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Results of the 1971 monitoring programme for mussel toxicity on the north-east coast of Britain

by

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INTRODUCTION

Following an outbreak of paralytic shellfish poisoning associated with mussels from the north-east coast of Britain in the summer of 1968 (McCollum et al. 1968, Wood and Mason 1968, Wood 1968) an annual monitoring programme was established, with special emphasis on the area of the 1968 outbreak. Results of this programme in 1969 and 1970 (Wood 1969, Wood and Ayres 1970) suggested that development of toxicity in the north-east might be an annual feature, only occasionally leading to clinical manifestations. Investigations in 1970 confirmed earlier suggestions that major concentrations of dinoflagellates were confined to offshore regions and that littoral molluscs were probably influenced only by the margin of this offshore population. The presence of low concentrations of dinoflagellates, particularly Gonyaulax tamarensis, suggested that toxicity of littoral molluscs resulted from movement of offshore water towards the coast. Because of the inherent difficulties in correlating toxicity of littoral mussels with phytoplankton observations in the near and offshore waters, the pattern of sampling for the current year has been modified from that used previously. As in previous years, samples of littoral mussels have been examined at intervals for toxicity, but, to establish the presence of phytoplankton organisms, water samples were abandoned in favour of direct examination of mussel gut contents in an attempt to find a more direct correlation between toxicity and phytoplankton content of the shellfish. This report summarizes the results of this programme and attempts a preliminary evaluation of the data collected.

The cooperation of the North-Eastern Sea Fisheries Committee, the Ministry's District Inspectors and others, in the collection of material, is gratefully acknowledged.

METHODS

(i) Determination of toxicity of shellfish

Arrangements were made, early in the year, for provision of samples at approximately weekly intervals from stations along 240 km of the north-east coast, an area stretching from Berwick in the north to Bridlington in the south. Since examination of the gut contents was to be a significant part of the investigation the practice of sampling tissues, removed from the shell at source, was discontinued, and samples of 12-18 mussels in the shell, packed in watertight containers and sent for analysis, were taken instead. In this way samples were usually received in a fresh condition at the laboratory the following day. Toxin was assayed by intra-peritoneal injection of acid extracts into female mice - 18 to 20 g body weight (McFarren, 1959). The concentration of toxin was expressed as mouse units/100 g of tissue, 400 m.u/100 g being regarded as the maximum safe concentration. Sampling commenced in March and continued until the end of August, pending any reappearance of toxicity.

(ii) Examination of gut contents

Six mussels were taken at random from each sample and opened carefully with a scalpel. The hind gut and stomach were dissected out separately and the contents expressed into a clean petri dish. The gut contents of six mussels were pooled together and mixed with 0.04 ml of sea water, previously filtered through a 0.4μm filter, and 2 drops of this mixture from a calibrated Pasteur pipette (total volume = 0.04 ml) were removed on to a slide for subsequent microscopical examination. Using a Zeiss photomicroscope, a preliminary examination under the x40 objective was made to identify the dominant genera of phytoplankton present in the samples. Where possible, identification of dinoflagellates was continued to species level. Once the genera and species had been established the sample was scanned under the x10 objective for 5 minutes, and counts were made of the organisms present. This did not yield a strictly quantitative assessment of the numbers and types present, but the examinations were made by one person under standard conditions and so enabled comparative assessments of types and abundance to be made between one sample and another. Although the extent of loss of the more fragile forms is difficult to assess, the variety of intact and identifiable cells found in the gut analysis suggests that this is an effective technique for further investigations.

RESULTS

(i) Toxicity of mussels

At the time of writing (middle of August) a total of 81 samples of mussels from the north-east coast have been examined and the results of the bioassay are summarized in Table 1. Sampling at Holy Island, the locus of the 1968 outbreak, commenced in March but toxicity was not detected until towards the end of May, when a sample contained 226 units/100 g. A similar level of toxicity was detected at this time in a sample of mussels from Berwick, 25 km north of Holy Island. A week later, toxicity at Holy Island had declined to 197 units/100 g and a new locus appeared at Whitby, 150 km to the south. The toxin level in Whitby mussels then reached 488 units and up to the present time this has not been exceeded at any sampling station; however, within two weeks toxin had declined to an undetectable level. The absence of toxicity in samples between Holy Island and Whitby, where the two loci appeared, is a phenomenon not observed in the sampling of previous years. Apart from these four samples, no further toxicity was detected until mid-July when another locus appeared at Hartlepool, 45 km north of Whitby. A toxicity of 454 units/100g had fallen to 415 units by the end of July, but no further samples have been examined from this station. During the current year's experiments, close observation of mice after injection revealed that mild paralytic responses (excitability, muscle twitches, gaping reflex), which although not fatal were indicative of sub-lethal toxin concentrations, gave advanced warning of increasing toxicity. It can be seen from Table 1 that this type of response occurred with samples from a number of stations at the same time, particularly from the Holy Island area. This suggests that small numbers of toxic organisms were widely distributed along the north-east coast during April and May, reaching concentrations causing measurable toxicity towards the end of May.

(ii) Examination of gut contents

The results of the phytoplankton examination are presented in Table 2, and levels of toxin observed in the mussels examined are included for comparison with the organisms present. No obvious correlation between toxicity and any one species or genera of dinoflagellate or diatom is evident, but a number of interesting points have emerged. It is likely that the majority of Gonyaulax recorded as Gonyaulax sp. were in fact G. tamarensis, but these have only been recorded as such when a positive identification was possible. If one assumes that the organism occurring

in the greatest number is the most significant, the importance of G. tamarensis observed in past years is not apparent in the current investigation. The appearance of toxicity at Holy Island was marked by the presence of increasing numbers of unidentified dinoflagellate cysts, which declined as toxin levels became undetectable. However, since Gonyaulax sp. also appeared at this time it is possible that the cysts observed were of this genus. It is perhaps significant that cysts were present with a greater frequency at all stations than any other organism observed, though the number recorded at Holy Island when toxicity appeared has not been exceeded elsewhere. However, at Berwick Gonyaulax sp. were dominant when a toxin level of 216 units/100 g was recorded, and this level was similarly not exceeded elsewhere. The toxicity of mussels at Whitby (the highest level recorded this year) could not be related to phytoplankton abundance, which was particularly low in samples from this station. At the remaining station yielding toxic samples, Hartlepool, samples showed some correlation between toxin and high numbers of Prorocentrum micans, 12 days prior to the detection of toxin. Two weeks later P. micans had virtually disappeared and toxicity had also declined. The Exuviaella sp. observed periodically, particularly in the Holy Island-Budle Bay area, were identified as Exuviaella baltica, but were replaced at the beginning of August by E. marina, when a count of 132 units (the highest count of any single species or genera recorded to date) was made in a sample from Holy Island. It is perhaps typical of the observations made this year that by the following week this species had been replaced by similar numbers of another dinoflagellate, Dinophysis acuta.

DISCUSSION

With the appearance of toxicity at the end of May, this year's observations promised to be similar to those made annually since 1968. However, toxicity did not increase, although three separate loci in turn appeared and declined to undetectable levels. In 1969-1970 toxicity developed in April-May, reaching a maximum in mid-June and declining during July. This year some factor or combination of factors prevented the increase in toxicity seen in previous years. Instead, three distinct areas of toxicity appeared (two in late May at Berwick/Holy Island and Whitby, and one in mid-July at Hartlepool), but at no time did levels of toxin reach those observed previously. No single species of dinoflagellate dominated the plankton, but sporadic outbursts of various species occurred

and supported the view that a considerable deviation from previous years (i.e. 1968-1970) had occurred. Climatically the month of June, when peak toxicity was expected to occur, was noted as being exceptionally wet and cold, and the daily meteorological records from Tynemouth (midway between Holy Island and Whitby) confirm this. Table 3 summarizes the June air temperature, sunshine and rainfall data for the years 1967-71 inclusive. The mean maximum air temperature for June 1971 was 2.7°C below the mean average for this station, and the lowest maximum and minimum temperatures over the period 1967-1971 for the month of June also occurred in 1971. Sunshine in June was only 78 per cent of the average for that month since records were started in 1937, and amounted to 56 hours less for the month than the previous lowest value for June during the period 1967-1971. Rain fell on more days than in any June since 1967, and the total was 136 per cent of the average for June observations at this station (since records started in 1864). Therefore it would appear that June 1971 was abnormal in having low air temperatures, low sunshine and high rainfall - conditions which could account in part for the sudden disappearance of toxicity at a time when it was expected to be increasing rapidly. The scattered outbursts of dinoflagellates which occurred, and the relative abundance of cyst forms, would support the apparent disruption of an established development sequence of phytoplankton off the north-east coast.

No unusual biological events associated with toxic phytoplankton were reported in the area, apart from two reports of discoloured water. The first, off Seahouses and Beadnell, coincided with the first toxic sample from Holy Island, but no material could be obtained for examination. A second bloom, reported from the Holy Island area at the beginning of June, revealed an almost pure culture of a pink pigmented copepod. The maximum levels of toxicity reached during 1971 (454-488 units) were much lower than those observed in 1970 (2100-4100 units) and 1969 (3800-6000 units) and barely significant compared with the 1968 values (20.000-27.000 units). No toxicity was observed at Blyth, where peak values had been obtained in previous years, but this is biased by the absence of many samples from this station.

From the data presented here it seems likely that the climatic conditions are the main factor influencing the development of toxicity. There would appear to be a need to extend these observations and to relate them to optimal conditions for growth of potentially toxic phytoplankton, as determined from field and/or laboratory experiments.

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Table 1 Levels of toxin in mussels, March to August 1971 (mouse units/100 g of tissue)

Station*	Week ending																						
	Mar		Apr					May				Jun				Jul					Aug	
	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25	2	9	16	23	30	6	13
Holy Island		-	-	-		+	-	-	+	266	197	-	-	-	+	-	-		-	-	-	+
Berwick		-			+	-	+	-	-	216			-					-		-		
Budle Bay	-	-			+		+	-	+	-			-					-		-		
Blyth											-		-									
Sunderland									+													
Hartlepool										-	-			-		-		454		415		
Redcar							-		-						+		-		-		-	
Whitby					-	-			-		488		-		Lost		-				+	
Bridlington	-	-	-	-			-	-	-	-	-			-		-		-		-		-

- negative sample; + non-fatal positive response

*For details of position, see earlier reports to ICES Shellfish and Benthos Committee (Wood and Mason 1968, Wood 1969, Wood and Ayres 1970)

Table 2 continued

Locality	Date 1971	Diatomaceae								Dinophyceae												Cysts			Others			Toxicity*
		Coscinodiscus sp.	Biddulphia sp.	Melosira sp.	Navicula sp.	Chaetoceros sp.	Skeletonema sp.	Nitzschia sp.	Rhizosolenia sp.	Pleurosigma sp.	Gonyaulax sp.	Gonyaulax tamarensis	Peridinium sp.	P. depressum	P. trochoideum	P. granii	Gymnodinium (large)	Gymnodinium (small)	Polykrikos schwarzii	Dinophysis acuta	Prorocentrum micans	Exuviaella sp.	Unidentified	Gonyaulax spp.	Gymnodinium lunula	Peridinium depressum	Other spp.	Favella sp.	Minute flagellates	Radiolaria	
Bridlington shore	23 Mar		✓						✓												✓						✓			
	31 Mar		2		3																						2			
	6 Apr		5	1	4			5																			19		✓	✓	
	22 Apr																						6							Poor sample
	5 May				4			3																			6				
	12 May				4					1	10					5											7			2	
	18 May																				1						14			2	
	25 May	5	3		8					1	1			13		7							2				5				
	15 Jun				2																3						3				
	28 Jun																				3					1	11				
	12 Jul														8	7															
	27 Jul		1		2												1										2				
	10 Aug				1						6										4		32				7				

✓ species observed but not counted

* poor sample - not received the day after collection

Table 2 continued

Locality	Date 1971	Diatomaceae									Dinophyceae											Cysts				Others			Toxicity*
		Cosinodiscus sp.	Biddulphia sp.	Melosira sp.	Navicula sp.	Chaetoceros sp.	Skeletonema sp.	Nitzschia sp.	Rhizosolenia sp.	Pleurosigma sp.	Gonyaulax sp.	Gonyaulax tamarensis	Peridinium sp.	P. depressum	P. trochoideum	P. granii	Gymnodinium (large)	Gymnodinium (small)	Polykrikos schwarzii	Dinophysis acuta	Prorocentrum micans	Exuviaella sp.	Unidentified	Gonyaulax sp.	Gymnodinium lunula	Peridinium depressum	Other spp.	Favella sp.	Minute flagellates	Radiolaria	
Hartlepool shore (continued)	14 Jun		1							1						13			1							5				
	30 Jun			1	1					1											75						1				
	12 Jul				1																3									454 m.u. toxin
	26 Jul													2							2									415 m.u. toxin
Redcar shore	29 Apr		3		8																	2			1		5		✓		
	13 May		2		13						6																4		✓		
	23 Jun																														Poor sample
	7 Jul			1	1						2						1				2						19				
	6 Aug		2							1	1			4													6				
Whitby shore	13 Apr				1	5																3					1		✓		
	23 Apr																					4					1				
	10 May					2																									
	24 May				1																1									488 m.u. toxin	
	7 Jun				1												4										8				
	7 Jul				2						4																				
	6 Aug				1									3	5															Weak +ve	

* Toxicity: m.u. toxin = mouse unit toxin; +ve = positive; ✓ = present

Table 2 Phytoplankton analysis of mussel gut contents (numbers seen in 0.04 ml)

Locality	Date 1971	Diatomaceae									Dinophyceae											Cysts				Others			Toxicity*
		Coscinodiscus sp.	Biddulphia sp.	Melosira sp.	Navicula sp.	Chaetoceros sp.	Skeletonema sp.	Nitzschia sp.	Rhizosolenia sp.	Pleurosigma sp.	Gonyaulax sp.	Gonyaulax tamarensis	Peridinium sp.	P. depressum	P. trochoideum	P. granii	Gymnodinium (large)	Gymnodinium (small)	Polykrikos schwarzii	Dinophysis acuta	Prorocentrum micans	Exuviaella sp.	Unidentified	Gonyaulax sp.	Gymnodinium lunula	Peridinium depressum	Other spp.	Favella sp.	Minute flagellates	Radiolaria	
Holy Island shore Oyster scarp	23 Mar		✓		✓			✓	✓						✓						✓		✓			✓	✓		✓	
	27 Mar		✓																							✓				Poor sample
	6 Apr		1	2	3			3						2							1		1							
	13 Apr		1		1																					3				
	19 Apr			2	3			4															40				2				Weak +ve
	26 Apr																														Poor sample
	5 May		3		6																2		3				5				
	11 May		1		3	7					5		4								3				1	1		✓	1		Weak +ve
	18 May		5		3	4								4												30			1		226 m.u. toxin
	25 May		3		2			1			7									4					1	8					197 m.u. toxin
	1 Jun				3	5			1		4										4						11				
	8 Jun		2	4							4		1									9				—	5		✓		
	15 Jun		3	5	1								3									4			1						
	21 Jun		1	2	2																						5				Weak +ve
	29 Jun																					3					17				
	6 Jul													1																	
	26 Jul											9									6		35								
	3 Aug													2							2	132									
	10 Aug			2								14		4	8				1		123		30								Weak +ve
Berwick shore	14 Apr		11		7			4					2	2		—							2				4		2		
	23 Apr		4		12																		7				6				Weak +ve
	28 Apr		5		12									1		3					3	5				1	20				

Table 2 continued

Locality	Date 1971	Diatomaceae									Dinophyceae												Cysts				Others			Toxicity*
		Coscinodiscus sp.	Biddulphia sp.	Melosira sp.	Navicula sp.	Chaetoceros sp.	Skeletonema sp.	Nitzschia sp.	Rhizosolenia sp.	Pleurosigma sp.	Gonyaulax sp.	Gonyaulax tamarensis	Peridinium sp.	P. depressum	P. trochoideum	P. granii	Gymnodinium (large)	Gymnodinium (small)	Polykrikos schwarzii	Dinophysis acuta	Prorocentrum micans	Exuviaella sp.	Unidentified	Gonyaulax sp.	Gymnodinium lumula	Peridinium depressum	Other spp.	Favella sp.	Minute flagellates	Radiolaria	
Berwick shore (continued)	7 May				6																2					1					Weak +ve
	12 May				6	1	1				9								8			4					1			1	
	21 May				5						18										2	7					3			5	216 m.u. toxin
	10 Jun		1							1																	2				Desmids present
	14 Jul		3		3									3									5								
Budle Bay shore	14 Apr		7		16																						3				Weak +ve
	23 Apr				5																						4				Weak +ve
	28 Apr				5																			1							
	7 May																														
	12 May				4						13											6								2	Weak +ve
	21 May				1	2					1			7							4	10					7			6	
	10 Jun			12							5											17					3				
	14 Jul										11								1			5	10				14				
Blyth shore	24 May	2		2	6						1																				
	10 Jun					4							1			1															
Sunderland shore	10 May	1		1	1	8							9										28								Weak +ve
Hartlepool shore	17 May				5						1												5								
	24 May	3		2			2							1							2		4				3			2	

Table 3 Weather records from Tynemouth for June 1967 to 1971

	1967	1968	1969	1970	1971
Air temperature					
Mean maximum (°C)	15.3	15.7	15.1	15.5	13.0
Difference from average	-0.4	0.0	-0.6	-0.2	-2.7
Mean minimum (°C)	9.7	10.1	9.5	10.4	8.8
Difference from average	-0.4	0.0	-0.6	+0.3	-1.3
Highest maximum (°C)	19.8	25.5	21.9	23.4	20.2
Date	19	30	12	20	24-25
Lowest minimum (°C)	6.9	7.5	4.5	7.0	5.5
Date	23	24	6	5	9
Sunshine*					
Days of no sunshine	1	2	0	3	7
Maximum daily duration (hours)	13.0	15.8	14.9	13.8	14.9
Date	23	13	12	1	1
Total for month (hours)	186	225	233	198	142
% of the average	102	124	128	109	78
Rainfall†					
Days of no rainfall	21	19	21	22	15
Maximum fall in 24 hours (mm)	14	18	27	10	14
Date	24	22	23	23	19
Total for month	43	52	57	24	61
% of the average	95	116	127	53	136

*First year of records 1937; the highest number of hours' sunshine for the month was 265, in 1949, and the lowest 104, in 1954.

†First year of records 1864; the highest rainfall for the month was 115 mm, in 1966 and the lowest 2 mm, in 1865.