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Uptake and Elimination of ^{14}C-Phenanthrene by the Blue Mussel *Mytilus edulis* L. at Different Algal Concentrations

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Dense mussel beds have an important ecological role in the removal and deposition of suspended particulate organic matter (Kautsky and Evans 1987; Dame and Dankers 1988). A high water processing potential, together with an effective retention of particulate organic matter, implies that filter-feeding mussels also may have an important role in the scavenging of hydrophobic organic contaminants (HOC) from the water column (Landrum *et al.* 1991). Accumulation of HOC in filter-feeding mussels is, as for other aquatic organisms, dependent on the concentration, bioavailability, and physical-chemical properties of the contaminant (Farrington 1991). However, since filter-feeders pump water to obtain food particles, regulation of pumping, filtration and ingestion rates in response to changes in particle concentration (see review by Winter 1978), is likely to influence contaminant accumulation as well. Thus, particulate organic matter may affect HOC accumulation in mussels by modifying bioavailability and route of exposure due to particle sorption (Schrap and Opperhuizen 1990), and/or by influencing physiological processes involved in contaminant accumulation.

This flow-through experiment was conducted to evaluate the uptake and depuration of ^{14}C-phenanthrene in filter-feeding blue mussels, *Mytilus edulis*, at three different levels of particulate organic carbon (POC). Our aims were to: (1) test if phenanthrene uptake and depuration in mussels is particle concentration dependent and (2) assess the relative contribution of freely-dissolved and particle-associated phenanthrene to the total uptake observed in the mussels.

MATERIALS AND METHODS

Blue mussels, *Mytilus edulis* L, were collected in October 1993 at a depth of 5 m in the vicinity of the Askö Field Station (S. Stockholm archipelago, northern Baltic proper, Sweden). Mussels with a shell length between 20 and 25 mm were used in this study, corresponding to an age of 6 to 8 yr (Kautsky 1982). Prior to the start of the experiment mussels were maintained in flow-through aquaria for 1 wk and fed with an unicellular green algae, *Chlamydomonas* sp.

The algae were cultured in 1.5 L transparent polythene bags in sterile filtered (0.22 µm, Millipore) natural brackish water with 1 mL L^{-1} algae culture medium B-1, (BioProcess, Denmark). Algae concentrations were analysed with a particle

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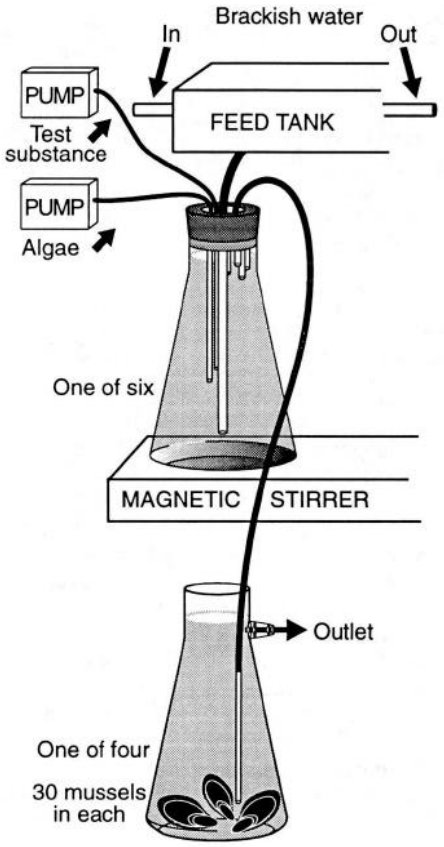

Figure 1. The design of the flow-through system.

counter (Elzone 280PC, Particle Data, USA) equipped with a 48 µm orifice. The average cell dry weight, 179 pg, was determined by vacuum filtering a set number of cells on pre-ashed GF/F-filters (0.7µm, Whatman) and drying to constant weight at 60°C. The carbon content, 100 pg C $cell^{-1}$, was measured with a carbon analyser (CHN-900, Leco, USA).

Phenanthrene, 9-^{14}C-labelled, with a specific activity of 8.3 mCi mM^{-1} and a purity of >98 %, was obtained from Sigma (St. Louis, USA). A 20 µg L^{-1} stock solution was prepared by gently mixing of phenanthrene and sterile water on a magnetic stirrer for 24 hr. The phenanthrene concentration in the stock solution, measured by liquid scintillation counting (LSC), was 18 µg L^{-1}, i.e., indicating an expected sorption to the glass walls. The stock solution was kept in the dark during the experiment and weekly LSC analyses confirmed a stable phenanthrene concentration.

The influence of particle concentration on the uptake and depuration of ^{14}C-labelled phenanthrene by the mussels was studied in a flow-through aquaria system (Figure 1). The system was set up to achieve an hierarchical experimental design with three different POC concentrations. Each treatment included two replicate mixing-chambers and each mixing-chamber four replicate exposure aquaria. The

system was operated by hydrostatic pressure except for two multi-channel peristaltic pumps (Alitea, Stockholm, Sweden) used for dosing of phenanthrene and algae, and magnetic stirrers in the mixing-chambers. The feed tank received sand filtered natural brackish water with an average POC concentration of 0.050 mg/L, pumped from a depth of 16 m outside the field laboratory. The brackish water flow rate to each mixing chamber was 5.72 ±0.04 L hr^{-1}, corresponding to a total water turnover of 17 exposure aquaria volumes every 24 hr. The background treatment (B) received brackish water only, whereas the brackish water in the low (L) and high (H) treatments were additionally enriched with POC in the form of algae. The algae were administered at a rate of 8.2 and 57.2 mL hr^{-1}, in the (L) and (H) POC treatment, respectively (Table 1). All treatments received phenanthrene from the stock solution at a rate of 1.5 mL hr^{-1} corresponding to a nominal concentration (bound and freely-dissolved) of 5 ng L^{-1} in the exposure aquaria.

Thirty random mussels were placed in each of 24 exposure aquaria in the flow-through system and exposed as described above for a period of 20 d. Thereafter, the addition of phenanthrene was ended and the mussels were kept in flowing uncontaminated water with the same water/algae ratios for a period of 14 d. During the experimental period the water temperature decreased steadily from 12°C to 10°C, and the salinity remained constant at 6.5‰.

Water samples were taken for ^{14}C-phenanthrene and particle analyses from each POC treatment on day 2, 6 and 13. Water samples of 500 mL were extracted with 2x50 mL hexane, concentrated to approximately 5 mL on a rotary evaporator, and counted in Lumagel-safe (Lumac, Olen, Belgium). Particle concentrations of the inlet and outlet waters of the exposure aquaria were analysed to assess the particle retention within the system.

Mussels were sampled for ^{14}C-phenanthrene analyses, one random individual from each exposure aquaria, on day 1, 2, 6, 13 and 20 during the exposure period and day 2, 6 and 14 during the depuration period. Mussels were also sampled for condition analyses prior to the start (20 random mussels) and at the termination of the experiment (2 random mussels from each exposure aquaria). Mussel condition was determined as a Body Condition Index (BCI); 100 x tissue dry weight (mg)/shell length (mm). All mussels were placed in capped glass vials and frozen at -20°C immediate after sampling. Before sample preparation, mussels were allowed to thaw for 15 min, the shell length was measured, and the tissue wet weight was determined after the soft tissue had been removed and blotted dry on soft paper. Tissue dry weight was obtained by drying to constant weight at 60°C. Wet mussel tissue for ^{14}C-phenanthrene analysis was placed in glass scintillation vials and

Table 1. *Mytilus edulis*. The effect of particle concentration on ^{14}C-phenanthrene wet tissue accumulation, mean (n = 8) [SE range], and first order elimination rate constants, k_a, mean (n = 8) [r^2] with calculated biological half-lives, $t_{1/2}$.

Treatment	Cells added (cells/L 10^6)	POC (mg/L)	Tissue conc. day 20 (ng/g)	(1%)	$t_{1/2}$ (d)
High POC (H)	1.00	0.150	2.1 [2.33-1.85]	0.047 [0.90]	14.7
Low POC (L)	0.14	0.064	0.85 [0.92-0.78]	0.050 [0.88]	13.9
Background (B)	0.00	0.050	1.3 [1.54-1.14]	0.051 [0.92]	13.6

*Particulate organic carbon

treated with Lumasolve (Lumac) 1 mL /100 mg at 50°C for 4 hr for tissue digestion. The samples were bleached to reduce color quenching by adding 0.2 mL of isopropanol followed by 0.2 mL of hydrogen peroxide per 100 mg of tissue, and counted in Hionic fluor (Packard, Groningen, The Netherlands).

All samples were counted on a liquid scintillation counter (Wallac 1214, Turku, Finland) after at least a 24 hr equilibration period in the dark. The samples were corrected for quenching by the external standard ratio method. For the mussel samples, a series of prepared tissue standards with internal ^{14}C-pellets (Wallac), was used to establish a channel ratio versus counting efficiency calibration curve which was used to correct the counts of the experimental samples.

Data was analysed by nested ANOVAs. Heterogeneity of variances was checked with Cochran's test and when needed data was log10 transformed. Aposteriori SNK tests were used in cases were significant main effects included more than two levels. The accumulation of phenanthrene from water and food was described as:

$$dC_a/dt = k_u C_w + F\ AE\ C_f - K_d C_a \quad (1)$$

Where:

C_a = tissue concentration of phenanthrene (ng g^{-1})
k_u = uptake rate constant for uptake from water (L g^{-1} d^{-1})
C_w = concentration of phenanthrene in water (ng L^{-1}),
F = feeding rate (mg C g^{-1} d^{-1})
AE = assimilation efficiency for phenanthrene uptake from food
C_f = concentration of phenanthrene in the food (ng mg-^{1}C)
K_d = depuration rate constant (d^{-1})

Thus, the uptake from food is:

$$F\ AE\ C_f \quad (2)$$

and elimination is described by:

$$d\ C_a/dt = k_d C_a \quad (3)$$

when uptake from food and water is zero.

Values for k_d were obtained by linear regression of 1n transformed tissue concentration data, and biological half-lives where calculate as: 1ln 0.5 k_d^{-1}.

RESULTS AND DISCUSSION

A significantly higher (P=0.004) phenanthrene tissue concentration was found in mussels exposed to phenanthrene at the high POC concentration (H), compared with mussels exposed at the low (L) and background (B) POC concentrations (Figure 2).The phenanthrene tissue concentration was apparently somewhat higher in the B treatment compared with the L treatment, however, this difference was not statistically significant (SNK aposteriori, p>0.05). The mussel tissue concentration measured at day 20, was approximately two times higher in the H mussels, compared with L and B mussels (Table 1). The results from our study are similar to the results obtained by Widdows et al. (1982), who found a significantly higher (1.5-2 times) tissue concentration of aromatic hydrocarbons in mussels simultaneously exposed to the water-accommodated fraction of North Sea oil and

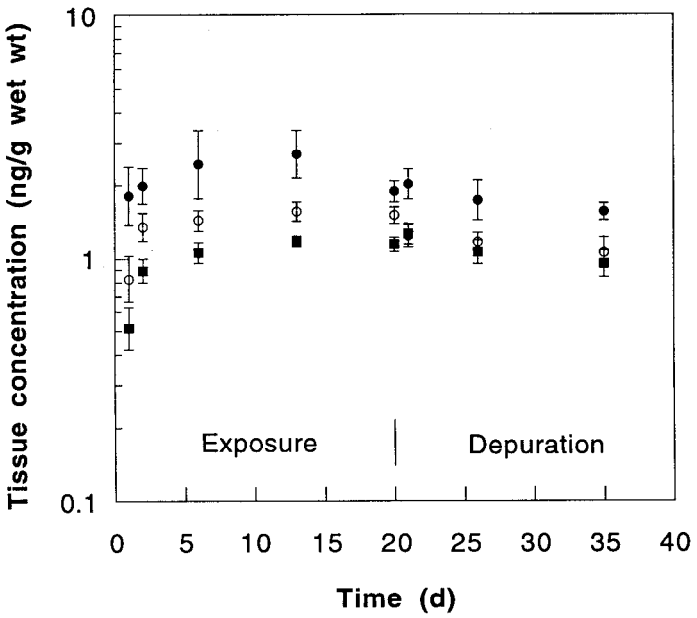

Figure 2. *Mytilus edulis.* ^{14}C-phenanthrene tissue concentration, mean (n=8) ±SE, at high (•), low (■) and brackish water background (o) POC concentration.

particulate algal cells, compared with mussels exposed to hydrocarbons without simultaneous food addition.

The initial uptake of phenanthrene in the mussels was rapid with an apparent equilibrium established within the exposure period. Studies by Widdows *et al.* (1982), Widdows *et al.* (1983), Broman and Ganning (1986), Pruell *et al.* (1986) and Pruell *et al.* (1987) also demonstrate this initial rapid uptake kinetics of hydrocarbons in blue mussels.

In our study a slight decrease in phenanthrene tissue concentration was found between days 13 and 20, possibly due to induction of PAH metabolising enzyme systems. Such a decline in mussel tissue concentration after a period of exposure to petroleum hydrocarbons has also been observed by Broman and Ganning (1986) and Pruell et *al.* (1987). The low phenanthrene concentration in our study, chosen to be close to realistic conditions, caused analytical problems for the water samples taken. The ^{14}C-phenanthrene concentration were close to the theoretical detection limit of 2 ng L^{-1}, and due to this analytical uncertainty, no bioaccumulation factors were estimated. However, based on the relationship between log BCF and log k_{ow} observed by Pruell *et al.* (1986), the BCF for phenanthrene is approximately 880, which is in agreement with the phenanthrene accumulation measured in our mussels.

No differences in phenanthrene elimination due to particle concentration could be detected during the depuration period (Figure 2 and Table 1). The ^{14}C-phenanthrene equivalent elimination was apparently of first order kinetics (equation 3). The depuration rate constants (slopes of the regression lines) for phenanthrene were 0.047 d^{-1}, 0.050 d^{-1} and 0.051 d^{-1}, for the H, L and B POC concentration, respectively. This corresponds to an average depuration half-life of 14.1 d (Table 1). This half-life is within the range of depuration half-lives, 11.9 to 29.9 d, for some PAHs (phenanthrene not included) in blue mussels, reported by Pruell et al. (1986).

Since the depuration rate constant (K_d) was not affected by POC concentration, the higher phenanthrene accumulation observed at the H treatment is attributable to a higher uptake rate. This higher uptake may be due to (1) an increased gastrointestinal uptake of particle-associated phenanthrene, (2) a higher uptake of freely-dissolved phenanthrene, or both.

Particle concentration analyses revealed that the mussels retained close to 100% of particulate matter added. Only a small fraction was found in the effluent water from the exposure aquaria and no significant differences in the effluent particle concentration could be detected between treatments. Body condition indices (BCI) decreased with time at all POC concentrations. The H and L mussels showed the smallest reduction in BCI and had significantly higher BCIs at the end of the study, compared to the B treatment (P=0.03). The POC concentration at the H treatment is approximately equal to the maintenance energy demand of *M. edulis* (Gilek et al. 1992). Hence, the mussels in the two other treatments experienced moderate starvation.

Is it then possible that differences in gastrointestinal uptake of particle-associated phenanthrene can explain the differences in phenanthrene accumulation observed? Assuming 100% retention of POC, the feeding rates of the mussels were 0.53, 0.26 and 0.22 mg C $g^{-1} d^{-1}$, in the H, L and B treatment, respectively, expressed on a wet weight basis. These feeding rates were used to assess the maximum theoretical contribution of particle-associated phenanthrene (equation 2) to the total tissue burdens by estimating the uptake of contaminants from the food (Table 2). The carbon normalised phenanthrene concentration in the particulate food (C_f) was calculated as $K_{oc} C_w$, where phenanthrene organic carbon normalised algae/water partitioning coefficients (K_{oc}) were derived from a log K_{oc}, log POC relationship (Björk and Gilek unpublished data, Table 2). Due to the problems with the water analysis, the nominal concentration of phenanthrene added to the system (5 ng/L) was used as C_w instead of the lower values indicated by the analysis. This was done to be sure not to underestimate the importance of food as a source of

Table 2. *Mytilus edulis*. The relative contribution of phenanthrene (phe) from food to the total uptake day 1. Food ration is given as the amount of particulate carbon added per day and g wet mussel tissue. C_f is the concentration of phenanthrene in the POC fraction.

Treatment	Food ration (mg/d/g)	log K_{oc}[1] (L/kg)	C_f (ng/mg)	Food uptake (ng phe/d/g)	Tissue conc. (ng phe/g)	% of total
H	0.53	4.56	0.18	0.077	2.25	3.4
L	0.26	4.76	0.29	0.060	0.58	10.4
B	0.22	4.82	0.33	0.058	0.97	6.0

[1] K_{oc} from the relationship: log K_{oc} = 5.74 - 0.54 log POC (Björk and Gilek unpublished data).

phenanthrene uptake. Finally, the assimilation efficiency (AE) of phenanthrene from food was set to 80% (Bruner *et al.* 1994). The resulting uptake rate constant for phenanthrene uptake from food was somewhat higher at the H POC treatment, compared with the L and B treatment (Table 2). However, the maximum theoretical contribution of the gastrointestinal uptake day 1 was only 3-10% of the total uptake, and lowest in the H treatment, assuming that the uptake of freely dissolved phenanthrene from water ($k_u C_w$) and the depuration ($K_d C_a$) was not altered at the different POC concentrations used (equation 1).

Thus, our calculation indicates that the higher phenanthrene accumulation observed in H mussels is not due to a higher uptake of particle-associated phenanthrene, but rather due to an increased uptake of freely-dissolved phenanthrene from water. Differences in the amount of particle-associated phenanthrene between the POC concentrations used in this study is expected to be quite low (Brownawell 1986). This was also confirmed in an equilibrium partitioning study where the fraction of phenanthrene sorbed to the algae *Chlamydomonas* sp. at POC concentrations of 0.52 and 0.13 mg L^{-1} was 1.0% and 0.6% respectively (Björk and Gilek unpublished data). Such a small expected difference in the concentration of freely-dissolved phenanthrene is not likely to significantly affect the gill uptake (Schrap and Opperhuizen 1990).

However, differences in the concentration of particulate organic matter in the water does not only influence phenanthrene partitioning, but also the physiology of the mussels. Mussels primarily pump water to obtain particulate food, not oxygen, and the particle concentration in the ambient water greatly influence the pumping rate of the mussels (Foster-Smith 1975; Widdows *et al.* 1979; Riisgård and Randløv 1981; Riisgård 1991). An apparent threshold for filtration has been observed at low particle concentrations and slightly above this threshold a maximum filtration rate is obtained (Riisgård and Randløv 1981). In this study the POC concentration in the H treatment was in the range where maximum pumping rates of the mussels were to be expected, and well below the level where pseudo-faeces production occur, whereas the lower POC concentrations (L and B) were close to the filtration threshold. Hence, particle dependent differences in pumping rate, i.e., the functional response, alters the exposure to freely dissolved phenanthrene, and is thus the most likely explanation to the significantly higher phenanthrene uptake observed in blue mussels at the high POC concentration.

The results of this study show that particle concentration dependent processes such as filtration and ingestion must be considered when assessing contaminant uptake in filter-feeding bivalves. In order to more accurately predict bioaccumulation in mussels, bioenergetic models incorporating functional response should be used instead of simple equilibrium partitioning models.

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