Quantitative biomonitoring of polycyclic aromatic compounds (PACs) using the Sydney rock oyster (*Saccostrea glomerata*)

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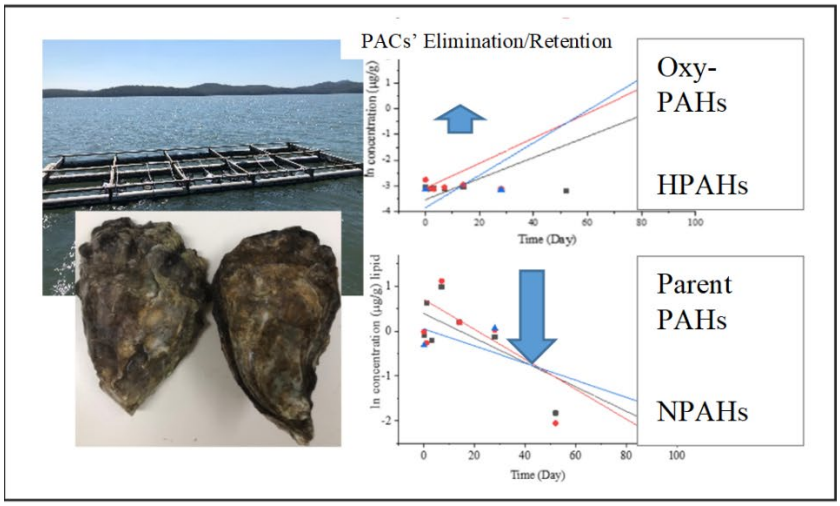
Highlights

- The fate of parent and polar PAHs in a biomonitoring species was assessed
- Quantitative monitoring of PACs was carried out on Sydney rock oysters for 86 days
- Parent PAHs, NPAHs and 9-FLO significantly depurated from oyster tissues
- There was no clear depuration of nearly all oxyPAHs and HPAHs

Graphical Abstract



Abstract

Increasing our understanding of the bioavailable fractions of polycyclic aromatic compounds (PACs) in an aquatic environment is important for the assessment of the environmental and human health risks posed by PACs. More importantly, the behaviour of polar polycyclic aromatic hydrocarbons (polar PAHs), which are metabolites of legacy PAHs, are yet to be understood. We, therefore, carried out a study involving Sydney rock oysters (*Saccostrea glomerata*) sourced from two locations, that had been exposed to PAH contamination, within an Australian southeast estuary. Biomonitoring of these oysters following relocation from the estuary to a relatively isolated waterway was done at 24 and 72 h after deployment, and subsequently at 7, 14, 28, 52 and 86 days. Control samples from Camden Haven River were sampled for PAC analyses just before deployment, after 28 days and at the end of the study (day 86). Lipid-normalised concentrations in oyster tissues across the 86-day sampling duration, elimination rate constants (k_2), biological half-lives ($t_{1/2}$), and time required to reach 95% of steady-state (t_{95}), were reported for parent PAHs and the less-monitored polar PAHs including nitrated/oxygenated/heterocyclic PAHs (NPAHs, oxyPAHs and HPAHs) for the three differently sourced oyster types. Most of the depurating PAHs and NPAHs, as well as 9-FLO (oxyPAH), had k_2 values significantly different from zero ($p < 0.05$). All other oxyPAHs and HPAHs showed no clear depuration, with their concentrations remaining similar. The non-depuration of polar PAHs from oyster tissues could imply greater human health risk compared to their parent analogues.

Keywords: *Polar PAHs, Saccostrea glomerata, Biomonitoring, Elimination rate constant, Biological half-lives, Aquatic environment*

1. Introduction

The fate of polycyclic aromatic compounds (PACs) in the natural environment is of critical importance from a human and environmental health risk perspectives. Exposure to PACs can result in numerous toxicological effects, including carcinogenesis, mutagenesis and teratogenesis [1-4]. Polar polycyclic aromatic hydrocarbons (polar PAHs) have varying physicochemical properties both as a group and in comparison to their parent analogues [5, 6]. These differing physicochemical properties result in varied behaviour and the eventual fate of PACs in the environment. To date, most emphasis has been on the environmental fate of the non-polar parent PAHs compared to polar PAHs such as oxygenated PAHs (oxyPAHs), nitrated PAHs (NPAHs) and heterocyclic PAHs (HPAHs) [7]. In recent years, and with the increasing knowledge of the potentially greater toxicity and bioavailability of polar PAHs, investigation of their fate and behaviour is increasing, albeit mostly focusing on soil and particulate matter in the air, rather than in aquatic environments [8-14].

Polycyclic aromatic compounds are amongst the most abundant contaminants in the aquatic environment [15, 16], which can be taken up by aquatic species. Exposure to PACs can arise from dissolved and particulate organic carbon fractions from the incomplete combustion of organic matter [17]. Alternatively, sediments are global sinks for PACs, which can be remobilised into water and ultimately bio-accumulate in living organisms[15, 18-20]. The bioavailability of PACs and other hydrophobic contaminants in aquatic organisms is of great essence since the bioavailable concentrations are directly related to the toxic effects in organisms including humans [15, 21]. The natural regenerative ability of water bodies, particularly rivers, makes water PAC concentrations, at any point in time, a poor predictor of bioavailability [17]. Accordingly, the evaluation of contaminant bioavailability in aquatic environments has commonly been made using benthic invertebrates such as mussels, clams and oysters [17, 22-26]. Monitoring of PAC concentrations in such organisms has provided a more definitive means of evaluating the

106 bioavailable fractions in an aquatic environment. Depending on their lipophilic characteristics,
107 PACs could be bio-accumulated in the lipid-rich tissues of oysters or remain in the water phase
108 [15, 17, 23]. The high rate of bioaccumulation compared to elimination through metabolism, is
109 mostly the reason for PACs' bioaccumulation in oyster and other sentinel organisms used as
110 aquatic biomonitors [17].
111 Apart from providing information about the health of an environment, bivalves are a popular
112 food source in many areas around the world, and global seafood consumption rates are rising
113 [27]. Early commercialisation of the Sydney Rock Oyster (*Saccostrea glomerata*) in New South
114 Wales (NSW) and Southern Queensland began in the late 1700s but the expansion and
115 development of the oyster industry that formed the basis of the current aquaculture industry
116 dates back to the early 1900s [28, 29]. Sydney rock oysters have historically been exposed directly
117 to petrogenic PACs through the use of coal tar and its derivatives in preserving the timber
118 infrastructure from marine borers [28]. Even though they have generally been phased out from
119 use, PACs are still present in the sediment beneath many farming areas.
120 Recycled plastic products are now being used in place of tarred hardwood infrastructure in
121 shellfish aquaculture of most NSW estuaries, due to their ease of use and durability [28].
122 However, this replacement could also have potential negative impacts on the surrounding
123 environment and oyster consumers as plastics could be a direct source of PACs [30, 31]. Other
124 important sources of PACs such as motor vehicle exhaust, forest and rangeland burning, oil
125 spills, industrial processes and run-off could have contributed to the estuary's sediment PAC
126 load [32, 33]. Oysters that have bio-accumulated environmental contaminants in their tissues may
127 cause chronic health anomalies in humans, especially if consumed over a long period of time
128 [32]. It, therefore, means that despite the ecological importance of bivalves, they could act as a
129 vector for PACs to humans.

130 Toxicokinetic parameters such as uptake rates (k_1), elimination rates (k_2) and biological half-
131 lives ($t_{1/2}$), have been used to describe parent PAH bioaccumulation/elimination patterns in
132 oysters [17, 23, 34]. Such studies provide information about the fate of PAHs in an organism
133 including its bio-accumulative potential, contaminant transformation possibilities, elimination
134 duration and toxicity. In this way, food regulatory authorities have been able to predict the
135 safety of consuming naturally sourced delicacies.
136 Unlike parent PAHs, the fate of the bioavailable fraction of polar PAHs in oysters and other
137 biomonitoring organisms that also serve as food has not been well researched. Further, most of
138 the studies for parent PAHs in the literature are laboratory-based, with very few field studies
139 on PAC bioaccumulation/elimination dynamics. In furtherance of our earlier study on the
140 bioaccumulation of parent and polar PAHs in the tissues of *S. glomerata* [35], the present study
141 set out to carry out on-field quantitative biomonitoring of the bioavailable fractions of parent
142 PAHs, oxyPAHs, NPAHs and HPAHs in the tissues of Sydney rock oysters sourced from a
143 south-east Australian estuary. To the best of our knowledge, this is the first study to
144 simultaneously investigate the elimination patterns of parent and polar PAHs in oysters from
145 an Australian aquatic environment.
146

2. Materials and Methods

2.1 Oyster sampling

Oyster samples were collected from two locations (A and B) close to the mouth of a south-east Australian estuary (Fig. 1). The choice of the estuary was based on recent testings by the NSW Food Authority (NSWFA) and our previously published article on the estuary sediment, oyster and water concentrations [35], which showed that it was experiencing higher than expected levels of PAC contamination. The oysters were collected around potential sources of PACs such as boat ramp, historical residual tar deposits and roadside drains (Fig. 1). A location non-disclosure agreement was entered into with NSW fisheries authorities during the sampling period, preventing us from showing the global positions on the map.

For the depuration study, approximately 200 sampled adult oysters of approximately equal sizes were relocated to a relatively isolated waterway, surrounded by a National Park, with low PAC background levels not exceeding those reported in the source estuary (NSWFA unpublished data). The relocated oysters were here held in three replicate batches in plastic cages on existing oyster culture infrastructure. Oysters translocated to the isolated waterway were tested 24 and 72 h after deployment and subsequently at Day 7, 14, 28, 52 and 86. On each sampling occasion, 15 randomly selected oysters were tested from each of the 3 replicate batches. Control oysters sourced from Camden Haven River, which has a history of low PAH concentrations (NSWFA unpublished data) were also monitored for concentration changes at the time of translocation (time 0), and again at 28 and 86 days later.

2.2. Extraction procedures and PAC analysis

The extraction of parent and polar PAHs from freeze-dried oysters was performed using the QuEChERS approach. A 2 g dried homogenised oyster tissue sample was transferred to a QuEChERS extraction tube, and 20 µl of 100 µg/ml acenaphthene-d10 and fluoranthene-d10 recovery standards and QuEChERS extraction salt (containing NaCl (1 g), $MgSO_4$ (4 g),

172 $Na_3C_6H_5O_7$ (1 g) and $C_6H_6Na_2O_7 \cdot 1.5H_2O$) were added. A mixture of (4:1 v/v) of
173 hexane/acetone (20 ml) was added as extraction solvent followed by shaking with the aid of a
174 vortex for 1 min and centrifugation (2000 xg at 4°C for 10 min). The supernatant was
175 subsequently transferred to a QuEChERS cleanup tube and vortexed (1 min), centrifuged at
176 2000 xg and 4 °C (10 min) and concentrated using a nitrogen concentrator at 35 °C and 12.5
177 psi to about 500 µl.
178 The concentrated extracts were applied to 2 g preconditioned 10% activated silica solid-phase
179 extraction cartridges connected to a manifold and operated under vacuum at 5 mmHg. The
180 fractionating procedure into parent and polar PAH fractions was done using 15 ml Hexane:
181 DCM (5:1) for parent PAHs and 8 ml DCM followed by 5 ml acetone, for polar PAHs. The
182 volume of eluent was concentrated to near dryness, solvent exchanged to hexane by adding 1
183 ml hexane and transferred to 1.5 ml GC vial for GC-MS analysis. Four deuterated internal
184 standard mix (naphthalene-d8, phenanthrene-d10, pyrene-d10 and perylene-d12) was added
185 prior to GC-MS analysis. The concentrations of the individual PACs were normalized against
186 the lipid fraction of the organism. Oyster percent lipid content (12.3) was determined by the
187 modification of a previously used method [36]. Extracted samples were centrifuged at 500 rpm
188 and concentrated to dryness using a nitrogen concentrator. The lipid content was the relative
189 weight of the dried residual. Further details on the analytical methods and quality control
190 procedures can be found in the Supplementary Information and previously published papers [33,
191 37].

192 2.3. Computation of kinetic parameters

193 Contaminant elimination from oyster is generally considered to follow first-order kinetics on a
194 natural log scale[17, 23]:

195 $$\frac{\mathrm{d}C_\mathrm{o}}{\mathrm{dt}} = -k_2 * C_\mathrm{o}$$
196

Where $\frac{dC_o}{dt}$ is the change in PAC concentration in oyster tissues over the change in time.

Integrating, the equation becomes:

$\ln C_{o,t} = - k_2 * t + \ln C_{o,0}$

Where $\ln C_{o,t}$ is the lipid normalized PAC concentration in oyster tissues at time t and initial time (t = 0); k_2 is elimination rate constant which is absolute value of the slope of a plot of $\ln C_o$ and t.

The k_2 values are useful in calculating biological half-lives ($t_{1/2}$) and time required to reach 95% of steady state (t_{95}). These are computed as:

$t_{1/2} = \ln 2 / k_2$

$t_{95} = - \ln 0.05 / k_2$

Elimination rate constants were evaluated through the determination of the slope of the ln-transformed PACs' lipid-normalized concentrations and time. The kinetic parameters were computed for PACs that showed visibly reduced concentrations in computed linear regression equations.

3. Results and Discussion

3.1. Concentration changes in polar and parent PAHs over the depuration period

As a build-up on our earlier published article where the concentrations of PACs in water, sediments and oyster tissue samples were reported for the contaminated site (locations A and B) [35], the depuration of parent and polar PAHs from the tissues of adult Sydney rock oysters were investigated in the current study.

The HPAH mean concentrations in oysters sourced from locations A and B of the estuary were relatively similar over the 86 days of study (Fig. 2A). For location A-sourced oysters, mean concentrations of 2-MBF, for example, at the beginning and end of the investigation, were 0.047 and 0.040 µg/g (Table S1, supplementary information). Mean concentrations of 2-MBF for locations A and B-sourced and control oysters were not significantly different from one another ($p > 0.05$) over the 86-day depuration study (Fig. 2A). The same trend of similar 'day 1' and 'day 86', not significantly different locations A and B- sourced and control oyster concentrations were observed for DBF, XAN, THIA, QUI, IND, 8-MQL and ACRI for the depuration period (Fig. 2A). Mean concentrations of HPAHs were generally less than 0.5 µg/g except for a few concentration spikes observed in few instances (Table S1).

Following the same trend as HPAH, the oxyPAH locations A and B-sourced and control oyster tissue mean concentrations were not significantly different ($p > 0.05$), and concentrations were relatively similar across the 86-day depuration period (Fig. 2B). Lipid-normalized concentrations of oxyPAHs were about 100 order of magnitudes lower than HPAHs concentrations (Table S2; Fig 2B.) with concentrations mostly below 0.1µg/g (Table S2). Out of the seven monitored oxyPAHs in this study, only 9-FLO concentrations showed slight decrease in oyster tissues. 9-FLO concentration in locations A and B-sourced oysters reduced to 0.0022 and 0.0024 µg/g from 0.0064 and 0.0058 respectively (Table S2).

236 The location A-sourced mean lipid-normalized concentrations of 1N-NAP in oyster were
237 significantly different from location B-sourced and control oyster concentrations ($p < 0.05$),
238 which in turn were not significantly different from each another (Fig. 2C). 1N-NAP
239 concentrations increased in location A-sourced and control oysters from 24.6 and 1.28 to 599
240 and 292 µg/g, respectively, within the 86 days of investigation (Table S3). Concentrations of
241 1N-NAP in location B-sourced oysters, within the same duration, were relatively dissimilar
242 (Fig. 2C). The increasing concentrations of 1N-NAP in oysters for locations A and B, may be
243 due to recurring fluxes of naphthalene from ready sources and the subsequent secondary
244 formation of 1N-NAP (Table S3), which is a metabolite of naphthalene. Declining
245 concentrations were however noticed in location A and B-sourced, and control oyster
246 concentrations for 2N-FLU and 9N-ANT (Table S3) with no significant difference ($p > 0.05$)
247 in mean concentrations (Fig. 2C). Mean concentrations of 2N-FLU in location A-sourced (0.91
248 µg/g), location B-sourced (0.98 µg/g) and control oyster (0.73 µg/g) were down to 0.20, 0.18
249 and 0.17 µg/g by the 86th day, respectively (Table S3). For 9N-ANT, initial concentrations in
250 locations A and B-sourced and control oysters were 0.75, 0.72 and 0.64 µg/g while the
251 concentrations by the last day of the study, declined to 0.15, 0.14 and 0.31 respectively (Table
252 S3).
253 Parent PAH lipid-normalized locations A and B-sourced, and control oyster tissue mean
254 concentrations, were not significantly different ($p > 0.05$) in this study (Fig 2D). PAH
255 concentrations showed marked decline within the depuration period (Fig 2D). Location A-
256 sourced oyster FLUA concentration (24,307 µg/g) was the highest PAH concentration at the
257 start of the depuration study, but it reduced considerably to 4.3 µg/g by the 86th day. Location
258 A-sourced oyster PYR and PHEN concentrations also declined from 14,802 and 6,542 µg/g to
259 2.7 and 1.9 µg/g, respectively (Table S4). The use of tar in infrastructure for shellfish
260 aquaculture may have been a contributing factor to the elevated concentrations at the start of

261 the study. Concentrations of low molecular weight (LMW) and high molecular weight (HMW)
262 PAHs in oyster were generally very low, at the start of the study, compared to the
263 concentrations of mid-range molecular size PAHs such as FLUA and PYR. High
264 concentrations of PAHs of mid-range molecular size and hydrophobicity in the tissues of oyster
265 could be as a result of the loss of smaller, volatile analytes and the insufficient partitioning of
266 strongly hydrophobic chemicals in water for uptake by the bivalve [23].
267 Overall, the concentrations of PACs in oyster tissues across the 86-day study was relatively
268 similar for polar PAHs while marked reduction in concentrations were recorded for parent
269 PAHs. The sustained low concentrations might imply a possible equilibrium between oyster
270 tissues and water polar PAH concentrations with potential environmental and human health
271 risks, especially because of the proven greater toxicity of polar PAHs compared to their parent
272 analogues [6, 38, 39].
273 3.2. Toxicokinetics of parent and polar PAHs in the Sydney rock oyster
274 The concentrations of the monitored parent PAHs in location A-sourced, location B-sourced
275 and control oyster samples of the estuary declined throughout the depuration period except for
276 FLU with seemingly rising concentrations (Fig. S2). For NPAHs, concentrations of 1N-NAP
277 in the tissues of oyster did not show a declining trend (locations B and control) (Fig. S2) like
278 2N-FLU and 9N-ANT concentrations (Fig. S1I and S1J). 9-FLO was the only oxyPAH with a
279 declining trend over the 86-day depuration study (Fig. S1K). Similarly, all HPAHs investigated
280 in this study did not exhibit visible elimination by the 86th day (Fig. S2). The non-declining
281 trend in HPAHs, oxyPAHs (all but one), 1N-NAP and FLU may be indicative of their possible
282 non-bioaccumulation in oyster tissues.
283 The elimination rate constants k_2, for bio-accumulated PAHs, 2N-FLU, 9N-ANT (NPAHs) and
284 9-FLO (oxyPAH), were evaluated by determining the slope of the linear regression between
285 their ln-transformed concentrations in oyster tissues and time [23]. For parent PAHs, only PHEN,

ANTH, FLUA, PYR, I[cd])P and D[a,h]A had k_2, values which were significantly different from zero ($p < 0.05$) (Fig. S1).

Elimination rate constant values, for parent PAHs, ranged from 0.02 – 0.11 day $^{-1}$ in location A-sourced oysters and 0.001 – 0.09 day $^{-1}$ in location B-sourced oysters (Table 2). 2N-FLU and 9N-ANT k_2 values of 0.03 and 0.02 day $^{-1}$, respectively, in location A-sourced oysters and 0.03 day $^{-1}$ in location B-sourced oysters (Table 2) were significantly different from zero ($p < 0.05$). Similarly, for 9-FLO, the k_2 value for both location A and location B-sourced oysters (0.01 day $^{-1}$) was significantly different from zero ($p < 0.05$). There was no significant value of k_2 recorded for any control oyster. Similarly, the k_2 values of ACENY, ACEN, B[a]A, CHRY, B[b+k]F and B[a]P were not significantly different from zero ($p > 0.05$) for all oyster types (Table 2). Elimination rate constant were in the order location A-sourced > location B-sourced > control oysters implying that elimination of PACs may be concentration dependent since PAC oyster concentrations followed the same order.

The $t_{1/2}$ for location A-sourced, location B-sourced and control oysters were not significantly different ($p > 0.05$) and ranged from 6.4 (PHEN and FLUA) to 53.9 days (9-FLO) for location A-sourced, 8.1 (PHEN) to 647.8 days (B[a]P) for location B-sourced and 9.7 (PYR) to 98.5 days (B[b+k]F) for control oysters (Table 2). Similarly, t_{95} values were not significantly different ($p > 0.05$) for location A and B-sourced oysters, and control. It ranged from 27.6 (PHEN and FLUA) to 232.8 days (9-FLO) for location A-sourced, 35.0 (PHEN) to 2800 days (B[a]P) for location B-sourced and 41.8 (PYR) to 425.5 days (PYR) for control oysters (Table 2).

The kinetic parameters for locations A and B-sourced, and control oysters, in this study, did not have the same values. This difference may be due to different environmental dynamics including varying PAC concentrations in water and sediment for the estuary (locations A and B), Camden Haven River and the isolated waterway. Varying values were particularly noticed

311 in PAHs (e.g. B[b+k] and B[a]P) whose slope were not significantly different from zero ($p > 0.05$) (Table 2).

313 Studies on the elimination of PACs from aquatic organisms are very scarce in the literature. As
314 far as we know, there are no past investigations on the elimination of polar PAHs from an
315 aquatic organism. Few studies have however investigated the elimination dynamics of PAHs
316 from bivalves. A comparison of the parent PAH kinetic parameters obtained in this study and
317 two previous studies revealed that location A-sourced computations were closest to the
318 literature values (Table 3). For example, location A-sourced oyster B[a]A k_2 value in this study
319 (0.095 day $^{-1}$) compares very well with the value of 0.092 day^{-1} for mussels (*Elliptio*
320 *complanata*) [17] (Table 3). Close comparison also existed in I[cd]P and D[a,h]h k_2 values of
321 0.039 and 0.046 day $^{-1}$ in this study, and 0.047 and 0.069 day $^{-1}$ respectively, for the study on
322 mussels [17] (Table 3). ACENY k_2 value was 0.034 in this study compared to 0.046 day $^{-1}$ in a
323 much ealier study also on *E. complanata* [23]. Arising from the similar k_2 values of this study and
324 the two previous studies, the half-life values were also similar (Table 3). Half-life values for
325 D[a,h]A in this study and one of the studies were both 15.1 days [17]. For I[cd]P, it was 14.7 days
326 in *E. complanata* [17] and 17.8 days in this study. PHEN, ANTH, FLUA, PYR and B[a]A values
327 in this study were just a little higher than values in *E. complanata* [23] (Table 3). Without
328 considering CHRY, which is the only parent PAH with a marked difference in k_2 and $t_{1/2}$ values,
329 the t_{95} values (for parent PAHs) ranged from 27.6 (PHEN and FLUA) to 95.5 days (ACEN) in
330 this study as well as 16.9 (PHEN) to 64.7 days (ACENY) and 12.6 (ACEN) to 63.7 days
331 (I[cd]P) respectively, in the two previous studies [17, 23] (Table 3). The reason for the difference
332 with CHRY is unclear.

333 The k_2, $t_{1/2}$ and t_{95} values for 1N-NAP, FLU, all oxyPAHs (except 9-FLO) and all HPAHs were
334 not computed because of thier variable behaviour as seen in their rising ln concentration with
335 time for all three differently sourced oysters (Fig. S2). The rising concentrations might have

resulted from higher bioavailability of these contaminants from water due to their less hydrophobic nature. The kinetic parameters for the polar PAHs have common attributes of lower k_2 values and longer $t_{1/2}$ and t_{95} compared to the parent PAHs. These imply their slower rate of elimination compared to parent PAHs. The PAC with the longest computed $t_{1/2}$ value (53.9 days) and t_{95} value (232.8 days), in this study, is 9-FLO: a polar PAH (Table 3).

3.3. Relationship between elimination rate constants (k_2) and log K_{ow} for parent and polar PAHs

The varying rates of elimination of bio-accumulated parent and polar PAHs from oysters may be related to their physicochemical properties and particularly log K_{ow}. The linear regression model of k_2 and log K_{ow} for parent PAHs and polar PAHs (Fig. S3) could provide a description of the possible relationship existing between these two parameters for the particular PAC type. Simple linear regression analysis of k_2 *versus* log K_{ow} for (A) parent PAHs and (B) polar PAHs (Fig. S3), for locations A and B-sourced and control oysters, yielded varying results. For parent PAHs, equations k_2 = -0.016 log K_{ow} + 0.26, -0.018 log K_{ow} + 0.12 and -0.014 log K_{ow} + 0.12 were obtained with r^2 values of 0.25, 0.34 and 0.47 for locations A and B-sourced and control oysters, respectively (Fig. S3). Though not significant ($p > 0.05$), the inverse relationship between k_2 and K_{ow} showed that the parent PAHs were being passively eliminated from the oyster tissues. Based on the r^2 values, the variability in k_2 that could be explained by log K_{ow} was as much as 25, 34 and 47% for location A-sourced, location B-sourced and control oysters respectively. These are high percentages considering the fact that the depuration study was conducted in the field and other competing factors could have influenced the rate of depuration. Similar r^2 values of k_2 and Kow for parent PAHs have been reported in the literature [17, 23, 40, 41]. Non-significant ($p > 0.05$) positive relationships were however observed in polar PAHs when a simple linear regression analysis was performed for k_2 and log K_{ow}, for locations A and B, indicating that the polar PAHs might not have been eliminated from oyster tissues possibly

because of their bioavailability from surrounding water. Very low r^2 values computed for locations A and B oysters (0.04 and 0.05), indicated that the proportion of variability in k_2 that could be explained by log K_{ow} was very low (Fig. S3). For the control oysters, a non-significant ($p > 0.05$) negative relationship existed between k_2 and K_{ow} (Fig. S3) implying possible passive depuration of polar PAHs from oyster tissues. This could probably be due to the prevalence of polar PAHs in control oysters particularly from parent PAH transformation, compared to oysters from the two other locations.

4.0 Conclusions

The toxicokinetics of parent PAHs and the less monitored polar PAHs (NPAHs, oxyPAHs and HPAHs) in *S. glomerata* were investigated with a first-order, one-compartment, linear model. Oysters relocated from a southeast Australian estuary to a comparably clean isolated waterway in NSW Australia, demonstrated varied elimination rates of parent and polar PAHs. Parent PAHs (except FLU) substantially bio-accumulated in oyster tissues and demonstrated impressive elimination rates in the isolated waterway. Similarly, two of the three investigated NPAHs had significant k_2 values implying strong depuration from oyster tissues. All oxyPAHs (except 9-FLO) and HPAHs exhibited low depuration with their concentrations remaining fairly constant. FLU, 1N-NAP and 9-FLO demonstrated varied behaviour compared to other members of their individual groups with rising concentrations of FLU and 1N-NAP, and reducing concentration of 9-FLO from oyster tissues. Unlike parent PAHs, polar PAH did not exhibit considerable depuration from oyster tissues as their k_2 values largely exhibited direct relationship with chemical hydrophobicity.

Acknowledgements

The authors acknowledge the support rendered by the Commonwealth of Australia and the University of Newcastle Australia through the Australian Government Research Training Program (RTP) Scholarship. The support of the NSW Food Authority and NSW Department of Primary Industries staff, during the fieldwork, is profoundly appreciated. The authors extend their appreciation to Mr Brand Archer and Mr Kyle Johnston (NSW DPI Fisheries) for their support and advice regarding the experimental setup and deployment of shellfish. The authors would also like to acknowledge the support of NSW shellfish industry members. O. Idowu appreciates the assistance rendered by Anthony Umeh during the laboratory work.

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Quantitative biomonitoring of polycyclic aromatic compounds (PACs) using Sydney rock oyster (*Saccostrea glomerata*)

Supplementary Information

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SI-Text 1: Chemicals and reagents

The following chemicals were purchased from Sigma Aldrich, Australia:

PAH mix containing: acenaphthylene (ACENY), acenaphthene (ACEN), fluorene (FLU), phenanthrene (PHEN), anthracene (ANTH), fluoranthene (FLUA), pyrene (PYR), benz[a] anthracene (B[a]A), chrysene (CHRY), benzo[b+k]fluoranthene, benzo[a]pyrene (B[a]P), indeno[1,2,3-cd]pyrene (I[cd]P) and dibenz[a,h]anthracene (D[ah]A); 7 carbonyl-OxyPAHs: 1,4-naphthoquinone (1,4-NQ), 9-fluorenone (9-FLO), 2-methyl anthraquinone (2-MAQ), 2-ethylanthraquinone (2-EAQ), 9,10-anthraquinone (9,10-ANQ), 2,3-dimethylanthraquinone (2,3-DMAQ), 7H-benz[d,e]anthracene-7-one (7H-BANT); 5 N-heterocycles: quinoline (QUI), 8-methylquinoline (8-MQL), indole (IND), acridine (ACR), carbazole (CBZ); 3 O-heterocycles: dibenzofuran (DBF), 2-methylbenzofuran (2-MBF), xanthene (XAN); 1 S-heterocycle: thianaphthene (THIA); 3 NPAHs: 1-nitronaphthalene (1N-NAP), 2-nitrofluorene (2N-FLU), 9-nitroanthracene (9N-ANT) and internal standards comprising of naphthalene-d8, phenanthrene-d10, chrysene-d12 and perylene-d12, as well as acenaphthene-d10 and flouranthene-d10 surrogate standards. Anhydrous sodium sulphate (99% purity), n-hexane, dichloromethane and acetone (99.8% purity) were also sourced from Sigma Aldrich, Australia. QuEChERS extraction tubes (50mL-Cat# 982-5650) and QuEChERS clean up, dispersive SPE tubes (15 mL-Cat# 5982-5156) were purchased from Agilent Technologies, Australia.

SI-Text 2: GC-MS analysis

The concentrations of PAHs, oxy-PAHs, NPAHs and HPAHs in extracts were measured by an Agilent 7890 B gas chromatograph (GC) coupled to a mass spectrometer (MS) with a HP-5MS (30 m x 0.25 mm x 0.25 μm) column. The GC oven parameters were according to (Idowu et al., 2019b; 2020). Sample volumes of 1 μl were injected into the system in splitless mode. The

mass spectrometer was operated in an electron impact ionisation mode, at 70 eV, for all the measured analytes, as well as under selected ion monitoring mode.

SI-Text 3: Quality assurance and quality control

Throughout the extraction and analysis processes, strict quality assurance and quality control procedures were followed. Amber coloured glass vials were used throughout to minimise PAH loss from photolysis. Cross-contamination was checked by analysing laboratory blanks after every batch of 10 samples during GC-MS analysis. Target polar and non-polar PAHs were either not detected or below detection limits in the solvent blanks. Triplicates samples were analysed and considering the variation in physicochemical properties of different polar PAHs, we conducted an exclusive recovery rate experiment under optimised extraction conditions. Oyster tissue samples were replicated five times for the experiment. Tissues (1g) were spiked with 20µl of 100µg/ml acenaphthene-d10/fluoranthene-d10 (parent PAHs) and individual polar PAHs, extracted according to the QuEChERS method, fractionated and analysed. Unspiked tissue samples were also extracted and analysed for polar PAHs and concentrations of both spiked and unspiked samples used to compute their recovery rates. The recovery results for parent and polar PAHs are presented in Table S5 (supplementary information).

Table S1

Concentration changes (μg/g d.w.) of HPAHs during the 86-day depuration study

Day	2-MBF			DBF			XAN			THIA			QUI		
	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control
0	0.048± 0.004	0.063± 0.021	0.044± 0.003	0.233± 0.173	0.258± 0.189	0.045± 0.014	0.144± 0.015	0.146± 0.016	0.148± 0.038	0.142± 0.010	0.305± 0.328	0.116± 0.011	0.048± 0.001	0.048± 0.002	0.047± 0.001
1	0.044± 0.002	0.044± 0.001		0.416± 0.139	0.204± 0.234		0.159± 0.004	0.154± 0.023		2.135± 0.327	0.111± 0.003		0.048± 0.002	0.048± 0.001	
3	0.045± 0.001	0.044± 0.001		0.181± 0.164	0.416± 0.244		0.163± 0.005	0.149± 0.004		0.125± 0.003	0.123± 0.016		0.048± 0.001	0.047± 0.001	
7	0.045± 0.002	0.047± 0.002		0.111± 0.120	0.040± 0.003		0.153± 0.015	0.203± 0.061		1.290± 1.100	0.594± 0.743		0.048± 0.001	0.048± 0.002	
14	0.048± 0.004	0.052± 0.009		0.184± 0.125	0.205± 0.235		0.334± 0.195	0.323± 0.129		0.183± 0.056	0.146± 0.021		0.049± 0.001	0.073± 0.023	
28	0.043± 0.001	0.044± 0.001	0.043± 0.000	0.328± 0.087	0.629± 0.001	0.289± 0.015	0.163± 0.008	0.188± 0.000	0.201± 0.019	0.635± 0.121	0.388± 0.002	0.459± 0.089	0.049± 0.001	0.049± 0.002	0.048± 0.000
52	0.041± 0.033	0.032± 0.037		0.020± 0.030	0.150± 0.147		0.190± 0.308	0.246± 0.127		0.689± 0.573	0.510± 0.571		0.013± 0.014	0.817± 1.045	
86	0.037± 0.059	0.046± 0.059	0.069± 0.003	0.028± 0.038	0.81±0.095	0.019± 0.017	0.542± 0.251	0.739± 1.035	0.249± 0.138	0.620± 0.581	0.489± 0.566	0.489± 0.278	0.109± 0.056	0.238± 0.361	0.035± 0.011

	IND			8-MQL			ACRI			CBZ		
	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control
0	0.066± 0.011	0.042± 0.003	0.040± 0.001	0.089± 0.005	0.088± 0.002	0.087± 0.000	0.239± 0.054	0.434± 0.228	0.185± 0.008	0.298± 0.022	0.260± 0.073	0.157± 0.006
1	0.021± 0.030	0.041± 0.001		0.091± 0.006	0.088± 0.000		0.368± 0.011	0.220± 0.006		0.241± 0.023	0.218± 0.025	
3	0.044± 0.005	0.043± 0.003		0.087± 0.003	0.089± 0.001		0.256± 0.047	0.304± 0.103		0.337± 0.199	0.221± 0.009	
7	0.042± 0.002	0.045± 0.001		0.087± 0.001	0.091± 0.002		0.263± 0.065	0.253± 0.016		0.202± 0.014	0.241± 0.021	
14	0.065± 0.014	0.064± 0.019		0.089± 0.004	0.093± 0.007		0.362± 0.079	0.395± 0.157		0.658± 0.465	0.426± 0.168	

28	0.043± 0.001	0.044± 0.002	0.045± 0.003	0.088± 0.005	0.086± 0.002	0.087± 0.003	0.375± 0.096	0.315± 0.002	0.292± 0.015	0.212± 0.032	0.189± 0.003	0.225± 0.103
52	0.002± 0.000	0.006± 0.001		0.008± 0.001	0.008± 0.000		0.015± 0.003	0.014± 0.001		0.012± 0.000	0.013± 0.003	
86	0.108± 0.001	0.787± 0.811	0.029± 0.004	1.683± 1.126	0.879± 0.470	1.074± 0.375	2.018± 1.142	0.838± 0.520	0.841± 0.354	2.506± 1.281	0.581± 0.407	0.432± 0.268

2-MBF (2-methylbenzofuran), DBF (dibenzofuran), XAN (xanthene), THIA (thianaphthene), QUI (quinolone), IND (indole), 8-MQL (8-methylquinoline), ACR (acridine) and CBZ (carbazole); values are mean±SD.

Table S2

Concentration changes (μg/g d.w.) of oxyPAHs during the 86-day depuration study

Day	1,4-NAQ			9-FLO			9,10-NQ			2-EAQ		
	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control
0	0.0061±0.0106	0.0038±0.0060	0.0144±0.0076	0.0064±0.0010	0.0058±0.0011	0.0047±0.0002	0.0126±0.0026	0.0133±0.0092	0.0091±0.0060	0.4453±0.7468	0.0049±0.0006	0.0115±0.0056
1	0.0037±0.0060	0.0046±0.0063		0.0042±0.0028	0.0052±0.0004		0.0082±0.0050	0.0082±0.0013		0.2525±0.4281	0.0070±0.0033	
3	0.0044±0.0076	0.0052±0.0055		0.0068±0.0026	0.0062±0.0010		0.0146±0.0109	0.0121±0.0036		0.0106±0.0115	0.0094±0.0088	
7	0.0024±0.0042	0.0006±0.0005		0.0054±0.0007	0.0065±0.0003		0.0075±0.0005	0.0091±0.0014		0.0102±0.0013	0.0124±0.0074	
14	0.0001±0.0001	0.0009±0.0001		0.0101±0.00027	0.013±0.002		0.0248±0.0245	0.0164±0.0057		0.1953±0.2035	1.0156±0.6871	
28	0.0001±0.0001	0.0005±0.0001	0.0001±0.0002	0.0050±0.0002	0.0053±0.001	0.0061±0.0017	0.0083±0.0061	0.0061±0.0010	0.0098±0.0090	0.0123±0.0072	0.0107±0.0001	0.0110±0.0065
52	0.0288±0.0394	0.0092±0.0021		0.0028±0.0011	0.0024±0.0004		0.0619±0.0891	0.0127±0.0072		0.0749±0.0854	0.0541±0.0492	
86	0.0169±0.0016	0.0448±0.0604	0.0124±0.0010	0.0022±0.0001	0.0024±0.0004	0.0023±0.0004	0.0430±0.0296	0.0438±0.0428	0.0136±0.0041	0.0882±0.0425	0.1037±0.0291	0.0878±0.0239

	2-MAQ			2,3-DMAQ			7H-BANT		
	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control
0	0.0072±0.002	0.0057±0.0003	0.0051±0.0001	0.0123±0.0063	0.0059±0.0016	0.0039±0.0002	0.0339±0.0006	0.0567±0.0519	0.0036±0.0005

1	0.0045±0.0022	0.0053±0.0001		0.0060±0.0003	0.0047±0.0012		0.0135±0.0135	0.0241±0.0042	
3	0.0056±0.0002	0.0054±0.0001		0.0061±0.0008	0.0047±0.0009		0.0223±0.0100	0.0167±0.0069	
7	0.0055±0.003	0.0057±0.0000		0.0050±0.0010	0.0059±0.0007		0.0136±0.0049	0.0163±0.0032	
14	0.0058±0.0001	0.0060±0.0006		0.0066±0.0010	0.0067±0.0021		0.1699±0.0334	0.3873±0.3639	
28	0.0058±0.0003	0.0056±0.00010	0.0055±0.0001	0.0046±0.0006	0.0046±0.0010	0.0048±0.0010	0.0112±0.0051	0.1337±0.0001	0.0906±0.0327
52	0.2535±0.3323	0.1546±0.1749		0.4485±0.6128	0.1248±0.0919		0.0308±0.0274	0.0967±0.0104	
86	0.4809±0.0657	0.0091±0.0022	0.0049±0.0011	0.1458±0.0257	0.0149±0.0068	0.0176±0.0031	0.0254±0.0236	0.6197±0.3653	0.4235±0.1846

1,4-NQ (1,4-naphthoquinone), 9-FLO (9-fluorenone), 9,10-ANQ (9,10-anthraquinone), 2-EAQ (2-ethylanthraquinone), 2-MAQ (2-methyl anthraquinone) 2,3-DMAQ (2,3-dimethylanthraquinone) and 7H-BANT (7H-benz[d,e]anthracene-7-one); values are mean±SD.

Table S3

Concentration changes (μg/g d.w.) of NPAHs during the 86-day depuration study

	1N-NAP			2N-FLU			9N-ANT		
Day	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control
0	24.6±35.9	0.02±0.014	1.275±0.606	0.914±0.065	0.983±0.163	0.728±0.033	0.750±0.047	0.720±0.087	0.640±0.073
1	204±74.2	0.550±0.009		1.861±1.310	0.769±0.044		0.886±0.150	0.638±0.052	
3	172.4±203.8	0.003±0.003		0.811±0.107	7.236±8.299		0.811±0.170	1.346±0.684	
7	153±135.6	0.003±0.007		2.688±2.034	3.054±1.898		0.920±0.360	1.026±0.164	
14	764.8±457.1	0.001±0.001		1.220±0.065	1.217±0.363		4.095±1.455	6.178±3.532	
28	108.8±31.8	0.007±0.001	72.6±21.4	0.875±0.052	1.023±0.001	1.068±0.304	0.726±0.016	1.047±0.001	0.744±0.067
52	507.2±96.6	0.002±0.0006		0.161±0.098	0.129±0.071		0.224±0.215	0.094±0.064	
86	598.9±59.4	0.002±0.009	292.4±29.2	0.197±0.012	0.181±0.022	0.170±0.033	0.147±0.017	0.135±0.032	0.314±0.227

1N-NAP (1-nitronaphthalene), 2N-FLU (2-nitrofluorene), 9N-ANT (9-nitroanthracene); values are mean±SD.

Table S4

Concentration changes (μg/g d.w.) of parent PAHs during the 86-day depuration study

	ACENY			ACEN			FLU			PHEN		
Day	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control
0	1.29±1.27	1.195±0.388	1.433±0.061	5.318±7.537	0.031±0.013	0.464±0.037	5.457±7.597	0.017±0.006	0.023±0.003	6542.0±7580.2	247.5±150.6	164.5±203.4
1	0.226±0.195	1.579±0.126		1.557±2.335	0.061±0.023		1.210±2.051	0.020±0.004		8624.7±13858.9	42.3±42.4	
3	1.549±1.171	2.590±0.417		0.567±0.718	0.553±0.419		0.565±0.587	0.059±0.002		527.0±458.1	10.2±14.4	
7	1.183±0..989	1.324±1.037		0.039±0.028	0.148±0.164		0.020±0.006	0.121±0.178		116.7±125.9	164.9±236.2	
14	10.725±14.385	2.865±0.494		1.494±2.280	0.865±0.167		6.854±1.638	0.044±0.017		66.4±25.3	26.6±21.6	
28	0.416±0.175	0.233±0.021	0.203±0.010	0.128±0.029	0.074±0.006	0.132±0.109	2.441±1.689	1.295±0.208	1.799±0.390	1.421±0.715	0.503±0.043	0.574±0.051
52	0.062±0.012	0.061±0.007		0.103±0.089	0.179±0.129		0.776±1.158	0.650±0.994		0.047±0.031	0.286±0.449	
86	0.111±0.055	0.036±0.020	0.028±0.014	0.095±0.011	0.259±0.110	0.116±0.064	1.086±0.833	0.749±1.057	0.109±0.060	1.913±0.887	0.102±0.080	0.235±0.072

	ANTH			FLUA			PYR			B[a]A			CHRY		
	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Upstream-sourced	Downstream-sourced	Control
0	1112.8±1434.4	52.5±34.5	17.5±7.7	24307.3±27698.4	366.4±332.9	531.2±574.9	14802.4±17013.5	237.6±227.1	367.4±418.8	31.6±27.0	0.104±0.180	0.0±0.0	29.0±24.9	0.073±0.126	0.0±0.0
1	1861.1±3127.2	16.4±20.3		17026.7±23758.9	123±131.3		10707.6±15224.7	55.9±42.6		2.2±1.6	0.0±0.0		1.973±1.481	0.0±0.0	
3	48.1±42.1	2.091±2.713		3198.7±2846.5	31.3±43.9		1928.3±1707.7	19.3±27.0		4.7±1.4	0.017±0.030		4.22±1.32	0.0±0.0	

7	24.5±13.6	176.1±272.7		867.7±1397.4	571.1±911.4		562.7±899.3	336.3±530.9		0.679±0.951	1.049±1.781		0.576±0.873	0.931±1.613	
14	74.4±28.5	29.9±24.2		226.1±172.6	135.8±108.9		128.3±109.9	50.0±34.0		1.92±1.556	0.170±0.173		1.686±1.447	0.100±0.147	
28	0.513±0.119	0.257±0.010	0.239±0.004	5.997±2.737	0.837±0.280	0.575±0.125	4.64±2.38	0.538±0.244	0.396±0.104	3.70±2.88	0.0±0.0	0.0±0.0	6.32±5.09	0.0±0.0	0.0±0.0
52	0.113±0.052	0.107±0.046		0.683±0.289	1.994±2.260		1.25±1.205	0.355±0.100		0.44±0.28	0.171±0.162		0.383±0.302	0.152±0.109	
86	0.242±0.051	0.076±0.005	0.224±0.142	4.255±2.854	0.647±0.036	0.449±0.154	2.67±1.71	0.386±0.027	0.281±0.094	0.42±0.20	0.152±0.148	0.118±0.043	0.847±0.568	0.412±0.293	0.250±0.121
		B[b/k]F			B[a]P			D[a,h]A			I[c,d]P				
	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control	Location A-sourced	Location B-sourced	Control			
0	74.3±94.0	0.027±0.035	0.044±0.025	71.8±52.3	0.075±0.072	0.150±0.012	8.13±5.14	0.468±0.338	1.02±0.459	35.1±23.0	2.11±0.980	3.77±0.818			
1	8.14±7.22	0.057±0.036		6.64±5.72	0.062±0.023		0.481±0.037	0.501±0.116		1.76±0.273	2.46±0.089				
3	4.80±1.18	0.773±0.092		4.45±1.49	0.076±0.013		0.683±0.189	0.920±0.403		2.20±0.481	2.49±0.392				
7	0.935±1.590	1.09±1.73		0.783±1.23	0.078±0.004		0.263±0.203	0.447±0.134		1.03±0.897	1.79±0.534				
14	1.78±1.70	0.374±0.175		1.60±1.39	0.024±0.042		0.343±0.271	0.682±0.215		1.03±0.908	1.88±0.573				
28	6.74±5.01	0.018±0.032	0.0±0.0	4.32±3.93	0.033±0.057	0.0±0.0	0.327±0.153	0.136±0.017	0.118±0.004	4.38±4.25	0.13±0.222	0.00±0.00			
52	0.247±0.188	0.204±0.184		0.225±0.084	0.037±0.034		0.034±0.015	0.068±0.007		0.069±0.116	0.0±0.0				
86	0.819±0.040	0.123±0.150	0.147±0.120	0.403±0.347	0.077±0.015	0.051±0.044	0.072±0.003	0.073±0.025	0.053±0.033	0.148±0.136	0.0±0.0	0.00±0.00			

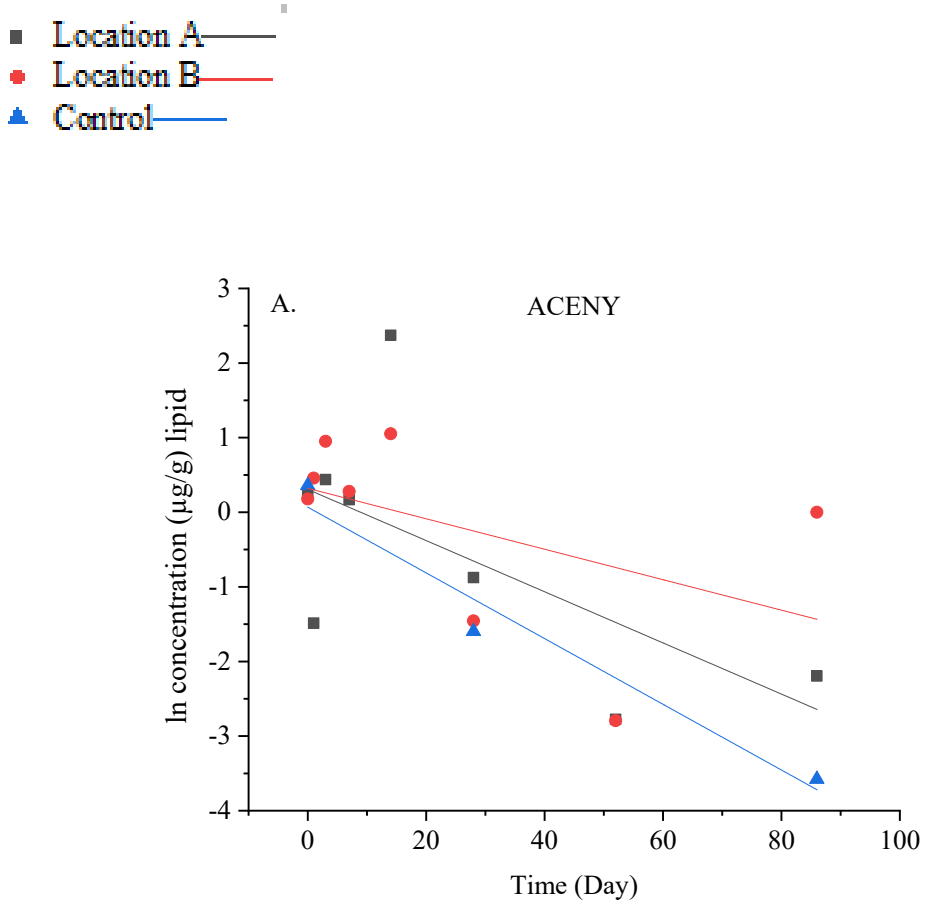
ACENY (acenaphthylene), ACEN (acenaphthene), FLU (fluorine), PHEN (phenanthrene), ANTH (anthracene), FLUA (fluoranthene), PYR (pyrene) B[a]A (benz[a] anthracene), CHRY (chrysene), B[b+k]F (benzo[b+k]fluoranthene), B[a]P (benzo[a] pyrene), I[cd])P (indeno[1,2,3-cd]pyrene) and D[a,h]A (dibenz[a,h]anthracene); values are mean±SD.

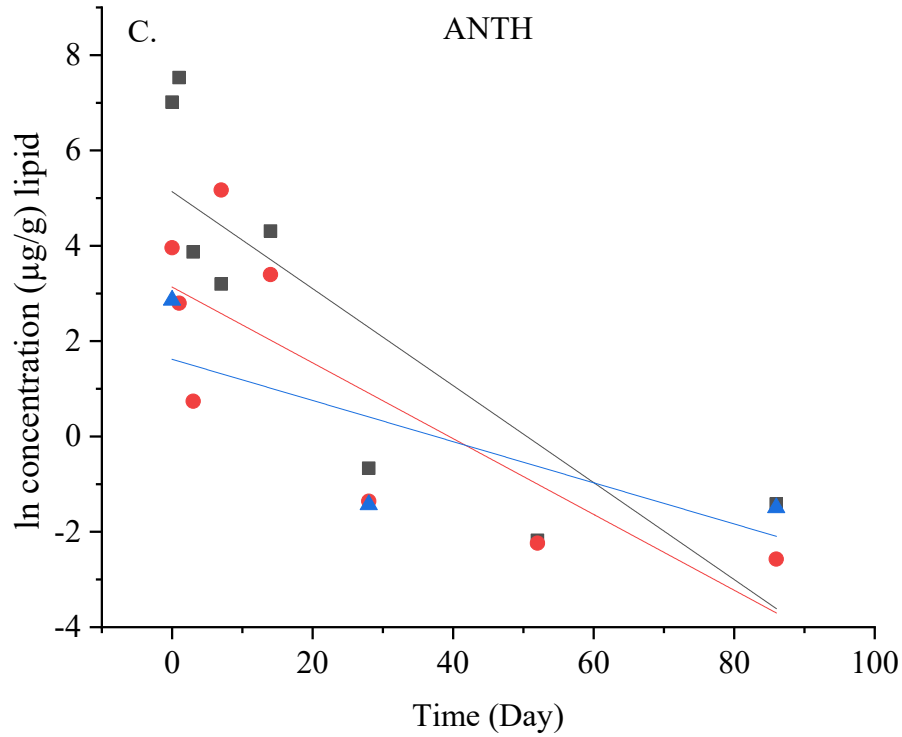
Table S5

Recoveries (%) of Acenaphthene-d10, Fluoranthene (d10) and individual NPAHs, oxy-PAHs and heterocyclic PAHs

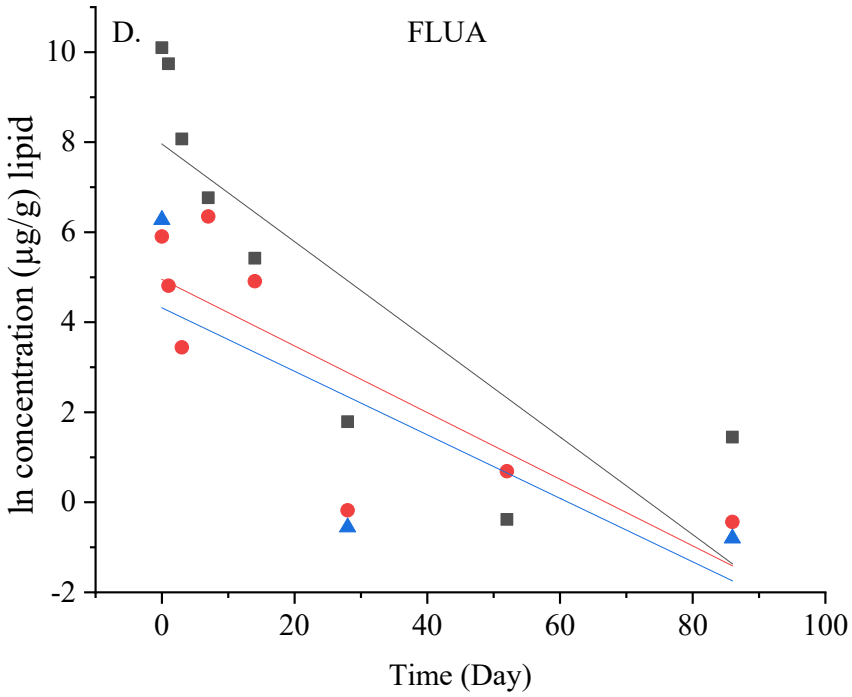
Parent PAHs	ACE-D10	FLU-D10						
	69.2	82.5						
Oxy-PAHs	1,4-NQ	9-FLO	9,10-ANQ	2-MAQ	2-EAQ	2,3-DMAQ	7H-BANT	
	44.6	92.8	90.9	79.1	82.1	81.7	81.8	
NPAHs	1N-NAP	2N-FLU	9N-ANT					
	66.6	79.8	92.1					
HPAHs	2-MBF	XAN	THIA	QUI	IND	8-MQL	ACR	CBZ
	106.9	63.5	77.6	101.9	101.1	102.3	66.9	76.8

ACE-D10 (Acenaphthene-d10), FLU-D10 (Fluoranthene-d10), 1,4-NQ (1,4-naphthoquinone), 9-FLO (9-fluorenone), 9,10-ANQ (9,10-anthraquinone), 2-MAQ (2-methyl anthraquinone), 2-EAQ (2-ethylanthraquinone), 2,3-DMAQ (2,3-dimethylanthraquinone), 7H-BANT (7H-benz[d,e]anthracene-7-one), 1N-NAP (1-nitronaphthalene), 2N-ANT (2-nitroanthracene), 9N-FLU (9-nitrofluorene), 2-MBF (2-methylbenzofuran), XAN (xanthene), THIA (thianaphthene), QUI (quinolone), IND (indole), 8-MQL (8-methylquinoline), ACRI (acridene) and CBZ (carbazole).

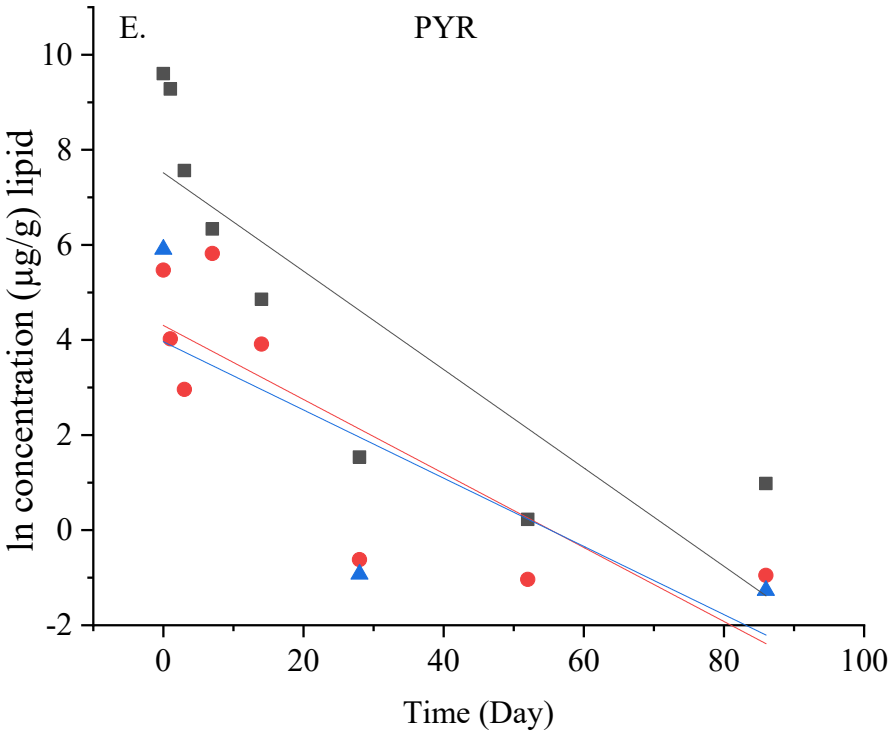

Location A
Location B
Control
A.
ACENY
ln concentration (µg/g) lipid
Time (Day)
B.
PHEN
ln concentration (µg/g) lipid
Time (Day)


C.
ANTH
ln concentration (µg/g) lipid
Time (Day)
8
6
4
2
0
-2
-4
0
20
40
60
80
100

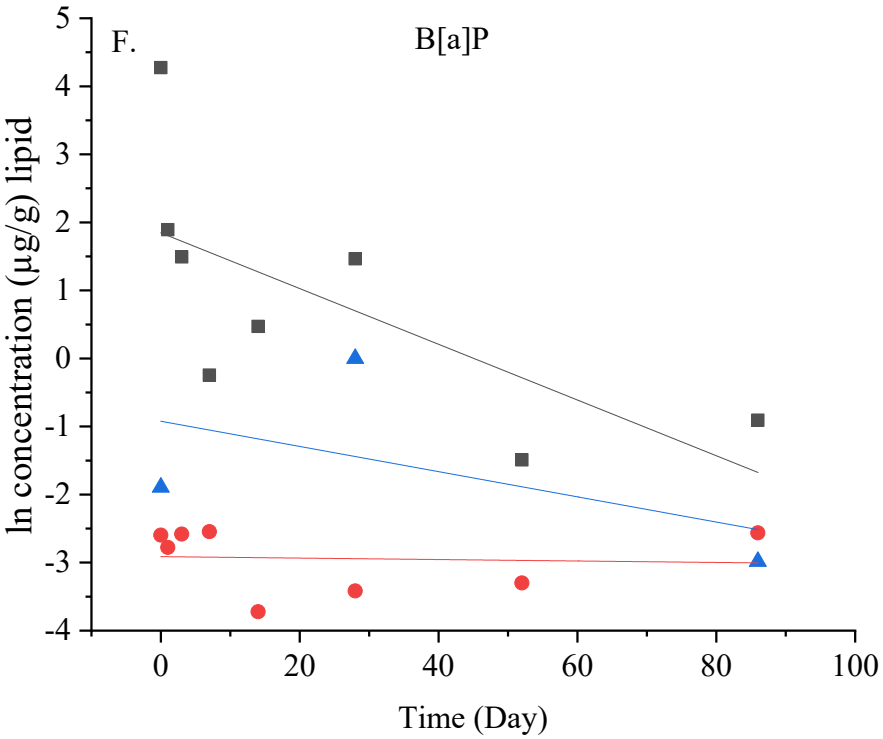
132


D.
FLUA
ln concentration (µg/g) lipid
Time (Day)
10
8
6
4
2
0
-2
0
20
40
60
80
100

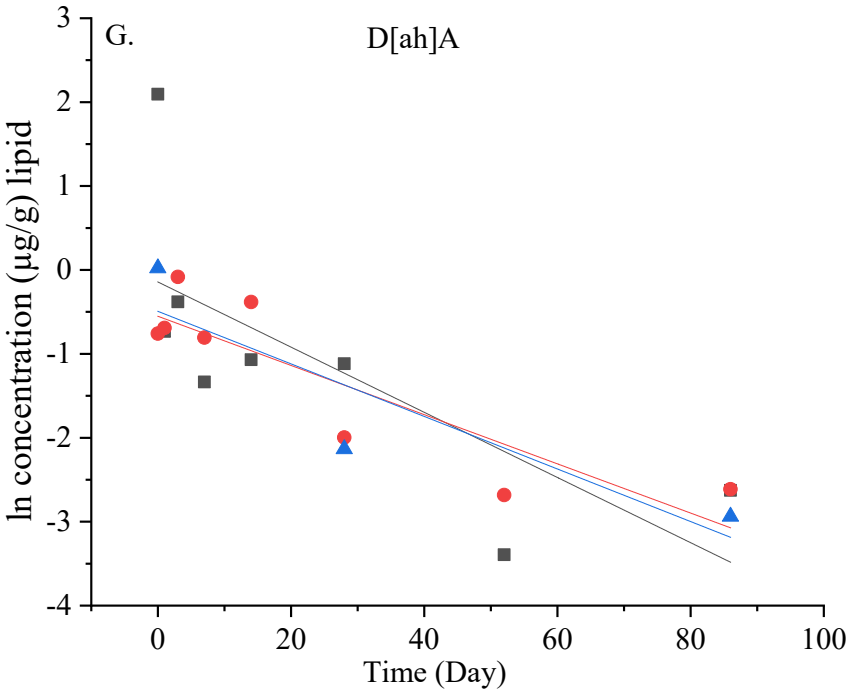
133


E.
PYR
ln concentration (µg/g) lipid
Time (Day)
10
8
6
4
2
0
-2
0
20
40
60
80
100

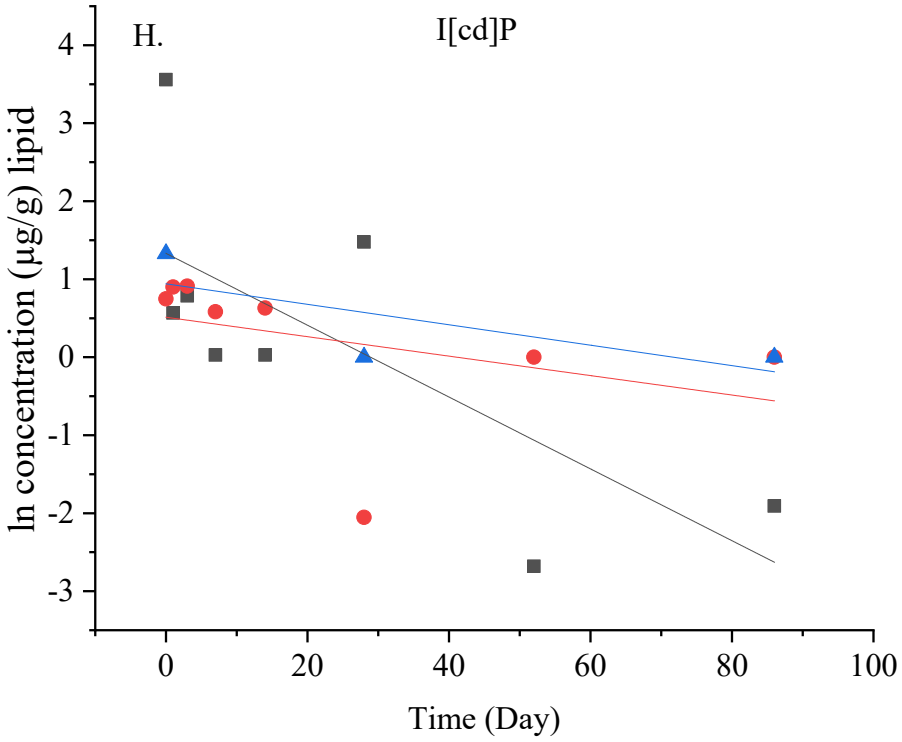
134


F.
B[a]P
ln concentration (µg/g) lipid
Time (Day)
5
4
3
2
1
0
-1
-2
-3
-4
0
20
40
60
80
100

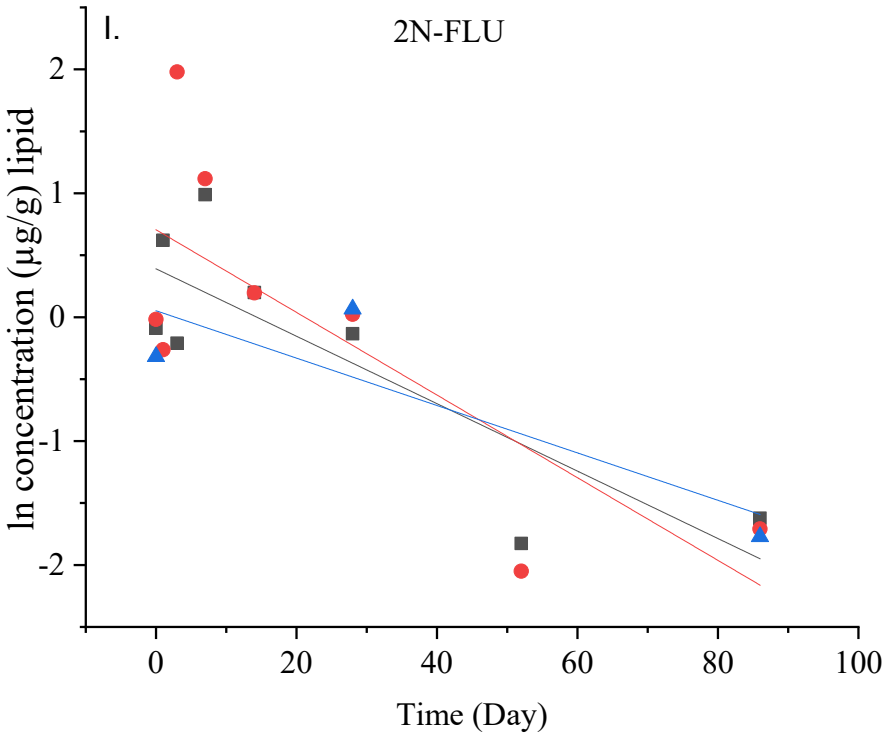
135


G.
D[ah]A
ln concentration (µg/g) lipid
Time (Day)
3
2
1
0
-1
-2
-3
-4
0
20
40
60
80
100

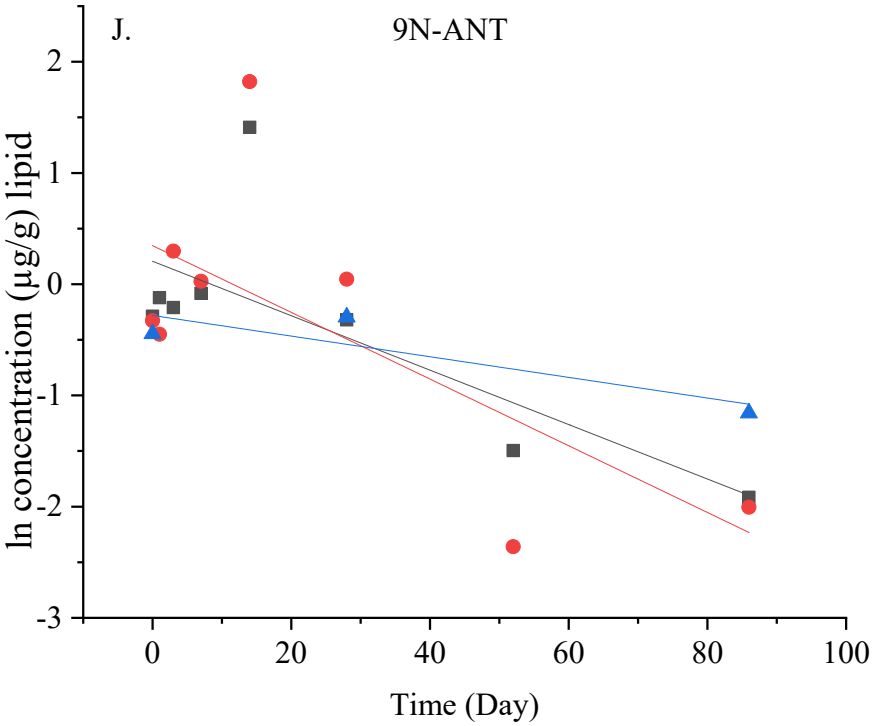
136


H.
I[cd]P
ln concentration (µg/g) lipid
Time (Day)
4
3
2
1
0
-1
-2
-3
0
20
40
60
80
100

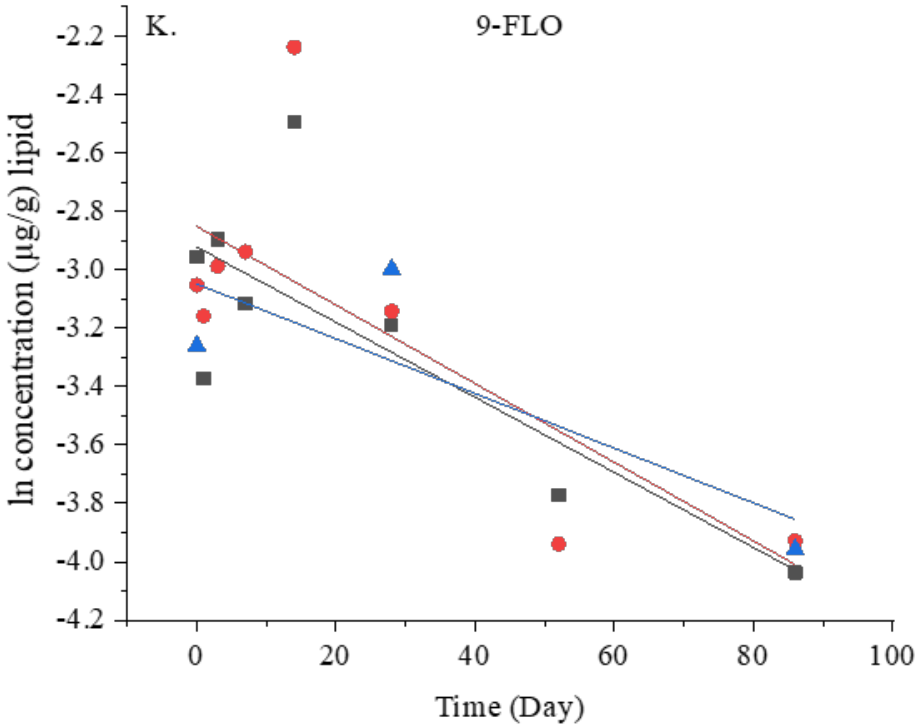
137


I.
2N-FLU
ln concentration (µg/g) lipid
2
1
0
-1
-2
0
20
40
60
80
100
Time (Day)

138


J.
9N-ANT
ln concentration (µg/g) lipid
2
1
0
-1
-2
-3
0
20
40
60
80
100
Time (Day)

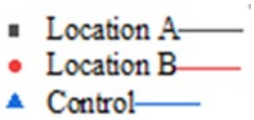
139



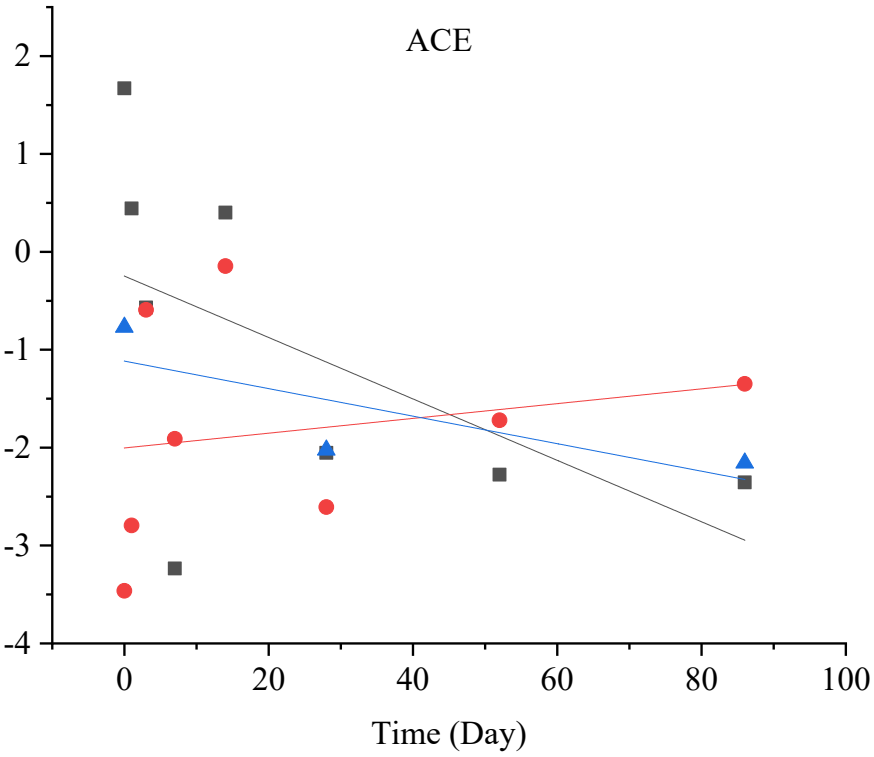
140

141 **Fig. S1**. Elimination kinetics of parent PAHs (bio-concentrated) (A – H), NPAHs (I – J) and
142 oxyPAH (9-FLO) (K) for location A/B-sourced and control oysters. ACENY (acenaphthylene)
143 PHEN (phenanthrene), ANTH (anthracene), FLUA (fluoranthene), PYR (pyrene), B[a]P (benzo[a]
144 pyrene), I[cd])P (indeno[1,2,3-cd]pyrene), D[a,h]A (dibenz[a,h]anthracene), 2N-ANT (2-
145 nitroanthracene), 9N-FLU (9-nitrofluorene), 9-FLO (9-filuorenone). The lines represent the linear
146 regression equations.
147

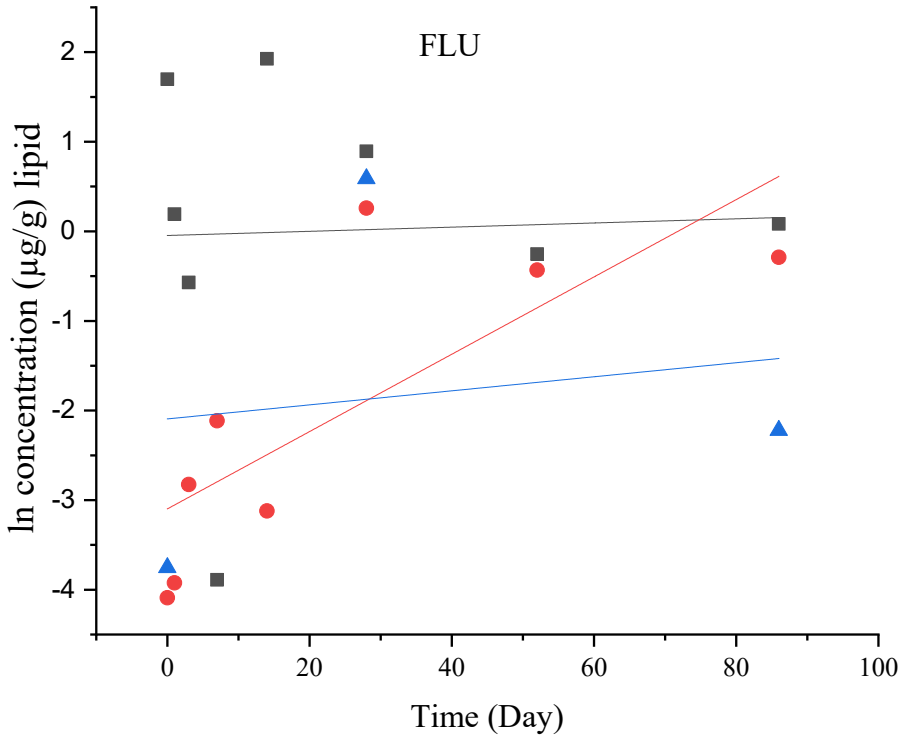
148


Location A
Location B
Control

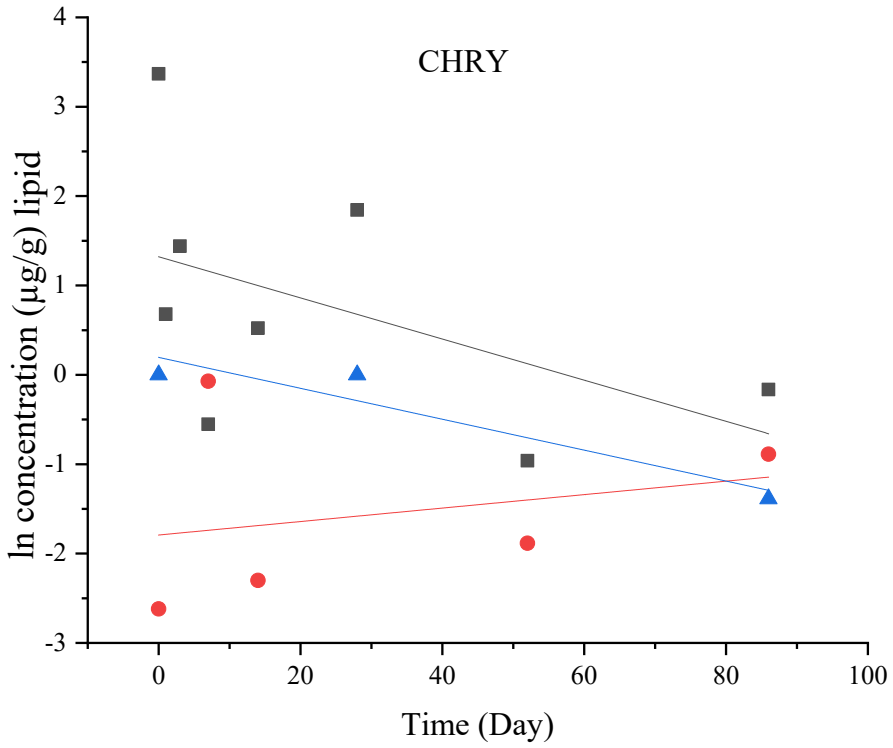
149


ACE
2
1
0
-1
-2
-3
-4
0
20
40
60
80
100
Time (Day)

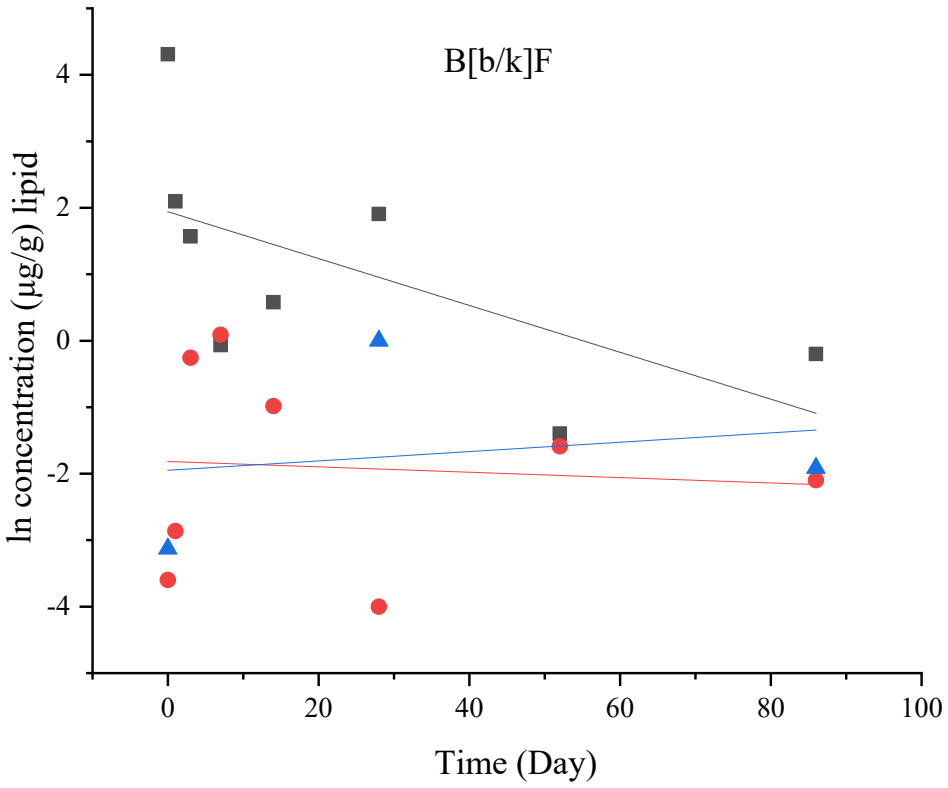
150


FLU
ln concentration (µg/g) lipid
2
1
0
-1
-2
-3
-4
0
20
40
60
80
100
Time (Day)

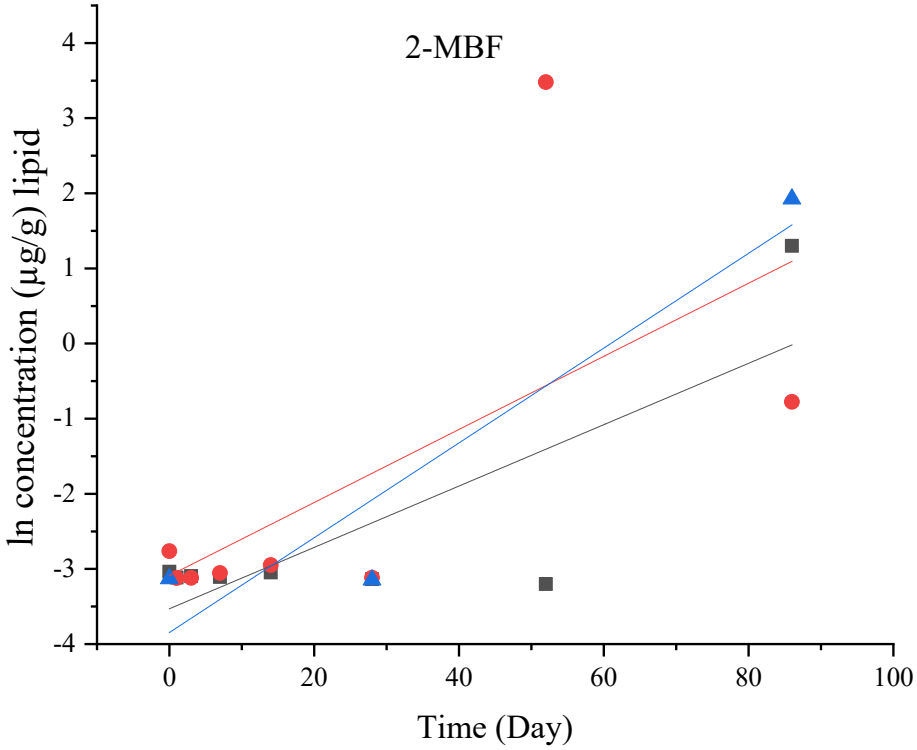
151


CHRY
ln concentration (µg/g) lipid
Time (Day)
4
3
2
1
0
-1
-2
-3
0
20
40
60
80
100

152


B[b/k]F
ln concentration (µg/g) lipid
Time (Day)
4
2
0
-2
-4
0
20
40
60
80
100

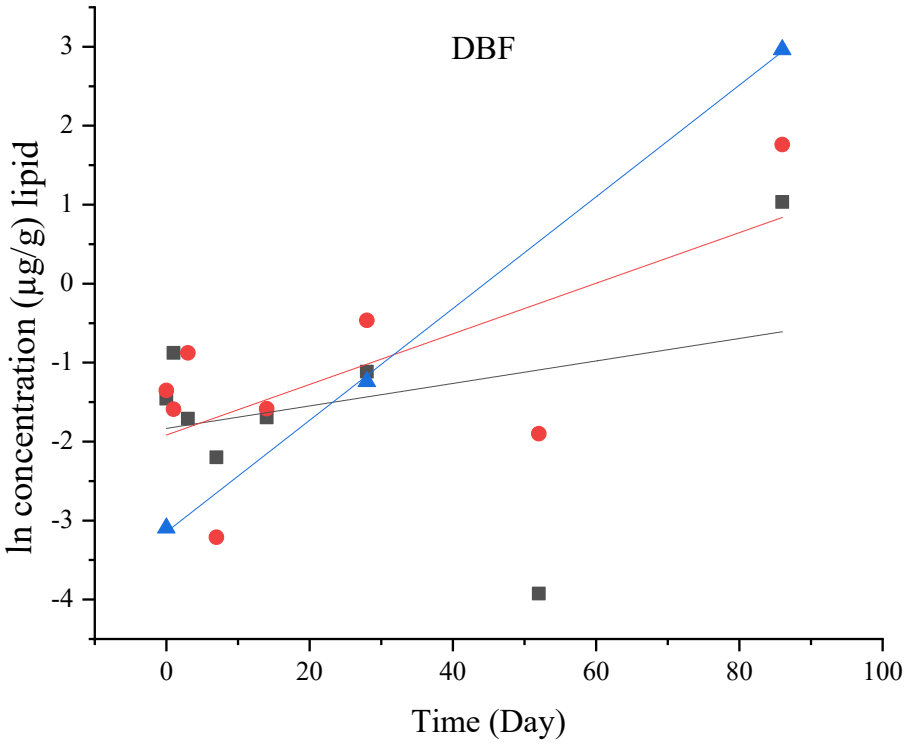
153



154

155

156



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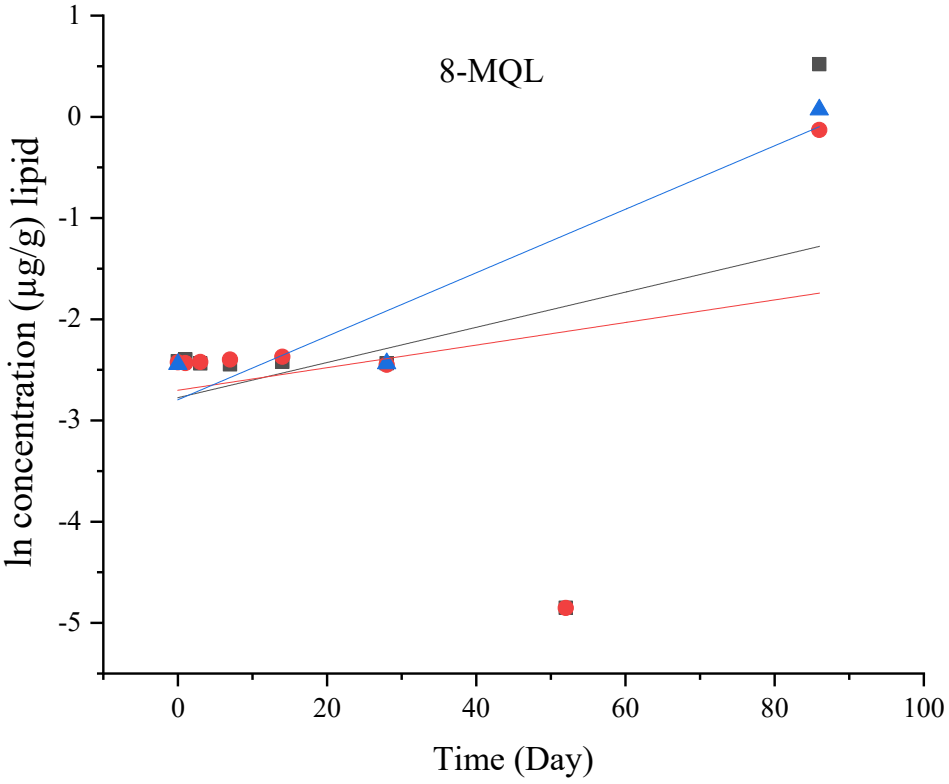
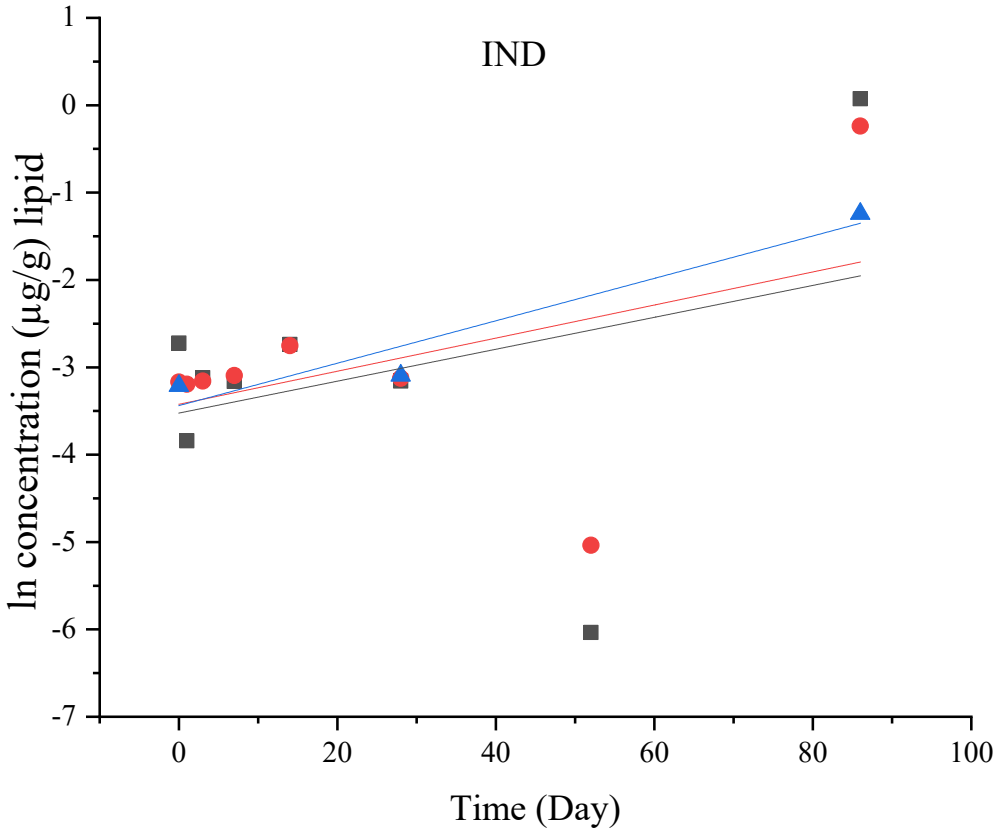
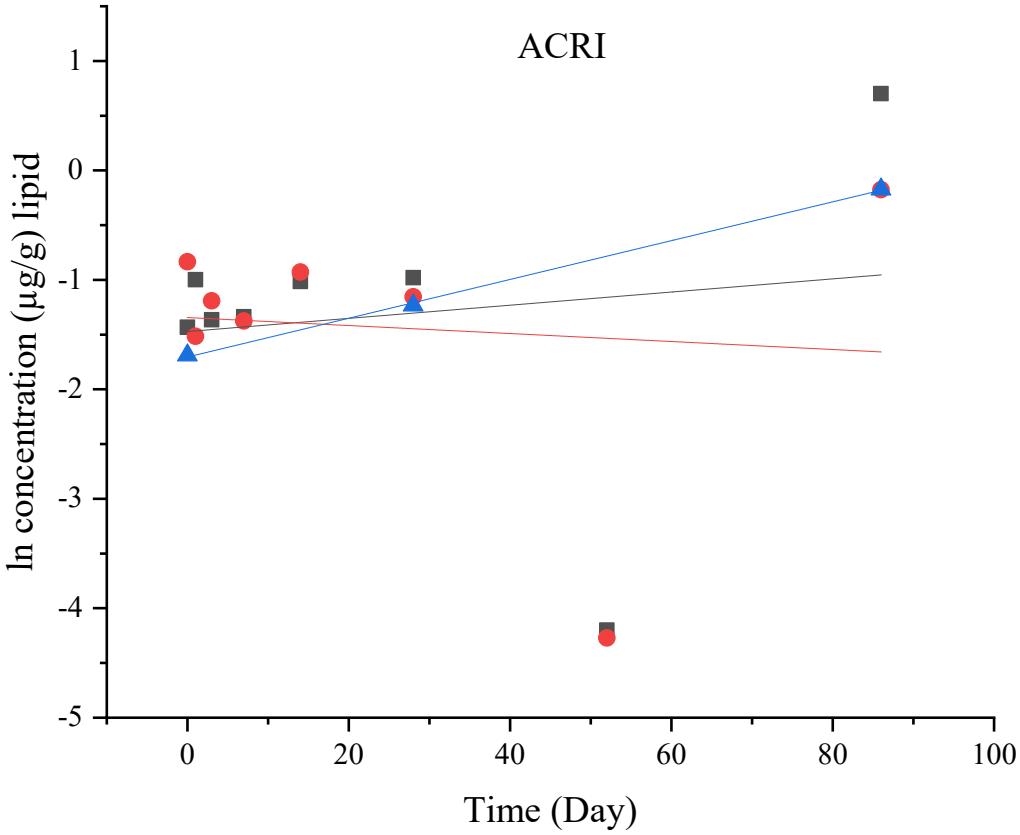

8-MQL
ln concentration (µg/g) lipid
Time (Day)
1
0
-1
-2
-3
-4
-5
0
20
40
60
80
100

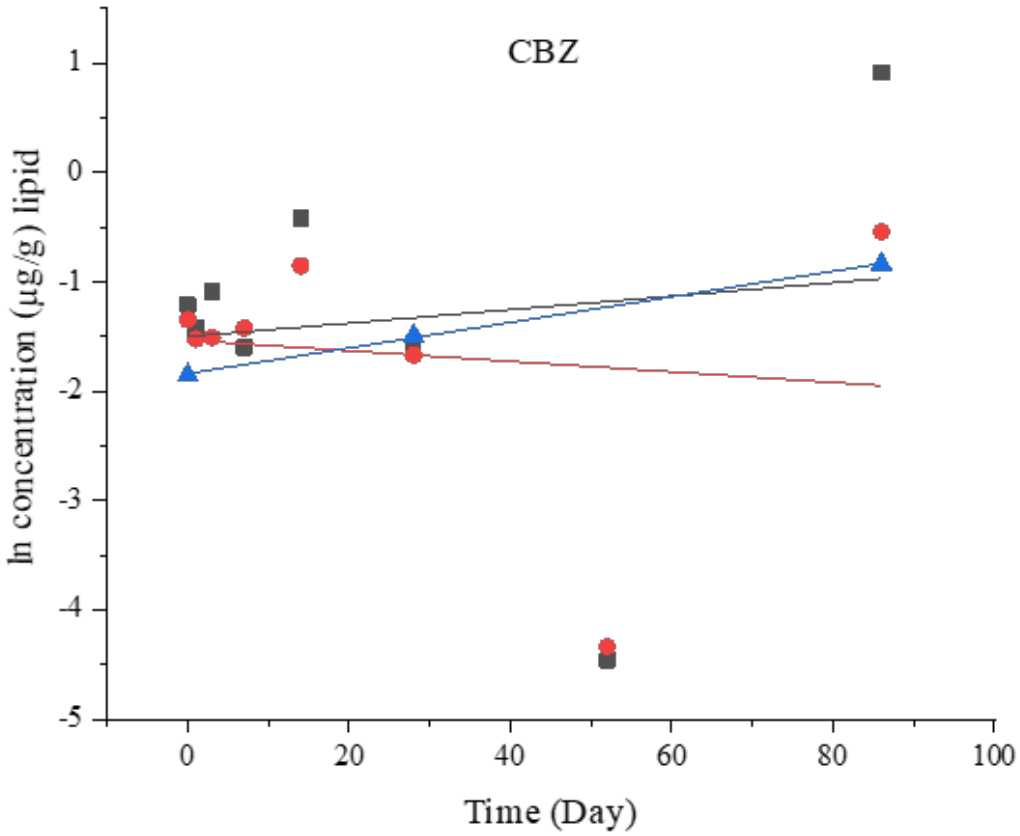
158


IND
ln concentration (µg/g) lipid
Time (Day)
1
0
-1
-2
-3
-4
-5
-6
-7
0
20
40
60
80
100

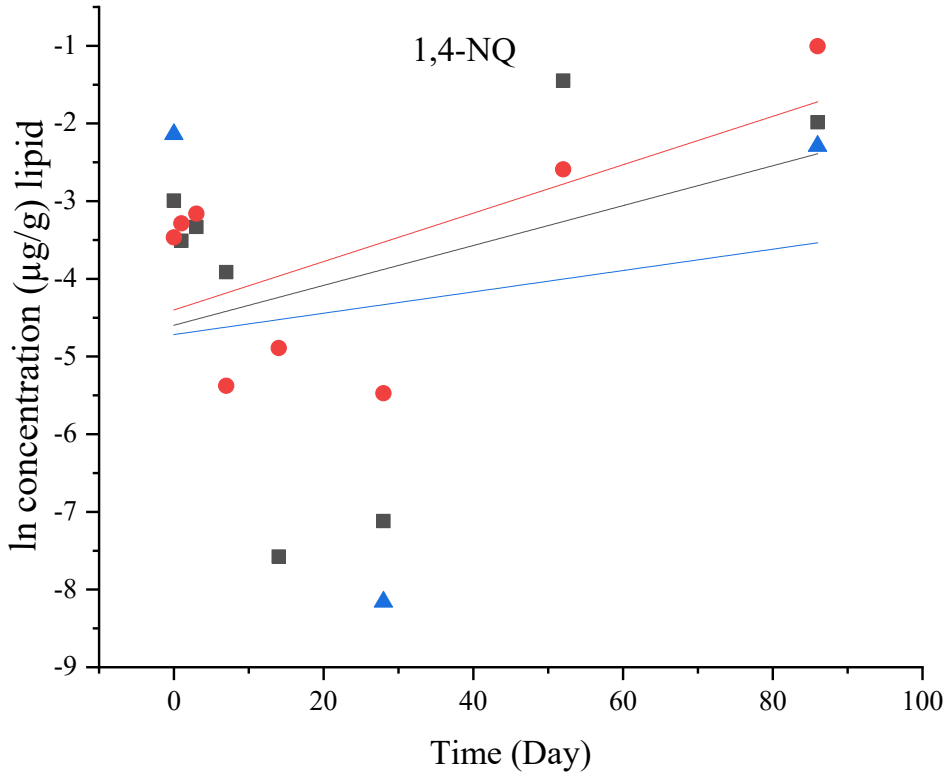
159


ACRI
ln concentration (µg/g) lipid
Time (Day)
1
0
-1
-2
-3
-4
-5
0
20
40
60
80
100

160

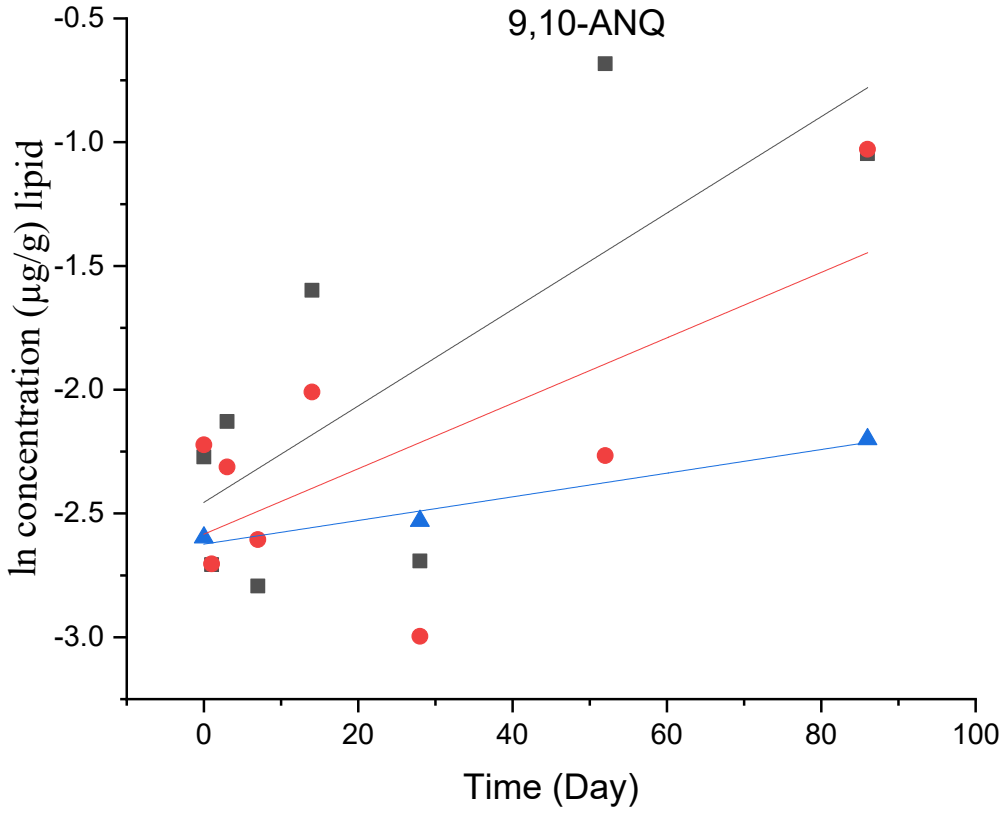

CBZ
ln concentration (µg/g) lipid
Time (Day)
1
0
-1
-2
-3
-4
-5
0
20
40
60
80
100

161

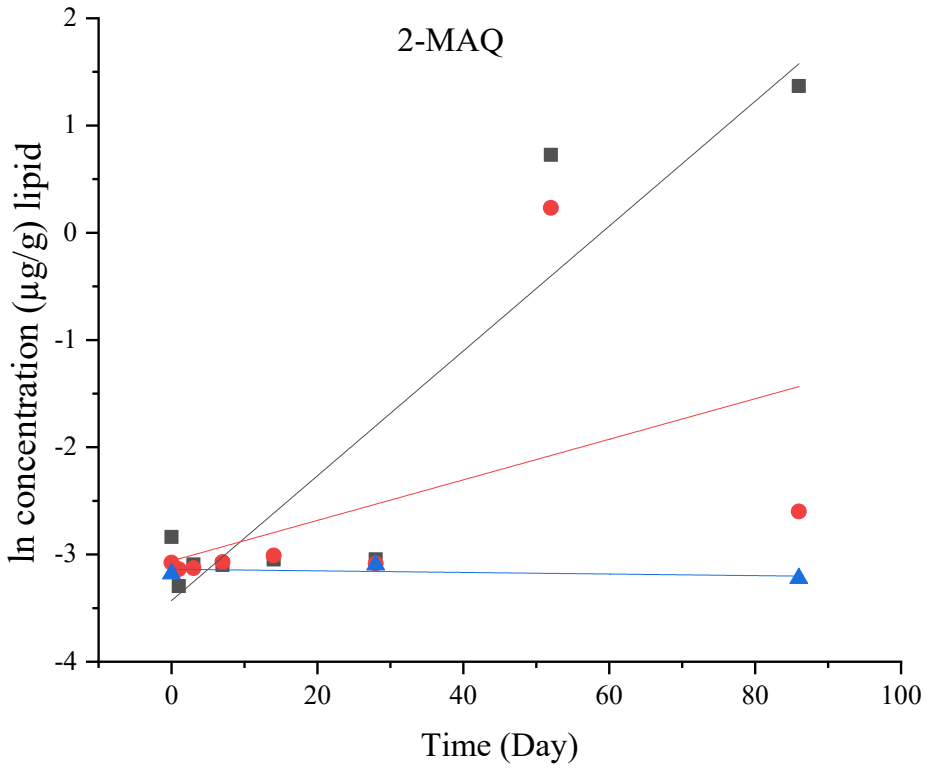

1,4-NQ
ln concentration (µg/g) lipid
-1
-2
-3
-4
-5
-6
-7
-8
-9
0
20
40
60
80
100
Time (Day)

162

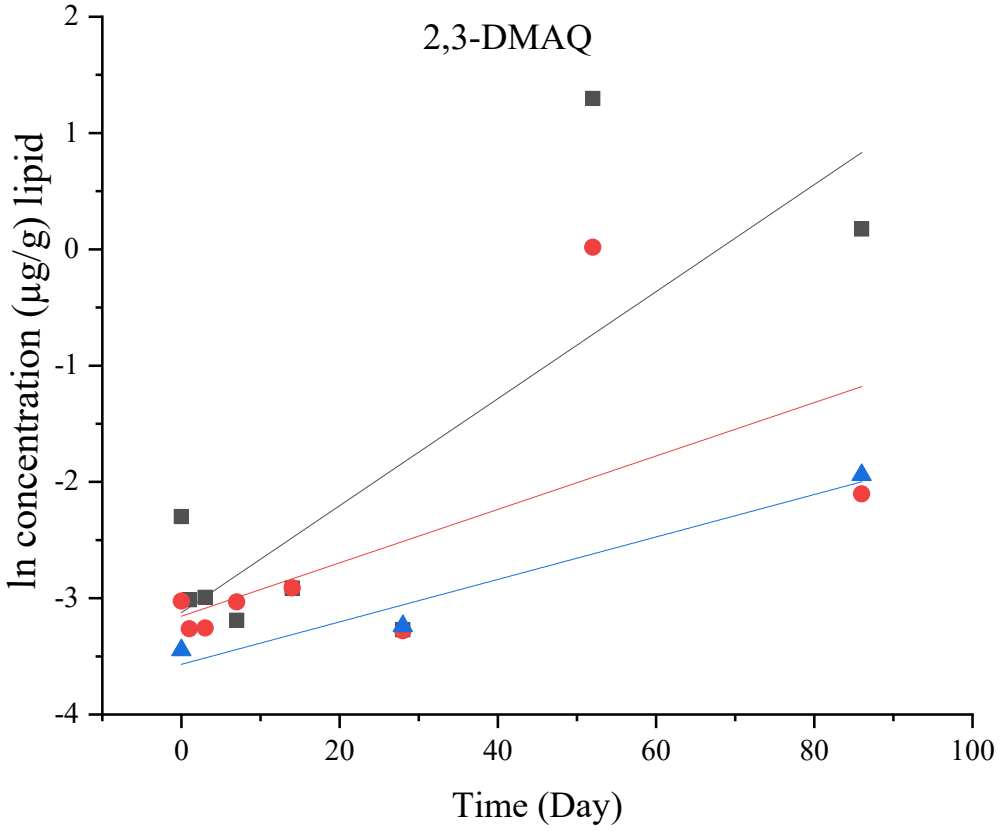
163


9,10-ANQ
ln concentration (µg/g) lipid
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
0
20
40
60
80
100
Time (Day)

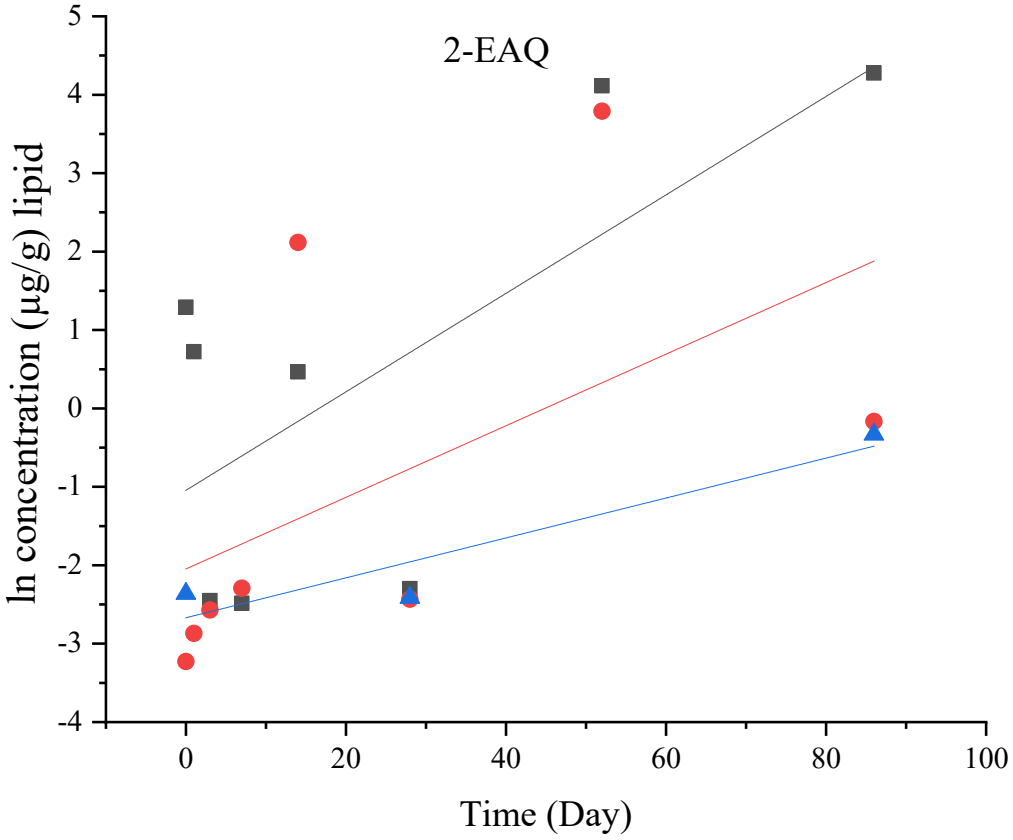
164


2-MAQ
ln concentration (µg/g) lipid
Time (Day)
2
1
0
-1
-2
-3
-4
0
20
40
60
80
100

165

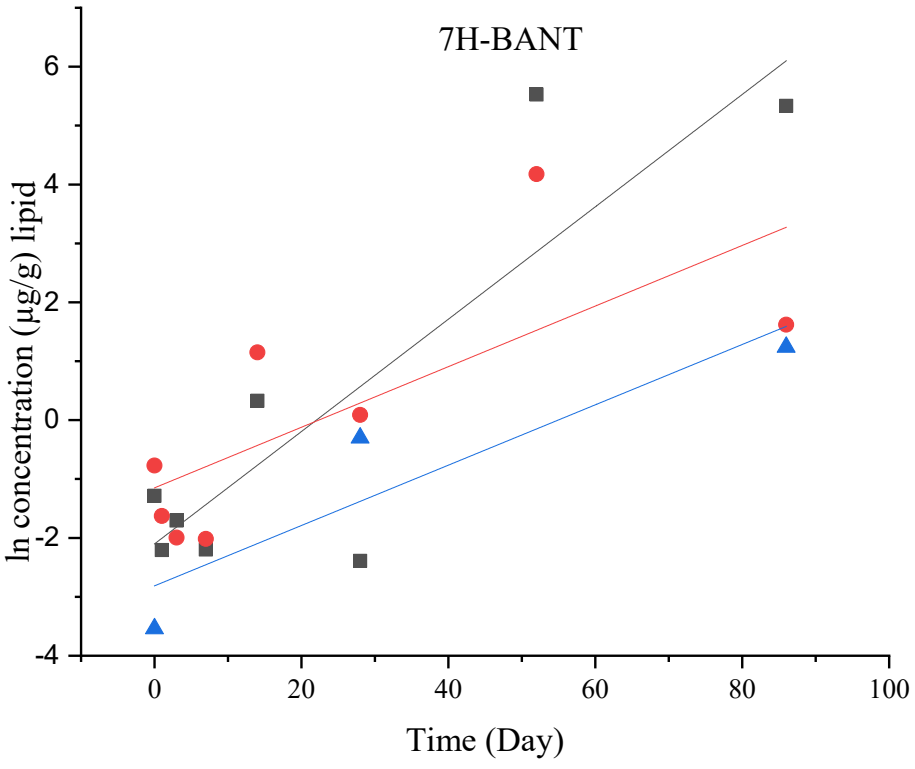

2,3-DMAQ
ln concentration (µg/g) lipid
Time (Day)
2
1
0
-1
-2
-3
-4
0
20
40
60
80
100

166


2-EAQ
ln concentration (µg/g) lipid
Time (Day)
5
4
3
2
1
0
-1
-2
-3
-4
0
20
40
60
80
100

167

168


7H-BANT
ln concentration (µg/g) lipid
Time (Day)
6
4
2
0
-2
-4
0
20
40
60
80
100

169

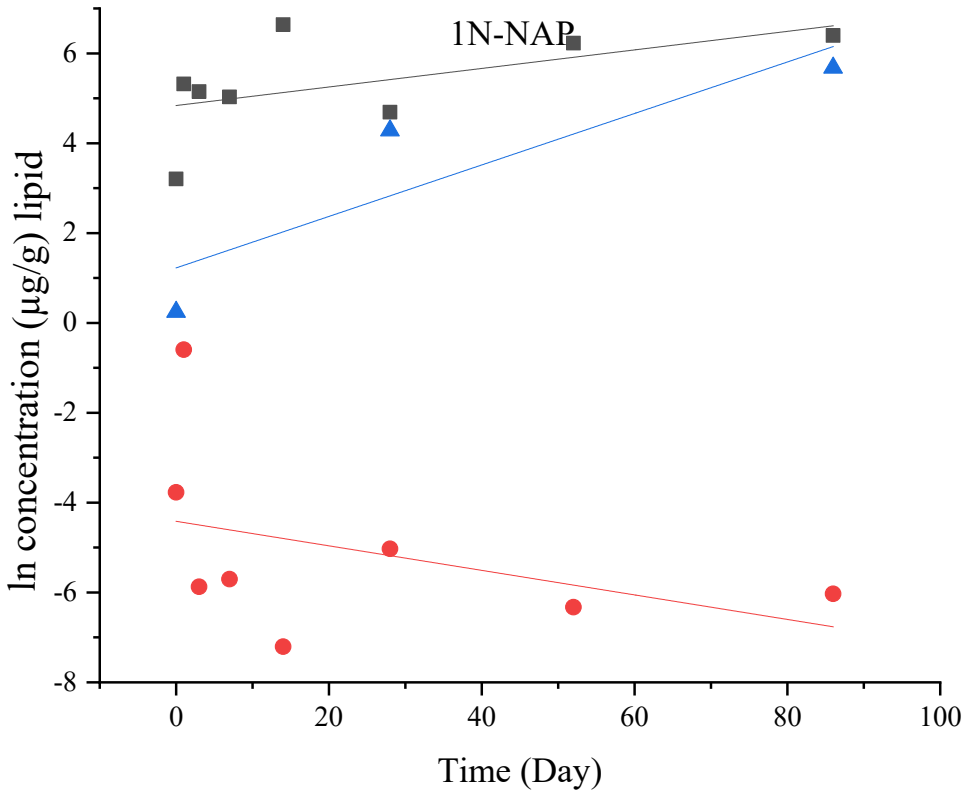


Fig. S2. Polar and non-polar PAHs showing variable relationship of ln concentration (µg/g) lipid with time.
ACEN (acenaphthene), FLU (fluorine), CHRY (chrysene), B[b+k]F (benzo[b+k]fluoranthene), 2-MBF (2-methylbenzofuran), DBF (dibenzofuran), 8-MQL (8-methylquinoline), IND (indole), ACR (acridine), CBZ (carbazole), 1,4-NQ (1,4-naphthoquinone), 9,10-ANQ (9,10-anthraquinone), 2-MAQ (2-methyl anthraquinone), 2,3-DMAQ (2,3-dimethylanthraquinone), 2-EAQ (2-ethylanthraquinone) 7H-BANT (7H-benz[d,e]anthracene-7-one) and 1N-NAP (1-nitronaphthalene).

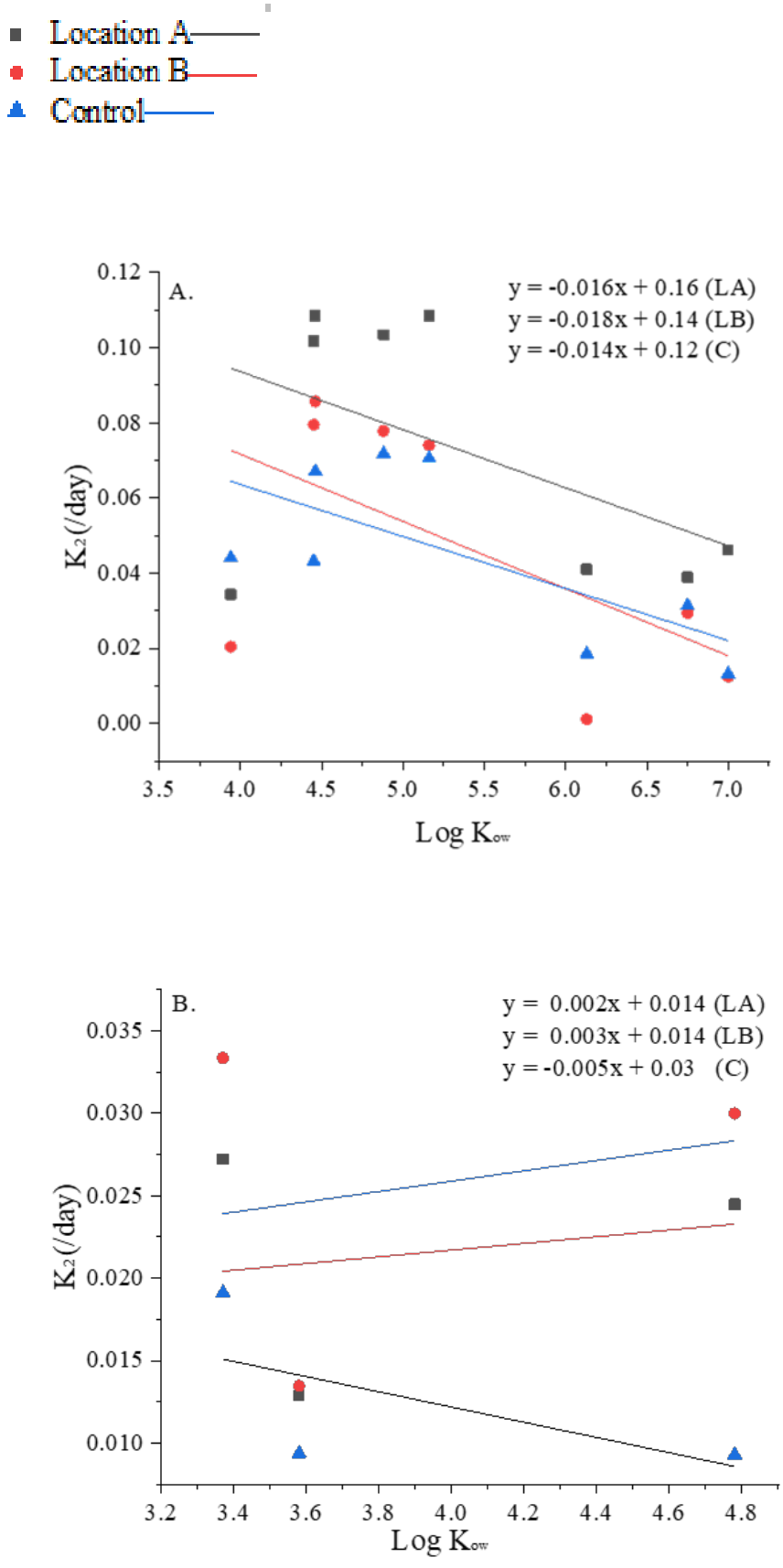
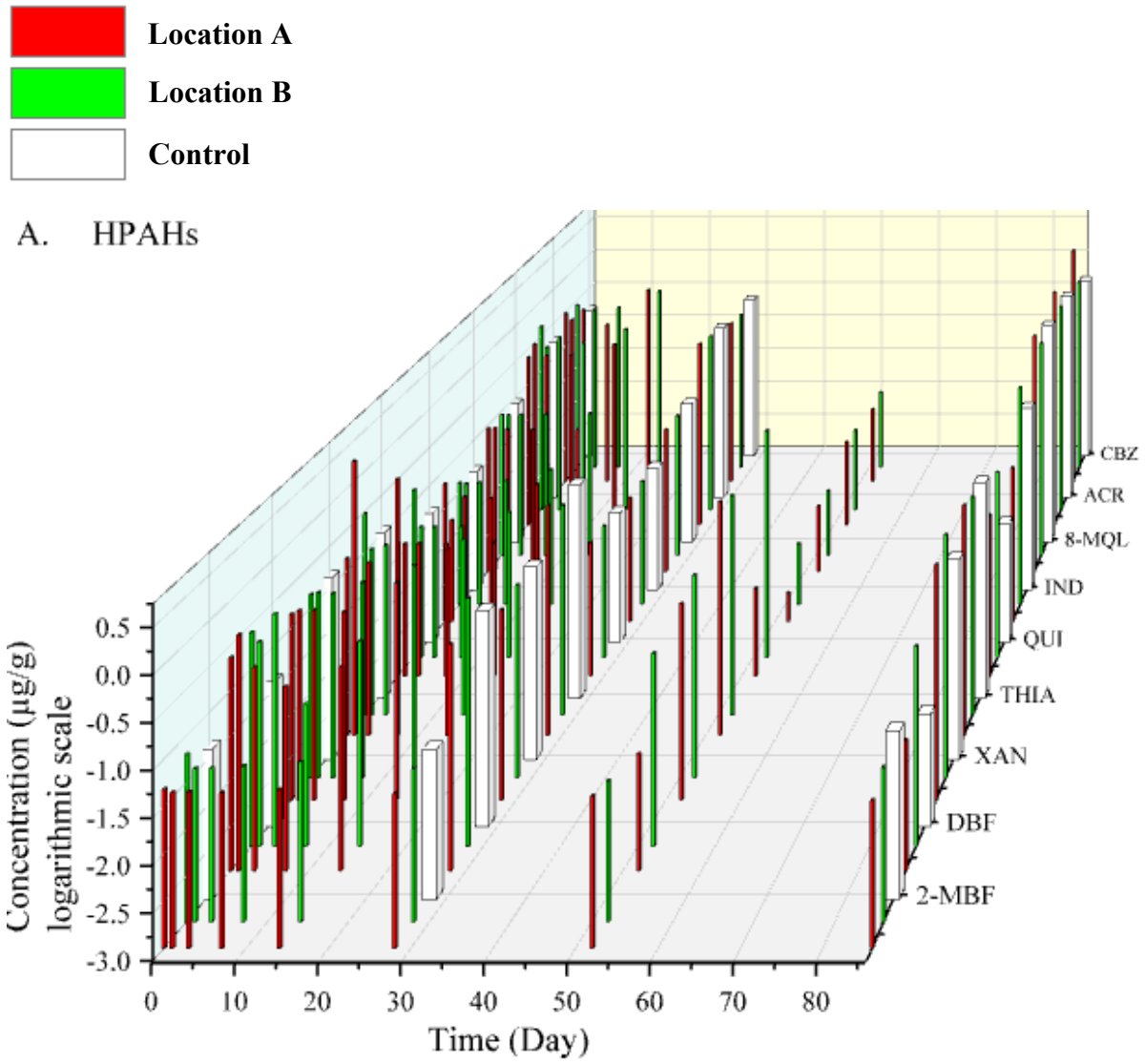
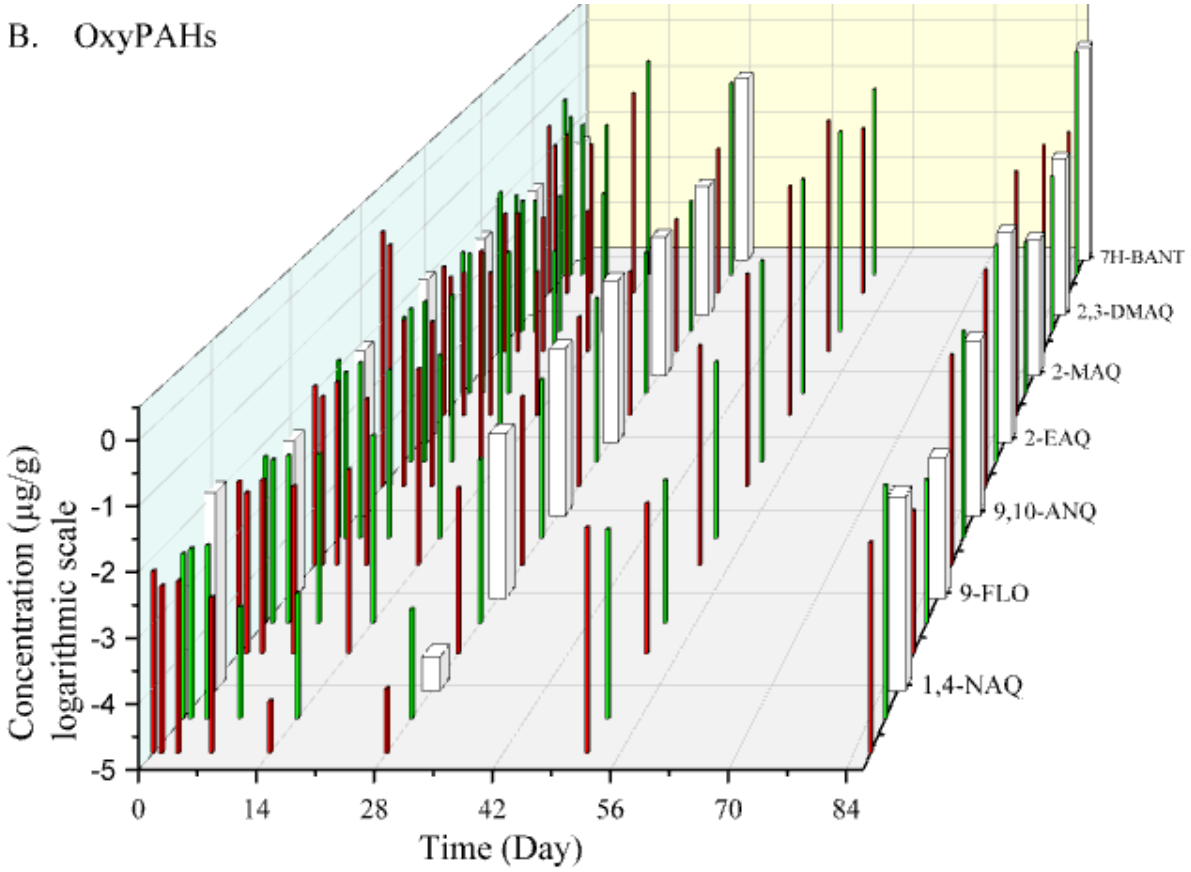


Fig. S3. Summary of k_2 versus Log K_{ow} following regression analysis for (A.) parent PAHs alone (B.) polar PAHs alone. Parent and polar PAHs subjected to regression analyses had k_2 values significantly different ($p < 0.05$) from zero. LA is location A-sourced; LB is location B-sourced; C is control.

Fig. 1. Map of the oyster-source estuary showing the sampling locations.


Location A
Location B
Control
A. HPAHs
Concentration (µg/g)
logarithmic scale
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
0
10
20
30
40
50
60
70
80
Time (Day)
CBZ
ACR
8-MQL
IND
QUI
THIA
XAN
DBF
2-MBF

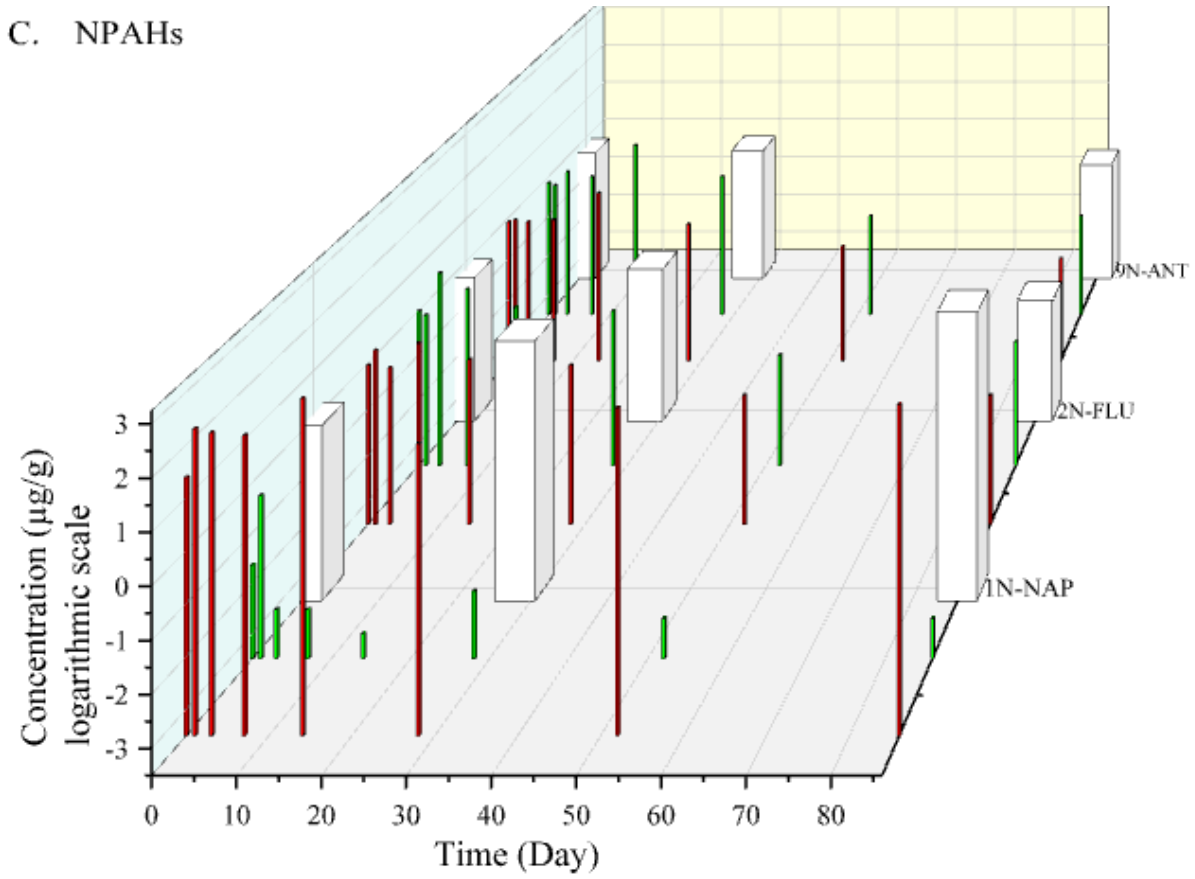

B. OxyPAHs
Concentration (µg/g)
logarithmic scale
0
-1
-2
-3
-4
-5
0
14
28
42
56
70
84
Time (Day)
7H-BANT
2,3-DMAQ
2-MAQ
2-EAQ
9,10-ANQ
9-FLO
1,4-NAQ

10

11

12

13


C. NPAHs
Concentration (µg/g)
logarithmic scale
3
2
1
0
-1
-2
-3
0
10
20
30
40
50
60
70
80
Time (Day)
9N-ANT
2N-FLU
1N-NAP

14

15

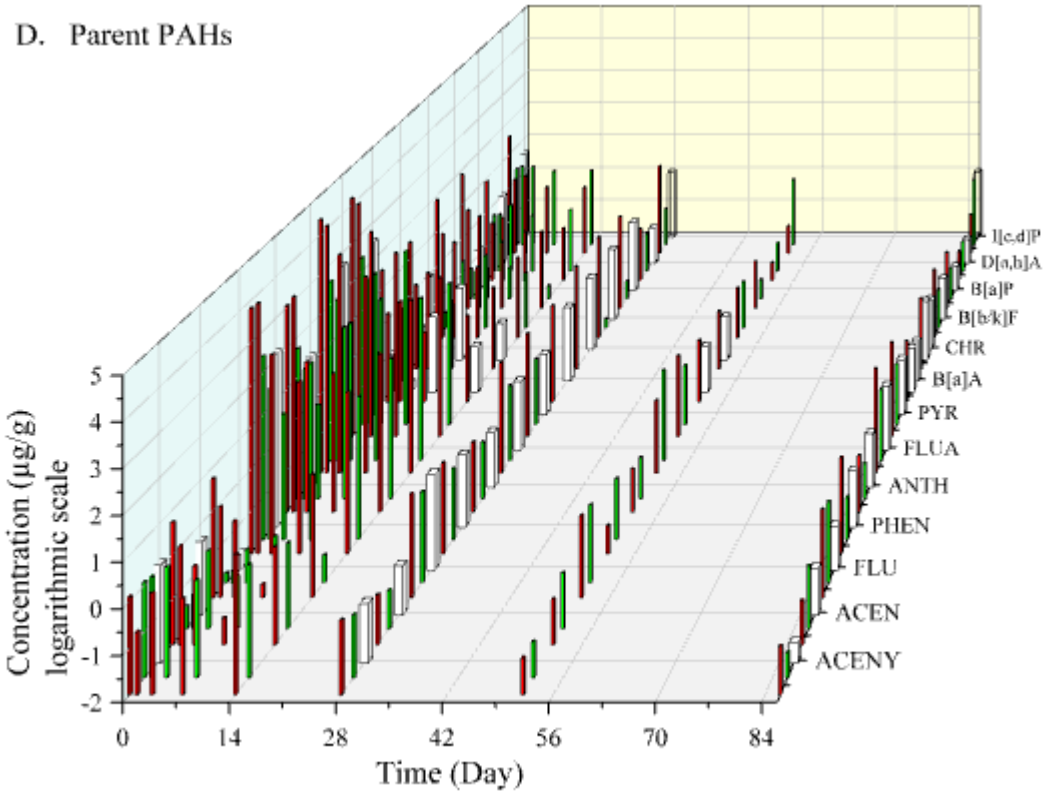


Fig. 2. Mean concentrations (µg/g lipid) in logarithmic scale of (A) heterocyclic (B) oxygenated (C) nitrated and (D) parent PAHs in oysters sampled from an isolated waterway during the 86-day depuration study. Oysters were initially collected from locations A and B of a southeast Australian estuary and transferred to the isolated waterway in NSW. The sampled oysters from locations A and B, and oysters from Camden Haven River (control) were used for an 86-day depuration study in the isolated waterway. Mean PAC concentrations were not significantly different ($p > 0.05$) (except for 1N-NAP), for the differently sourced oysters. HPAHs are: 2-MBF (2-methylbenzofuran), DBF (dibenzofuran), XAN (xanthene), THIA (thianaphthene), QUI (quinolone), IND (indole), 8-MQL (8-methylquinoline), ACR (acridine) and CBZ (carbazole). OxyPAHs are: 1,4-NQ (1,4-naphthoquinone), 9-FLO (9-fluorenone), 9,10-ANQ (9,10-anthraquinone), 2-EAQ (2-ethylanthraquinone), 2-MAQ (2-methyl anthraquinone), 2,3-DMAQ (2,3-dimethylanthraquinone) and 7H-BANT (7H-benz[d,e]anthracene-7-one). NPAHS are: 1N-NAP (1-nitronaphthalene), 2N-FLU (2-nitroanthracene) and 9N-ANT (9-nitrofluorene). Parent PAHs are: ACENY (acenaphthylene), ACEN (acenaphthene), FLU (fluorine), PHEN (phenanthrene), ANTH (anthracene), FLUA (fluoranthene), PYR (pyrene), B[a]A (benz[a] anthracene), CHRY (chrysene), B[b+k]F (benzo[b+k]fluoranthene), B[a]P (benzo[a] pyrene), D[a,h]A (dibenz[a,h]anthracene) and I[cd])P (indeno[1,2,3-cd]pyrene).

Table 1

Monitored PACs and their abbreviations

Parent PAHs	abbr.	OxyPAHs	abbr.	HPAHs	abbr.
acenaphthylene	ACENY	1,4-naphthoquinone	1,4-NQ	quinoline	QUI
acenaphthene	ACEN	9-fluorenone	9-FLO	8-methylquinoline	8-MQL
fluorene	FLU	2-methyl anthraquinone	2-MAQ	indole	IND
phenanthrene	PHEN	2-ethylanthraquinone	2-EAQ	acridine	ACR
anthracene	ANTH	9,10-anthraquinone	9,10-ANQ	carbazole	CBZ
fluoranthene	FLUA	2,3-dimethylanthraquinone	2,3-DMAQ	dibenzofuran	DBF
pyrene	PYR	7H-benz[d,e]anthracene-7-one	7H-BANT	2-methylbenzofuran	2-MBF
benz[a]anthracene	B[a]A	NPAHs		xanthene	XAN
chrysene	CHRY	1-nitronaphthalene	1N-NAP	thianaphthene	THIA
benzo[b+k]fluoranthene	B[b+k]F	2-nitrofluorene	2N-FLU	INTERNAL STANDARDS	
benzo[a]pyrene	B[a]P	9-nitroanthracene	9N-ANT	naphthalene-d8	
indeno[1,2,3-cd]pyrene	I[cd]P	RECOVERY STANDARDS		phenanthrene-d10	
dibenz[a,h]anthracene	D[ah]A	Acenaphthene-d10	ACE-d10	chrysene-d12	
		flouranthene-d10	FLU-d10	perylene-d12	

Table 2

Kinetic parameters of PACs in oyster tissues during the depuration study

PACs [a]	Log K_{ow} [b]	Elimination rate constant k_2 (day^{-1})			Half-life ($t_{1/2}$)			t_{95}		
		Location A	Location B	Control	Location A	Location B	Control	Location A	Location B	Control
ACENY	3.94	0.034	0.020	0.044	20.2	34.0	15.739	87.365	146.778	68.023
PHEN	4.46	0.109	0.086	0.067	6.4	8.1	10.4	27.6	35.0	44.7
ANTH	4.45	0.102	0.080	0.043	6.8	8.7	16.1	29.5	37.7	69.4
FLUA	5.16	0.108	0.074	0.071	6.4	9.4	9.8	27.6	40.5	42.5
PYR	4.88	0.103	0.078	0.072	6.7	8.9	9.7	29.0	38.5	41.8
B[a]A	5.76	0.095	0.051	0.051	7.3	13.5	13.5	31.4	58.2	58.5
CHRY	5.81	0.023	0.008	0.017	30.1	92.1	40.1	130.1	397.8	173.2
B[b+k]F	5.78	0.035	0.004	0.007	19.7	171.6	98.5	85.0	741.5	425.5
B[a]P	6.13	0.041	0.001	0.019	16.9	647.8	37.4	73.2	2799.8	161.8
I[cd])P	6.75	0.039	0.029	0.031	17.9	23.6	22.1	77.1	102.2	95.7
D[a,h]A	7	0.046	0.012	0.013	15.1	55.6	52.9	65.1	240.2	228.5
2N-FLU	3.37	0.027	0.033	0.019	25.5	20.8	36.3	110.1	89.8	156.7
9N-ANT	4.78	0.024	0.030	0.009	28.3	23.1	74.7	122.4	99.9	322.8
9-FLO	3.58	0.013	0.013	0.009	53.9	51.5	74.0	232.8	222.6	319.7

[a] PACs: ACENY (acenaphthylene), ACEN (acenaphthene), FLU (fluorene), PHEN (phenanthrene), ANTH (anthracene), FLUA (fluoranthene), PYR (pyrene), B[a]A (benz[a] anthracene), CHRY (chrysene), B[b+k]F (benzo[b+k]fluoranthene), B[a]P (benzo[a] pyrene), I[cd])P (indeno[1,2,3- cd]pyrene), D[a,h]A (dibenz[a,h]anthracene), 2N-FLU (2-nitrofluorene), 9N-ANT (9-nitroanthracene).
k_2 (elimination rate constant), $t_{1/2}$ (half-life), t_{95} (time required to reach 95% steady-state).

[b] Log K_{ow} values from Idowu *et al*., (2019) except I[cd])P and D[a,h]A which were reported in Gewurtz *et al*., 2002.

Table 3

Comparison of PACs kinetic parameters in this study and two previous studies

PACs [a]	This study			Gewurtz *et al*., 2002			Thorsen *et al*., 2004		
	k_2 (day^{-1})	$t_{1/2}$	t_{95}	k_2 (day^{-1})	$t_{1/2}$	t_{95}	k_2 (day^{-1})	$t_{1/2}$	t_{95}
ACENY	0.034	20.2	87.4		0.046	15.1	64.7	0.185	3.8
ACEN	0.031	22.1	95.5	0.095	7.3	31.6	0.237	2.9	16.2
PHEN	0.109	6.4	27.6	0.177	3.9	16.9	0.171	4.1	12.6
ANTH	0.102	6.8	29.5	0.163	4.3	18.4	0.179	3.9	17.6
FLUA	0.108	6.4	27.6	0.130	5.3	23.0	0.126	5.5	16.7
PYR	0.103	6.7	29.0	0.144	4.8	20.8	0.164	4.2	23.9
B[a]A	0.095	7.3	31.4	0.148	4.7	20.2	0.092	7.5	18.3
CHRY	0.023	30.1	130.1	0.105	6.6	28.6	0.084	8.3	32.5
B[b+k]F	0.035	19.7	85.0	0.103/0.037	6.7/18.7	29.1/81.8	0.083/0.059	8.4/11.8	35.9
B[a]P	0.041	16.9	73.2	-	-	-	0.076	-	36.3/50.9
I[cd])P	0.039	17.8	77.1	0.162	4.3	18.5	0.047	14.7	-
D[a,h]A	0.046	15.1	65.1	0.048	14.4	63.0	0.069	15.1	63.7
2N-FLU[b]	0.027	25.5	110.1	-	-	-	-	-	43.7
9N-ANT[b]	0.024	28.3	122.4	-	-	-	-	-	-
9-FLO[b]	0.013	53.9	232.8	-	-	-	-	-	-

[a] PACs: ACENY (acenaphthylene), ACEN (acenaphthene), FLU (fluorine), PHEN (phenanthrene), ANTH (anthracene), FLUA (fluoranthene), PYR (pyrene), B[a]A (benz[a] anthracene), CHRY (chrysene), B[b+k]F (benzo[b+k]fluoranthene), B[a]P (benzo[a] pyrene), I[cd])P (indeno[1,2,3-cd]pyrene), D[a,h]A (dibenz[a,h]anthracene); 2N-FLU (2-nitrofluorene) and 9N-ANT (9-nitroanthracene). k_2 (elimination rate constant), $t_{1/2}$ (half-life), t_{95} (time required to reach 95% steady-state). Location A-sourced values used for comparison. B[b]F and B[k]F values were reported separately in the previous studies. [b] Comparative values not found in the literature.