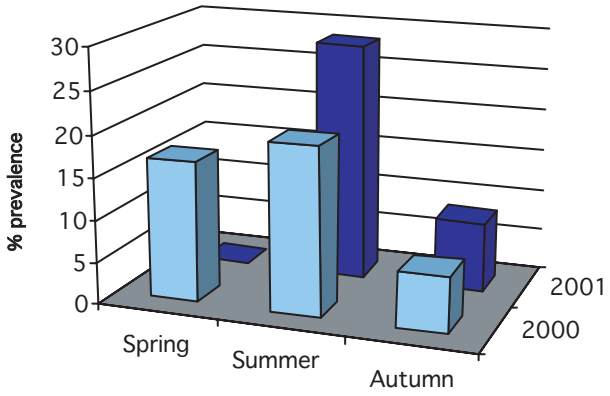

Fig. 4. Temporal distribution of *Perkinsus* sp., over the two-year period (2000-2001).

increased mortality in the stock, suggesting that Galician *R. phillipinarum* were weakened by a high prevalence of other potential pathogens that act synergically with *V. tapetis* increasing clam susceptibility to BRD.

Other histopathologically related pathogens were Rickettsia-like organisms, protistans and metazoan parasites, although Rickettsiae were detected with highest prevalences on 3 out of the 4 reported mortality episodes. Rickettsia-like organisms have been directly associated with severe mortalities of mollusc species (Le Gall et al. 1991; Norton et al. 1993; Renault and Cochennec 1994; Villalba et al. 1999).

Surprisingly, *Perkinsus* sp., were detected with the highest prevalence in the mortality episode reported in winter of the year 2000. Perkinsosis affects a wide variety of molluscs in the five continents with dramatic consequences in many areas (Burreson et al. 1994; Ford 1996; Hine and Thorne 2000; Choi et al. 2002; Casas et al. 2004; Navas et al. 1992; Ordas et al. 2001; Leite et al. 2004; Villalba et al. 2005). Among molluscan diseases, it is likely that perkinsosis has resulted in the most severe economic losses (Villalba et al. 2004). Therefore, other potentially pathogenic agents were also detected in all the mortality reported episodes and it could not be concluded that mortalities were due to the Rickettsiae and *Perkinsus* sp., respectively.

In the present work we have studied the temporal distribution of the potentially pathogenic agents of carpet- shell clams, *R. decussatus* from Galicia (NW Spain) and we have compared it with the pathogenic burden of suffering mortalities stocks populations of *R. decussatus*. No significant differences were detected among all the analyzed clam stocks and although it could be concluded that each pathogen by itself would not probably be lethal for the host, the simultaneous presence of all of them in the same population could weaken individuals endangering clam production that under unfavorable environmental conditions (sudden changes in temperature, salinity or food availability) might lead to a high mortality rate of the clam populations. This is of great importance in Galicia, an area in which aquaculture is one of the most relevant economical activities and where the clam production has been significantly diminished over the recent years. Moreover, the possible presence of pathogenic agents only detectable by ultrastructural analysis (i.e. virus) might influenced negatively clam production. Further studies on the incidence of these factors are needed to improve clam production and to design management strategies.

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