

Long term monitoring of polycyclic aromatic hydrocarbons (PAHs) in blue mussels (*Mytilus edulis*) from a remote Scottish location

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Introduction

Blue mussels (*Mytilus edulis*) have been used extensively as sentinel indicator species for monitoring uptake and accumulation of hydrophobic contaminants in the marine environment, including polycyclic aromatic hydrocarbons (PAHs). PAHs are mainly produced by pyrolysis, but can also be of petrogenic origin from crude oils or refinery products. There is a continuing concern over the occurrence of PAHs in the marine environment due to their toxicity and persistence. In general, the 2- and 3-ring compounds are acutely toxic and can cause tainting of fish and shellfish while some of the heavier, 4- to 6-ring, PAHs are mutagenic and carcinogenic. The more hydrophilic 2- and 3-ring PAHs, will be more readily available to mussels through the water column than the heavier, more hydrophobic, PAHs (4- to 6- ring), associated with particulate matter.

Following any environmental incident, such as an oil spill, a monitoring programme is put in place to assess the environmental impact of the incident. Following the *Braer* oil spill on Shetland in 1993, PAHs were measured in mussels, collected both within and out with an Exclusion Zone (EZ). Despite there being no petrogenic taint, the EZ could not be lifted as PAH concentrations in mussels harvested from within the EZ were consistently higher than concentrations found in the reference mussels¹. Not until March 2000, when further work demonstrated that the higher PAH concentrations were not due to contamination from Gullfaks crude oil, could the EZ finally be lifted for mussels. High total PAH concentrations have also been found in farmed mussels from Loch Leven, in the western Highland region of Scotland, as a result of discharges from an aluminium smelter². Monitoring demonstrated that concentrations were considerably higher than had previously been reported in mussels sampled from elsewhere around the Scottish mainland.

These environmental incidents have highlighted the need to establish background concentrations for contaminants in a range of matrices so as to have a benchmark against which sites of potential contamination can be assessed. Therefore, mussels from a remote Scottish location, Loch Etive, have been collected regularly over several years so as to provide data on background PAH concentrations in mussels.

Methods

Since June 1999 mussel samples have been collected at monthly intervals from a mussel farm in Loch Etive, situated in the western highland region of Scotland, UK. The soft tissue from between 20 and 30 mussels was homogenised. Deuterated aromatic standards were added to the mussel sample (10 g) which was then saponified and the non-saponifiable material extracted into *iso*-hexane. The extract was washed with methanol:water (1:1 v/v) followed by water, dried over Na₂SO₄ and concentrated to about 500 µl. The *iso*-hexane solution was fractionated by isocratic, normal phase HPLC, to separate the aliphatic and aromatic components.

The concentration of the PAHs (2- to 6-ring parent and branched) was determined by gas chromatography- mass spectroscopy (GC-MS) using an HP6890 Series gas chromatograph interfaced with an HP5973 MS and fitted with a cool on-column injector and a HP5 MS column (30 m x 0.25 mm id, 0.25 µm film thickness). The MSD was set for selective ion monitoring (SIM). A total of 29 ions plus the six internal standard ions were measured over the period of the analysis. The analysis incorporated 2- to 6-ring, parent and branched PAHs. The analytical method is accredited to ISO 17025 by the United Kingdom Accreditation Services (UKAS).

Results and Discussion

Total PAH concentrations (2- to 6-ring parent and branched) in Loch Etive mussels collected over the six year (1999-

2004 inclusive) period ranged from 12.5 $\mu\text{g kg}^{-1}$ wet weight in a sample collected in August 2004 to 151.2 $\mu\text{g kg}^{-1}$ wet weight in a sample collected in March 2000. Seasonal trends in PAH concentrations have been well documented^{1, 3-5} and are clearly seen at Loch Etive (Fig. 1). Seasonal mean total PAH concentrations were 67.4, 62.8, 31.7 and 33.7 $\mu\text{g kg}^{-1}$ wet weight for winter, spring, summer and autumn, respectively; concentrations increased over the winter and started to decrease in spring. From autumn to spring lipids are saved for gameteogenesis and the increase in lipid results in an increase in the uptake of hydrophobic contaminants such as PAHs. Spawning generally occurs in late spring/early summer, although the exact timing is dependent on external factors such as the water temperature. Spawning is associated with a decrease in PAH burden.

PAH concentrations and sources were previously investigated in mussels from a range of coastal locations in Scotland⁶. Mussel samples were collected during March and April 1998. The majority of the rope grown mussels from remote locations had low total PAH concentrations (defined as $< 50 \mu\text{g kg}^{-1}$ wet weight) whereas mussels from estuarine sites had higher total PAH concentrations (defined as $> 150 \mu\text{g kg}^{-1}$ wet weight). Only three Loch Etive samples gave total PAH concentrations close to 150 $\mu\text{g kg}^{-1}$ wet weight. These were collected in March 2000 (151.2 $\mu\text{g kg}^{-1}$ wet weight) and January and February 2001 (150.2 and 148.1 $\mu\text{g kg}^{-1}$ wet weight respectively).

Provisional OSPAR Background Concentrations (BCs) have been established for ten parent PAHs in mussels. Observed concentrations are said to be 'near background' if the mean concentration is statistically significantly below the corresponding Background Assessment Concentration (BAC). BACs were occasionally exceeded for the 4- and 5-ring PAHs in mussels collected in spring and winter from Loch Etive.

PAH Sources

PAH distributions and concentration ratios can be used to distinguish PAHs of petrogenic and pyrolytic origin. A high proportion of the heavier parent PAHs is indicative of a predominately pyrolytic input whereas a high proportion of alkylated 2- and 3-ring PAHs would suggest a petrogenic source. However, this approach should be treated with caution when looking at mussels as different PAHs will have quite different uptake and depuration rates which will alter their profiles. In addition, farmed rope grown mussels (in low turbidity water) would be expected to accumulate a higher proportion of the lower molecular weight, more water soluble PAHs through uptake from the water column. Natural mussels collected from mussel beds, or rope grown mussels in more turbid water, may accumulate a higher proportion of the more hydrophobic, higher molecular weight PAHs, associated with particulate material⁵.

The naphthalenes, DBTs and 6-ring PAHs contributed the least to the total PAH concentrations, with the proportion of 3-ring (phenanthrenes), 4-ring and 5-ring PAHs making the largest contribution (Fig. 2). The PAH profiles of Loch Etive mussels were investigated further using principal component analysis (PCA). The parent and alkylated PAH concentrations were first normalised to the total PAH concentration. The first component was a contrast between the heavier (4- to 6-ring) and lighter (2- and 3-ring) PAHs with the alkylated PAHs being positively correlated with the corresponding parent compounds (Fig 3a). The score plot showed a separation by season on the first component (Fig. 3b). The majority of samples collected in the spring and winter months gave a negative component 1 indicating these samples had a higher proportion of the heavier PAHs. This may be due to the increased turbidity of the water due to storms, resulting in sediment being re-suspended in the water column; any PAHs associated with this suspended particulate material (mainly the heavier 4- to 6-ring PAHs) would be more bioavailable to the mussels.

References

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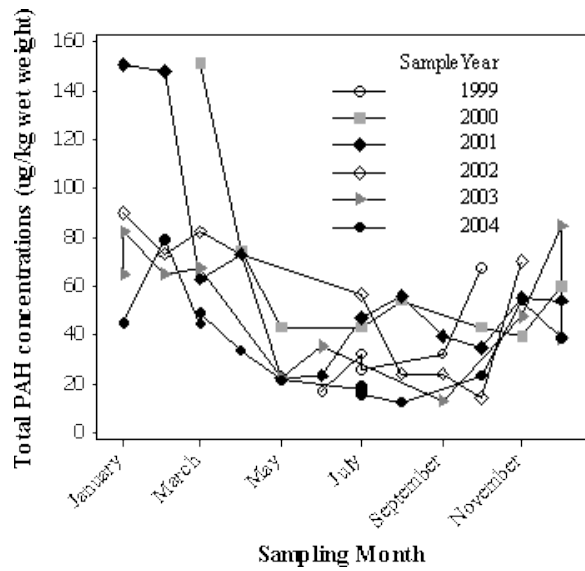


Fig. 1 Total PAH (2- to 6-ring parent and branched) concentrations in mussels collected from Loch Etive.

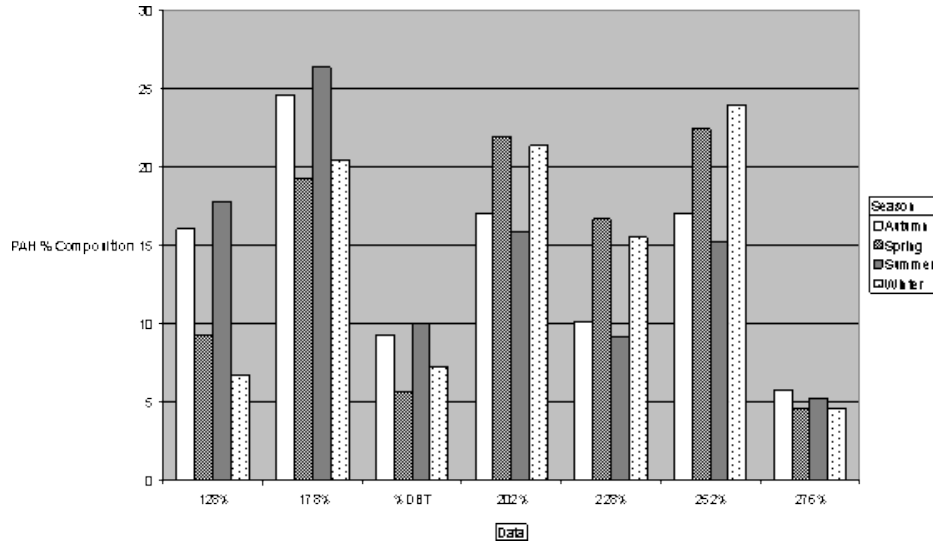
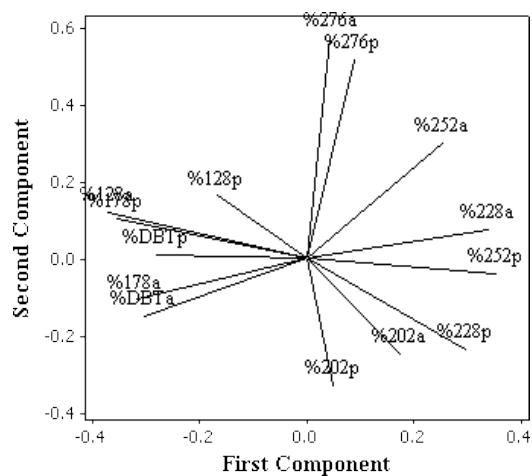


Fig. 2 PAH composition of Loch Etive mussels collected in spring, summer, autumn and winter between 1999 and 2004.

(a)



(b)

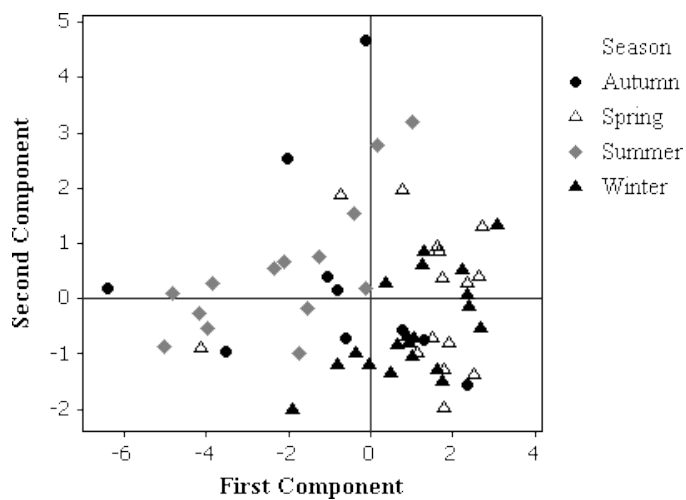


Fig. 3 (a) Loading plot and **(b)** score plot for the PCA of the parent (p) and alkylated (a) concentrations in each PAH group, expressed as proportions of the total PAH concentration.