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THEMATIC ISSUE

Bioaccumulation of PAHs in marine zooplankton: an experimental study in the copepod *Pseudodiaptomus marinus*

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Abstract Laboratory exposure experiments were performed on the marine coastal zooplankton species *Pseudodiaptomus marinus*—a keystone species of vast Atlantic and Pacific marine ecosystems, tracing the accumulation of three polycyclic aromatic hydrocarbons (PAHs): phenanthrene, anthracene and pyrene. The experiment was designed to study the bioconcentration of PAHs in phytoplankton (*Rhodomonas baltica*) and accumulation in copepods (*P. marinus*), through two different pathways: food and water uptake. For this purpose, water and organism subsamples were collected, ASE (accelerated solvent extraction) extracted, and analyzed for PAHs by GC/MS technique. As a result, experimental bioconcentration (BCF) and bioaccumulation (BAF) factors which showed a significant correlation with the octanol–water partition coefficient (K_{ow}) were presented for *P. marinus*. The active feeding route of exposure showed no significant differences versus passive uptake, highlighting the importance of dietary exposure as a major pathway for POPs accumulation in zooplankton; in particular for those which cannot be metabolized.

Keywords Copepod · *Pseudodiaptomus marinus* · Phenanthrene · Fluoranthene · Pyrene · Accumulation · Organic compounds

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Introduction

Polycyclic aromatic hydrocarbons (PAHs), are currently considered as one of the major groups of environmental contaminants, but unlike other harmful organic chemicals that have been banned or regulated in terms of their discharge, PAHs continue to be released into the environment due to their widespread formation after fossil fuel burning and leakage during petroleum recovery, transportation and use (McCready et al. 2006; Riccardi et al. 2013; Zhu et al. 2005; Bajt 2014). In fact, their continuous release is caused by the ongoing utilization of fossil fuels on a global basis (Wang et al. 1999; Mandalakis et al. 2005). In addition, the present fossil fuel industry expansion poses an increasing risk of accidental spills to the marine environment, where the impact on native organisms has only rarely studied (Jensen et al. 2012).

As a general environmental pathway, PAHs entering the water system can firstly be accumulated in fine-grained sediments and suspended particles; they remobilize later in the seawater and become bioavailable to local organisms (Wetzel and Van Vleet 2004). While higher molecular weight PAHs are relatively immobile because of their large

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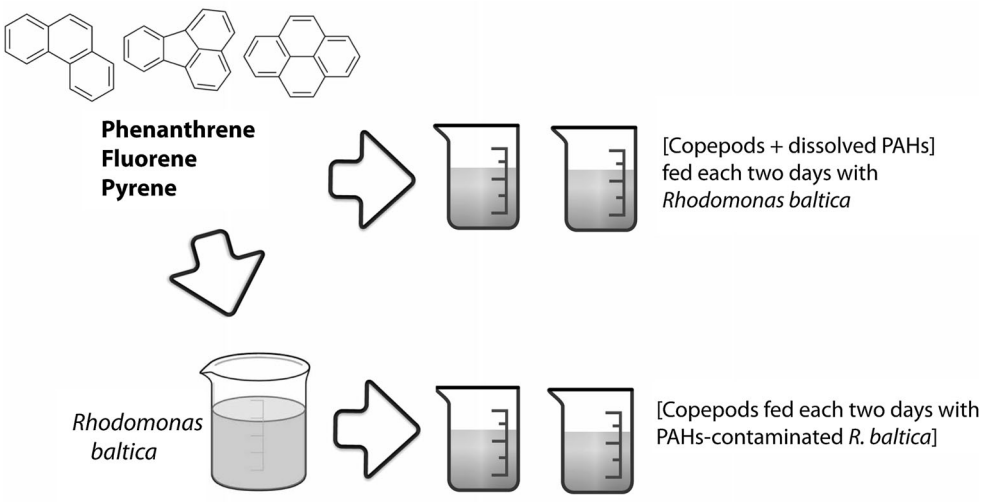

Fig. 1 Experimental set up of the different PAHs exposure treatments, including the generation of contaminated algae to feed the copepod *Pseudodiaptomus marinus* during the experiments

molecular volumes and their extremely low volatility and solubility, the lower molecular weight unsubstituted PAH compounds, containing 2–3 rings such as naphthalenes, fluorenes, phenanthrenes, and anthracenes, provide a significant acute toxicity to some organisms.

Transport and partitioning of PAHs in the environment are determined to a large extent by physicochemical properties such as water solubility, vapor pressure, Henry's law constant, octanol–water partition coefficient (K_{ow}), and organic carbon partition coefficient (K_{oc}). As for the air/water transition, Henry's law constant provides the partition coefficient that expresses the ratio of the concentration of a chemical in air and water at equilibrium and is used as an indicator of its potential to volatilize. In general, PAHs have low water solubilities. As for the air/sediment transition, K_{oc} indicates its potential to bind to organic carbon in soil and sediment, while K_{ow} is used to estimate the potential for an organic chemical to move from water into lipid and is frequently correlated with the bioconcentration/bioaccumulation in aquatic organisms (Barron 1990; Mackay 1982; Hamelink et al. 1994). With regard to this last issue, there is still a gap in the current knowledge about the bioaccumulation of PAHs at lower trophic levels (Meador et al. 1995). The bioaccumulation of PAHs by zooplankton is little understood as yet. For instance, the response of planktonic communities to petroleum hydrocarbons and other POPs have been carried out in the field (Mackie and Adron 1978; Middleditch et al. 1979; Serrazanetti et al. 1991; Kowalewska and Konat 1997, Cailleaud et al. 2007) and at experimental conditions (Skjoldal et al. 1982; Stange and Swackhamer 1994; Sibley et al. 2004; Gerofke et al. 2005 -PCBs and other POPs; Magnusson and Tiselius 2010 -PCBs/PBDE; Zhang et al. 2011-dioxins). Concerning the interaction between PAHs and copepods, Cailleaud et al. (2009) studied the uptake and elimination of some of these compounds in *Eurytemora affinis* under laboratory controlled conditions, while Lotufo (1998) performed PAHs uptake and elimination experiments with marine benthic copepods. In general, the scarce literature on this issue considers that the bioaccumulation of hydrophobic organic compounds in marine zooplankton occurs predominantly by an uptake from water, leaving a marginal role for uptake from diet. The present study tackles these two major contaminant pathways in marine copepods by investigating the ability of the marine copepod *Pseudodiaptomus marinus* to bioaccumulate a mixture of PAHs, namely phenanthrene (PHE), fluoranthene (FLA) and pyrene (PYR), from a water culture medium, either exposed through water (dissolved PAHs) or through contaminated food (algae). Despite the technical challenges inherent to set a marine culture, *P. marinus* was chosen because of its ubiquitous occurrence in several oceans (Pacific, Atlantic, Mediterranean and North Sea) (Sabia et al. 2014, 2015). Here, it has a key position in the food web and acts in the removal and transfer of contaminants within food webs.

Chemicals

A PAHs solution was prepared under laboratory conditions simulating the real PAH levels described for the Seine estuary (Cailleaud et al. 2007), including three different major PAH compounds that were found in the Atlantic Ocean: phenantrene, fluoranthene and pyrene (Sigma-Aldrich). The PAHs were dissolved in acetone that was allowed to evaporate for 30 min in experimental bottles before adding seawater. Deuterated internal standards, phenanthrene-d_{10} and pyrene-d_{10} were purchased from Protochem. HPLC grade solvents (hexane, dichloromethane, methanol and acetonitrile) were purchased from *Dislab* (Lens, France). No significant amount of target

compounds could be detected in procedural blanks. Ultrapure water (Milli-Q) was produced by a Millipore apparatus with 18.2 MΩ/cm resistivity. Merck silica gel 60 (70–230 mesh ASTM) activated at 450 °C was heated at 120 °C for 12 h prior to use. Glassware was washed with detergent (Decon), rinsed with ultrapure water and acetone and was dried at 120 °C prior to use.

Copepod cultivation

Pseudodiaptomus marinus is a typical estuarine coastal copepod, living only in shallow inshore waters, often highly eutrophicated and is reported as herbivorous and detritivorous (Uye and Kasahara 1983). This species is known to live near the bottom during the day (Valbonesi and Harada 1980; Fleminger and Kramer 1988; Liang and Uye 1997), feeding on detritus through the creation of feeding currents (Uye and Kasahara 1983), while at night, it moves along the water column, likely exploiting different food sources (Valbonesi and Harada 1980; Uye and Kasahara 1983). The feeding behavior is quite similar for both adult sexes that exhibit different swimming behaviors (Sabia et al. 2014), but is different between the copepodite and naupliar stages (Uye and Kasahara 1983). *Pseudodiaptomus marinus* is now commonly found in several Pacific and Indian bays (e.g., Grindley and Grice 1969; Fleminger and Kramer 1988; Islam and Tanaka 2009), and recently, its presence has been recorded in the North Sea (Brylinski et al. 2012) as well as in the Mediterranean Sea (Sabia et al. 2014, 2015).

For the purpose of this study, *P. marinus* was cultured in the Marine Station of Wimereux—Laboratory of Oceanology and Geosciences (UMR 8187 LOG), France, where this strain is maintained since 2011. The copepods were grown in non-food limiting conditions in constant and optimal temperature and salinity. The protocol of maintaining the copepods during several generations was slightly modified from those used for the estuarine copepod *Eurytemora affinis* (Souissi et al. 2010, 2015): copepod strains were maintained in 2 L beakers in duplicate and maintained in an incubator at constant temperature of 18 °C and a photoperiod of 12L:12D cycle (Souissi et al. 2015). In this occasion, copepods were fed every two days using mainly *Rhodomonas sp* species, (Fig. 1) but *P. marinus* can feed and develop very well if other microalgae are used (e.g. *Isochrysis galbana, Pavlova lutheri* and *Porphyridium cruentum*). The protocol of feeding copepods in 2 L beakers is detailed in Souissi et al. (2010). The sea water obtained in the Marine Station of Wimereux (see Table 1 in Tlili et al. 2016) was used after filtration and UV treatment to renew the strains every week. When the density becomes high after visual check the population was split into two beakers or transferred to a larger beaker of 5 L. For our experiments we used transparent Nalgene bottles of 10 and 20 L to upscale copepod production. Generally, 4–6 weeks of culture were needed to transfer copepods from small volume (2 L) up to 20 L. Then, copepods can be grown during several weeks in 20 L bottles but if the experiment required higher number of copepods, Plexiglass tanks of 40 L were also used occasionally to produce copepods. In all volumes, copepods were fed excess of microalgae every 2 days. The water of copepod cultures was changed regularly to avoid any accumulation of organic matter and the increase of levels of ammonia that can be toxic to copepods. The individuals used for all the present experimental settings belonged to the late copepodite and adult stages.

Generation of PAH contaminated algae

A commercial *Rhodomonas baltica* strain was cultured at n phase at the Marine Station of Wimereux-France to obtain PAHs free algae. The axenic stock cultures were routinely maintained at two temperatures, 18 and 25 °C, at 12:12 h light and dark (L:D) cycle in a light- and temperature-controlled incubator for more than three dilutions in a semicontinuous culture mode during the log phase of growth in filtered, aged seawater. The strain of *R. baltica* was maintained in the laboratory in 250 ml glass Erlenmeyer flasks and was shaken by hand daily. The culture was kept in an incubator (SANYO model MLR) at 18 °C and at a photoperiod of 12L:12D cycle under a fluorescent light with an intensity of 2500 lx. Batch cultures in 2–6 L flasks were used to grow the microalgae used as copepod food during the exposure experiments. The culture flasks were filled with autoclaved seawater (salinity 33 psu) and enriched with Conway medium. Cultures were aerated with sterile air and incubated in the same condition as the strain *inoculum*. Then, the experimental incubation with PAHs (PHE, FLA and PYR) was conducted by transferring cells during the log-phase into the incubation bottles to ensure subsequent exponential growth. Cell density was maintained by shaking daily to ensure optically favorable conditions. Subsamples for cell counts were fixed using a 5 % formaldehyde solution and stored in the dark at 4 °C until further analysis (Iwasawa et al. 2009). Cell counting was performed in triplicate under an inverted microscope. Cell density was determined using a hemocytometer (Erma, depth 0.1 mm, Tokyo, Japan) under a compound microscope (Olympus, IMT-2, Tokyo, Japan) at 100× magnifications using the method that was previously described by Guillard and Sieracki (2005). PAHs were spiked to the media at the start of the incubation period and algae were fed each 2 days with Conway medium and counted each day in triplicate (Fig. 1). Water subsamples were collected from each beaker immediately before the addition of *R. baltica* to analyze both dissolved organic carbon and the initial

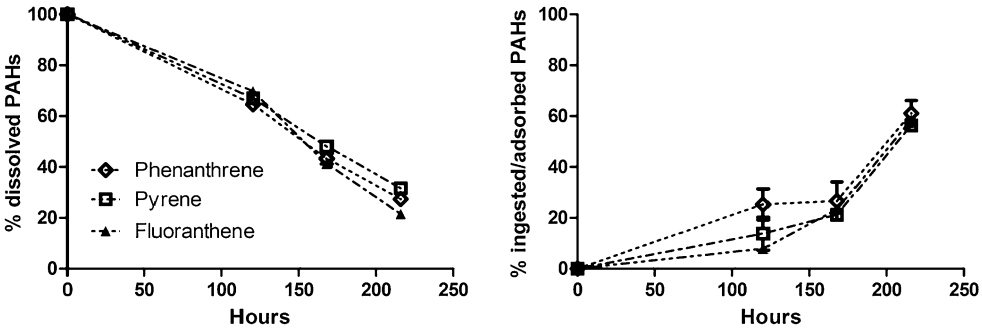

Fig. 2 The percentage of dissolved PAHs, percentage of PAHs taken up by cells in the *Rhodomonas baltica* culture system in relation to incubation time

(To) PAHs concentration. Subsequently, the water and algae subsamples were sampled during the exponential phase, from the 5th to the 9th day of the culture. From each bottle, an average of 750 mL of water containing the algae were filtered through a precombusted GF/F filter (cut off size 0.7 um). Alternatively, algae (filters) and filtered medium were spiked with the PAHs surrogate standards, extracted (ASE and L/L extraction) and analyzed for PAHs by GC/MS.

After Chan et al. (2006) the PAH percentages in the medium and cells were calculated as follows:

$$\% \text{ in medium} = (\text{amount remaining in the medium/amount of PAHs added}) \times 100\%$$

$$\% \text{ in cells} = (\text{amount accumulated in the cells/amount of PAHs added}) \times 100\ \%$$

PAHs contaminated algae used as food were sampled and filtered at the end of the log-phase, when the PAHs adsorption to the algae was quantitatively assured and well defined (Fig. 2).

Experimental setting for copepod exposure

These set of experiments were designed to study the PAHs bioaccumulation by copepods, either through the media or contaminated algal cells during the normal exponential stage of a culture (Fig. 1). Experiments were performed as static exposures with the organisms kept in 5 L beakers. Copepods were stored at the optimal species temperature at dimmed light and aeration of the volume was provided through gentle air bubbling. The animals where fed twice each 2 days (three times during the exposure) and the exposure phase lasted for 5 days (120 h). Then, duplicates of incubation bottles were prepared using 5 L glass bottles and three conditions were set-up: control, PAH exposure through the medium and PAH exposure through the administration of contaminated food (algae). Copepods were fed with either pollutant free axenic algae ("dissolved PAHs" condition) or PAHs contaminated algae ("PAHs contaminated food" condition). Water and copepods subsamples were collected from each beaker immediately before the addition of the animals to investigate both DOC and the initial (T_o) PAHs concentration. Subsequently, medium and copepods were sampled at the end of the exposure period (T_f, 5 days). Copepods were filtered through a precombusted GF/F filter (cutoff size 0.7 μm), after that they were spiked with PAHs subrogate standards, ASE extracted (Accelerated Solvent Extraction) and analyzed for PAHs by GC/MS.

Extraction procedures

After the experimental phase, each sample was kept in clean glass bottles, capped with a Teflon-lined lid. Samples were rapidly filtered using 0.7 μm Whatman GF/C glass microfiber filters and both particulate and dissolved phases were kept. While the filtered water was extracted using liquid–liquid extraction (LLE) technique, the algae retained in the filters were extracted using accelerated solvent extraction (ASE 200, Dionex Corp., USA). The applications of LLE in water and other liquid matrixes have been widely accepted in standard methods for various classes of organic contaminants such as PAHs, pesticides, and PCBs analysis (Barceló 1993; USEPA 2007). Regarding ASE, also known as pressurized liquid extraction (PLE), it is well known that remains an efficient tool for different solid sample extraction which has received an increasing interest because of its efficiency, facility to implement, solvent and time saving. In addition, ASE maintains constant extraction conditions and gives a good repeatability by its automation (Hubert et al. 2001; Schantz 2006).

Water extraction The samples were first spiked with internal standard (Phe-d_{10} and Pyr-d_{10}) and then extracted using LLE extraction technique. Each water subsample (1 L) was extracted with 80 mL of dichloromethane, repeated three times. The extracts were then pooled and dried using Na_2SO_4. Finally, the extract was concentrated

using a rotary evaporator followed by a slight stream of nitrogen before GC–MS analysis.

Algae and copepods extraction each biomass subsample was spiked with deuterated internal standards (Phe-d_{10}, Pyr-d_{10}). After a delay of equilibration, the subsamples were extracted using ASE. The extraction operational conditions were: heated: 5 min; static solvent extraction time: 5 min ($n = 2$) at 100 °C; purged 3 min, 115 % flushed, 1500 psi and dichloromethane was used as extraction solvent. High purity nitrogen was employed as the purge gas. Extraction procedure afforded a total volume of extract of 40 mL. The extracts were concentrated, the solvent was exchanged to hexane, and then purified and fractioned by liquid chromatography on a silica column to eliminate organic interferences. The elution was performed using hexane, and then with the mixture of hexane/dichloromethane (3/1 v/v and 1/1 v/v). The sample was concentrated using a rotary evaporator followed by a slight stream of high purity nitrogen before GC–MS analysis.

GC–MS analysis

The extracts were analyzed using a Varian 3900 gas chromatograph (GC), equipped with a deactivated fused silica guard column (5 m, 0.53 mm i.d.) and a fused silica capillary Phenomenex XLB (60 m length, 0.25 mm i.d., 0.25 µm film thickness), coupled with a Varian Ion Trap Saturn 2000 mass spectrometer (MS). The carrier gas was helium held at a constant flow rate of 1 mL/min. Samples were injected in the splitless mode at 280 °C and the injector was purged with helium after 1 min. Each group of organic compounds was analyzed separately. The transfer line and the ion trap were, respectively, held at 260 and 220 °C. Each contaminant was identified based on the retention time and the mass spectrum from chromatogram of standard solutions acquired in full scan mode. Quantification was then performed in the single ion storage (SIS) mode for better selectivity. Response factors were determined relative to the deuterated internal standards response and to standard mixtures. Deuterated standards were chosen to better fit to the properties of each group of contaminants. Quality assurance procedural blanks for water, filters, glass materials and solvents were conducted throughout all the experiment. The effectiveness of the different analytical procedures was evaluated by analyzing NIST Reference Material (SRM 1944 and 2978 for PAHs). The mean recoveries for PAHs compared with the certified concentrations were in the range of 90–110 %. The limits of detection for PHE, FLA, and PYR were 0.86, 1.23 and 1.12 µg/L, respectively; whereas the corresponding limits of quantification were 2.87, 4.08 and 3.72 µg/L, respectively.

Equilibrium partitioning and Bioaccumulation Factors (BAFs)

The equilibrium partitioning (EqP) theory applied to PAHs assumes the equilibrium of their concentrations between the dissolved and particulate phases (Weber and Gould 1966; Hamelink et al. 1971; Burgess et al. 2003). This concept can be expressed as follows:

$$K_p = C_p/C_d, \quad (1)$$

where K_p is the partition coefficient (L/kg) of a PAH between particulate (C_p) and dissolved phases. In this case, PAHs will preferably adsorb to the organic matter coating the particles; then, the partition coefficient can be often normalized by the particulate organic carbon concentration (f_{oc}, kg organic carbon/kg sediment) resulting in the organic carbon normalized partition coefficient K_{oc} (in L/kg organic carbon), where:

$$K_{oc} = K_p/f_{oc}. \quad (2)$$

To efficiently predict the relationship between environmental concentrations and expected tissue PAH's burden, Bioconcentration/bioaccumulation factors are used commonly (Gobas 2000; Mackay and Fraser 2000). For the present study, bioconcentration factor (BCF) was defined as:

$$BCF = C_t/C_w, \quad (3)$$

where C_t was the PAHs concentration in the copepods and C_w was the dissolved concentration, while bioaccumulation factor (BAF) was defined as:

$$BAF = C_t/C_a, \quad (4)$$

where C_t was the concentration in the copepods (biomass) and C_a was the concentration in the algae (food). All concentrations were calculated as dry weight.

Results and discussion

Considering the PAHs water exposure experiments, the BCF (120 h) of phenanthrene in *P. marinus* was 6362 (SD = 3549). For fluoranthene, the BCF was 18,774 (SD = 6958) while for pyrene 53,022 (SD = 14,229), Table 1. There is a significant correlation between BCFs and K_{ow} values ($p < 0.01$; slope = 1.50), Fig. 3. In the case of PAH's food exposure experiments, the BAF (120 h) for phenanthrene was 7234 (SD = 1852), 13,439 (SD = 3234) for fluoranthene and finally 89,854 (SD = 35,920) for pyrene, Table 1. In this case, there is also a significant correlation between BAFs and K_{ow} values ($p < 0.01$; slope = 1.75), Fig. 3. This is consistent with other studies that have reported a near 1:1 relationship for

Table 1 Bioaccumulation (BAFs) and bioconcentration (BCFs) factors at 120 h for *Pseudodiaptomus marinus* exposed to phenanthrene, fluoranthene and pyrene and the respective log values

	log K_{ow}[a]	$BAF_{120\ h}$	log BAF	log BAF/log K_{ow}	$BCF_{120\ h}$	log BCF	log BCF/log K_{ow}
Phenanthrene	4.57	7234	3.86	0.84	6362	3.80	0.83
Fluoranthene	4.9	13,439	4.13	0.84	18,774	4.27	0.87
Pyrene	5.18	89,854	4.95	0.95	53,022	4.72	0.91

The log octanol–water partitioning coefficient (log K_{ow}) for PAHs is given as well as the relative proportion between log BAF/BCF (empirical) and log K_{ow}. BAF refers to the bioaccumulation factor between copepods and food (algae) while BCF refers to the relationship of copepods and dissolved PAHs

Chiou et al. (1998)

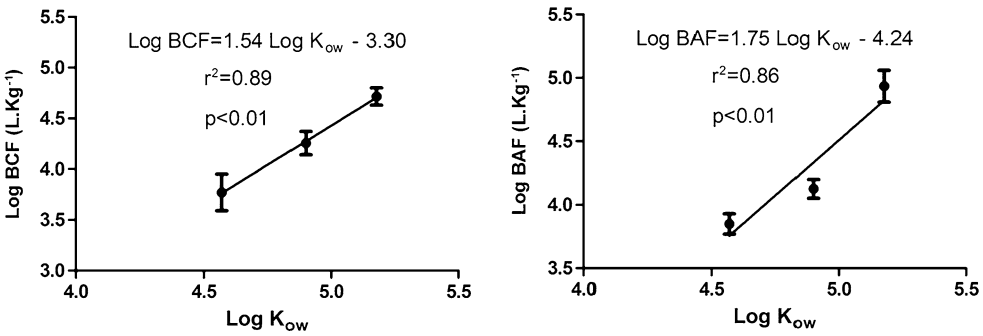

Fig. 3 Bioconcentration factors (BCF) for treatments with *Pseudodiptomus marinus* on its own and bioaccumulation factor (BAF) for treatment made with *P. marinus* in the presence of *Rhodomonas baltica* for each PAH related to the octanol–water partitioning coefficient (K_{ow})

BCF/BAF-K_{ow} in zooplankton. For example, Berrojalbiz et al. (2009) performed 40 h experiments measuring PAHs concentrations in samples of *Paracartia grani* and found a linear correlation between BCF and K_{ow} for these compounds (slope = 1.52 and 1.27). In addition, Fisk measured HCH and PCB concentrations in samples of the Arctic marine copepod *Calanus hyperboreus* (Fisk et al. 2001) and described a significant linear correlation between BAF and K_{ow} (slope = 0.72) while Hoekstra et al. (2002) found a similar relationship between BAF and K_{ow} of different organochlorine contaminants (slope = 1.04) in *Calanus hyperboreus.*

BAF expressed bioaccumulation is only attributable to food uptake. Hence, it can be assumed that the active feeding process at 120 h achieves similar PAHs bioaccumulation levels than those predicted by the literature equilibrium partitioning and have no appreciable differences to the BCFs (*t* test, $p < 0.05$). As pointed out by Berrojalbiz et al. (2009), the lack of significantly higher BAF than BCF values does not mean that PAH could not be transferred from food to the organism, but that depuration and other elimination processes can be fast enough in a way that the final PAH levels in the organism are dominated by water-zooplankton partitioning and/or metabolism. Then, bioavailability, degradation and excretion of PAHs should be further considered and discussed.

Bioavailability has been emphasized as a main factor leading to lower BAF values compared to organic compounds' theoretical K_{ow} (Landrum 1989; van Hattum et al. 1998). In fact, hydrophobic compounds tend to adsorb to surfaces such as the walls of experimental beakers, possibly leading to a reduction in the bioavailable fraction of the compound. In the present experiment, the initial concentrations of phenanthrene, fluoranthene, pyrene and the dissolved organic carbon (DOC) were similar in the experimental setup, discarding these factors as reducers of PAHs bioavailability. Furthermore, bioavailability could also be lowered by the presence of particles since high K_{ow} PAHs readily bind to particles (Means et al. 1980; Jensen et al. 2012). In our experimental setting, this factor has been controlled by using the same filtered seawater (filtered in series mode, starting with sand filters and ending with 1 μm filters). Then, the order of bioaccumulation found (pyrene > fluoranthene > phenanthrene) over the 120 h exposure period considering both exposure ways (food and water, Table 1) is, therefore, not attributable to potential differences in the bioavailability of these compounds.

Active uptake via food vs. water exposure

In this study, results showed similar copepod bioaccumulation yields for PAHs food exposure (contaminated algae) than for PAHs from water (dissolved PAHs, Fig. 4).

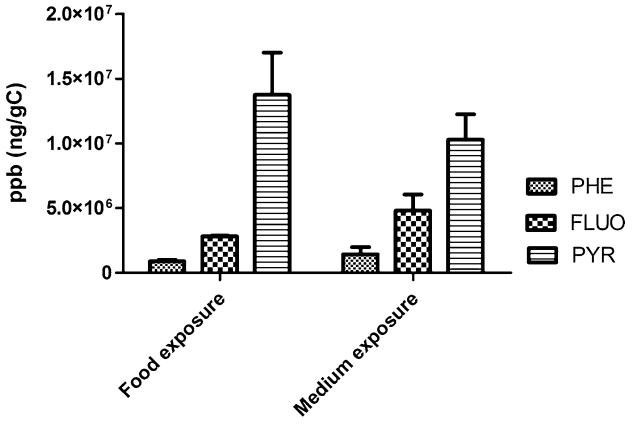

Fig. 4 PAHs loads of *Pseudodiaptomus marinus* after 120 h of either water vs. food exposure. *Error bars* indicate SE

Despite different experimental procedures, were similar results obtained in previous research. For instance, Magnusson et al. (2007) examined the uptake of PCB in *Calanus finmarchicus*, obtaining log bioaccumulation factors (BAFs) that exceeded their corresponding log K_{ow} values for "fed" vs. "overwintering" copepods. In contrast, the BAF determined for active but unfed copepods was lower than their corresponding log K_{ow}, thus suggesting that the food uptake process favors the uptake of contaminants here. In a similar study of various PCB congeners, Magnusson and Tiselius (2010) observed a higher bioaccumulation in fed organisms relative to passive partitioning in *Acartia clausi*; however, this can be valid only for extremely recalcitrant compound like PCBs. In the case of PAHs –which are able to be at least partially metabolized—Berrojalbiz et al. (2009) observed no significant differences in experimental BAF values between food uptake and water treated copepods exposed to various PAHs of the same concentrations. Further, a study on *Mysis relicta* concluded that "feeding" lowered the accumulation of Benz[a]pyrene (a PAH) but increased hexachlorobiphenyl accumulation (a PCB), a compound which this species cannot metabolize (Landrum et al. 1992). As noted by Jensen et al. (2012), regarding bioaccumulation copepods may not behave like mysids. However, these studies outline the role of dietary exposure as a pathway which favors the accumulation of recalcitrant organic compounds. Hence, the "feeding mode" plus the "exposure pathway" and the suitability of the compound to undergo metabolization shall not be undervalued when comparing BCF values.

In summary, this study shows that zooplankton plays a critical role for the cycling of PAHs and its fate: if PAHs are diffusively accumulated, PAHs would stay in the water column following *P. marinus* cycle while if ingested they will end up at as fecal pellets commonly accumulating at the sea bottom in shallow waters. This process will ultimately depend on the trophic status of zooplankton, an issue that will require further research in the future.

Concluding remarks

The present outcome reveals new baseline information regarding PAHs bioaccumulation for *P. marinus*—including empirical BAF and BCF values for the selected compounds. When examined in context with other studies, there is evidence that active and feeding *P. marinus* bioaccumulate PAHs either through contaminated food or from the medium. PAHs that are accumulated by dietary intake can undergo metabolization. The recorded BAF and BCFs would have useful implications for future environmental risk assessments involving *P. marinus,* and represent new baseline information able to support future analyses on the fate and behavior of PAHs inputs, crude oil spills or accidental releases into the ocean, particularly the North Sea and/or Atlantic and Pacific waters where this species is endemic.

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