PERKINSUS, A PROTISTAN THREAT TO BIVALVE CULTURE IN THE MEDITERRANEAN BASIN

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Perkinsus marinus. formerly Dermocystidium marinum Labyrinthomyxa marina, was first noted and identified as a bivalve pathogen in the American oyster, Crassostrea virginica cultured in the estuaries adjacent to the Gulf of Mexico (Mackin et al., 1950). This organism has been implicated as the causative agent of numerous serious ovster mortalities in Gulf and East Coast oyster populations in North America (Andrews and Hewatt, 1957; Kern et al., 1973). Alderman and Grass (1969) and Cousserans et al. (1974) allude to the presence of similar parasites in oysters from the atlantic and mediterranean coasts of France.

In the summer of 1978 we noted the presence of a protistan parasite, quite similar to Perkinsus marinus Levine 1978, in histological preparations of live Venerupis decussata (Figure 1). These clams had been held in heated running sea water since the previous autumn at the Stazione Idrobiologica in Chioggia, as part of a gonad maturation study (Breber, 1980). The clams had been obtained from a local shellfish dealer and the probable origin was the Laguna Veneta (Northeast Italy). Considerable mortality was subsequently noted in this group during their maintenance in the laboratory. As a point of reference, we should indicate that a survey, using the thioglycollate procedure, was conducted in the late summer of 1976 in the lower Laguna. Almost 200 living Crassostrea gigas were sampled at that time, but none of these proved to be positive for *Perkinsus*. This raises the question as to whether the parasite is a very recent introduction or of the possibility of cyclic abundance or virulence.

During the winter of 1978 this parasite was noted in flat oysters imported from Greece in the Northern Adriatic (Figure 2). These findings stimulated a more intensive search for Perkinsus-like organisms in the period 1979 to 1983. The survey of bivalve molluscs has been the conducted using thioglycollate "culture" procedure of Ray (1952) applied to samples of both living and moribund individuals of various species (Table 1).

During summer 1979 considerable mortalities of *V. decussata* in the Laguna Veneta were observed and samples from the heated conditioning tanks at the Stazione Idrobiologica exhibited very high prevalence of *Perkinsus* in moribund individuals. High mortalities of *Venerupis aurea* were also noted and samples of gapers (moribund individuals) of this species had high levels of the parasite as demonstrated by culture assay. These clams had been dredged just inside the port entrance to Chioggia in the lower Laguna Veneta.

Perkins (1976) recently reviewed the life cycle and pathology of this protistan disease agent. Mackin and Wray (1952), Andrews (1965) and Canzonier (1966) have reported on the epizootiology of the

parasite in Crassostrea. Infection and subsequent development of the disease are favoured by temperatures in excess of 25°C, high salinities and proximity of host individuals. All of these conditions exist in several bivalve populations in the Laguna Veneta and other estuarine areas of the Mediterranean Basin, Transfer of the disease is enhanced by high population densities as would occur in certain types of culture practices (e.g. tray or hanging culture). Certain culture procedures might be modified minimize losses e.g. reduction of parasite load in infected stocks by transfer of certain bivalve species (e.g. Crassostrea spp) to low salinity waters is an example of an effective procedure that could be used to protect valuable brood stock. Although the definitive identification of the organism, or organisms, found in bivalve molluscs in Italian estuaries must await further study including electron microscopic examination, it reasonable to tentatively identify it as Perkinsus marinus on the basis of its behaviour in culture and on the features noted in histological preparations. In any case, it is probably closely allied to P. marinus if not identical to this notorious parasite of commercial bivalves.

Summary

As a result of a monitoring program *Perkinsus sp.* was found in live and dying individuals of several species of commercially important bivalves from different areas of the Italian Peninsula. The identification of the organism by culture method was subsequently confirmed by histological examination.

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Note: Alderman and Gras indicate resemblance of organism in Ostrea to Labyrinthomyxa marina and claim that this has been cultured in the case of oysters from Cancale, France.

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Table 1. Observations of Perkinsus-like organism in various bivalve molluscs collected in Italy and adjacent waters. The number of positive individuals is noted above the total number examined in each sample; the "G" designates that the individual was moribund or a gaper. The tissues were cultured according to the method of Ray (1952). The column marked WP gives the Weighted Prevalence according to the system of Ray in which each infection is related for intensity on a scale of 0 to 5

Veneru	nis	decussata

Date	Origin	Laguna Veneta	WP	Laguna di Grado	WP	Laguna di Orbetello	WP	Turkey	WP	Egypt	WP
VIII.79		5/5	4.2								
IX.79		8/8	3.6								
X.79		15/15	2.2								
X.79		5/5 G	3.1								
XI.79		19/19	2.7			6/7	1.4	0/6	0		
XI.79		5/5 G	2.2								
XII.79		5/10	1.9	0/5	0			0/2 G	0		
XII.79		4/5 G	2.6								
1.80		2/2	0.7	1/1	4.0						
II.80		1/1	4.0					0/2	0		
IV.80		6/11	1.4								
VI.83		10/10	1.6							0/4	0
VII.83		19/33	1.7								
VII.83		6/6 G	2.6								
VIII.83		0/3	0								
IX.83		19/19	3.2								
IX.83		16/19 G	2.4								
X.83		6/6	4.5								

Ostrea edulis

Date	Origin	Laguna Veneta	WP	Adriatic Sea	WP	Greece	WP
I.80		0/37	0	0/15	0	0/30	0
11.80		0/1	0	_			
IV.80		_		_		0/8	0
V.80				0/1 G	0	_	
IX.80		0/20	0	_		0/20	0
VIII.81		_		0/3	0		
VIII.82		0/10	0	_			
VI.83		_		0/16	0	-	
VII.83		_		0/15	0		
IX.83		2/6	0.2	0/2	0	_	
X.83		11/12	2.0	_			

Crassostrea gigas

Date	Origin	Laguna Veneta	WP	Adriatic Sea	WP
X.79		0/6	0		
X.79		1/1 G	0.5		
11.80		0/2	0		
11.80		0/22 G	0		
VIII.81		0/8	0		
VIII.82		0/10	0		
VI.83		_		0/13	0
VII.83		_		0/16	0
IX.83		0/7	0		
IX.83		0/1 G	0		
X.83		1/3	0.2		

Venerupis aurea

Date	Origin	Laguna Veneta	WP
IX.79		5/6	1.7
VIII.80		2/6	0.5
VIII.81		6/8	2.0
VIII.81		6/7 G	0.8
IX.83		22/23	2.8

Mytilus galloprovincialis

Date	Origin	Laguna Veneta	WP
VII.82		0/3	0
VI.83		0/3	0
VII.83		0/20	0
IX.83		0/10	0

Venus verrucosa

Date	Origin	Laguna Veneta	WP
XI.79		1/10	0.1
XII.79		1/11	0.2
IV.80		2/9	0.3
VI.83		4/4	2.2

Cerastoderma edule

Date	Origin	Laguna Veneta	WP
11.80		0/25	0
II.80		0/22 G	0
IX.83		2/3	1.2

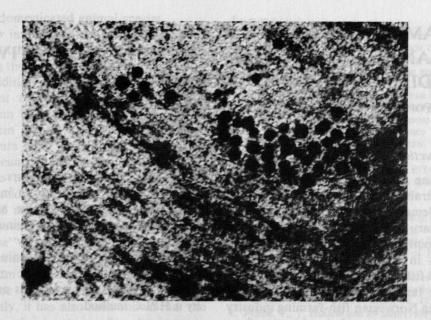


Fig. 1. Iodine stained "culture" stages of Perkinsus sp. in pieces of Venerupis decussata tissue maintained in thioglycollate medium according to the procedure of Ray (1952).

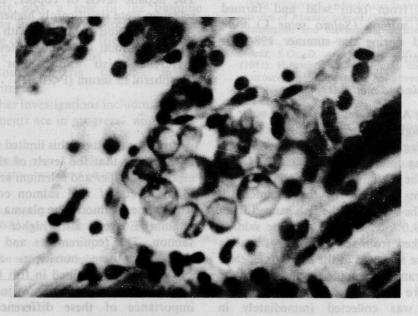


Fig. 2. Stained tissue section showing a typical hypnospore stage in Ostrea edulis from Greece.