Reprint 188

ISSN 0308-5589

MINISTRY OF AGRICULTURE FISHERIES AND FOOD
DIRECTORATE OF FISHERIES RESEARCH

FISHERIES RESEARCH TECHNICAL REPORT

No. 40

Paralytic shellfish poisoning

An account of investigations into mussel toxicity in England 1968-77

P. A. AYRES and MARY CULLUM

LOWESTOFT, 1978

The authors:

P A Ayres, MI Biol MRSH is a Senior Scientific Officer and Mary Cullum is a Scientific Officer in the Marine Environment Protection Division of the Directorate of Fisheries Research. Both are stationed at the Fisheries Laboratory, Burnham-on-Crouch, Essex.

Fish. Res. Tech. Rep., MAFF Direct. Fish. Res., Lowestoft, 40, 23pp.

FISHERIES RESEARCH TECHNICAL REPORT NUMBER 40

PARALYTIC SHELLFISH POISONING
An account of investigations into mussel toxicity in England 1968-77

by

P. A. Ayres and Mary Cullum

Introduction

At the end of May 1968, 78 persons living near the north-east coast of England were admitted to hospital with a paralytic illness following the consumption of locally gathered mussels *(Mytilus edulis)*. Of those affected, most had purchased cooked mussels from a retail outlet and a few had cooked the mussels at home. Subsequent investigations (McCollum *et al*, 1968) demonstrated that the symptoms were typical of paralytic shellfish poisoning (PSP). This form of shellfish poisoning is extremely rare in Britain, only ten outbreaks having been recorded since 1828 (Ayres, 1975) all of which were associated with the consumption of mussels. This type of poisoning, which results from the ingestion by bivalve molluscs of large numbers of toxin-producing dinoflagellates, has been comprehensively reviewed by Halstead (1965). Dinoflagellates may form an important component of the phytoplankton and in favourable conditions undergo an explosive increase in numbers, resulting in a 'bloom' or visible discolouration of the sea. Such events have led to the description of 'red-tides' often associated with fish mortalities and outbreaks of PSP, although the species of dinoflagellate responsible for fish-kills and PSP are now recognised as being different (Halstead, 1965).

The investigations carried out following the 1968 outbreak, which will be reviewed later, suggested that peak toxicity arose off the Northumberland coast and extended both north and south in decreasing concentrations. Results from offshore sampling of shellfish further suggested that toxicity also extended for some miles off the coast. In view of the public health and fishery implications of this incident, a monitoring programme was established within the area from which toxic shellfish had been found. This monitoring has continued on an annual basis from March to August, when dinoflagellate blooms are most likely to occur in the area (Wood, 1969; Wood and Ayres, 1970; Ayres, 1971; Ayres and Cullum, 1975). The present paper reviews the results of annual monitoring of the north-east coast 1968 to 1977, includes details of some special investigations and concludes with a discussion of the results in the light of investigations recorded elsewhere.

Methods

Shellfish sampling

Samples of molluscan shellfish were collected from littoral regions, as near to low water mark as possible, at various sites along the coast of north-east England (Figure 1) and sent to the laboratory for examination. Wherever possible, mussels *(Mytilus edulis)* were sampled, for it had been established (Ingham, *et al*, 1968) that they accumulated substantially more toxin than other species of mollusc. In 1968 and 1969 shellfish were also taken from areas other than the north-east coast. Sampling was carried out by staff of the Ministry's Fisheries Inspectorate, Sea Fisheries Committees, local fishermen and, during 1976 and 1977, by local environmental health officers of various local authorities along the coast. In the light of experience gained during the period 1968-1970, the programme for 1971 and subsequent years was modified and samples of whole shellfish (rather than tissue only) were taken so that gut contents could be examined for the presence of phytoplankton.

Determination of toxicity

The tissues of mussels examined during 1968-1970 were removed from the shell when collected and dispatched to the laboratory for examination. From 1971 onwards each sample consisted of 18 whole shellfish in the shell, 12 of which were used for toxin assay and the remaining six for gut content analysis. Tissue extracts were prepared and assayed by the methods described by McFarren (1959). Acid extracts were injected intraperitoneally into female mice of 18 to 20 g body weight and the time to death recorded to the nearest second. Extracts from toxic samples caused the mice to become restless with rapid arching movements of the body and periodic leaps into the air. Immediately before death these animals rolled on to their side, jerking and gasping spasmodically. Death time was recorded to the last gasping breath and toxin concentration, as mouse units (mu)/100 g of tissue, calculated using Sommers' table given by McFarren (1959). Sommer and Meyer (1937) recommended that death times of between five and seven minutes should be aimed at in order to achieve an accurate bioassay, and where necessary the sample extracts were diluted with 0.1 N HCl to obtain death times within this range.

Examination of gut contents

Six mussels were taken at random from each sample collected during the 1971 and subsequent sampling programmes. The mussels were opened carefully with a scalpel and the hind gut and stomach dissected out separately. The gut contents of six mussels were pooled together in a clean petri dish and mixed with 0.04 ml sea

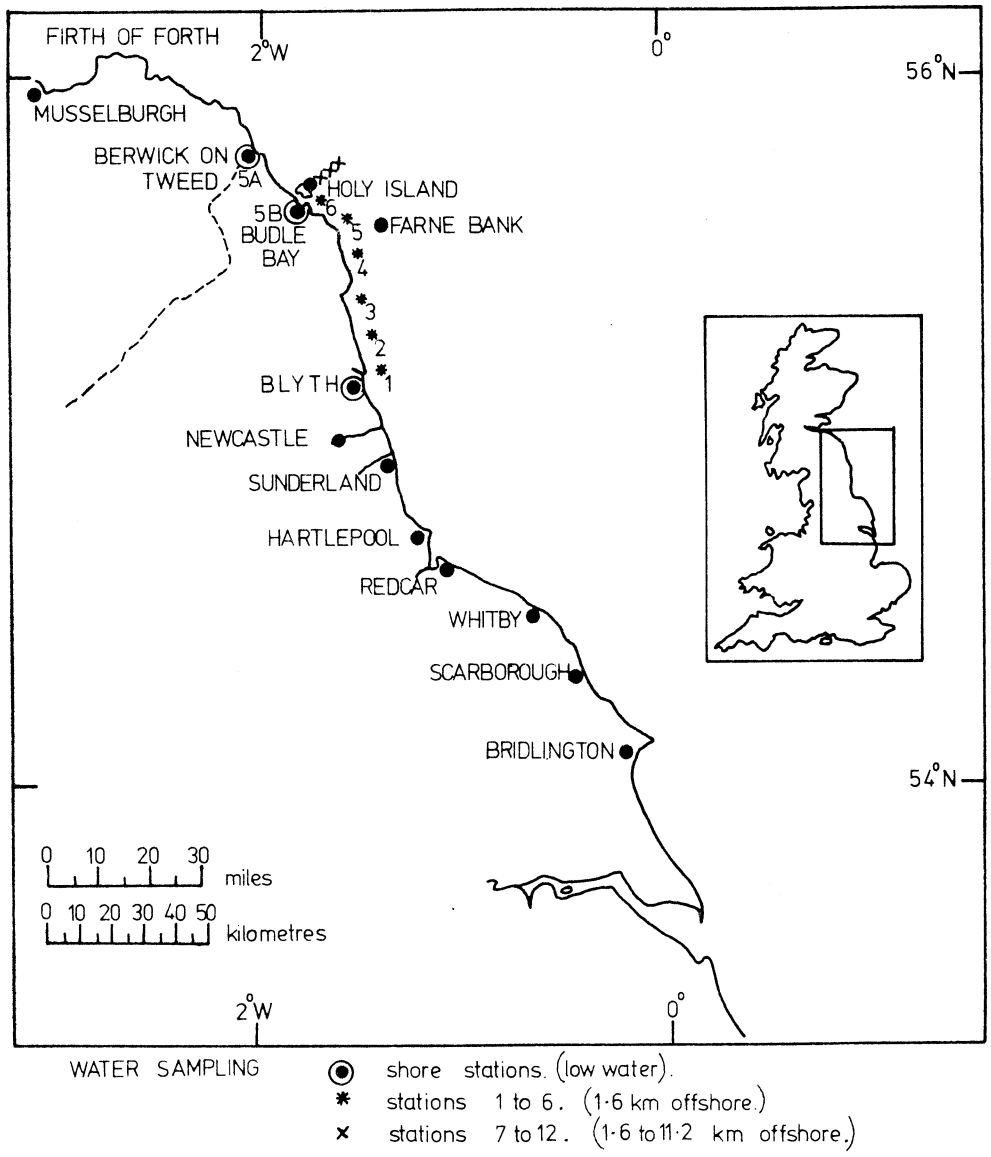


Figure 1. Location of major sampling areas during the period 1968-1977

water (previously filtered through a 0.4 µm filter). Two drops (0.04 ml) of this mixture from a calibrated Pasteur pipette were placed on a slide for microscopical examination. Preliminary observations at 480 magnification were made to identifify the dominant genera of phytoplankton, and where possible, to allow identification of dinoflagellates to species level. Once the genera and species had been established the sample was scanned at x120 magnification for five minutes and counts made of the organisms present. This did not yield a strictly quantitative assessment of the numbers and types present, but by standardizing the method as much as possible comparative assessments between one sample and another were achieved. Although the loss of the more fragile cells was difficult to assess, the variety of intact and identifiable cells found in the gut analysis suggested that this was a satisfactory technique.

Collection and examination of water samples

Prior to 1970 water samples for phytoplankton analysis were collected at various localities within the area where toxicity occurred in 1968, particularly in the vicinity of visible plankton blooms. In 1970 an attempt was made to link water and shellfish examination by simultaneous sampling of each at three sites. Additionally, surface water samples were taken weekly from six stations 1.6 km offshore between Holy Island and Blyth, and at stations up to 11.2 km offshore of Holy Island. Duplicate samples were taken in 100 ml polythene containers, one being fixed with Lugol's iodine at the time of collection. On receipt at the laboratory 10 ml aliquots from each sample were centrifuged at 2,500 rpm for 10 minutes and the sediment examined microscopically. Diatoms were identified to generic level and the dinoflagellates, where possible, to species. When sufficient dinoflagellates were present they were counted by means of an inverted microscope, using a 10 ml sample settled for two hours in a modified sedimentation chamber. Water sampling and examination was abandoned as part of the monitoring programme after 1970 in favour of mussel gut analysis.

Results

Toxicity of molluscs

Figure 1 shows the area sampled during the course of monitoring investigations between 1968 and 1977. For comparative purposes the results of toxin bioassay for the period 1968 to 1970 are presented in Figures 2a and 2b.

Low level sporadic incidents of toxicity and changes in sampling stations precludes the presentation of all the data in this manner and the results for 1971-1976 are presented in table form (Tables 1 to 6) 1977 results are in Appendix Table 1. It should be noted that the maximum safe concentration of toxin in mussels for consumption is 400 mu/100 g.

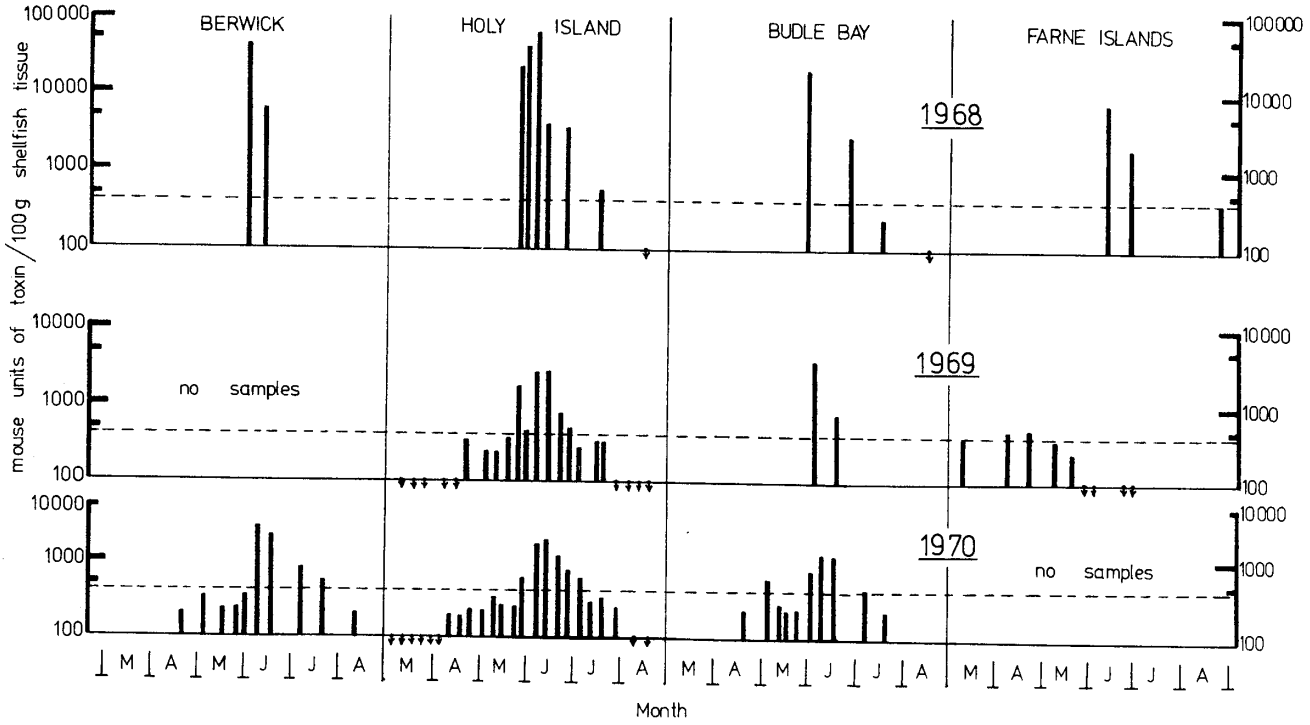


Figure 2a. Results of toxin bioassay; shellfish sampling 1968-70

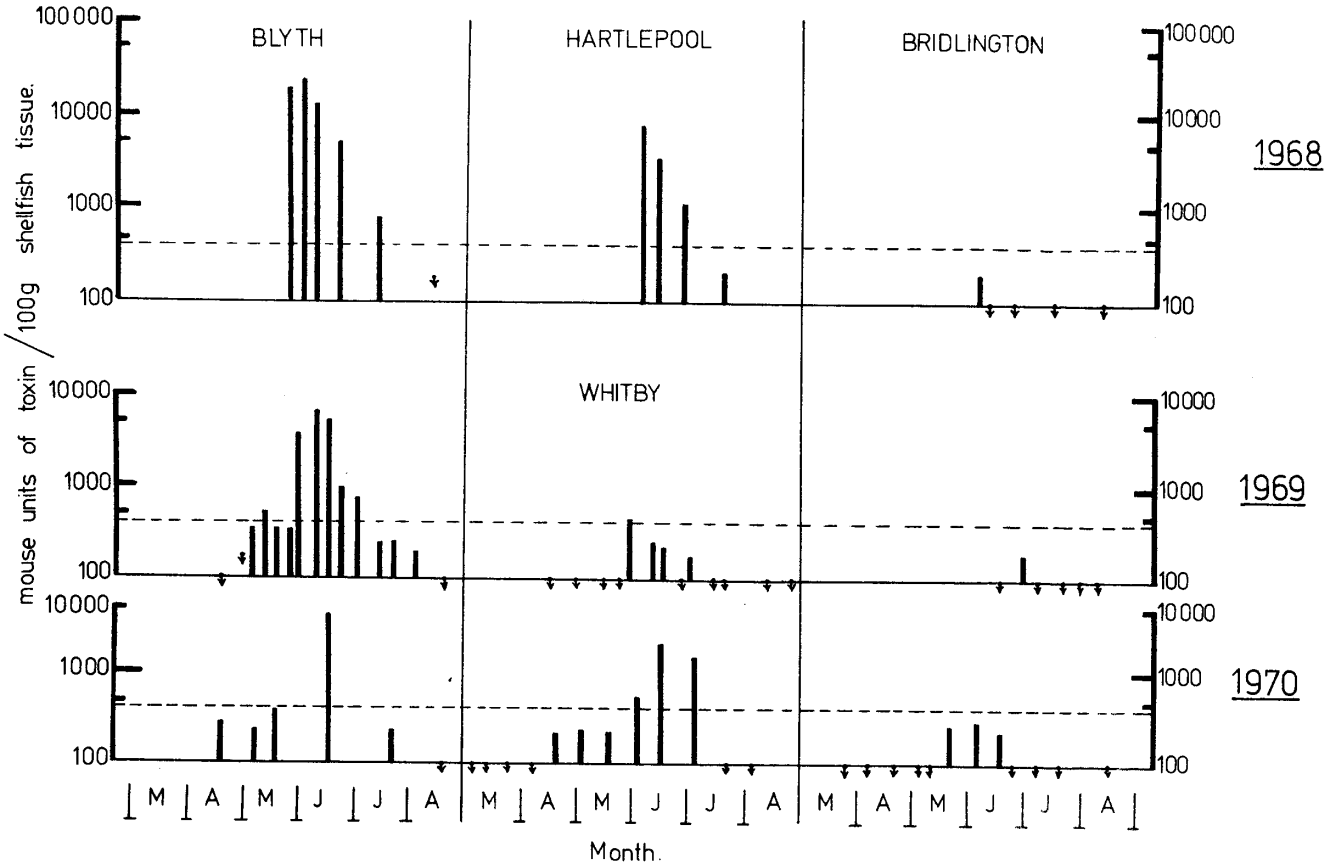


Figure 2b. Results of toxin bioassay; shellfish sampling 1968-70

The 1968 outbreak, and subsequent monitoring has been described elsewhere (McCollum *et al* 1968; Wood, 1968). However, brief notes on the main findings are of relevance here in order to set the background for the results obtained in subsequent years. In the 1968 incident consumption of the mussels produced neurological symptoms on which the diagnosis of PSP was based. Injection of acid extracts of mussel tissue into mice produced the characteristic neurotoxic symptoms described by Sommer and Meyer (1937) and the dose mortality curve closely resembled similar curves for *Gonyaulax* and *Prorocentrum* toxins observed in incidents elsewhere (Medcof e*t al*, 1947; Pinto and Silva, 1956). High concentrations of the dinoflagellate *Gonyaulax tamarensis* were reported in the North Sea prior to the outbreak (Robinson, 1968) and it seemed likely that cases of poisoning were attributable to this organism.

Samples of littoral mussels collected between 29 May and 14 June 1968 from an area extending from Bridlington in the south, northwards to Rosehearty in Scotland, contained toxin (see Wood and Mason, 1968). Peak toxicities greater than 20,000 mu/100 g of shellfish tissue were found at Eyemouth, Holy Island, Alnmouth and Blyth (Figures 2a and 2b). No toxicity could be detected in winkles *(Littorina littorea)*, whelks *(Buccinum undatum)*, crabs *(Cancer pagurus)*, lobsters *(Homarus gammarus)*, flounders *(Platichthys flesus)*, plaice *(Pleuronectes platessa)*, haddock *(Melanogrammus aegelfinus)* or herring *(Clupea harengus)* taken in this area during the same period. Offshore stocks of the filter feeding escallop *(Pecten maximus)* from the Farne Bank and the queen scallop *(Chlamys opercularis)* taken off Flamborough Head accumulated between 7840 and 1280 mu/100 g when tested on 10 and 24 June. The maximum toxicity observed in Holy Island mussels (50,000 mu/100 g) in early June was not exceeded elsewhere although toxicity persisted at all stations north of Scarborough until late July. Following the 1968 outbreak, the programme for 1969 was extended to include shellfish from the principal commercial bivalve fisheries of England and Wales. Early in March, red discoloured water was reported in the Holy Island area and mussel samples were found to contain low levels of toxin (< 175 mu/100 g). A sample of escallops taken at the same time from the offshore Farne Bank contained 498 mu toxin. Subsequently, the sampling programme was extended southwards to Bridlington with samples taken at 7 to 14 day intervals from each site. The results shown in Figures 2a and 2b indicate an increase in toxicity at most stations with peaks in June followed by a decline during July. Samples collected by the Department of Agriculture and Fisheries Marine Laboratory, Aberdeen and examined by Central Microbiological Laboratories, Edinburgh demonstrated the presence of toxin (2,384 mu/100 g) in mussels from Lower Largo on the north bank of the Firth of Forth. Further sampling indicated toxicity extending northwards to Aberdeen where a maximum toxicity of 237 mu was recorded towards the end of June (Conn and Farrand, 1970).

A total of 166 samples was taken from principal mollusc fisheries of England and Wales in addition to those taken from the north-east coast. The majority of samples collected were mussels, but other species including European flat oysters *(Ostrea edulis)*, Portuguese oysters *(Crassostrea angulata)* and cockles *(Cardium edule)* were sampled where they represented the main commercial shellfishery. Toxin, characteristic of that identified on the north-east coast, was not detected in any of the samples, but occasionally on injection of acid extracts, mice died overnight without neurotoxic symptoms. The majority of such samples were oysters and there is some evidence that deaths were caused by copper and zinc, concentrated by the shellfish, and extracted by the techniques employed.

The results of the 1969 investigations indicated that toxicity was limited to the north-east coast where the 1968 incident occurred. The programme for 1970 therefore limited sampling effort to this area and a total of 88 samples was collected. Sampling started in March but no toxicity was detected until mid-April when a sample from Holy Island, the locus of the 1968 outbreak, contained 200 mu of toxin/100 g. Measurable toxicity then appeared at five stations between Berwick and Whitby (ie along 150 km of coast) and in an escallop sample taken offshore in the Seahouses area. Figures 2a and 2b show that although minor fluctuations in toxicity continued through April and May, no substantial increase was observed until June when maximum values were observed at most stations, eg 2594 mu at Berwick, 1840 mu at Holy Island, 4100 mu at Blyth and 2100 mu at Whitby. During May the affected area spread northwards to Musselburgh on the south side of the Forth (reaching a maximum of 265 mu/100 g). As in 1968 and 1969, peak toxicity at all sampling stations occurred in June with maximum values at Blyth.

In 1971, sampling again commenced in March and during the period up until August, 91 samples from between Berwick and Bridlington were collected and examined. During the mouse bioassay close observation of test animals immediately following injection revealed positive, but sublethal responses to certain samples. It was noted that mice injected with non-lethal extracts became subdued and remained quietly in a crouched position, often for some hours. In contrast, the injection of samples containing lethal concentrations of toxin led to immediate, erratic and jerky movements, alternating with brief quiescent periods; the ears were held forward, the coat ruffled, and the animals responded to the slightest external stimuli. In the terminal phase test animals were observed to leap in the air and twitch involuntarily before rolling over, gasping periodically, and dying.

Results of the toxin monitoring in 1971 are summarised in Table 1; measurable toxicity did not appear until mid-May, with 216 mu/100 g at Berwick and 226 mu at Holy Island. Toxin persisted at Holy Island until the end of May when a sample from Whitby contained 488 mu/100 g. No further measurable toxicity occurred at any station until a sample from Hartlepool in mid-July was found to contain 454 mu which fell to 415 mu two weeks later. Sub-lethal traces of toxicity were detected at all stations where lethal levels of toxin has occurred but also at Redcar, Sunderland and Budle Bay. The most southerly station at Bridlington was sampled regularly from March to August but no traces of toxicity were observed. Elsewhere trace levels appeared in mid-April and were recorded sporadically, and often simultaneously until sampling ceased.

During 1972 sampling was carried out between March and August and a total of 66 samples was examined; results are shown in Table 2. Some sub-lethal trace responses were observed during March and April but measurable toxicity did not appear until early May when a sample from Holy Island gave a result of 193 mu/100 g and one from Hartlepool, 120 km to the south, 212 mu/100 g. No further measurable toxin was recorded though occasional trace responses were observed until early August.

The procedure of recording sub-lethal responses first adopted in 1971 was continued and the results are summarised in Table 2. Sub-lethal effects were detected prior to, and following the detection of lethal levels of toxin and also at other stations where toxicity was not detected. Only at Holy Island, where samples were obtained at regular intervals, was it possible to demonstrate the value of such observations in predicting potential development of toxicity. The simultaneous observation of sub-lethal toxicity at several points along the coast was again evident in 1972.

In 1973 more comprehensive coverage of the coast resulted in the collection and examination of 135 samples but toxin development was limited and only three samples contained measurable toxin (Table 3). Trace, sub-lethal responses occurred sporadically during the period March to May but were not recorded prior to measurable toxicity which first appeared in late June at Hartlepool (218 mu/100 g) followed a week later by 198 mu/100 g at Berwick. At the end of July a sample from Whitby contained 192 mu/100 g after which no further toxicity was detected at any station sampled.

Results of the 1974 monitoring programme are summarised in Table 4. Of 99 samples examined 10 contained measurable toxin and for the first time since 1971 the adopted safe level of 400 mu/100 g sample was exceeded. Toxin appeared first at Berwick in early May and had reached a maximum value of 2730 mu/100 g at Hartlepool by the end of June. Unfortunately, further samples could not be obtained from Hartlepool to determine whether an upward trend continued or if toxicity declined. From 1974 onwards records of trace responses were discontinued because time spent on detailed observation necessary did not yield any useful information.

A total of 87 samples was taken during 1975 and following the upward swing in toxin development during 1974 a number of samples exceeded the safe limit of 400 mu/100 g (Table 5). Toxin was first detected in early May, and the maximum value of 6146 mu/100 g recorded at Berwick at the end of May was the highest obtained since 1969. Some toxin was still evident in mussels from Budle Bay in July but lack of samples from other sites prevented a more detailed appraisal of the decline of toxicity in the area as a whole.

In 1976, an attempt was made to obtain more frequent samples to overcome problems experienced in the previous year and this met with a measure of success, reflected in the 131 samples examined (Table 6). Ironically perhaps, toxicity at detectable levels was only recorded at two stations, Sunderland and Hartlepool and even this was very patchy and sporadic. The maximum value observed was 869 mu/100 g at Sunderland in early July. 1976 was unusual in that toxicity extended over a period of almost four months, appearing in late April and still being evident at the end of August when sampling ceased.

Phytoplankton observations

During 1968 plankton observations were commenced soon after reports of dead sea birds appeared (Coulson *et al,* 1968) and at sites where toxicity was detected in mussels. Phosphorescence was noted in water off the Farne Islands (14 May) and off Staithes (23 May), where water samples contained up to 74,000 cells/l of the dinoflagellate *Gonyaulax tamarensis.* On 24 May, the Dove Marine Laboratory, Cullercoats, reported discoloured water 4 miles (6.5 km) off Blyth which also contained *G. tamarensis*. Similar reports of discoloured water came from 16 km north-east of the Tyne and 4 km off St Abbs Head at the end of May. In early June, small numbers of *G. tamarensis* were observed over a wide area of the north-east coast and the Firth of Forth, extending up to 24 km offshore. Continuous plankton recorder samples (Robinson, 1968) indicated that *G. tamarensis* first appeared off the Forth Estuary in mid-April; the numbers of cells per unit volume of water and the body of water containing *G. tamarensis* both appeared to gradually increase and move southwards until mid-May. Maximum concentrations were detected 16 to 24 km off the coast between Eyemouth and the Farne Islands during the period 11-19 May. Observations were not made within 8 km of the coast, and only a few were made to the south of the Farnes, but *G. tamarensis* was detectable up to 60 miles (97 km) off the coast until the end of May. Thus it was established that *Gonyaulax tamarensis* was widely distributed immediately before and during the sea bird deaths and the occurrence of the first cases of mussel poisoning. In the absence of significant concentrations of other dinoflagellates it appeared that *G. tamarensis* was the source of toxicity observed.

In May and June 1969 continuous plankton recorder

Table 1 Levels of toxin in mussels sampled from the north-east coast during March to August 1971

		Mar		Apr					May				Jun				Jul					Aug	
Station	Week ending	19/3	26/3	2/4	9/4	16/4	23/4	30/4	7/5	14/5	21/5	28/5	4/6	11/6	18/6	25/6	2/7	9/7	16/7	23/7	30/7	6/8	13/8
Berwick		-	*	-	-	+	*	+	*	*	216	-	*	-	-	-	-	-	*	-	*	-	-
Holy Island		-	*	*	*	-	+	*	*	+	266	197	*	*	*	+	*	*	-	*	*	*	+
Budle Bay		*	*	-	-	+	-	+	*	+	-	-	-	*	-	-	-	-	*	-	*	-	-
Blyth		-	-	-	-	-	-	-	-	-	-	*	-	*	-	-	-	-	-	-	-	-	-
Sunderland		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Hartlepool		-	-	-	-	-	-	-	-	-	*	*	-	-	*	-	*	-	454	-	415	-	-
Redcar		-	-	-	-	-	-	*	-	*	-	-	-	-	-	+	-	*	-	*	-	*	-
Whitby		-	-	-	-	*	*	-	-	*	-	488	-	*	-	Lost	-	*	-	-	-	+	-
Bridlington		*	*	*	*	-	-	*	*	*	*	*	-	-	*	-	*	-	*	-	*	-	*

- no sample
* sample negative
+ trace response (non fatal)
216 etc mouse units of toxin/100g sample

Total samples examined 75

Table 2 Levels of toxin in mussels sampled from the north-east coast during March to August 1972

		Mar				Apr					May				Jun				Jul					Aug			
Station	Week ending	4/3	11/3	18/3	25/3	1/4	8/4	15/4	22/4	29/4	6/5	13/5	20/5	27/5	3/6	10/6	17/6	24/6	1/7	8/7	15/7	22/7	29/7	5/8	12/8	19/8	26/8
Berwick		*	-	+	-	-	*	*	*	-	-	+	-	-	*	-	-	-	*	-	-	-	*	-	-	-	-
Holy Island		*	+	+	*	*	*	*	*	+	193	+	+	*	-	+	*	-	*	*	*	*	*	*	*	*	-
Budle Bay		*	*	*	-	-	*	*	*	-	+	*	-	-	+	-	-	-	*	-	-	-	-	*	-	-	-
Sunderland		-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	*	-	-	-	-	-	+	-	-	-
Hartlepool		-	-	-	-	-	-	*	-	-	212	-	-	+	-	-	-	-	-	*	-	*	-	-	-	-	*
Redcar		-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	*	-	*	-	*	-	-	-	-	-	-
Bridlington		-	-	+	*	-	-	-	+	*	-	*	+	*	-	-	+	-	-	-	*	-	-	-	-	-	-

- no sample
* sample negative
+ trace response (non fatal)
193 etc mouse units of toxin/100g sample

Total samples examined 66

Table 3 Levels of toxin in mussels sampled from the north-east coast during March to Agust 1973

Station	Week ending	Mar				Apr				May				Jun					Jul				Aug			
		10/3	17/3	24/3	31/3	7/4	14/4	21/4	28/4	5/5	12/5	19/5	26/5	2/6	9/6	16/6	23/6	30/6	7/7	14/7	21/7	28/7	4/8	11/8	18/8	25/8
Berwick		-	*	-	-	+	*	+	*	*	+	*	*	*	*	*	*	-	198	*	*	*	*	*	*	-
Budle Bay		-	*	*	*	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	*	-
Holy Island		*	*	*	*	+	*	+	*	+	+	+	+	*	*	*	*	*	*	*	*	*	*	*	*	*
Sunderland		-	-	-	-	-	-	-	-	-	-	*	-	-	*	-	-	-	*	-	-	-	-	-	-	-
Seaham Harbour		-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hartlepool		-	-	-	*	-	*	-	*	-	+	-	-	-	-	-	*	218	-	*	-	*	-	*	-	-
Redcar		*	-	*	-	+	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	-
Saltburn		-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-
Whitby		*	+	*	*	+	*	*	*	*	*	*	+	*	*	*	*	*	*	-	192	*	*	*	*	*
Scarborough		-	-	-	-	+	*	*	*	*	*	*	-	*	*	*	*	*	-	*	*	*	*	*	*	*
Bridlington		-	*	-	+	*	-	-	-	-	-	*	-	-	-	*	-	-	-	-	-	-	-	-	-	-

- no sample
* sample negative
+ trace response (non fatal)
198 etc mouse units of toxin/100g sample

Total samples examined 135

Table 4 Levels of toxin in mussels sampled from the north-east coast during March to August 1974

		Mar					Apr				May				Jun					Jul				Aug			
Station	Week ending	2/3	9/3	16/3	23/3	30/3	6/4	13/4	20/4	29/4	4/5	11/5	18/5	28/5	1/6	8/6	15/6	22/6	29/6	6/7	13/7	20/7	27/7	3/8	10/8	17/8	24/8
Berwick		-	*	*	*	*	*	*	*	*	*	240	242	210	194	+	*	*	*	*	*	*	*	*	-	-	-
Holy Island		-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-
Budle Bay		-	-	-	-	-	-	-	-	-	-	-	386	217	-	-	-	-	-	-	-	-	-	-	-	-	-
Blyth		-	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sunderland		-	-	-	-	*	-	-	-	*	-	-	-	-	-	-	*	-	192	-	-	-	-	-	-	-	-
Hartlepool		-	-	-	-	-	*	-	*	-	*	-	-	259	-	-	1130	-	2730	-	-	-	-	-	-	-	-
Redcar		-	-	-	-	*	-	-	-	-	-	-	-	*	-	*	-	-	*	-	-	-	*	-	-	*	-
Whitby		-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Scarborough		-	*	*	*	*	*	*	*	*	*	*	-	-	-	*	*	*	*	*	*	*	-	*	-	-	-
Bridlington		-	-	*	-	*	*	-	-	*	-	*	*	*	*	*	*	*	*	-	*	-	-	-	-	-	-

- no sample
* sample negative
+ trace response (non fatal)
192 etc mouse units of toxin/100g sample

Total samples examined 99

Table 5 Levels of toxin in mussels sampled from the north-east coast during March to July 1975

Station	Week ending	Mar			Apr				May					Jun				Jul			
		15/3	22/3	29/3	5/4	12/4	19/4	26/4	3/5	10/5	17/5	24/5	31/5	7/6	14/6	21/6	28/6	5/7	12/7	19/7	26/7
Berwick		*	-	*	*	*	*	*	*	184	341	476	6146	1080	283	284	201	*	*	-	-
Budle Bay		*	*	*	*	*	*	*	*	*	575	1200	3918	1382	1290	462	414	-	212	*	*
Holy Island		-	-	-	-	-	-	-	-	-	-	-	-	-	-	286	1149	-	-	-	-
Seahouses		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1657	-	-	-	-
Amble		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	206	-	-	-	-
Blyth		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1450	-	-	-	-
Cullercoats		-	-	-	-	-	-	-	-	-	-	-	-	-	-	727	-	-	-	-	-
Sunderland		*	-	-	-	*	*	-	*	-	*	-	*	3918	-	-	-	-	-	-	-
Seaham Harbour		-	-	-	-	-	-	-	-	-	-	-	3915	-	-	-	-	-	-	-	-
Hartlepool		*	-	-	*	*	*	-	*	-	*	*	-	3068	-	-	-	-	-	-	-
Redcar		-	*	-	*	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Whitby		*	*	-	*	-	*	-	*	-	*	*	-	-	-	220	-	*	-	-	*
Scarborough		*	-	-	-	*	-	*	-	-	214	-	-	+	251	-	*	*	*	-	-
Bridlington		*	*	-	*	-	-	*	-	-	-	*	-	*	-	*	-	-	-	-	-

- no sample
* sample negative
+ trace response (non fatal)
184 etc mouse units of toxin/100g sample

Total samples examined 87

Table 6 Levels of toxin in mussels sampled from the north-east coast during March to August 1976

		Mar		Apr				May					Jun				Jul					Aug			
Station	Week ending	20/3	27/3	3/4	10/4	17/4	24/4	1/5	8/5	15/5	22/5	29/5	5/6	12/6	19/6	26/6	3/7	10/7	17/7	24/7	31/7	7/8	14/8	21/8	28/8
Berwick		***	*	*	*	*	-	-	*	*	*	-	-	-	-	-	-	+	+	-	*	*	*	-	-
Holy Island		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-
Budle Bay		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-
Wansbeck		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	-
South Shields		-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	*	*	*	*	*	*	*	*	-
Sunderland		-	-	-	*	-	-	-	*	-	-	-	-	-	*	-	869	-	*	-	194	-	387	329	-
Seaham H.		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	*	*	*	-	-
Hartlepool		-	*	*	*	-	-	206	*	-	*	224	*	686	-	-	*	622	*	*	*	*	*	*	-
Redcar		-	-	-	*	-	-	-	-	-	*	-	*	-	-	-	-	-	*	-	-	-	-	-	-
Saltburn		-	-	*	-	*	-	*	-	-	-	*	-	-	*	-	-	-	-	-	*	-	-	-	-
Staithes		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-
Whitby		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	**	*	*	*	*	-	-
Scarborough		*	*	*	*	-	-	*	-	*	-	*	-	-	*	-	-	*	-	-	*	-	-	-	-
Bridlington		-	*	*	*	-	-	*	-	-	*	-	-	-	*	*	-	*	*	-	-	-	*	-	-

- no sample

* sample negative

\+ trace response (non fatal)

206 etc mouse units of toxin/100g sample

Total samples examined 131

samples showed *G. tamarensis* was present in an area extending up to 60 miles (97 km) off the coast between the Firth of Forth and Whitby (Robinson, 1969). Dr J Gray of the Wellcome Marine Laboratory reported discoloured water 5 to 12 miles (8 to 19 km) off Whitby during the period 14-27 May. The plankton was dominated by *Peridinium depressum* and *Dinophysis acuta*, with *Peridinium ovatum* (or *conicum?*) and *Gonyaulax* species occasionally present. Between 13 May and 16 July samples were taken fortnightly at coastal stations between the Tyne and Holy Island and offshore of Holy Island for a distance of 11.2 km. A variety of dinoflagellate species was identified in the samples fixed with Lugol's iodine, although no species was found to be present in concentrations exceeding 100/l prior to 30 June, when toxicity in mussels was at its peak. Thereafter, some species increased in number, particularly *Ceratium furca* which reached a maximum of 13,600 cells/l off Holy Island on 7 August. Numerically this species consistently exceeded the records of any other dinoflagellate at any station during the sampling period. The maximum concentration of *Gonyaulax tamarensis* (400/l) was recorded at a station 3.2 km east of Holy Island at the end of June.

Results of the phytoplankton examination of water samples collected in 1970 are presented in Table 7 which shows the frequency of occurrence of diatoms and dinoflagellates in the samples examined. Only the diatom *Leptocylindrus danicus* was observed in large numbers, reaching a maximum of 1.4×10^6 cells/l at station 1A (Blyth) on 1 July. A variety of dinoflagellates was recorded including two species of *Gonyaulax*, one being *tamarensis* and the other a larger, unidentified form. It is possible that the larger form was an intermediate stage of cyst formation of this organism, since cysts were common at times, reaching 15,000/l off Holy Island when peak mussel toxicity was recorded. A protozoan, *Favella* sp was frequently observed to contain cells of *Gonyaulax*, and *Gymnodinium* spp Needler (1949) reported that *Favella ehrenbergi* was the principal predator of *Gonyaulax tamarensis* in Canadian waters.

Samples of water taken along the shore at stations 1A, 5A and 5B (see Figure 1), did not contain any dinoflagellate species in excess of 500/l even during June when maximum mussel toxicities were recorded. Offshore samples from stations 1 to 6 (Figure 1) contained dinoflagellates taxonomically and numerically similar to those found in samples taken at the water's edge. In contrast, stations east-wards and offshore of Holy Island (numbers 7 to 12 in Figure 1) yielded a more diverse and numerous dinoflagellate flora with some evidence to suggest that both numbers and diversity increased with distance offshore. Peak concentrations occurred at station 11 (9.6 km offshore) with a total of 23,600/l on June 19; of this about 8,400/l were *Gonyaulax tamarensis*. Maximum numbers of species and of individuals coincided with peak toxicity of Holy Island mussels. Several coastal blooms of diatoms were reported during the sampling period, but no unusual biological events were recorded.

As described previously, the 1971 monitoring programme saw the introduction of the analysis of mussel gut contents to replace the water sampling carried out in previous years. At Holy Island, dinoflagellate numbers oscillated at low levels before increasing rapidly in early June. Toxicity in mussels coincided with the appearance of a *Gonyaulax* sp and of unidentified cysts (possibly also of this genus). Cysts were generally more abundant than identifiable cells at all stations; the number observed at Holy Island was not exceeded elsewhere. Sampling at Berwick was less frequent but a peak of dinoflagellates in the gut contents, predominantly *Gonyaulax*, coincided with the only measureable toxicity at this station. The apparently ubiquitous *Prorocentrum micans* was present in small numbers throughout this period. Dominance of this organism preceeded toxicity in mussels at Hartlepool, and although still present when toxin was detectable actual numbers had declined considerably.

Two reports of patches of red water were received; the first off Seahouses and Beadnell coincided with the appearance of mussel toxicity at Holy Island but water samples could not be obtained for examination. The second, off Holy Island in June, consisted of high concentrations of a pink pigmented copepod.

In 1972 the appearance and decline of low toxicity in mussels at Holy Island coincided with observations of *Gonyaulax* spp and a small ($< 30\ \mu m$) dinoflagellate. Toxicity at Hartlepool appeared in early May when few diatoms or dinoflagellates were observed in the gut analysis. *Peridinium depressum* was the commonest dinoflagellate observed but smaller numbers of *Gonyaulax* spp and *Exuviaella* spp were recorded. *Prorocentrum micans* was present at all stations when traces of toxicity were recorded but there appeared to be no direct correlation with cell numbers. Counts of a *Phalacroma* sp (possibly *P. rotundatum*) at all stations were generally in excess of those of any other species or genus at any station during the sampling period. Dinoflagellate cysts were often evident throughout the sampling but did not exhibit any apparent correlation with toxin development.

Low and isolated cases of measurable toxicity in 1973 were reflected by low levels of both diatoms and dinoflagellates and when toxicity was detected at Berwick, mussel gut contents were almost devoid of identifiable phytoplankton. Toxicity at Hartlepool was coincident with the appearance of *Gonyaulax* spp and at Whitby with the appearance of *Gonyaulax tamarensis*. No reports of unusual biological events were recorded.

Again in 1974 very little identifiable phytoplankton was seen in mussel gut contents. However, toxicity at Sunderland and Hartlepool was marked by the presence of *Gonyaulax* spp and *Gonyaulax tamarensis* in numbers not observed at other stations or at any other time during the sampling programme. Toxicity at Berwick was recorded in association with a dominance of *Peridinium* spp.

In 1975 toxin levels were considerably higher and although no phytoplankton blooms were reported, the diversity and numbers of organisms present in the gut analysis had increased substantially. Both *Gonyaulax* spp and *Exuviaella* spp were dominant when toxicity appeared but there was no correlation between levels of toxin and relative abundance of these, or any other dinoflagellates. For the second time since 1968 reports were made of sea bird deaths on the coast, coincident with maximum toxicity at Berwick. The relationship between dinoflagellates and bird mortality remains uncertain.

In an earlier section dealing with results of toxin monitoring, 1976 was high-lighted as an unusual year because of the persistence of toxicity. It was also unusual in that the summer was exceptionally long, hot and dry and it would have been tempting to postulate a 'record' year for dinoflagellate blooms. To an extent this was so, though not reflected in high or widespread toxicity, some high counts of dinoflagellates were obtained from mussel guts. Toxicity at Hartlepool, and Sunderland, was coincident with a marked dominance of *Peridinium trochoideum* (up to 222/0.04 ml gut contents) but also at Hartlepool and Budle Bay, *Exuviaella* spp were present in similar concentrations. As toxicity at Sunderland decreased *Peridinium trochoideum* was replaced by even greater numbers of *Prorocentrum micans* (587 in 0.04 ml gut contents). *Gonyaulax* spp were only recorded in the gut analysis at the time maximum toxicity was observed at Sunderland though numbers were relatively low (50 in 0.04 ml gut contents).

The nature and origin of toxicity

When the original outbreak of mussel poisoning occurred in 1968, consumers exhibited symptoms characteristic of paralytic shellfish poisoning, and this feature, coupled with evidence of *Gonyaulax tamarensis* in the area of coast where the mussels were taken, suggested similarities with outbreaks in Canada (Needler, 1949; Prakash, 1963). Samples of toxic mussels and acid extracts prepared from this material were sent to Dr Edward Schantz in the USA, a world authority on PSP toxin (called saxitoxin in the USA) and to Dr Martin Evans of Cambridge, who was interested in the pharmacological properties.

Schantz (pers. comm.) found that the acid extract contained a poison with properties similar to those of a substance obtained from axenic cultures of *Gonyaulax tamarensis* grown in the laboratory. Evans (1970) examined mussels which had been frozen for six months following the 1968 incident and extracted two toxic fractions. The minor fraction seemed to be identical with saxitoxin which has been identified by Schantz (1967) as the toxic principle in *Gonyaulax catenella* and in species of mollusc implicated in PSP outbreaks in North America. The major fraction obtained by Evans exhibited similar properties to those of saxitoxin but had a poor affinity for ion exchange resin (Amberlite CG-50) in comparison with saxitoxin. These results suggest that either *Gonyaulax tamarensis* produces both saxitoxin and another (the major factor found by Evans) or that the major toxin originated elsewhere. Attempts to use standard saxitoxin purification procedures to extract poison from Bay of Fundy (Canada) scallops, where *Gonyaulax tamarensis* was implicated as the causative organism, also failed (Schantz, 1960).

Throughout this present paper the name *Gonyaulax tamarensis* has been retained in keeping with earlier publications on the 1968 outbreak. However, Braarud (1945) designated the toxic form *G. tamarensis* as *G. tamarensis var. excavata* to distinguish it from the original Tamar culture of Lebour which was never shown to be toxic. More recently Balech (1971) raised this to specific level, ie *Gonyaulax excavata* (Braarud) Balech.

Discussion

The monitoring programme carried out annually on the north-east coast of England since 1968 has revealed the presence of dinoflagellate toxins in littoral mussels in each of the years sampled (Figure 3). The extent of the area affected and the levels of toxicity observed in 1968 when human cases of paralytic shellfish poisoning reported have not been observed since. However, toxicity has been detected at varying levels, sometimes over a wide area, sometimes within apparently very limited localities and sporadically. No further mussel poisoning incidents have occurred and toxicity has not been detected elsewhere in England and Wales up to the end of 1976. This suggests that the appearance of dinoflagellate toxicity is an annual feature only on the north-east coast, although toxins rarely reached levels which resulted in the clinical syndrome observed in 1968 and which led to the setting up of the annual monitoring described in this paper.

The collection of water and mussel samples from the shore, together with offshore water sampling yielded some interesting results particularly during 1970. Offshore transect samples indicated that large numbers of diatoms and dinoflagellates were present in concentrations which increased further offshore, out to 16 km. In contrast, those samples taken at low water mark contained a variety of diatoms and dinoflagellates but in low numbers even during periods of peak mussel toxicity. This distribution of dinoflagellates coincident with mussel toxicity suggest that the shellfish became toxic as a result of continued exposure to low numbers of dinoflagellates rather than short term exposure to bloom concentrations. From the limited sampling of offshore molluscs (e.g. escallops from the Farne Bank) it was evident that they became toxic three to four weeks earlier than mussels in the littoral regions, coincident with the appearance of dinoflagellates in the offshore area.

During three consecutive years (from 1968 to 1970) maximum toxicity of mussels was observed at Blyth; peak values at all stations occurring in June. It was also evident that toxicity at stations to the north and south of Blyth did not reach such high levels; peak values declining with increasing distance in both northerly and southerly directions. No toxicity was detected at Bridlington

Table 7 Occurrence of phytoplankton in water samples from the north–east coast examined in 1970*

			DIATOMACEAE									DINOPHYCEAE																				CYSTS						
Station (see figure 1)	Sampling period	Number of samples examined	*Coscinodiscus* sp	*Biddulphia* sp	*Navicula* spp	*Gyrosigma* spp	*Chaetoceros* spp	*Skeletonema* sp	*Nitzschia* spp	*Rhizosolenia* sp	*Leptocylindrus danicus*	*Gonyaulax* spp	*Gonyaulax tamarensis*	*Peridinium* spp	*Peridinium depressum*	*Peridinium trochoideum*	*Heterocapsa triquetra*	*Gymnodinium* sp (large)	*Gymnodinium* sp (small)	*Gymnodinium* spp	*Phalacroma* sp	*Polykrikos* sp	*Nematodinium* sp	*Amphidinium* spp	*Dinophysis* spp	*Oxyrrhis* sp	*Ceratium* spp	*Ceratium furca*	*Exuviaella* spp	*Gonyaulax* spp	*Gymnodinium lunula*	*Peridinium depressum*	Others	Dominant organism	Maximum numbers per litre of water	Date observed
Blyth St 2 Shore LW	Apr 15 to Jun 18	5	1	3	1	0	2	2	3	0	0	2	2	1	3	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	2	*Gonyaulax* sp	2.1×10^6	Apr 15
Blyth St 1A 1.6 km off	May 18 to Jul 13	6	0	3	2	0	6	3	1	3	2	3	4	3	2	2	1	1	4	0	0	0	0	2	3	0	2	0	0	0	0	0	5	*Leptocylindrus danicus*	1.4×10^6	Jul 1
Snab Pt St 2 1.6 km off	May 18 to Jul 13	6	0	3	4	2	6	1	3	2	2	1	3	3	3	3	0	1	4	1	1	0	0	2	2	2	0	0	0	0	0	0	5	None greater than 500/l		
N E Coquet Buoy St 3 1.6 km off	Jun 3 to Jul 13	5	0	2	1	2	4	2	3	2	2	1	3	2	3	2	0	1	1	0	0	0	0	4	3	1	0	3	1	1	1	0	3	None greater than 500/l		
Cullernose Pt St 4 1.6 km off	May 18 to Jul 13	6	0	3	1	1	5	2	3	1	1	2	2	3	2	2	0	1	3	0	0	0	0	4	1	2	0	2	0	0	0	0	2	None greater than 500/l		
N Sunderland Buoy St 5 1.6 km off	May 18 to Jul 13	6	0	0	0	1	4	1	1	1	2	4	4	2	2	5	0	2	2	1	0	1	0	1	0	1	0	2	0	0	0	0	3	None greater than 500/l		
Budle Bay St 5A Shore LW	Apr 21 to Jul 8	5	0	0	5	1	0	1	4	2	0	2	0	0	0	0	0	0	1	2	0	0	0	2	0	3	0	0	1	0	0	0	3	*Gonyaulax* sp	2.1×10^3	May 14
Berwick St 5B Shore LW	Apr 21 to Jul 8	7	0	0	5	0	0	1	5	1	0	1	1	0	1	1	0	0	3	0	0	0	0	0	0	0	0	0	2	0	0	0	5	None greater than 500/l		
Ridge Buoy St 6 1.6 km off	May 18 to Jul 13	6	0	1	1	0	5	0	1	0	2	3	1	2	2	3	0	2	1	0	0	0	0	1	1	1	0	0	0	0	0	0	3	None greater than 500/l		
Station 7 3.2 km off	May 18 to Jul 13	6	0	3	0	0	6	3	2	0	3	1	4	3	1	4	1	0	3	1	1	1	1	2	0	0	0	2	0	1	0	0	2	None greater than 500/l		
Station 8 4.8 km off	May 18 to Jul 13	6	0	1	1	1	6	1	0	2	2	1	4	2	1	4	0	0	2	1	0	0	0	2	2	0	0	2	0	0	0	0	3	*Gonyaulax tamarensis*	4.9×10^3	Jun 10
Station 9 5.4 km off	May 18 to Jul 13	6	0	2	0	0	4	1	1	2	2	1	3	3	2	5	0	3	4	0	1	1	1	5	2	0	0	3	0	0	1	1	5	*Gonyaulax tamarensis*	5.3×10^3	Jun 10
Station 10 8.0 km off	May 18 to Jul 13	6	1	1	2	0	4	0	2	0	2	3	3	5	1	5	3	3	0	1	1	2	0	3	3	1	2	4	0	2	1	1	5	*Gonyaulax tamarensis*	6.3×10^3	Jun 19
Station 11 9.6 km off	May 18 to Jul 13	6	0	2	1	0	5	1	2	1	2	1	4	5	1	4	1	3	2	1	0	0	2	3	0	0	2	2	0	0	1	0	4	*Gonyaulax tamarensis*	8.4×10^3	Jun 19
Station 12 11.2 km off	May 18 to Jul 13	6	0	1	1	1	4	1	2	1	2	1	3	5	4	5	5	3	2	1	1	0	3	2	1	0	2	2	0	1	0	0	4	*Gonyaulax tamarensis*	5.6×10^3	Jun 19

*Numbers show frequency of occurrence in number of samples examined (column 3)

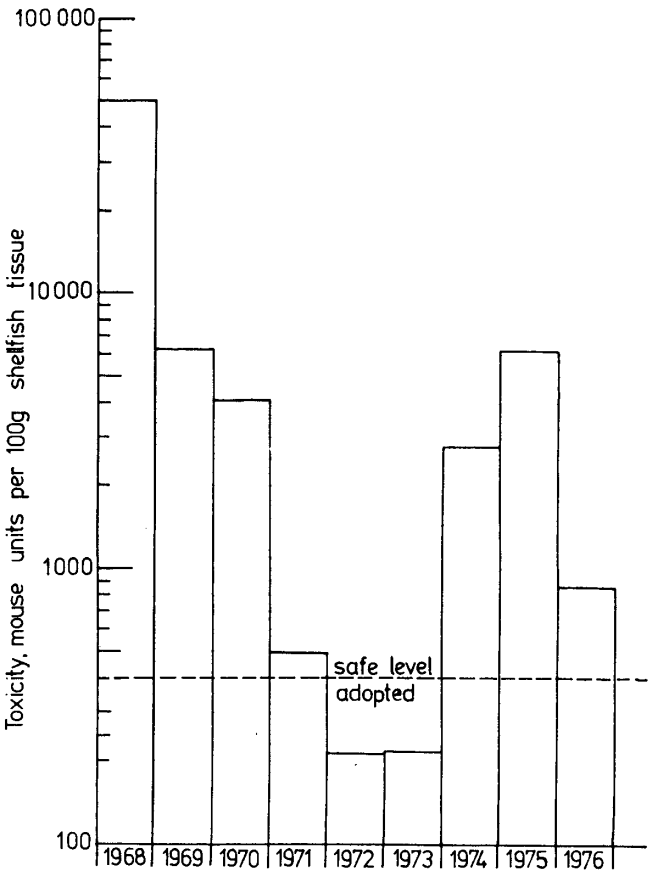


Figure 3 Mussel toxicity on the north-east coast of England: maximum values recorded 1968-1976 (see addendum)

(148 km south of Blyth) during 1968-1969 but at times, toxicity extended up to 270 km north of Blyth into Scottish waters. This suggests either that there was a general movement northwards of the body of water containing the toxic organism, or alternatively that the primary development of toxic dinoflagellates took place further north, in the region of the Firth of Forth. In 1968, the continuous plankton recorder samples suggested that the latter explanation was the most likely, for the first appearance of *Gonyaulax tamarensis* occurred in the Forth area (Robinson 1968).

In 1968, *Gonyaulax tamarensis* was widely distributed off the north-east coast during the period of mussel toxicity and was detectable up to 97 km (60 miles) offshore in small numbers; in inshore waters it occurred at concentrations up to 74,000 cells/l. No other species was detected in similar numbers though *Peridinium depressum* was occasionally abundant. In 1969, *Gonyaulax tamarensis* was again present up to 97 km (60 miles) off the coast but only low concentrations (up to 700/l) were detected at the waters edge where mussels were toxic. A similar picture emerged in 1970 with *G. tamarensis* the only species observed at concentrations greater than 1000 cells/l. However it appeared that mussel toxicity coincided with a peak in total dinoflagellate numbers and that both numbers and diversity of types increased with distance offshore.

In the years 1971-1972, only small, localized areas of toxicity appeared sporadically on the north-east coast, suggesting local phytoplankton development rather than dispersion of a single offshore concentration of dinoflagellates.

During this period water sampling was replaced by mussel gut analysis and although only low levels of toxicity developed in both years, some degree of correlation between toxicity and total dinoflagellate numbers was apparent. Toxicity at Holy Island in 1972 coincided with the appearance of *Gonyaulax tamarensis* in small numbers and these were also observed concurrent with toxicity at Hartlepool although here, *Peridinium depressum* dominated the phytoplankton.

Low toxin levels were also evident during 1973-1974 and phytoplankton was sparse. In both years however, *Gonyaulax tamarensis* and *Gonyaulax* spp coincided with appearance of toxicity at the majority of stations.

In 1975 toxin levels were higher than at any time since 1969 and the diversity and number of dinoflagellates was also notably increased in comparison with the period 1971-1974. *Gonyaulax* spp were again evident when toxicity appeared, but in 1976 were only recorded at Sunderland, and then only when maximum toxicity occurred.

A broad analysis of the results obtained during the period 1968-1976 suggests that on the north-east coast high levels of toxin are accumulated by mussels only during those years when toxicity is detected during March and April.

Later development leads either to low levels of toxin or sporadic and fluctuating levels. In 1971 and 1972, the first measurable toxicity did not appear until May, although trace reactions were recorded during the March-April period. It is possible that the early development of toxicity, i.e. in March-April is associated with high off-shore concentrations of dinoflagellates, characteristic of the period 1968-1970 whereas the more sporadic, low level of fluctuating toxicity observed in recent years is indicative of smaller local blooms. *Gonyaulax* spp or the *Gonyaulax* referred to here as *Gonyaulax tamarensis* have been present, and usually dominant when toxicity has been at its peak but have often not been identified during the early development of toxicity except when this occurs in the March-April period. Other dinoflagellates, particularly *Prorocentrum micans*, the *Peridinium* spp *trochoideum* and *depressum, Polykrikos*, spp *Phalacroma* spp and *Exuviaella* spp have all been observed in concentrations far exceeding those of *Gonyaulax* spp but normally appear after toxicity in mussels, has reached maximum values. On balance the evidence suggests that the dinoflagellate referred to as *Gonyaulax tamarensis*, in this study, was responsible for observed toxicity in mussels, but if this is so, then very low concentrations in water appear to be capable of producing detectable toxicity in mussels.

The continuous uptake, by mussels, of toxins from small numbers of dinoflagellates will lead to an increase in toxicity, only when the rate of uptake exceeds the rate of loss or decomposition. Mussels containing toxin transferred to an area free of toxin phytoplankton in the River Crouch, Essex retained 34% of their toxin after seven days immersion (ie 9400 mu/100 g down to 3000 mu/100 g) and after two weeks only 20% of the original toxin still remained. Thus, the low rate of loss suggests that only low numbers of toxic dinoflagellates need to be present for toxin to be accumulated or maintained. This is in contrast to the widely held view that molluscs only become toxic as a result of proximity to large dinoflagellate blooms.

In trying to determine which factors were important in the annual appearance of toxicity on the north-east coast, two approaches were considered; (a) what factors had been linked with similar occurrences elsewhere, (b) what factor or factors could be related to the trends in toxicity seen during 1968-1976.

Conditions favourable to dinoflagellate development are generally found in coastal waters where there is a rapid turnover of nutrients. In areas where the water is deep and the shore steep, upwelling may have an advantageous effect by bringing nutrients to the surface. Primary production is favoured by formation of a thermocline which may result from the exposure to sunlight of a shallow layer of warm nutrient-rich water particularly under conditions of little wind and turbulence. Brongersma-Sanders (1948) stated that water temperature probably does not limit production of phytoplankton but usually affects its composition. In her studies on *Gonyaulax tamarensis* in Canadian waters, Needler (1949) concluded that the chief physical factor affecting growth was water temperature, and that the principal competitors were diatoms. Prakash and Medcof (1962) however, found that shellfish toxicity was more closely related to the amount of sunlight that to water temperature. These authors also found that summer and winter temperatures affected subsequent bloom and toxin production; low temperatures favoured encystment and dinoflagellates remained in this form until temperatures high enough for germination were reached. Among the other factors to be considered are water turbulence, transparency, surface illumination, passive sinking of the organism below the photic zone and grazing by zooplankton.

From an examination of meteorological data recorded at Tynemouth on the north-east coast there appeared to be no direct correlation between dinoflagellate production, total solar radiation, surface water temperature or air temperatures. However, when the sunshine data was analysed in more detail by plotting the net increase or decrease in hours of sunlight between mean values for one month and the month preceeding it, there appeared to be some marked differences between the years 1968-1972 (Figure 4). There appeared to be a relationship between net increases and decreases in monthly mean sunshine values and the levels of toxicity recorded each year. During the years 1968-1972, where this possible relationship was examined, high toxin levels occurred when there had been net increase in sunlight above the mean during March and April, May-June periods, alternating with pronounced decreases during April/May and June/July. As these fluctuations become less marked so similar changes are noted in the extent and degree of shellfish toxicity. Deviation from the alternating pattern observed prior to 1971 led to apparent disruption of dinoflagellate development so that relatively low levels and sporadic incidents of shellfish toxicity were recorded. Evidence of toxicity occurring annually over a period of nine years would suggest that nutrients are not a limiting factor on the north-east coast.

There is at present no practical way of predicting the development of toxic blooms, although the annual appearance of toxicity on the north-east coast would indicate that this is an area where the risk has been clearly established. Furthermore, it is not possible to prevent mussels becoming toxic and it is therefore necessary to maintain a monitoring programme and which can alert public health authorities and the general public of possible health dangers as they arise.

Summary and Conclusions

1. Shellfish toxins associated with PSP have been demonstrated annually since May 1968 when 78 people were affected after consuming mussels from the north-east coast of England.

2. A survey of all major commercial molluscan shell-

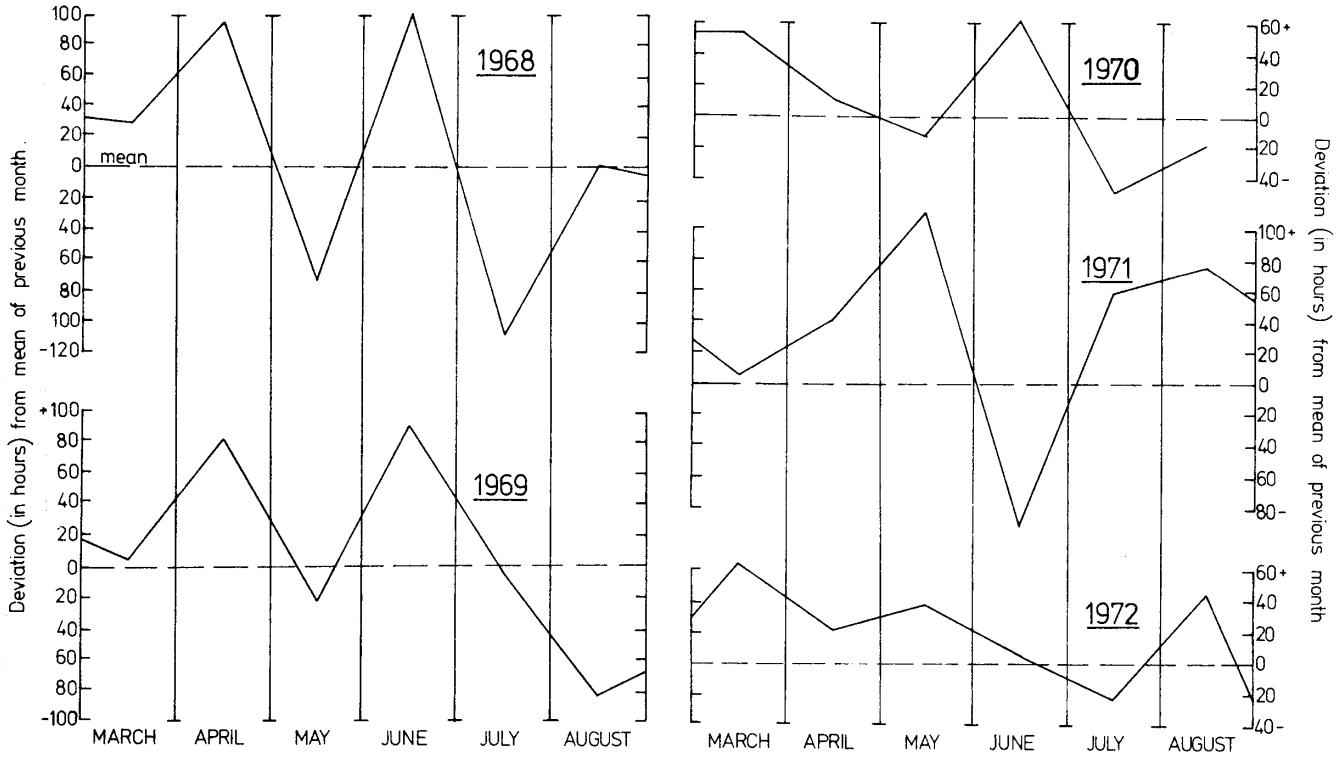


Figure 4 Sunshine at Tynemouth: change (in hours) over mean figure for previous month

fisheries in England and Wales failed to demonstrate toxicity in other areas; no clinical cases have been reported in the United Kingdom since 1968.

3. Examination of phytoplankton samples and of toxicity data suggests that the dinoflagellate *Gonyaulax tamarensis (G. excavata?)* was the source of toxin but examination of the toxin itself revealed two toxic fractions, one or both of which may originate from this organism.

4. Shellfish appeared to become toxic following continual exposure to low concentrations of toxin producing organisms, rather than a short-term exposure to "bloom" concentrations.

5. High levels of toxin in littoral shellfish were preceeded by the presence of toxin in offshore shellfish stocks and the appearance of dinoflagellates in offshore waters as early as April.

6. Toxicity has developed annually but the levels recorded and the area affected have not reached the proportions observed in 1968.

7. The use of mussel gut examination for phytoplankton appears to be a technique worthy of future consideration and development.

8. A tenuous but possible association may exist between the degree and extent of toxin accumulation and a characteristic pattern of solar radiation.

Acknowledgements

The organisation and implementation of this investigation could not have been achieved without the assistance of many people. Whilst it is not possible to name all those individuals and organisations involved, special thanks are due to Sheila Bensilum (née Halls) for practical help in sample analysis and to fishermen, MAFF inspectorate, DAFS Aberdeen, North-Eastern Sea Fisheries Committee, Dove and Wellcome Marine Laboratories.

References

AYRES, P.A., 1971. Results of the 1971 monitoring programme for mussel toxicity on the north-east coast of Britain. ICES CM 1971/E:27, 6pp (mimeo).

AYRES, P.A., 1975. Mussel poisoning in Britain with special reference to Paralytic Shellfish Poisoning. Review of cases reported 1814-1968. Envir. Hlth., 83 (7), 261-265.

AYRES, P.A. and CULLUM, M., 1975 A monitoring programme for Paralytic Shellfish Poisoning (PSP) on the north-east coast of England. Notes on the 1975 programme with a review of work during the period 1968-1974. ICES CM 1975/K:29, 4pp (mimeo).

BALECH, E., 1971. Microplancton del Atlantico equatorial oeste (Equalent I). Rep. Argentina, Armada Argentina, Sev. Hidrografia Naval, Publico H654, 1-103, pls 1-12.

BRAARUD, T., 1945. Morphological observations on marine dino-flagellate cultures *(Porella perforata, Goniaulax tamarensis, Prorocentium reticulatum).* Avh. Norske Vidensk Akad. Oslo Mat -Naturv. Kl, 1944, 11, 1-18.

BRONGERSMA-SANDERS, M., 1948. The importance of upwelling water to vertebrate paleontology and oil geology. Berhandel. Koninkl. Ned. Akad. Wetenschap (Tweede sect) 45 (4) 59 : 68-72.

CONN, N.K. and FARRAND, R.J., 1970. Shellfish toxicity in Scotland, 1969. Scott. Med. J., 15, 169-171.

COULSON ,J.C., POTTS, G.R., DEANS, I.R. and FRASER, S.M., 1968. Exceptional mortality of shags and other seabirds caused by paralytic shell-fish poisoning. Br. Birds., 61, 381-404.

EVANS, M.H., 1970. Two toxins from a poisonous sample of mussels (*Mytilus edulis*). Br. J. Pharmac., 40 (4), 847-865.

HALSTEAD, B.W. (ed), 1965. Poisonous and venomous marine animals of the world, Vol. 1. U.S. Govt. Printing Office, Washington, D.C., 994pp.

INGHAM, H.R., MASON, J. and WOOD, P.C., 1968. Distribution of toxin in molluscan shellfish following the occurrence of mussel toxicity in north-east England. Nature, Lond., 220, 25-27.

McCOLLUM, J.P.K., PEARSON, R.C.M., INGHAM, H.R., WOOD, P.C. and DEWAR, H.A., 1968. An epidemic of mussel poisoning in north-east England. Lancet, Oct. 5, 767-770.

McFARREN, E.F., 1959. Report on collaborative studies of the bio-assay for paralytic shellfish poison. J. Ass. off. agric. Chem., 42, 263-271.

MEDCOF, J.C., LEIM, A.H., NEEDLER, A.B., NEEDER, A.W., GIBBARD, J. and NAUBERT, J., 1947. Paralytic shellfish poisoning on the Canadian Atlantic Coast. Bull, Fish. Res. Bd Can.,(75), 32pp.

NEEDLER, A.B., 1949. Paralytic shellfish poisoning and *Gonyaulux tamarensis*. J. Fish. Res. Bd Can., 7 (8), 490-504.

PINTO, J. dos S., SILVA, E. de S., 1956. The toxicity of *Cardium edule* L. and its possible relation to the dinoflagellate *Prorocentrum micans*. Ehr. Notas. Estud. Inst. Biol. mar., Lisb. (12), B20pp.

PRAKASH, A. and MEDCOF, J.C., 1962. Hydrographic and meteorological factors affecting shellfish toxicity at Head Harbour, New Brunswick. J. Fish. Res. Bd Can., 19, 101-112.

PRAKASH, A., 1963. Source of paralytic shellfish toxin in the Bay of Fundy. J. Fish. Res. Bd Can., 20, 983-996.

ROBINSON, G.A., 1968. Distribution of *Gonyaulax tamarensis* Lebour in the Western North Sea in April, May and June 1968. Nature, Lond., 220, 22-23.

ROBINSON, G.A., 1969. Distribution of *Gonyaulax tamarensis* Lebour in the Western North Sea in 1969. ICES CM 1969/L:17, 2pp (mimeo).

SCHANTZ, E.J., 1960. Biochemical studies on paralytic shellfish poisons. Ann. NY. Acad. Sci., 90, 843-855.

SCHANTZ, E.J., 1967. Biochemical studies on purified *Gonyaulax catenella* poison. pp91-95. In, Animal Toxins (ed. F.E. Russell and P.R. Saunders). Pergamon Press Ltd, Oxford and New York, 444pp.

SOMMER, H. and MEYER, K.F., 1937. Paralytic shellfish poisoning. Archs Path., 24, 560-598.

WOOD, P.C., 1968. Dinoflagellate crop in the North Sea. Introduction. Nature, Lond., 220, 21.

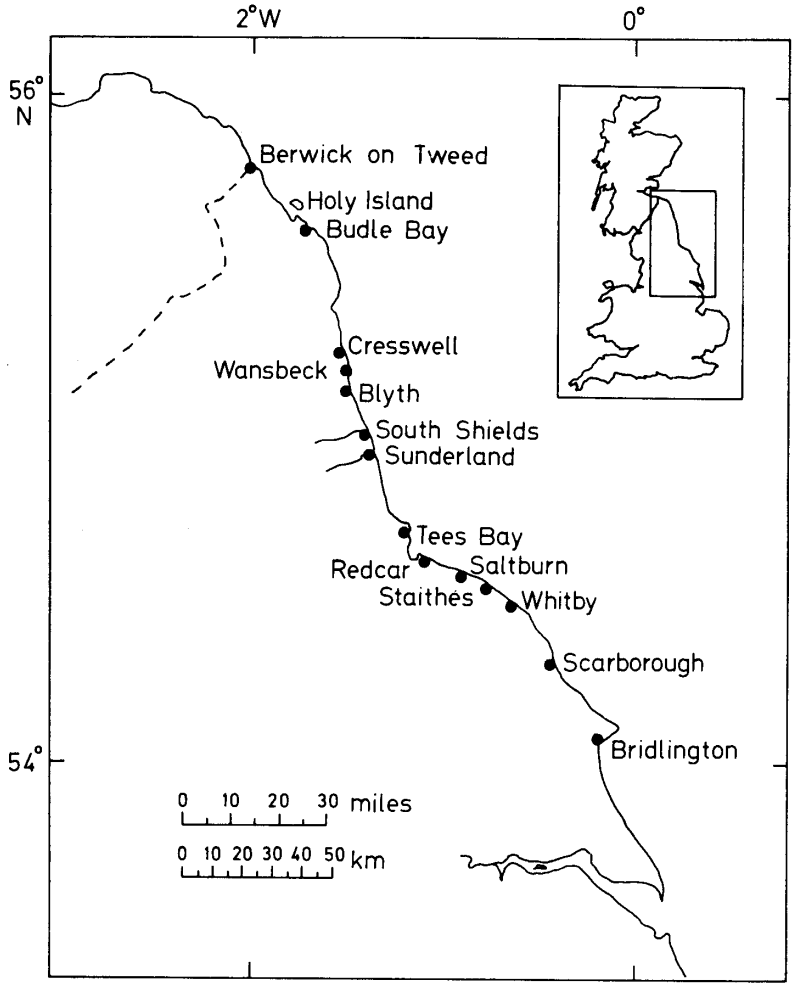
WOOD, P.C. and MASON, J., 1968. Paralytic shellfish poisoning: a short account of an outbreak occurring on the north-east coast of Britain in May 1968. ICES CM 1968/K:16, 6pp (mimeo).

WOOD, P.C., 1969. Mussel toxicity in Great Britain, 1969. ICES CM 1969/K:25, 4pp (mimeo).

WOOD, P.C. and AYRES, P.A., 1970. Mussel toxicity and dinoflagellate distribution off north-east Britain in 1970. ICES CM 1970/K:12, 6pp (mimeo).

APPENDIX

Mussel Toxicity on the North-east Coast of England, 1977



Appendix Figure 1 Map showing area sampled and location of sampling sites during 1977

Monitoring of toxicity levels of mussels, as developed in 1968-76, was continued in 1977 (Figure A1). During the sampling period March-early August, mussel samples were taken weekly where possible. The 1977 survey also included some additional stations not monitored in previous years; samples from these were obtained with the co-operation of the Environmental Health Departments of local authorities. Sampling was also initiated at some sites but later abandoned because only small, seed mussels, unsuitable for analysis, were available.

Results

Toxicity levels of mussels in the 135 samples taken are given in Appendix Table 1.

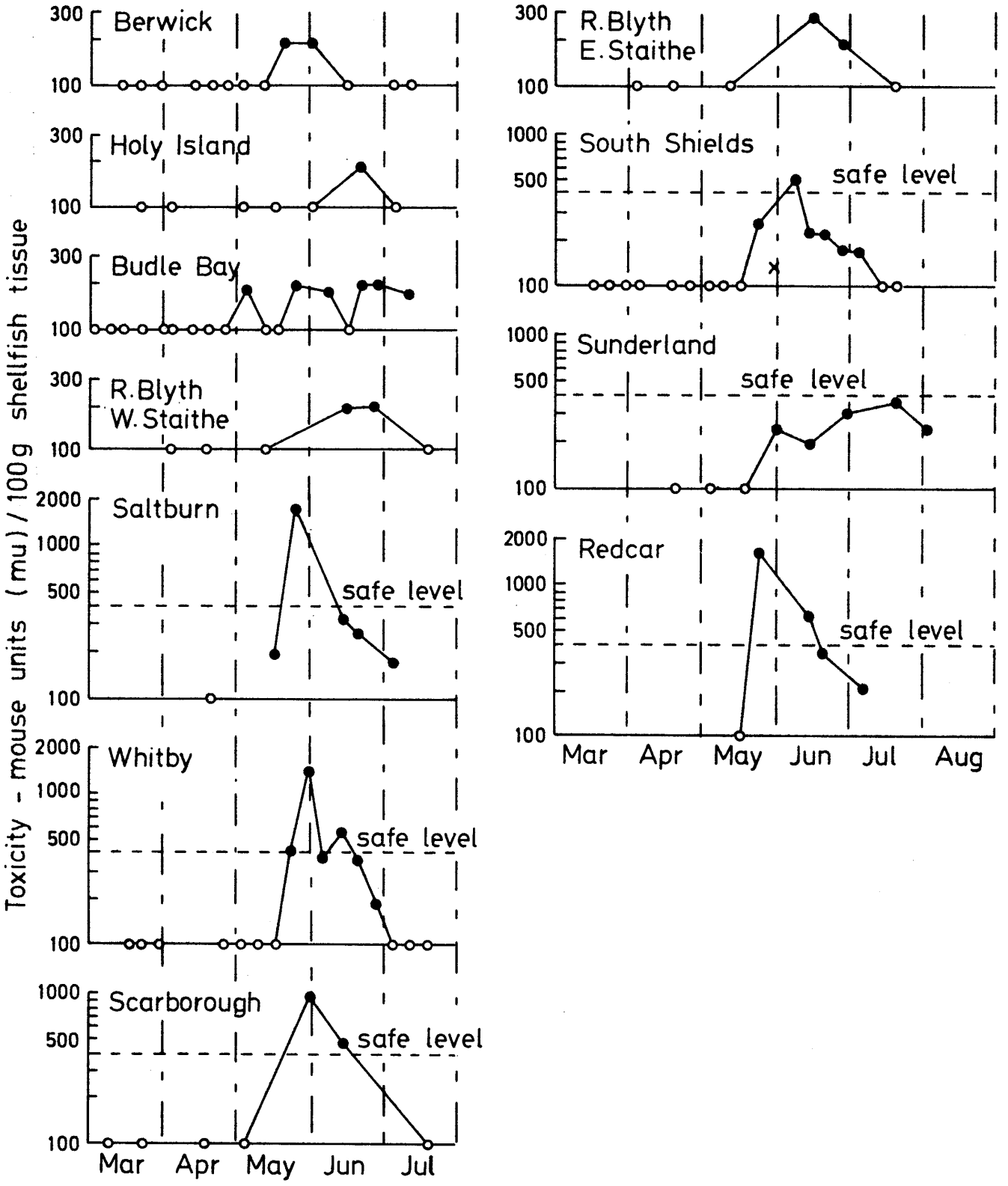
Toxicity was detected at low levels at Budle Bay in the first week of May (188 mu/100 g) and was evident at stations along the coast between Berwick and Scarborough by the end of the month. Sampling at some sites was sporadic, but at other sites where sampling was more regular it was possible to follow in some detail the development and decline of toxicity in mussels. The levels of toxin at these stations are shown in Appendix Figure 2.

Toxicity was detected at stations north of South Shields but remained at low levels throughout the sampling period and did not exceed the adopted safe level of 400 mu/100 g of shellfish tissue. At South Shields one sample in early June exceeded the safe level with a bioassay result of 420 mu/100 g. Marked toxin development was evident at stations south of Tees Bay (Hartlepool area), with peak toxicity occurring at the end of May. The maximum level found was 1792 mu/100 g at Saltburn. During June and July toxicity declined throughout this southern area but remained at low, fluctuating levels at Budle Bay and Sunderland. Sampling cased in July at all stations except Sunderland where sampling continued until toxin levels declined in early August.

Appendix Table 1 Levels of toxin in mussels from the north-east coast of England, 1977 (mouse units/100 g)

		Mar				Apr					May				Jun				Jul					Aug	
Station	Week ending	5/3	12/3	19/3	26/3	2/4	9/4	16/4	23/4	30/4	7/5	14/5	21/5	28/5	4/6	11/6	18/6	25/6	2/7	9/7	16/7	23/7	30/7	6/8	13/8
Berwick		-	-	*	*	*	-	*	*	*	*	*	181	-	187	-	*	-	-	*	-	-	-	-	-
Budle Bay		*	*	*	*	*	*	*	*	*	188	*	*	214	*	180	*	198	196	-	177	-	-	-	-
Holy Island		-	-	-	*	-	*	-	-	-	-	-	*	-	*	-	-	190	-	*	-	-	-	-	-
Cresswell		-	-	-	-	*	-	-	-	-	-	-	*	-	-	*	-	*	-	-	-	-	-	-	-
Wansbeck		-	-	-	-	-	*	-	*	-	-	*	-	-	-	-	180	-	*	-	-	*	-	-	-
R. Blyth East Staithe		-	-	-	-	-	*	-	*	-	-	*	-	-	-	-	274	-	180	-	-	*	-	-	-
West Staithe		-	-	-	-	-	*	-	*	-	-	*	-	-	-	-	192	-	194	-	-	*	-	-	-
South Shields		-	-	*	*	*	*	*	-	*	*	*	*	260	*	420	227	229	173	+ 178	*	*	-	-	-
Sunderland		-	-	-	-	-	-	-	*	-	*	-	*	-	233	-	194	-	310	-	-	365	-	-	254
Hartlepool		-	-	-	*	*	*	-	-	-	-	-	-	-	-	-	-	175	-	-	-	-	-	-	-
Redcar		-	-	-	-	-	-	*	-	-	*	-	*	1624	-	-	638	364	-	204	-	-	-	-	-
Saltburn		-	-	-	-	-	-	*	-	-	-	-	192	1792	-	-	330	268	-	175	-	-	-	-	-
Staithes		-	-	-	-	-	-	*	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Whitby		-	-	*	*	*	-	-	-	*	*	*	*	413	1344	362	525	186	-	*	*	*	-	-	-
Scarborough		-	*	-	*	-	-	*	-	-	*	-	-	1020	970	-	464	-	-	-	-	*	-	-	-
Bridlington		-	-	-	-	-	*	-	*	-	*	-	-	-	*	-	-	*	-	*	-	-	-	-	-

* negative response
\+ sublethal response
\- no sample

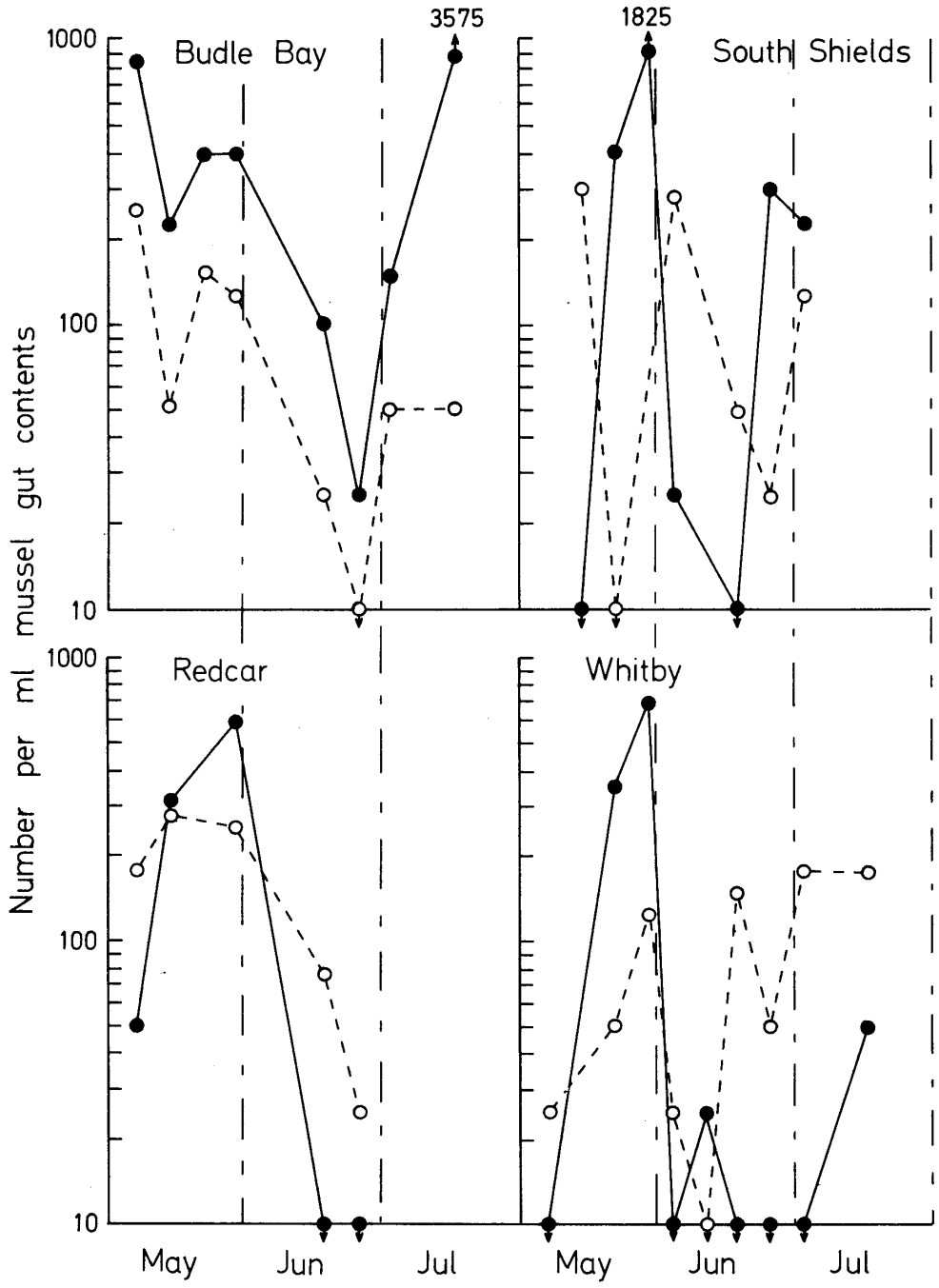


Appendix Figure 2 Toxin levels in mussels from the north-east coast of England

In order to examine possible origins of the toxicity observed, the phytoplankton results from mussel gut analysis were taken from four stations representative of the area sampled and where sampling was frequent enough to permit graphical presentation. These results are shown in Appendix Figure 3 as the total numbers of dinoflagellates and of dinoflagellate cysts per ml of mussel gut contents for the period May to July.

At Budle Bay low but fairly persistent levels of toxin were marked by the early appearance of dinoflagellates and cysts which showed a general decline during the period of toxicity. Initital development of toxicity was marked by a dominance of *Peridinium* and *Gonyaulax* spp these genera were replaced by an *Exuviaella* sp which accounted for almost all the dinoflagellate total recorded in mid-July. The pattern exhibited by dinoflagellate numbers was closely paralleled by that of cysts, although the latter were less numerous.

At South Shields a different pattern of dinoflagellate development was apparent and peak numbers were observed at the end of May just prior to the development of maximum toxicity in mussels. Dominant dinoflagellates were again *Peridinium* and *Gonyaulax* spp the later peak in dinoflagellate numbers observed in late June was due to an



Appendix Figure 3 Numbers of dinoflagellates and dinoflagellate cysts in mussels at selected stations, May-July 1977

- ● Total dinoflagellates
- o Dinoflagellate cysts

Exuviaella sp. Numbers of cysts observed were almost inversly proportional to dinoflagellate numbers.

Maximum toxicity recorded at Redcar and Whitby at the end of May coincided with the appearance of maximum numbers of *Peridinium* and *Gonyaulax* spp, although at Redcar the dominant dinoflagellate was an *Exuviaella* sp. Numbers of cysts at the two stations showed different trends; at Redcar they followed the appearance and decline of total dinoflagellate numbers, and at Whitby they fluctuated at low levels during the May-July period.

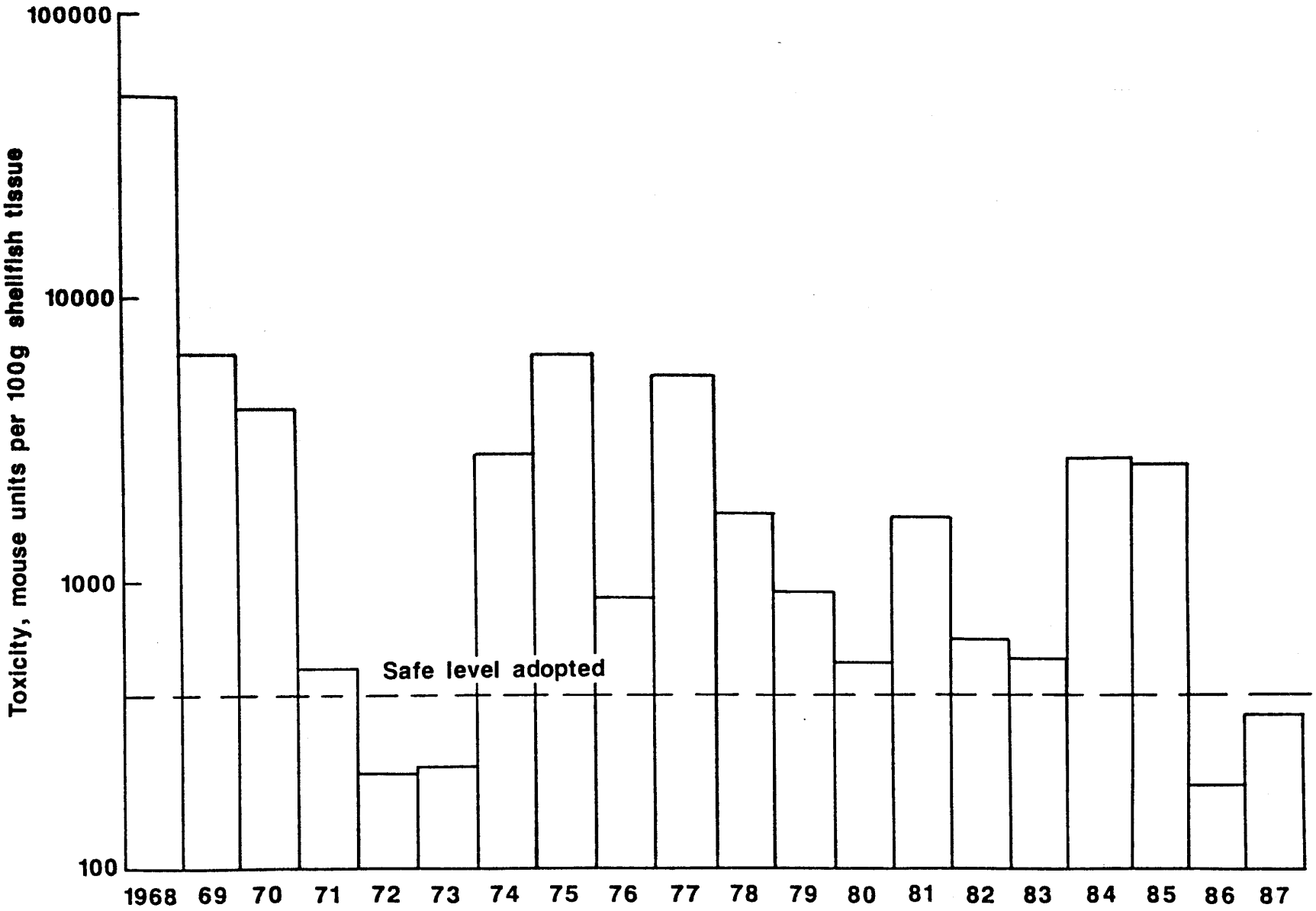
Taking a broad view of the results from all stations sampled and comparing phytoplankton and toxin observations, *Gonyaulax* was the only genus represented at all stations at the time when toxicity reached maximum values. At the majority of stations *Peridinium* spp and an *Exuviaella* sp were also present, often in numbers exceeding those of *Gonyaulax* spp. As toxin levels declined both *Gonyaulax* and *Peridinium* spp disappeared, but the *Exuviaella* sp persisted or increased towards July.

Discussion

The precise identification of dinoflagellates in mussel gut contents is fraught with difficulties, primarily due to the very small amount of material available and also to damage sustained in the gut. Additionally many of the *Peridinium* spp and *Gonyaulax* spp. are superficially very similar in gross morphology with only very subtle distrinctions between species within the two genera. However, from the results obtained during 1977 and experience of previous years' monitoring, both *Gonyaulax* and *Peridinium* spp appear in the phytoplankton about the same time, although individual species of *Peridinium* eg *P. depressum* may precede the *Gonyaulax* spp by a few weeks. As shown both here and previously there is an obvious association between these two genera of dinoflagellates and the development of toxicity in mussels. Although the *Exuviaella* sp was also present, numbers of this organisms often increased while toxin levels in shellfish declined. The results for 1977 suggest the development of toxin-producing dinoflagellates in the area south of Tees Bay, ie along the Yorkshire coast, with sporadic and possibly unrelated minor centres of toxin production elsewhere. The maximum level of toxin recorded during 1977 was 1792 mu/100 g at Saltburn, compared with 869 mu/100 g at Sunderland in 1976.

Acknowledgements

The monitoring programme described would not have been possible without the valued assistance of various Environmental Health Officers, Fishery Officers, fishermen and others who provided samples.



Revised Figure 3 Mussel toxicity on the north-east coast of England: maximum values recorded 1968-87