Bull. Environm. Contam. Toxicol. 29, 326–333 (1982)

Accumulation of the Polychlorinated Biphenyl Aroclor 1242 from Contaminated Detritus and Water by the Saltmarsh Detritivore, *Uca pugnax*

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Detritus of Spartina alterniflora is the base of many estuarine food chains (MARINUCCI 1982). During the formation of detritus from Spartina litter, polychlorinated biphenyls (PCBs) are accumulated by this detritus from surrounding contaminated water (MARINUCCI and BARTHA 1982). This PCB accumulation is amplified by the litter microbiota, which are also a principle food source for detritivores. We could then expect that consumption of PCB-laden detritus would result in accumulation of the PCB by these detritivores. Furthermore, this vector of transfer of PCB to higher trophic levels may be greater than direct absorption from water, because of the low water solubility of PCB. In the present study we attempted to answer this question by comparing PCB uptake by the fiddler crab, Uca pugnax, from ingested detritus and from contaminated water.

Uca pugnax is a common inhabitant of Spartina alterniflora marshes and consumes large quantities of detritus during its active periods. It requires litter associated algae to grow (HAINES 1976). Uca pugnax accumulates PCBs (KREBS et al. 1974) and DDT (ODUM et al. 1969) from contaminated saltmarsh environments with a 50 to 100-fold concentration of these materials in animal tissue. PCBs are chronically toxic to fiddler crabs, and inhibit molting (FINGERMAN and FINGERMAN 1979, 1977), cause abnormal color changes in the animal (FINGERMAN and FINGERMAN 1978) and cause decreases in general field populations of the crab (KREBS et al. 1974). Nevertheless, the animal can survive low level exposure to PCBs and is easy to handle in the laboratory studies.

METHODS

Animals. Uca pugnax were dug from a southern New Jersey saltmarsh late in November. The animals were reacclimated to 20°C and were held in large plastic trays containing both marsh mud and brackish water (WELSH et al. 1968). The animals were placed on 12 hr/light - 12 hr/dark photoperiod and fed Spartina detritus supplemented with a high protein (ca. 50%) tropical fish food (R. E. LOVELAND, personal communication). We were unable to collect a sufficient number of female crabs, so only male

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This work was performed as part of NJAES Project No. 1501, and was supported and is a part of the Journal Series of the New Jersey Experiment Station, Cook College, Rutgers University, New Brunswick, New Jersey.

0007-4861/82/0029-0326 $01.60

crabs were used in these experiments. Photoperiod and temperature parameters described here were used in all subsequent experiments.

PCB-laden Detritus. PCBs were incorporated into Spartina detritus by passing seawater containing soluble PCBs, as Aroclor 1242, over a column containing freshly collected Spartina litter (see MARINUCCI and BARTHA 1982 for details). After this treatment, the PCB-laden detritus contained 17 ng/g wet weight of PCB (85 ng/g by dry weight).

Extraction and Analysis of PCBs. Analytical techniques used here were similar to those previously described for extraction and analysis of litter and water (see MARINUCCI and BARTHA 1982). After mixing and grinding of the animal and anhydrous sodium sulfate to a free flowing powder, fiddler crabs were extracted with nanograde hexane in 10 ml micro-soxhlet extractors for 1 hr. The hexane extracts were concentrated to 10 ml in a micro-concentrator tube and were then placed on a micro-Florisil™ column and eluted with 20 ml of 92:6:2 petroleum ether:ethyl ether:ethyl alcohol (ERNEY 1974). This effluent was concentrated to 1 ml for gas-liquid chromatographic analysis.

Uptake of PCBs from Detritus (Fed Treatment). Five healthy male Uca pugnax were placed in 8 in (20 cm) diameter Carolina culture dishes containing Ottawa sand (ca. 1 cm) and 200 ml of 24°/oo artificial seawater (Utility Chemical Co., Patterson, NJ). Ten g by wet weight of PCB-laden detritus was placed into each culture system. Because detritus was depleted rapidly in these systems, new culture dishes were prepared as above and, after 19 days, animals were transferred to these. Dishes with crabs were periodically analyzed for PCB concentrations in crabs, and sand. Crabs were removed from the culture system, killed in a desiccator with chloroform fumes, and weighed. The dead animals were measured in all three dimensions of the body (carapace width, length, and height) and the large chela (chela length, height, and width) in order to construct wet weight-body dimension relationships. (Note that the order of the chela and the carapace axes are from the largest axis to the smallest axis for each animal part.) Animals were then dissected, and the hepatopancreas and chela muscle were removed and weighed. All animal parts, sand, and any remaining detritus in the culture dish were extracted for PCBs by the procedure described above.

Uptake of PCBs from Water (Unfed Treatment). Since PCB leached continually from PCB-laden detritus into the surrounding water during the above feeding study, a control system was designed to evaluate PCB uptake by crabs from water alone. Prior to setting up these controls, the static equilibrium concentration of PCB, as Aroclor 1242, in water that contained PCB-laden detritus was determined. Ten g wet weight of detritus was placed in 200 ml of artificial seawater and, after vigorous agitation for several hours the mixture was allowed to equilibrate for 24 hrs. Water was removed without disturbing the settled detritus and was analyzed for PCB. This preliminary measurement showed that the PCB concentration in the water in contact with PCB-laden detritus was 14 to 15 ng/ml. This value is the same PCB concentration obtained in water leaving the PCB solubilizers used in preparation of PCB-laden detritus (see MARINUCCI and BARTHA 1982). Since only 0.3 ug dissolved PCB was available from static water

(200 ml x 15 ng/ml), the PCB solubilizers used in previous experiments, were employed here to maintain a constant PCB concentration in the water. The uptake of PCB from water by the crabs was measured using 5 male crabs in culture dishes containing sand and 200 ml of water as in the feeding experiment. PCB-laden water was, however, continually added to this system from the PCB solubilizers at a rate of 0.42 ml/min. A constant volume was maintained in the culture system by the removal of the excess water with a pump intake line that was cut to the 200 ml water line of the culture dish. The outflow pump rate exceeded the inflow rate which assured that no buildup of water could occur in these dishes. Because of the limited number of animals only two replicates were prepared for the measurement of PCB uptake from water. These systems were terminated at the first and last sampling periods of the study. The animals were measured and analyzed as in the previous feeding treatment.

RESULTS AND DISCUSSION

Length-Weight Relationships. Carapace and chela dimensions fed animals from the fed treatment were equated to the wet weight of the male fiddler crabs with a power function model (TEISSIER 1960).

$$[1] \quad W = K L^a$$

where W = dry weight, K = weight at unit length, L = reference dimension, a = coefficient of increase.

This model was transformed to a linear form and expanded to encompass all reference dimensions:

$$[2] \quad \text{Log } W = a \log L_a + b \log L_b + \ldots + n \log_n + \log K$$

The model in Equation 2 was subjected to stepwise linear regression in order to single out the major dimensional components which predict animal weight. The results of this analysis showed that carapace width and the chelae height (the largest carapace dimension and the second largest chelae dimension) predicted the weight of the Uca pugnax males most accurately (Table 1), and allowed the calculation of male crab weight from the following equation:

Table 1. Results of stepwise regression analysis of power function growth model for male Uca pugnax. The model had 14 degrees of freedom. Shown here are only significant models.

Number of Components	Component Description	Slope	Intercept (g/mm)	F values Component	F values Model
1	Carapace width	2.79	-3.10	281	281
2	Carapace width,	1.50	-2.69	34	460
	Chela height	1.18		29	

[3] $W = .002042\ (g/mm^2) * CW^{1.50} * CLH^{1.18}$

where, W = weight wet (g), CW = carapace width (mm)(largest carapace axis), CLH = chela height (mm)(second larges chela axis).

Equation 3 does not represent any allometric relationship between the various body components of these crabs, but was primarily used as a predictive equation (LAIRD 1965). Equation 3 was subsequently used for calculation of normal wet weight. The calculated value was related to PCB absorption from water and PCB uptake from detritus in animals subjected to the respective treatments. This indirect approach was necessary because unfed animals that absorbed PCB from water, lost weight in relation to volume and surface area.

Animal dry weight could be calculated from wet weight by multiplication of the wet weight by 0.68. This factor is taken from a study of weight and chemical changes on Uca pugilator throughout its post-larval life (GUYSELMAN 1953). The 67.9 ± 4.7% water value is the average water weight of the animal during its post-larval life and is mean water content for the intermolt phase of this crab, which is 50 to 70% of the total life time of the animal.

PCB Accumulation from Water and Detritus. Aroclor 1242 was accumulated by Uca pugnax from PCB-laden detritus at more rapid rates than from water alone (Figure 1). Approximate linear accumulation rate of PCB by these crabs from litter was 1 ug PCB/day/animal. The rate from water was only about 1/10 of this (0.1 ug PCB/day/animal). In the detritus

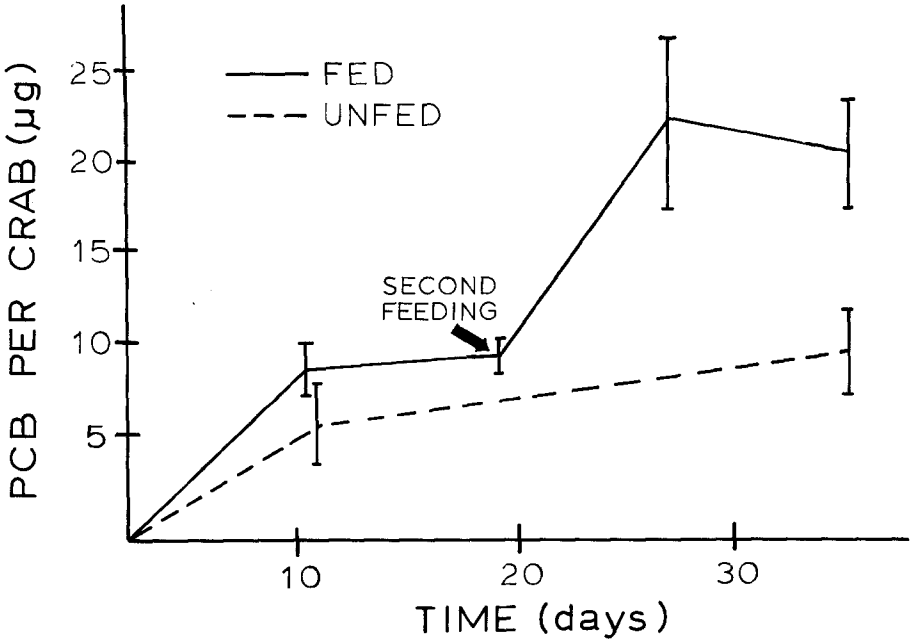

Figure 1. Total accumulation of Aroclor 1242 by the male fiddler crab, Uca pugnax.

feed treatments, 26.2 ± 2.9% of all the PCB in the detritus eventually was incorporated in the animals. Residual PCB in the remaining detritus and sand was 52 ± 9.8% of the initial PCB in the system, which results in an overall recovery for the experiment of 78.2 ± 11.2%. Losses in this open system were probably the result of co-distillation of PCB with water vapor during the experiment (GIAM et al. 1980).

Animals rapidly depleted the PCB from the available portion of the detritus as can be seen from the total uptake data (Figure 1). Uptake of PCB by the crabs was essentially complete prior to the first sampling after introducton of the food to the animals. The subsequent period did not result in a further increase in the PCB content of the animals.

Aroclor 1242 was highly concentrated in the hepatopancreatic tissue of these animals with a concentration of 9.5 ± 3.5 times the PCB concentration in whole animals and chela tissue. PCB concentrations of chela muscle were essentially the same as whole body values.

The concentration of PCB in animals of fed and unfed treatments did not show as clear a difference as the total PCB per animal values (Figure 2). However, if we apply a power function model to correct for differences in animal weights between fed and unfed treatments, a clearer relationship emerges (ZEUTHEN 1960).

Assuming that PCB uptake is a function of respiration in unfed animals and respiration and feeding rate in feed treatments, one can use the equation:

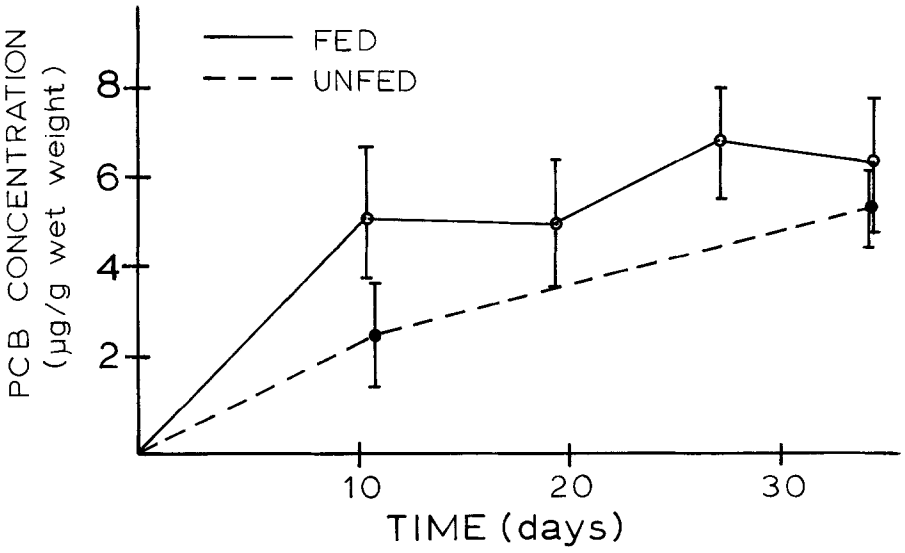

Figure 2. Concentration changes of Aroclor 1242 in the male fiddler crab, Uca pugnax.

[4] $PCB = a\ W^{b}$

where, PCB = total PCB (ng), a = PCB quantity at unit weight (ug PCB/g wet wt), W = wet weight of the animal (g), b = proportionality exponent.

Then PCB concentration is:

[5] $[PCB] = \frac{PCB}{W} = a\ W^{b-1}$

Logarithmic transformation of 5 to a linear form is then:

[6] $\log [PCB] = (b-1) \log W + \log a$

Regression of transformed PCB concentration data and animal weight data was used to determine (b-1) and (a) values for the above model for each sampling period (Figure 3). Because animals starved for 36 days had a different (b-1) value than the 10-day starved animals when measured wet weights were used in calculations, all starved animal weights were calculated from body dimensions and Equation 3.

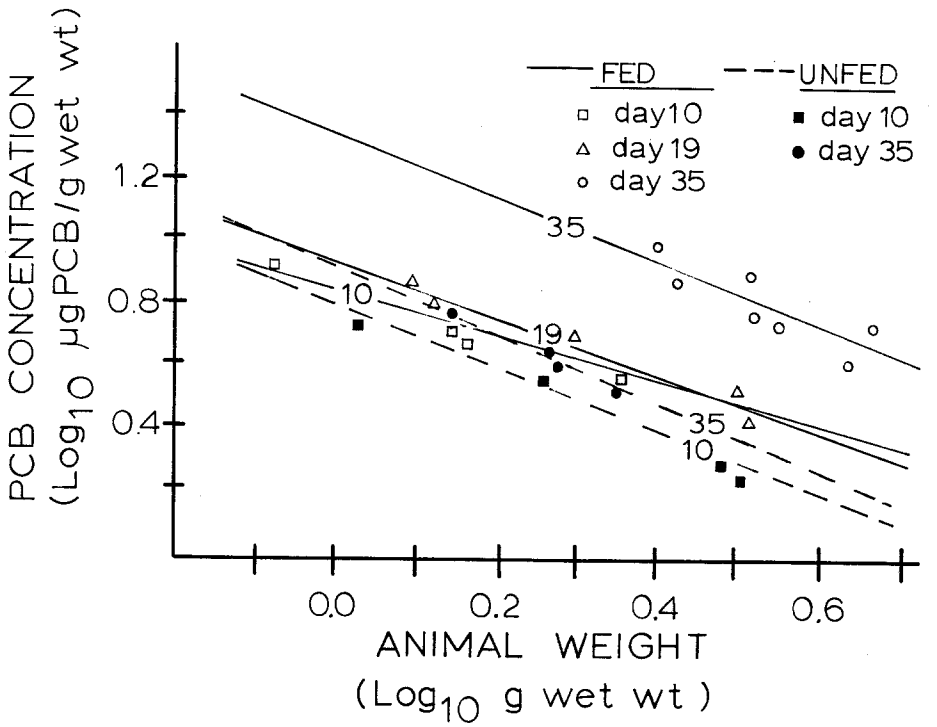

Figure 3. Relationship between concentration of PCB and crab weight in male fiddler crabs exposed to PCBs in detritus and water. Days of exposure are indicated on respective regression line.

The results of the regression analysis of transformed concentration data and weight data showed that (b-1) values for both fed and unfed animals, which range from 1.25 to 4.46 g wet wt, at each sampling period were about -1 (Table 2). The b term was then equal to 0. These values indicated that total PCB accumulation by the animals was independent of the weight of the animal and that PCB concentration of the animals was inversely related to the animals' weight. This latter relationship was the result of PCB dilution in larger crabs when compared to smaller crabs. Comparison of PCB uptake from water with PCB uptake from detritus, in terms concentration increases in the animals, was best made through the regression intercepts of the power function analysis of PCB concentration and animal weights (Table 2). The intercepts are the PCB concentrations for animals of the same weight. These data showed that PCB uptake from water was about 1/2 that of uptake from PCB-laden detritus after 34 days. If one assumes that uptake from both water and detritus occurred in the fed treatment, then one could determine uptake of PCB from detritus alone by subtraction of the accumulated PCB in the unfed treatment from the fed system. Such a calculation showed that PCB uptake from detritus was equal to uptake from water in this system.

Respiration and egestion rates may not be the only factors controlling the PCB accumulation and concentration by the fiddler crabs. The concentration of PCB in the detritus may have a major effect of PCB accumulation by the crabs. Egestion rate b value for these animals is 0.59 and the respiration rate b value is 0.71 (CAMMEN et al. 1980). Uptake of a pollutant form water and from a food source is shown to also be dependent on relative differences in egestion and respiration rates coupled with the water concentration of the pollutant (NORSTROM et al. 1976). Results of this study illustrated the relative contribution of these two routes of uptake at one PCB concentration level. These relationships may change if PCB levels in the water were raised or lowered. This manipulation may be difficult since the water PCB concentration when PCB-laden detritus was present approached the Aroclor 1242 solubility.

Table 2. Analysis of relationship between male Uca pugnax weight and concentration of Aroclor 1242 in the animal tissue. The model used was: (Log of PCB concentration) = (b-1) log (wet weight) + log (PCB concentration at unit weight). Slope = (B-1). Intercept = ug PCB/g wet wt for 1 gram animal.

Time of Exposure (Days)	TREATMENT: Experimental (PCB in detritus and water)		Control (PCB in Water)	
	Slope	Intercept	Slope	Intercept
10	-0.72 ± 0.09	6.2 ± 0.2	-1.02 ± 0.23	6.1 ± 0.9
19	-0.92 ± 0.08	8.5 ± 0.2	-----	-----
34	-1.02 ± 0.25	21.4 ± 2.1	-1.11 ± 0.05	8.1 ± 0.1

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Accepted June 20, 1982