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Long-term Monitoring of Polycyclic Aromatic Hydrocarbons in Mussels (*Mytilus edulis*) Following the *Braer* Oil Spill†

Lynda Webster[a], Lindsay Angus[b], Graham Topping[a], Eric J. Dalgarno[a] and Colin F. Moffat*[a]

[a] *FRS Marine Laboratory Aberdeen, P.O. Box 101, Victoria Road, Aberdeen, UK AB11 9DB*
[b] *North Atlantic Fisheries College, Port Arthur, Scalloway, Shetland, UK ZE1 0UN*

On January 5, 1993, 84 700 t of Norwegian Gullfaks crude oil was released into the coastal region of south Shetland when the tanker *MV Braer* grounded at Garths Ness. A Fisheries Exclusion Zone was designated under the Food and Environment Protection Act 1985 (FEPA), prohibiting the taking or harvesting of fish or shellfish within the Zone so as to prevent contaminated products reaching the marketplace. The criteria set for lifting of the Order were that the particular species must be free from any petrogenic taint and the concentration of polycyclic aromatic hydrocarbons (PAHs) must be within the range for reference samples. Between April 1993 and February 1995 the Order was progressively lifted for wild fish, salmon, crustacea, excluding *Nephrops norvegicus* (Norway lobster), and molluscs, with the exception of mussels. As part of the monitoring exercise, mussels from a reference site were transplanted in June 1995 to three sites within the Zone, where they were suspended in plastic mesh boxes from rafts to a depth of 5 m. Samples were collected at regular intervals over the following 12 months and the concentration and composition of PAHs were determined by gas chromatography with mass spectrometric detection. The total measured PAH concentration at the control site increased from 13.7 to 66.1 ng g^{-1} wet mass of tissue between June 1995 and February 1996. This trend was reversed by July 1996 when the PAH concentration was 12.8 ng g^{-1}. The mean across the year for the control site was 24.0 ng g^{-1} (SE = 8.9 ng g^{-1}, *n* = 6). A similar seasonal trend in PAH concentration over the year was observed at all sites within the Zone, but the PAH concentration was consistently greater at these sites, reaching a maximum concentration of 316 ng g^{-1} in February 1996. Although no taint was detected in any of the mussels, these results meant that it was not possible to lift the Prohibition Order for mussels. Further monitoring at three sites outwith the Zone and three sites within the Zone is under way together with investigations into the specific source of the PAHs.

Keywords: *Polycyclic aromatic hydrocarbons; mussels; gas chromatography–mass spectroscopy; oil spill*

The tanker *MV Braer* grounded on Garths Ness, Shetland, on January 5, 1993. Over the following 7 d the entire cargo of approximately 85 000 t of Norwegian Gullfaks light crude oil, a naturally biodegraded oil resulting in a more naphthenic and aromatic crude, was released from the ship, together with some bunker fuel oil. A Fisheries Exclusion Zone was designated on January 8, 1993, by Order under the Food and Environment Protection Act 1985 (FEPA). The Order prohibited the harvesting of farmed or wild fish or shellfish within the Zone to prevent contaminated products reaching the marketplace. The Zone was extended 5 miles westward on January 27, 1993 (see Fig. 1).[1] The Scottish Office Agriculture, Environment and Fisheries Department (SOAEFD) instituted a programme of

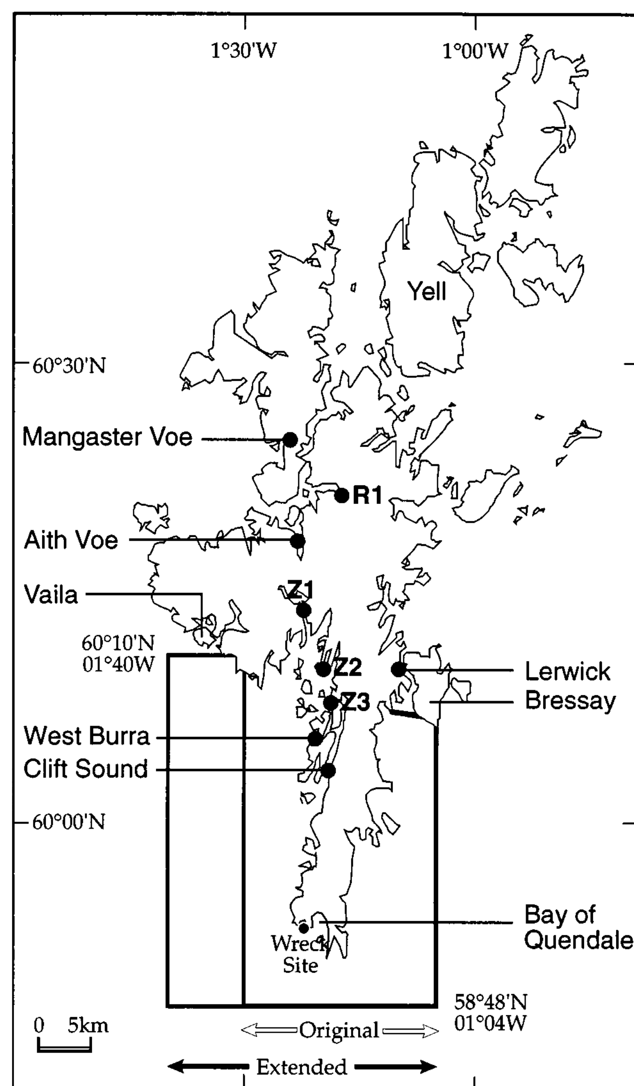

Fig. 1 Map of Shetland showing the FEPA Exclusion Zone together with the location of the reference site at Olna Firth (R1) and the sites within the Zone (Z1–Z3). Z1, Sandsound Voe; Z2, Stromness Voe, which contained a northerly (N) and southerly (S) site; and Z3, Merry Holm/Trondra. The *Braer* grounded at Garths Ness on the southerly tip of the Shetland Islands close to the Bay of Quendale.

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marine monitoring with the aims of ensuring that the original decision to institute the FEPA Zone was a sensible one, that its limits had been correctly drawn and to provide support for any decision-making process with regard to the possible extension or future lifting of the Zone.

The criteria for revoking the FEPA Order for fish and shellfish were first, that the fish and shellfish from within the Zone should not contain taint that is associated with crude oils and petroleum fractions, and second, that the concentration of aliphatic hydrocarbons and PAHs in fish and shellfish from within the Zone should fall within the background range of values for fish and shellfish outside the Exclusion Zone.[2]

It was apparent from the first analytical measurements of hydrocarbons in fish and shellfish that the concentration of aliphatic hydrocarbons in samples from within the Zone was generally low and that the distribution of these hydrocarbons in Gullfaks crude was not distinctive. Therefore, in terms of the aims of the programme, attention was focused on the PAHs. Naphthalenes (75.6%), phenanthrenes (12.5%) and dibenzothiophenes (5.4%) were the major PAHs in Gullfaks crude oil with lesser amounts of the fluoranthenes and pyrenes (4.2%), benzophenanthrenes, benzanthracenes and chrysenes (1.5%) and five- and six-ring PAHs (0.7%).[2] As a result of the monitoring programme, the Order was lifted for wild fish on April 23, 1993, farmed salmon on December 8, 1993, crustaceans, with the exception of *Nephrops norvegicus* (Norway lobster), on September 30, 1994, and molluscs, with the exception of mussels, on February 9, 1995.

The blue mussel (*Mytilus edulis*), a circumboreal species,[3] is farmed from rafts in the sea lochs around the Shetland Islands. Some of these mussel farms were within the Exclusion Zone. Mussels from the western area of the Exclusion Zone were sampled during 1993 and 1994 and the PAH concentration, defined as the combined concentration of the two- to six-ring parent and branched PAHs, was determined in the edible tissue. A total measured PAH concentration of 1450 ng g^{-1} wet mass of tissue was determined in a sample taken from Stromness Voe in March 1993, this being the greatest concentration found in mussels.[2] The mean PAH concentration in the samples collected from various locations within the Zone during October 1994 was 221 ng g^{-1} (SE = 60 ng g^{-1}, n = 5). This contrasts with a mean for reference samples, collected from a mussel farm in Olna Firth (March 1993) and from Aith Voe (February, June and October 1994), of 56.8 ng g^{-1} (SE = 17.2 ng g^{-1}, n = 4).[2] Mussels are known to accumulate trace contaminants, such as heavy metals and hydrocarbons, present in the water column.[4] As such, it was concluded that mussels could be utilized as an organism for monitoring long-term hydrocarbon pollution in marine waters.[5] For these reasons, the decision was taken to transplant animals from a site well outside the Zone to various sites within the Zone and to monitor the change in PAH concentration with time.

Experimental

Reagents

Methanol, isohexane, dichloromethane and acetone were glass-distilled reagents specifically prepared for hydrocarbon analysis by Rathburn Chemicals (Walkerburn, UK). HPLC-grade water was also supplied by Rathburn Chemicals. Individual batches of all solvents were checked for contaminants as described previously.[6] Analytical-reagent grade nitric acid was purchased from BDH (Poole, Dorset, UK). Sodium chloride, sodium hydroxide and anhydrous sodium sulfate were analytical-reagent grade reagents from Fisons Scientific Equipment (Loughborough, UK). Deuteriated naphthalene, biphenyl, dibenzothiophene, anthracene, pyrene and benzo[*a*]pyrene were obtained from C/D/N/Isotopes through K&K-Greeff (Croydon, UK).

Preventative Measures for Reducing Casual PAH Contamination

PAHs are ubiquitous in the environment and great care must be taken to avoid adventitious contamination of samples. To this end, all glassware was washed and dried in a GW 4000 glassware washer (Camlab, Cambridge, UK). Prior to use, the glassware was rinsed twice with dichloromethane and then twice with isohexane, the latter being allowed to evaporate before proceeding. The columns used for the sodium sulfate filtration were soaked at regular intervals in concentrated nitric acid to clean the frits. The columns were then flushed with copious volumes of water before being washed as described above. The sodium sulfate was cleaned by washing with isohexane in an ultrasonic bath for 10 min. The solvent was decanted to waste and the sodium sulfate placed in an oven at 110 °C overnight. The use of Socorex PTFE-lined pipettes (Camlab) with disposable glass Pasteur pipettes, the minimum presence of any plastics, a strict regime for storage of samples, environmental control of the laboratory and assignment of all equipment to specific areas of analysis are all further precautions taken to avoid such contamination.

Test Sites

The test sites included a reference site at Olna Firth (Site R1, Fig. 1) and three sites within the Exclusion Zone: Sandsound Voe (Site Z1), Stromness Voe (Site Z2, two farms) and Merry Holm (Site Z3). Three of the sites, R1, Z1 and Z2, were located within discrete voes which were remote from any urban or industrial areas, were associated with only one minor road and would have only small-boat traffic. In contrast, Merry Holm (Z3) was a more open, in-shore site approximately 2.5 km south west of Scalloway harbour. Samples of sediment were collected at each site, from the Fisheries Research Vessel *Clupea*, using a Day grab. The sediments at Z3 were characterised as medium sand/shell sand whereas those at Sandsound (Z1) were a mixture of fine sand and mud. The reference site and Stromness Voe (Z2) contained muddier sediments. Total organic carbon concentration (mean ± s, n = 6) for the sediments, determined using a Model 2400 CHN Elemental Analyser (Perkin-Elmer, Beaconsfield, Bucks., UK), was 5.080 ± 0.807%, 5.155 ± 1.082% and 6.160 ± 0.678% for Sandsound Voe, Stromness Voe and Olna Firth, respectively. The total organic carbon content of the sediment around Merry Holm was lower at 1.384 ± 0.124%.

Mussels

Mussels (*Mytilus edulis*), of uniform age, were transplanted from the reference mussel farm in Olna Firth (Site R1) in mid-June 1995 to the three sites within the Zone (Fig. 1). Samples of approximately 70 mussels were placed in numbered plastic mesh boxes and suspended from rafts to a depth of 5 m. Sampling, which comprised collection of one of the mesh boxes, took place during August 1995, October 1995, February 1996 and June/July 1996. The mussels were thoroughly iced and dispatched to Aberdeen by overnight ferry. On arrival in Aberdeen, approximately 20 mussels were removed for sensory assessment. The tissue was removed from the shell of the bulk of the remaining animals, combined and homogenised. A portion was taken for chemical analysis. All residual material was packaged and stored at −30 °C in case repeat analysis was required.

Isolation of Hydrocarbons

The method was based on that of Grimmer and Böhnke.[7] To a homogenised sample of mussel (10 g) were added the aliphatic hydrocarbon internal standards heptamethylnonane and squalane (approximately 3.2 μg of each). A mixture of deuteriated

naphthalene, biphenyl, dibenzothiophene, anthracene, pyrene and benzo[*a*]pyrene (100 μl; approximately 1 μg ml^{-1} each) was then added. This was mixed with sodium hydroxide (10% m/v) in methanol–water (9 + 1 v/v; 40 ml) and 3–5 pre-washed anti-bumping granules. The mixture was refluxed for 3 h 45 min before the addition of water (10 ml) and then refluxing was continued for a further 15 min. The resulting hot solution was extracted with isohexane (80 ml) following the addition of methanol–water (4 + 1 v/v; 40 ml). A second extraction of the aqueous solution with isohexane (80 ml) was performed. The first organic extract was washed with methanol–water (1 + 1 v/v; 40 ml) and this aqueous solution was then used to wash the second organic extract. The two isohexane extracts were combined and washed with water (3 × 40 ml). The resulting organic solution was dried by passage through a column (11 × 1.5 cm id) containing sodium sulfate (approximately 60 g). The column was washed with isohexane (50 ml) and the combined solvent concentrated to approximately 300 μl by rotary evaporation (water bath, <30 °C). The concentrate was transferred into a vial and concentrated back to approximately 300 μl under a stream of scrubbed nitrogen.

The PAHs were isolated from the aliphatic hydrocarbons by isocratic normal-phase HPLC. An aliquot (150 μl) of the concentrated isohexane solution was injected on to a Genesis SIL 4 μm HPLC column (25 × 0.46 cm id) (Jones Chromatography, Hengoed, UK) and eluted with isohexane at 2 ml min^{-1}. The aliphatic fraction was collected between 0 and 2.75 min and the aromatic fraction between 2.75 and 20 min. The resulting eluates were separately concentrated under reduced pressure prior to transfer to a chromatographic vial insert (Hewlett-Packard, Stockport, UK), where they were further concentrated to approximately 15–20 μl under a stream of scrubbed nitrogen. The sides of the vial insert were carefully washed down with the concentrate before being capped. A procedural blank was analysed with each batch of samples.

Determination of Polycyclic Aromatic Hydrocarbons

The concentration and composition of the PAHs were determined by gas chromatography with mass spectrometric detection (GC–MS). Samples (1 μl) were chromatographed on an HP 5890 Series gas chromatograph equipped with an HP 7673A on-column injector and fitted with a fused silica capillary column (25 m × 0.2 mm id) coated with a 0.33 μm film of Ultra 1, a cross-linked methylsilicone gum (Hewlett-Packard). Injections were made at 50 °C and the oven temperature was held constant for 3 min, after which it was increased at 20 °C min^{-1} to 100 °C. This was followed by a slower ramp of 4 °C min^{-1} up to a final temperature of 270 °C. The oven temperature remained constant until the end of the analysis. Helium (10 lb in^{-2}) was used as the carrier gas. The gas chromatograph was interfaced with an HP 5970 Series mass selective detector (Hewlett-Packard), which was set for selective ion monitoring (SIM) with a dwell time of 50 ms. A total of 25 ions plus the six internal standard ions were measured over the period of the analysis, as detailed previously.[2] Thus, the analysis incorporated two- to six-ring, parent and branched PAHs. This does not cover all of the many PAH compounds that exist. Thus, all references to PAH concentrations and distributions relate to the measured PAHs, details of which are presented in Table 1. Perfluorotributylamine was used as the mass spectrometric calibrant.

Standards for all the parent and branched PAHs cannot be obtained, but the limit of detection, calculated as three times the standard deviation of the mean value from six procedural blanks, was found to be <0.2 ng g^{-1} for benzo[*k*]fluoranthene and benzo[*a*]pyrene and <0.3 ng g^{-1} for chrysene. Good reproducibility was generally obtained for individual PAHs (Table 1). Further quality control was assured through participation in the PAH programme of QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe).

Assessment of Taint

The mussels were steamed for approximately 4 min or until the shells were completely open. At this point the meat was transferred to a lidded casserole and kept warm on an electric hot-plate for the duration of the tasting session (15–20 min). The mussels were assessed 'blind' by each member of a panel

Table 1 Duplicate determination of the PAHs (ng g^{-1} wet mass of tissue) in a sample of mussel tissue. The numbers following the name refer to the molecular mass. The sum of the molecular mass groups is also presented. The total PAH concentration was 316.3 and 328.5 ng g^{-1} for samples 1A and 1B respectively

	Sample			Sample	
PAH	1A	1B	PAH	1A	1B
Naphthalene	0.9	0.6	C_3 202	11.5	11.4
C_1 Naphthalenes	1.0	0.9	Sum of 202s	55.0	69.1
C_2 Naphthalenes	6.1	5.3	Benzo[*c*]phenanthrene (228)	0.8	0.9
C_3 Naphthalenes	16.4	17.3	Benz[*a*]anthracene (228)	1.7	4.4
C_4 Naphthalenes	0.8	0.5	Chrysene + triphenylene (228)	4.7	1.3
Sum of naphthalenes	25.2	24.6	Benz[*a*]anthracene (228)	nd	nd
Phenanthrene	5.5	5.6	C_1 228	7.2	6.3
Anthracene	nd*	nd	C_2 228	3.1	3.4
C_1 178	31.8	32.6	Sum of 228s	17.5	16.3
C_2 178	63.9	60.4	Benzofluoranthene (252)	8.0	6.7
C_3 178	50.3	49.7	Benzo[*e*]pyrene (252)	3.7	4.0
Sum of 178s	151.5	148.3	Benzo[*a*]pyrene (252)	1.0	0.9
DBT†	0.5	0.6	Perylene (252)	1.4	1.3
C_1 DBT	8.3	8.1	C_1 252	3.1	3.1
C_2 DBT	22.2	21.7	C_2 252	nd	2.0
C_3 DBT	16.2	18.3	Sum of 252s	17.2	18.0
Sum of DBTs	47.2	48.7	Indenopyrene (276)	1.2	1.2
Fluoranthene (202)	4.2	4.8	Benzoperylene (276)	1.5	1.8
Pyrene (202)	4.0	4.6	C_1 276	nd	0.5
C_1 202	17.3	14.8	C_2 276	nd	nd
C_2 202	18.0	33.5	Sum of 276s	2.7	3.5

* nd, Not detected. † DBT, dibenzothiophene.

of 8–10 staff trained to recognise petroleum-derived taints by odour and taste and scored as described by Whittle *et al.*[1]

Results and Discussion

It is essential in any environmental monitoring programme to have a benchmark against which the sites of potential contamination can be assessed. Consideration must also be given to the fact that the test matrix is a living, growing animal. The objective of transferring samples from a single reference site to several locations within the Zone was to ensure that there was a commonality for the test matrix. The total measured PAH concentration of the mussels at time of transfer was 13.7 ng g^{-1}. All the individual parent PAHs and associated branched groups were present at <2 ng g^{-1} and many were not detected. The largest individual grouping was the three-ring compounds (phenanthrene, molecular mass 178, and the C_1–C_3 substituted compounds) which comprised 38% of the PAHs. The naphthalenes comprised 24% of the PAHs and the five-ring compounds 18%. The concentration of PAHs at the control site increased progressively over 8 months to a maximum of 66.1 ng g^{-1} in the sample collected in February 1996 (Fig. 2). This was a result of an increase in the concentration of the four- to six-ring compounds, the concentration of the naphthalenes and three-ring PAHs remaining relatively consistent over the year. The greatest PAH group concentration was 19.7 ng g^{-1}, determined for the five-ring PAHs isolated from the February sample.

The observed increase in PAH concentration over autumn and winter was reversed by July 1996, when the PAH concentration was found to be 12.8 ng g^{-1}. This resulted from a decrease in the concentration of the larger ring PAHs. Thus, the proportion of two- and three-ring compounds again dominated the PAH profile. The mean PAH concentration across the year for the control site was 24.0 ng g^{-1} (SE = 8.9 ng g^{-1}, n = 6). No taint was detected in any of the mussels from the reference site.

After 2 months, an increase in the PAH concentration was detected in the mussels from both sites in Stromness Voe (Site Z2) but not the more northerly site at Sandsound Voe (Site Z1, Fig. 2). This change was a result of an increase in the concentration of all PAH groupings but there was a relative decrease in the proportion of naphthalenes present. The PAH distributions at the two sites in Stromness Voe were very similar. The three-ring compounds still dominated at 38% but the naphthalenes comprised only 18% and 19% of the PAHs at the northerly and southerly sites, respectively. In contrast, the proportion of four-ring compounds had increased from 15% at the time of transplanting to 26% at the northerly site and 25% at the southerly site.

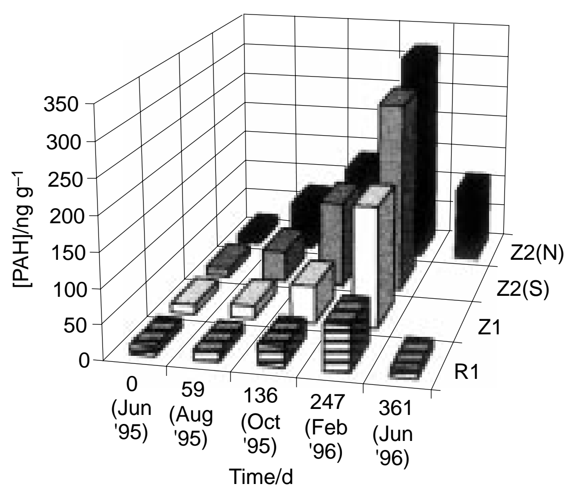

Fig. 2 Variation in PAH concentration (ng g^{-1} wet mass of tissue) with time for mussels collected from a reference site (R1) and from sites within the Zone. In all cases there is a progressive increase in PAH concentration with time over the autumn and winter months. The final samples from both the reference site and northerly site in Stromness Voe [Z2(N)], collected in early summer, show a decrease relative to the February sample. The PAH concentrations are consistently greater at sites within the Zone relative to the reference site.

All sites within the Zone displayed a progressive increase in PAH concentration between August 1995 and February 1996 with a maximum concentration of 316 ng g^{-1} in Stromness Voe (Fig. 2). Neither this, nor any other sample from within the Zone, was found to be tainted. The dominant group was the C_{16} four-ring PAHs (pyrene, fluoranthene and C_1–C_3 substituted compounds), which comprised approximately 30% of the PAHs (Table 2). Although there was no August sample for site Z3, samples were obtained from this location during October 1995 and February 1996 when the PAH concentrations were determined to be 133 and 279 ng g^{-1}, respectively. Thus, the trend was maintained at this site. Although the concentration in the October sample from Z3 was similar to that of the northerly site in Stromness Voe, the PAH distribution was distinct, having 35% naphthalenes. In contrast, the PAH distribution of the February sample from Z3 was similar to the other sites within the Zone (Table 2). Indeed, the percentage compositions of the PAHs across the sites within the Zone for the samples collected in February were very similar. The mean percentage (with standard error) for the various PAH groupings at the four sites were: naphthalenes 4% (0.6%); 178, 18% (0.5%); DBTs 2% (0.3%); 202, 30% (0.6%); 228, 18% (0.4%); 252, 24% (0.8%); 276, 5% (0.4%).

Severe storms resulted in the loss of some of the mesh boxes from the rafts. This meant that there was only one Zone sample left, at the northerly site in Stromness Voe, in the summer of 1996. As with the equivalent reference sample, a decrease in PAH concentration was observed, relative to the February sample, but the value of 108 ng g^{-1} was greater than that at the reference site. As with the mussels from Olna Firth, the dominant PAH grouping was the three-ring compounds at 40% with the naphthalenes comprising 20% of the measured PAHs.

There was an apparent seasonal trend for the PAH concentration in mussels, the concentration increasing over winter and declining in the spring. From autumn until spring lipids may be saved for gametogenesis[8] and this increase in lipid content would permit the retention of increased amounts of lipophilic compounds such as PAHs. The maturation of the gametes is

Table 2 Percentage distribution of the various PAH groupings for the PAHs isolated from mussels collected at sites within the Exclusion Zone during February 1996

	Site within the Exclusion Zone			
PAH group*	Z1	Z2 (North)	Z2 (South)	Z3
Naphthalenes	4	6	3	4
178	17	19	18	19
DBT	1	1	2	2
202	31	29	30	28
228	19	17	18	18
252	25	23	22	25
276	4	5	6	5

* Naphthalenes, naphthalene and C_1–C_4 branched compounds; 178, phenanthrene/anthracene and C_1–C_3 branched compounds; DBT, dibenzothiophene and C_1–C_3 branched compounds; 202, C_{16} four-ring PAHs and C_1–C_3 branched compounds; 228, C_{18} four-ring PAHs and C_1–C_2 branched compounds; 252, C_{20} five-ring PAHs and C_1–C_2 branched compounds; 276, C_{22} six-ring PAHs and C_1–C_2 branched compounds.

under several exogenous (*e.g.*, water temperature, lunar periodicity, depth, food abundance and availability, light intensity) and endogenous (*e.g.,* genetic, hormonal) controls. Of all the external controls, temperature is generally regarded as the most important and the act of spawning is initiated when the water temperature generally exceeds 10–12 °C for *Mytilus edulis,*[9] although in Shetland the relevant temperature range is regarded as 8–12 °C. The principal spawning time is, thus, late spring/early summer, but it is obviously affected by the prevailing weather conditions in any year. During 1996, some mussels in Shetland were observed to spawn in early June and this coincides with the decrease in PAH concentration. The seasonal trend can, therefore, be related to physical and chemical changes in the animals.

In order for bioaccumulation of PAHs to occur, these compounds must be bioavailable. PAHs may be present dissolved in the water or in the sediment where they are incorporated in the biota, associated with particulate organic and inorganic matter, associated with dissolved organic matter and truly dissolved in the interstitial water. Filter-feeding molluscs are exposed to both water and suspended particulate material (SPM), which would include resuspended sediments. The relative importance of dissolved and particulate PAHs as a source for PAH accumulation in mussels is uncertain. Pruell *et al.*[10] concluded that the dissolved phase was the direct source of contaminants accumulated by mussels. In contrast, Naes *et al.*[11] suggested that particulate PAH is the main source for mussels. This being so, winter storms would result in a degree of resuspension of the sediments, which, if they contained PAHs, would mean an increase in the exposure of the animals to these organic xenobiotics over the winter. The higher concentrations observed for sites within the Zone could result from either a greater exposure to SPM or a greater concentration of PAHs within the SPM.

The primary objective of the monitoring programme was to provide data that enabled pertinent decisions to be made with regard to a change in status of the Exclusion Zone. It was evident that the PAH concentration for mussels within the Zone was consistently greater than for the reference site, hence it was not possible to lift the Zone for mussels.

Further monitoring is necessary and a second, 3 year, mussel experiment was initiated in September 1996. The seasonal variation, evident for the PAHs, illustrates the clear need for pertinent reference sites which must be sampled at the same time as the test sites and to this end the new experiment incorporates a further two reference sites, at Mangaster Voe and Vaila (Fig. 1). Studies into the source of the PAHs have been initiated and additional investigations into the association of fat content with PAH concentration are under way.

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