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ROLE OF RESPIRATION IN THE ACCUMULATION OF ORGANIC XENOBIOTICS BY THE AMPHIPOD *DIPOREIA* sp.

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Abstract—Accumulation of hydrophobic organic xenobiotics is thought to occur by passive diffusion across the respiratory membrane of aquatic organisms. This route has been confirmed with fish. However, aquatic invertebrates tend to remove organic xenobiotics from water much more efficiently than oxygen, based on the relative uptake clearances. Uptake clearance is the rate coefficient that describes the volume of water stripped of analyte per g of organism per h. For the amphipod *Diporeia* sp., formerly classified as *Pontoporeia hoyi*, ratios of the contaminant uptake clearances to oxygen clearance were essentially constant at 3.9 ± 0.4 (X + SE) for benzo[*a*]pyrene (BaP), 3.8 ± 0.3 for hexachlorobiphenyl (HCBP), and 4.2 ± 0.6 for phenanthrene (Phe). Therefore, based on the membrane transport efficiencies of nonpolar xenobiotics (60–80%) and oxygen (approximately 63%) in fish, and the uptake clearance for organic xenobiotics by *Diporeia* sp., either xenobiotics are accumulated through routes other than across the respiratory membrane or the accumulation efficiency of oxygen from water is much lower in amphipods than it is in fish. The variability in the uptake clearance for both BaP and HCBP was best described by regressions with the surface area-to-volume ratio, whereas the uptake clearance for the more hydrophilic Phe was best described by a total surface area relationship.

Keywords—Uptake kinetics Hexachlorobiphenyl PAH *Diporeia* sp. Respiration

INTRODUCTION

Accumulation of hydrophobic organic xenobiotics by aquatic organisms is thought to occur through passive diffusion across the respiratory membrane, and studies with fish have confirmed this hypothesis [1–8]. In fish, the accumulation efficiency for compounds with a log octanol/water partition coefficient (log K_{ow}) of 3 to 6.5 was approximately the same as the accumulation efficiency for oxygen and was directly associated with ventilation rate [4]. For aquatic invertebrates, we initially assumed that the rate of contaminant accumulation would be directly related to the rate of oxygen accumulation. However, in recent work with lower organisms, the uptake clearance for organic xenobiotics relative to oxygen ranged from 5% for *Mysis relicta* [9] to 200 to 300% for *Stylodrilus heringianus* [10] for compounds with log K_{ow} from 4 to 6.5 and assuming the oxygen consumption values reported in the literature. (Note: Uptake clearance is the rate coefficient that describes the volume of water stripped of analyte per gram of organism per hour and can be used for common analytes such as oxygen or xenobiotics.)

Because of the large differences in the invertebrates' relative accumulation of contaminant compared with literature values for oxygen consumption, we simultaneously measured the respiration rate and contaminant accumulation rate for the amphipod *Diporeia* sp., formerly classified as *Pontoporeia hoyi* [11]. The model compounds for this study included two polycyclic aromatic hydrocarbon (PAH) congeners and one polychlorinated biphenyl (PCB) congener. In addition to examining the relative accumulation of the hydrophobic contaminants, the potential effects of the carrier and exposure to simple two-component contaminant mixtures were examined.

METHODS AND MATERIALS

Animal collection

Diporeia were collected from Lake Michigan approximately three miles west of Grand Haven, Michigan, at a water depth of 24 to 29 m by PONAR grab. The animals were screened from the sediment, placed in lake water, and transported on ice. At the laboratory, the animals were placed in

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aquaria with 5 cm of lake sediment and 10 cm of lake water at 4°C under red darkroom lights [12].

Radioactive materials

Both ^{14}C-phenanthrene (Phe) (specific activity 21.5 mCi/mmol) and ^{14}C-2,4,5,2′,4′,5′-hexachlorobiphenyl (HCBP) (specific activity 13.09 mCi/mmol) were obtained from Sigma Radiochemicals; ^{3}H-benzo[*a*]pyrene (BaP) (specific activity 23.8 Ci/mmol) was obtained from Amersham, Inc. Prior to use, the radiopurity of all compounds was determined by a combination of TLC and liquid scintillation counting (LSC). When required, compounds were purified by the same TLC procedure. TLC purity checks and purification were performed on silica gel plates (E. Merck, 250-μm coating) with a hexane:benzene (8:2 v/v) solvent. All compounds used for experimental work were at least 98% pure. All analytical and preparatory work was performed under gold fluorescent light ($\lambda > 500$ nm) to prevent photochemical degradation of the PAH.

Uptake and respiration experiments

Accumulation of the xenobiotics and respiration were measured at 4°C with 60-ml BOD bottles containing two animals. Before the start of each experiment, lake water was filtered through Gelman AE glass-fiber filters (nominal pore size 1 μm) and dosed with the appropriate compound using methanol as a carrier. Diporeic respiration was examined in five simultaneous experiments: in lake water alone; in lake water with the methanol carrier; and in the presence of the contaminants BaP, HCBP, and either BaP and HCBP in combination or PHE. The accumulation of the contaminants in the organisms was also determined. All studies were run in the dark because *Diporeia* is light sensitive [13]. After 24 h exposure, the animals were removed, blotted dry, weighed, and placed in vials containing 12 ml scintillation cocktail (3a70b; Research Products International, Inc.). The animals were kept in the cocktail a minimum of 12 h before LSC so the cocktail could extract the contaminant from the organisms. This method for determining amounts of nonpolar radiolabeled contaminants in *Diporeia* is as effective as predigestion or solubilizing the organism or tissue for small samples where there is no metabolism [14]. Water samples (1 ml) were also taken to determine total activity of the compound under study. Separate water samples were collected and passed through a C_{18} Sep-Pak cartridge. The eluate was measured for bound compound [15] to determine PAH and PCB binding to dissolved organic matter. Calculated uptake clearances (K_u) were corrected for contaminant binding to dissolved organic matter that reduces the contaminant's biological availability [16,17]. Tritium and ^{14}C activity were determined on an LKB 1217 Rackbeta scintillation counter. Samples were corrected for quench by using the external standards ratio method after subtracting background. Dissolved oxygen concentrations were determined by Winkler titrations [18].

Computations

Kinetics. The uptake clearance coefficient (K_u) for the contaminants was determined with a mass balance model. The model assumes that the mass of contaminant in the system is conserved and exists only in the organism or in the water (Eqn. 1).

$$Q_a = \frac{K_{1\,m}A}{(K_{1\,m} + K_2)} - \frac{K_{1\,m}Ae^{-(K_{1\,m}+K_2)t}}{(K_{1\,m} + K_2)} \quad (1)$$

where A = total mass of contaminant in the system (nmol), Q_a = the mass of contaminant in the animal (nmol), Q_w = the mass of contaminant in the water (nmol), and the mass balance equation $A = Q_w + Q_a$ is assumed. In addition, $K_{1\,m}$ = conditional uptake rate constant (h^{-1}), K_2 = the depuration rate constant (h^{-1}), and t = time (h).

Applying the assumption that $K_2 \ll K_{1\,m}$, which is defensible from previously determined toxicokinetics for *Diporeia* [19], then Equation 1 may be simplified to

$$Q_a = A(1 - e^{-K_{1\,m}t}) \quad (2)$$

and solving for $K_{1\,m}$:

$$K_{1\,m} = \frac{-\ln(1 - Q_a/A)}{t} \quad (3)$$

$K_{1\,m}$ is a system-dependent conditional rate constant that describes the fractional change in the amount of contaminant in the water per unit time for a particular size organism in a specific BOD bottle. This rate constant must be converted to a clearance coefficient to be comparable between bottles and experiments. The conversion to the clearance coefficient (K_u) requires that $K_{1\,m}$ be multiplied by the ratio of the volume of the water divided by the mass of the animal (Eqn. 4).

$$K_u = K_{1\,m}\left[\frac{\text{volume of the water (ml)}}{\text{mass of the organism (g)}}\right] \quad (4)$$

This clearance coefficient is system independent and can be compared between individual bottles and experiments. However, the coefficient must be considered conditional on such environmental factors as the experimental temperature and such physiological factors as organism size.

Oxygen consumption and clearance. Equations for determining oxygen consumption ($C\text{-}O_2$) ($\mu g\ O_2\ mg^{-1}\ h^{-1}$) and oxygen clearance ($K\text{-}O_2$) ($ml\ g^{-1}\ h^{-1}$) are based on Winkler determinations:

$$C\text{-}O_2 = \frac{(Cml - Aml)\cdot v\cdot 0.16\cdot Tmol}{Awt\cdot t} \quad (5)$$

$$K\text{-}O_2 = \frac{(Cml - Aml)\cdot v\cdot 1{,}000}{(Cml\cdot Awt\cdot t)} \quad (6)$$

where Cml = ml of thiosulfate titrant for 50-ml control sample, Aml = ml of thiosulfate titrant for 50-ml animal sample, v = total volume of bottle containing the organisms (ml), $Tmol$ = molarity of thiosulfate, Awt = weight of animal (mg), and t = time (h). The equation for $K\text{-}O_2$ is analogous to a one-compartment uptake model:

$$C_a = K_u C_w t \quad (7)$$

where $(Cml - Aml)/Awt\cdot 1{,}000$ is equivalent to the amount of contaminant/g organism C_a and Cml/v is equivalent to C_w concentration in the water. The factor of 1,000 converts mg to g to yield consistent units for model calculations. The 0.16 comes from the 50-ml sample measured for oxygen concentration, the 4:1 ratio of thiosulfate to oxygen required for equimolar titration, and the 32-g mol^{-1} molecular weight of oxygen, to yield $32/(4\cdot 50) = 0.16$.

Estimation of surface area. The surface area of *Diporeia* was estimated on the assumptions that the density of the organisms was 1 g cm^{-3} and that the geometry of the organism was a sphere. From these two assumptions, a value proportional to the true surface area can be obtained by using the following formula when the mass of the organism is known:

$$SA = 4\pi[(3\cdot M)/4\pi]^{0.67} \quad (8)$$

where SA is the estimated surface area in cm^2 and M is the mass of *Diporeia* in grams. This estimate (SA) is expected to deviate from the true surface area only by a proportionality constant. Finally, the surface area-to-volume ratio (SA/V) was estimated as SA/M because mass (M) is proportional to volume through the assumed unit density.

Statistics. The differences between means and slopes were compared by using the Student's t test. Differences were considered significant when $p < 0.05$. Linear regressions were performed with the linear regression package in SAS® [20].

Data qualifications. In some of the regression relationships developed below, nine HCBP observations and eight BaP measures were omitted because they were outliers in the data base. These data points were removed only when they lay outside the 95% C.I. of the regression equation. However, when these outlier points are included, the trends in the data do not change.

RESULTS

Respiration

All respiration and contaminant accumulation samples were taken at the 24-h time interval because previous studies had demonstrated that effects on oxygen consumption that might be attributable to handling disappear after 24 h (M.A. Quigley, personal communication). An additional factor that might have affected both the oxygen consumption and subsequently the accumulation of contaminants was the partial depletion of oxygen over the course of the 24-h exposure (the O_2 depletion was generally less than 20% of the initial concentration) and the potentially different initial oxygen concentrations between experiments. However, the respiration rate for *Diporeia* is well regulated and declines 50% only when the oxygen concentration falls to approximately 3 mg L^{-1} (M.A. Quigley, personal communication). Thus, over the range of initial oxygen concentrations of 7.6 ± 0.75 mg L^{-1} and the final concentrations of generally more than 6 mg L^{-1}, the expected variation in $C\text{-}O_2$ or $K\text{-}O_2$ should be no more than 10 to 15% between experiments.

Originally, the data collected for the juvenile animals were treated separately from those for the fertile females. The two groups seemed to exhibit different $K\text{-}O_2$ and $C\text{-}O_2$ values (Table 1). This division of the data was abandoned when the differences between the groups were found to be solely a matter of organism size.

The size of animals often dictates the rate of respiration. *Diporeia* is no exception, and their $K\text{-}O_2$ decreased with increasing organism size (mass) for the water-only control exposures (Fig. 1) ($K\text{-}O_2 = 64.3[7.0] - 3{,}322.6[895.6]$ g *Diporeia*, $r^2 = 0.34$, $n = 29$, $p < 0.001$). The clearance coefficient for oxygen ($K\text{-}O_2$) can be converted to the mass specific respiration ($C\text{-}O_2$) through a linear regression relationship: $C\text{-}O_2 = -0.009(0.0025) +$

Table 1. Oxygen clearance and consumption for *Diporeia* sp. controls at 4°C

	Clearance (ml g^{-1} h^{-1})	Oxygen consumption (μg mg^{-1} h^{-1})	N
Juveniles			
Water only	47.4 ± 23[a]	0.38 ± 0.019[a]	21
Methanol	34.8 ± 10	0.27 ± 0.08	19
Fertile females			
Water only	24.3 ± 4	0.20 ± 0.04	9
Methanol	15.7 ± 3	0.12 ± 0.02	9

[a]Mean ± SD.

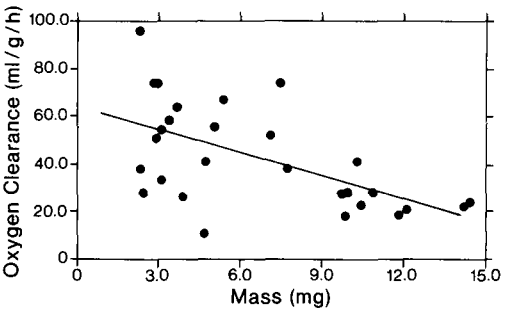

Fig. 1. Oxygen clearance (ml g^{-1} h^{-1}) was best described as a linear decline with increasing mass for the water-only controls.

0.008(0.0007) K-O_2, $r^2 = 0.99$, $n = 142$. Thus, not only does the clearance decline with increased mass, but due to the proportionality between K-O_2 and C-O_2, the mass-specific respiration declines as well. Further, respiration is often thought to be more a function of organism surface area than of organism mass across a wide range of different size organisms [21]. Regressions of oxygen clearance on the surface area or on surface area-to-volume ratio were examined as alternate models to better explain the variation in the respiration rate with changes in organism size. Neither of the surface area models was better than mass for representing the variation in respiration rate based on the r^2 values for the various regressions.

Oxygen clearance (K-O_2) varies in relation to the increasing mass of the organisms in the presence of the methanol carrier (46 ± 12 μg ml^{-1}, averaged over all experiments) (Fig. 2) (K-O_2 = 47.8[3.4] − 2,474.0[412.8] g *Diporeia*, $r^2 = 0.59$, $n = 27$, $p < 0.0001$). The expected maximum oxygen clearance represented by the intercept at mass equals zero was significantly lower for the methanol controls ($p < 0.01$), using a Student's t test, compared to the water-only controls. Further, the slope of the regression line was shallower for the methanol carrier controls ($p < 0.05$). Thus, the methanol carrier reduced the respiration, as represented by K-O_2, for *Diporeia* slightly but significantly. (Remember from above, K-O_2 is directly proportional to C-O_2, the mass-specific respiration rate.) However, the comparisons made in the presence of the contaminants should be internally consistent, as all tests with contaminants were performed in the presence of the methanol carrier and can be compared to the methanol carrier control.

K-O_2 values determined in the presence of either BaP, HCBP, or both in combination provided regression relationships that were more variable but not significantly different from *Diporeia* in the methanol carrier control (K-O_2 = 52.6[5.2] − 2,862.0[632.2] g *Diporeia*, $r^2 = 0.34$, $n = 41$, $p < 0.0001$ for oxygen clearance in the presence of BaP; and K-O_2 = 50.6[4.0] − 2,585.1[501.5] g *Diporeia*, $r^2 = 0.39$, $n = 43$, $p < 0.0001$ in the presence of HCBP). In the presence of Phe, K-O_2 did not exhibit any apparent relationship with organism size. This absence of a relationship was attributed in part to the small data set available (n = 11) — "one very high clearance measurement" — and the natural variability of the data. Because the BaP and HCBP regressions did not differ from the methanol control regression, we conclude that the contaminants had no significant effect on the animals' respiration. Further, the decline in respiration compared to the water-only control was likely due only to the presence of the methanol carrier.

Contaminant clearance

As with the oxygen clearances, the contaminant clearance coefficients (K_u) between juveniles and

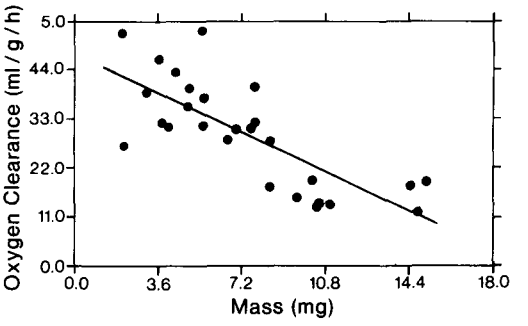

Fig. 2. Oxygen clearance (ml g^{-1} h^{-1}) for the methanol carrier control declined linearly with increasing mass.

Table 2. Clearance of the contaminants for juvenile and fertile female *Diporeia* sp.

Compound	Contaminant clearance (ml g^{-1} h^{-1}) Juveniles	Fertile females
Benzo[*a*]pyrene	192 ± 109[a] (n = 18)	74 ± 18[a] (n = 9)
Hexachlorobiphenyl	170 ± 84 (n = 20)	68 ± 27 (n = 9)
Benzo[*a*]pyrene (BaP/HCBP)[b]	147 ± 67 (n = 9)	66 ± 39 (n = 8)
Hexachlorobiphenyl (BaP/HCBP)[b]	110 ± 57 (n = 9)	49 ± 26 (n = 8)
Phenanthrene	119 ± 41 (n = 11)	NM

NM = not measured.
[a]Mean ± SD.
[b]Individual contaminant clearances measured with exposure to both contaminants.

fertile females differed significantly (Table 2). These differences were again attributed to differences in organism size and not to some other physiological parameter. The clearances of the contaminants measured in the mixtures were not significantly different from the clearances measured independently for either the juveniles or the fertile females. Thus, the data for each compound, whether measured in juveniles or fertile females, could be combined to examine the variability.

The possible relationships between the clearance of the contaminants and organism size were examined by using mass, ln mass, surface area, or surface area-to-volume ratio. Both BaP and HCBP exhibited significant linear regressions with mass, ln mass, and surface area-to-volume ratio. The best model for the clearances was the regression with SA/V (Figs. 3a and 3b) ($K_{u_{BaP}} = -145.4[40.8] + 10.4[1.5]\ SA/V$, $r^2 = 0.54$, $n = 41$, $p < 0.0001$; $K_{u_{HCBP}} = -208.4[27.3] + 12.7[1.1]\ SA/V$, $r^2 = 0.80$, $n = 38$, $p < 0.0001$). Neither the slopes nor the intercepts of these lines are significantly different from each other. For Phe, all the relationships between K_u and mass, ln mass, and SA/V were only marginally significant with p slightly larger than 0.05.

The clearance coefficient is typically expressed on a mass-specific basis, i.e., milliters cleared per mass of organism per hour. If the functional unit for clearance is the surface area of the organism, then modifying the clearance coefficient to a surface area basis (milliters cleared per surface area of the organism per hour) might provide insight into the important organism characteristics for contaminant accumulation. When the clearance coefficient for Phe was expressed on an area basis instead of a mass basis (i.e., ml cm^{-2} h^{-1} instead of ml g^{-1} h^{-1}), the best model was a linear regression of the area-based clearance coefficient (K_{ua}) on mass. This unit conversion for the clearance coefficient resulted in a significant improvement in r^2 by almost a factor of two (Fig. 4) ($K_{ua} = 0.54[0.93] + 983.7[264.0]\ M$, $r^2 = 0.61$, $n = 11$, $p < 0.005$). A similar regression between K_{ua} and SA exhibited essentially the same r^2 value. An attempt to fit the BaP and HCBP to an area-based clearance model significantly reduced the fits of the data for all of the above potentially independent variables. Thus, the uptake of the more nonpolar BaP and HCBP is apparently explained by a different model than the model underlying the uptake of Phe.

Mass-based comparisons between oxygen clearance and contaminant clearance for BaP and HCBP yielded significant linear relationships (Figs. 5a and 5b) ($K_u[\text{BaP}] = 53[19.2] + 1.7[0.5]\ K\text{-}O_2$,

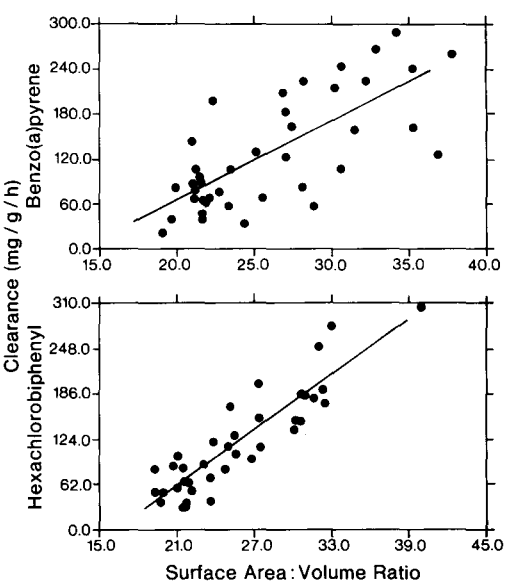

Fig. 3. Clearances for the contaminants BaP (top) and HCBP (bottom) were both directly proportional to the surface area-to-volume ratio of the organism.

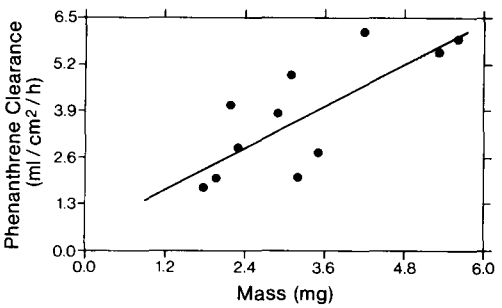

Fig. 4. Phenanthrene clearance was best described on a surface area basis as a direct proportion with the mass of the organism.

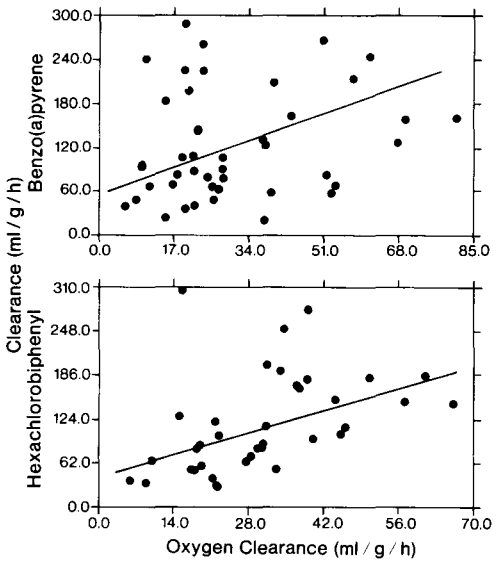

Fig. 5. Clearances for BaP (top) and HCBP (bottom) were both directly proportional to the oxygen clearances measured in the same organisms.

$r^2 = 0.25$, $n = 36$, $p < 0.002$ and K_u(HCBP) = 40.1[19.9] + 2.24[0.56] K-O_2, $r^2 = 0.32$, $n = 37$, $p < 0.001$). The variability between the clearances for the contaminants and oxygen is only partially explained by the regression equations, in spite of their statistical significance. When a similar relationship between Phe clearance and oxygen clearance was examined, no significant relationship was found, whether clearance of Phe was expressed on a mass or area basis. This might have been due to the small number of Phe measurements. To examine this possibility further, the contaminant clearances (mass-based) were divided by the oxygen clearances and averaged. These were found to produce values that were relatively constant, with relative standard errors in the range of 10% (Table 3). None of the ratios is significantly different from any other.

DISCUSSION

Respiration rate and oxygen clearance

Previous work indicates that *Diporeia* respiration is well regulated, with a half-saturation constant for respiration of 3.0 mg L^{-2} at 4°C. Further, at oxygen saturation, the mass-specific respiration rate was greater than that reported for the closely related species *Pontoporeia femorata* and *Monoporeia affinis* (see Bousfield [11] for reclassification from *Pontoporeia affinis*) (M.A. Quigley, personal communication). In addition to the above characteristics, *Diporeia* respiration exhibits a strong dependence on mass. Reduced respiration is correlated with increased size for many organisms [21] and has been observed specifically for amphipods [22]. This reduction of mass-specific respiration with increasing size reflects a general reduction in tissue respiration demands. This reduction may occur, in part, due to changes in the composition of the organisms. Specifically, as *Diporeia* ages, the relative lipid content of the organisms increases from approximately 21% of the nonlipid dry weight (NLDW) for juveniles to 31% of the NLDW for adult females [23]. The relative increase in percent lipids, which are nonrespiring mass, would reduce mass-specific respiration.

The usual relationship between mass and respiration is a power function of organism weight. However, regressing the log of the mass on respi-

Table 3. Ratio of contaminant clearance to oxygen clearance for *Diporeia* sp.

Compound	Ratio (K_u/K-O_2)[a]	Log K_{ow}
Phenanthrene	4.2 ± 0.6 (n = 11)	4.16
Benzo[*a*]pyrene	3.9 ± 0.4 (n = 36)	6.2
2,4,5,2′,4′,5′-Hexachlorobiphenyl	3.75 ± 0.3 (n = 37)	6.77

[a]Mean ± SE.

ration or log of the respiration resulted in less significant regressions than the linear regressions described. This result is consistent with the failure of our attempts to use surface area in place of mass to describe the variability in the K-O_2 values. Thus, within the range of examined organism sizes, the best descriptions of the data variability are regressions on mass.

The methanol carrier produced a slight reduction in K-O_2 for *Diporeia*, compared to the water-only control. This result was unexpected at the low levels of methanol employed, less than 50 mg L^{-1}. The reduced respiration in the presence of methanol is not apparent when the mean values of the oxygen clearance or oxygen consumption data for the carrier controls are compared respectively to those of the water-only controls. The variation in the clearances with organism size must be accounted for to observe a significant deviation between the two control measurements. Finding reduced respiration with small amounts of the water-soluble carrier was a surprise, because the toxicity of methanol requires much greater concentrations to produce observed effects [24]. Although the effect is small, this finding suggests that the role of even small amounts of water-soluble solvents could have some ecosystem-level effects and/or affect laboratory bioassays.

PAH congeners such as naphthalene reduce respiration in a variety of aquatic species, but the levels required for this effect are in the 1-μg ml^{-1} or greater range [24–30]. A similar depression of respiration was observed with Phe at 1.1 mg L^{-1} [30]. The levels of PAH employed were in the low μg- to ng L^{-1} range, and the respiration rates in the presence of the HCBP and BaP were the same as those of the methanol carrier controls. Thus, neither hydrophobic compound produced a measurable effect on *Diporeia* over the range of concentrations examined. For Phe, the data base was too small to obtain good relationships between size and respiration; therefore, statistical comparisons could not be made with the methanol control. However, the mean respiration values for Phe did not differ from those for the methanol control. This suggests that Phe also did not likely affect respiration at the employed concentrations.

Contaminant clearance

If K_u equaled K-O_2, the accumulation efficiency for oxygen would equal that of the contaminants, and those values should not be significantly different. Yet the average ratio of K_u divided by K-O_2 is approximately four (Table 3). Thus, the data suggest that either the passive diffusion of the contaminant across the gill membrane cannot be the sole method of contaminant uptake in the animal, or the efficiency of oxygen accumulation in *Diporeia* is much lower than that found in fish if the accumulation of the organic contaminants has the same accumulation efficiency in both.

The presence of a chitinous cuticle in both terrestrial and aquatic organisms was suggested to impart sensitivity to DDT because the cuticle acts as site of transport for this nonpolar hydrophobic compound [31]. Further research demonstrated that the chitin cuticle was a major site for insecticide accumulation in insects [32]. If passive diffusion of hydrophobic contaminants across the integument contributes significantly to the total flux of contaminants into the animal, and if the cuticle does not pass oxygen, then the model of K_u equal to K-O_2 is no longer appropriate, even if the efficiency of transfer is the same over the respiratory portion of the organism's surface. Instead, the model for contaminant accumulation should include some function of body size to incorporate the potential passage of the contaminant across the chitin cuticle. From the regressions, the surface area-to-volume ratio yields the most significant regressions among the comparisons. This result indicates that the total surface area is important for the uptake of these contaminants.

Based on the Gobas et al. model [33] for the range of compounds examined, membrane permeation control is suggested as the basic mechanism governing the accumulation of the compounds, because the rate coefficients generally increase with increasing contaminant lipophilicity. The model employs several potential rate-determining steps to describe the accumulation of contaminants. First, an aqueous diffusion layer must be crossed. Second, a transfer across the membrane barrier may act as the rate-determining step. Third, but not considered in the Gobas model, distribution of contaminants away from the membrane barrier to maintain a chemical activity gradient may also be rate-determining. This third potential rate-determining step was important for the accumulation of insecticides by various insects [32]. For BaP and HCBP, the clearance was proportional to the surface area-to-volume ratio of the organisms. This relationship suggests that the distribution away from the membrane could be rate-limiting because the distribution will slow as the volume of the organism increases. BaP and HCBP are very hydrophobic compounds; once they are in the membrane, they are likely to resist dissolution into the

body water for distribution to the rest of the organism. Thus, the rate-determining step for contaminant distribution likely includes the desorption rate from the membrane. This type of mechanism has also been suggested as a rate-limiting process for oxygen accumulation by amphipods [22]. For Phe, the rate-determining step is apparently the transfer rate across the membrane barrier, as is suggested by the regression of Phe uptake clearance coefficient on surface area. Redistribution is apparently not rate-limiting for Phe due to the reduced ability to account for the variance in the uptake clearance data on the basis of mass rather than surface area. Further, the greater aqueous solubility of Phe compared to that of BaP and HCBP would be less of a hindrance for Phe dissolution out of the membrane for redistribution than for BaP and HCBP.

The suggested change in the rate-determining step for the contaminant accumulation's varying with changes in contaminant characteristics is further supported by accumulation data measured under flow-through conditions with a wider range of contaminants [19]. Reanalysis of the flow-through experiments indicates that uptake clearances for all compounds with a log K_{ow} of 3.2 to 4.5 are better described by surface area clearances than by mass-based clearances. From this same work, BaP and tetrachlorobiphenyl (TCB) remain best described by mass-based clearances when accounting for variation due to organism size. Thus, the apparent cutoff between the two potential rate-determining steps for accumulation occurs with compounds that are more lipophilic than anthracene but less lipophilic than TCB.

A second potential mechanism to account for differences between K_u and K-O_2 would be a difference in the accumulation efficiency of oxygen compared to that of the nonpolar organic contaminants. For fish, the respiratory membrane is apparently the main site for accumulation of waterborne contaminants, and a similar assumption has been suggested for other aquatic species. Thus, if all the organic compounds were accumulated across the respiratory membrane, only then would differences in the clearances represent differences in the efficiencies of membrane transfer. The difference in efficiency for the two types of compounds, i.e., oxygen and nonpolar organics, would have to be about a factor of four, the approximate ratio between K_u and K-O_2. This may not be unreasonable, as the accumulation efficiency for oxygen by other amphipods, *Gammarus* sp., has been suggested to be in the range of 1 to 5% [22]. If 5% accumulation efficiency is assumed, then the accumulation efficiency for the organic contaminants would be approximately 20%. This would be much lower than that observed for fish [1–8].

Although making the above assumptions would not result in accumulation efficiencies that are too large to be considered, there is no reason to believe that the relative efficiencies for oxygen and contaminants should be drastically different between organisms. For fishes, the accumulation efficiencies of compounds with a log K_{ow} ranging from 4 to 6.5 are 61 to 80%, and the efficiency for oxygen is about 63% [4]. Although the overall accumulation efficiency may be lower for amphipods than it is for fish, as suggested by Sutcliff [22], it is reasonable to suggest that the accumulation efficiencies for the organic contaminants should be equally low. If this is the case, the arguments that a second site is required for nonpolar contaminant accumulation relative to the site for oxygen uptake remain valid and the mechanistic discussions remain reasonable.

The initial goal of this study was to examine relationships between respiration rates and contaminant accumulation rates so that respiration could be used as a predictive tool to estimate uptake clearances. Both BaP and HCBP clearances were in fact linearly related to the clearance of oxygen. Neither the slopes nor intercepts were significantly different between the regressions for these two compounds. Although the uptake clearances between the two compounds are somewhat different when regressions are made with surface area-to-volume ratios, due primarily to differences in the intercept of the linear regressions, the fairly constant ratio of contaminant clearance to oxygen clearance for both BaP and HCBP indicates that an estimate of the contaminant clearance could be obtained from respiration measurements.

Because regressions between Phe clearance and oxygen clearance were not significant, a better estimate of contaminant clearance may be obtained from respiration by using the ratio of K_u to K-O_2 for the various compounds (Table 3). The calculated ratios were relatively constant and had small standard errors (approximately 8–14%). Similar ratios between oxygen clearance and uptake clearance for HCBP, BaP, and Phe were calculated for *Hexagenia limbata* [34]. The ratios for *H. limbata* were more variable than the ratios for *Diporeia*, perhaps because the oxygen and contaminant clearances for *H. limbata* were not measured in the same organisms but with organisms collected at the same time. However, the ratios for BaP (2.78 ±

0.51, $n = 5$) (mean ± SE) and HCBP (2.35 ± 0.81) were fairly constant over the course of a season. The ratios for Phe exhibited considerable variability (2.04 ± 0.93) in *H. limbata*. Thus, these ratios may also be useful for making initial estimates of the accumulation of contaminants from respiration. However, the physiological characteristics of various organisms must be taken into account before applying such ratios.

SUMMARY

The respiration of *Diporeia* was strongly dependent on the mass of the organisms. Oxygen clearance was directly proportional to the uptake clearance for HCBP and BaP but did not exhibit any relationship with Phe clearance, expressed as a function of mass or surface area. The variability in the uptake clearance for HCBP and BaP was best described by a regression based on an estimate of the surface area-to-volume ratio, suggesting that distribution within the organism may be the rate-determining step in the accumulation. The uptake clearance variability for Phe was best described as a total surface area phenomenon, which implies that membrane permeation is the rate-limiting step in accumulation. These results indicate that the mechanisms for contaminant and oxygen accumulation within aquatic organisms such as *Diporeia* must be better understood before respiration can be effectively used as a possible surrogate for measuring the uptake of contaminants.

REFERENCES

1. **Murphy, P.G.** and **J.V. Murphy.** 1971. Correlations between respiration and direct uptake of DDT in the mosquito fish *Gambusia affinis. Bull. Environ. Contam. Toxicol.* **6**:581–588.
2. **McKim, J.M.** and **H.M. Goeden.** 1982. A direct measure of the uptake efficiency of a xenobiotic across the gills of brook trout (*Salvelinus fontinales*) under normoxic and hypoxic conditions. *Comp. Biochem. Physiol.* **C72**:65–74.
3. **McKim, J.M.** and **E.M. Heath.** 1983. Dose determinations for waterborne, 2,5,2′,5′-[^{14}C]tetrachlorobiphenyl and related pharmacokinetics in two species of trout (*Salmo gairdneri* and *Salvelinus fontinalis*): A mass balance approach. *Toxicol. Appl. Pharm.* **68**: 177–187.
4. **McKim, J.M., P. Schmieder** and **G. Veith.** 1985. Absorption dynamics of organic chemical transport across trout gills as related to octanol-water partition coefficient. *Toxicol. Appl. Pharm.* **77**:1–10.
5. **McKim, J.M., P.K. Schmieder, R.W. Carlson, E.P. Hunt** and **G.J. Niemi.** 1987. Use of respiratory-cardiovascular responses of rainbow trout (*Salmo gairdneri*) in identifying acute toxicity syndromes in fish: Part 1. Pentachlorophenol, 2,4-dinitrophenol, tricaine methanesulfonate and 1-octanol. *Environ. Toxicol. Chem.* **6**:295–312.
6. **McKim, J.M., P.K. Schmieder, G.J. Niemi, R.W. Carlson** and **T.R. Henry.** 1987. Use of respiratory-cardiovascular responses of rainbow trout (*Salmo gairdneri*) in identifying acute toxicity syndromes in fish: Part 2. Malathion, carbaryl, acrolein and benzaldehyde. *Environ. Toxicol. Chem.* **6**:313–328.
7. **Rhead, M.M.** and **J.M. Perkins.** 1984. An evaluation of the relative importance of food and water as sources of *p,p′*-DDT to the goldfish, *Carassius auratus* (L.). *Water Res.* **18**:719–725.
8. **Black, M.C.** and **J.F. McCarthy.** 1988. Dissolved organic macromolecules reduce the uptake of hydrophobic organic contaminants by the gills of rainbow trout (*Salmo gairdneri*). *Environ. Toxicol. Chem.* **7**:593–600.
9. **Frez, W.A.** and **P.F. Landrum.** 1984. Species-dependent uptake of PAH in Great Lakes invertebrates. In M.W. Cooke and A.J. Dennis, eds., *Polynuclear Aromatic Hydrocarbons: Ninth International Symposium on Chemistry, Characterization and Carcinogenesis.* Battelle Press, Columbus, OH, pp. 291–304.
10. **Frank, A.P., P.F. Landrum** and **B.J. Eadie.** 1986. Polycyclic aromatic hydrocarbon rates of uptake, depuration, and biotransformation by Lake Michigan *Stylodrilus heringianus. Chemosphere* **15**:317–330.
11. **Bousfield, E.L.** 1989. Revised morphological relationships within the amphipod genera *Pontoporeia* and *Gamaracanthus* and the "glacial relict" significance of their post-glacial distributions. *Can. J. Fish. Aquat. Sci.* **46**:1714–1725.
12. **Landrum, P.F.** 1982. Uptake, depuration and biotransformation of anthracene by the scud, *Pontoporeia hoyi. Chemosphere* **11**:1049–1057.
13. **Donner, K.O.** and **M. Lindstrom.** 1980. Sensitivity to light and circadian activity of *Pontoporeia affinis* (Crustacea, Amphipoda). *Ann. Zool.* **17**:203–212.
14. **Landrum, P.F.** 1983. The effect of co-contaminants on the bioavailability of polycyclic aromatic hydrocarbons to *Pontoporeia hoyi.* In M.W. Cooke and A.J. Dennis, eds., *Polynuclear Aromatic Hydrocarbons: Formation, Metabolism and Measurement. Seventh International Symposium.* Battelle Press, Columbus, OH, pp. 731–743.
15. **Landrum, P.F., S.R. Nihart, B.J. Eadie** and **W.S. Gardner.** 1984. Reverse-phase separation method for determining pollutant binding to Aldrich humic acid and dissolved organic carbon of natural waters. *Environ. Sci. Technol.* **18**:187–192.
16. **Landrum, P.F., M.D. Reinhold, S.R. Nihart** and **B.J. Eadie.** 1985. Predicting the bioavailability of organic xenobiotics to *Pontoporeia hoy* in the presence of humic and fulvic materials and natural organic matter. *Environ. Toxicol. Chem.* **4**:459–467.
17. **Landrum, P.F., S.R. Nihart, B.J. Eadie** and **L.R. Herche.** 1987. Reduction in bioavailability of organic contaminants to the amphipod *Pontoporeia hoyi* by dissolved organic matter of sediment interstitial waters. *Environ. Toxicol. Chem.* **6**:11–20.
18. **Grasshoff, K.** 1983. Determination of oxygen. In K. Grasshoff, M. Ehrhardt and K. Kremling, eds., *Methods of Seawater Analysis.* Verlag Chemie, Weinheim, Federal Republic of Germany, pp. 61–72.
19. **Landrum, P.F.** 1988. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: Role of

physiological and environmental variables. *Aquat. Toxicol.* **12**:245–271.

20. **SAS Institute.** 1985. *SAS® User's Guide: Statistics. Version 5 Edition.* Cary, NC.
21. **Prosser, C.L.** 1973. Oxygen: Respiration and metabolism. In *Comparative Animal Physiology*, 3d ed. W.B. Saunders, Philadelphia, PA, pp. 190–196.
22. **Sutcliffe, D.W.** 1984. Quantitative aspects of oxygen uptake by *Gammarus* (Crustacea, Amphipoda): A critical review. *Freshwater Biol.* **14**:443–489.
23. **Quigley, M.A., J.F. Cavaletto** and **W.S. Gardner.** 1989. Lipid composition related to size and maturity of the amphipod, *Pontoporeia hoyi. J. Great Lakes Res.* **15**:601–610.
24. **Verschueren, K.** 1983. *Handbook of Environmental Data on Organic Chemicals*, 2d ed. Van Nostrand Reinhold, New York, NY.
25. **Tatem, H.E.** 1977. Accumulation of naphthalenes by grass shrimp: Effects on respiration, hatching and larval growth. In D.A. Wolfe, ed., *Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms.* Pergamon Press, Elmsford, NY, pp. 201–209.
26. **Harmon, H.J.** and **M.R. Sanborn.** 1982. Effect of naphthalene on respiration in heart mitochondria and intact cultured cells. *Environ. Res.* **29**:160–173.
27. **Sabourin, T.D.** 1982. Respiratory and circulatory responses of the blue crab to naphthalene and the effect of acclimation salinity. *Aquat. Toxicol.* **2**:301–318.
28. **Crider, J.Y., J. Wilhim** and **H.J. Harmon.** 1982. Effects of naphthalene on the hemoglobin concentration and oxygen uptake of *Daphnia magna. Bull. Environ. Contam. Toxicol.* **28**:52–57.
29. **Russell, L.C.** and **M. Fingerman.** 1984. Exposure to the water soluble fraction of crude oil or naphthalenes alters breathing rates in gulf killifish, *Fundulus grandis. Bull. Environ. Contam. Toxicol.* **32**:363–367.
30. **Geiger, J.G.** and **A.L. Buikema.** 1981. Oxygen consumption and filtering rate of *Daphnia pulex* after exposure to water soluble fractions of naphthalene, phenanthrene, No. 2 fuel oil and coal-tar creosote. *Bull. Environ. Contam. Toxicol.* **27**:783–787.
31. **Richards, A.G.** and **L.K. Cutkomp.** 1946. Correlation between the possession of a chitinous cuticle and sensitivity to DDT. *Biol. Bull.* **90**:97–108.
32. **Gerolt, P.** 1983. Insecticides: Their route of entry, mechanism of transport and mode of action. *Biol. Rev.* **58**:233–274.
33. **Gobas, F.A.P.C., A. Opperhuizen** and **O. Hutzinger.** 1986. Bioconcentration of hydrophobic chemicals in fish: Relationship with membrane permeation. *Environ. Toxicol. Chem.* **5**:637–646.
34. **Landrum, P.F.** and **R. Poore.** 1988. Toxicokinetics of selected xenobiotics in *Hexagenia limbata. J. Great Lakes Res.* **14**:427–437.