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Accumulation and Clearance of PAHs and CYP1A Levels in Farmed Green Mussels (*Perna viridis* L.) from a Coastal Industrial Area in Thailand

Varaporn Cholumpai [a], Malin C. Celander [b] and Praparsiri Kanchanopas-Barnette [c, d]

[a] *Environmental Science Programme, Faculty of Science, Burapha University, Chonburi 20131, Thailand*
[b] *University of Gothenburg, Department of Biological and Environmental Sciences, P.O. Box 463, SE 405 30, Göteborg, Sweden*
[c] *Department of Aquatic Science, Faculty of Science, Burapha University, Chonburi 20131, Thailand*
[d] *The Center of Excellence on Environmental Health and Toxicology, Bangkok, Thailand*

Abstract

Green mussels (*Perna viridis* L.) that inhabit along coastal areas with established petro-chemical industries are likely to be exposed to petroleum hydrocarbons. In year 2011, polycyclic aromatic hydrocarbons (PAHs) accumulated (*i.e*. total of 16 PAH congeners) in three different sizes of farmed mussels in the Maptaphut industrial estate which is an industrial park in the Gulf of Thailand. The levels of mean total PAHs (0.4303 ± 0.3067 µg/g dry weight) in large sized (consumable size) mussels were 16 and 8 times higher than medium and small sized mussels. Levels of total carcinogenic PAHs (0.0311 ± 0.0310 µg/g dry weight) in consumable size mussels were 15 and 11 times higher compared to medium and small sized mussels. Two carcinogenic PAHs (*i.e*. chrysene and benzo[*a*]anthracene) were detected in all sized mussels. The ratio of high molecular weight versus low molecular weight PAHs in all sized mussels indicated the presence of pyrogenic PAHs contamination over petrogenic PAHs in this coastal area. Further studies were carried out in year 2012 involved depuration in consumable sized mussels and effects on the cytochrome P450 1A (CYP1A) biomarker were analyzed over a 30 day depuration period. The half-life was five days for total PAH burden (0.4765 ± 0.0615 µg/g dry weight), which included four non-carcinogenic PAHs. After 10, 15 and 30 days depuration in clean water, the mean total PAHs levels decreased gradually but yet significantly (0.2501 ± 0.0186, 0.1350 ± 0.0122 and 0.1554 ± 0.0353 µg/g dry weight, respectively) compared to the PAH levels at day 0. Levels of CYP1A declined accordingly and at 30 days depuration CYP1A protein levels were significantly reduced by almost 3-fold compared the PAH levels in mussels from the Maptaphut industrial estate. The results show that farmed green mussels reared for human consumptions are exposed to PAHs including carcinogenic PAHs and that clearance of these PAHs is evident at 30 days depuration. This study demonstrates the importance of analysing PAHs in mussels and the usefulness of the CYP1A biomarker to assess exposures of PAHs in Thailand coastal waters. Therefore, a continuous monitoring and evaluation of PAHs contamination in marine species is a priority in this area and other petro-chemical estate areas of Thailand in order to reduce the risk of dietary exposures to carcinogenic PAHs from consumption of green mussels as well as to endorse reduced anthropogenic releases of PAHs into the marine ecosystem.

Keywords: green mussel (*Perna viridis* L.); Maptaphut; PAH; CYP1A; tropical waters

1. Introduction

Green mussels (*Perna viridis* L.) and other bivalves are commonly used in order to assess water contamination and in environmental monitoring programs. These filter-feeding and sessile organisms are exposed to complex mixture of pollutants in their natural habitat and are therefore ideal species for studies on effects of pollutants in coastal areas. Polycyclic aromatic hydrocarbons (PAHs) represent one of the most common classes of pollutant that are continuously released into coastal waters worldwide. In Thailand, various industrial estates are spread out over the whole country, while others are located along the coast and thereby pose a threat to the marine ecosystem. Earlier investigations between 1994 and 1999 in five coastal locations (bay, canal, and river) in Thailand reveal industrial contamination of PAHs in green mussels were in the range of 0.1000 - 0.2110 µg/g dry weight, classified as low levels of hydrocarbon pollution (Isobe *et al*., 2007). On the other hand, levels of total PAHs were much higher in farmed green mussels collected in year 2007 from the Chonburi Province, on the East coast of Thailand. The levels of PAHs ranged between 2.33 to 3.06 µg/g dry weight during the dry season and between 2.49 to 3.15 µg/g dry weight during the rainy season (Mokkongpai *et al*., 2010).

The Rayong Province, close to the Chonburi province, has petrochemical industries located in

Maptaphut Industrial Estate. The physical conditions of marine sediments around this area appeared to be black sediments (35 cm depth) with a composition of total petroleum hydrocarbons in a wide range of 0-170 mg/kg dry weight and mercury at a range of 0.15 - 1.61 mg/kg dry weight (Water Quality Management Bureau, 2009). However, in this industrial area fishery activities and farming of green mussels for human consumptions are also important. Some of PAHs congeners are known carcinogens or are strongly suspected to act as carcinogens (IARC, 2012). Four substances of carcinogenic PAHs (benzo[*a*]anthracene, chrysene, benzo[*a*]pyrene and benzo[*k*]fluoranthene) were found in mussels (*Mytilus galloprovinclalis*) in canals of Venice, Italy (Wetzel and Van Vleet, 2004).

Determination of the cytochrome P450 1A (CYP1A) biomarker is widely used either alone or in combination with chemical analyses to indicate the exposures of PAHs on living organisms. Tropical fish species caught from coastal Chonburi Province had detectable CYP1A protein levels, which is indicative of exposures to PAHs (K-Barnette *et al*., 2010). Since mussels populations reside and reproduce along the coastal area and are sessile, they are suitable indicator organisms for studies on point source pollution along coastal areas. For instance, Solé *et al*. (1995) found CYP1A expression in blue mussels (*Mytilus edulis* L.) after the oil spill in the Galician coastline in Spain. In addition, levels of PAHs (Σ of 13 PAHs) in these mussels positively correlated with levels of CYP1A in their digestive glands. The integrated analyses of PAHs and CYP1A levels in mussels (*M. galloprovincialis*) from NW Spain proved the existence of petrogenic and pyrogenic hydrocarbons from polluted site (Porte *et al*., 2001). The main important factor for using the biomarker is the benefits of having it as early warning parameter (Sarkar *et al*., 2006). In the Mediterranean Sea, CYP1A expression in digestive gland of *M. galloprovincialis* from contaminated sites revealed the highest CYP1A when compared to mussels from intermediate contaminated sites and a reference site by 1.67 and 2.27 times respectively (Shaw *et al*., 2004).

The purpose of this study was to examine accumulation of total PAHs in relationship to their size of cultured green mussels in the Maptaphut industrial estate. In addition, the impact of depuration over a 30 day time-period on PAH clearance and on CYP1A protein levels in the digestive glands were addressed in green mussels from the polluted Maptaphut industrial estate in the Rayong province transferred to a non-polluted area in the Trat province.

2. Materials and methods

2.1. Experimental sites

Two experimental sites of coastal water in this study were selected, Maptaphut Industrial Estate, Rayong Province (polluted) and Trat Province (non-polluted) as shown in Fig. 1.

It is well-known that non-polluted area is suitable for green mussel culture. Some of the major chemical environmental data of non-polluted site in 2011 (wet season), obtained from Petsut *et al*. (2012), include salinity (15-27 ppt), total inorganic nitrogen (34.3-280.0 μg/L) and phosphate (3.0-67.0 μg /L). On the other hand, data of the polluted site in 2011(August-December) as obtained from Water Quality Management Office (2013) include salinity (33.50-30.20 ppt), dissolved oxygen (5.6-6.2mg/L), nitrate-nitrogen (0.1-10.7μg nitrogen/L), phosphate-phosphorous (0.2-70.0 μg- phosphorous/L), ammonia-nitrogen (5.5-3.5 μg nitrogen/L) mercury (0.01-0.01 μg/L), arsenic (1.6-1.9 μg/L) and petroleum hydrocarbon (0.1-4.5 μg/L).

2.2. Sample collection and preparation

Three sizes of green mussels were randomly collected from raft-cultured mussels (3 km^2 long) from Maptaphut Industrial Estate in November in 2011 for PAHs analyses. Samples were transported into the laboratory within 1 hour. Mussels were divided into large (consumable size), medium, and small sizes: mean sizes in length were 6.6186 ± 0.0910 cm, 4.8934 ± 0.2099 cm and 3.4950 ± 0.1249 cm respectively. For depuration experiment in year 2012, consumable size in length was at 6.3940 ± 0.1444 cm from polluted site (Maptaphut industrial estate) and 7.3931 ± 0.1583 cm from non-polluted site (Trat). For PAHs analysis, the whole mussel tissues from each size were pooled from 20 individuals per sample and wrapped in clean aluminum foil and stored at -20^{o}C. For CYP1A measurement, digestive glands were immediately dissected and stored in liquid nitrogen.

2.3. Experimental design for depuration study

Mussels on a rope from polluted site were transferred to hang on raft in non-polluted site. Afterwards, mussel sample in both sites was collected at the same time at 0, 5, 10, 15, and 30 days. PAHs level and CYP1A biomarker were analyzed. Five replicates of samples (20 individuals per replicate) were analyzed for ∑16 types of PAHs and its level by analyzing its body tissue by using GC-MS. Another five replicates of samples (20 individual per replicate) were analyzed for CYP1A biomarker (CYP1A protein levels) in

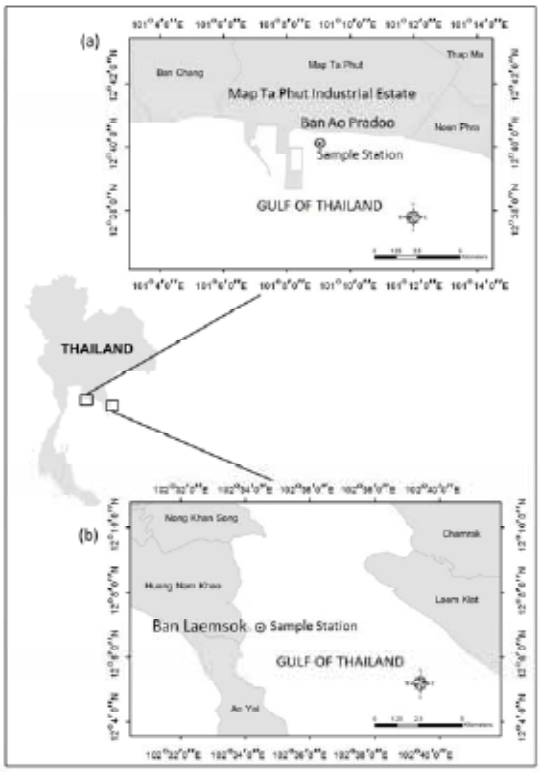

Figure 1. Map of Thailand, showing two coastal water sites: (a) Maptaphut Industrial Estate, Rayong Province and (b) Trat Province.

the digestive gland by using antibody technique and polyclonal antibodies raised against rainbow trout CYP1A with Western blot method (K-Barnette *et al*., 2010).

2.4. Chemical analysis

The pollutants of ∑16 PAHs were analyzed, as classified into 16 types of PAHs by the US-EPA (2000) as potent chemicals and prioritized in the pollutant list (Ribeiro *et al*., 2012). Mussel samples were performed extraction, clean-up process and analysis following the method of Kelly *et al*. (2000) with minor modification. Briefly, two grams of homogenised samples (freeze dried) were soxhlet extracted for 24 hours and saponified with 1 N potassium hydroxide in methanol. Then, the extract was purified and separated by using silica gel-florisil column chromatography. PAHs in the purified samples were analyzed by gas chromatography (HP 6890 N GC equipped with a split/splitless injector) coupled with mass spectrometry. It should be noted that the method detection limits (MDLs) were calculated based on the calibration curves of each PAH. The formula for MDLs is to 3.3 SD/S, where SD corresponds to the standard deviation of the mean value and S to the slope of the calibration curve. The obtained MDLs are presented in Table 1.

2.5. CYP1A protein analyses

Microsomal fractions were prepared from the digestive glands by using differential centrifugation by a modification of the method described in Livingstone (1988). Briefly, the digestive glands samples were homogenized, and then centrifuged at 10,000 and 100,000 x g (Optima L100 XP Ultracentrifuge, Beckman Coulter, USA). The final microsomal pellets were re-suspended in microsomal buffer (20 mM Tris-HCl pH 7.6, 20% glycerol). All procedures were carried out at 4 ºC. Microsomal CYP1A protein levels were electrophoresed by Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and determined by Western blot analyses using standard protocols and detected as described in Celander and Förlin (1991), with minor modifications. Here, 20 µg microsomal proteins were loaded in each lane and transferred to nitrocellulose membranes. The polyclonal antibodies raised rainbow trout CYP1A in rabbits (Celander and Förlin, 1991) were diluted 1:1000 in blocking solution (5% fat free dry milk (w/v) in TRIS-buffer saline, pH 7.4). As secondary antibodies, goat anti-rabbit IgG conjugated with horseradish peroxidase were used and diluted 1:1000 in blocking solution. The immunoblots were stained with 0.03% 3,3´diaminobenzidine-4-HCl, 0.006% H_2O_2, 0.05 % $CoCl_2$ in phosphate buffer saline, pH 7.4. The CYP1A protein levels are presented as arbitrary units (AU) using the ImageMaster 2D Elite 4.01 analysis software (Amersham Pharmacia Biotech AB, Uppsala, Sweden). It should be noted that these antibodies raised against rainbow trout CYP1A also cross-react with CYP1A proteins in mammals, fish and mussels (Celander, unpublished data).

2.6. Statistical analyses

The results were expressed as mean ± SD. Differences of PAHs among green mussel sizes were compared by one-way ANOVA followed by a Tukey post-hoc test. Differences of levels of PAHs and levels of CYP1A proteins among time points at any stations were compared by one-way ANOVA followed by a Tukey post-hoc test. Differences of PAHs and CYP1A among stations at any times were compared by *t*-test. For all statistical tests, differences were considered significant at $p<0.01$. The statistical software IBM SPSS Statistic 20 was used.

3. Results

In year 2011, nine PAHs congeners were found in medium and consumable sized mussels namely,

phenanthrene, fluoranthene, pyrene, chrysene, fluorene, acenaphthylene, anthracene, benz[*a*]anthracene and acenaphthene. Two of these PAHs are classified as possibly carcinogenic (*i.e*. chrysene and benzo[*a*] anthracene). The mean concentrations of total PAHs in mussels of three different sizes: small, medium and consumable size were 0.0507 ± 0.0606, 0.0263 ± 0.0345 and 0.4303 ± 0.3067 µg/g dry weight, respectively. As for the carcinogenic PAHs, the mean concentrations were 0.0028 ± 0.0045, 0.0020 ± 0.0052 and 0.0311 ± 0.0310 µg/g dry weight, respectively (Table 1).

Levels of total PAHs in consumable size (0.4303 ± 0.3067 µg/g dry weight) were 16 times higher than medium size (0.0263 ± 0.0345 µg/g dry weight) and 8 times higher than that in small size mussels (0.0507 ± 0.0606 µg/g dry weight). The levels of total PAHs in consumable size mussels were significantly higher than the levels of PAHs in medium and small size mussels with $p<0.01$. Levels of carcinogenic PAHs in consumable size mussels (0.0311 ± 0.0310 µg/g dry weight) were 15 times higher than that in medium size (0.0020 ± 0.0052 µg/g dry weight) and 11 times higher than levels of carcinogenic PAHs in small size mussels (0.0028 ± 0.004 µg/g dry weight). The levels of carcinogenic PAHs in consumable size mussels were significantly higher than the levels of carcinogenic PAHs in medium and small size mussels with $p<0.01$. However, there were no statistically significant differences in levels of total PAHs or carcinogenic PAHs between medium and small size mussels.

In small size mussels, the distribution between low molecular weight (LMW) PAHs (*i.e*. two- and three-ring PAHs) and high molecular weight (HMW) PAHs (*i.e*. four-ring PAHs) was 83.17% and 16.83% of total PAHs analyzed (=100%) (Fig. 2). The ratio between HMW/LMW in small size mussels was 0.20. In medium size mussels, the distribution between LMW and HMW PAHs was 84.41% and 15.59% (Fig. 2). The

Table 1. Concentrations of low and high molecular weight PAHs (µg/g dry weight) in whole body tissue and physical characteristics of small, medium and large sized mussels collected from Maptaphut in 2011.

∑16 PAHs	PAHs concentration (µg/g dry weight)			MDLs
	small size	medium size	large size	(µg/ml)
Low molecular weight (LMW) PAHs				
Naphthalene (NAP)*	nd	nd	nd	0.0009
Acenaphthylene (ACY)	0.0010	0.0006	0.0056	0.0006
Acenaphthene (ACE)	nd	0.0002	0.0003	0.0002
Fluorene (FLO)	0.0012	0.0006	0.0060	0.0003
Phenanthrene (PHE)	0.0165	0.0097	0.1883	0.0007
Anthracene (ANT)	0.0007	0.0004	0.0047	0.0004
Fluoranthene (FLA)	0.0229	0.0107	0.1247	0.0005
High molecular weight (HMW) PAHs				
Pyrene (PYR)	0.0057	0.0022	0.0695	0.0002
Benzo[*a*]anthracene (BaA)*	nd	0.0006	0.0046	0.0006
Chrysene (CHR)*	0.0028	0.0014	0.0264	0.0007
Benzo[*b*]fluoranthene (BbF)*	nd	nd	nd	0.0005
Benzo[*k*]fluoranthene (BkF)*	nd	nd	nd	0.0004
Benzo[*a*]pyrene (BaP)*	nd	nd	nd	0.0002
Indeno[1,2,3-*cd*]pyrene (IcdP)*	nd	nd	nd	0.0003
Dibenzo[*a,h*]anthracene (DahA)*	nd	nd	nd	0.0009
Benzo[*ghi*]perylene (BghiP)	nd	nd	nd	0.0006
Number of mussels	14	22	11	
Σ Total PAHs (x±SD)	0.0507 ± 0.0606	0.0263 ± 0.0345	0.4303 ± 0.3067	
Σ Total carcinogenic PAHs (x±SD)	0.0028 ± 0.0045	0.0020 ± 0.0052	0.0311 ± 0.0310	
Mussel Length, cm. (x±SD)	3.4950 ± 0.1249	4.8934 ± 0.2099	6.6186 ± 0.0910	
Mussel Weight, g. (x±SD)	3.7997 ± 0.4356	10.0885 ± 1.4621	19.0982 ± 0.8762	
HMW PAHs/ LMW PAHs ratio	0.20	0.18	0.31	

* = carcinogenic PAHs; Data are presented as mean ± SD; nd = not detected (below level of detection)

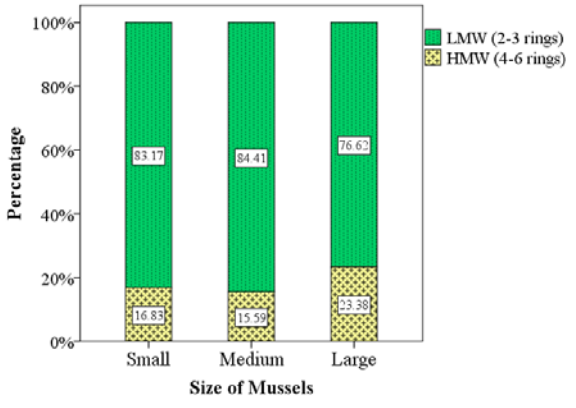

Figure 2. Distribution between HMW/LMW as % of total PAHs analyzed in the whole body tissue of small, medium and large sized mussels collected from bamboo raft farms along the Maptaphut coast in 2011.

ratio between HMW/LMW in medium size mussels was 0.18. In large size, the distribution between LMW and HMW PAHs was 76.62% and 23.38% (Fig. 2). The ratio between HMW/LMW in large size mussels was 0.31.

In year 2012, PAHs were analyzed in consumable size mussels from the polluted Maptaphut industrial estate. Levels of total PAHs were 0.4765 ± 0.0615 µg/g dry weight. Four different PAH congeners were detected (*i.e*. fluorene, fluranthrene, pyrene, and phenanthrene) with mean concentrations of 0.0515 ± 0.0183, 0.0896 ± 0.0120, 0.0985 ± 0.0159 and 0.2369 ± 0.0311 µg/g dry weight, respectively (Table 2). The distribution of HMW and LMW was 79.33% and 20.67% and the HMW/LMW ratio was 0.26. For comparison, levels of PAHs levels were also analyzed in consumable size mussels from the non-polluted Trat province. No PAHs were detected in these mussels.

Mussels from polluted site of consumable size were next transferred to the non-polluted site and sampled 30 days after transfer to the non-polluted site. Levels of total PAHs and CYP1A protein levels were analyzed 10, 15 and 30 days after transfer. Mean total PAHs concentrations in depurated mussels had significantly decreased over the 30 day time period (Table 2). The levels of total PAHs (4 congeners) at 0 day (0.4765 ± 0.0615 µg/g dry weight) was up to 2 times higher than the mean values at 5, 10, 15, and 30 days (0.2383 ± 0.0231, 0.2501 ± 0.0186, 0.1350 ± 0.0122 and 0.1554 ± 0.0353 µg/g dry weight, respectively) with statistically significance difference of $p<0.01$ (Table 2 and Fig. 3(b)). The half-lives ($t_{½}$) of phenanthrene, pyrene, fluoranthrene and fluorene were 5.46, 4.98, 5.03 and 4.82 days, respectively (calculated according to Sericano *et al*., 1996). The concentrations of the four PAHs congeners detected were 0.2369 ± 0.0311, 0.0985 ± 0.0159, 0.0896 ± 0.0120 and 0.0515 ± 0.0183 µg/g dry weight, respectively. After 30 days depuration in non-polluted site the corresponding levels had declined to 0.1180 ± 0.0175, 0.0480 ± 0.0036, 0.0450 ± 0.0038 and 0.0273 ± 0.0044 µg/g dry weight, respectively (Table 2). On the other hand, the levels of total PAHs can still be detected in consumable size mussels at polluted site within 30 days (Table 2 and Fig. 3(a)).

A comparison between two sites at 5, 10, 15 and 30 days, the mean total PAHs concentration in consumable size at polluted site (0.4941 ± 0.0827, 0.4324 ± 0.0725, 0.4400 ± 0.0863 and 0.6413 ± 0.0413 µg/g dry weight respectively) was higher than mean total PAHs concentration in depurated mussels equivalent to 1, 1, 2, and 3 times higher, respectively with statistically significant difference of $p<0.01$ (Fig. 4).

In addition to PAHs chemical analyses, CYP1A protein levels were analyzed in the digestive gland of consumable sizes mussels from both sites using Western blot analyses. A CYP1A immunoreactive band at 56 kDa was detected and the intensities on the protein bands were analyzed using densitometry. A CYP1A immunoreactive protein was detected in all samples analysed. Highest CYP1A levels were detected in mussels collected from the polluted site at day 0 (68.26

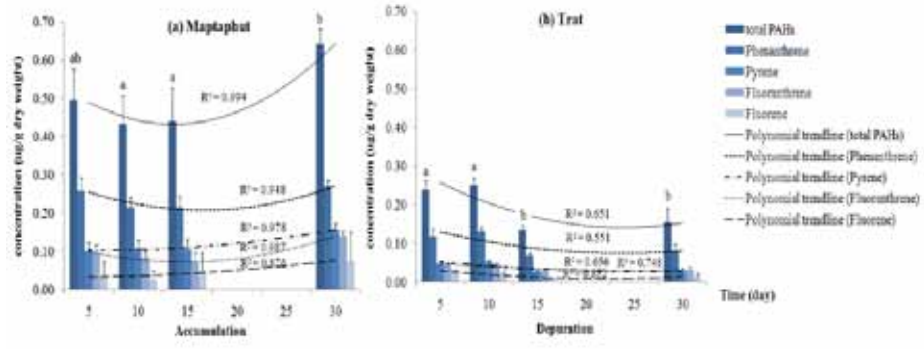

Figure 3. Total PAHs, phenanthrene, pyrene, fluoranthrene and fluorene concentration (µg/g dry weight) in consumable sized mussel tissues at any times and trend line. Error bars represent one standard deviation from the mean (n = 5). A) Consumable sized mussels at Maptaphut and B) consumable sized mussels transferred from Maptaphut to Trat.

Table 2. Mean PAHs concentrations (µg/g dry weight) in whole body tissues and physical characteristics of consumable sized mussels at Maptaphut and depurated mussels (Trat) for up to 30 days.

PAHs	PAH concentration (µg/g dry weight)								
	day 0	day 5		day 10		day 15		day 30	
	Maptaphut	Maptaphut	Trat	Maptaphut	Trat	Maptaphut	Trat	Maptaphut	Trat
LMW PAHs									
NAP*	nd	nd	nd	nd	nd	nd	nd	nd	nd
ACY	nd	nd	nd	nd	nd	nd	nd	nd	nd
ACE	nd	nd	nd	nd	nd	nd	nd	nd	nd
FLO	0.0515± 0.0183	0.0361± 0.0078	0.0273± 0.0044	0.0249± 0.0130	0.0224± 0.0025	0.0483± 0.0260	0.0095± 0.0019	0.0753± 0.0128	0.0139± 0.0072
PHE	0.2369± 0.0311	0.2588± 0.0347	0.1180± 0.0175	0.2142± 0.0273	0.1306± 0.0112	0.2149± 0.0291	0.0689± 0.0061	0.2700± 0.0161	0.0819± 0.0154
ANT	nd	nd	nd	nd	nd	nd	nd	nd	nd
FLA	0.0896± 0.0120	0.0979± 0.0186	0.0450± 0.0038	0.0842± 0.0173	0.0451± 0.0034	0.0706± 0.0264	0.0299± 0.0027	0.1400± 0.0120	0.0312± 0.0072
HMW PAHs									
PYR	0.0985± 0.0159	0.1013± 0.0231	0.0480± 0.0036	0.1090± 0.0201	0.0520± 0.0040	0.1061± 0.0274	0.0267± 0.0026	0.1559± 0.0163	0.0283± 0.0071
BaA*	nd	nd	nd	nd	nd	nd	nd	nd	nd
HR*	nd	nd	nd	nd	nd	nd	nd	nd	nd
BbF*	nd	nd	nd	nd	nd	nd	nd	nd	nd
BkF*	nd	nd	nd	nd	nd	nd	nd	nd	nd
BaP*	nd	nd	nd	nd	nd	nd	nd	nd	nd
IcdP*	nd	nd	nd	nd	nd	nd	nd	nd	nd
ahA*	nd	nd	nd	nd	nd	nd	nd	nd	nd
ghiP	nd	nd	nd	nd	nd	nd	nd	nd	nd
Σ Total PAHs (x±SD)	0.4765± 0.0615	0.4941± 0.0827	0.2383± 0.0231	0.4324± 0.0725	0.2501± 0.0186	0.4400± 0.0863	0.1350± 0.0122	0.6413± 0.0413	0.1554± 0.0353
Length (x±SD)	6.3940± 0.1444	6.3330± 0.0936	6.3020± 0.1316	6.5470± 0.4275	6.4420± 0.1217	6.6240± 0.3499	6.4710± 0.2091	6.6560± 0.5132	6.6750± 0.2436
Weight (x±SD)	17.6535 ±1.8136	17.1581 ±1.5012	13.7500 ±0.9854	17.4670 ±2.5068	14.8450 ±1.2215	19.1083 ±3.4479	16.5150 ±1.2513	18.7658 ±3.0980	17.4840 ±1.2379

* = carcinogenic PAHs; Data are presented as mean (n=5) ± SD; nd = not detected (below limit of detection)

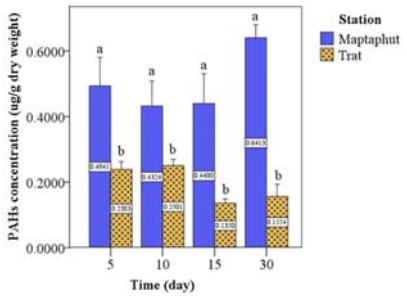

Figure 4. Total PAH concentrations (µg/g dry weight) in consumable size mussel tissues from the polluted site (Maptaphut) and from the non-polluted site (Trat). Error bars represent standard deviation from the mean (*n=5*). For each context (each set of two bars), bars with the different letters are statistically significantly with *p<0.01*.

± 13.35 AU). After transfer to the clean site, CYP1A levels significantly declined at 5, 10, 15, and 30 days depuration (27.64 ± 13.47, 27.18 ± 7.40, 17.53 ± 10.79 and 8.96 ± 8.52 AU, respectively, (Fig. 5) with *p<0.01*. Hence, the mean value of CYP1A protein level in mussels at polluted site (26.35 ± 9.23 AU) at 30 days was higher than the value in depurated mussels (8.96 ± 8.52 AU) or equivalent to 2 times higher with statistically significant difference of p<0.01 (Fig. 6).

4. Discussion

Green mussels farmed for human consumption that were sampled from the Maptaphut industrial estate in year 2011 contained several different PAHs. Two of these PAHs (*i.e.* chrysene and benzo[*a*]anthracene) are

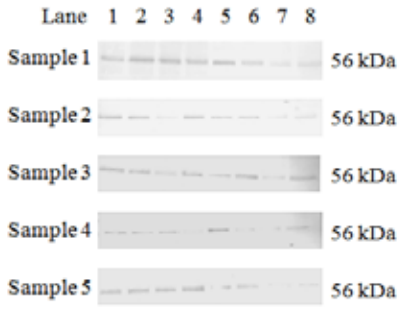

Figure 5. The 56 kDa band represents CYP1A protein levels in consumable size mussels from two different at different time points and detected using polyclonal antibodies raised in rabbits againts rainbow trout CYP1A1 and Western blot technique. Lane 1) Maptaput day 5; Lane 2) Maptaput day 10; Lane 3) Maptaput day 15; Lane 4) Maptaput day 30; Lane 5) Trat day 5; Lane 6) Trat day 10; Lane 7)Trat day 15; Lane 8) Trat day 30.

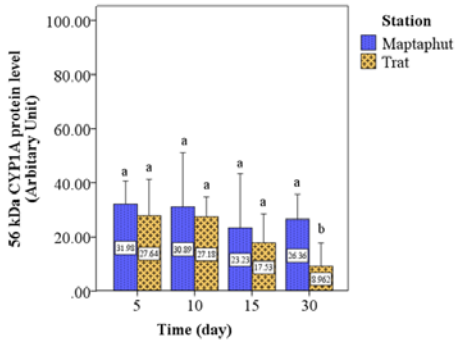

Figure 6. The 56 kDa band, CYP1A protein levels (AU) in digestive gland tissue of consumable sized mussels for two sites at any times within 30 days. See Fig. 4 for details.

classified as group 2B carcinogens or possibly carcinogenic to humans (IARC, 2012). Therefore, consuming mussels from this area would pose a health hazard for humans, especially the consumption of large consumable size mussels that contains significantly higher levels of PAHs including carcinogenic PAHs compared to smaller size mussels. According to the European Commission Regulation 208/2005, the proposed limitation of total PAHs in fish and shellfish that may present risks to consumers is more than 10 ng/g (wet weight) for benzo[*a*]pyrene in bivalves, while the US EPA (2000) proposed the safety level of 6 ug/g wet weight or equivalent to 44.4000 ug/g dry weight. Fortunately, the maximum levels of total PAHs in mussels at Maptaphut had levels below these safety levels. Mussels were collected from the same area one year later, 2012, and this time a reduced number of PAHs were detected. Besides, no carcinogenic PAHs were detected in these mussels. These results illustrate the importance of regular monitoring of PAHs contamination in mussels exposed to petrogenic emissions and accidently oil spills in order to reduce adverse toxic effects of PAHs contamination in the food-web. Consequently, the Pollution Control Department (PCD) of Thailand should be monitoring and evaluating the contamination of PAHs in mussels and other marine organisms regularly. Contaminations of PAHs in mussels from tropical waters have been reported in other studies. For example in Brazil, moderate contamination of 16 different PAHs by petrogenic sources were suggested from farmed mussels (*Perna perna*) in Southern Brazilian Bay and was also below safety levels (Yoshimine and Carreira, 2012).

Mussels have low biotransformation capacities compared to fish and other vertebrates, resulting in bioaccumulation of foreign substances, especially hydrophobic contaminants such as PAHs. Thus, the level of pollutants detected in mussels reflects the pollutants in the surrounding environment where mussels sedentary live. Therefore, mussels are commonly used as a biomonitor to evaluate the quality of the environment (Baumard *et al*., 1998; Yoshimine and Carreira, 2012). Baumard *et al*. (1998) proposed to organize levels of aquatic pollution according to PAHs found in mussels or dividing into three groups as: low level of pollution (0 - 0.1 µg/g dry weight), moderate level of pollution (0.1 - 1 µg/g dry weight) and high level of pollution (1 - 5 µg/g dry weight). The large consumable size mussels from Maptaphut contained total PAHs of 0.4303 ± 0.3067 µg/g dry weight, which can be classified as moderate contamination. On the other hand, the medium and small sized mussels contained much lower total PAHs, which can be classified as low level. This finding shows how large sized mussels are more suitable as a biomonitor. The HMW/LMW ratios in small, medium and large sized mussels were 0.2, 0.18 and 0.31, respectively, which is higher than in crude oil samples (0.02-0.13) collected from the Malaysian coasts (Zakaria *et al*., 2001). Therefore, our results indicate the contribution from pyrogenic PAHs in Maptaphut coastal water.

Interestingly, similar results concerning level of mean total PAHs and HMW/LMW ratio were found in mussels collected from the Maptaphut area in 2012. These mussels had levels of total PAHs considered as moderate level of pollution. The HMW/LMW ratio in consumable sized mussels indicates that there was more contamination of pyrogenic PAHs than petrogenic PAHs in Maptaphut in 2012 similar to in 2011. In the mussels collected in 2012 from the polluted Maptaphut site, the concentrations of four PAHs (fluorene, fluoranthrene, pyrene, and phenanthrene) declined over a 30 day time-period when these mussels were transferred to a clean site. The half-lives of the four detected PAHs was approximately five days. According to another study

on uptake and depuration of four PAHs substances in green mussels, the reported half-lives of anthracene, fluoranthene, pyrene and benzo[a]pyrene were two days (Richardson *et al*., 2005). In that study, natural water was used, which may does not contain other pollutants that could interfere with PAH clearance. There was another study on accumulation and depuration of PAHs in American oysters (*Crassostrea virginica*) from the Galveston Bay, Texas, where indigenous American oysters were transferred from contaminated area to non-contaminated area. This finding suggest the ability of CYP enzyme to metabolize fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*e*]pyrene, benzo[a]pyrene and indeno[1,2,3-*cd*] pyrene by 50% or equivalent to 32, 12, 15, 16, 16, 10 and 11 days, respectively (Sericano *et al*., 1996). The reason for the difference in half-lives for PAHs in bivalves between different species is not clear. It is possible, that the rate of PAH depuration in mussels is increased when the ambient temperature increases as a result of increased respiration and filtration (Meador *et al*., 1995). Thus, mussels in tropical waters are able to eliminate pollutants including PAHs faster than mussels in cooler waters.

In the present study, levels of PAHs declined during a 30 day depuration period and this was also reflected on the CYP1A biomarker response which declined in mussels following clearance. Hence, the concentrations of PAHs in mussels was 3 times higher mussels sampled directly from the polluted site compared to mussels that had been transferred to a clean site and depurated for up to 30 days. Furthermore, CYP1A protein levels were 2 times higher in mussels sampled directly from the polluted compared to mussels that had been depurated in the clean site. This also illustrates the usefulness of the CYP1A biomarker response in green mussels to assess exposure to PAH in the coastal zone. Our results show that 0.6413 ± 0.0413 µg/g dry weight remarkable induced CYP1A protein levels in green mussels. However, in another study in NW Spain using *M. galloprovincialis* no statistically significant effect on CYP1A protein levels were seen in mussels with PAH concentrations at 0.2028 µg/g wet weight (Porte *et al*., 2001). In order to use CYP1A protein in mussels as a biomarker to evaluate the quality of the environment appropriately, levels of contamination and types of pollutants should be considered. Ideally, CYP1A analyses should be combined with analyses of concentrations of PAHs, especially in areas with low contamination of PAHs.

5. Conclusion

Accumulated PAHs in consumable sized green mussels (*P. viridis L*.) at Maptaphut coastal water in 2011 and 2012 were found to be lower than the threshold values stated in guidelines of the US-EPA, but PAHs carcinogenic type were found in 2011. The level of accumulated PAHs indicates moderate level of pollution from pyrogenic sources. The induction of CYP1A protein in green mussels can be used as a biomarker for monitoring and evaluation of mussels exposed and clearance to pollutants in the aquatic environment. The induction of CYP1A protein levels declined with declining PAH levels after 30 days depuration. There is considerable risk of contamination of PAHs and other classes of pollutants in Maptaphut coastal water. Therefore, this coastal zone should regularly monitor the contamination of PAHs in consumable green mussels and other marine organisms.

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Correspondence to
Dr. Praparsiri Kanchanopas-Barnette
Department of Aquatic Science,
Faculty of Science,
Burapha University,
Chonburi 20131
Thailand
Phone: +668 7026 2612
E-mail: praparsi@buu.ac.th; mayschonicm@yahoo.com