Bioavailability of polycyclic aromatic compounds (PACs) to the Sydney rock oyster (*Saccostrea glomerata*) from sediment matrices of an economically important Australian estuary

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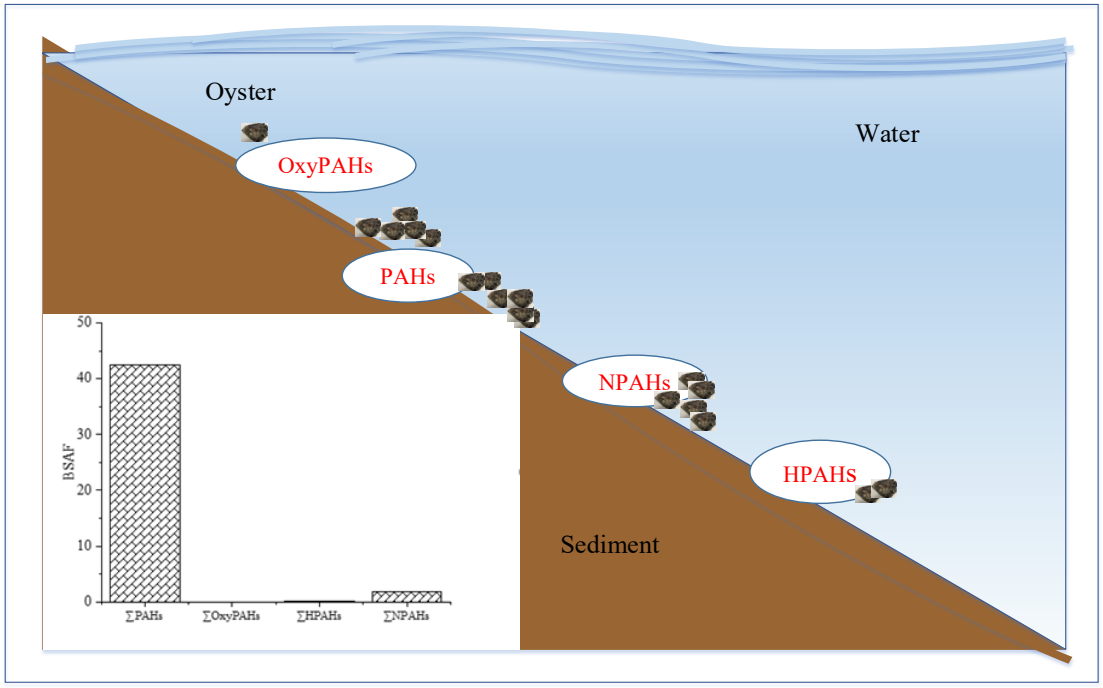
Highlights

- First study on the fate of polar PAHs in an Australian aquatic environment
- Parent PAHs exhibited the highest levels of bioaccumulation in oyster tissues
- NPAHs were the only polar PAHs that highly bio-accumulated in oyster tissues
- HPAHs and most oxyPAHs showed relatively low levels of bioaccumulation

Graphical Abstract



Abstract

Improving risk assessment and remediation rests on better understanding of contaminant bioavailability. Despite their strong toxicological attributes, little is known about the partitioning behaviour and bioavailability of polar polycyclic aromatic hydrocarbons (PAHs) in aquatic environments. The present study provides an insight into the bioavailable fractions of polar PAHs and their parent analogues in the tissues of the Sydney rock oyster, *Saccostrea glomerata,* a model aquatic bio-indicator organism. The concentration and distribution patterns of parent and polar PAHs including oxygenated PAHs (oxyPAHs), nitrated PAHs (NPAHs) and heterocyclic PAHs (HPAHs) were determined in water, sediment and oysters from an ecologically and economically important estuary of New South Wales, Australia. Total concentrations of PAHs, oxyPAHs, NPAHs and HPAHs were higher in sediments compared to oyster tissue and water. For most polar PAHs, total concentrations for water, sediment and oyster samples were less than 1 μg/g (μg/l for water) while parent PAH concentrations were several orders of magnitude higher. Computed biota-sediment accumulation factors (BSAFs) on lipid-normalized oyster concentrations revealed that while ∑oxyPAHs and ∑HPAHs exhibited low accumulation from sediment to oyster tissues (BSAF < 1), ∑PAHs and ∑NPAH were found to be accumulated at high levels (BSAF > 1). BSAF individual computation showed that bioaccumulation of nine investigated HPAHs in oyster tissues were relatively low and only 2-EAQ (oxyPAH) and 1N-NAP (NPAH) showed high levels of accumulation in oyster tissues, similar to parent PAHs. To the best of our knowledge, this is the first known study on the bioavailability of polar and non-polar PAHs in an Australian aquatic environment. The outcome of this study might be a useful indicator of the potential risks of polar PAHs to humans and other living organisms.

Keywords: *Polar PAHs, Bioavailability, Sydney rock oyster, Aquatic environment, Human health risk*

1. Introduction

Sediments play an important ecological role in providing crucial ecosystem functions and habitats to many aquatic organisms [1]. However, they can serve as both sink and potential source of toxic persistent environmental contaminants [2]. Generally, polycyclic aromatic compounds (PACs), a group of relatively hydrophobic contaminants, have strong bioaccumulation and biomagnification properties [1]. Sediment perturbation (bioturbation, storms, tidal changes, dredging, acidification etc.) provides the channel through which PACs can remobilise into the water column, enter the food chain, build up and ultimately exert adverse effects on humans [3-6] . Chronic exposure to PACs as a result of dietary intake of contaminated foods can result in kidney and liver damage, lung malfunctioning, skin inflammation, teratogenicity, carcinogenicity and genotoxicity [7]. However, compared to the sediment contaminant concentrations, only a fraction of PACs is accumulated in organisms. This is referred to as the bioavailable fraction, and it is the most relevant concentration in environmental and human health risk assessment [8, 9]. The bioavailable fraction serves a useful purpose of human adverse effect prediction.

Evaluation of contaminant bioavailability in the aquatic environment has been done using invertebrate benthic bivalves such as mussels, clams and oysters [10-15]. Due to the relatively lipophilic characteristics of PACs, they could bioaccumulate in fatty and lipid-rich organs and tissues of filter feeders [16, 17]. Bioaccumulation by bivalves can occur through the absorption of water-solubilized PAC by the gills or assimilation of smallest grain-sized sediment fraction by digestive tracts [18]. Bivalves are important biomonitoring organisms because of their unique characteristics such as abundance and distribution, sedentary nature, tolerance to various environmental contaminants and other types of stress, long life span and low rate of contaminant transformation [16]. Oysters are suspension bivalve species mostly feeding on

104 phytoplankton and suspended organic matter through the filtration of water. They live on solid
105 surfaces and man-made habitats in intertidal zones [16] and are prized food source in Australia.
106 The bioaccumulation of PACs in oysters provides a more realistic measure of pollution from
107 the risk perspective as the total amount of contaminants in sediment and water determined with
108 routine chemical methods may not indicate the true environmental or human health risk [13]. The
109 total sediment concentration could be an overestimation of possible risk because of the
110 tendency of PACs to be less bioavailable when bound to sediments [9]. Although the PACs'
111 concentrations in the water compartment of an aquatic system represent the most soluble,
112 mobile and bioavailable concentrations, measured PACs concentration may be an
113 underestimation because of the natural regenerative properties of natural water systems such
114 as streams and rivers [2, 19]. Contaminants bioaccumulation in oysters and other bivalves is
115 therefore the best indices of bioavailability.

116 In addition to the sediment and water properties such as organic matter contents, the
117 partitioning behaviour of PACs could be related to the physicochemical characteristics of
118 individual PACs. Parent and polar polycyclic aromatic hydrocarbons (parent and polar PAHs),
119 for example, demonstrate varied behaviour in water-sediment systems [20-23], as their
120 hydrophobicity and lipophilicity are different. Generally, polar PAHs are less hydrophobic and
121 sorb less to soil organic matter and tissues of aquatic organisms compared to their parent
122 analogues [23]. Further, the specific behaviours of polar PAHs in the water-sediment system vary
123 as they demonstrate different levels of polarity [23]. For example, nitrated PAHs (NPAHs) might
124 not partition significantly into water in an aquatic environment because of their relatively low
125 solubility. High concentrations of NPAHs, for example, have been reported in the Swedish part
126 of Baltic Sea and urban sediments of Denmark [22]. Oxygenated PAHs (oxyPAHs) and
127 heterocyclic PAHs (HPAHs) exhibit higher polarity and partition more into the water segment

128 of the water-sediment system. The physicochemical characteristics of polar PAHs have been
129 reviewed elsewhere [22, 23].

130 The bioavailable fractions of PACs provide information about the concentrations of dissociated
131 and sediment-bound PACs that pass through a biological membrane into a living system [24].
132 Once in the living system the contaminant becomes metabolically processed and excreted or
133 remain in the organism to exert its toxic effects. Of particular interest to human health are
134 organisms, such as oysters, that bioaccumulate contaminants. Bio-accumulated contaminants
135 could pose greater danger directly to oysters through protracted exposure or become available
136 to humans when contaminated oysters are consumed [24]. More importantly, bio-accumulated
137 polar PAHs could elicit greater direct toxic effects in living systems, at lower concentrations
138 compared to their parent analogues [23, 25].

139 In the present study, we investigated the bioavailability of parent and polar PAHs in the tissues
140 of Sydney rock oyster (*Saccostrea glomerata*) collected from a southeast Australian estuary.
141 Oyster, sediment and water samples were collected, in conjunction with the NSW Food
142 Authority (NSWFA), from the estuary. The estuary, like many in NSW, has a history of
143 commercial use of coal tar that dates back to the early 1900s when the rapid expansion of the
144 aquaculture industry started [26, 27]. Traditional farming practices, in operation at that time, used
145 tarred hardwood to curtail marine stem borers from destroying the timbers used in aquacultural
146 constructions [26, 27]. Even though they have largely been phased from use, PACs are still present
147 in the sediment underneath many farming areas. There was a need for a concomitant
148 investigation of the concentrations of parent and polar PAHs in the water-sediment system as
149 well as biological matrices of the estuary. This will provide better insights about the
150 bioavailability of sediment-borne contaminants in the river and the possible environmental and
151 human health risks. To the best of our knowledge, this is the first study to simultaneously

152 investigate the bioavailability of parent and polar PAHs from sediments of any Australian
153 aquatic environment.

154 **Materials and Methods**

155 2.1. Oyster, sediment and water sampling

156 Sampling sites were selected based on the results of a recent investigation by the New South
157 Wales Food Authority (NSWFA), which indicated a higher than expected levels of PAH
158 contamination of the estuary. Accordingly, oysters, water and sediment samples were collected
159 from 33 sites across the estuary (Table 1, Fig. 1). A location non-disclosure agreement was
160 entered into with NSW fisheries authorities during the sampling period, preventing us from
161 showing the global positions on the map.

162 Oyster samples were collected from within ~ 2 km of the estuary mouth from locations A and
163 B (Fig. 1). Three samples of 15 oysters were collected from each of the sampled locations.
164 Water and superficial sediment samples (0-5 cm) were also collected from same sites where
165 the oysters were sampled (Fig. 1). Oyster samples were kept frozen at -20 °C and
166 water/sediment samples at 4 °C until, extraction, the following day.

167 2.2. Determination of sediment and oyster parameters

168 The pH and electrical conductivity of sediment samples were determined as previously
169 described [28]. Total organic carbon (TOC) for oven-dried sediment samples was determined by
170 the use of LECO CR-412 Carbon Analyzer with 1350 °C furnace temperature for 60 s after the
171 removal of inorganic carbon through the acidification by 1-2 ml of 1M HCl. Oyster lipid
172 content was determined by the modification of the method of Bligh and Dyer, 1959 [29].
173 Extracted samples were centrifuged at 500 rpm and concentrated to dryness using a nitrogen
174 concentrator. The lipid content was the relative weight of the dried residual.

175

176 2.3. Chemicals and reagents

177 The following chemicals were purchased from Sigma Aldrich, Australia:

178 PAH mix containing: acenaphthylene (ACENY), acenaphthene (ACEN), fluorene (FLU),
179 phenanthrene (PHEN), anthracene (ANTH), fluoranthene (FLUA), pyrene (PYR), benz[a]
180 anthracene (B[a]A), chrysene (CHRY), benzo[b+k]fluoranthene, benzo[a]pyrene (B[a]P),
181 indeno[1,2,3-cd]pyrene (I[cd]P) and dibenz[a,h]anthracene (D[ah]A); 7 carbonyl-OxyPAHs:
182 1,4-naphthoquinone (1,4-NQ), 9-fluorenone (9-FLO), 2-methyl anthraquinone (2-MAQ), 2-
183 ethylanthraquinone (2-EAQ), 9,10-anthraquinone (9,10-ANQ), 2,3-dimethylanthraquinone
184 (2,3-DMAQ), 7H-benz[d,e]anthracene-7-one (7H-BANT); 5 N-heterocycles: quinoline (QUI),
185 8-methylquinoline (8-MQL), indole (IND), acridine (ACR), carbazole (CBZ); 3 O-
186 heterocycles: dibenzofuran (DBF), 2-methylbenzofuran (2-MBF), xanthene (XAN); 1 S-
187 heterocycle: thianaphthene (THIA); 3 NPAHs: 1-nitronaphthalene (1N-NAP), 2-nitrofluorene
188 (2N-FLU), 9-nitroanthracene (9N-ANT) and internal standards comprising of naphthalene-d8,
189 phenanthrene-d10, chrysene-d12 and perylene-d12, as well as acenaphthene-d10 and
190 flouranthene-d10 surrogate standards. Anhydrous sodium sulphate (99 % purity), n-hexane,
191 dichloromethane and acetone (99.8 % purity) were also sourced from Sigma Aldrich, Australia.
192 Bond Elut C18 (500 mg) and QuEChERS extract and dispersive SPE tubes (15 ml) were
193 purchased from Agilent Technologies, Australia.

194 2.4. Extraction Procedure

195 2.4.1. Sediment

196 A 2 g freeze-dried sample of sediment was transferred to a clean 50 ml centrifuge tube and
197 spiked with 20 µl of 100 µg/ml acenaphthene-d10 and fluoranthene-d10 as recovery standards.
198 An aliquot of 20 ml hexane: acetone (4:1) was then added into the sample tube. The mixture
199 was vortexed for 1 min and then subjected to ultrasonic treatment for 15 min followed by
200 centrifugation at 2000 x g for 10 min. The organic layer was transferred into 60 ml amber vials

after centrifugation. The extraction process of solvent addition, vortexing and centrifugation were repeated two more times and the organic extracts combined followed by concentration to approximately 500 µl.

2.4.2. Sydney rock oysters

The extraction of parent and polar PAHs from oysters samples was performed using the QuEChERS approach. A 2 g freeze-dried and homogenised sample of oysters tissue was transferred to a 50 ml 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), $Na_3C_6H_5O_7$ (1 g) and $C_6H_6Na_2O_7 \cdot 1.5H_2O$) were added. A mixture of (4:1 v/v) of hexane/acetone (20 ml) was added into the sample tube as extraction solvent followed by shaking with the aid of a vortex for 1 min and centrifugation (2000 x g, 4 °C, 10 min). The supernatant was subsequently transferred to a QuEChERS clean-up tube and vortexed (1 min), centrifuged at 2000 x g and 4 °C (10 min) and concentrated using a nitrogen concentrator at 35 °C and 12.5 psi to about 500 µl.

2.4.3. Water

The method used for parent and polar PAH extraction of water was a modification of a previously used method [30]. The preconditioned 500 mg Bond Elut (C18) cartridges were equilibrated with 10 ml ultrapure water. The water sample (250 ml) was loaded to the sorbent at the flow rate of 5.0 ml/min after which the cartridge was kept in vacuum for 30 min to remove residual water.

2.5. Clean-up procedure

For the sediment and oyster samples, the concentrated extracts were applied to 2 g preconditioned 10 % activated silica solid-phase extraction cartridges connected to a manifold and operated under vacuum at 5 mmHg. The loaded C18 cartridges were utilized for the water

samples. The fractionating procedure into parent and polar PAH fractions for all samples was done using 15 ml Hexane: DCM (5:1) for parent PAHs and 8 ml DCM followed by 5 ml acetone, for polar PAHs [31, 32]. The volume of eluent was concentrated to near dryness, solvent exchanged to hexane by adding 1 ml hexane and transferred to 1.5 ml GC vial for GC-MS analysis. Four deuterated internal standard mix (naphthalene-d8, phenanthrene-d10, pyrene-d10 and perylene-d12) was added prior to GC-MS analysis. Further details on the analytical methods and quality control procedures can be found in the Supplementary Information (SI-Text 2) and previously published papers [31, 32].

2.6. Biota-Sediment Accumulation Factor

The bioavailability of PACs in the environment is often assessed by comparing the concentrations of individual PACs in the benthic organisms to those in sediment [33]. This is expressed as biota-sediment accumulation factor (BSAF). It is the ratio of PAC concentration in biota (C_b) to that in sediment (C_s). The concentration of the individual PACs are normalized against the lipid fraction (fl) of the organism and the organic carbon content (foc) of the sediment. A contaminant is considered bio-accumulated when the BSAF is ≥1 [34, 35].

$$\mathrm{BSAF} = \frac{\mathrm{C_b}/fl}{\mathrm{C_s}/foc}$$

When many sampling points are involved, the relative bio-concentration factor (RBSAF) becomes useful in comparing PAH accumulation from sediments across the stations.

$$\mathrm{RBSAF} = \frac{\mathrm{BSAF}}{\sum \mathrm{BSAF}} * 100$$

The RBSAF for all the sampled points were determined in this study.

2.7. Data Analysis

Hierarchical cluster analysis (HCA) was used to determine the association between parent and polar PAH concentrations in sediment, oyster and water samples. Turkey post-hoc test was

248 used to separate the mean of BSAF values obtained in this study (Origin Lab Crop.,
249 Northampton, MS, USA, 8.5 software).

250 **3. Results and Discussion**

251 In order to develop a comprehensive understanding of PAC concentration dynamics and
252 possible contaminant bioavailability in the estuary, we carried out an investigation of the
253 concentrations of parent and polar PAHs in water, sediment and biota (oyster) compartments:

254 3.1 Distribution of parent PAHs in water, sediment and oyster of the estuary

255 Rivers have a natural regenerative ability since they are not static. Direct measurement of total
256 water concentrations of contaminants may, therefore, not represent a true contaminant
257 concentration estimation. For this study, the determination of contaminants' water
258 concentrations was imperative to emphasise the high partitioning capacities of PACs into
259 sediments and biota in an aquatic environment. Parent PAH concentrations in water, across the
260 sampling points of the estuary, ranged from 0.05 – 9.64 µg/l (Table 2). Concentrations were
261 generally lower than 0.50 µg/l except for noticeable spikes mostly around the shoreline sand-
262 verge, boat ramp and shoreline-historical deposit (Table 2, Fig. 1). This could be because of
263 high concentration of contaminants in the shoreline sand-verge and historical residual tar
264 deposits as well as petrogenic PAHs from gasoline powered boat engines.

265 The concentrations of PAH in sediments were several orders of magnitudes higher than
266 concentrations in water. This confirms PAHs' ability to partition into sediment organic matter
267 in an aquatic water-sediment system [4, 18, 32, 36, 37]. The highest concentrations of parent PAHs
268 were recorded at the shoreline-sand verge (S28), historical residual tar deposits (S7 – S9) and
269 locations of recent coal tar usage (S22 – S23) (Tables 1 & 2). The highest sediment parent PAH
270 concentrations across the estuary was 44330 µg/g (S23). Coal tar was believed to have been
271 recently used in this location and this was reflected in the total concentration of parent PAHs
272 (212617 µg/g) (Table 2). Sediment parent PAHs of the 33 sampled sites were dominated by

fluoranthene and pyrene with average concentrations of 2449 and 2218 µg/g, respectively (Table S3, supplementary information). High sediment concentrations of PAHs were reported for Australian southeast estuaries in studies conducted in the 80s and 90s [38, 39], confirming the long years of PAH contamination of the estuaries. In addition to the use of coal tar in infrastructure for shellfish aquaculture, dominant signatures of sediment PAHs might be indicative of possible coal combustion source(s) of contamination. For the oysters, the locations A/B-sampled parent PAH concentrations were 5761 µg/g and 111.2 µg/g, respectively (Table 2).

3.2 Distribution of polar PAHs in water, sediment and oyster of the estuary

The total concentration of oxyPAHs in analysed water samples from the estuary was 9.9 µg/l with concentration trends relatively similar to that of parent PAHs. Concentration spikes were recorded in few sampling points located at the roadside drain, boat ramp and shoreline-historical deposits. All concentrations, except S1 (2.2 µg/l) were less than 1.0 µg/l (Table 2). OxyPAH concentrations in sediments followed a similar trend as parent PAH concentrations with concentration peaks around shoreline- sand verge and historical deposit (Table 1, Fig. 1). Overall, the highest oxyPAH concentration of 63.5 µg/g was recorded at S22 (shoreline-historical residual coal tar deposit), and the total sediment oxyPAH concentration was 409.3 µg/g (Table 2). Oyster oxyPAH mean concentration at location A (5.2 µg/g), was higher than the location B concentration (0.96 µg/g) (Table 2).

Water concentrations of HPAH and NPAH were mostly about ten orders of magnitudes lower than parent PAH and oxyPAH concentrations (≤ 0.1 µg/l) (Table 2). The only exception was the water sample (S12), around the boat ramp, with an NPAH water concentration of 6.6 µg/l. Total estuary HPAH and NPAH sediment concentrations were 37.2 and 237.1 µg/g, respectively (Table 2). For oysters, HPAH locations A and B-sourced oyster concentrations

were similar at 0.16 and 0.20 µg/g, respectively. NPAH oyster concentrations were 3.22 µg/g (location A) and 5.5 µg/g (location B) (Table 2).

The highest mean oxyPAH concentration in sediments was 7H-benz[d,e]anthracene-7-one (5.4 µg/g) followed by 9,10-anthraquinone (3.8 µg/g) (Table S4). For HPAHs, highest mean concentrations were recorded in carbazole (0.5 µg/g), DBF (0.3 µg/g) and ACR (0.2 µg/g) (Table S5). For NPAHs, the highest mean concentration was for 9N-ANT (6.0 µg/g) (Table S6). These signatures all implicate coal combustion/coal tar as the main source of contamination.

Polar PAH concentrations in this study, for the three environmental media, were higher than the few reported concentrations found in the literature. For example ∑oxyPAHs reported in urban stream sediments for eight oxyPAHs from Conodoquinet Creek Watershed Pennsylvania, United States was 17.2 µg/g (409.9 µg/g in this study) while ∑HPAHs of four HPAHs was 4.4 µg/g [40] as against 37.2 µg/g in this study. In our previous study of Lake Macquarie Australia, we reported total sediment concentrations of 15.6, 0.44 and 0.06 µg/g for oxyPAHs (7), HPAHs (9) and NPAHs (3) respectively [32]. The use of coal tar in the treatment of timber poles used in shellfish infrastructure within the study area could be the reason for the high sediment concentrations in this study.

Compared to the 9.9 µg/l ∑oxyPAHs water concentration in this study, the concentration of ∑oxyPAHs reported for Chaobai River, northern China, which is reputed for high level of anthropogenic activities, was 0.3 µg/l [41]. Similarly, ∑oxyPAHs concentration for the combination of dissolved and particulate phase samples from a water-shortage area of Haihe River system China was 1.35 µg/l while NPAHs were undetected [42].

As far as we know, few studies in the literature investigated the bioaccumulation of polar PAHs in aquatic organisms [43]. For example, oysters and mussels sampled from Osaka Bay, Japan,

321 recorded ∑NPAHs (8) concentrations that ranged from 2380 – 24,688 and 2672 – 25,961 pg/g,
322 respectively [44]. The concentrations of ∑oxyPAHs (15) reported in fish species from the West
323 African country of Ghana averaged 422,000 pg/g [43]. In a similar study on lake trout fish from
324 Lake Michigan, USA, ∑NPAHs (9) concentrations ranged from 0.2 – 31 pg/g [45] .
325 Hierarchical cluster analysis (HCA) was used to explain the spatial distribution of PAHs,
326 oxyPAHs, HPAHs and NPAHs in water (Fig. S1, supplementary information), sediment (Fig.
327 S2) and oyster tissue (Fig. S3) samples. The sampling sites were clustered into groups based
328 on polar and non-polar contamination with the HCA yielding two groups each for the water,
329 sediment and oyster samples. The water samples were clustered mainly in group 2, implying
330 that polar and non-polar PAH concentrations were generally similar for all water samples
331 except S7, S10 and S19 with much higher concentrations and S12 with a much lower
332 concentration (Fig. S1). The high concentrations could be the result of historical residual tar
333 deposits (S7 and S19) and boating activities in the boat ramp (S10) where S12 was also sampled
334 (Table 1). The low concentration of S12 could have resulted from dilution through active water
335 mixing. The sediment group 1 sample had higher concentrations compared to group 2 samples.
336 This could be due to the upstream location and closeness of most of the group 1 sampling sites
337 to shoreline-historical residual tar deposits compared to group 2 sites (Fig. S2). Similarly,
338 oyster samples classified as group 2 were more contaminated, by polar and non-polar PAHs,
339 compared to group 1 (Fig. S3) reflecting the results for the sediment samples.
340 3.3 Bioaccumulation of parent and polar PAHs in estuary oysters
341 The BSAF and RBSAF values for total and individual polar and non-polar PAHs were
342 computed for the 33 sampled sites in order to have a better understanding of the mechanism of
343 polar and non-polar bioaccumulation in *S. glomerata*.

344 Total parent PAH was highly bio-accumulated in oyster tissues (mean BSAF = 42.5) (Fig. 2).
345 For polar PAHs, only ∑NPAH had a value of BSAF > 1. Mean BSAF values for ∑oxyPAHs

and ∑HPAHs in this study were 0.002 and 0.094, respectively indicating very low accumulation of oxyPAHs and HPAHs in oyster tissues (Fig. 2). This result is in accordance with the common understanding that NPAHs, because of their relatively lower solubility, partition more into sediments and tissues of aquatic organisms. On the other hand, oxyPAHs and HPAHs partition more into the water segment as against tissues, in an aquatic system, due to their higher polarity. The RBSAF values compare total parent and polar PAH accumulation from sediments into oyster tissue across the stations with the highest percentages in sites 1 – 6; 26, 27 and 29, 30 (Table S8). The sites were all relatively upstream.

The individual BSAF values of parent and polar PAHs provided a more detailed description of their bioaccumulation in oyster (Fig. 3(A-D)). All the investigated HPAHs had mean BSAF values < 1. Their order of bioavailability based on BSAF was: THIA > 2-MBF > QUI > 8-MQL > XAN > ACRI > IND > CBZ > DBF (Fig. 3A). The mean BSAF was only significantly different ($p < 0.05$) for DBF and 2-MBF; DBF and THIA; DBF and QUI; DBF and 8-MQL; and, CBZ and QUI.

For oxyPAHs, only 2-EAQ had a mean BSAF > 1 (Fig. 3B). The order of bioavailability was: 2-EAQ > 7H-BANT > 2, 3-DMAQ > 2MAQ >9-FLO > 9, 10-ANQ > 1, 4-NQ (Fig. 3B). Mean BSAF was not significantly different ($p > 0.05$) except for 2-EAQ and each of, 1, 4-NQ, 9-FLO, 9, 10-ANQ, 2-MAQ and 2, 3-DMAQ where mean BSAF were significantly different ($p < 0.05$).

Mean BSAF for 1N-NAP was 4.7 compared to 2N-FLU and 9N-ANT, which were 0.2 and 0.3, respectively (Fig. 3C). The 1N-NAP mean BSAF value was significantly different ($p < 0.05$) from the other two NPAHs, which on the other hand, were not significantly different from each other. Similar to the result of this study, residues of nitronaphthalenes were higher in bivalves from intertidal areas of Osaka Bay Japan, compared to other NPAHs [44].

All 13 parent PAHs investigated in this study had values of mean BSAF > 1 and at greater order of magnitudes compared to polar PAHs (Fig. 3D). Low molecular weight ACENY and ACEN had the least values of 1.4 and 1.7 respectively occupying the lower end of the bioavailability order: FLUA > PYR > ANTH > CHRY > B[a]A > I[cd]P > B[b+k]F > B[a]P > D[a,h]A > PHEN > FLU > ACEN > ACENY (Fig. 3D). Mean BSAF for FLUA was significantly different ($p < 0.05$) from ACENY, ACEN, FLU, PHEN, FLUA, B[b+k]F, B[a]P, I[cd]P, D[a,h]A. Mean BSAF for other PAHs were not significantly different ($p > 0.05$) from one another (Fig. 3D). Highest PAH BSAF value compared to other PAHs was recorded for FLUA in a similar study [46].

Individual BSAF results showed that apart from 1N-NAP and 2-EAQ, polar PAHs did not bio-accumulate in oyster tissue. Conversely, all analysed PAHs bio-accumulated in oyster tissue. The BSAF values of parent and polar PAHs provide information about the possible bioavailability of the contaminants. Bioavailable concentration is of great importance being the final concentration that becomes available to living systems that could elicit toxic effects [9, 47] . From a human health risk point of view, oysters with accumulated PACs may be a ready source of contaminants that could result in devastative chronic health effects [7, 47].

Most of the studies on the bioaccumulation of PACs in the tissues of biomonitoring organisms, found in the literature, focused on parent PAHs. Biota-sediment accumulation factors, in such studies just like this study, indicated a prevalence of 3-4 ringed PAHs compared to HMW PAHs in tissues of the organisms [16, 33, 48].

Bio-accumulated PACs in *S. glomerata* may have been from sediment and suspended particle ingestion and passive diffusion of freely dissolved contaminants present in water [49]. Literature results showed that the dominant contamination source (sediment or water) of bivalves is an important factor determining the PAC distribution pattern in their tissue [30]. This was the case

394 in the differences noticed in the BSAF values of mussels sampled from the Mediterranean Sea
395 and Arcachon Bay [34]. The PAH distribution in the tissue of *S.glomerata*, in this study, mostly
396 reflected the dominant PAHs in the estuary sediments particularly the parent PAHs, 1N-NAP
397 (NPAH) and 2-EAQ (oxy-PAH) (Fig. 4).

398 This implies that the PACs' oyster concentrations could have been in thermodynamic
399 equilibrium as the sediment concentrations [34, 35] (Fig. 4). Consequently, the bio-accumulated
400 PACs in oyster tissue might have largely originated from the amorphous organic carbon of the
401 sediment [49]. Contaminants accumulation in benthic aquatic organisms have essentially been
402 through sediment ingestion. For example, about 95 % of benzo[a]pyrene and 61 % pyrene
403 accumulated by *Ilyodrilus templetoni* and *Lumbriculus variegatus*, respectively were through
404 sediment ingestion [50, 51]. Based on the results, sediment ingestion is the major bioaccumulation
405 route in this study.

406 In addition, bioaccumulation of PACs is highly dependent on the octanol-water partition
407 coefficient- K_{ow} [16] as seen in the general less partitioning of the polar PAHs investigated in
408 this study into oyster tissue (Fig. 4). The associated low K_{ow} values of polar PAHs make them
409 partition more into water, in a water-sediment-tissue system [23]. The only group of polar PAHs
410 that could exhibit evidently varied partitioning behavior into tissue, according to the literature
411 and as confirmed in this study, are NPAHs [22, 23]. The varied behavior is due to the relative
412 hydrophobic nature of NPAHs compared to other polar PAHs. The bioaccumulation potential
413 of NPAHs to biota in an aquatic environment has been discussed elsewhere [22].

414 3.4 Implications of PACs' (non) bioaccumulation in oyster tissue

415 Bioavailability provides an estimation of the actual contaminant uptake by a living organism
416 and a better understanding of the possible risks [9, 24]. Based on the concentrations of parent and
417 polar PAHs in the water, sediment and oysters from the polluted estuary in this study, the

418 partitioning behavior of the PACs under investigation were revealed. Only parent PAHs and
419 NPAHs bio-accumulated significantly in oyster tissues. OxyPAHs and HPAHs demonstrated
420 low levels of bioaccumulation. The bioaccumulation of parent PAHs and NPAHs in oyster
421 tissue could imply greater risk of these types of PACs because of the increased potential of
422 their slow release from the oyster tissues and the possibility of protracted exposure of
423 contaminants to other living organisms in the food web [9, 24]. In spite of the higher bio-
424 accumulated concentrations of parent PAHs in oyster tissue compared to NPAHs, the NPAH
425 protracted toxic effects on organisms might be higher than the toxicity of the parent analogues.
426 This is because NPAHs elicit direct toxic effects at much lower concentrations relative to
427 parent PAHs. The toxicity of parent PAHs is indirect as it is derived from the cytochrome P450
428 mediated detoxification mechanisms of cells and tissues, which often result in the formation of
429 highly reactive genotoxic metabolites such as diol epoxides and quinones [23, 52-54] and
430 subsequent adducts with DNA. The developmental toxicity malformation profile in zebrafish
431 embryos exposed to NPAHs, for example, was similar or higher in the transformation products,
432 when compared to the parent PAHs [55, 56].
433 Notwithstanding their lower bioaccumulation, the potential risks of oxyPAHs and HPAHs to
434 living organisms could be profound especially when there is chronic exposure. In an
435 elimination rate study by our group, the concentrations of polar PAHs in the tissue of Sydney
436 rock oyster was relatively constant, over a 3-month period, implying a possible equilibrium
437 between the oyster tissue and water polar PAH concentrations [57]. Such sustained low
438 concentrations, particularly of oxyPAHs, could elicit greater toxic effects in humans and other
439 living organisms compared to parent PAHs [23, 25, 58]. OxyPAHs like NPAHs do not require
440 transformation to intermediate metabolites to elicit their toxic effects and have shown adverse
441 effects on observed toxicological endpoints in aquatic organisms such as zebrafish embryos at
442 very low concentrations [56, 59].

4. Conclusion

This study was executed to ensure a better understanding of the partitioning behaviour and bioavailability of PACs and particularly polar PAHs in a model aquatic biomonitoring organism. This is important because polar PAHs have superior ability to exert greater toxic effects in living organisms, at relatively lower concentrations, compared to their parent analogous. Consequently, the concentrations and distribution of parent and polar PAHs in the sediment and water of an economically important Australian southeast estuary were determined as well as their partitioning into the tissue of the Sydney rock oyster (*S. glomerata*)**.** Concentrations of parent and polar PAHs in water were generally low. Parent PAH concentrations in sediments were moderate to very high and moderate to high in the tissues of *S. glomerata*. Comparatively lower concentrations were analysed in sediment and oyster tissues for polar PAHs. Bioavailability determination using BSAF revealed that all investigated PAHs bio-accumulated in oyster tissues unlike polar PAHs, which were mostly non-bioaccumulated. Only 1N-NAP and 2-EAQ recorded values of BSAF > 1. As far as we know, this is the first study in Australia that simultaneously looked at the partitioning dynamics of polar and non-polar PAHs in water, sediment and a biomonitoring organism. The overall findings will be useful in future monitoring and risk assessment efforts.

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Fig. 1. Map of the estuary showing the sampling locations for water, sediment and oyster. A and B represent locations where oysters were collected. Lettered numbers represent locations where water and sediments were sourced.

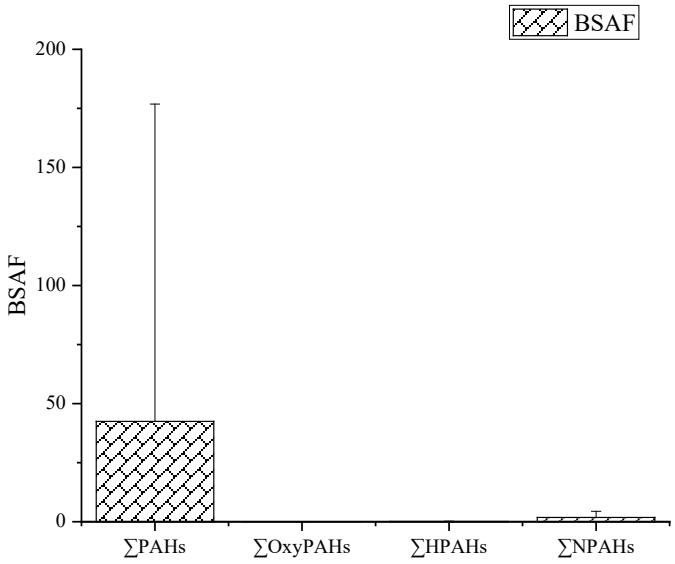
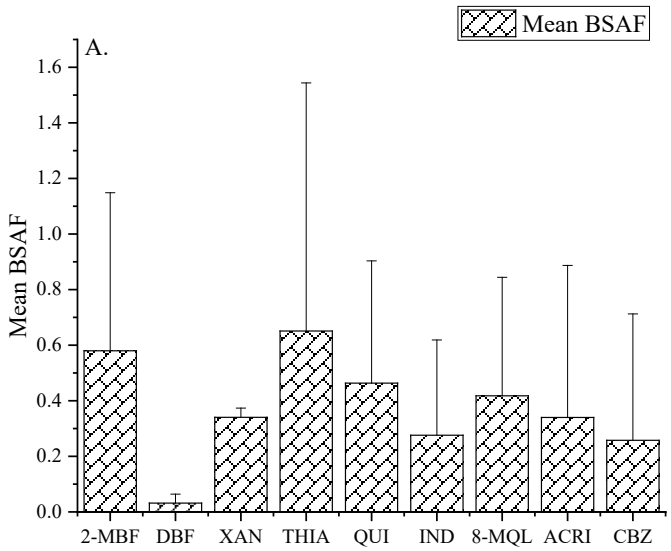


Fig. 2. Biota-sediment accumulation factor (BSAF) of total parent PAHs, oxyPAHs, HPAHs and NPAHs computed from sediment/oyster analyte concentrations in this study.


Mean BSAF
A.
Mean BSAF
1.6
1.4
1.2
1.0
0.8
0.6
0.4
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DBF
XAN
THIA
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8-MQL
ACRI
CBZ

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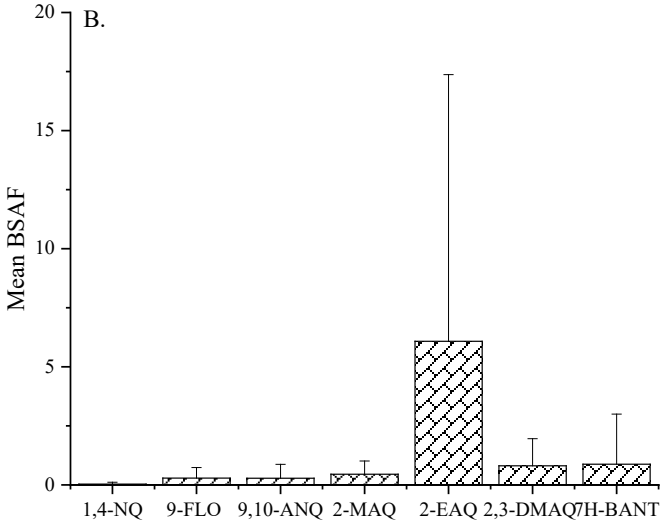
B.
Mean BSAF
20
15
10
5
0
1,4-NQ
9-FLO
9,10-ANQ
2-MAQ
2-EAQ
2,3-DMAQ
7H-BANT

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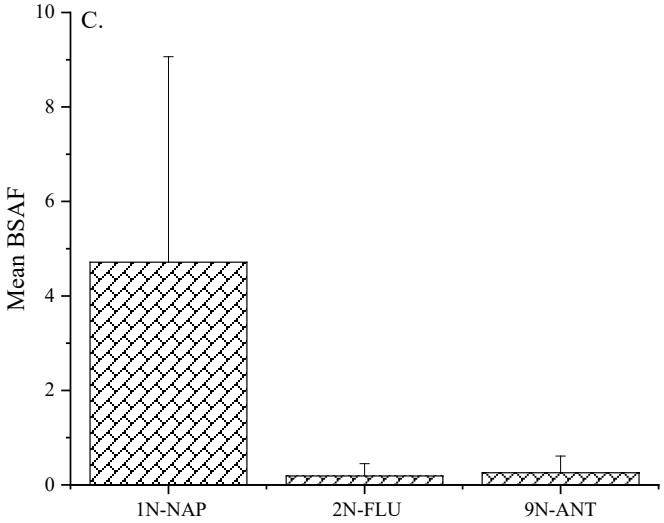

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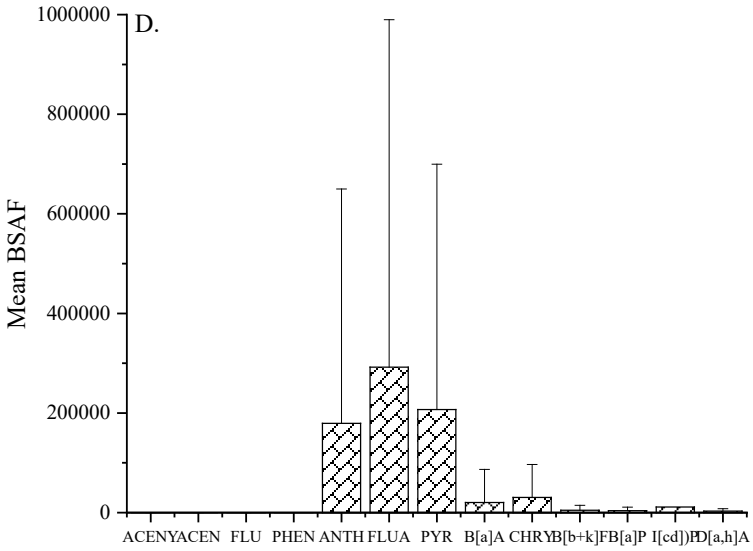

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38 **Fig. 3.** Biota-sediment accumulation factor (BSAF) of (A) HPAHs (B) oxyPAHs (C) NPAHs
39 and (D) parent PAHs computed from sediment/oyster analyte concentrations in this study.
40 2-MBF, 2-methylbenzofuran; DBF, dibenzofuran; XAN, xanthene; THIA, thianaphthene;
41 QUI, quinoline; IND, indole; 8-MQL, 8-methylquinoline; ACR, acridine; CBZ, carbazole; 1,4-
42 NQ, 1,4-naphthoquinone; 9-FLO, 9-fluorenone; 9,10-ANQ, 9,10-anthraquinone; 2-EAQ, 2-
43 ethylanthraquinone; 2-MAQ, 2-methyl anthraquinone; 2,3-DMAQ, 2,3-
44 dimethylanthraquinone; 7H-BANT, 7H-benz[d,e]anthracene-7-one; 1N-NAP, 1-
45 nitronaphthalene; 2N-ANT, 2-nitroanthracene; 9N-FLU, 9-nitrofluorene; ACENY,
46 acenaphthylene; ACEN, acenaphthene; FLU, fluorene; PHEN, phenanthrene; ANTH,
47 anthracene; FLUA, fluoranthene; PYR, pyrene; B[a]A, benz[a] anthracene; CHRY, chrysene;
48 B[b+k]F, benzo[b+k]fluoranthene; B[a]P, benzo[a] pyrene; I[cd])P, indeno[1,2,3-cd]pyrene;
49 D[a,h]A, dibenz[a,h]anthracene.

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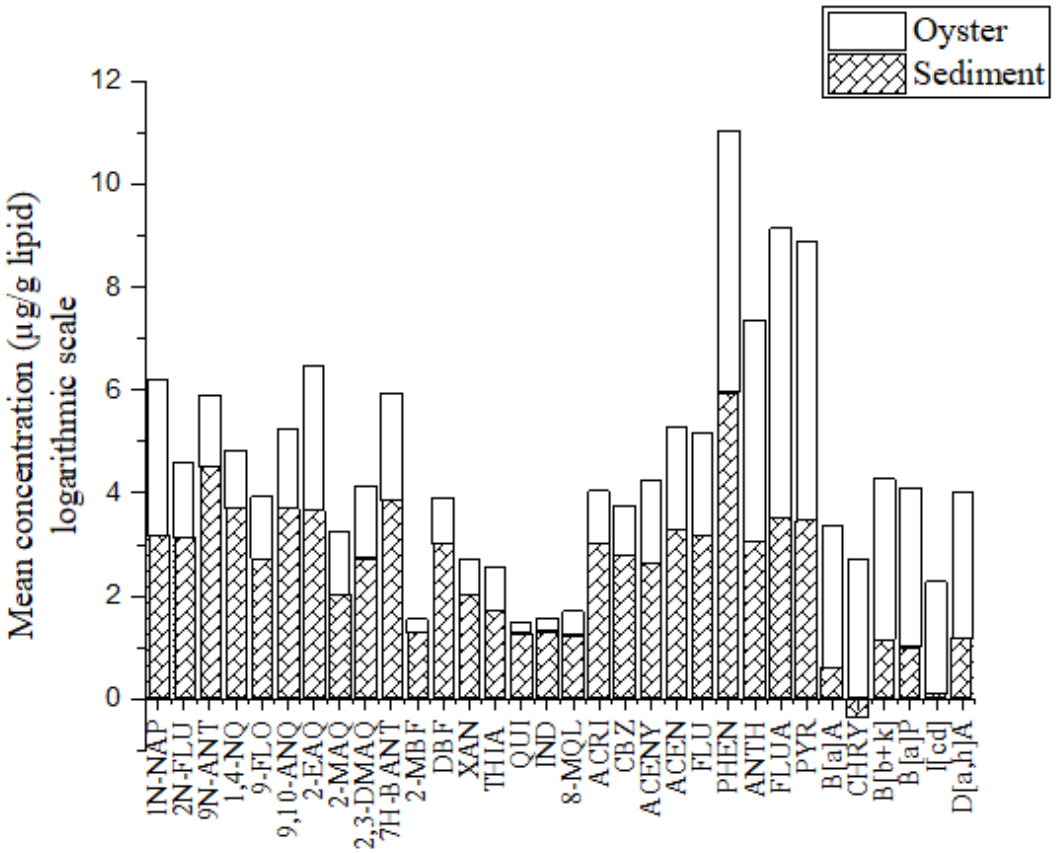

Fig. 4. Sediment-oyster analyte concentration dynamics in this study.

1N-NAP, 1-nitronaphthalene; 2N-FLU, 2-nitrofluorene; 9N-ANT, 9-nitroanthracene; 1,4-NQ, 1,4-naphthoquinone; 9-FLO, 9-fluorenone; 9,10-ANQ, 9,10-anthraquinone; 2-EAQ, 2-ethylanthraquinone; 2-MAQ, 2-methylanthraquinone; 2,3-DMAQ, 2,3-dimethylanthraquinone; 7H-BANT, 7H-benz[d,e]anthracene-7-one; 2-MBF, 2-methylbenzofuran; DBF, dibenzofuran; XAN, xanthene; THIA, thianaphthene; QUI, quinoline; IND, indole; 8-MQL, 8-methylquinoline; ACR, acridine; CBZ, carbazole; 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.

Supplementary Information

Bioavailability of polycyclic aromatic compounds (PACs) to the Sydney rock oyster (*Saccostrea glomerata*) from sediment matrices of an economically important Australian estuary

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SI-Text 1: 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 an 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*., 2019; 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 2: 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. Tissues (1g), sediments (1g) and water (200 ml) were spiked with 20µl of 100µg/ml acenaphthene-d10/ fluoranthene-d10 (parent PAHs) and individual polar PAHs. Afterwards, the samples were extracted, fractionated and analysed. Unspiked 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 S2.

Table S1

Physicochemical properties of sediments from the southeast Australian estuary

Site location	EC mS/cm	pH	N (%)	TOC
S1	25.8	6.9	0.61	8.48
S2	21.3	6.7	0.7	10.56
S3	20.6	7.0	0.07	4.96
S4	8.3	7.1	0.26	4.69
S5	11.0	7.1	0.45	7.29
S6	16.4	7.0	0.55	8.31
S7	6.4	7.0	0.25	3.1
S8	7.0	6.9	0.14	1.9
S9	7.7	7.1	0.21	2.52
S10	2.7	6.8	0.06	0.56
S11	2.6	7.0	0.03	0.33
S12	2.5	7.1	0.08	0.81
S13	5.2	7.3	0.07	0.92
S14	5.1	7.5	0.09	1
S15	5.2	7.4	0.05	0.77
S16	2.4	7.1	0.03	0.36
S17	3.3	7.0	0.04	0.54
S18	2.9	7.1	0.08	0.79
S19	3.1	7.3	0.05	0.46
S20	2.8	7.2	0.08	0.92
S21	2.9	7.1	0.05	0.57
S22	8.4	7.5	0.23	6.95
S23	8.8	7.6	0.24	4.61
S24	7.9	7.6	0.26	4.58
S25	6.8	7.1	0.08	1.24
S26	6.2	7.1	0.09	1.17
S27	5.9	7.3	0.1	1.28
S28	15.4	6.9	0.27	5.19
S29	11.3	7.0	0.28	3.95
S30	14.8	7.2	0.19	3.65
S31	4.3	7.3	0.07	1.42
S32	4.9	7.2	0.09	1.9
S33	5.4	7.2	0.06	1.29

Table S2

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

Sediment							
1,4-NQ	9-FLO	9,10-ANQ	2-MAQ	2-EAQ	2,3-DMAQ	7H-BANT	
85.8	102.9	66.2	97.0	61.1	90.8	103.5	
ACE-D10	FLU-D10	1N-NAP	2N-FLU	9N-ANT			
63.6	80.3	84.8	108.4	72.8			
2-MBF	XAN	THIA	QUI	IND	8-MQL	ACR	CBZ
107.2	73.2	73.5	122.3	109.5	108.2	97	70.2
Oyster							
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	
ACE-D10	FLU-D10	1N-NAP	2N-FLU	9N-ANT			
69.2	82.5	66.6	79.8	92.1			
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
Water							
1,4-NQ	9-FLO	9,10-ANQ	2-MAQ	2-EAQ	2,3-DMAQ	7H-BANT	
60.0	63.2	56.6	64.9	65.8	65.8	74.9	
ACE-D10	FLU-D10	1N-NAP	2N-FLU	9N-ANT			
61.9	73.9	81.2	63.1	70.5			
2-MBF	XAN	THIA	QUI	IND	8-MQL	ACR	CBZ
98.7	63.9	53.3	101.9	71.2	108.5	64.0	68.2

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; ACE-D10, Acenaphthene-d10; Fluoranthene (d10), FLU-D10; 1N-NAP, 1-nitronaphthalene; 2N-ANT, 2-nitroanthracene; 9N-FLU, 9-nitrofluorene; 2-MBF, 2-methylbenzofuran; XAN, xanthene; THIA, thianaphthene; QUI, quinoline; IND, indole; 8-MQL, 8-methylquinoline; ACRI, acridene; CBZ, carbazole. Acenaphthene-d10 and Fluoranthene-d10 were the recovery standards for parent PAHs.

Table S3

Concentrations of parent PAHs in sediments of the southeast Australian estuary (μ/g d.w.)

	ACEN	ACEN	FLU	PHEN	ANTH	FLUA	PYR	B[a]A	CHRY	B[b+k]F	B[a]P	I[cd])P	D[a,h]A
S1	0.0	0.0	0.0	50.1	9.8	305.6	297.4	0.2	0.2	0.3	0.3	0.0	0.2
S2	0.0	0.0	0.0	73.3	15.7	425.8	409.0	0.2	0.2	0.4	0.4	0.0	0.6
S3	0.0	0.0	0.0	61.8	14.3	348.6	338.1	0.1	0.1	0.4	0.4	0.1	0.9
S4	0.0	0.1	0.0	120.9	34.5	343.6	284.6	0.1	0.1	0.1	0.1	0.0	0.2
S5	0.0	0.0	0.0	31.4	7.9	82.4	72.7	0.0	0.0	0.0	0.0	0.0	0.1
S6	0.0	0.1	0.0	70.8	32.2	216.9	182.5	0.0	0.0	0.1	0.1	0.0	0.2
S7	1.1	8.5	3.3	3581.3	2192.0	10947.7	10196.0	26.7	24.6	42.4	9.4	2.0	61.7
S8	0.5	5.1	2.1	4912.2	2319.9	15841.2	14606.8	70.1	64.5	14.9	43.7	1.4	36.0
S9	1.2	6.6	4.1	4126.9	4624.9	12375.8	10905.9	24.1	22.2	35.7	8.3	1.3	48.9
S10	0.0	0.0	0.0	6.6	1.7	12.6	17.1	0.0	0.0	0.0	0.0	0.0	0.0
S11	0.0	0.1	0.0	33.0	8.2	58.3	46.9	0.0	0.0	0.0	0.0	0.0	0.0
S12	0.0	0.0	0.0	37.2	9.3	118.0	94.3	0.0	0.0	0.1	0.1	0.0	0.1
S13	0.0	0.0	0.0	15.6	4.4	36.6	32.3	0.0	0.0	0.0	0.0	0.0	0.0
S14	0.0	0.0	0.0	18.4	4.5	39.9	37.1	0.0	0.0	0.0	0.0	0.0	0.0
S15	0.0	0.0	0.0	18.0	4.7	34.5	30.0	0.0	0.0	0.0	0.0	0.0	0.0
S16	0.2	0.2	0.3	3.1	1.1	8.1	7.0	0.1	0.1	1.6	1.7	0.1	3.4
S17	0.2	0.5	0.5	5.7	1.6	13.8	12.5	0.2	0.2	1.6	1.7	0.2	2.3
S18	0.3	0.2	0.3	3.1	0.8	7.7	6.7	0.1	0.1	2.0	2.2	0.3	4.7
S19	0.0	0.0	0.0	0.4	0.2	2.5	2.1	0.7	0.6	0.8	0.8	0.1	1.2
S20	0.0	0.1	0.0	0.3	0.1	1.5	1.3	0.4	0.4	0.4	0.4	0.0	0.3
S21	0.0	0.1	0.0	28.8	9.2	135.3	113.2	0.1	0.1	0.3	0.7	0.0	1.0
S22	2.3	5.9	7.4	5329.0	5972.1	10741.3	10481.5	20.5	18.9	25.0	13.9	2.9	62.2
S23	1.3	4.9	5.5	7369.1	2925.9	18727.3	15162.1	29.2	26.9	37.9	34.3	2.9	2.8
S24	1.4	2.9	3.4	29.0	11.7	86.0	70.1	33.4	30.8	39.3	13.8	3.3	3.1
S25	0.0	0.0	0.0	11.9	3.2	49.2	40.4	0.0	0.0	0.3	0.3	0.0	0.5
S26	0.0	0.0	0.0	0.2	0.0	0.7	0.7	0.0	0.2	0.3	0.3	0.1	0.5
S27	0.0	0.2	0.2	0.5	0.2	1.3	1.1	0.0	0.4	0.6	0.6	0.1	0.9
S28	0.3	13.4	13.2	5754.7	6449.1	9774.0	9694.7	26.3	24.2	38.2	27.3	2.3	38.9
S29	0.0	0.0	0.0	0.2	0.1	0.7	0.7	0.0	0.2	0.2	0.2	0.0	0.1

S30	0.1	0.1	0.1	0.8	0.3	1.9	1.7	0.1	0.4	0.4	0.4	0.0	0.5
S31	0.2	1.6	1.4	7.9	3.1	19.1	15.5	0.4	4.9	3.6	3.4	0.3	2.7
S32	0.5	2.4	1.9	9.7	3.0	25.8	21.1	35.9	138.7	145.9	141.5	21.6	187.3
S33	0.4	2.8	2.1	12.6	4.6	27.8	24.0	7.6	13.2	93.5	89.0	5.7	54.3
Mean	0.3	1.7	1.4	961.4	747.6	2448.8	2218.4	8.4	11.3	14.7	12.0	1.4	15.6

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

Table S4

Concentrations of oxyPAHs in sediments of the southeast Australian estuary (μ/g d.w.)

	[a]1,4-NQ	9-FLO	9,10-ANQ	2-EAQ	2-MAQ	2,3-DMAQ	7H-BANT
S1	2.0	0.1	0.4	0.2	0.0	0.0	0.6
S2	1.9	0.0	0.4	0.1	0.0	0.0	0.6
S3	0.6	0.0	0.1	0.0	0.0	0.0	0.2
S4	0.7	0.0	0.0	0.0	0.0	0.0	0.1
S5	1.2	0.0	0.0	0.1	0.0	0.0	0.0
S6	0.1	0.0	0.0	0.1	0.0	0.0	0.0
S7	1.0	0.9	13.6	1.3	0.1	0.1	19.2
S8	0.7	0.6	4.9	0.5	0.1	0.0	7.3
S9	2.1	1.4	15.0	1.7	0.1	0.1	23.2
S10	1.5	0.0	0.0	0.1	0.0	0.0	0.0
S11	0.3	0.0	0.0	0.1	0.0	0.0	0.0
S12	0.3	0.0	0.0	0.1	0.0	0.0	0.0
S13	0.7	0.0	0.1	0.1	0.0	0.0	0.0
S14	2.0	0.0	0.0	0.2	0.0	0.0	0.0
S15	1.6	0.0	0.0	0.1	0.0	0.0	0.0
S16	0.5	0.1	0.5	0.1	0.0	0.0	1.5
S17	7.9	0.5	2.9	21.5	0.1	2.5	3.0
S18	2.4	0.1	0.6	0.1	0.0	0.0	0.9
S19	1.7	0.1	0.1	0.1	0.0	0.0	0.3
S20	2.9	0.0	0.2	0.0	0.0	0.0	0.6
S21	2.0	0.1	0.3	0.1	0.0	0.0	0.7
S22	1.5	1.6	23.0	2.0	0.1	0.2	35.1
S23	3.1	1.8	15.8	2.0	0.2	0.2	23.7
S24	1.9	0.7	6.8	0.7	0.1	0.1	13.3
S25	3.3	0.1	0.3	0.0	0.0	0.0	0.5
S26	0.1	0.0	0.0	0.0	0.0	0.0	0.2
S27	1.1	0.1	0.3	0.0	0.0	0.0	0.4
S28	2.0	0.5	14.9	1.0	0.1	0.0	14.0
S29	1.5	0.0	0.2	0.1	0.0	0.0	0.4
S30	0.0	0.0	0.3	0.1	0.0	0.0	0.8
S31	0.9	0.3	3.7	0.4	0.0	0.0	5.1
S32	2.0	0.7	9.1	0.9	0.1	0.1	15.8
S33	2.3	1.1	11.0	1.1	0.1	0.1	12.0
Mean	1.6	0.3	3.8	1.1	0.0	0.1	5.4

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; 7H-BANT, 7H-benz[d,e]anthracene-7-one

Table S5

Concentrations of HPAHs in sediments of the south-east Australian estuary (μ/g d.w.)

	2-MBF	DBF	XAN	THIA	QUI	IND	8-MQL	ACR	CBZ
S1	0.00	0.18	0.01	0.01	0.00	0.00	0.01	0.02	0.03
S2	0.00	0.20	0.02	0.01	0.00	0.01	0.01	0.02	0.04
S3	0.00	0.17	0.02	0.01	0.00	0.01	0.01	0.01	0.02
S4	0.00	0.14	0.01	0.01	0.00	0.00	0.01	0.02	0.01
S5	0.00	0.38	0.02	0.01	0.00	0.00	0.01	0.02	0.01
S6	0.00	0.51	0.01	0.01	0.00	0.01	0.01	0.02	0.01
S7	0.00	0.15	0.01	0.01	0.00	0.01	0.01	0.05	0.48
S8	0.00	0.18	0.01	0.01	0.00	0.02	0.01	0.06	0.24
S9	0.00	0.22	0.01	0.04	0.00	0.01	0.01	0.18	0.44
S10	0.00	0.12	0.01	0.01	0.00	0.00	0.01	0.02	0.01
S11	0.00	0.27	0.02	0.01	0.00	0.00	0.01	0.03	0.01
S12	0.00	0.83	0.02	0.01	0.00	0.00	0.01	0.02	0.01
S13	0.00	0.07	0.01	0.01	0.00	0.00	0.01	0.02	0.01
S14	0.00	0.22	0.02	0.01	0.00	0.00	0.01	0.02	0.01
S15	0.00	0.21	0.02	0.01	0.00	0.00	0.01	0.02	0.02
S16	0.00	0.26	0.02	0.01	0.00	0.00	0.01	0.02	0.13
S17	0.07	0.32	0.34	0.14	0.06	0.01	0.01	4.71	0.14
S18	0.00	0.09	0.01	0.01	0.00	0.00	0.01	0.05	0.10
S19	0.00	0.16	0.02	0.01	0.00	0.00	0.01	0.03	0.06
S20	0.00	0.44	0.02	0.01	0.00	0.01	0.01	0.04	0.04
S21	0.00	1.41	0.04	0.01	0.00	0.02	0.01	0.02	0.04
S22	0.00	0.21	0.02	0.14	0.00	0.05	0.01	0.33	5.21
S23	0.00	0.23	0.01	0.08	0.00	0.02	0.01	0.26	2.17
S24	0.00	0.20	0.01	0.01	0.00	0.01	0.01	0.21	1.29
S25	0.00	0.78	0.03	0.02	0.00	0.02	0.01	0.04	0.05
S26	0.00	0.33	0.02	0.01	0.00	0.00	0.01	0.02	0.03
S27	0.00	0.44	0.01	0.01	0.00	0.00	0.01	0.03	0.19
S28	0.00	0.46	0.01	0.01	0.00	0.02	0.01	0.48	1.53
S29	0.00	0.22	0.01	0.02	0.00	0.00	0.01	0.02	0.04
S30	0.00	0.17	0.01	0.01	0.00	0.00	0.01	0.04	0.06
S31	0.00	0.34	0.01	0.01	0.00	0.01	0.01	0.14	0.77
S32	0.00	0.82	0.01	0.03	0.00	0.03	0.01	0.29	1.80
S33	0.00	0.22	0.01	0.01	0.00	0.01	0.01	0.27	1.41
Mean	0.00	0.33	0.03	0.02	0.00	0.01	0.01	0.23	0.50

2-MBF, 2-methylbenzofuran; DBF, dibenzofuran; XAN, xanthene; THIA, thianaphthene; QUI, quinoline; IND, indole; 8-MQL, 8-methylquinoline; ACR, acridine; CBZ, carbazole

Table S6

Concentrations of NPAHs in sediments of the southeast Australian estuary (μ/g d.w.)

	1N-NAP	2N-FLU	9N-ANT
S1	0.2	0.2	0.1
S2	0.2	0.2	0.1
S3	0.2	0.1	0.0
S4	0.2	0.1	0.1
S5	0.3	0.1	0.0
S6	0.2	0.1	0.0
S7	0.2	2.4	0.2
S8	0.2	1.0	0.1
S9	0.1	3.2	0.2
S10	0.1	0.0	0.1
S11	0.2	0.1	0.0
S12	0.4	0.0	0.1
S13	0.1	0.0	0.0
S14	0.2	0.0	0.1
S15	0.2	0.1	0.1
S16	0.2	0.1	0.1
S17	2.9	1.8	175.3
S18	0.1	0.2	0.7
S19	0.2	0.1	0.0
S20	0.4	0.1	0.1
S21	1.6	0.1	0.0
S22	0.2	5.0	8.6
S23	0.3	3.3	5.2
S24	0.2	1.4	4.4
S25	1.9	0.1	0.1
S26	0.2	0.1	0.0
S27	0.2	0.1	0.1
S28	0.2	2.0	0.3
S29	0.1	0.1	0.1
S30	0.1	0.1	0.1
S31	0.2	0.7	0.1
S32	1.0	1.8	0.2
S33	0.2	2.3	0.2
Mean	0.4	0.8	6.0

1N-NAP, 1-nitronaphthalene; 2N-FLU, 2-nitrofluorene; 9N-ANT, 9-nitroanthracene

Table S7
Computed lipid content of oyster tissue

S/N	Initial weight (mg)	Final weight (mg)	Lipid weight	Tissue weight	% lipid content
1	22.6	22.6	0.1	1.0	9.0
2	23.4	23.5	0.1	1.0	13.0
3	23.2	23.3	0.1	1.0	15.0
4	23.1	23.2	0.1	1.0	9.0
5	23.2	23.3	0.1	1.0	15.0
6	22.8	23.0	0.2	1.0	17.0
7	23.1	23.2	0.2	1.0	15.0
8	23.0	23.1	0.1	1.0	10.0
9	22.7	22.9	0.2	1.0	15.0
10	23.2	23.3	0.1	1.0	13.0
11	23.0	23.1	0.1	1.0	7.0
12	23.4	23.5	0.1	1.0	11.0
13	23.0	23.1	0.1	1.0	8.0
14	23.0	23.2	0.1	1.0	14.0
15	22.8	22.9	0.1	1.0	13.0
16	22.7	22.8	0.1	1.0	11.0
17	23.0	23.2	0.1	1.0	12.0
18	22.9	23.0	0.1	1.0	10.0
19	22.8	22.9	0.1	1.0	13.0
20	22.8	22.9	0.1	1.0	15.0
				Mean	12.25

Table S8

Relative bio-concentration factor (RBSAF) of total PAHs, oxyPAHs, HPAHs and NPAHs across all sites.

	∑PAHs	∑OxyPAHs	∑HPAHs	∑NPAHs
S1	0.4	9.1	13.2	8.3
S2	0.4	12.2	14.8	9.9
S3	0.2	17.2	8.4	5.9
S4	0.0	3.4	11.9	10.5
S5	0.0	3.2	8.4	14.0
S6	0.0	17.0	7.7	17.4
S7	0.0	0.1	2.3	0.8
S8	0.0	0.1	1.9	1.1
S9	0.0	0.0	1.5	0.5
S10	0.0	0.2	1.6	1.6
S11	0.0	0.4	0.5	0.9
S12	0.0	1.1	0.5	1.4
S13	0.0	0.6	3.5	4.9
S14	0.0	0.3	1.8	2.4
S15	0.0	0.3	1.4	1.8
S16	0.0	0.1	0.4	0.6
S17	0.0	0.0	0.0	0.0
S18	0.0	0.1	1.6	0.4
S19	1.6	0.7	0.7	0.6
S20	6.1	0.9	0.7	0.6
S21	0.1	0.6	0.2	0.1
S22	0.0	0.4	0.5	0.2
S23	0.0	0.4	0.7	0.2
S24	0.5	0.7	1.1	0.3
S25	0.4	1.0	0.5	0.3
S26	12.2	9.9	1.2	1.7
S27	7.1	2.3	0.8	1.4
S28	0.0	0.6	0.9	0.9
S29	51.7	6.4	5.2	5.2
S30	18.1	9.9	5.2	4.9
S31	0.7	0.5	0.5	0.6
S32	0.1	0.2	0.3	0.3
S33	0.1	0.2	0.3	0.2

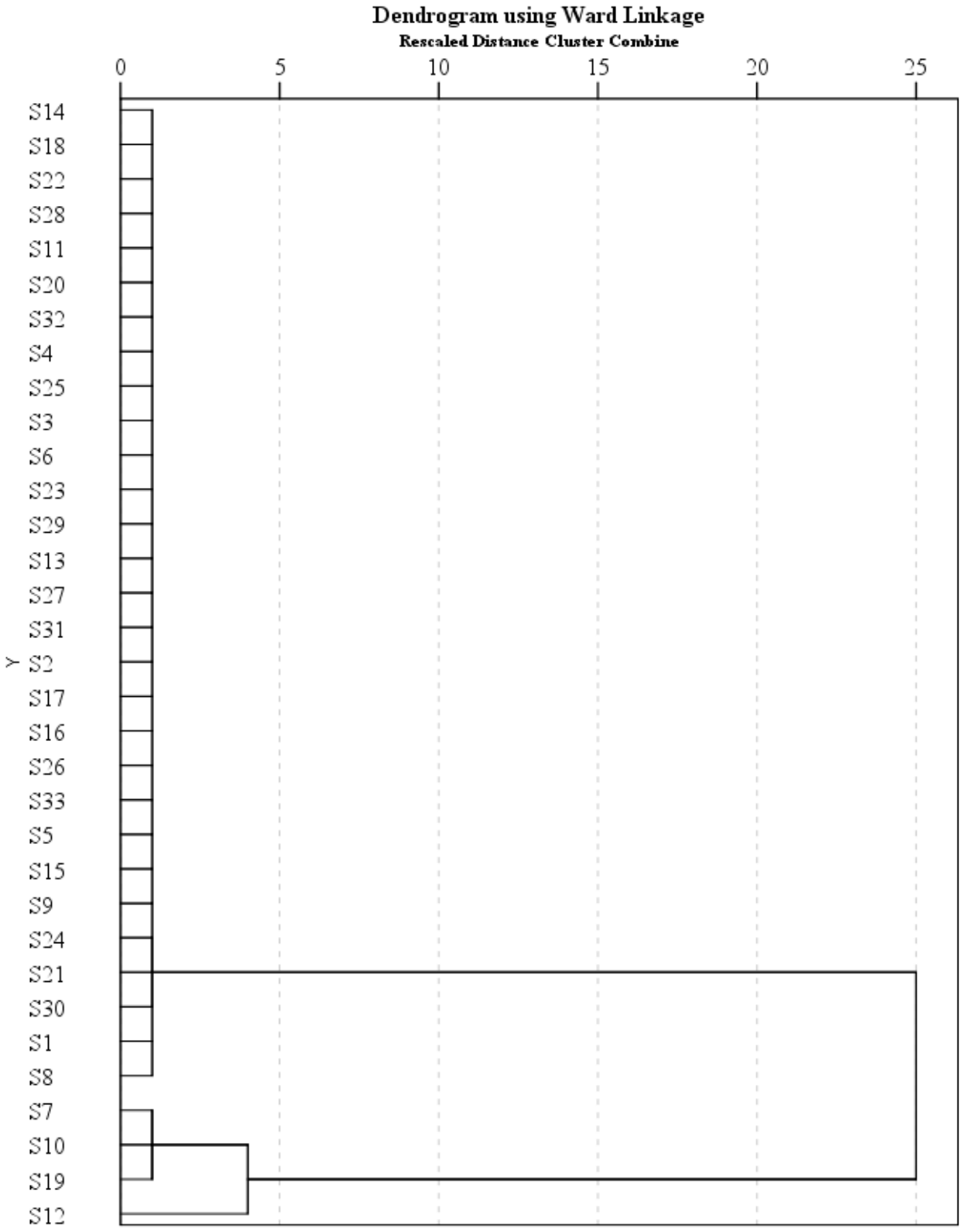

Fig. S1. Dendogram of hierarchical cluster analysis (HCA) of the spatial distribution of parent and polar PAHs in water samples.

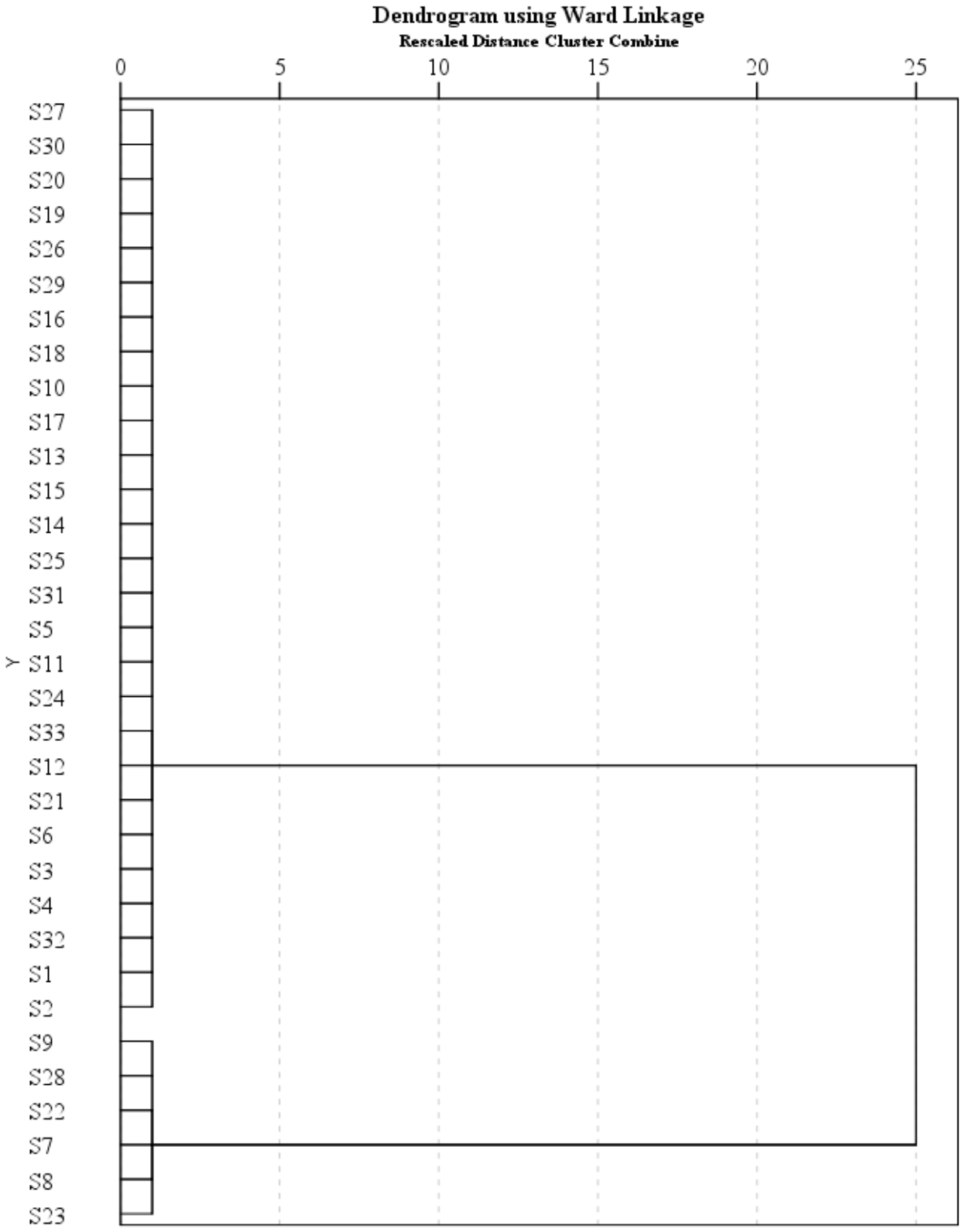

Fig. S2. Dendogram of hierarchical cluster analysis (HCA) of the spatial distribution of parent and polar PAHs in sediment samples.

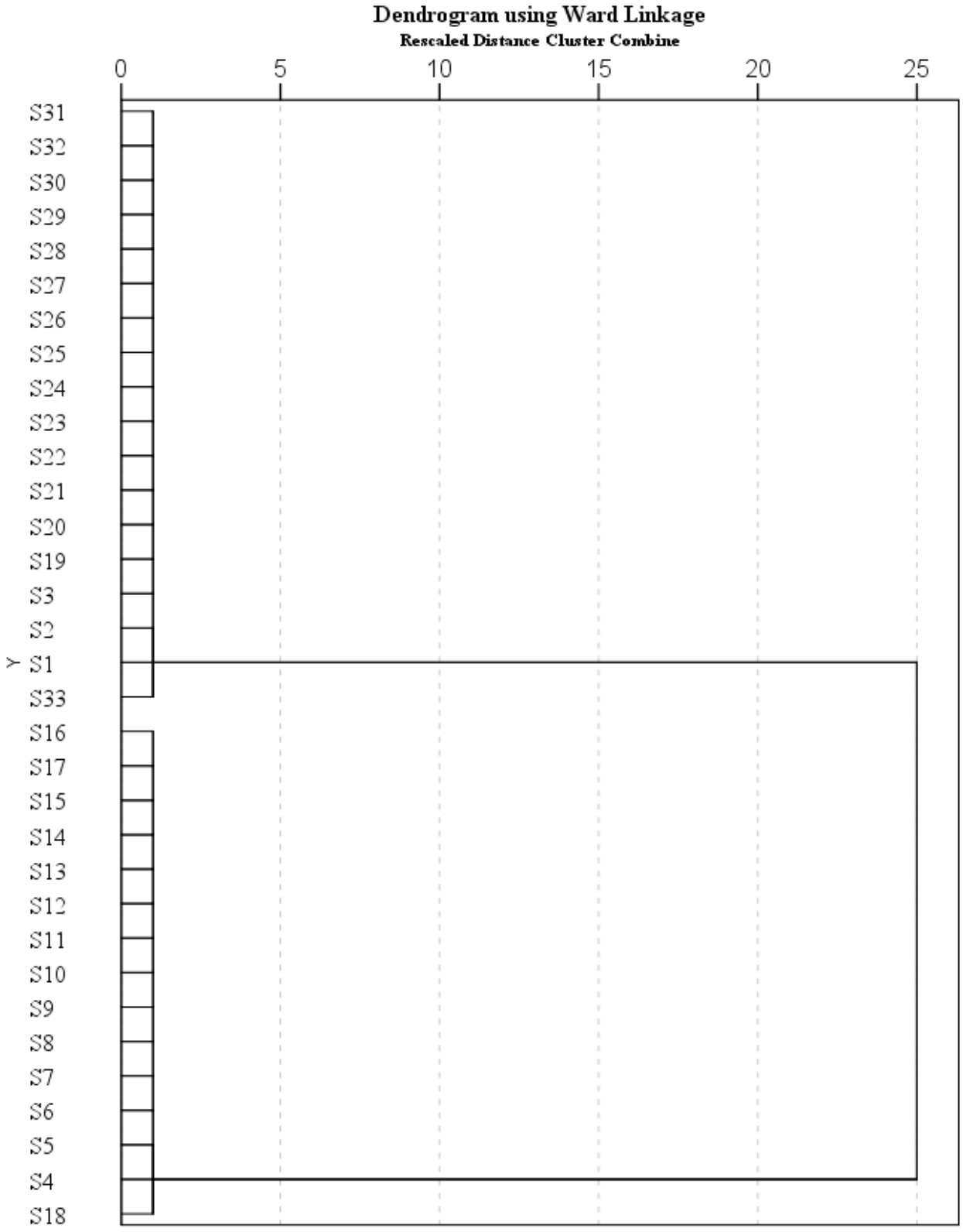

Fig. S3. Dendogram of hierarchical cluster analysis (HCA) of the spatial distribution of parent and polar PAHs in oyster samples.