BONAMIA OSTREAE PRESENT IN FLAT OYSTERS (*OSTREA EDULIS*) DOES NOT INFECT MUSSELS (*MYTILUS GALLOPROVINCIALIS*)

BY A. FIGUERAS (*) AND J. A. F. ROBLEDO.

Introduction.

Bonamia ostreae is a serious parasite of the flat oyster (*Ostrea edulis*) that caused high mortalities in several European countries during the 1980's (Hervio, 1992). *Bonamia* was introduced into Galicia with flat oyster spat supplied by French producers due to the lack of production by local growers (Figueras, 1991). This has contributed to the almost total disappearance of the culture of this species being the flat oyster present, at the moment, in Galicia a result of an import business. Since the beginning of mussel culture (*Mytilus galloprovincialis*) in Galicia no mass mortalities have been reported (Figueras, 1989). The 200.000 metric tons of mussel production per year establishes this culture as the world leader in its type.

Table 1. Presence of *Bonamia ostreae*. in the mussels and oysters cultured in the same raft in Domayo (Ría de Vigo, NW Spain).

Year/ Month	*Mytilus galloprovincialis*			*Ostrea edulis*		
1988						
	N. exam.	**N. Bon.**	**Preval.**	**N exam.**	**N. Bon.**	**Preval.**
V	-	-	-	33	13	39
VII	-	-	-	30	7	23
IX	-	-	-	30		23
XI	-	-	-	27		7
1989						15
I	30	0	0	30	17	56
III	27	0	0	25	18	72
V	24	0	0	22	17	77
VII	26	0	0	28	18	64
IX	25	0	0	25	21	84
XI	18	0	0	25	20	80
1990						
II	18	0	0	25	15	60
IV	22	0	0	-	-	-
VI	25	0	0	-	-	-
VII	18	0	0	-	-	-
X	27	0	0	-	-	-
TOTAL	260			300		

N. Exam: number of individuals examined.
N. Bon.: number of individuals with *Bonamia*.
Preval. (Prevalence): number of individuals with *Bonamia* divided by number of individuals examined multiplied by 100.

Under the EEC Council Directive 91/67 *Bonamia ostreae* is included in list II of Annex A. This list includes serious pathogens that should be obligatorily declared. An area where flat oysters are infected with *Bonamia* will not be allowed to export to another one free of this disease. Grizel *et al.* (1988) reported that tests conducted with several genera of molluscs (*Mytilus edulis* among them) living in a *Bonamia*-contaminated habitat did not result in infections with this parasite. Despite of the lack of references on mussels being infected with *Bonamia* some doubts have been shed on its role acting as carriers. It is very important and urgent to give all the information available on this question to clarify the safety of the commercial movements of mussels coming from *Bonamia* infected areas.

Materials and Methods.

From January 1988 to October 1990, mussels and oysters were maintained in the same raft in Domayo (Ría de Vigo, NW of Spain). The animals were kept on experimental baskets hung on ropes that were less than one meter away from each other. Half of the raft was used for mussel culture and the other half for oyster culture.

Only during 1989 and in February 1990, were examined animals of both species because of other scientific objectives, than the present infection experiment, that were pursued with both species. 30 oysters and/or mussels were sampled and processed for histology following the usual protocols. A total of 260 mussels and 300 flat oysters were examined in this way.

Results and Discussion.

The data are summarised in Table 1. The prevalence of *Bonamia* in the flat oysters reached the 84% causing a mortality of 34% (Figueras, 1991). Despite the high prevalence recorded in the flat oysters, no presence of the *Bonamia* or anything resembling this parasite was found in the haemocytes or any of the examined organs of the mussels that were cultured side by side in the same raft.

The present findings suggest that the *Bonamia* found in flat oysters from Galicia does not infect neighbour mussels. With these results the mussel should not be considered as a *Bonamia* susceptible species, following the international regulations on the health status of animals coming from aquaculture such as the EEC Council Directive 91/67.

Another experiment needed would be one in which mussels that had been held together with *Bonamia* infected oysters for a time, at least as long as we report here, were maintained, in the same tank, with *Bonamia* free oysters testing then if these mussels transmit or not *Bonamia*. Unfortunately this experiment has serious problems difficult to solve.

Although a microcell-like organism, which closely resembles *Bonamia* in histological section was found in a single mussel (*Mytilus edulis*) out of a total of 93 from New Jersey (USA) (Figueras *et al.*, 1991a) nothing similar has been reported in any of the papers dealing with Galician mussel pathology (Figueras *et al.*, 1991b; Mourelle, 1993;Robledo, *et al.*, 1994).

Summary:

Flat oysters (*Ostrea edulis*) infected with *Bonamia ostreae* and uninfected mussels (*Mytilus galloprovincialis*) were cultured in the same raft for two years. After examination of histological slides of mussels collected bimonthly no presence of the parasite was detected in these animals.

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A SIMPLE PROCEDURE FOR THE ISOLATION OF *PERKINSUS MARINUS* MEROZOITES, A PATHOGEN OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*

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Introduction.

The protozoan *Perkinsus marinus* (Apicomplexa) causes heavy mortalities of the eastern oyster, *Crassostrea virginica*. Studies of the pathogenesis of this parasite and the host defense responses have been hampered by the absence of a standard protocol to isolate and enrich the tissue-associated parasitic stages.

The main histozoic stage of *P. marinus*, the merozoite (uninucleate coccoid cells, 2-4µm), multiplies within oyster tissues following enlargement and division by schizogony (Perkins, 1991). It has recently been demonstrated that *P. marinus* merozoites can be propagated *in vitro* (La Peyre *et al.*, 1993; La Peyre and Faisal, Submitted). It is unknown, however, if the culture procedure and conditions influence the virulence of *P. marinus*. In order to make this evaluation, it is necessary to isolate merozoites of known high pathogenicity as the reference in comparisons with cultured merozoites. In the present study, we report on a relatively simple procedure that allows the isolation of *P. marinus* merozoites directly from heavily infected oysters in a relatively pure form.

Materials and Methods.

Haemolymph from individual oysters (*Crassostrea virginica*) was withdrawn from the adductor muscle sinus and examined for the presence of *P. marinus* merozoites with phase contrast microscopy. Oysters that had at least 25% infected haemocytes with 5 or more merozoites per haemocyte, were selected (Figure 1). The visceral mass of each individual oyster was excised, minced finely into 0.1mm^3 fragments, and suspended in sterile filtered (0.2µm) York River water (FYW) using 25 ml/g of oyster tissue wet weight. The infected oyster tissue was then homogenized with a blender (Virtis 200, Virtis Company, Gardiner, NY) for 45 seconds. The homogenate was then filtered through a series of Nytex screens (53, 35, 15µm mesh size) (Tetko Inc., Briarcliff Manor, NY) into a 500 ml beaker. In order to separate the larger oyster cells

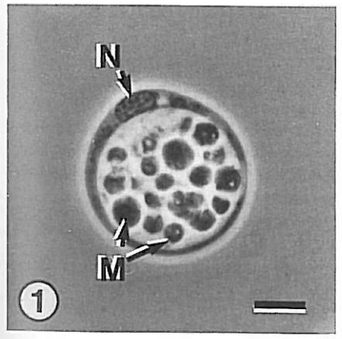

Figure 1. Phagosome of a haemocyte filled with *Perkinsus marinus* merozoites (M). Haemocyte nucleus (N). Bar = 10µm.

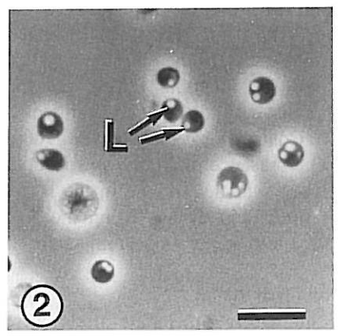

Figure 2. Light micrograph of isolated and partially purified *Perkinsus marinus* merozoites with typical lipid droplets (L). Bar = 10µm.