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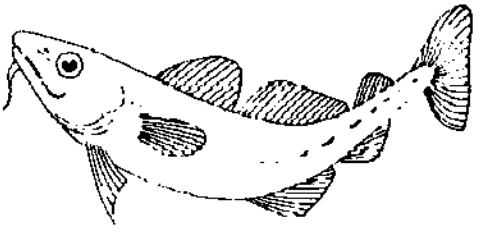
AQUATIC ENVIRONMENT MONITORING REPORT

Number 41



Monitoring for diseases in marine and freshwater fish, 1991

MAFF

Directorate of Fisheries Research
Lowestoft, 1994

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD
DIRECTORATE OF FISHERIES RESEARCH

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LOWESTOFT
1994

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FOREWORD

A monitoring programme for marine fish diseases has been conducted for several years by the Fish Diseases Laboratory (FDL) of the Directorate of Fisheries Research (DFR) to assess spatial and temporal variability of disease prevalence, and to explore possible links with pollution. This work is conducted in response to concern that sewage and industrial waste materials entering the sea might cause increased levels of disease in fish. The Ministry of Agriculture, Fisheries and Food (MAFF) has reponsibilities for controlling and monitoring waste disposal by dumping at sea under the Food and Environment Protection Act, 1985 (Great Britain - Parliament, 1985(a)), and point discharges under the Environmental Protection Act, 1990 (Great Britain - Parliament, 1990). The emphasis of the work in 1991 was to collect fish disease data in two areas of the North Sea and to collate these with similar data collected by other North Sea countries as part of the North Sea Task Force, Quality Status Report (NSTF, QSR) 1993 (North Sea Task Force, 1993). The UK data were also integrated with other biological and chemical sampling conducted at the instigation of the Marine Pollution Monitoring Management Group (MPMMG) (Department of the Environment, 1991(a),(b)).

A separate monitoring programme for diseases in fish, molluscs and crustacea has been conducted to fulfil statutory requirements of the Diseases of Fish Act, 1937 (Great Britain - Parliament, 1937), Diseases of Fish Act, 1983 (Great Britain - Parliament, 1983) and the Sea Fisheries (Shellfish) Act, 1967 (Great Britain - Parliament, 1967). The work in 1991 involved monitoring, diagnosing and providing advice for the control and prevention of notifiable diseases in cultivated stocks of fish and shellfish. The remit also included a surveillance of the relationship between diseases of cultivated and wild stocks of fish and shellfish. Additional duties were related to the statutory requirements for registration of fish farms, for which Inspectors were charged with checking registration and stock movement details.

The accumulated data from the fish and shellfish monitoring programmes provided background evidence for the EC Directive 91/67 (European Comunities, 1991) concerning the animal health conditions and the safeguard of health status zones free of certain diseases for the UK, which came into force on 1 January 1993.

The above programmes are backed by research and development projects at FDL, Weymouth, to continually enhance the techniques and facilities for diagnosis of diseases, and assessment of their significance.

The 1991 programme was successfully carried out in spite of interruptions caused by the transfer of the staff and equipment from the site on the Nothe to a temporary facility nearby in Weymouth. This is to enable a new laboratory to be built on the Nothe site (due for completion mid-1994).

P. W. Greig-Smith
Deputy Director
(Aquatic Environment Protection)
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MARINE FISH DISEASES MONITORING PROGRAMME

1. THE MONITORING PROGRAMME FOR DETERMINATION OF DISEASE IN MARINE FISH

1.1 Introduction

Previous Aquatic Environment Monitoring Reports (MAFF, 1991, 1993) provided detailed background information on MAFF's Marine Fish Diseases Monitoring Programme. The main emphasis in 1991 was to monitor prevalences of diseases in marine fish from a number of sampling stations in the North Sea to provide data for the North Sea Task Force (NSTF) Quality Status Report 1993 (North Sea Task Force, 1993). Additionally, reports of diseases in marine fish from MAFF District Sea Fisheries Inspectorate offices and other bodies were investigated.

1.2 Monitoring programme for DFR research cruises

Marine fish disease surveys were conducted during cruises on MAFF's research vessels:

RV CORYSTES Cruise 3b, 28 March-5 April, 1991;
RV CIROLANA Cruise 8a, 1-5 October 1991; and
RV CORYSTES 10a, 9-14 October, 1991.

Some additional data were collected on a charter vessel, MV INA-K, 16-18 July 1991 (this survey was part of a German programme – 'Investigations of Fish Diseases in the North Sea', in which MAFF participated as observer).

1.3 RV CORYSTES Cruise 3b, 28 March-5 April 1991

The aims of this cruise were to investigate diseases in dab, the species selected by the International Council for the Exploration of the Seas (ICES) for standardised disease monitoring in the North Sea, and to record the prevalence of diseases of other commercial fish species (ICES, 1989).

1.3.1 Methods and results

RV CORYSTES Cruise 3b made 33 x 1 h tows using a Granton trawl fitted with tickler chains and a small mesh cod-end liner. The trawls were made on clear ground within the selected ICES rectangles, which included relevant NSTF stations. Several hauls were made in each ICES rectangle in order to obtain sufficient numbers of dab for examination. The catch from each haul was sorted, weighed and sub-samples measured for stratified length distribution. Otoliths were sampled for ageing profiles. The dab were subsequently separated into 3 length-size groups (15-19 cm, 20-24 cm and ³25 cm). Samples of the dab were examined for gross lesions, including lymphocystis, epidermal ulcerations, epidermal hyperplasia, liver nodules and, for this study only, epidermal melanosis. Cod and other commercial fish species were examined for disease when suitable numbers were available. Samples of livers from dab showing lesions were also preserved for histological examination in order to diagnose neoplasms. Examples of other lesions were preserved for histological examination.

Table 1 details information on the areas fished, catch data, and disease prevalence, including degrees of severity. Figure 1 shows the % prevalences and distribution for individual diseases recorded from the 5029 dabs examined. The severity of diseases in dab was recorded according to the categories recommended in the ICES Training Guide for the Identification of Common Diseases and Parasites of Fish in the North Atlantic (Bucke *et al*., in press). Briefly, this is as follows:

Epidermal ulcerations:
stage 1 = acute ulceration,
stage 2 = partially-healed ulceration,
stage 3 = healed ulceration.

Lymphocystis:
stage 1 = 2-10 nodules on body surface,
stage 2 = clusters of nodules covering up to twice the area of spread-out caudal fin,
stage 3 = as for 2 but covering a larger area of the body surface.

Epidermal hyperplasia/papilloma:
stage 1 = 1-4 lesions ³ 2 mm dia.,
stage 2 = ³ 4 lesions ³ 2 mm-1 cm dia.,
stage 3 = lesions ³ 1 cm dia.

Figure 2 shows the length size frequencies for dab sampled on this survey. Table 2 details numbers and categories of liver lesions in dab. Table 3 details numbers of gross diseases in cod.

From Table 1 it can be seen that although numbers of gross lesions appear high, the severity of the lesions were, in fact, of the lowest category, and epidermal ulcerations were mostly present as healed lesions. The

Table 1. Catch data and disease prevalence in dab (Limanda limanda) by size categories on stations sampled in the North Sea for fish disease monitoring (1 hour tows using a Granton trawl), RV CORYSTES Cruise 3b/91

ICES Rectangle	No. of Hauls	Size Range (cm)	Average Depth (m)	Numbers examined		Total catch (unsexed)		No. and severity of diseases recorded according to ICES (1989)												
				Male	Female	Total no.	Weight (kg)	LY			E/P			U			MEL			LN	LIP
								1	2	3	1	2	3	1	2	3	1	2	3		
33F2	5	15-19	40.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NR	0
		20-24		0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0
		>25		0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0
37F4	5	15-19	47.9	316	184	3955	198.4	13	0	0	8	1	0	1	1	3	2	0	0	NR	1
		20-24		55	178			14	0	0	11	1	0	1	1	5	0	0	0	6	0
		>25		8	13			0	0	0	0	0	0	0	0	0	0	0	0	0	0
39F3	5	15-19	30.8	320	180	4956	395.8	27	1	0	14	0	0	5	3	12	2	1	1	NR	2
		20-24		132	313			30	10	0	15	7	0	13	5	35	3	0	1	9	0
		>25		8	122			3	1	0	2	4	0	1	1	9	3	0	0	9	0
37F2	10	15-19	97.5	577	423	17457	976.78	22	1	0	21	1	1	4	1	16	3	0	0	NR	6
		20-24		123	518			35	3	1	17	7	0	1	2	50	11	1	0	12	0
		>25		0	60			2	0	0	1	1	0	1	0	7	0	0	1	8	0
37F0	4	15-19	57	211	189	7888	604.1	17	1	0	7	2	0	1	0	10	10	11	1	NR	0
		20-24		129	226			15	9	3	8	6	1	10	4	42	24	25	12	9	1
		>25		4	129			3	6	2	6	9	0	6	1	29	10	4	23	23	0
35F0	4	15-19	30.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NR	0
		20-24		0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0
		>25		0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key: *LY=Lymphocystis*
E/P=Epidermal papilloma
U=Ulcers
MEL=Melanosis
LN=Liver nodules
LIP=Lipoma
NR=Not recorded due to fish size

skin melanosis study was included in order to investigate whether there was any relationship between this condition and hepatic lesions. Highest prevalences of both conditions were recorded in samples from stations on ICES rectangle 37F0 (Flamborough Off Ground).

Insufficient dab were present for examination in ICES rectangles 34F2 and 35F0. Numbers of the largest category size of dab were very low on stations in ICES rectangles 37F4 and 37F1. Consequently, spatial comparisons of liver lesions, usually present in higher numbers in the largest sized group (older) dab, was not possible. However, it can be seen in Table 2 that numbers of hepatic foci and adenoma, as well as dab exhibiting epidermal melanosis, were higher in dab sampled from ICES rectangle 37F0.

Macroscopical examination of other commercial fish species showed fish to be generally in good condition. The exception was the high prevalence (25%) of vertebral compressions recorded in small cod sampled from stations in the Wash (ICES rectangle 34F0) (Table 3). According to DFR records, these abnormalities have been a common feature in juvenile cod sampled from this area for at least 20 years. No cause has been identified.

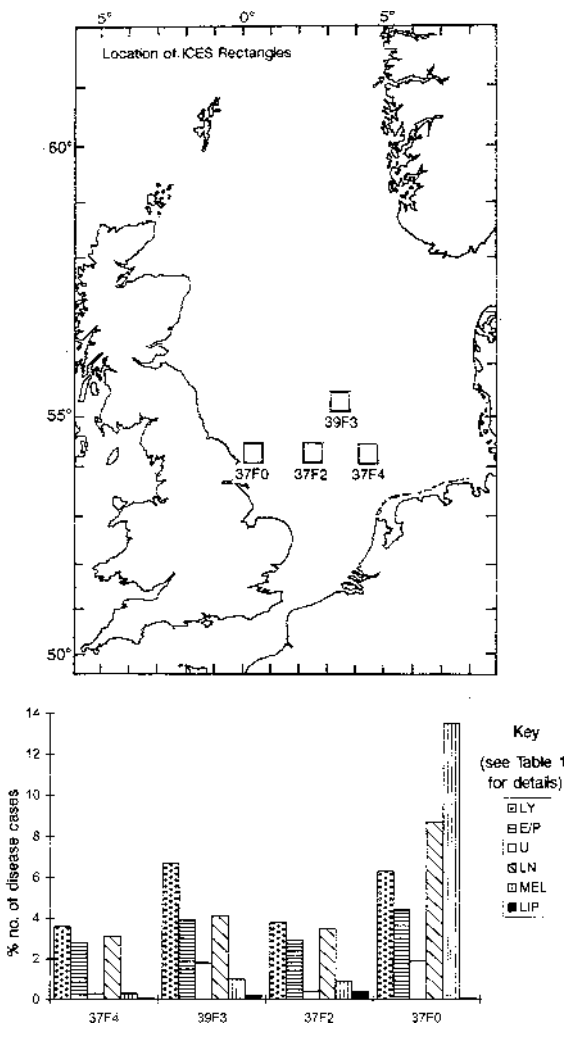

Figure 1. % prevalences of total cases of lymphocystis, epidermal hyperplasia/ papilloma, ulceration, liver adenoma, subcutaneous lipoma and epidermal melanosis in dab sampled from 4 ICES rectangles in the North Sea, RV CORYSTES Cruise 3b, 28 March-5 April 1991

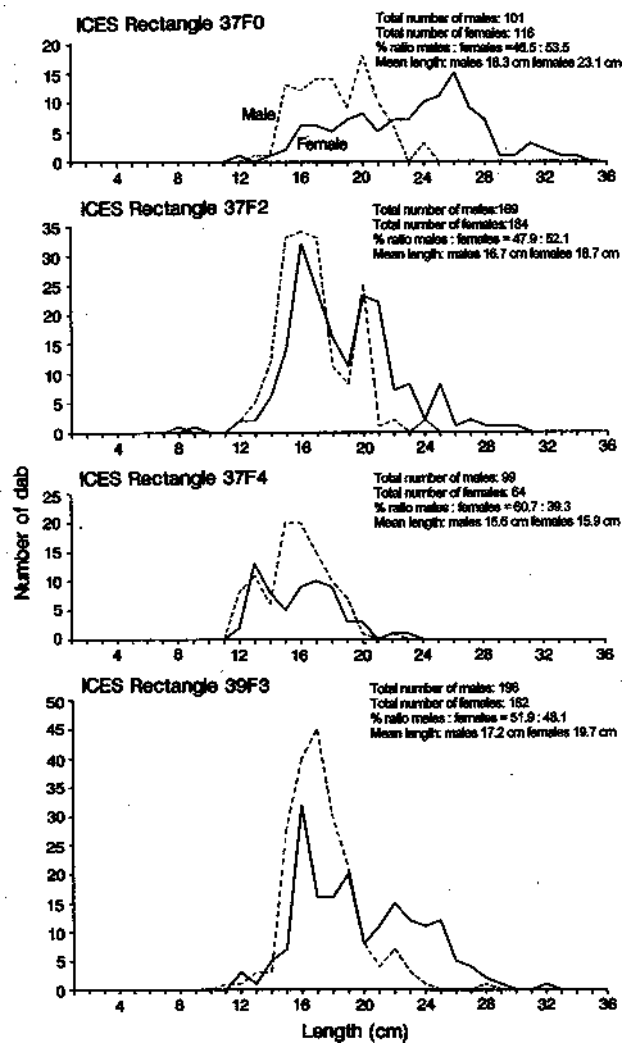

Figure 2. Mean length frequency for dab on stations sampled in 4 ICES rectangles in the North Sea, RV CORYSTES Cruise 3b, 28 March-5 April 1991

Table 2. Histological results of dab liver samples, pooled from hauls within ICES rectangles which included NSTF stations on RV CORYSTES Cruise 3b/91

	ICES Rectangle			
	37F4	39F3	37F2	37F0
No. of livers examined	6	18	22	31
Necrosis	2	0	0	0
Melano macrophage centre +	0	1	1	1
Storage cell change foci	4	3	7	8
Adenoma	2	4	1	8
Adenosarcoma	0	1	2	1
Other *	0	1	2	1

*Key: * other category includes parasite infestation, cysts, and peliosis (haemorrhages)*

+ represents percentage cases of prominent and excessive macrophage centres

Note: only livers showing lesions >2 cm diameter were examined

1.4 RV CIROLANA Cruise 8a, 1-5 October 1991

The specific aim of this short survey was to examine dab for diseases in ICES rectangle 37F0 (Flamborough Off Ground), ICES rectangle 36F0 (Humber Off Ground) and ICES rectangles 38F1 and 37F1 (Western Dogger).

1.4.1 Methods and results

RV CIROLANA Cruise 8a made 8 x 1 h tows with a Granton trawl fitted with tickler chains and a small mesh cod-end liner. Sampling followed the standard procedures detailed above.

Table 3. Catch data and disease prevalence in cod (Gadhus morhua) on stations sampled in the North Sea for fish disease monitoring (1 hour tows using a Granton trawl), RV CORYSTES Cruise 3b/91

ICES Rectangle	Number of hauls	Total catch numbers	Diseases				Remarks
			U	SKD	PBT	CR	
37F4	2	2	0	0	0	0	None
37F0	2	3	0	0	0	0	None
37F2	2	10	0	0	0	0	None
33F2	4	8	0	0	0	0	1 eye missing
35F0	4	98	0	21	0	0	Vertebral compression

Key: U=ulcers; SKD=skeletal deformity; PBT=pseudobranchial tumour; CR=cryptocotyle sp.

Table 4. Catch data and disease prevalence in dab (Limanda limanda) on stations sampled in the North Sea for fish disease monitoring (1 hour tows using a Granton trawl), RV CORYSTES Cruise 8a/91

ICES Rectangle	Number of hauls	Average depth (m)	Total catch (unsexed)		Size range (cm)	Numbers examined		Number of disease cases			
			Total no.	Weight (kg)		Male	Female	LY	E/P	U	LN
36F0	2	46	272	76	15-19	134	66	9	1	3	NR
					20-24	20	25	3	0	0	0
					>25	1	26	1	0	2	6
37F0	2	60.75	388	39.3	15-19	64	136	9	2	2	NR
					20-24	26	141	4	6	3	0
					>25	1	20	2	0	0	4
38F1	2	37.5	259	336.5	15-19	119	63	5	1	3	NR
					20-24	28	37	5	0	2	0
					>25	0	5	1	0	0	1
37F1	2	57.5	288	687	15-19	166	34	13	3	1	NR
					20-24	46	42	2	0	2	3
					>25	0	0	0	0	0	0

Key: LY=Lymphocystis *E/P=Epidermal papilloma*
U=Ulcers *LN=Liver nodules*
NR=Not recorded due to fish size

Table 4 provides information on the areas sampled, numbers of hauls, and the catch and disease data for dab. Assessment of the severity of diseases and categorisation of liver histopathology were not attempted from this survey. Disease prevalence in dab was found to be low on all stations sampled (Figure 3) and is consistent with dab predominantly from the smallest size category (15-19 cm length) (Figure 4). Of particular note were the results of the histological examination of dab livers in which numbers of neoplasms were reduced in samples from ICES rectangle 37F0 (Flamborough Off Ground) compared to the levels in samples taken from this area earlier in the year (RV CORYSTES Cruise 3b/91) (Table 4).

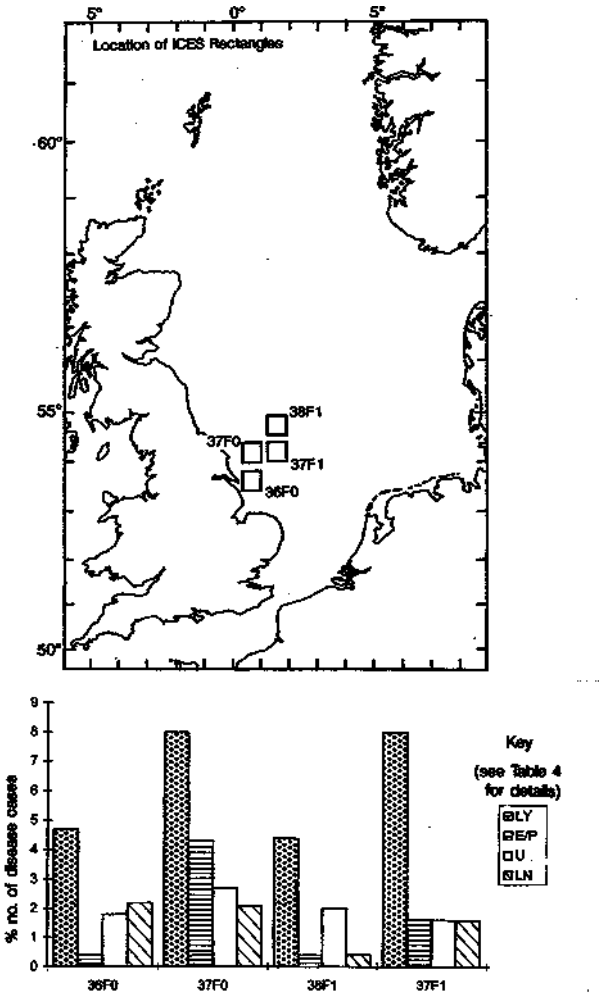

Figure 3. % prevalences of total cases of lymphocystis, epidermal hyperplasia/ papilloma, ulceration and liver adenoma in dab sampled from 4 ICES rectangles in the North Sea, RV CIROLANA Cruise 8a, 1-5 October 1991

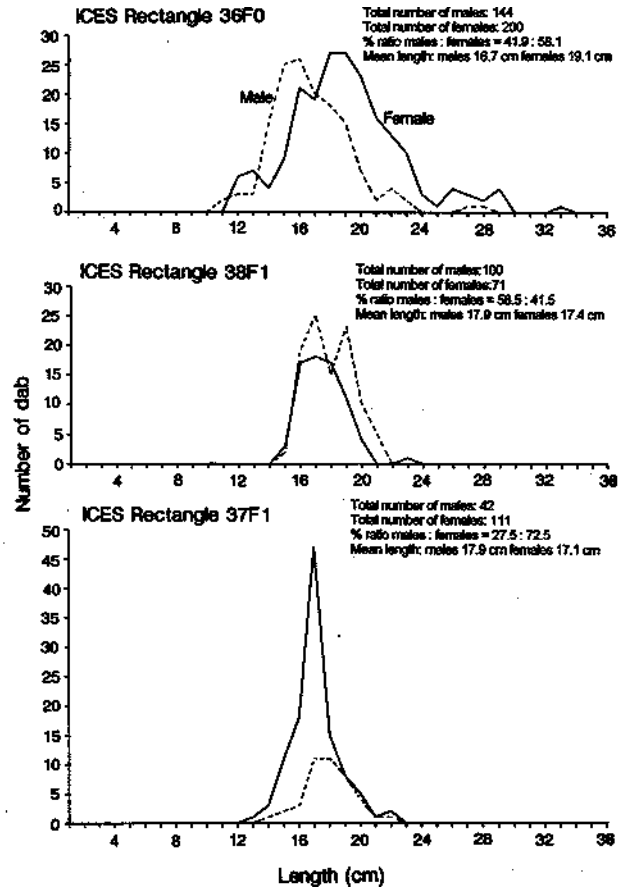

Figure 4. Mean length frequency for dab sampled from 3 ICES rectangles in the North Sea, RV CIROLANA Cruise 8a, 1-5 October 1991

1.5 RV CORYSTES Cruise 10a, 9-14 October 1991

The aim of this survey was to investigate the prevalence of diseases of dab, cod and other commercial fish at stations in the western part of the North Sea.

1.5.1 Methods and results

RV CORYSTES Cruise 10a made a total of 24 x 1 h tows with a Granton trawl, equipped as in the previous studies. Sampling followed the standard procedure. Catches of dab were generally adequate, except for stations in ICES rectangle 33F2 (Smiths Knoll). A total number of 4430 dab were examined. Table 5 details information on sample area, catch and disease data for dab. Generally, diseases were found to be in the low severity categories for all sampling areas (Figure 5). All liver nodules from the samples of the larger size category of female dab were examined histologically. Very few lesions (6%) were diagnosed as neoplasms (Table 6). Figure 6 shows the mean length size frequencies for dab sampled on this survey.

Diseases in cod and other species were present in insufficient numbers for meaningful comparison between areas. For example, 102 cod were caught from only 2 ICES rectangles sampled and only one case of skeletal deformity was found (Table 7).

Table 5. Catch data and disease prevalence in dab (Limanda limanda) by size categories on stations sampled in the North Sea for fish disease monitoring (1 hour tows using a Granton trawl), RV CORYSTES Cruise 10a/91

ICES Rectangle	Number of hauls	Total catch (unsexed)		Size range (cm)	Numbers examined		Number and severity of diseases recorded according to ICES (1989)										
		Total no.	Weight (kg)		Male	Female	LY			E/P			U			LN
							1	2	3	1	2	3	1	2	3	
37F4	6	906	68.3	15-19	314	385	14	1	0	11	0	0	0	0	1	NR
				20-24	92	211	11	1	0	4	0	0	5	0	0	13
				>25	0	15	1	0	0	1	0	0	0	0	1	0
39F3	5	1038	192	15-19	290	290	18	5	0	5	1	0	30	2	16	NR
				20-24	94	365	23	3	0	7	0	0	13	3	38	7
				>25	3	94	1	0	0	0	0	0	3	1	9	13
38F1	5	861	66.5	15-19	300	196	15	1	0	9	0	0	6	6	6	NR
				20-24	80	220	6	3	0	1	1	0	2	2	11	6
				>25	1	50	0	0	0	0	0	0	1	0	4	6
37F0	5	1062	NR	15-19	316	182	12	2	0	1	1	0	4	1	10	NR
				20-24	168	329	15	0	0	8	3	0	9	6	14	4
				>25	3	50	0	0	0	0	0	0	2	0	2	8

Key: LY=Lymphocystis
U=Ulcers
NR=Not recorded due to fish size
E/P=Epidermal papilloma
LN=Liver nodules

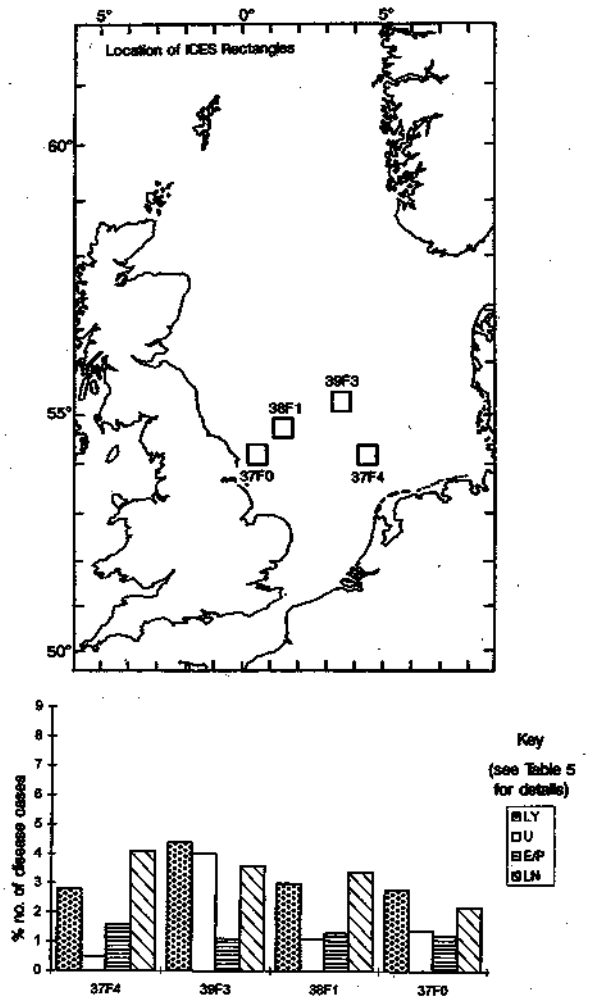

Figure 5. % prevalences of total cases of lymphocystis, epidermal hyperplasia/ papilloma, ulceration and liver adenoma in dab sampled from 4 ICES rectangles in the North Sea, RV CORYSTES Cruise 10a, 9-14 October 1991

Table 6. Histological results of dab liver samples, pooled from hauls within ICES rectangles which included NSTF stations on RV CORYSTES Cruise 10a/91

	ICES Rectangle			
	37F4	39F3	38F1	37F0
No. of livers examined	13	20	12	14
Necrosis	0	0	1	0
Melano macrophage centre +	2	3	0	2
Storage cell change foci	13	6	10	6
Adenoma	3	7	5	2
Adenosarcoma	0	0	0	1
Other*	1	4	2	3

*Key: *other category includes parasite infestation, cysts, and peliosis (haemorrhages)*

+represents percentage cases of prominent and excessive macrophage centres

Note: only livers showing lesions >2 cm diameter were examined

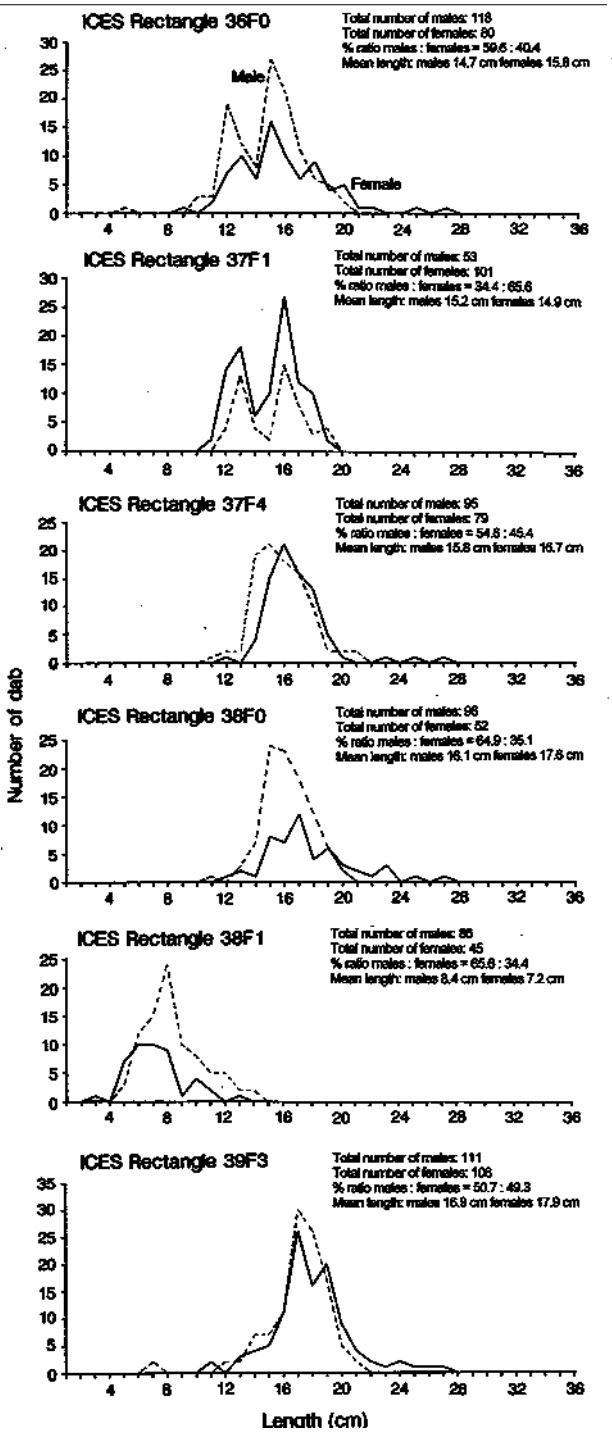

Figure 6. Mean length frequency for dab on stations sampled from 6 ICES rectangles in the North Sea, RV CORYSTES Cruise 10a, 9-14 October 1991

Table 7. Disease data for cod taken on RV CORYSTES Cruise 10a/91

	ICES Rectangle	
	37 F0	34 F2
Number of hauls	1	3
Total number of fish examined	87	15
Average length (cm)	42.3	67.3
Size range (cm)	10-85	39-87
Number of disease cases recorded	1	0
Remarks	SKD	-

Key: SKD=skeletal deformity

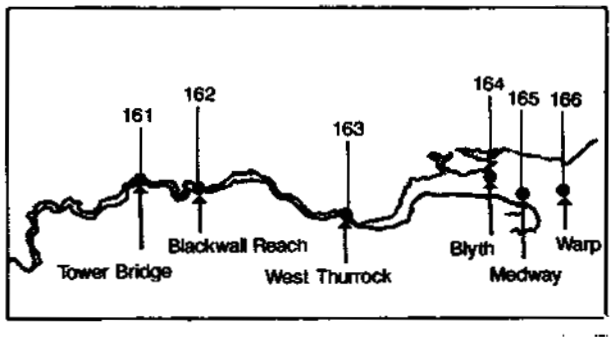

Figure 7. Thames Estuary sampling stations (161-166), MV INA-K, 16-18 July 1991

1.6 MV INA-K, 16-18 July 1991

1.6.1 Methods and results

MV INA-K made 7 x ½ h hauls with 4 m beam trawls on stations in ICES rectangle 32F0 (outer Thames Estuary), and a further 11 hauls with modified 2 m beam trawls on stations upstream to Tower Bridge (Figure 7).

The target fish species was flounder but, as catches of this species were low, all fish species caught were visually examined for gross lesions.

Table 8 details areas fished, catch and disease data. It can be seen that gross lesions were very low in numbers and, on the upstream stations, insufficient fish were caught for any meaningful assessment.

Table 8. Data for fish disease investigation on 6 stations in the tidal part of the River Thames, MV INA-K, 16-18 July 1991

Station	Haul	ICES Rectangle	Site	Total number of fish examined	Species	Diseases			Remarks
						LY	U	Other	
166	3	32FO	Warp	192	Flounder	3	8	1	Fin rot
				51	Dab	0	0	1	E/P
				198	Plaice	0	0	0	
				5	Lemon sole	0	0	0	
				137	Dover sole	0	0	0	
				103	Pout	0	0	22	LE
				19	Pogge	0	0	0	
165	2	32FO	Medway	70	Flounder	1	1	0	
				37	Dover sole	0	0	0	
				73	Pout	0	0	19	LE
				7	Whiting	0	0	6	LE
164	2	32FO	Blyth	12	Flounder	0	2	1	Fin rot
				16	Dover sole	0	1	0	
				6	Pout	0	0	2	LE
				6	Whiting	0	0	3	LE
163	5	32FO	West Thurrock	150	Flounder	0	0	2	Fin rot
				252	Dover sole	0	0	0	
				5	Smelt	0	0	1	Deformity
162	4	32FO	Blackwall Reach	1	Flounder	0	0	0	
				14	Eel	0	0	2	Damaged
161	2	32FO	Tower Bridge	10	Eel	0	0	1	Damaged
				2	Smelt	0	0	0	

Key: LY=Lymphocystis U=epidermal ulceration
E/P=Epidermal papilloma LE= Lerneocera

1.7 Disease reports from MAFF Sea Fisheries District Inspectorate Offices (SFIs) and from other bodies

1.7.1 Results from MAFF SFIs

Since 1979, continual reports have been received about low numbers (1-2 per day) of large cod (³40 cm length) landed at the port of South Shields (north-east coast) exhibiting an epidermal ulcerative condition (Bucke, 1989). Fish with the condition were recorded during the routine random sampling of landed fish at the port by the Sea Fisheries Inspectorate. The affected cod in 1991 were mostly caught from a fairly small area of the North Sea (see MAFF, 1993). As in previous years, its prevalence remains low and is regarded as insignificant in relation to population impact. The cause of this condition remains unknown.

1.7.2 Other marine fish disease monitoring

Diseases in other marine fish reported to FDL Weymouth, during the year have been relatively sparse (20-30). Most of the reports involve fish caught by rod and line angling, and appear to be more numerous during October to December, which is the time when cod populations appear in coastal waters in southern England and anglers' catches increase. Cod examined revealed skeletal deformities, eye defects, skin lesions and nodules on visceral organs. The causes of most of the anomalies could not be diagnosed with any degree of certainty, but some (e.g. the nodules on visceral organs, eye and skin lesions) probably have an infectious aetiology (van Banning, 1987; Bucke, 1989).

STATUTORY MONITORING FOR FISH AND SHELLFISH DISEASE

2. THE MONITORING PROGRAMME FOR THE CONTROL OF DISEASE IN FARMED STOCKS AND ITS TRANSMISSION TO WILD STOCKS

2.1 Introduction

Statutory controls, to prevent the introduction and spread of fish disease in Great Britain, have existed for more than 50 years. These are derived from the Diseases of Fish Act (1937) (Great Britain - Parliament, 1937) as amended by the Diseases of Fish Act (1983) (Great Britain - Parliament, 1983). The controls relate to salmon, trout and other freshwater fish and operate to prevent the introduction and spread of disease. They prohibit the importation of live salmonids into Great Britain, except from Northern Ireland, and imports of salmonid ova are permitted only under licence with supporting health certification. Similar licensing controls also apply to the importation of other types of freshwater fish and their ova which are likely to be released into the wild. The Acts also give the regulatory authorities powers to control the spread of serious 'notifiable' diseases. The following diseases are the only ones which at present come into this category:

viral haemorrhagic septicaemia (VHS);
infectious haematopoietic necrosis (IHN);
infectious salmon anaemia (ISA);
gyrodactyliasis caused by *Gyrodactylus salaris;*
bacterial kidney disease (BKD);
infectious pancreatic necrosis (IPN);
whirling disease (WD);
enteric redmouth (ERM) (Scotland only);
furunculosis in salmon; and
spring viraemia of carp (SVC).

Apart from SVC, these are diseases of trout and salmon and the effects are much more pronounced under intensive farming conditions. The viral diseases VHS and IHN are by far the most serious of the diseases on this list but both of them are absent from British waters as are gyrodactyliasis and ISA.

Marine shellfish are regulated under the Sea Fisheries (Shellfish) Act (1967) (Great Britain - Parliament, 1967). The Act controls the depositing of molluscan shellfish and lobsters in coastal waters. The mollusc controls operate on shellfish of UK or imported origin and cover the bivalve pests, Mytilicola, Crepidula and American tingle and a range of diseases such as *Bonamia*, *Marteilia* and Haplosporidia. Controls on

lobsters were specifically designed to prevent the spread of Gaffkaemia. This is a bacterial septicaemic condition caused by *Aerococcus viridans*, commonly imported in North American lobsters and which may be transmitted to our native stocks.

FDL has responsibility for fulfilling MAFF's statutory inspection and diagnostic duties in England and Wales under these Acts. Inspectors visit farm, river and large open water sites to examine fish or shellfish where notifiable or other serious diseases are suspected and samples are taken for laboratory testing. This inspection, together with programmes for monitoring certain other serious diseases, provides for an assessment of the incidence, prevalence and significance of diseases of fish and shellfish generally in England and Wales. This information provides a scientific basis, *inter alia*, for governmental policies and the application of statutory measures for such diseases.

When notifiable diseases have been confirmed by testing, or are suspected, an Inspector has the powers to enter sites and prohibit or regulate the movement of live fish, ova and foodstuffs into and out of the designated infected area and to regulate the removal and disposal of dead and dying fish. Inspection and laboratory testing of cultivated and wild stocks of fish and shellfish are also undertaken for health certification to facilitate exports.

Since 1985, under the Registration of Fish Farming and Shellfish Farming Businesses Order (1985) (Great Britain - Parliament, 1985(b)), it has been a legal requirement that all fish and shellfish farming businesses in England and Wales register with MAFF in order to assist efforts to prevent the spread of disease. There are currently some 710 registered fish farms and 258 registered shellfish farms. Inspectors are charged with checking the registration details and movement records which must be kept by such registered businesses.

From 1 January 1993, the single European market became operative and trade barriers for live fish and shellfish, as well as other animals, will be removed. However, EC Directive 91/67 concerning the animal health conditions governing the placing on the market of aquaculture animals and products (European Communities, 1991(a)) has been agreed and this should safeguard the health status of zones free of certain diseases. Following outbreaks of IHN in France and Italy in 1987 (and latterly in Belgium and Germany), an EC Decision 90/495 (European Communities, 1990) was agreed requiring Member States to undertake a VHS/IHN survey to establish the distribution of these diseases in the Community. In the UK, a programme of testing all drainage areas with salmonid farms was agreed by the Commission; in England and Wales this required testing of 52 individual sites (see Figure 8).

2.2 Materials and methods

2.2.1 Sampling methods

The way in which samples are selected is important, whether the samples are being taken for monitoring or for investigating the cause of outbreaks of clinical disease. Prior to sampling, an assessment of the site and husbandry conditions which prevail may be needed, as well as an inspection of the fish or shellfish present. For monitoring purposes, sites are generally sampled to a statistical standard which provides for a 95% confidence level of detecting a 2% incidence of disease (Ossiander and Wedemeyer, 1973). This means that, in practice, 150 animals are sampled on site from across the population. With 'wild' populations, which are at lower densities and where sample size is limited, sampling to give a 95% confidence level of detecting disease at the 10% level is acceptable (Ossiander and Wedemeyer, 1973). Where overt clinical disease is manifested, sampling will normally be limited to 5-10 affected animals.

Because of the long history of freedom from IHN and VHS in the UK, the testing undertaken to monitor for these diseases in the survey under EC Decision 90/495 was limited to a 30 fish sample from each farm site tested.

2.2.2 Laboratory tests

***(a) Virology*:** Testing of fish for viruses depends primarily on the isolation of the virus through passage in tissue culture, but an enzyme-linked immunosorbent assay (ELISA) for early identification of viral antigen is used in cases of clinical disease. For isolation of disease organisms, viscera (liver, kidney, spleen and pyloric caeca for salmonids; liver, spleen, kidney and brain for cyprinids) are pooled from 5 fish and homogenised with sterile sand and balanced salt solution (Hank's or Earl's) using a pestle and mortar. To eliminate contamination, the homogenates are diluted with a high-level balanced salt solution (containing the antibiotics penicillin at 1000 i.u. ml^{-1}, streptomycin at 1000 mg ml^{-1} and mycostatin at 50 i.u. ml^{-1}). Cell cultures are inoculated with extracts prepared from pooled homogenates and incubated for one week before a repeat passage and a further week's incubation. The type of cell culture used is dictated by the virus under investigation. Passage of a virus through a tissue culture is normally accompanied by cytopathological effects (CPE) and confirmation of the identity of a virus causing CPE is achieved by means of a serum neutralisation test or ELISA (Way and Dixon, 1988).

In drawing up the protocols for virological testing to be undertaken throughout the European Community (EC)

from 1 January 1993, a common methodology was agreed under EC Decision 92/532 (European Communities, 1992) and this will be used for all future testing undertaken to meet the requirements of EC Directive 91/67. This same methodology was used in the European Community survey testing programme conducted in 1991.

(b) Bacteriology: In testing for *Renibacterium salmoninarum*, the causative agent of BKD, sterile swabs are used to take fresh kidney samples which are plated onto specific kidney disease medium (Austin *et al*., 1983). Plates are incubated at 15°C for up to 6 weeks and examined weekly. Confirmatory tests for the organism include an immuno-fluorescent antibody test (Bullock and Stuckey, 1975), the API enzyme test (Austin *et al*., 1983), and the co-agglutination test (Dixon, 1987).

Tests for *Aerococcus viridans*, the causative agent of gaffkaemia, are based on isolation of the organism from haemolymph samples in presumptive gaffkaemia broth or directly on brain-heart infusion and blood agar plates following incubation at 28°C for 3-5 days (Stewart *et al*., 1966). Confirmation of any presumptive gaffkaemia organisms which exhibit the typical tetrad form, are Gram-negative, and catalase-negative is obtained using the co-agglutination test.

***(c) Parasitology*:** Tests for WD are based on the examination of Giemsa-stained histological sections of cranial cartilage and bone for evidence of spores of *Myxobolus cerebralis*, the causative agent.

Tests for the oyster parasite *Bonamia ostreae* also rely on histological examination. A 5 mm steak through the gills and digestive tract is fixed in Davidson's seawater fixative (Shaw and Battle, 1957). Sections are stained by Gomori's one-step trichrome method (Drury and Wallington, 1973).

(d) Crayfish plague: The tests used for the identification of *Aphanomyces astaci* are those described by Alderman and Polglase (1986).

2.3 Statutory field investigations for fish diseases

The fish disease testing in 1991 included:

(a) tests on fish from sites where notifiable or serious disease was suspected;
(b) the annual monitoring of trout hatcheries and salmon sites for IPN;
(c) re-tests on sites already designated for SVC and BKD; and
(d) tests for IHN and VHS on salmonids from farms in each drainage area with salmonid farms.

2.3.1 Investigations into the cause of disease outbreaks in fish

In 1991, 105 cases of fish mortality were investigated, including 4 where a notifiable disease was diagnosed. BKD was confirmed on a rainbow trout farm, IPN in a trout hatchery, and SVC in 2 coarse fisheries. A further 71 cases were investigated where SVC was suspected, but virological tests proved negative. Two cases of ERM and 5 cases involving bacterial septicaemia were also diagnosed, and trout fry anaemia was confirmed in one trout hatchery. Water shortages during the year exacerbated problems caused by parasite infestations and proliferative kidney disease (PKD).

2.3.2 Investigations to monitor for specific diseases of fish

***(a) Infectious pancreatic necrosis*:** An annual programme of monitoring of trout hatcheries and salmon sites for IPN has operated following a relaxation in policy in 1984, concerning the control of fish movements from sites designated for this disease. All registered sites holding salmon are now tested annually, along with a selection of trout hatchery sites. In 1991, monitoring was undertaken at 6 rainbow trout hatcheries and at all 15 registered sites holding salmon; all the tests on the salmon sites proved negative, but tests on fish from 2 of the rainbow trout hatcheries confirmed the presence of IPN and the sites were designated.

***(b) Whirling disease*:** Monitoring for WD was also instigated following the 1984 policy changes. However, following provisional agreement within the EC that WD is not of sufficient seriousness to warrant movement controls, no monitoring for this disease was undertaken in 1991 other than on sites with a specific requirement to facilitate exports to North America.

2.3.3 EC survey for IHN and VHS

Some 52 farm sites, of which 21 held broodstock, representing individual drainage areas as shown in Figure 8 were visited as part of the EC survey for IHN and VHS. Virological tests on all of the samples proved negative, confirming the absence of these diseases from England and Wales.

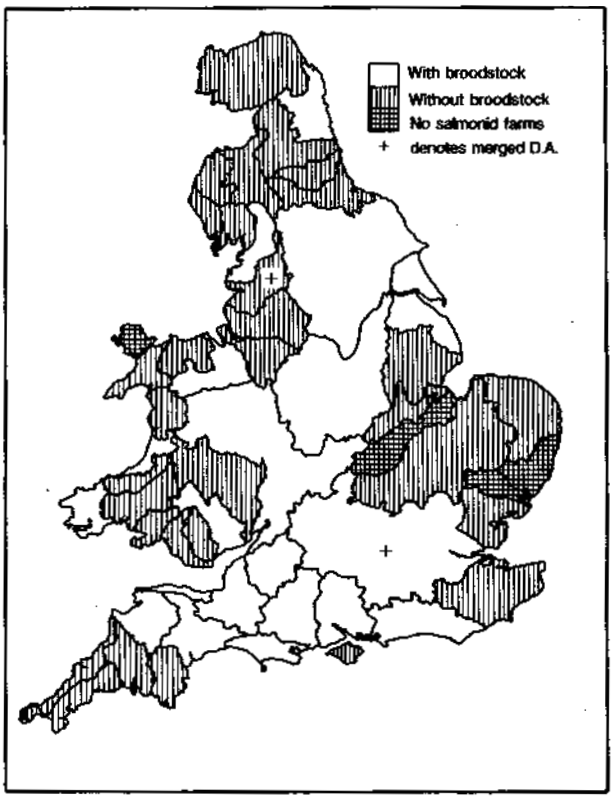

Figure 8. Drainage areas in England and Wales sampled in IHN/VHS survey

2.3.4 Re-tests for specific diseases of fish on designated sites

***(a) Bacterial kidney disease*:** Sites where BKD is confirmed are designated as diseased and controls are imposed on movement of stock. Re-tests in 1991 on 2 BKD-designated sites continued to give negative results, and on the basis of these findings their designated status was revoked. A third designated site remained non-operational and could not be tested. Following the first confirmation of BKD on a fourth site, it was designated and checks on contacts resulted in the disease being confirmed on a cage farm site in the same area, which was subsequently designated along with the surrounding lake. Tests on a further 6 contact sites proved negative.

***(b) Spring viraemia of carp*:** Following outbreaks of SVC in 1988 and early 1989, some 40 sites were found to be infected and had restrictions imposed on movement of stock. Eighteen undertook clearance and/or disinfection, and by the end of 1990, 22 sites remained designated for SVC. These were generally large open waters, used as fisheries, where clearance and disinfection was not possible. During 1991, a major final check programme, based on negative testing and the use of sentinel fish, was successfully completed on those sites which had also undergone testing over a 3-year period and, as a result, the Designated Area Orders were removed.

A total of 82 suspected cases of SVC were also investigated during this period. From this, 2 new fishery sites were found to be infected and were designated, but none of the 9 contact sites for these proved positive.

2.4 Diseases and anomalies reported in wild freshwater fish

Reports of barbel (*Barbus barbus*) with a number of lesions, including raised skin nodules, ocular blindness and bifurcation of barbules, have been described by the press during the year. Rivers where fish with the abnormalities occur include the River Lea (a tributary of the River Thames) and River Avon (Hampshire). Most reports refer to the larger (3-4 kg) fish being affected. A preliminary examination of a small number of samples of skin lesions on these fish presented to the laboratory for histological examination showed that they had the characteristics of either epidermal hyperplasia or papilloma (Barnes *et al*., 1993). These lesions are common benign conditions in certain species of both marine and freshwater fish, and have been described for many years, although their aetiology has not been resolved and, as with other diseases in wild fish, will continue to be monitored.

2.5 Statutory field investigations into diseases of shellfish under the Sea Fisheries (Shellfish) Act, 1967

Under this Act, the coast of England and Wales is divided into 27 'Control of Deposit' areas, as shown in Figure 9, and transfer of molluscan shellfish into and between these areas for deposit is controlled by licensing. Shellfish testing, under this Act, includes examinations of shellfish stocks where serious disease is suspected and the tracing of contacts when disease has been confirmed. Annual monitoring of *Ostrea edulis* stocks for *Bonamia* is also undertaken in coastal areas with a past history of disease, to establish the levels of infection and the implications for both wild and farmed stocks and to permit control of its spread by movement, licensing movement of stock. Details of the impact and spread of *Bonamia* in the UK has recently been published (Hudson and Hill, 1991; MAFF, 1993).

2.5.1 Investigations to monitor for specific diseases of shellfish

Because of the development of the new duties arising from EC Directive 91/67 on fish and shellfish disease

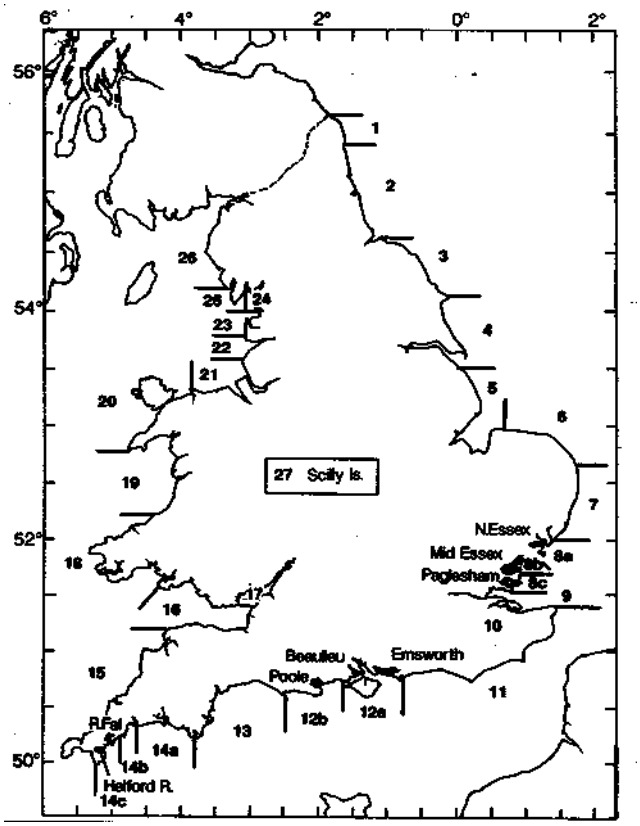

Figure 9. Control of Deposit areas and Bonamia ostreae-infected sites in England and Wales

(European Communities, 1991(a)), and EC Directive 91/492 on shellfish hygiene (European Communities, 1991(b)), staff time available for *Bonamia* monitoring was limited in 1991. Most effort was invested in testing hatchery suppliers and shellfish growing areas adjacent to known infected areas monitoring for disease spread. A total of 1471 *Ostrea edulis* making up 13 sample groups were tested in 1991 and details of the results are given in Table 9. No additional locations were found to be infected during this testing. Testing undertaken included samples from a hatchery on the north Kent coast and the several fisheries at Chilling, Stanswood and Calshot in the Solent, which are all important sources of stock for on-growing. Eight further samples from the Solent, which were also negative, included 3 taken from the Lepe grounds adjacent to the Beaulieu infected area. Samples were also taken from wild stocks in Poole Harbour adjacent to known infected areas, to establish the rate of spread and intensification in these stocks.

2.5.2 Test for Gaffkaemia

One American lobster (*Homarus americanus*) was caught from the Skerries Bank, South Devon, but tests for Gaffkaemia proved negative. Following a Gaffkaemia outbreak in a lobster-holding site in 1990, a sample of European lobsters caught in the adjacent coastal area in North Wales was tested, but no evidence of Gaffkaemia infection was found. A survey of current lobster storage sites has been undertaken and 6 new sites, which should have had individual licences, have been identified. A comprehensive list of all lobster storage facilities is being prepared and 90 have been identified to date.

2.5.3 Tests for crayfish plague

During 1991 crayfish mortalities occurred in 5 new catchments. Crayfish plague has been confirmed in samples from Wycombe Dyke (High Wycombe), and the River Clun (Teme, Severn catchment). These represent major extensions to the disease's distribution in England and Wales.

Table 9. Bonamia testing during 1991

Area		Site	Number of oysters	Results				
				Number positive	Number negative	Av. infection rate (%)	min. %	max. %
9	Whitstable	1 commercial site	50	0	50	0	0	0
12a	Solent	Calshot	150	0	150	0	0	0
		Chilling	320	0	320	0	0	0
		Stanswood	150	0	150	0	0	0
		Osborne	150	0	150	0	0	0
		Pennington	150	0	150	0	0	0
		Newtown	150	0	150	0	0	0
		Ryde	150	0	150	0	0	0
		Sowley Boom	150	0	150	0	0	0
12b	Poole	Holes Bay	4	0	4	0	0	0
		South deep	35	3	32	8.5	8.5	8.5
		Plot AA	12	0	12	0	0	0

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