Polycyclic aromatic hydrocarbon contamination and recovery characteristics in some organisms after the Nakhodka oil spill

Jiro Koyama a,*, Seiichi Uno a, Kumiko Kohno b

a Faculty of Fisheries, Kagoshima University, Shimoarata, Kagoshima 890-0056, Japan
b National Research Institute of Fisheries and Environment of Inland Sea, Ohnoch, Hiroshima 739-0452, Japan

Abstract
Following the oil spill from the Russian tanker Nakhodka in 1997 in the Sea of Japan, polycyclic aromatic hydrocarbons (PAH) were monitored for three years in some molluscs from the Mikuni-cho shore in Japan. Total PAH concentrations in marine organisms except for spiny top shell, ranged from 5.3 to 32.7 ng/g wet weight, but no trends were evident. Total PAH concentration in spiny top shell (Turbo cornutus) was 44 ng/g w.w. in the first month after the oil spill. However, it rapidly decreased to less than 5.4 ng/g w.w. from the second month. Spiny top shell, which was exposed to dietary Nakhodka heavy fuel oil, concentrated benzo(a)pyrene to 17.1 ng/g w.w. after two weeks of exposure and then rapidly eliminated it during an elimination phase. These results suggest that spiny top shell accumulates PAHs because of their low ability to metabolize PAH, but it can excrete parent PAHs rapidly when removed from the source of contamination. Thus it is suitable as an indicator organism in monitoring oil contamination. It can also be inferred from these field and laboratory investigations that, in three years, organisms from the Mikuni-cho shore seem to have adequately recovered from the Nakhodka oil spill contamination.

Keywords: Nakhodka heavy fuel oil; PAHs; Spiny top shell; Blue mussel; Limpet; Bioaccumulation

1. Introduction

Heavy fuel oil, 6200 kl, was spilled from the Russian tanker Nakhodka on January 4, 1997 in the Sea of Japan. The Japan shoreline was heavily impacted, especially at the Mikuni-cho shore, where a huge volume of oil and the bow of the tanker drifted ashore. Although many volunteers cleaned up the contaminated shoreline, the spilled oil was not completely removed. Oil spilled from the Exxon Valdez persisted in the environment for a long time (Wolfe et al., 1994; Hayes and Jacqueline, 1999; Jacqueline and Hayes, 1999), and the intertidal community took 3–5 years to recover from the impact (Skalski et al., 2001). The Nakhodka oil may also have persisted in the coastal area with sustained impact on the intertidal community. The recovery of the intertidal community in the area was assessed (Komatsu et al., 2003; Yamamoto et al., 2003), although the long-term recovery of organisms has not been well understood.

Due to PAH toxicity to organisms and persistence in the environment (Malins et al., 1988; Moore et al., 1989; Carls et al., 2001; Cohen et al., 2001; Wang et al., 2001), environmental contamination by spilled oil has often been assessed by analyzing PAH concentrations in the spill site’s water (Koyama et al., 1998; Reddy and Quinn, 2001), sediments (Lee and Page, 1997), and organisms (Malins et al., 1988; Moore et al., 1989).

* Corresponding author. Tel./fax: +81 99 286 4743.
E-mail address: koyama@fish.kagoshima-u.ac.jp (J. Koyama).
To assess the long term impact of the Nakhodka oil spill, the accumulation of PAH from the oil was monitored for three years in the spiny top shell (*Turbo cornutus*, an important commercial snail in Japan), in the blue mussel (*Septifer virgatus*), goose barnacle (*Capitulum mitella*), limpet-1 (*Cellana toreuma*), and limpet-2 (*Cellana grata*) in the present study.

Meador et al. (1995), and Nandini and Menon (1999) pointed out that marine organisms take up PAHs from food, as well as from water and sediments. In oil spills, some of the oil adsorbed on food surfaces should be ingested along with the food. Thus PAH bioaccumulation characteristics from food by spiny top shell were also investigated in the present study.

2. Materials and methods

2.1. Collection of organisms

Blue mussel (*S. virgatus*), goose barnacle (*C. mitella*), limpet-1 (*C. toreuma*), and limpet-2 (*C. grata*) were collected every March from 1998 to 2000 at the Nakhodka oil spill site in Mikuni-cho on the Japan coast (Fig. 1). The spiny top shells (*T. cornutus*) were also collected one and two months after the oil spill in March and April 1997 at same site. They were also collected every May/June and August/September from 1998 to 2000. After collection all the organisms were frozen at −20 °C until analyses.

The blue mussel is a filter feeder in the intertidal zone while goose barnacles and limpets are grazers, but the spiny top shell is a grazer in the subtidal zone.

2.2. PAH accumulation test with spiny top shell

Spiny top shells of shell average height 81 ± 9 mm, and average shell width 65 ± 6 mm were collected from an oil uncontaminated area near the National Research Institute of Fisheries Science laboratory facing the Saganami Bay. They were acclimatized for one week in an aquarium with flowing sand-filtered seawater flowing through it, and fed seaweed (*Ecklonia cava*).

After acclimatization, they were divided into three aquaria each holding 30 animals, still with sand filtered seawater flowing through them, but the food was changed. One group, which served as the control, was fed five filter papers everyday with 5 mg of lecithin dissolved in 0.1 ml ether as appetizer. A second group was fed five filter papers each with 5 mg of Arabia crude oil and 5 mg of lecithin in 0.1 ml ether. The third group was fed five filter papers each with 5 mg of Nakhodka heavy fuel oil and 5 mg of lecithin dissolved in 0.1 ml ether. In every case, the filter papers were kept overnight to allow the ether to completely evaporate before feeding to the snails.

They were fed the filter papers for two weeks followed by an elimination period of 10 days during which they were fed uncontaminated seaweed (*Ecklonia cava*). Five shells were sampled from each group for PAH concentration determination on the seventh day (only in the two groups with oil), and the 14th day of the accumulation phase. During the elimination phase they were sampled on the second, sixth day (only in the two groups with oil), and 10th days. Based on observations of higher PAH concentrations in the hepatopancreas relative to other organs in the preliminary analysis, PAH analysis was done only for the hepatopancreas in this test.

Fig. 1. Location of a sampling site.
2.3. PAH analysis

The soft tissue of all the organisms collected were individually analyzed for their PAH concentrations. Two grams of the sample was spiked with surrogates (fluorene–d10 and benzo(a)pyrene–d12) and saponified by shaking for one night in 20 ml 1 N KOH/methanol at room temperature. PAHs were extracted with 20 ml of n-hexane three times. These extracts were dehydrated and concentrated to 1 ml using Turbo-Vap II (Zymerk, USA) at 40 °C under streams of nitrogen gas. Column chromatography on silica gel was performed. PAHs were eluted with 10 ml of n-hexane and 15 ml of 1% acetone n-hexane. After concentration with Turbo-Vap II to 1 ml, PAH concentrations were determined by gas chromatography/mass spectrometry (GC/MS). PAH recovery from fish tissue was around 100% (Ministry of Environment, 2000).

2.4. Gas chromatography

PAH measurements were individually conducted using a gas chromatograph (HP-5891 II; Hewlett Packard, USA) equipped with a mass spectrometer (HP-5971A, SIM mode). The peak area was used for quantification. Two microlitres of sample was injected by a HP-7673A automatic sampler into a DB-5 capillary column (0.25 mm ID X 30 m, film thickness 0.25 μm, J&W Scientific, USA) under splitless injection mode. The injection and detection temperatures were 270 and 300 °C respectively. The oven temperature of column for measurement of PAHs was incrementally increased as follows: 60 °C held for 1 min; 10 °C/min to 160 °C held for 1 min; 2 °C/min to 280 °C held for 10 min.

2.5. Statistical method

Statistical differences for the concentration changes among sampling periods were evaluated by Dunnett’s multiple comparison tests at the p < 0.05 significance level. Statistical differences of the PAH concentrations between crude oil and heavy fuel oil exposure groups were evaluated using student’s t-test at the p < 0.05 significance level.

3. Results

3.1. PAH concentrations in spiny top shell collected at Mikuni-cho

The concentrations of total PAHs (fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, and benzo(a)pyrene) in the spiny top shell from the contaminated Mikuni-cho shore is shown in Fig. 2. Each PAH concentrations in the spiny top shell and their deviations are shown in Table 1. Unfortunately, we determined PAH concentrations of one and two sample composed of three shells collected in 1997 and 1999. Therefore, the PAH concentrations could not be statistically compared. However, the 44 ng/g w.w. PAH concentrations recorded after one month of the oil spill decreased to less than 5.4 ng/g w.w. from the second month. Chrysene had the highest concentration among the PAHs in the spiny top shell at 31.1 ng/g w.w. recorded one month after the spill. Benzo(a)pyrene, a very carcinogenic PAH, was detected at less than 0.5 ng/g w.w. in every year of sampling.

Fig. 2. PHA concentrations in spiny top shells collected at Mikuni-cho from one month to three years after the oil spill.
3.2. PAH concentrations in other organisms collected at Mikuni-cho

Total PAH concentrations (fluorene, dibenzothiophene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, and benzo(a)pyrene) in the other organisms are shown in Fig. 3. Due to the small weight of limpets (C. toreuma and C. grata), their PAH concentrations were determined from a pool of five individuals. Mean total PAHs concentration of blue mussel, goose bar-
3. PAH accumulation test in the spiny top shell

Table 2 shows the PAH concentration in the filter papers fed to spiny top shells in the accumulation test. While fluorene, dibenzothiophene and phenanthrene had higher concentrations in the filter papers with Arabia crude oil, those with Nakhodka heavy fuel oil had higher concentrations of anthracene, fluoranthene, pyrene, chrysene, and benzo(a)pyrene.

Fig. 4 shows the PAH accumulation results in the spiny top shells from the test. Most PAHs reached steady state accumulation within one week. Significantly higher concentrations of fluoranthene, pyrene, chrysene, and benzo(a)pyrene were detected in the shells exposed to dietary Nakhodka heavy fuel oil than those exposed to dietary Arabia crude oil. Their mean concentrations after two weeks of exposure were 4.4, 12.6, 41.7 and 17.1 ng/g w.w. respectively.

PAH concentrations from the elimination period, after the accumulation, when the shells were fed uncontaminated seaweed (E. cava) are presented in Fig. 4. While most PAH concentrations in the shell hepatopancreas rapidly decreased and reached normal levels within 10 days, pyrene concentration did not reach normal level. Due to higher concentrations of dibenzothiophene in the food with crude oil, their concentrations in spiny top shell hepatopancreas during the exposure and elimination phase were higher than those exposed to heavy fuel oil.

4. Discussion

Total PAH concentrations in spiny top shell from the oil spill site decreased from 44 ng/g one month after the oil spill to less than 5.4 ng/g one month later (i.e. two months after the oil spill). This rapid decrease suggests that the PAHs were derived from the Nakhodka oil. The Sea of Japan had no recent oil spill until the Nakhodka spill.

The accumulation test has shown that the spiny top shell accumulates PAHs, especially PAHs with more than four rings like pyrene, chrysene, and benzo(a)pyrene. Relatively high concentrations of chrysene and benzo(a)pyrene were detected in the hepatopancreas in this study, however, Uno et al. (2003) did not detect any in the flounder (Paralichthys olivaceus) exposed to a similar dietary concentration of chrysene and benzo(a)pyrene. While Oshima et al. (2003) could detect relatively high EROD activity in the flounder, they could not detect any EROD activity in the spiny top shell exposed to dietary PAHs, suggesting that spiny top shell accumulates PAHs but cannot metabolize PAHs as reviewed in mollusk by James (1989). This accounts for why PAH accumulation reached steady state so fast in this experiment.

As PAHs decreased so rapidly in the elimination phase, it is reasonable to conclude that they will also be eliminated in the spiny top shell very fast in an oil spill situation if the spilled oil disappears from the environment. Uptake and elimination rates and half-lives could not be computed in this study because PAH concentrations were measured only in the hepatopancreas but not in the whole body. However, it can be deduced from the concentration changes during elimination that most PAHs were eliminated so fast. But it took more than 10 days to decrease pyrene concentrations to normal levels.

Total PAH concentrations in the other organisms were less than 33 ng/g w.w. all three years after the oil spill. Excluding fluorene and dibenzothiophene, Knutzen and Sortland (1982) reported the same total PAHs in mussels (Mytilus edulis) from an open coast to range from 172 to 326 ng/g dry weight and 207 ng/g d.w. in the common periwinkle (Littorina littorea). Baumard et al. (1998a) reported 103 ng/g d.w. total PAHs in the mussel (M. edulis) from the Arcachon Bay, while from the coast of Spain and France, Baumard et al. (1998b) reported a range of 15.9–59.2 ng/g d.w. in the mussel (M. galloprovincialis). When approximately converted to wet weights by dividing by five (Meador et al., 1995), these values become 34.4–65.2 ng/g for M. edulis, 41.4 ng/g for the periwinkle, and 20.6 ng/g for

<table>
<thead>
<tr>
<th>PAHs</th>
<th>Filter papers PAHs conc. (ng/g w.w.)</th>
<th>Control</th>
<th>Crude oil</th>
<th>Heavy fuel oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorene</td>
<td>2.7</td>
<td>56.5</td>
<td>30.7</td>
<td></td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>3.0</td>
<td>737</td>
<td>20.8</td>
<td></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>24.1</td>
<td>236</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.6</td>
<td>ND</td>
<td>95.3</td>
<td></td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>3.7</td>
<td>17.1</td>
<td>20.8</td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>4.2</td>
<td>17.5</td>
<td>80.8</td>
<td></td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.8</td>
<td>54.4</td>
<td>152</td>
<td></td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>ND</td>
<td>1.1</td>
<td>63.7</td>
<td></td>
</tr>
</tbody>
</table>
M. galloprovincialis from the Arcachon bay; while that for M. galloprovincialis from the coasts of Spain and France becomes a range of 3.2–11.8 ng/g. Onduka et al. (2003) also reported a PAH concentration range of 1.37–30.7 ng/g w.w. in mussels from the northern and eastern coasts of Japan. PAH concentrations reported for the shells in this study are within the ranges mentioned above, suggesting that the organisms in this study...
have recovered from the Nak hodka oil spill of 1997. Therefore, while the spiny top shell seemed to have recovered in two months, the other organisms have recovered within one year.

Oil persistence in the environment has been observed on the sandy beach of the Persian Gulf (Koyama and Kurosima, 1998) and the gravel beach of Prince William Sound after the Exxon Valdez oil spill (Hayes and Jacqueline, 1999) where weathered oil was also found deep on the beaches. Though PAH was not analyzed from the shore in this study, the shore is mostly rocky and spilled oil may not persist. The low PAH concentrations in the spiny top shell from the area also seem to lead to this summation. Additionally, there seem to be lower concentrations of PAHs in subtidal than in tidal sediments (Lee and Page, 1997). High molecular weight PAHs adsorb onto particles in water, but spiny top shells are distributed in subtidal areas where they feed on macroalgae, hence they were not exposed to the higher water borne and dietary PAHs.

These results show that the spiny top shell accumulates PAHs because of its low ability at PAH metabolism. Just like fish, which excretes parent PAHs (Pointet and Milliet, 2000), spiny top shell also seems to excrete parent PAHs very fast. Oil contamination causes an initial increase in tissue PAH concentration, but in recovery, when the source of contamination is removed, they are eliminated very fast. The spiny top shell (Turbo cornutus) therefore, seems to be suitable for monitoring oil contamination. The results of this study also indicate that organisms from the Mikuni-cho shore have sufficiently recovered from the Nak hodka oil spill of 1997.

References


Pointet, K., Milliet, A., 2000. PAHs analysis of fish whole gall bladders and liver from the Natural Reserve of Camargue by GC/MS. Aquatic Toxicology 45, 63–69.


