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Effect of the water-soluble fraction of petroleum on microsomal lipid metabolism of *Macrobrachium borellii* (Arthropoda: Crustacea)

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Abstract

The effect of the water-soluble fraction of crude oil (WSF) on lipid metabolism was studied at critical metabolic points, namely fatty acid activation, enzymes of triacylglycerol and phospholipid synthesis, and membrane (lipid packing) properties in the freshwater prawn *Macrobrachium borellii*. To determine the effect of the contaminant, adults and embryos at different stages of development were exposed to a sublethal concentration of WSF for 7 days.

After exposure, microsomal palmitoyl-CoA synthetase (ACS) showed a two-fold increase in adult midgut gland. Embryo's ACS activity was also affected, the increment being correlated with the developing stage.

Endoplasmic reticulum acylglycerol synthesis was also increased by WSF exposure in adults and stage 5 embryos, but not at earlier stages of development. Triacylglycerol synthesis was particularly increased (18.5%) in adult midgut gland.

The microsomal membrane properties were studied by fluorescent steady-state anisotropy, using the rotational behavior of the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH). Microsomes from midgut gland of WSF-exposed prawn showed no differences in fluidity. Nevertheless, microsomes incubated with WSF *in vitro* increased their fluidity in a temperature- and WSF concentration-dependent fashion. Both, aliphatic and aromatic hydrocarbons individually tested elicited an increase in membrane fluidity at 10 mg/l, but at 4 mg/l only *n*C10–C16 aliphatics did.

In vivo results indicate that WSF increased the activity of microsomal enzymes that are critical in lipid metabolism, though this change was not due to direct alterations in membrane fluidity, suggesting a synthesis induction, or an enzyme-regulatory mechanism. Nevertheless, hydrocarbons elicited membrane fluidity alterations in *in vitro* experiments at concentrations that could be found in the environment after an oil spill.

Keywords: Prawn; Crustacea; Toxicity; WSF; Hydrocarbon; Lipid metabolism; Membrane fluidity; Acyl-CoA synthetase; Acylglyceride; Pollution; ACS; Palmitoyl-CoA; Invertebrate

1. Introduction

The water-soluble fraction of petroleum hydrocarbons (WSF) is generated by oil spills or industrial discharges, and it is responsible for the toxic and carcinogenic effects of crude oil on aquatic biota (GESAMP, 1993). This complex mixture of environmental contaminants preferentially accumulates in membrane lipids and other lipidic compartments (Di Toro et al., 2001); thus it could disturb the physicochemical and physiological properties of membranes causing toxic effects (Sikkema et al., 1994). Changes in the composition and packing of vertebrate membranes can cause changes in enzymatic and receptor activities (Castuma and Brenner, 1983; Spector and Yorek, 1985). Moreover, previous studies from our laboratory have shown that microsomal membranes exposed *in vitro* to a lipophilic pesticide significantly altered lipid packing in *Macrobrachium borellii* (González Baró et al., 1997). It was, therefore, interesting to explore whether WSF could affect enzymatic characteristics in crustaceans as there are indications that these changes are also present in aquatic organisms. For example, mollusks exposed to polyaromatic hydrocarbons (PAH) showed an increase in membrane permeability and a decrease in certain enzymatic activities (Moore and Farrar, 1985). Freshwater crustaceans are currently employed to monitor the environment pollution status because of their advantageous features: they are the major invertebrate component in most aquatic ecosystems; their populations are often numerous and they are easily cultured in the laboratory

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(Shigehisa and Shiraishi, 1998; Fossi et al., 2000; Gerhardt et al., 2002). Nevertheless, little work has been done on the alterations in lipid metabolism and energetics elicited by petrogenic hydrocarbons in these arthropods (Capuzzo et al., 1984; Wang and Stickle, 1988).

Though there are studies on the effect of hydrocarbons on microsomal membrane biochemistry in mollusks and crustaceans, all of them are focused on the use of detoxification enzymes as biomarkers, especially the monoxygenase system dependent on cytochrome P450 (Livingstone et al., 1985; Moore et al., 1987; García Martinez and Livingstone, 1995; Solé et al., 1996). There are no available reports dealing with the effect of hydrocarbons on membrane-enzymes involved in lipid anabolism.

The aim of our work was to study the effect of WSF on microsomal lipid metabolism, focusing on fatty acid activation, enzymes of triacylglycerol (TAG) and phospholipid (PL) synthesis as well as membrane structural properties in the endoplasmic reticulum.

2. Materials and methods

2.1. Sample collection

Adults of *M. borellii* were sampled in spring and summer (October–February) in an uncontaminated watercourse close to the Rio de La Plata river, Argentina (20 km SW from La Plata). They were taken to the laboratory and kept in dechlorinated tap water at $22 \pm 2\,^{\circ}C$, and 14:10 h L:D photoperiod for at least a week before experiments (Collins and Petriella, 1999). Eggs were removed from the pleopods of ovigerious females and checked under a stereoscopic microscope to determine the stage of development (Lavarías et al., 2002).

2.2. Test media

Punta Loyola light crude oil, obtained from Santa Cruz, Argentina, stored at 4 °C, was used to prepare the water-soluble fraction. The procedure used was a modification of that applied by Heras et al. (1992). Crude oil was stirred in a 10 l stainless steel mixing vessel equipped with a mechanical stirrer, a bottom drain, and kept in a cold room at 4 °C. Crude oil and freshwater in a ratio of 1:100 (v/v) were stirred for 24 h and allowed to settle for additional 48 h (Lavarías et al., 2004). During experiments, fresh WSF batches were prepared every 2 days to avoid changes in hydrocarbon composition by volatilization (Heras et al., 1995), and a sample was extracted and analyzed by gas liquid chromatography (GLC) with a Hewlett Packard HP6890 capillary gas chromatograph equipped with a flame ionization detector (FID) following the method described in (Lavarías et al., 2004). Typical WSF hydrocarbon concentration was 3.4 mg/l in the stock solution. The detailed WSF composition has been previously reported (Lavarías et al., 2004).

2.3. Adult and embryo toxicity tests

Assays were performed using a sublethal concentration of WSF as determined previously for this prawn (Lavarías et al., 2004). Embryogenesis lasts 39 days in this prawn, and it has been divided into 7 stages according to major morphological characteristics; stages 4 and 5 evidenced the major yolk consumption and enzymatic activity (Heras et al., 2000; González Baró et al., 2000; Lavarías et al., 2002).

Groups of 8 adults (56–60 mm long, 1.6–2.0 g weight, approx.) or 6 ovigerous females carrying eggs at developmental stage 4 or 5 in their pleopods (Lavarías et al., 2002) were exposed for 7 days to a sublethal level of WSF (0.6 mg/l) at $22 \pm 2\,^{\circ}C$ and a 14-h light:10-h dark cycle. Prawns were kept in a 2.5 l aquarium with the lid sealed to avoid hydrocarbon loss, with daily change of media. Control groups were kept in clean water. Temperature, pH, and dissolved oxygen were measured in the control containers (Lavarías et al., 2006).

2.4. Preparation of microsomal fractions

After a week exposure to WSF, egg clutches and midgut gland from adults were immediately removed, weighed and cooled on ice. They were homogenized in 0.25 mol l^{-1} sucrose containing 1.4 mmol l^{-1} *N*-acetyl-L-cysteine, 0.15 mol l^{-1} ClK, 5 mmol l^{-1} $MgCl_2$, 0.4 mmol l^{-1} EDTA and 62 mmol l^{-1} potassium phosphate buffer, pH 7.4 by using a glass-Teflon potter homogenizer (Thomas, Philadelphia, PA) as described by González Baró et al. (2000). The crude homogenate was centrifuged at 10,000 × *g* for 20 min in a Sorvall RC-2 (Newtown, CT) refrigerated at 4 °C using an SS-1 rotor to remove egg envelopes, cellular debris, nuclear fraction and mitochondrial fraction. The supernatant was then centrifuged at 100,000 g for 50 min at 4 °C in a Beckman L8M ultracentrifuge (Beckman, Palo Alto, CA) using a Ti 70.1 rotor to obtain the microsomal pellet. Total protein was determined by colorimetry using BSA as standard (Bradford, 1976).

2.5. Long-chain fatty acyl-CoA synthetase assay

The procedure used was a modification of that applied by Singh et al. (1988). The reaction mixture in a total volume of 0.5 ml contained 0.18 μCi (6.4 μmol l^{-1}) [1-^{14}C] palmitic acid in the presence of cofactors and conditions detailed in a previous work (González Baró et al., 1990). The reaction was started by the addition of microsomes (60 μg protein ml^{-1} of reaction mixture), and it was incubated at 32 °C for 10 min. Afterwards, the reaction was stopped with a mixture of isopropylic alcohol/*n*-heptane/H_2SO_4 (200:50:5, v/v/v) (Dole, 1956), a partition was produced and the lowest phase was washed with *n*-hexane to remove free fatty acids. Palmitoyl-CoA was quantified in the aqueous phase in a Pharmacia LKB Wallac 1219 Rackbeta liquid scintillation counter (Uppsala, Sweden).

2.6. Biosynthesis of acylglycerols

Ten nanomoles (0.57 μCi) of [1-^{14}C] palmitic acid were incubated with microsomes (400 μg protein ml^{-1} of reaction mixture) in a total volume of 0.5 ml at 32 °C for 60 min as optimized for this prawn previously (González Baró and Pollero,

1993; González Baró et al., 2000). The reaction was stopped by the addition of lipid extraction solvents; lipids were separated by high performance thin layer chromatography (HPTLC) on silica gel 60 plates (Merck, Darmstadt, Germany) with hexane–ethyl ether–acetic acid (80:20:1.5, v/v/v), and the radioactivity associated with each lipid class was quantified by proportional scanning counting in a Berthold LB2723 apparatus (Wildbad, Germany).

3. Fluorescent measurements

Fluorescence anisotropy measurements are useful techniques that provide information on the rigidity of molecular environments that can be used to measure the fluidity of biological membranes (Lakowicz, 1991).

Measurements were made in a Perkin-Elmer LS55 luminescence spectrometer with a polarization accessory (Norwalk, CT, USA). Samples were prepared using 67 μg microsomal protein/ml in 50 mmol l^{-1} potassium phosphate buffer, pH 7.4. For labeling, samples were mixed with a few microliters of concentrated DMSO solution of either 1,6-diphenyl-1,3,5-hexatriene (DPH) (final concentration 2 μmol l^{-1}) or 3-(*p*-(6-phenyl)-1,3,5-hexatrienyl) phenylpropionic acid (DPH-PA). Blanks were prepared similarly but without the fluorescent probe, adding the same volume of DMSO to correct for scattering and non-specific fluorescence. Samples were gently swirled at 20 °C in the dark for at least 2 h to allow a complete equilibration of DPH with the microsomal lipid bilayer. Before starting the final assays the quenching effect of WSF on DPH was tested. Fluorescence intensity was compared between the ethanol solution containing the probe alone and the probe with different WSF concentrations. No differences in the intensity pattern were observed between them. Experiments were performed using either microsomes from the *in vivo* exposure assays or control microsomes incubated with two concentrations of WSF (1.3 and 3.2 mg/l) and several individual WSF components dissolved in ethanol added to samples prior to equilibration (see Table 3). Samples were measured at 10, 20 and 30 °C which are within the range that can be usually found in the habitat of this prawn.

Steady-state anisotropy (r_s) was measured according to Lakowicz et al. (1979) with modifications (Garda et al., 1994; Tricerri et al., 1994). The excitation wavelength was 361 nm, and the emitted light passed through a sharp cut-off filter (Schott K V 389) to eliminate light of wavelengths below 389 nm.

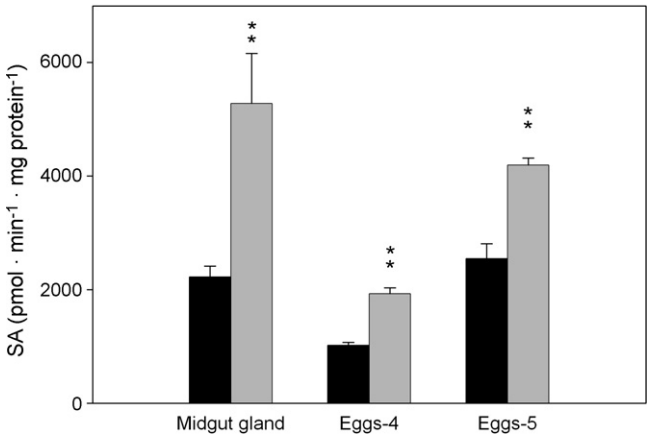

Fig. 1. Effect of WSF on the activity of microsomal palmitoyl-CoA synthetase (ACS) in adult midgut gland and embryos of *Macrobrachium borellii* after 7-day exposure to 0.6 mg/l WSF. (■) Control; (▭) WSF exposed. Values represent the mean of three determinations ±1 S.D. Bars marked on top are significantly different ** ($p < 0.001$). Eggs-4: stage 4 embryos; Eggs-5: stage 5 embryos. SA: specific activity.

The steady state fluorescence anisotropy was calculated as:

$$\mathbf{r}_s = \frac{\mathbf{I}_{\parallel} - \mathbf{G} \cdot \mathbf{I}_{\perp}}{\mathbf{I}_{\parallel} + 2\mathbf{G} \cdot \mathbf{I}_{\perp}}$$

where: $\mathbf{I}_{\parallel} = \mathbf{I}_{vv}$ and $\mathbf{I}_{\perp} = \mathbf{I}_{vh}$.

$\mathbf{G} = \mathbf{I}_{hv}/\mathbf{I}_{hh}$, is a correction factor for the monochromator transmission efficiency for vertically and horizontally polarized light. The value is given by the ratio of the fluorescence intensities of the horizontal to vertical components when the exciting light is polarized in the horizontal direction.

3.1. Statistical analyses

Data were analysed either by Student's *t*-test or analysis of variance using Instat v.2.0. Results were considered significant at 5% level.

4. Results

4.1. Effect of WSF on microsomal enzymes

After 7-day exposure to WSF, adult and embryo prawns showed significant increases in microsomal ACS activity. Embryos at stage 4 of development showed 89% increase, while at stage 5 the increase was 64% (Fig. 1). Microsomes from

Table 1
Synthesis of lipid classes in midgut gland and eggs from *Macrobrachium borellii* after 7-day exposure to 0.6 mg/l WSF (% radioactivity incorporation)

	Midgut gland		Eggs-4		Eggs-5	
	Control	WSF	Control	WSF	Control	WSF
Triacylglycerol	45.4 ± 3.8	53.8 ± 1.5*	18.8 ± 1.2	21.9 ± 4.4	18.9 ± 2.2	18.5 ± 3.2
Phospholipid	13.2 ± 2.5	10.7 ± 2.7	55.3 ± 3.7	52.3 ± 13.7	52.8 ± 10.1	45.0 ± 9.3
Sterol esters	6.9 ± 0.5	6.9 ± 1.0	Tr	Tr	Tr	Tr
Mono and diacylglycerol	34.4 ± 4.5	28.7 ± 1.2	29.9 ± 2.8	25.8 ± 16.0	30.3 ± 7.9	34.6 ± 6.2

Values are the mean of three determinations ±1 S.D.
* Significant ($p < 0.05$). Tr: trace. Eggs-4: stage 4 embryos; Eggs-5: stage 5 embryos.

adult midgut gland showed almost twice the increase observed in embryos (137%) compared with the control group (Fig. 1).

The incorporation of palmitic acid into acylglycerols is shown in Table 1. After 1 h incubation with radiolabeled palmitic acid, this precursor was noticeably incorporated into different microsomal lipids according to the life stage analyzed. In adult midgut gland, most of the label was found in triacylglycerol (TAG) while that of eggs was incorporated mainly into phospholipids (PL). When we analyzed if the WSF exposure modified the precursor incorporation into different lipids, significant differences in adult midgut gland were observed, particularly the TAG fraction was increased at the expense of the other lipids. On the contrary, eggs did not change their pattern of incorporation into lipid classes (Table 1).

Besides, the specific activity of the enzymatic systems of acylglycerol synthesis was affected at both life stages. Adults increased acylglycerol synthetic activity by 12% in midgut gland of exposed organisms as compared to control. On the other hand, while embryos at stage 4 did not show significant differences, stage 5 embryos showed the highest rate of incorporation, which increased by 61% compared to that of non-exposed organisms (Fig. 2).

4.2. *Effect of WSF on physical properties of microsomal membranes*

When the effect of the WSF on midgut gland microsomal membranes was analyzed, no significant changes in the steady-state anisotropy (r_s) of DPH between control and WSF-exposed prawn microsomes were observed. Microsomes were not significantly affected by temperature within the tested range either (Table 2).

In those assays where control microsomes were exposed *in vitro* to different WSF concentrations, the presence of hydrocarbons caused an increase in the fluidity of the lipid environment of the probe, as shown by the significant decrease in the r_s values (Fig. 3). Results showed that changes in anisotropy were temperature- and WSF concentration-dependent. Changes in r_s for the assay at the lowest temperature were the most marked ones (about 10%). To further localize the effect of hydrocarbons in the membrane, we also performed an assay using the fluorescent probe DPH-PA, the propionic acid derivative of DPH. This amphipathic probe locates in a region different to that of DPH,

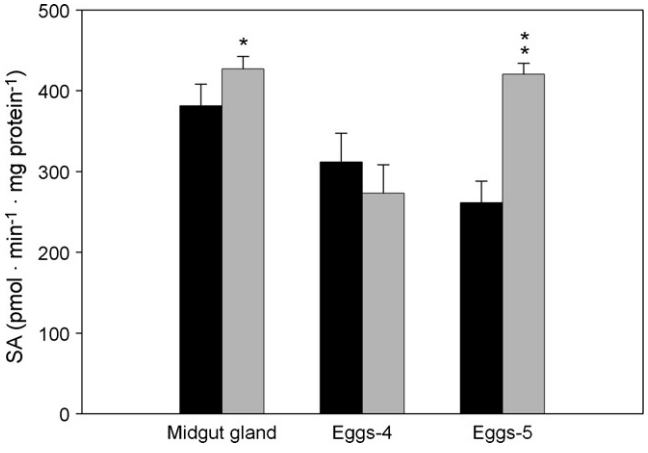

Fig. 2. Effect of WSF on acylglycerol synthesis activity in adult midgut gland and embryos of *M. borellii* after 7-day exposure to 0.6 mg/l WSF. (■) Control; (□) WSF exposed. Values represent the mean of three determinations ±1 S.D. Bars marked on top are significantly different * ($p < 0.05$); ** ($p < 0.001$). Eggs-4: stage 4 embryos; Eggs-5: stage 5 embryos. SA: specific activity.

Table 2
DPH fluorescence anisotropy in midgut gland microsomes obtained after 7-day exposure of *M. borellii* to 0.6 mg/l de WSF

Temperature (°C)	Control	WSF	*p*
10	0.189 ± 0.040	0.186 ± 0.040	NS
20	0.164 ± 0.032	0.170 ± 0.039	NS
30	0.148 ± 0.023	0.154 ± 0.040	NS

Values are the mean of three determinations ±1 S.D. NS: not significant at $p < 0.05$ level.

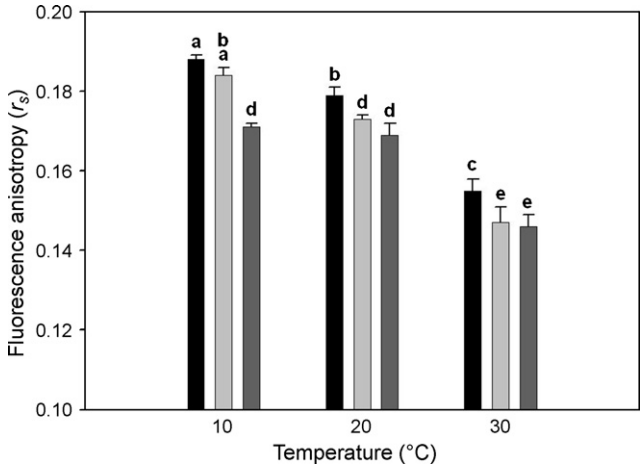

Fig. 3. Fluorescence anisotropy of DPH in microsomes from *M. borellii* midgut gland treated *in vitro* with different WSF levels. (■) Control; (□) 1.3 mg/l WSF; (▒) 3.2 mg/l WSF. Values represent the mean of three determinations ±1 S.D. Bars holding different letters on top have significantly different values ($p < 0.05$).

Table 3
Decrease of DPH fluorescence anisotropy in midgut gland microsomes from *M. borellii* treated *in vitro* with different levels of the major hydrocarbons classes present in WSF

Hydrocarbon	r_s decrease (%)	
	4 mg/l	10 mg/l
Xylene	<1%	6.6 ± 1.1*
Toluene	<1%	12.5 ± 0.6*
Naphthalene	<1%	8.8 ± 2.5*
Benzene	<1%	12.8 ± 3.4*
Aliphatics (a)	27.7 ± 9.7*	29.1 ± 4.9*
Hydrocarbon mix (b)	22.9 ± 10.0*	31.4 ± 2.9*

Microsomes were preincubated at 20 °C with 4 mg/l and 10 mg/l hydrocarbons for 2 h. Values represent the mean of three determinations ±1 S.D. (a) Aliphatics: an equimolar mixture of *n*C10–*n*C16; (b) hydrocarbon mix: an equimolar mixture of naphthalene, toluene, benzene, ethylbenzene, *o*-xylene, *m*-xylene, *n*-propylbenzene, isopropylbenzene, 1,2,3-trimethylbenzene, 1,2,4-trimethylbenzene, 1,2,3,4-tetramethylbenzene, *o*-diethylbenzene, *m*-diethylbenzene, *p*-diethylbenzene; even-number *n*C10–*n*C22.

* Significant ($p < 0.05$).

aligning its carboxylate group with the PL's polar head. Unlike DPH, the r_s values obtained using this probe were not changed by WSF treatment.

Considering that WSF is a complex mixture of hydrocarbons (Lavarías et al., 2004) we performed a series of experiments to discriminate the influence of its major components on membrane fluidity. Table 3 shows that all the hydrocarbons at 10 mg/l significantly reduced the r_s, but when microsomes were exposed to 4 mg/l, only short aliphatic hydrocarbon and the mixture of aliphatic, mono and diaromatic did.

5. Discussion

5.1. Effect of WSF on microsomal enzymes

WSF exposure affected both the lipid synthetic pathways of prawn (present paper) and the catabolic ones (Lavarías et al., 2006). ACS activity increased at all the life stages studied probably due to enzymatic induction (see below). It is known that long-chain fatty acids could be channeled into pathways of β-oxidation or the acylglycerol synthesis and storage; the fate is globally regulated by the balance between energy intake and expenditure. As we found that mitochondrial and microsomal ACS activities are increased by WSF (Lavarías et al., 2006), we may suggest that in *M. borellii*, regardless of the subcellular location of the long-chain ACSs, synthesized acyl-CoA would be incorporated into a common cytosolic pool from which they are possibly channeled not only into acyglycerol synthesis, but also towards the degradative pathway of mitochondrial β-oxidation, as both pathways are increased simultaneously. This is also supported by the fact that the increase in microsomal ACS activity is higher than that of the mitochondrial ACS isoform which would be the natural candidate to increase its activity in an energy-demanding situation such as WSF-exposure (Lavarías et al., 2006). This long-chain acyl-CoA cytosolic pool has been postulated in insects but without any experimental data (Haunerland, 1997). If this arthropod mechanism is confirmed, it will contrast with mammals where the current knowledge suggests that the fate of a particular acyl-CoA depends on which ACS isoform catalyzed its synthesis (Coleman et al., 2002).

ACS could also conjugate hydrocarbons with CoA in crustaceans subjected to hydrocarbon pollution. This has been evidenced by studies on the xenobiotic detoxification metabolism in mammals, that have shown that liver microsomal long-chain ACS can catalyze the conjugation of CoA with carboxylic-containing toxicants (Watkins, 1997; Knights and Drogemuller, 2000). Considering that hydrocarbon detoxification mechanisms in crustaceans involve the formation of carboxylated products (Di Giulio et al., 1995; Livingstone, 1998), they are likely to be conjugated with CoA by the activated microsomal ACS, and then used as an alternative substrate and channeled into the TAG synthesis pathways as suggested in mammals (Sallustio et al., 1988; Dodds, 1991).

The simultaneous increase of the synthesis and degradative pathways would reflect an active exchange of lipids under stress, possibly to keep metabolic homeostasis (Rafi et al., 1991). Midgut gland is very active in lipid metabolism, where TAG synthesis is the prevailing anabolic pathway in adults (González Baró and Pollero, 1993). This route was significantly increased by exposure to WSF. On the other hand, the labeled precursor was incorporated preferentially into PL in embryos at stages 4 and 5, because at these developing stages there is a great demand for these lipids for membrane synthesis and organogenesis (González Baró et al., 2000). This differential incorporation related to life-stage agreed with the PL/TAG ratio that diminished in adult midgut gland and increased in eggs due to WSF exposure (Lavarías et al., 2005).

5.2. Effect of WSF on physical properties of microsomal membranes

To determine if differences in microsomal enzyme activities could be attributed to differences in microsomal membrane structure, some of its physical properties were studied. We observed that microsomes obtained from organisms exposed *in vivo* to WSF showed no fluidity changes in the membrane, a fact similar to that reported in microsomes of the same species exposed to the lipophilic pesticide fenitrothion (González Baró et al., 1997). We have previously shown that prawn hydrocarbon concentration exposed to a constant concentration of WSF achieves equilibrium with the surrounding water within 24 h, reaching the maximal hydrocarbon concentration attainable in its tissues (Lavarías et al., 2004). Considering that these prawns in their environment could be exposed to higher hydrocarbon concentrations (Colombo et al., 1989), the effect of elevated concentrations was tested using *in vitro* experiments where the effect of aromatic as well as aliphatic hydrocarbon on membranes was studied. Both hydrocarbon groups triggered changes on membrane properties. Moreover, after an oil spill, hydrocarbon concentrations can reach several times the *in vivo* exposure levels tested (Ritchie and O'Sullivan, 1994; Gallego et al., 1995), and they are within the range of the hydrocarbon levels selected for the *in vitro* assays. We can thus suggest that the 0.6 mg/l WSF used for the *in vivo* experiments was not enough to alter membrane structure, causing undetectable changes in membrane fluidity. Besides, the effect produced by hydrocarbon on microsomes *in vitro* was dependent on the hydrocarbon class assayed, being the effect of aliphatics more fluidifying than that of the aromatic ones. In this regard, Sikkema et al. (1994) showed that the accumulation of cyclic hydrocarbon in artificial membranes led to a fluidity increase evoked by a surface area increase. These results confirmed the findings in other biological membranes where there was a strong correlation between the lipophilicity of a compound and its accumulation, which after a critical concentration affected membrane integrity and functioning. Moreover we have previously shown that aromatics are rapidly lost after 24 h enriching the WSF in alkanes (Heras et al., 1995).

Hydrocarbons in lipid bilayers are located in the inner region in contact with the hydrocarbon chains of lipids. Several studies have reported this location for aromatic as well as for aliphatic hydrocarbon (McIntosh et al., 1980; White et al., 1981; Sikkema et al., 1995). Due to this fact, DPH-PA did not show any changes in r_s values, as this probe senses the most external region of lipid bilayers. Microsomes of this prawn exposed to lipophilic xeno-

biotics of higher polarity behaved differently, showing changes in the membrane packing in the inner (DPH-sensed) as well as in the outer (close to the polar heads, DPH-PA-sensed) regions, supporting the present findings (González Baró et al., 1997). Moreover, some studies have shown that the major changes in membrane properties were caused by small aliphatic hydrocarbons (such as hexane in comparison with the aromatic ones), that could increase the thickness of the bilayer (White et al., 1981). This agrees with our results, as aliphatic hydrocarbon caused the major fluidifying effect in *M. borellii* microsomes.

Regarding aromatics, *m*-xylene showed subtle but measurable increases in microsomal membrane fluidity. This contrast with a microsomal membrane fluidity study in rats orally supplied with *m*- and *p*-xylene where only the *p*-xylene-ingested group showed a decrease in fluidity (Stickney et al., 1989).

Therefore, we can conclude that *in vivo* exposure to WSF increased the activity of microsomal enzymes assayed in adult prawn. This effect would not result from a direct alteration in membrane fluidity, but probably from either an induction of enzyme synthesis or a regulatory mechanism. In fact, induction mechanisms in response to hydrocarbons have been suggested for invertebrate detoxification enzymes (Livingstone, 1998). Ongoing research deals with the effect of WSF on the antioxidant defense system to gain further knowledge about the biochemical effect of hydrocarbon pollution on crustaceans.

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References

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–274.

Capuzzo, J.M., Lancaster, B.A., Sasaki, G.C., 1984. The effects of petroleum hydrocarbons on lipid metabolism and energetics of larval development and metamorphosis in the american lobster (*Homarus americanus*, Milne Edwards). Mar. Environ. Res. 14, 201–228.

Castuma, C.E., Brenner, R.R., 1983. Effect of fatty acid deficiency on microsomal membrane fluidity and cooperativity of UDP-glucuronyltransferase. Biochim. Biophys. Acta 729, 9–16.

Coleman, R.A., Lewin, T.M., Van Horn, C.G., González Baró, M.R., 2002. Do long-chain Acyl-CoA synthetases regulate fatty acid entry into synthetic versus degradative pathways? Rec. Adv. Nutr. Sci., 2123–2126.

Collins, P.A., Petriella, A., 1999. Growth pattern of isolated prawns of *Macrobrachium borellii* (Crustacea, Decapoda, Palaemonidae). Invert. Reprod. Dev. 36, 1–3.

Colombo, J.C., Pelletier, E., Brochu, C., Khalil, M., Catoggio, J.A., 1989. Determination of hydrocarbon sources using *n*-alkane and polyaromatic hydrocarbon distribution indexes. Case study: Rio de La Plata estuary, Argentina. Environ. Sci. Technol. 23, 888–894.

Di Giulio, R.T., Benson, W.H., Sanders, B.M., Van Veld, P.A., 1995. Biochemical mechanisms: metabolism, adaptation, and toxicity. In: Rand, G.M. (Ed.), Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment. Taylor & Francis, Bristol, USA, pp. 523–561.

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ. Toxicol. Chem. 20, 2383–2396.

Dodds, P.F., 1991. Incorporation of xenobiotic carboxylic acids into lipids. Life Sci. 49, 629–649.

Dole, V.P., 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. 35, 350–357.

Fossi, M.C., Casini, S., Savelli, C., Corbelli, C., Franchi, E., Mattei, N., Sanchez-Hernández, J.C., Corsi, I., Bamber, S., Depledge, M.H., 2000. Biomarker responses at different levels of biological organisation in crabs (*Carcinus aestuarii*) experimentally exposed to benzo(a)pyrene. Chemosphere 40, 861–874.

Gallego, A., Cargill, L.H., Heath, M.R., Hay, S.J., Knutsen, T., 1995. An assessment of the immediate effect of the Braer oil-spill on the growth of herring larvae using otolith microstructure analysis. Mar. Pollut. Bull. 30 (8), 536–542.

García Martinez, P., Livingstone, D.R., 1995. Benzo(a)pyrene-dione-stimulated oxyradical production by microsomes of digestive gland of the common mussel, *Mytilus edulis* L. Mar. Environ. Res. 39, 185–189.

Garda, H.A., Bernasconi, A.M., Brenner, R.R., 1994. Possible compensation of structural and viscotropic properties in hepatic microsomes and erythrocyte membranes of rats with essential fatty acid deficiency. J. Lipid Res. 35, 1367–1377.

Gerhardt, A., Janssens de Bisthoven, L., Mo, Z., Wang, C., Yang, M., Wang, Z., 2002. Short-term responses of *Oryzias latipes* (Pisces: Adrianichthyidae) and *Macrobrachium nipponense* (Crustacea: Palaemonidae) to municipal and pharmaceutical waste water in Beijing, China: survival, behaviour, biochemical biomarkers. Chemosphere 47, 35–47.

GESAMP, 1993. Impact of oil and related chemicals on the marine environment. Reports and Studies No. 50. IMO, London.

González Baró, M.R., Garda, H.A., Pollero, R.J., 1997. Effect of fenitrothion on hepatopancreas microsomal membrane fluidity in *Macrobrachium borellii*. Pestic. Biochem. Physiol. 58, 133–143.

González Baró, M.R., Heras, H., Pollero, R.J., 2000. Enzyme activities involved in lipid metabolism during embryonic development of *Macrobrachium borellii*. J. Exp. Zool. 286, 231–237.

González Baró, M.R., Irazú, C.E., Pollero, R.J., 1990. Palmitoyl-CoA ligase activity in hepatopancreas and gill microsomes of the freshwater shrimp *Macrobrachium borellii*. Comp. Biochem. Physiol. 97B, 129–133.

González Baró, M.R., Pollero, R.J., 1993. Palmitic acid metabolism in hepatopancreas of the freshwater crustacean *Macrobrachium borellii* during an annual cycle. Comp. Biochem. Physiol. 106B, 71–75.

Haunerland, N.H., 1997. Transport and utilization of lipids in insect flight muscles. Comp. Biochem. Physiol. 117 B, 475–482.

Heras, H., Ackman, R.G., Macpherson, E.J., 1992. Tainting of Atlantic salmon (*Salmo salar*) by petroleum hydrocarbons during a short-term exposure. Mar. Pollut. Bull. 24 (6), 310–315.

Heras, H., González Baró, M.R., Pollero, R.J., 2000. Lipid and fatty acid composition and energy partitioning during embryo development in the shrimp *Macrobrachium borellii*. Lipids 35, 645–651.

Heras, H., Zhou, S., Ackman, R.G., 1995. Plastic bags for stable storage of the water-soluble fraction of crude petroleum used in aquatic environment toxicity and tainting studies. Bull. Environ. Contam. Toxicol. 55, 597–602.

Knights, K.M., Drogemuller, C.J., 2000. Xenobiotic-CoA ligases: kinetic and molecular characterization. Curr. Drug Metab. 1, 49–66.

Lakowicz, J.R., 1991. Principles of Fluorescence Spectroscopy. Plenum Press, New York.

Lakowicz, J.R., Prendergast, F.G., Hogen, D., 1979. Differential polarized phase fluorometric investigations of diphenylhexatriene in lipid bilayers. Quantitation of hindered depolarizing rotations. Biochemistry 18, 519.

Lavarías, S., Dreon, M.S., Pollero, R.J., Heras, H., 2005. Changes in phosphatidylcholine molecular species in the shrimp *Macrobrachium borellii* in response to a water-soluble fraction of petroleum. Lipids 40, 487–494.

Lavarías, S., Heras, H., Demichelis, S., Portiansky, E., Pollero, R.J., 2002. Morphometric study of embryonic development of *Macrobrachium borellii* (Arthropoda: Crustacea). Invert. Reprod. Dev. 41, 157–163.

Lavarías, S., Heras, H., Pollero, R.J., 2004. Toxicity, uptake, and release of the water-soluble fraction of crude oil in different developing stages of the

prawn *Macrobrachium borellii*. Arch. Environ. Contam. Toxicol. 47, 215–222.

Lavarías, S., Pollero, R.J., Heras, H., 2006. Activation of lipid catabolism by the water-soluble fraction of petroleum in the crustacean *Macrobrachium borellii*. Aquat. Toxicol. 77, 190–196.

Livingstone, D.R., 1998. The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. Comp. Biochem. Physiol. 120A, 43–49.

Livingstone, D.R., Moore, M.N., Lowe, D.M., Nasci, C., Farrar, S.V., 1985. Responses of the cytochrome P-450 monooxygenase system to diesel oil in the common mussel, *Mytilus edulis* L., and the periwinkle, *Littorina littorea* L. Aquat. Toxicol. 7, 79–91.

McIntosh, T.J., Robertson, J.D., Ting-beall, H.P., Walter, A., Zampighi, G., 1980. Structure of the hemocyanin channel in lipid bilayers. Biochim. Biophys. Acta 601, 289–301.

Moore, M.N., Farrar, S.V., 1985. Effects of polynuclear aromatic hydrocarbons on lysosomal membranes in molluscs. Mar. Environ. Res. 17, 222–225.

Moore, M.N., Livingstone, D.R., Widdows, J., Lowe, D.M., Pipe, R.K., 1987. Molecular, cellular and physiological effects of oil-derived hydrocarbons on molluscs and their use impact assessment. Environ. Effects North Sea Oil Gas Dev. 316B, 603–623.

Rafi, G.M., Srinivas, T., Reddy, S.J., Reddy, D.C., Ramamurthi, R., 1991. Acute and chronic toxicity of endosulfan to crab: effect on lipid metabolism. Bull. Environ. Contam. Toxicol. 47, 918–924.

Ritchie, W., O'Sullivan, M., 1994. ESGOSS, The Environmental Impact of the Braer. The Scottish Office, Edinburgh, United Kingdom.

Sallustio, B.C., Meffin, P.J., Knights, K.M., 1988. The stereospecific incorporation of fenoprofen into rat hepatocyte and adipocyte triacylglycerols. Biochem. Pharmacol. 37, 1919–1923.

Shigehisa, H., Shiraishi, H., 1998. Biomonitoring with shrimp to detect seasonal change in river water toxicity. Environ. Toxicol. Chem. 17, 687–694.

Sikkema, J., De Bont, J.A., Poolman, B., 1994. Interactions of cyclic hydrocarbons with biological membranes. J. Biol. Chem. 269, 8022–8028.

Sikkema, J., De Bont, J.A., Poolman, B., 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 59, 201–222.

Singh, I., Bhushan, A., Relan, N.K., Hashimoto, T., 1988. Acyl-CoA ligase from rat brain microsomes: an immunochemical study. Biochim. Biophys. Acta 963, 509–514.

Solé, M., Porte, C., Biosca, X., Mitchelmore, C.L., Chipman, J.K., Livingstone, D.R., Albaiges, J., 1996. Effects of the Aegean Sea oil spill on biotransformation enzymes, oxidative stress and DNA-adducts in digestive gland of the mussel (*Mytilus edulis* L.). Comp. Biochem. Physiol. 113C, 257–265.

Spector, A.A., Yorek, M.A., 1985. Membrane lipid composition and cellular function. J. Lipid Res. 26, 1015–1035.

Stickney, J.A., Roberts, A.E., Silverman, D.M., Schatz, R.A., 1989. The effect of *m*-xilene on rat lung benzo[a]pyrene metabolism and microsomal membrane lipids: comparison with *p*-xylene. Toxicology 58, 155–165.

Tricerri, M.A., Garda, H.A., Brenner, R.R., 1994. Lipid chain order and dynamics at different bilayer depths in liposomes of several phosphatidylcholines studied by differential polarized phase fluorescence. Chem. Phys. Lipids 71, 61–71.

Wang, S.Y., Stickle, W.B., 1988. Biochemical composition of the blue crab *Callinectes sapidus* exposed to the water-soluble fraction of crude oil. Mar. Biol. 98, 23–30.

Watkins, P.A., 1997. Fatty acid activation. Prog. Lipid Res. 36, 55–83.

White, S.H., King, G.I., Cain, J.E., 1981. Location of hexane in lipid bilayers determined by neutron diffraction. Nature 290, 161–163.