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Dietary Accumulation of Dimethylnaphthalene by the Grass Shrimp *Palaemonetes pugio* Under Stable and Fluctuating Temperatures[1]

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Chronic ingestion of oil-contaminated food by aquatic organisms has received little attention relative to contamination via oiled seawater or sediments. SINGER et al. (1980) suggested that the uptake of aromatic hydrocarbons through the diet may be a major route of contamination because of their lipid solubility. NEFF (1979), in summarizing the importance of oil-contaminated food on aquatic crustaceans, concluded that they did rapidly assimilate petroleum but it was eventually depurated from the tissues. All of the work which NEFF (1979) reviewed utilized a single dose of contaminated food. At present, there is little comparable information on the chronic dietary accumulation of oil by estuarine crustaceans.

There is a body of evidence which indicates that the effects of whole oil, on a variety of marine organisms, is dependent on the relative size of the aromatic fraction, especially the aromatic naphthalenes (ANDERSON et al. 1974). Laboratory studies with aquatic crustaceans have shown that dietary naphthalene is taken up more efficiently, is metabolized more slowly, and is depurated from the tissues at a slower rate than naphthalene in solution (CORNER et al. 1976; HARRIS et al. 1977). Dimethylnaphthalene (DMN), an alkylated homolog of naphthalene, is more toxic than naphthalene to many marine organisms and is persistent in oil-contaminated environments and organisms (TATEM et al. 1978; ANDERSON 1979). This paper reports on experiments in which the estuarine grass shrim, Palaemonetes pugio, was fed DMN-contaminated food for 32 days under both stable and fluctuating temperature conditions and then allowed 16 days to depurate in stable temperatures.

MATERIALS AND METHODS

Grass shrimp, P. pugio, were collected by dip net in the York River near Gloucester Point, VA. They were maintained in 17 °/oo salinity York River water at 22-23°C for 1-2 days before use. For

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all experiments, artificial seawater was prepared with sea salts (Aquarium Systems, Mentor, OH) diluted with well water for 17 °/oo. Salinities were determined with a hand-held refractometer.

A DMN-contaminated food source was prepared by exposing freshly hatched Artemia sp. (San Francisco Bay) nauplii to 2,6-DMN (Chemical Samples Co., Columbus, OH) at a concentration of 266 ± 8 µg/L (n=3). After 8 h exposure, Artemia sp. were removed with fine Nitex screen, rinsed in 17 °/oo, drained and frozen in trays partitioned into 10 mm cubes. The resultant food cubes contained 0.24 ± 0.06 µg DMN/g wet wt (n=9). This concentration is approximately one-third the concentration of DMN in seawater which is acutely toxic to P. pugio (TATEM et al. 1978). Artemia sp. prepared in a similar manner, but without DMN exposure, served as food for shrimp receiving an uncontaminated food source. All food cubes were kept frozen until needed.

To help quantify the feeding regime, the caloric content of Artemia sp. nauplii and the daily caloric requirement of P. pugio were calculated to be 330 calories/food cube and 7.83 calories/shrimp/day, respectively based on caloric data reported by ANDERSON (1977). One food cube is sufficient to supply the caloric requirements of 40 shrimp. This ration was doubled to help eliminate effects of food deficiency.

Details of the general experimental method have been described elsewhere (DILLON 1981). Shrimp were exposed for 32 days in four aquaria (110 shrimp/aquarium) to the four experimental treatments of: stable temperature (20°C) and clean food (ST/CLEAN); stable temperature (20°C) and DMN-contaminated food (ST/DMN); fluctuating temperature (18-22°C) and clean food (FT/CLEAN); fluctuating temperature (18-22°C) and DMN-contaminated food (FT/DMN). Clean or contaminated food was provided on a daily basis at about 1200 h. Each of the four treatment aquaria contained a charcoal corner filter and 37 L of 17 °/oo seawater. New filters and seawater were provided midway (16 day) through the 32 day exposure period.

After the exposure period, a number of the shrimp were transferred to aquaria each containing a charcoal filter and 37 L of new 17 °/oo seawater. They were given equal daily rations of uncontaminated Artemia sp. food cubes and maintained at a constant temperature of 20°C. This recovery period was designed to evaluate the persistence of DMN in shrimp tissues.

DMN in tissue and water samples was extracted and analyzed according to the method of NEFF & ANDERSON (1975). Three Artemia sp. food cubes and one 250-mL water sample were analyzed for DMN in three of the Artemia sp. 8 h exposures. Six to 8 grass shrimp/treatment were routinely removed for DMN analysis after each of the six separate exposure and recovery periods. All tissue samples were frozen on tared aluminum sheets at -20.0°C. After thawing, wet weights were determined just prior to analysis. Grass

shrimp were first cut into several pieces with dissecting scissors. Two shrimp were extracted and analyzed together in each sample. Tissues were homogenized by hand in a consistent manner in the presence of 5 ml spectroscopic grade hexane (Burdick and Jackson Laboratories) with all glass hand homogenizers. The hexane layer was poured into 20-mL glass vials. Approximately 1 g Florisil was added to remove coextracted lipids. Vials were sealed with polyethylene-lined caps and stored at 5°C for no longer than 1 wk. Absorbance at 228 nm was determined. Concentrations were determined from DMN standards made up in hexane. Tissue interference was corrected by subtracting the absorbance of extracts of uncontaminated tissues. Concentrations are expressed as µg DMN/L water and µg DMN/g wet wt. tissue.

Statistical Analysis. Mean DMN concentrations among the six exposure/recovery periods were initially compared with a one-way analysis of variance. Since no significant differences were detected, concentrations were pooled into overall means. The effects of the two thermal regimes and the recovery period on these overall means were examined with a two-way analysis of variance (STEELE & TORRIE 1960). All significant differences were examined at the 95% confidence level.

RESULTS

The overall means calculated within each treatment after the exposure and recovery periods are shown in Table 1. After ingesting contaminated food for 32 days, Palaemonetes pugio accumulated approximately an order of magnitude greater concentration of DMN than in the Artemia sp. food cubes (0.24 µg DMN/g wet wt.). The overall means of DMN in shrimp from the fluctuating and stable temperature regimes were 5.26 and 7.20 µg DMN/g wet wt., respectively. Results of the two-way ANOVA indicated that there was a significant decrease in DMN concentrations for shrimp from both stable and fluctuating temperature regimes after the recovery period to levels of 1.27 and 2.60 µg DMN/g wet wt., respectively. In addition, there were no significant differences in DMN concentrations in shrimp from the two thermal treatments either after exposure or recovery.

DISCUSSION

Accumulation of petroleum hydrocarbons through the diet has received very little attention. NEFF (1979), in reviewing the environmental fate of polycyclic aromatic hydrocarbons, concluded that although aquatic crustaceans readily assimilate dietary aromatics, the release was also rapid and the potential for food-web contamination was therefore minimal. However, under field conditions, where the source of petroleum may not be easily eliminated, the potential for biological harm and food-web contamination may exist. This may be especially important for crustaceans which, relative to vertebrates, have a limited ability to metabolize aromatic compounds (MALINS 1977). Such a situation has been reported for the fiddler crab Uca pugnax ingesting

Table 1. Concentrations of dimethylnaphthalene (µg DMN/g wet wt.) in Artemia sp. and P. pugio from the six exposure and recovery periods. $\bar{x} \pm 1$ SE, n = number of samples analyzed.

	Palaemonetes pugio[b]	Artemia sp.[c]
Exposure		
Stable Temperature	5.26[a] ± 1.37 n=20	0.24 ± 0.06 n=9
Fluctuating Temperature	7.20[a] ± 1.00 n=20	0.24 ± 0.06 n=9
Recovery		
Stable Temperature	1.27[a] ± 0.35 n=27	0.00[d]
Fluctuating Temperature	2.60[a] ± 0.48 n=27	0.00[d]

[a] overall means
[b] each sample analyzed contained two shrimp
[c] each sample analyzed contained one food cube
[d] uncontaminated food source

contaminated detritus in a heavily oiled marsh (BURNS & TEAL 1979). Large reductions in the crab population were observed.

Because Palaemonetes pugio did accumulate DMN in the present study, the potential for biological harm and food-web contamination may exist. However, there are two arguments opposing this interpretation. First, previous work has shown that the ingestion of DMN-contaminated food has only a minimal detrimental effect on P. pugio (DILLON 1981). This is especially true when compared to the highly stressful effects of daily fluctuating temperatures. If the competitive fitness of grass shrimp was only slightly altered by the ingestion of DMN-contaminated food, one would not expect wholesale changes in the population structure and function.

Another reason for suggesting that the accumulation of DMN observed in this study may not represent serious biological harm for P. pugio relates to its feeding behavior. In the field, grass shrimp ingest a wide variety of food types. In the present study, shrimp were fed only a single food type. If a food source became contaminated in the field and was noxious to P. pugio, the grass shrimp could presumably behaviorally select another food type if

available. Some crustaceans can detect very low concentrations of naphthalene and DMN (PEARSON et al. 1980) and it is a reasonable assumption that P. pugio has a similar capability. The observed pattern of reduced tissue concentrations in shrimp once the contaminated food was removed indicates that DMN, if accumulated, would not remain in the tissues very long once the ingestion of uncontaminated food resumed.

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