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Interim report on investigations into gill disease of Portuguese oysters

by

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Introduction and method

During February 1968 notification was received from French biologists of a disease which appeared to be the cause of abnormally high mortalities of Portuguese oysters in the oyster producing basins of the French Atlantic coast. The mortalities appeared to be associated with damage to the gills. As a result of these reports British oyster stocks were examined to see if there was evidence of the presence of the disease in this country.

Samples from a number of consignments of Crassostrea angulata imported by private merchants from Portugal and from France since April 1966 were examined for gill damage. In addition, Brittany oysters (Ostrea edulis) imported by the Ministry of Agriculture, Fisheries and Food (M.A.F.F.) during April 1967, and small samples of Ostrea edulis and Crassostrea gigas, reared at the Conway Laboratory, North Wales, were also examined.

To assist in the recording of data obtained from the examination of these oysters and to maintain a standard for comparison, the following classification of gill damage has been devised. These categories, however, are arbitrary and are not intended to represent successive stages of a single destructive agency.

Light damage Gills showing no signs of active degeneration with the margin entire though irregular. Indentations in the gill surface not penetrating more than half the width of the gill and with not more than a total of five indentations on all gills and not more than three on any one gill.

Plates A and B show gills which are slightly damaged; the former showing a slight but marked indentation of a single gill and the latter marked undulations of the gill margin.

Moderate damage Gills showing no signs of active degeneration with the margin entire though irregular. One or more indentations of the gills extending beyond half the width of the gill or more than five "light" indentations on all gills or more than three on any one gill (plates C and D).

Heavy damage Gill edge ill-defined and tattered with degenerating gill tissue, but less than half total gill area eroded away.

Plate E shows an oyster with heavy damage and also with areas of decaying tissue bordered by an area of yellow discoloration. Also with the yellowed area on the underside of the mantle.

Severe damage Gill edge ill-defined and tattered with degenerating tissue. More than half of the total gill tissue missing.

Plate F is an example of an oyster in this condition. This oyster also displayed numerous yellow pustules on the body.

Results of Examination

1. Occurrence of gill damage

(a) Typical damage

The results of all examinations made are shown fully in Table 1. All samples examined contained some oysters with gill damage though in the majority of cases this was predominantly of the light and moderate types (plates A-D) with no active breakdown of gill tissue being apparent, the margins of the gills were entire and all tissue appeared healthy under the microscope. Only the two samples from Emsworth and sample D of Tagus oysters imported into the Blackwater in 1967 showed substantial proportions of heavy and severe damage (plates E-H). The Emsworth oysters were imported from France during Spring 1967.

It became apparent during the examination of the oysters that such features as protrusions in the shell in the region of the gills produced irregularities in gill shape. It is believed also that certain organisms such as the pea-crab, Pinnotheres sp. can cause gill damage. It would appear therefore that there is a background incidence of gill damage which has no connection with disease. The fact that small samples of Ostrea edulis and Crassostrea gigas reared at the Conway laboratory contained 50 per cent with light damage further suggests that there may also be, under some conditions, a larger incidence of gill damage due to causes other than disease.

(b) Occurrence of yellow discoloration on gill tissue

Decaying gill tissue bordered by an area of yellow discoloration, as clearly shown in photograph E, was observed in the Emsworth sample examined on 26 March when one oyster displayed this symptom and in the Blackwater A (1967) Tagus oysters, where 25 our of a sample of 200 oysters (12.5 per cent) showed this feature to varying degrees. In this latter sample the extent of

discoloration and decay varied from a mere pin point of yellowed tissue to large areas of brown degenerating gill tissue bordered by a yellowed area (plate F) giving the impression of a sequence which started with the invasion of the gill at one point which then extended to adjacent gill tissue.

By 30 April some other samples from the rivers Blackwater and Roach had shown this condition and it appeared significant that all these samples had been stored either dry or in tanks of sea water for a period of days before examination.

To determine the effect of dry storage on the condition of the gills, an experiment was conducted with Portuguese oysters imported by M.A.F.F. in 1967. Five batches of 50 oysters were packed in polythene bags and stored dry in the laboratory. A further 65 oysters from the same sample were examined on arrival at the laboratory and the amount and extent of gill damage was recorded. Each batch of 50 was opened and examined at intervals during a period of 20 days following the start of the experiment. Heavy mortalities had occurred by the fifteenth day and the two remaining samples were combined and placed in a tank of running sea water and examined on the twentieth day.

There was no consistent increase in the amount or extent of gill damage during the course of the experiment, though the last sample examined contained 16 per cent with areas of yellow discoloration on the gill tissue (Table 2).

A further experiment to test the effect of storing oysters was started on 2 May. Oysters were stored (i) in aerated sea water, (ii) out of water, (iii) for varying periods out of water and then immersed in sea water for a further 5 days. The results of this experiment, shown in Table 3, indicated that no significant change in either the per cent damage or yellow discoloration occurred.

On 1 May a further sample of M.A.F.F. 1967 Tagus imports was examined and 28 per cent showed varying degrees of gill damage and 3 per cent of the sample showed gills with yellow discoloration and decaying gill tissue. Previously these oysters had shown only 14 per cent with light damage and had not displayed any yellow discoloration. Later samples from this population have shown a lower incidence of damage and no discoloration in the gills.

It is clear from these experiments that Portuguese oysters can be kept out of water for many days without any increase in the incidence of damage to the gills, but when the oysters are approaching death damage to the gills can occur, including the form which shows yellow discoloration.

The latest indications are that the yellow symptoms occur when water temperatures are fairly low, samples containing yellow discoloration of the

gill having so far only been obtained when river temperatures have been between 8°C and 13°C.

From observations on yellow discoloured gills it appears that the accumulation of leucocytes in the damaged area may be the cause of the discoloration.

Black pigmentation often occurs along the gill margin of healthy oysters but there does not appear to be any association between this pigmentation and disease.

(c) Gills with separated filaments

In addition to the typical damage found in oyster gills as shown in plates A to D, there also occurred oysters with gills having single or multiple separation of gill filaments down to the base, in some cases heavily damaged, (plates F to H) and these were included in the numbers shown in Table 1.

II Functioning of damaged gills

Damaged gills which did not show yellow discoloration were examined to discover if they could behave in a normal manner, by observing the gills in situ after removing the flat valve.

Damaged gill edges often appeared to be able to function in carrying material towards the mouth even though the oral groove had disappeared. In some instances material was seen to drop to an undamaged gill below the damaged one, but in others the damaged gill edge appeared to take on the function of the oral groove.

Oysters with yellow discoloration did not show this ability to transfer material over the damaged portions. It is thought that oysters recovering from the disease may be able to re-establish an oral tract on the damaged edges.

III Histology and Culture

Tissues which showed yellow discoloration and those showing damage but no discoloration have been examined for causative organisms by the following methods.

1. Zenkers fixative.

   Tissues teased and treated as follows, but not sectioned.

   (a) K O H (conc.)

   (b) (i) 70 per cent alcohol and iodine

   (ii) K O H (conc.)

2. Bouin's fixative

   Tissues sectioned and stained with

   (a) Delafield's Haematoxylin/Eosin

   (b) Harris' Haematoxylin/Eosin

Table 1. Details of oyster samples examined for gill damage

	Source	Year imported	Pits/Lays	Date of dispatch	Date examined	Sample size	% Gill damage									
							Without yellow discoloration					With yellow discoloration				
							Light	Moderate	Heavy	Severe	Total	Light	Moderate	Heavy	Severe	Total
Roach A	Sado	1967	Lays			200	12.0	0	0	0	12.0					0
Roach B	Tagus	1967	Lays			200	14.0	0	0	0	14.0					0
Blackwater A	Sado	1968	Lays	23 March 1968	26 March 1968	200	1.0	5.5	0.5	0.5	7.5					0
Blackwater B	Tagus	1968	1 week Lays	26 March 1968	27 March 1968	200	3.0	4.0	1.0	0	8.0					0
Blackwater C	Tagus	1966	Lays	26 March 1968	29 March 1968	200	16.5	15.5	1.0	0	33.0					0
Blackwater D	Tagus	1967	Lays (5 days in sink)	26 March 1968	1 April 1968	199	11.0	5.5	0.5	1.0	18.0	5.0	0	7.5	0	12.5
Emsworth	France	1967	Lays	19 March 1968	26 March 1968	54	5.5	11.0	7.4	7.4	31.3	0	0	2.0	0	2.0
Orford	Tagus	1967	Lays	31 March 1968	2 April 1968	100	29.0	8.0	1.0	0	38.0					0
Blackwater B	Tagus	1968	Lays	5 April 1968	5 April 1968	188	12.5	2.5	0	0	15.0					0
Roach C	Sado	1968	New Import	5 April 1968	5 April 1968	100	7.0	1.0	0	0	8.0					0
Roach D	Sado	1966	Lays	5 April 1968	5 April 1968	100	21.0	4.0	0	0	25.0					0
Roach C	Sado	1968	New Import	5 April 1968	9 April 1968	100	8.0	8.0	0	0	16.0					0
Roach D	Sado	1966	Lays	5 April 1968	9 April 1968	100	16.0	7.0	0	0	25.0	0	2	0	0	2.0
Emsworth	France	1967	Lays	21 April 1968	24 April 1968	56	25.0	16.0	9.0	2.0	52.0					0
Blackwater E	Setubal	1967	Pits	25 April 1968	25 April 1968	17	23.5	0	0	0	23.5	0	12	6	0	18.0
Blackwater F	Sado	1968	Lays 3 weeks Tanks 4 days	25 April 1968	26 April 1968	50	16.0	4.0	0	0	20.0	2	2	0	0	4.0
Blackwater F	Sado	1968	"	25 April 1968	30 April 1968	50	24.0	4.0	0	0	28.0					0
Roach B	Tagus	1967	Lays	1 May 1968	2 May 1968	250	17.0	8.0	0.4	0	25.4	0	0	3	0	3.0
O. edulis	Conway		Trays		3 April 1968	10	50.0	0	0	0	50.0					0
C. gigas	Conway		Trays		3 April 1968	10	50.0	0	0	0	50.0					0
O. edulis	Brittany	1967	Pits	28 March 1968	2 April 1968	150	11.0	0	0	0	11.0					0
Roach B	Tagus	1967	Lays	5 June 1968	10 June 1968	152	7.9	3.9	0.7	0	12.5					0
Roach B	Tagus	1967	Lays	8 July 1968	9 July 1968	205	5.8	7.8	2.9	0.5	17.0					0
Blackwater D	Tagus	1967	Lays		25 July 1968	215	6.5	9.3	0.9	0	16.7					0
Emsworth	France	1967	Lays	26 July 1968	29 July 1968	79	17.7	7.6	6.3	0	31.6					0
R. Helford	Tagus	1968	Lays		9 May 1968	200	9.0	5.0	0.5	0	14.5					0

(c) Periodic acid - Schiff technique

(d) Heidenhain's Haematoxylin

3. Staining for fungal hyphae

Lactophenol/cotton blue

4. Culture techniques

(a) Thioglycolate medium (specific for Dermocystidium marinum)

(b) Agar/sea water - for fungi

(c) Yeast/peptone in sea water

(d) Nutrient agar - standard bacteria test

None of the above techniques has revealed any positive causative organism of the disease.

Mr. D. Alderman, of the Portsmouth College of Technology, has made tests for fungi from material supplied from this laboratory and by Dr. Marteil in France. Tests using thioglycolate medium have been made at the Portsmouth and Burnham-on-Crouch laboratories but these have not produced the characteristic results expected when Dermocystidium is present.

Mr. Alderman has isolated a fungal hypha from diseased tissue but it is thought likely to be a secondary infection, and re-infection experiments have yet to be made.

Discussion and conclusion

It cannot be said with certainty that any of the symptoms described here have arisen from the same cause that has resulted in heavy mortalities among Portuguese oysters in France; however it is significant that the Portuguese oysters imported from France and laid at Emsworth on the south coast of England in 1967, which might be expected to be carrying the disease, showed the highest incidence of "heavy" and "severe" gill damage, suggesting that the disease is already in England. However similar gill damage was also found among Portuguese oysters imported from Portugal in 1967, and laid in the river Roach and the Blackwater on the east coast.

There have been no reports of above average mortalities among Portuguese oysters during 1967 or 1968.

Results of oyster storage experiments

Table 2 Experiment started 4 April 1968

Sample	Storage period (days)	No.	% showing damage					
			Light	Moderate	Heavy	Severe	Yellow	Total
Original	0	65	18.5	-	-	-	-	18.5
I	4	50	18.0	12.0	4.0	2.0	-	36.0
II	7	50	16.0	4.0	-	-	-	20.0
III	12	50	14.0	4.0	-	-	-	18.0
IV, V	20	55	14.0	10.0	-	-	16.0 (heavy)	40.0

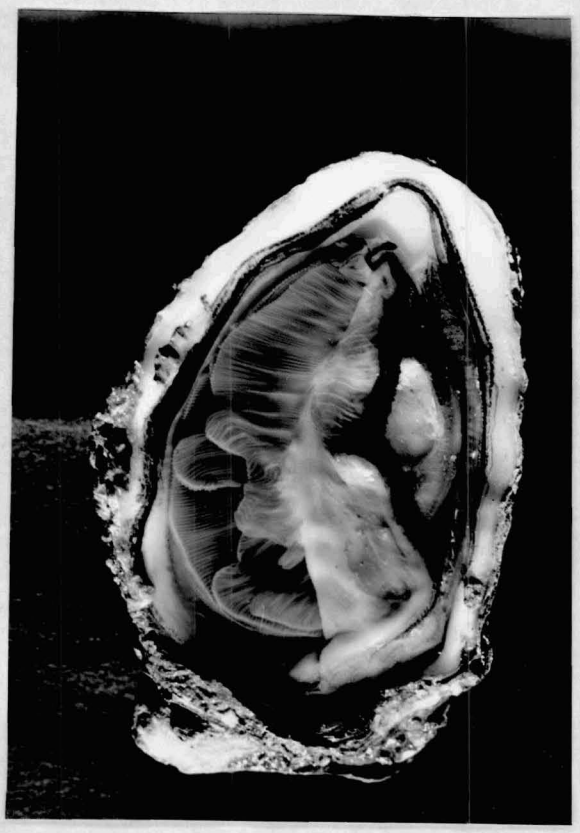
Table 3 Experiment started 1 May 1968

Sample	Storage period (days)		No. of oysters	% showing damage					
	Dry	In water		Light	Moderate	Heavy	Severe	Yellow*	Total
I	0	0	50	16.0	10.0	-	-	2.0(h)	28.0)
	0	0	50	12.0	8.0	2.0	-	4.0(h)	26.0)
	0	0	50	14.0	8.0	-	-	2.0(h)	24.0) 28.5
	0	0	50	20.0	8.0	-	-	4.0(h)	32.0)
	0	0	50	24.0	6.0	-	-	2.0(h)	32.0)
II	0	5	81	12.3	6.2	-	-	2.0(s)	21.5
III	5	0	100	13.0	8.0	1.0	-	4.0(h)	26.0
IV	5	+ 5	109	13.8	2.8	-	-	3.9(h)	20.7
V	0	10	82	8.5	3.7	-	-	4.9(h)	17.1
VI	10	0	100	8.0	8.0	1.0	-	1.0(l) 4.0(h)	22.0
VII	10	+ 5	98	19.0	8.0	3.0	-	1.0(m)	31.0
VIII	0	15	74	23.0	5.5	-	-	5.5(h)	34.0
IX	15	0	100	23.0	6.0	1.0	-	1.0(l)	31.0
X	15	+ 5	ALL DEAD						
XI	0	20	79	15.2	3.8	-	-	2.5(h)	21.5
XII	20	0	ALL DEAD						

*l = light m = moderate h = heavy s = severe

A

B



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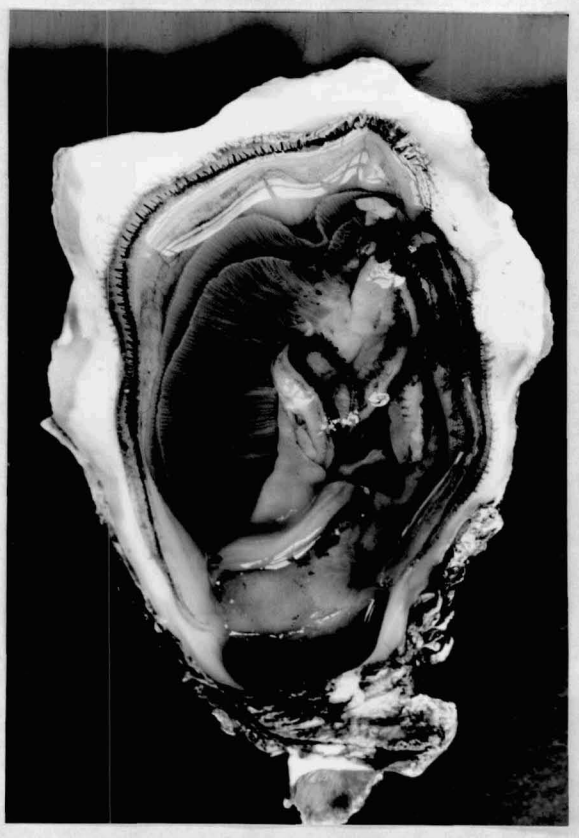

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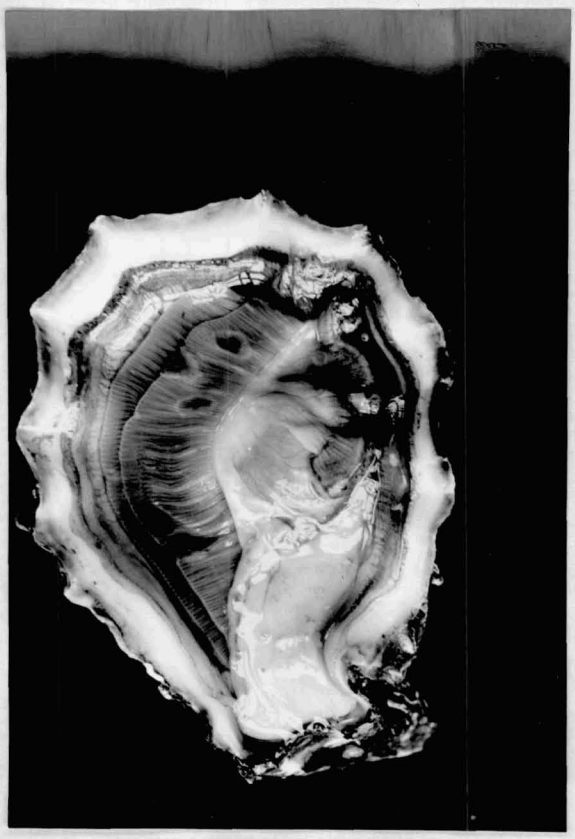

E

F

G

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