

Toxicity, Uptake, and Release of the Water-Soluble Fraction of Crude Oil in Different Developing Stages of the Prawn *Macrobrachium borellii*

S. Lavarías, H. Heras, R. J. Pollero

Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET—Cátedra de Bioquímica, UNLP, Calles 60 y 120, (1900) La Plata, Argentina

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Abstract. The effects of a water-soluble fraction of light crude oil dissolved in freshwater (WSF) on *Macrobrachium borellii* exposed at three life stages was evaluated. Adults, larvae (PL), and embryos were exposed to five levels of WSF for 96 h. At 48 and 72 h PL were significantly more sensitive to WSF than adults, though values for 96-h LC50 were not significantly different (1.56 and 1.41 mg/L, respectively). Mortality was never high enough to allow LC50 calculation in embryos, but chronic exposure to WSF increased the mortality near hatching and significantly decreased PL survival compared to the control group. The uptake, accumulation, and depuration of WSF were evaluated in adult prawns (lipid content 1.5% wet wt) exposed to a sublethal concentration for 96 h followed by a 10-d depuration period. Hydrocarbons were rapidly taken up, and after 24 h most of them reached an equilibrium concentration. Increases in the number and alkylation of the aromatic rings enhanced both their accumulation and their retention. When returned to clean freshwater, levels of lighter aromatics dropped rapidly over 12 h. From these studies we conclude that early life stages of *M. borellii* are not very vulnerable to WSF toxicity, while adults have a rapid uptake and release of most of the contaminating hydrocarbons, probably due to their low lipid levels.

Aquatic pollution by oil spills is especially critical in rivers and coastal waters. Freshwater oil spills are often complicated by proximity to shorelines, stronger water currents, smaller volumes of water, unidirectional flow, and greater potential for floods than those occurring in a marine environment (Walker *et al.* 1995). Oil is retained much longer in low-energy environments than on marine, wave-swept coasts (Bhattacharyya *et al.* 2003). The Rio de La Plata estuary in Argentina is a region where there is an urgent need to evaluate the ecotoxicological effects of hydrocarbons (HCs). The area is exposed to several contaminants, and it is the most contaminated region in the

country in terms of HCs (FCS 1994), polyaromatics plus alkanes being 0.7 mg/L in superficial water of some areas (Colombo *et al.* 1989). As in other waters the major sources of crude oil input are industrial and domestic discharges as well as accidents during transport (SHN-SOHMA 1989). Although the most visible part of an oil spill is the surface slick or mousse, the fraction responsible for the toxic effects is the water-soluble hydrocarbon fraction (WSF). The WSF closely resembles the aromatic hydrocarbon composition of diesel or domestic furnace oil fuels (Ackman *et al.* 1997).

We studied the effect of the WSF on the caridean prawn *Macrobrachium borellii* (Nobili), chosen partly because it is known that benthic organisms may accumulate water-column hydrocarbons directly from the water column or from the interstitial water in the sediment (Bhattacharyya *et al.* 2003). This species is a widespread South American lecithotrophic freshwater prawn living in turbid, temperate water streams (Boschi 1981; Morrone and Lopretto 1995). Its reproductive season lasts from spring to early summer (November to February). Breeding females produce large, yolky eggs that are cemented to the pleopods in a brooding chamber. The species has a highly abbreviated development stage hatching directly as PL (Boschi 1981). Clutches are small, 30 to 100 eggs that take around 41 days to hatch (pers. obs.), and hatchlings are translucent larvae of benthonic habits which are 5–6 mm long and omnivorous–carnivorous (Collins and Paggi 1998). There are neither data on the effect of the WSF on species of the area nor data on the detailed uptake and depuration of major HC aromatic groups of WSF in aquatic invertebrates.

In the present work, we determined the toxicity of the WSF at different life stages of *M. borellii* and, also, analyzed the aromatic HC uptake and biodepuration of adults in order to contribute with data that could be employed for environmental risk assessment in a region that is heavily polluted.

Materials and Methods

Sample Collection

Adults of *M. borellii* were sampled during spring and summer (October to February) in an uncontaminated watercourse close to the Rio de

La Plata River, Argentina (20 km SW from La Plata), with temperatures ranging from 21 to 28°C. They were taken to the laboratory and kept in dechlorinated tap water (CaCO₃ hardness, 160 mg/L) at 22 ± 2°C, and under a 14:10 L:D photoperiod for at least a week before experiments (Collins and Petriella 1999). Eggs were removed from the pleopods of ovigerous females for the experiments and checked under a stereoscopic microscope to determine stage of development (Lavarías *et al.* 2002). PL were hatched in the laboratory and 2- to 4-day-old individuals were used for the experiments.

Preparation of the WSF of Crude Oil

Punta Loyola light crude oil, obtained from Santa Cruz, Argentina, stored at 4°C, was used to prepare the WSF. It was stirred in a 10-L stainless-steel mixing vessel equipped with a mechanical stirrer and a bottom drain, and kept in a cold room at 4°C. Crude oil and freshwater at a ratio of 1:100 (v/v) were stirred at low speed for 24 h and allowed to settle for an additional 48 h (lower settling times rendered a fraction with dispersed oil droplets). WSF was collected daily using the bottom drain. During the 96-h and chronic experiments, fresh WSF batches were prepared every 2 days using several 10-L vessels.

Adult and Larval Toxicity Tests

The experiments were designed to establish LC50 for the WSF and to select WSF concentration for sublethal studies on uptake and biodegradation. All experiments for LC50 over 96 h were done in triplicate, without feeding, at 20–22°C and a 14-h light:10-h dark cycle.

Experimental animals were randomly placed for each of five WSF concentrations and one control. The nominal concentrations for exposures were 0.34–0.69–1.37–2.06–2.75 mg/L hydrocarbon, plus a control group held in clean water. Groups of three adults (mean wet weight, 1.05 g) were placed into glass flasks containing 600 ml of test solution with airtight screw-lids. For each level, five flasks were used and WSF was replaced daily. Before each daily medium change, temperature, pH, and dissolved oxygen were measured in the control containers. These results were within normal ranges for *M. borellii*. Two- to four-day-old PL animals were tested in a similar way except that groups of 15 individuals were placed in 125-ml flasks.

Statistical analysis of data to establish median lethal concentrations (LC50) with 95% confidence limits (CL) were analyzed by PROBIT using the program Toxdat (USEPA). LC50 was calculated for each experiment and then an average taken. Analysis of variance was used to determine if significant group differences ($p < 0.05$) existed within treatments.

Embryo Test

The 96 h exposures of egg embryos were performed near the end of development (stage 5, 6, or 7) at 24°C (Lavarías *et al.* 2002). Embryonic coat and exoskeleton are transparent, making it possible to evaluate embryo malformations. Eggs were removed from the pleopods of ovigerous females with forceps, rinsed with filtered water, and placed individually into 24-well, 2-ml plastic tissue culture plates, according to Rayburn *et al.* (1996). After 24 h acclimation eggs were checked again and dead embryos discarded. The same five WSF dilutions as for the mortality tests and a control held in clean water were conducted within a single 24-well tissue culture plate ($n = 24$ embryos for each treatment level). WSF was replaced every 24 h and embryos examined daily under a stereoscopic microscope for abnormalities, survival, stage of development, and hatching. In another set of experiments, eggs were exposed continuously to a sublethal nominal concentration

of WSF (2.7 mg/L) under conditions similar to those in the LC50 experiment, with daily replacement of WSF. Just-laid eggs (synchronized in their development at stage 1) were followed throughout development until hatching (approx. 40 days). Microscopic observations were made every second day and mortality, teratogenesis, and hatching time were recorded.

Uptake and Release of Hydrocarbons

Adult prawns ($n = 100$) were placed in several 2-L sealed glass tanks and exposed to 0.3 mg/L WSF for 96 h, followed by a depuration period of 10 days without feeding, at 20–22°C and a 14-h light:10-h dark cycle. WSF exposure concentration was chosen from results of toxicity studies. Before each daily medium change, temperature, pH, and dissolved oxygen were measured in the control containers. Control prawns were kept in uncontaminated water under the same conditions. During the uptake and depuration, groups of 10 prawn were taken at 3, 6, 12, 24, and 96 h for the uptake experiment and at 0, 3, 6, 24, 48 and 96 h and 10 days for the depuration experiment. The whole organism was frozen at –70°C for lipid and hydrocarbon analyses.

Hydrocarbon Analysis

WSF analysis. Analysis of the WSF was performed on water samples from the stock WSF every time a WSF batch was prepared. The HCs in the WSF were extracted according to the Murray extraction procedure (Murray *et al.* 1984). All HCs analyses were conducted with a Hewlett Packard HP6890 capillary gas chromatograph (GLC) equipped with a flame ionization detector (FID) and a split injection system. The chromatography was conducted on a DB-1 column (60 m, 0.25- μ m film thickness). Conditions and temperatures were as follows: FID, 280°C; injector, 250°C; split, 1:20. The column temperature was programmed as follows: initial temperature, 45°C, held for 15 min, then increased at a rate of 12°C min⁻¹; final temperature, 280°C, held for 25 min. The carrier gas was helium at a flow of 0.8 ml/min and hydrogen, and air flows were 40 and 450 ml/min, respectively. The concentration of HC in the samples was calculated with respect to the internal standard (nC21) and corrected for GLC response, combined with the recovery efficiency of the extraction technique. Blank readings were made daily and all solvents were HPLC grade. The specimen components were identified by comparison of retention times with those of external standards and with a WSF identified by GLC-MS (Heras *et al.* 1992).

Tissue analysis. HCs in the prawn were extracted by the steam distillation procedure of Ackman *et al.* (1997) with modifications as follows. Steam distillations were carried out in 250-ml round-bottom flasks. The flask was attached to a Barret-type distilling receiver, which was connected to a cool water condenser. All the apparatus and glasswares were carefully washed and subsequently rinsed with distilled water, acetone, and twice with methylene chloride (Carlo Erba, Italy). The apparatus and glasswares were dried at 100°C for 2 h and rinsed again with methylene chloride just before used. Forty milliliters of distilled water was placed in the flask and heated to boiling point using an electric heating mantle. This preliminary distillation was over when 10 ml of water condensate was collected. The flask was cooled at room temperature and the water condensate was discarded.

The frozen prawn was thawed in a domestic refrigerator and immediately minced upon softening. About 5 g of the minced tissue was added to the remaining water in a clean 250-ml flask for distillation, then 2 ml of methylene chloride was added to the flasks. The graduated receiver was immersed in an ice/water bath to avoid the loss of HCs

during distillation. The distillation was continued for about 25 min until 10 ml condensate were collected.

The condensate was received in a 12-ml precooled centrifuge tube. Methylene chloride, 200 μ l, containing *n*-heneicosane (nC21) as internal standard, was added to each centrifuge tube. The water and methylene chloride in the centrifuge tube were vortexed for 1 min and centrifuged at 1000 rpm for 5 min. The methylene chloride layer was removed with a syringe and directly injected into a capillary GLC or concentrated under a nitrogen flow of 0.2 L/min before injecting into the GLC. Samples were distilled in duplicate and each collected methylene chloride was also injected twice. Distillation of control samples was performed following exactly the same procedure. The recovery efficiency on hydrocarbon distillation was evaluated by spiking 20 HC standards into the sample, the extraction being performed as described above. The amount of hydrocarbons was calculated according to the internal standard plus the recovery efficiency and the GLC response factor of individual HCs.

Lipid content in aliquots from each sample was determined gravimetrically after the $\text{Cl}_3\text{CH}-\text{MeOH}$ extraction (Bligh and Dyer 1959).

Results

WSF Composition

Table 1 shows the hydrocarbon composition of the WSF employed in the experiments. The WSF was mainly composed of single-ring aromatic hydrocarbons (toluene, benzene, xylene, and di- and trimethylbenzenes) and very low concentrations of $\text{C}_{12}-\text{C}_{24}$ *n*-alkanes, reflecting the low water solubility of these compounds. In the stock solution lower concentrations of naphthalene and methyl-naphthalenes and higher levels of methyl-cyclohexane were found, compared to WSF prepared from other light crude oils (Heras *et al.* 1992).

Adult and Larval Toxicity Tests

As is standard practice in acute tests, prawns were not fed during the tests. The 96-h bioassays allowed an evaluation of the sensitivity of prawn life stage to the WSF. They also provided data that allowed us to select the appropriate WSF concentrations for the uptake and depuration study. During the course of these toxicity tests PL were more sensitive to WSF exposure than adults, resulting in an earlier onset of mortality (Table 2). Thus, 48- and 72-h LC50 values for adults were significantly higher than values determined for PL (Figures 1A and B, Table 2). Nevertheless, at the end of the test the 96-h LC50 values determined for adults and PL were 1.56 and 1.41 mg/L, respectively, these values not being significantly different from each other. There was no mortality in unexposed specimens. Temperatures ranged between 20 and 22°C, pH between 6.6 and 6.9, and dissolved oxygen between 4.5 and 5 mg/L for both experiments.

Tests with Embryos

In all embryo LC50 assays no significant differences in mortality were observed between the control and the WSF-treated eggs, and mortality was never high enough to allow calculation of 96-h

Table 1. Typical composition of the WSF of Punta Loyola light crude oil after 24 h of shaking and 48 h of settling as determined by capillary GLC

Hydrocarbon	mg/L
Benzene	0.192 \pm 0.007
Cyclohexane	0.035 \pm 0.004
2-Methylhexane	0.004 \pm 0.000
Isooctane	0.036 \pm 0.001
nC7	0.018 \pm 0.000
Methylcyclohexane	0.227 \pm 0.006
Toluene	0.292 \pm 0.034
Dimethylcyclohexanes	0.082 \pm 0.003
nC8	0.038 \pm 0.000
Ethylcyclohexane	0.047 \pm 0.000
Ethylbenzene	0.124 \pm 0.016
<i>m</i> - + <i>p</i> -Xylene	0.713 \pm 0.097
<i>o</i> -Xylene	0.239 \pm 0.032
Isopropylbenzene	0.016 \pm 0.002
Propylbenzene	0.020 \pm 0.002
1-Ethyl-3-methylbenzene	0.256 \pm 0.031
nC9	0.000 \pm 0.000
1,3,5 Trimethylbenzene	0.146 \pm 0.020
1-Ethyl-2-methylbenzene	0.062 \pm 0.009
1,2,4-Trimethylbenzene	0.229 \pm 0.028
nC10	0.039 \pm 0.001
1,2,3-Trimethylbenzene	0.075 \pm 0.009
nC11	0.018 \pm 0.010
1,2,4,5 + 1,2,3,5-Tetramethylbenzenes	0.018 \pm 0.002
C4 alkylbenzenes	0.184 \pm 0.017
Naphthalene	0.061 \pm 0.008
nC12	0.004 \pm 0.000
nC13 + 2-methylnaphthalene	0.057 \pm 0.009
1-Methylnaphthalene	0.017 \pm 0.003
nC14 + Ethylnaphthalene	0.011 \pm 0.003
Dimethylnaphthalenes	0.066 \pm 0.024
C3 naphthalenes	0.010 \pm 0.006
PAH	0.007 \pm 0.001
nC15–nC23	0.027 \pm 0.016
Unknown	0.068 \pm 0.003
Total	3.436 \pm 0.018

Note. Values represent the mean of triplicate analysis \pm 1 SD. C4 alkylbenzenes—a group containing $\text{C}_1 \times 4$, $\text{C}_2 \times 2$, $\text{C}_1 + \text{C}_3$ and $\text{C}_1 \times 2 + \text{C}_2$ benzenes. C3 naphthalenes—several $\text{C}_1 \times 3$ and $\text{C}_2 + \text{C}_1$ and C_3 naphthalenes. PAH, polyaromatic hydrocarbon.

LC50, thus being >3.4 mg/L. Nevertheless, when embryos were chronically exposed from oviposition to hatching to 2.7 mg/L WSF, a significant increase in mortality in exposed embryos became evident. In these experiments, the overall embryo survival until hatching was 77.3 and 46.3% for the control and exposed groups, respectively, and this increased mortality became evident near hatching time (stage 6 onward) (Figure 2A). There was also a difference in the hatching time. While all control PL hatched on days 42 and 43, 42% of the WSF-treated embryos hatched earlier and over a wider range of days (i.e., from day 40 to day 43) (Figure 2B). Then PL performance was studied, and we observed that the majority of control PL survived the process of hatching (64.7%), while only 10.5% of the treated ones were able to complete this process. When PL were then immediately transferred to clean water we observed that 50% of the control larvae and 5% of the treated ones were able to reach the first molt. We could not observe differences throughout development in any of

Table 2. LC50 values for adult and 2- to 4-day larvae of *Macrobrachium borellii* continuously exposed to WSF for 96 h

Time of exposure	LC50 (95% CL)		<i>p</i>
	Adult	Larvae	
24 h	>3.4 ^a	2.52 (2.11–>3.4)	ND
48 h	3.14 (2.23–>3.40)	1.73 (1.46–2.0)	<0.05
72 h	2.04 (1.24–3.24)	1.46 (1.13–1.48)	<0.05
96 h	1.56 (0.74–2.61)	1.41 (1.22–1.56)	NS

Note. Values are expressed as mg/L. NS, not significant; ND, not determined. *p*: Student *t*-test values.

^aNo mortality observed using 100% WSF test solutions.

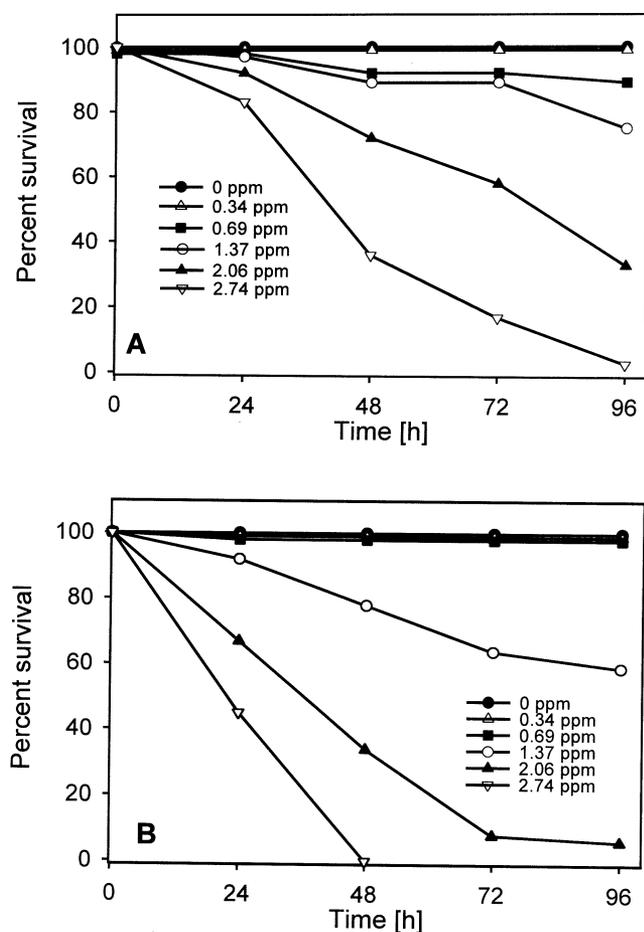


Fig. 1. Typical survival curves of adult (A) and postlarvae (B) exposed to different concentrations of WSF

the other variables measured including morphometry and overall morphology under optical microscopy. No teratogenic effects were observed.

Uptake and Release of Hydrocarbons

There was no mortality during the 96-h exposure. Lipid content in control and exposed samples did not show any significant differences and values were $1.5 \pm 0.03\%$ wet wt.

A pilot experiment to optimize conditions for sublethal ex-

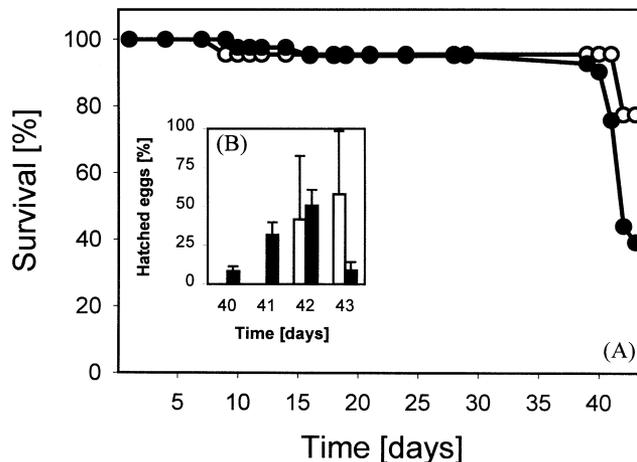


Fig. 2. Survival percentage of *Macrobrachium borellii* eggs throughout development until hatching (A) and percentage of hatched eggs per day (inset, B). ○ and open bars, control; ● and filled bars, WSF exposed

posure was performed, where adult prawns were exposed for a short time (24 h) to different concentrations of HC. It was observed that the accumulation was dose dependent, increasing in the range assayed (0–3.0 mg/L). At an exposure medium of 0.3 mg/L, tissue HC levels were $0.9 \mu\text{g/g}$, a bioconcentration factor on a wet weight basis (BCF) of 3. Based on this result and the LC50 values, adult *M. borellii* were exposed to a sublethal concentration of 0.3 mg/L for 96 h. HCs were rapidly taken up from the medium for the first 24 h, after which equilibrium was reached by the majority, and accumulation remained the same over the entire exposure period (Figure 3). Values after 24-h exposure ranged from 0.05 to $0.37 \mu\text{g/g}$ tissue, the highest concentration observed corresponding to propylbenzene and other C3 alkylbenzenes and the lowest to the higher molecular weight naphthalenes and methylnaphthalenes. Differences in the uptake rate constants were observed among HC (Table 3). In general, bioaccumulation increased along with the substitution and number of aromatic rings. Thus, BCF of methylnaphthalenes is two and one-half times higher as that for the one-ring benzene (Table 3). BCF of methylcyclohexane was unusually higher than that of aromatic HCs of equivalent molecular weight. As expected, there was a linear relationship between $\log K_{ow}$ and BCF ($\log \text{BCF} = 0.2084K_{ow} - 0.2597$; $R^2 = 0.65$; $n = 7$). Organisms showed selective properties concerning the accumulation of low molecular weight HCs (Table 3; discrimination factor).

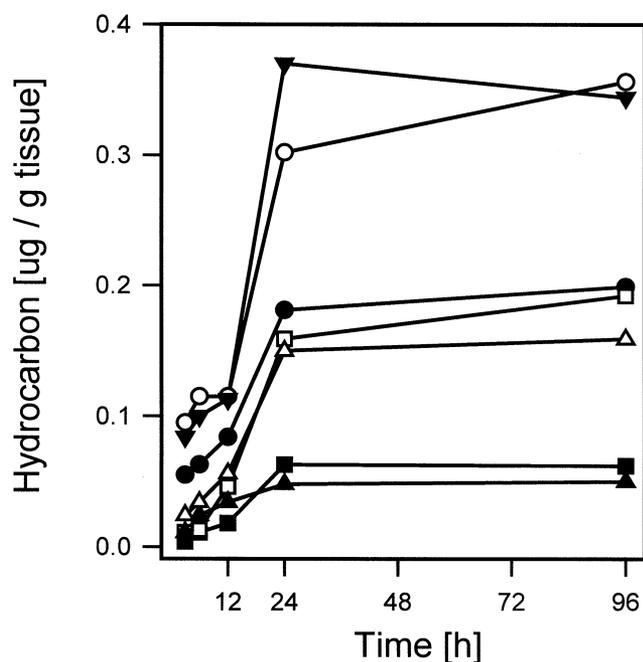


Fig. 3. Bioaccumulation of major hydrocarbons in *Macrobrachium borellii* exposed to 0.3 mg/L WSF for 96 h. (●) Benzene; (△) toluene; (○) ethylbenzenes + xylenes; (▼) propylbenzenes + C3 alkylbenzenes; (□) C4 alkylbenzenes; (▲) naphthalene; (■) methylnaphthalenes

After 96-h exposure, prawns were transferred to clean water and HC depuration from tissues followed for 10 d, showing a typical biphasic kinetics where the transport process takes place through the two-compartment model, where uptake and elimination by the body occur only through the fast or central compartment. Based on this model the rates constants were calculated by a graphical method (Spacie and Hamelink 1995). HCs were released at different rates in two phases as reflected by the elimination rate constants (Figure 4, Table 3). First, there was a rapid-release phase for the first 12 h, where HC loss accounted for more than 80% of the total amount accumulated. The second phase of depuration (between 24 h and 10 days) was slow. It is interesting to note that after 10 days of depuration, organisms were almost clean and had less than 0.07 $\mu\text{g/g}$ HCs. The half-life of all HCs was below 12 h and the depuration of individual HCs was related to their alkyl substitution and number of aromatic rings (Figure 4, Table 3). Benzene and toluene were mainly completely depurated in a single day and the rates of individual HCs sharply slowed down with increasing alkyl substitutions. The depuration of methylnaphthalenes was very slow, and after 10 days more than 40% was still present in prawn tissues.

Discussion

Toxicity of WSF to Adults and Larvae

Biological response to HC exposure is closely dependent upon precise exposure conditions, which makes generalizations and comparisons from different laboratories difficult (Kühnhold

1977). It is essential to measure not only the WSF concentration in the water and in the organism, but also the HC compositions. To avoid WSF changes in the concentration and composition that occur during storage (Heras *et al.* 1995), two batches of WSF were prepared for the 4-day experiments, so that it was never stored for more than 36 h. Light aromatic HCs of molecular weight lower than that of naphthalene, especially benzene, toluene, xylenes, and dimethyl and trimethyl benzenes, were the main HC components. The physicochemical properties of monoaromatic hydrocarbons (MAH) render them more soluble in fresh water than in seawater, therefore stock WSF was expected to be more concentrated than the seawater counterpart (Whipple *et al.* 1981).

PL were significantly more sensitive to the WSF than adults at shorter times. In fact all PL died after 48 h at high WSF concentrations. However, 96-h LC50 values were not significantly different between adults and PL, agreeing with observations on other aquatic invertebrate species (Rice *et al.* 1977). Higher 96-h LC50 values were reported for other palaemonidae such as *Palaemonetes pugio* adults (3.5 mg/L) and *Penaeus aztecus* larvae (4.9 mg/L) (Anderson *et al.* 1974). However, those results were obtained employing a different parent oil and quantitation and WSF preparation method, and it is known that the toxicity of an oil is a function of its mono-, di-, and triaromatic HC content, which depends on the parent oil composition (Kühnhold 1977). Therefore, values should be carefully extrapolated from other similar oils, taking into consideration that the way of applying the oil in the experiments also varies. The test medium composition affects the final WSF composition; therefore the 96-h LC50 values are difficult to compare with WSF prepared using other crude oils. Toxicity of WSF to *M. borellii* was also less than that reported for larvae of *P. pugio* exposed to No. 2 fuel oil, which agrees with the fact that refined oils have larger quantities of moderately volatile aromatic compounds that contain the most toxic and persistent WSF components of petroleum oils. Given the LC50 determined, these prawn are normally exposed to sublethal concentration in their environment (Colombo *et al.* 1989), but in the weeks following an oil spill, hydrocarbon concentrations can reach several times this level (Gallego *et al.* 1995; Ritchie and O'Sullivan 1993). Different life strategies of the organism assayed also affect the toxicity. For example, *P. pugio*, compared with *M. borellii*, has smaller eggs, hatching as small planktrophic larvae after 12 days, and this may also account for the higher sensitivity of *P. pugio* PL (Fisher and Foss 1993; Hartman 1992), reflecting the relatively larger surface area-to-volume ratio and, hence, relatively high pollutant uptake, as well as different physiology, in smaller and less developed larvae.

Toxicity of WSF to Embryos

Embryos were tested at stages 5–7, as these developing stages are being reported to have a high metabolic rate and the greatest vitellus lipid consumption (Gonzalez Baró *et al.* 2000; Heras *et al.* 2000), and they were, therefore, expected to be most affected by WSF as lipids are the main energy source during embryogenesis. However, survival of embryos acutely exposed to WSF was not affected by the contaminant compared

Table 3. Major hydrocarbon bioconcentration^a (BCF), discrimination factor^b (DF), and rate constants^c after exposure of *Macrobrachium borellii* adults to 0.3 mg/L WSF for 96 h followed by 10 days of depuration

Hydrocarbon	BCF	DF	logK _{ow}	k ₀₁	k ₂₁	k ₁₀
Benzene	1.4	0.7	2.13	0.2999	-0.1627	-0.0227
Toluene	2.9	1.4	2.73	0.2552	-0.0946	-0.0102
Ethylbenzene + xylenes	1.8	0.9	3.15	0.3434	-0.0991	-0.0013
Propylbenzene + C3 Alkylbenzenes	2.5	1.2	3.65	0.3333	-0.1340	-0.0047
C4 alkylbenzenes	4.5	2.2	4.04	0.1857	-0.1097	-0.0002
Naphthalene	3.0	1.5	3.30	0.2300	-0.1315	-0.0024
Methylnaphthalenes	3.6	3.5	3.87	0.1736	-0.0032	-0.0010

Note. C3 alkylbenzenes: several C3 and C1 × 3 and C2 + C1 benzenes. C4 alkylbenzenes: see Table 1 for references.

^a Calculated as the ratio of hydrocarbon concentration in tissue divided by hydrocarbon concentration in exposure water.

^b Discrimination factor was calculated as the ratio of the individual hydrocarbon concentration to the total hydrocarbon concentration in tissue divided by the equivalent ratio in the exposure water.

^c Calculations by a graphical method from the biphasic kinetics model: k₀₁, uptake rate constant of the HC from water; k₂₁, elimination rate constant of the HC from the "slow" to the "fast" compartments; k₁₀, elimination rate constant of the HC from the "fast" compartment to the medium. All k values in ng/g h⁻¹.

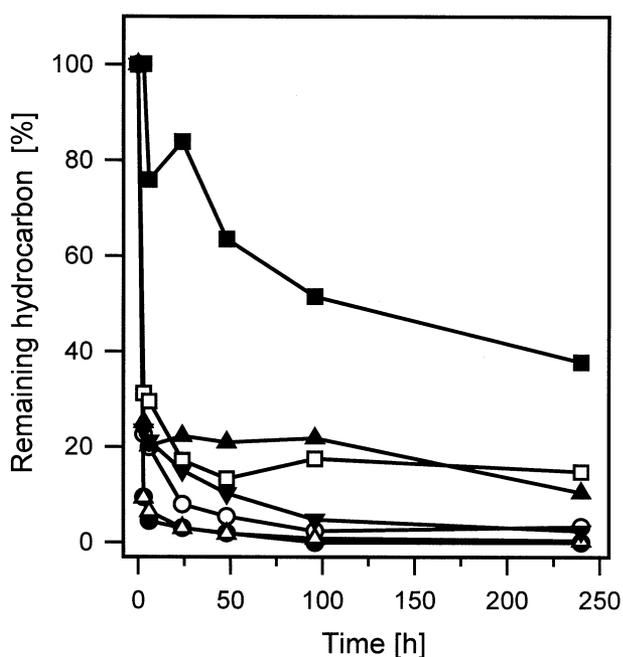


Fig. 4. Biodepuration of major hydrocarbons followed for a 10-day period in adult *M. borellii* previously exposed to 0.3 mg/L WSF for 4 days. See Figure 3 for references

to the control group. It is generally assumed that some crustacean eggs are more tolerant than other life stages to oil exposures (Rice *et al.* 1977), probably due to the protective effect of the egg coat (Rodríguez and Pisanó 1993). Nevertheless, a chronic exposure of eggs to WSF from oviposition until hatching showed an increase in mortality in the WSF-treated embryos compared to the control group, especially just before or during the hatching process. This may be due to the fact that the embryonic coat restricts the entry of the chemicals until later stages, as occurs in *P. pugio* (Rayburn and Fisher 1997). Moreover, Glas *et al.* (1997) demonstrated a rapid increase in permeability of *P. pugio* embryonic coat 2–3 days before

hatching. *M. borellii* belongs to the same family and it may be possible that the slower development of the latter gives the embryo the opportunity to induce some protective mechanism altering the permeability of the coat. The chronically exposed eggs showed a trend to hatch earlier than the control organisms. Moreover, hatching survival of exposed PL was six times less than that of controls, many prawns dying with the egg coat attached to the body, and from this reduced group, only 5% of those exposed survived the stress of the first molt. The exposure to WSF somehow affects the embryos in such a way that they hatch earlier and are not capable of surviving for a long period of time after hatching.

Hydrocarbon Uptake and Release

Petroleum HCs in crustaceans have no biological functions, hence their deposition in different compartments is only determined by their lipid:water partition coefficients and diffusion processes (Neely *et al.* 1974) and not by biological selectivity. Therefore, the kinetics of bioaccumulation and release from prawn tissues is mainly controlled by the physicochemical properties of the cells and the flow of hemolymph around them. Membranes are a key factor in the partitioning of HCs (Boryslawskyj *et al.* 1988), diffusion probably being the major limiting step controlling the accumulation rate.

Based on the LC50 value, we selected a sublethal, realistic WSF concentration and exposed the prawn for a short period of time. The WSF composition in the exposure flasks was reflected in the HCs taken up by the prawn after 1-day exposure. The GLC analysis showed that the aromatic HCs accounted for almost 60% of the total HCs bioconcentrated in prawn after the 4-day exposure, though in different proportions as reflected by the individual HC BCF. Aromatic HC level peaked after 1 day readily and rapidly bioaccumulated from water, probably via the gills. When prawns were returned to HC-free freshwater, the lipid:water partition favored a more gradual release of HCs from two tissue compartments (lipid-poor and lipid-rich) to the water. The pool of low molecular weight HCs was responsible for the rapid decrease in HCs observed for the first 12 h of

deuration (Figure 4). They were probably transported from a lipid-poor compartment by hemolymph to gills or integument for excretion or to hepatopancreas to be metabolized (Stegeman and Hahn 1994). The lean muscle of cod and scallops is a compartment very similar to this compartment. The rapid uptake (minutes–hours) and relatively fast deuration (few days) of the HCs of this compartment in those organisms confirm its characteristics (Ernst *et al.* 1987, 1989). Lipid-rich compartments, on the other hand, played the most important role in the uptake and long-term release of HCs, especially the more hydrophobic HCs that showed a slow release during deuration. Lipidic compartments probably became the only storage site for the remaining HCs after less than 4 days of deuration.

We also observed that increasing the number of aromatic rings and substitutions on the rings favored the bioaccumulation of individual HCs and decreased their deuration rate constants. In addition, there is a positive relationship between the BCF and the K_{ow} of the compounds, indicating that lipid-based bioconcentration factors increase with hydrophobicity and that K_{ow} is a good descriptor of bioconcentration as observed in other aquatic organisms (Baussant *et al.* 2001). The selective accumulation of HCs is the result of the combined effects of detoxification metabolism and diffusion of individual HCs according to their solubility in water. HC deuration is usually slower than xenobiotics already containing functional groups, though crustaceans generally metabolize HCs faster than other invertebrates such as mollusks (Livingston 1998). Polycyclic aromatic HCs (PAH) such as methyl-naphthalenes were still present in samples after 10 days in HC-free water. A similar result has been observed in mussel and fish (Baussant *et al.* 2001). Generally, in acute bioassays, the PAH appears to be more toxic and more persistent in tissues than the MAH (Whipple *et al.* 1981). The effect of molecular size of aromatic HC molecules (increased number of aromatic rings or substitution in aromatic rings) has been known to increase toxicity (Thomas and Rice 1981). The accumulation and retention of parent compound and metabolites by coho salmon also increase in this fashion (Zhou *et al.* 1997).

From this study we conclude that adult prawns have a rapid uptake and release of most of the WSF contaminating HCs, this fast process probably being related to their low lipid levels. An increase in the number of aromatic rings and substitutions on the rings favored the bioaccumulation of individual HCs and decreased their deuration rates. Early life stages of *M. borellii* are not very vulnerable to WSF. The lower sensitivity of PL and eggs to WSF compared to that of other crustaceans may be related to their developmental strategy. The direct development of this species has the advantage of (a) increasing the volume-to-surface ratio of PL and eggs alike (decreased permeability) and (b) increasing the period of probable protection within the egg coat. This strategy may have evolved to facilitate the colonization of the freshwater environment by diminishing osmotic stress (Anger 1996) and is probably secondarily helping this species to cope with contaminants. Further comparative work is needed to evaluate these possibilities.

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