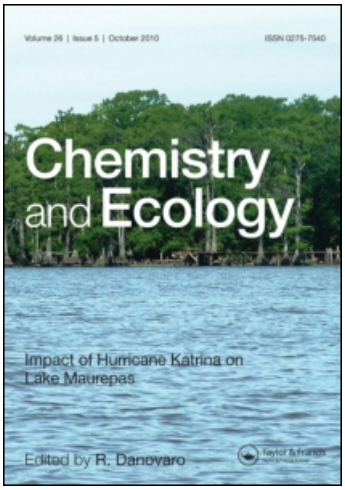

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Uptake and Dupuration of Petroleum Hydrocarbons By the Backwater Clam, *Meretrix Casta* Chemnitz

A. J. A. Sophia[a]; T. Balasubramanian[a]
[a] Centre of Advanced Study in Marine Biology Parangipettai (Porto Novo), Tamil Nadu, India

To cite this Article Sophia, A. J. A. and Balasubramanian, T.(1991) 'Uptake and Dupuration of Petroleum Hydrocarbons By the Backwater Clam, *Meretrix Casta* Chemnitz', Chemistry and Ecology, 5: 4, 249 — 262
To link to this Article: DOI: 10.1080/02757549108035254
URL: http://dx.doi.org/10.1080/02757549108035254

Chemistry and Ecology, 1991, Vol. 5, pp. 249–262
Reprints available directly from the publisher.
Photocopying permitted by license only
Printed in Singapore

UPTAKE AND DUPURATION OF PETROLEUM HYDROCARBONS BY THE BACKWATER CLAM, *MERETRIX CASTA* CHEMNITZ

A.J.A. SOPHIA and T. BALASUBRAMANIAN

Centre of Advanced Study in Marine Biology Parangipettai (Porto Novo) 608 502 Tamil Nadu, India.

(Received 21 March, 1991)

Meretrix casta were experimentally exposed to water soluble fractions of refined and crude oil and their rate of accumulation of the petroleum hydrocarbons including total and individual aromatics viz., naphthalene, methylnaphthalene and dimethylnaphthalene, was studied. Subsequent transfer to clean waters in the laboratory and field resulted in depuration of the accumulated hydrocarbons from tissues. In general, the rate of discharge was found to be dependent on the concentration that the animals had been earlier exposed to and also the alkylation of aromatic hydrocarbons. Occasional increases were observed in the hydrocarbon values of clams which were placed in the field, compared to their laboratory counterparts, suggesting an intermittent source of petroleum hydrocarbon input into the environment. The influence on the rate of uptake, release and retention of hydrocarbons in the clams is discussed.

KEY WORDS: Uptake, depuration, petroleum hydrocarbons, *Meretrix casta*

INTRODUCTION

Bivalve molluscs are known for their ability to accumulate hydrocarbons from trace amounts in water (Erhardt, 1972; Disalvo *et al.*, 1975; Fossato, 1975; Mix *et al.*, 1977; Fossato *et al.*, 1979; Farrington *et al.*, 1980; Clarke and Law, 1981; Boehm *et al.*, f1982) and are able depurate them over a period of time (Lee *et al.*, 1972; Stegeman and Teal, 1973; Fossato and Canzonier, 1976; Boehm and Quinn, 1977; Nunes and Benville, 1979; Clement *et al.*, 1980; Farrington *et al.*, 1982), proving useful biomonitors of a specific environment. With a paucity of such information for Indian waters (Rajan, 1986; Sophia, 1987) the commercially important clam, *Meretrix casta*, widely distributed along the coasts of India, was chosen to study the extent of uptake and depuration of aromatic hydrocarbons on exposure to water-soluble fractions of refined and crude oils.

MATERIALS AND METHODS

Uniform sized individuals of *Meretrix casta*, 3.5±1 cms) were obtained from clam beds in the Vellar estuary situated at Porto Novo (11°29'N Lat. and 79°47'E Long.) and acclimated for a week in filtered sea water that was renewed every day in the laboratory. Salinity (32‰) and temperature (29±1°C) of the water was adjusted to field conditions; feeding and cleaning procedures were done regularly.

The test oils employed in this study included refined oils (diesel and engine oil — Servo 30) which were used locally by fishing trawlers, and Kuwait crude,

representative of a type transported in domestic foreign waters. Water soluble fractions (WSF) of these oils were prepared according to the method of Anderson *et al.* (1974). A 25% dilution of the LC50 value (10 days) of the refined oils and 25% dilution of the stock WSF of crude oil (as even full strength WSF failed to produce 50% mortality in the animals) were used as sublethal concentrations for the experiment. This dilution was designated as 1 sublethal toxic unit (1 STU) while half and one tenth this value were 0.5 and 0.1 STU, respectively. These dilutions were prepared by mixing filtered sea water with the stock solutions of the WSF. The actual hydrocarbon concentrations in the different exposure media were monitored by adopting the procedure of Clement *et al.* (1980) and analysed in an Aminco Brown fluorescence spectro-photometer at an excitation and emission wavelength of 310/360 nm respectively (IOC/WMO, 1976). Naphthalenes and their alkyl derivatives in the media were determined by a UV/VIS spectrophotometer, Hitachi 220S Model, by following the method of Neff and Anderson (1975).

Uptake

The acclimated clams were exposed to the various sublethal concentrations of WSF in large fibre-glass tanks ($40 \times 35 \times 35$ cms) with exposure media renewed every 24 hours over a period of 5 days. Salinity and temperature were maintained at levels similar to that of the acclimatization period. On the 2nd and 5th days a few animals were removed, shucked and held frozen at $-20°C$ for analysis. The rest were transferred to clean water for depuration studies.

Depuration

Laboratory and field studies were conducted simultaneously. Laboratory studies were carried out for 23 days in clean fibre-glass tanks where water was changed regularly without disturbing the animals and also maintaining salinity and temperature, 32‰ and $29 \pm 1°C$ respectively. Animals were removed for analysis after 1, 2, 5, 12 and 23 days. Parallel field studies were conducted for a period of 38 days with clams placed in nylon meshed three-tiered iron cages ($30 \times 30 \times 30$ cms) and suspended just one foot above the substrate to ensure that they remained submerged at all times. These enclosures were constructed in a relatively clean zone of the Vellar estuary. The clams were removed for analysis after 1, 2, 5, 12, 23 and 38 days.

Frozen degutted whole body tissue was analysed using the UV/VIS spectrophotometer and the UVF spectrophotometer. The ordinary UV analysis was done according to the method of Neff and Anderson (1975). Standards used were naphthalene, 2 methylnaphthalene and 1, 2– dimethylnaphthalene. The samples were analysed by the UVF spectrophotometer according to the method of Clement *et al.* (1980). They were thawed, digested with 4 N NaOH and extracted with hexane. These extracts were dried and concentrated in a nitrogen atmosphere. This was then eluted through a silica gel column and concentrated as before. UVF was done at 310/360 nm for assessing the whole aromatic content of the tissue.

RESULTS

The actual concentrations of petroleum hydrocarbons that the animals were exposed to are given in Tables 1 and 2.

Table 1 Petroleum hydrocarbon concentration (mg/l) in three sublethal units of exposure among the three oil types.

Oil Type	*Exposure levels*		
	0.1 STU	*0.5 STU*	*1.0 STU*
Diesel	0.27	1.22	2.34
Engine oil (Servo-30)	0.18	1.01	1,93
Kuwait Crude	0.34	1.31	3.18

Table 2 Actual concentration (mg/l) of naphthalene (N), methylnaphthalene (MN) and dimethylnaphthalene (DMN) in different exposures (STU) of the WSF of the three oils.

Exposure	*Oil Type*								
	Diesel			*Engine Oil—30*			*Kuwait Crude*		
	N	*MN*	*DMN*	*N*	*MN*	*DMN*	*N*	*MN*	*DMN*
0.1 STU	0.028	0.025	0.024	0.024	0.013	0.014	0.023	0.016	0.008
0.5 STU	0.131	0.117	0.067	0.135	0.070	0.076	0.083	0.056	0.020
1.0 STU	0.241	0.215	0.123	0.257	0.134	0.145	0.202	0.136	0.066

Uptake

(i) *Aromatic hydrocarbons*: Accumulation reached zenith around 48 and 60 hours of exposure in all concentrations of the three oil types. Controls were more or less steady at 0.9 ppm (Figure 1). Among diesel dosed clams, those in the higher concentrations of WSF (0.5 and 1 STU, i.e. 1.22 and 2.34 ppm WSF) accumulated the most (Figure 3 and 4). In engine oil treated animals, the rate of accumulation was proportional to the concentration in the medium (Figure 2, 3 and 4), while animals exposed to the WSF of crude showed higher accumulatory rates in 0.1 STU (0.37 ppm WSF) than 0.5 STU (1.31 ppm WSF) but clams in 1 STU (3.18 ppm WSF) accumulated the most (Figure 2, 3 and 4).

(ii) *Specific aromatics* (Figure 5): Control animals exhibited more or less constant levels of these aromatics (naphthalene, methyl naphthalene and dimethylnaphthalene) throughout the uptake period.

(a) Naphthalene (N): the refined oil exposed clams showed higher rates of accumulation in 0.5 STU followed by the 1 STU and 0.1 STU exposed animals. Those in crude revealed accumulatory patterns which were more in concordance with the concentration gradient of the test medium.

(b) Methylnaphthalene (MN): here again the refined oil exposed animals in general showed higher accumulation than crude dosed animals.

(c) Dimethylnaphthalene (DMN): the diesel and engine oil treated clams resembled each other in their pattern of uptake, being proportional to the concentration of the medium while crude treated forms showed a slight variation with those exposed in the lowest concentration accumulated the most. In general, the crude exposed clams were seen to accumulate DMN to a greater extent than refined oil treated forms.

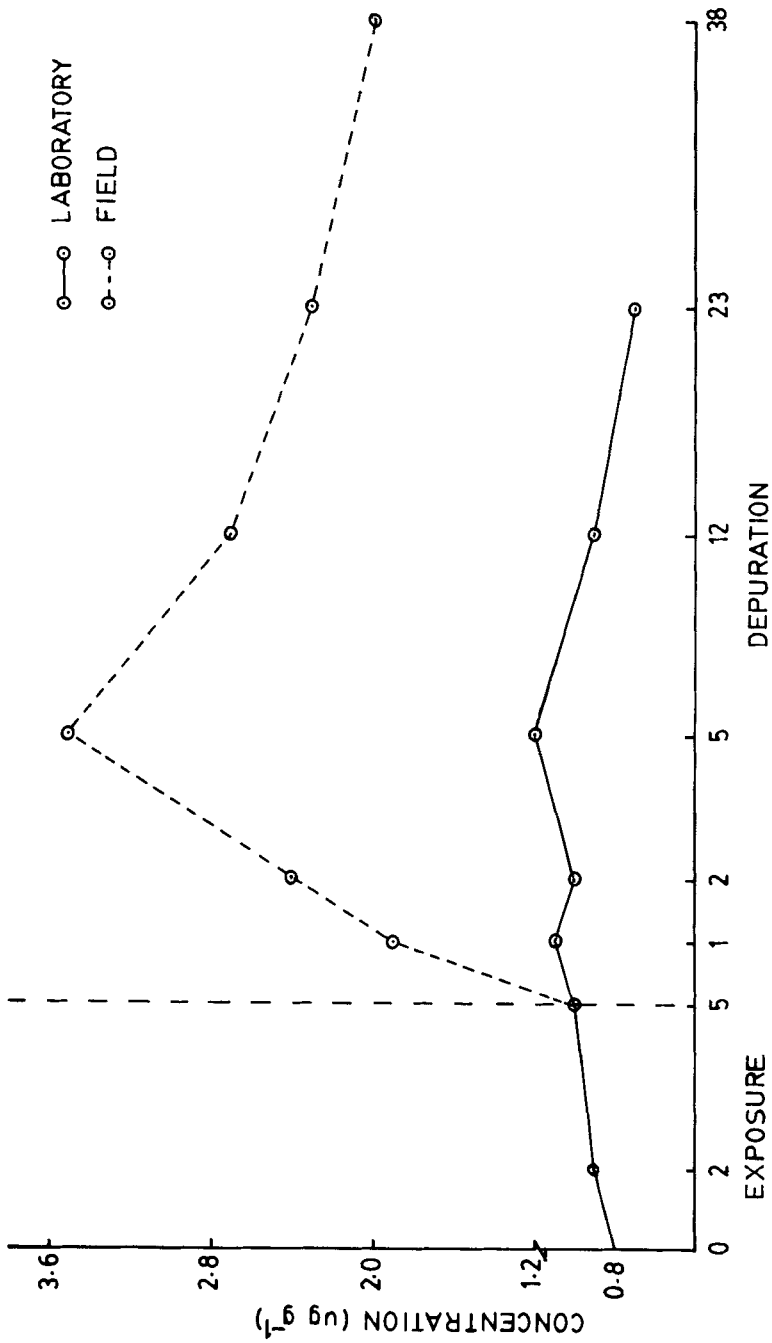

Figure 1 Uptake and depuration of aromatic hydrocarbons in *Meretrix casta* held as controls.

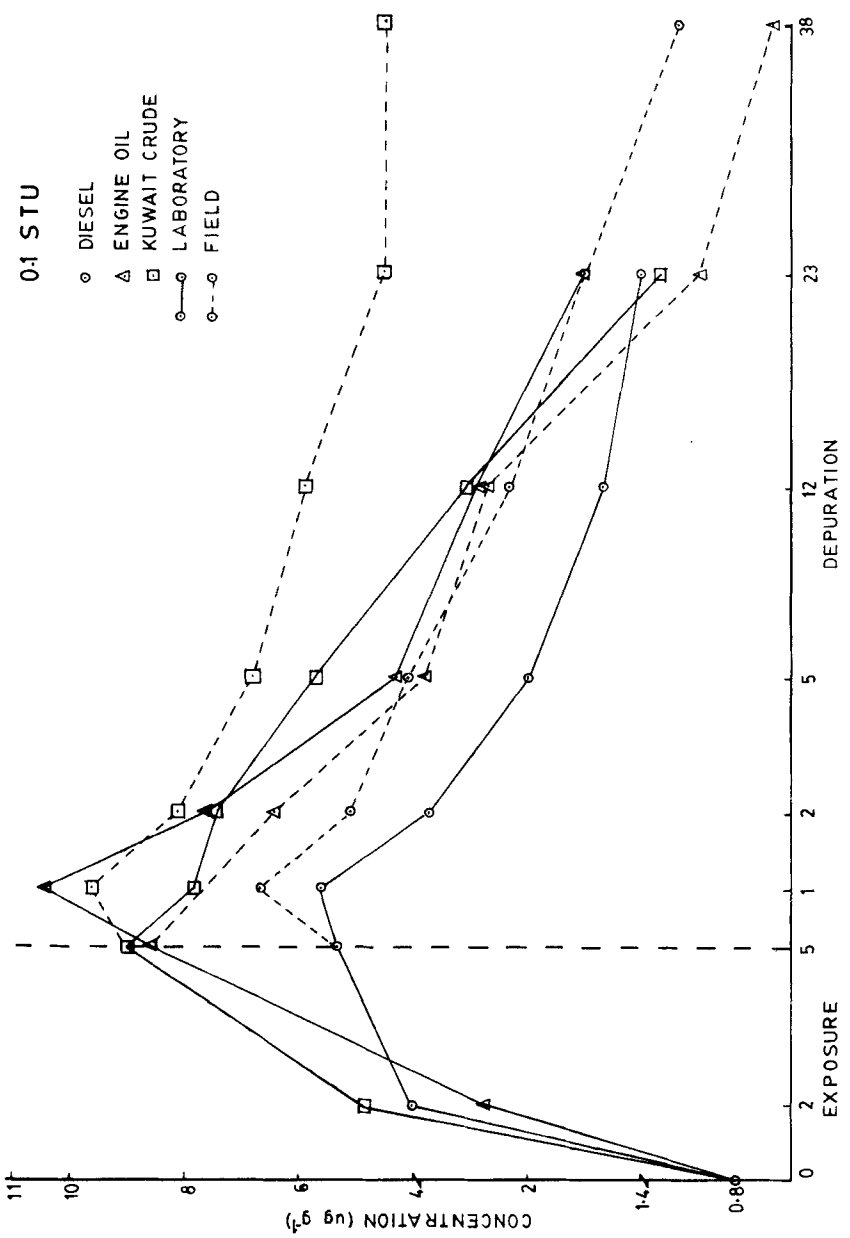

Figure 2 Uptake and depuration of petroleum hydrocarbons in *Meretrix casta* exposed to 0.1 STU (Diesel = 0.27 ppm; Engine oil = 0.13 ppm and crude = 0.37 ppm) of WSF.

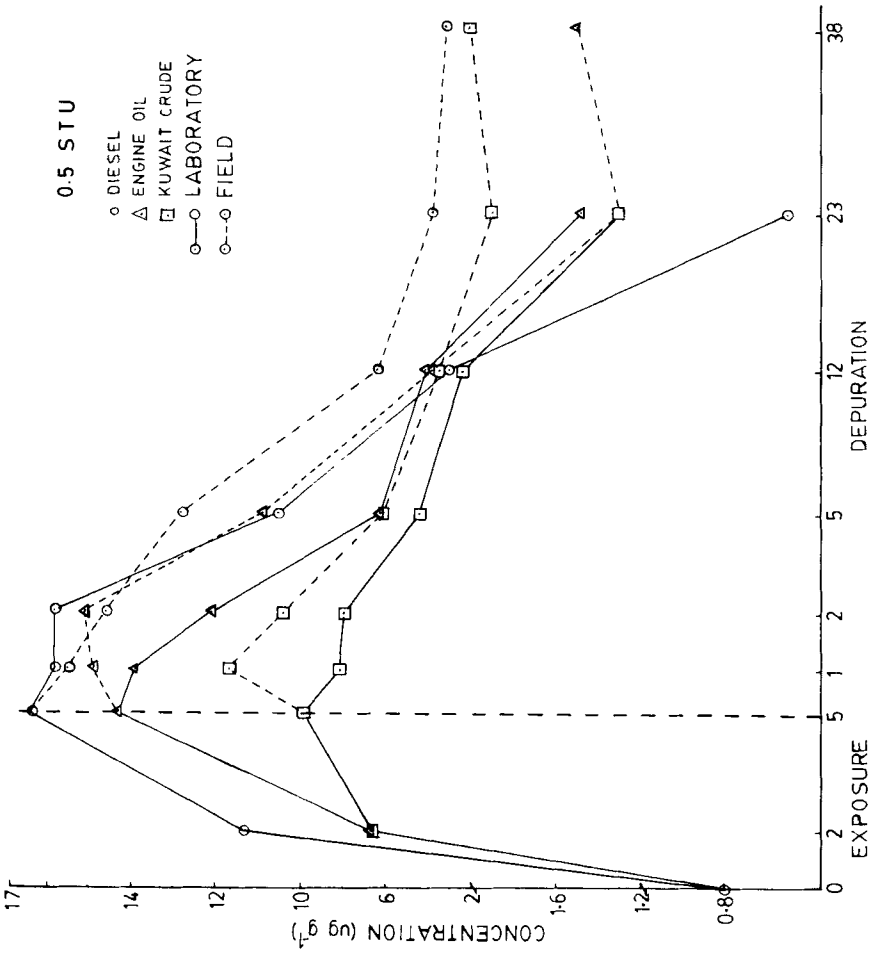

Figure 3 Uptake and depuration of aromatic hydrocarbons in *Meretrix casta* exposed to 0.5 STU (Diesel = 1.22 ppm, Engine oil = 1.01 ppm and crude = 1.31 ppm) of WSF.

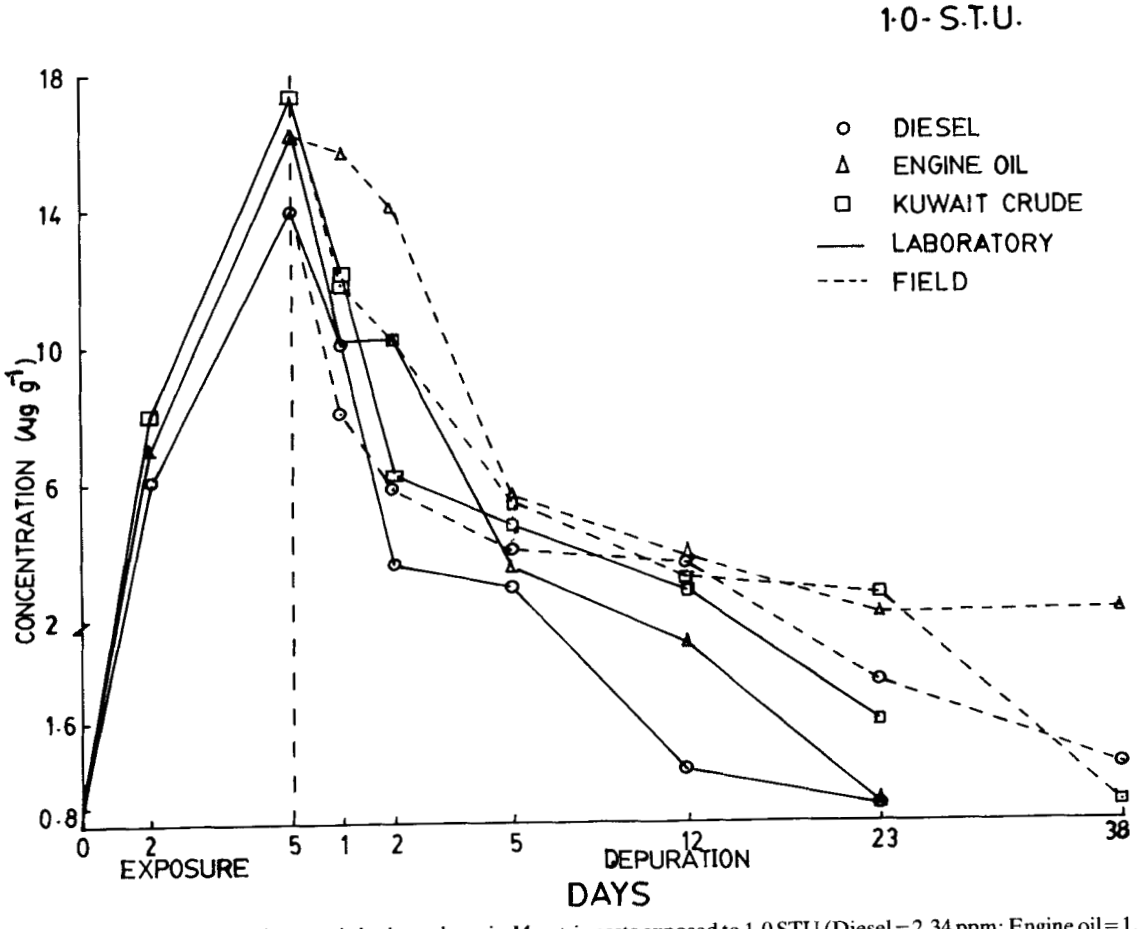

Figure 4 Uptake and depuration of aromatic hydrocarbons in *Meretrix casta* exposed to 1.0 STU (Diesel = 2.34 ppm; Engine oil = 1.93 ppm and crude = 3.18 ppm) of WSF.

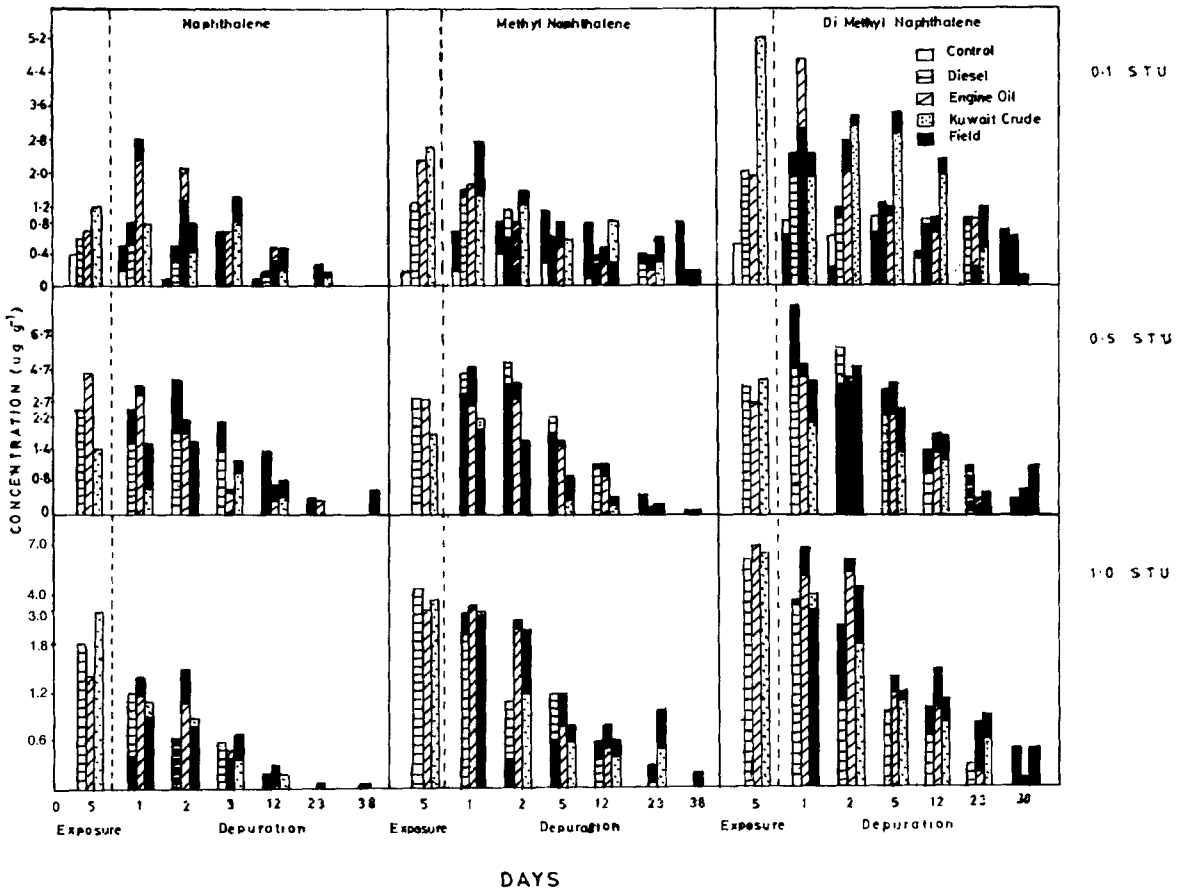

Figure 5 Uptake and depuration of specific aromatic hydrocarbons (N, MN and DMN) in *Meretrix casta* exposed to different concentrations of WSF of the test oils.

The bioaccumulation factor (ratio of petroleum hydrocarbons in tissue to that in water) revealed that clams in lower concentration levels (0.1 and 0.5 STU) of all oils exhibited greater rates of accumulation than animals exposed to the highest sublethal toxic unit (1 STU) although the actual amount accumulated was greater in this latter group. This pattern was found for both total aromatics (Table 3) and individual aromatics (Table 4) assessed.

Table 3 Bioaccumulation factors for clams exposed for 5 days to WSF of diesel engine oil and crude, with respect to aromatic hydrocarbons (general).

Oil Type	*Exposure levels*		
	0.1 STU	*0.5 STU*	*1.0 STU*
Diesel	19.6	12.9	5.9
Engine oil-30	47.8	14.3	8.4
Kuwait Crude	27.0	6.7	5.4

Table 4 Bioaccumulation factors for clams exposed for 5 days to WSF of diesel, engine oil and crude, with respect to specific aromatics, viz. N, MN and DMN.

Exposure	*Oil Type*								
	Diesel			*Engine Oil—30*			*Kuwait Crude*		
	N	*MN*	*DMN*	*N*	*MN*	*DMN*	*N*	*MN*	*DMN*
0.1 STU	21.4	52.0	79.2	29.2	176.9	135.7	56.5	156.3	662.5
0.5 STU	18.3	24.8	55.2	32.6	45.7	48.7	16.1	30.4	21.0
1.0 STU	7.5	21.9	51.2	5.5	23.1	50.3	14.4	29.9	101.5

Depuration

(i) *Aromatic hydrocarbons*: Release of the accumulated aromatic hydrocarbons by the clams occurred within a period of 23 days after transfer to clean water in the laboratory. In the field it was slower, with some hydrocarbons persisting in the tissue even after 23 days.

Control clams kept in the laboratory had a negligible quantity of these compounds but those in the field were found to accumulate PHC up to 3.5 ppm, nearly three times greater than that found originally (Figure 1). While the diesel and engine oil treated clams resembled each other in their pattern of laboratory depuration, those treated with WSF of crude retained PHC in their tissues even after 23 days. In the field, aromatic hydrocarbons were not completely depurated from the 0.5 STU diesel dosed clams (Figure 3). In the case of engine oil treated forms depuration started only after 48 hours in the lower concentration levels (Figure 2 and 3). The field depuration pattern of crude treated animals resembled the refined oils but the final PHC levels were far higher than in their laboratory counterparts. In general, animals in the higher concentration levels (Figure 3 and 4) depurated faster than those in the lower concentration (Figure 2).

(ii) *Specific aromatics*: The depuration patterns of N, MN and DMN by control and exposed animals are represented in Figure 5. Control animals which were found initially to possess trace quantities of N, MN and DMN lost all traces in the laboratory but MN and DMN persisted in the tissues of clams kept in the field, throughout the period of study.

(a) Naphthalene: The rate of depuration in diesel exposed clams was rapid in the highest concentration but in the two lower STU levels they showed fluctuations eventually (23 days), resulting in total release. In engine oil treatment, the laboratory and field depuration tests showed initially traces of enhancement in the 0.1 STU treated animals, while in the other two exposure concentrations the depuration was more or less steady. However, in the field, the rate was evident but slow. In clams treated with WSF of crude, the depuration of N was rapid in the higher concentration exposures than in the lower STU. Transfer to the field reduced the rate even further.

(b) Methylnaphthalene: In diesel and crude treated clams, those exposed at 1 STU depurated faster than those exposed at lower concentrations. In fact, those in 0.5 STU WSF started depuration only after 5 days' transfer to 'clean water' in both the laboratory and the field. In engine oil exposed animals depuration was steady in all levels of concentration. Similar patterns were observed in the field but at a slower rate.

(c) Dimethylnaphthalene: Animals which had been exposed to higher concentrations of WSF of diesel depurated faster than those exposed to lower concentrations. The laboratory depuration was generally faster than in the field. A similar pattern was observed in engine oil exposed clams in which actual depuration started only after 5 days in 0.5 and 0.1 STU exposed animals. In crude treatments depuration was slow in the 0.1 STU group of animals, while in 0.5 STU and 1 STU it was faster. Nevertheless, it remained incomplete even after 23 days in the laboratory and 38 days in the field.

DISCUSSION

Uptake:

An increase in the uptake rate of aromatic hydrocarbons in the clams occurred mainly during 48 and 60 hours and this suggests an initial tardiness in their accumulatory capacity of PHC from the medium, as reported by Neff *et al.* (1976) and Neff (1979). Moreover, as reported by Stegeman (1974) and Clement *et al.* (1980) a reduction in siphon activity with increase in hydrocarbon concentration of the ambient medium could have caused the inverse relationship observed in the present investigation between the rate of uptake and the concentration of the exposure medium. The findings, however, suggest that *Meretrix casta* individuals were more efficient in retaining accumulated hydrocarbons in their tissues over a long period of time in spite of their brief period of exposure to the pullutant.

In comparing the accumulation of specific aromatics, DMN were the compounds that accumulated to the greatest extent, although their availability in the medium was less than N or MN compounds (Table 2). In brief, as alkylation increased, the uptake rate decreased but retention time increased, similar to that postulated by Clement *et al.* (1980) and Widdows *et al.* (1982) in clams and mussels, respectively.

Fossato and Canzonier (1975) reported bioaccumulation factors as high as 1000 in mussels that had been polluted by oil. Higher accumulation was also observed by Stegeman and Teal (1973) and DiSalvo *et al.* (1975) under conditions approximating a chronic pollution situation. The highest accumulation recorded in our studies was 662 in crude treated clams and indicates that *Meretrix casta* seems to restrict its uptake of PHC from water.

According to Blumer *et al.* (1970) and Stegeman and Teal (1973), the PHC taken up by the organisms becomes a part of their lipid pool. However, the lipid content in these animals from another experiment dealing with biochemical correlates in *Meretrix casta* showed no marked increase during 5 days' exposure, but on extended exposures (30 days) a marked increase did develop especially in crude treated forms and to a lesser extent in diesel and engine oil exposed animals (Figure 6).

Depuration:

A definite variation in the rate of depuration may be related to the duration of exposure and composition of the pollutant hydrocarbon. First, considering the rate of depuration of aromatic hydrocarbons in general, it was found to be rapid with clams attaining complete depuration within 23 to 38 days in clean sea water. In a study on clams and oysters that had accumulated aromatics and aliphatic hydrocarbons following twenty four hours exposure to an experimental oil spill, Bieri and Stamoudis (1977) reported that the rate of release of polycyclic aromatic hydrocarbons (PAH) was spectacular, with complete depuration being achieved within 10 hours' transfer to clean water. Thus, the shorter the exposure period, the more rapid is depuration (Balouet *et al.*, 1985). This view had been postulated nearly two decades ago by Lee *et al.* (1972), following studies on *Mytilus edulis*.

Aromatic hydrocarbons are generally retained for a long time. However, it is the larger compounds such as DMN and other PAHs, which though accumulated slowly, are retained longer than monoaromatics (Rice *et al.*, 1979). Thus, in the present study, the order of release conforms to the above view with DMN being better retained in the tissue than N or MN.

The increase in traces of aromatics observed in control clams, placed in the field for depuration studies, denotes a definite input of PHC into the estuary. This intermittent input may not be revealed by analysing the water, as tides help to disperse oily wastes discharged to the estuary by fishing vessels; clams being more sensitive may trap and accumulate PHCs in their tissues and retain them over a period of time. This probably explains the consistent difference in aromatic hydrocarbon concentrates between laboratory and field depurating animals. The accumulation of PHC is a passive process dependent on the lipid/water partition (Stegeman and Teal, 1973; Neff *et al.*, 1976; Balouet *et al.*, 1985). In short term exposures possibly the PHC may not get included into the lipid fraction as effectively as in long term exposures, thereby being released to the environment on transfer to clean water, but not completely. In our study the maximum retention of aromatics was seen in crude dosed clams after 23 days' depuration, but as alkylation of aromatics (as seen in DMN and MN) increased, the refined oils (diesel and engine oil — 30) treated forms slowed down their rate of depuration, retaining these hydrocarbons in their tissues to a greater extent than crude exposed animals. Similar observations have also been reported in other bivalve molluscs (Cox *et al.*, 1975; Neff *et al.*, 1976; Neff, 1979; Farrington *et al.*, 1982; Balouet *et al.*, 1985).

Temperature and salinity are seen to play an important role in the uptake and

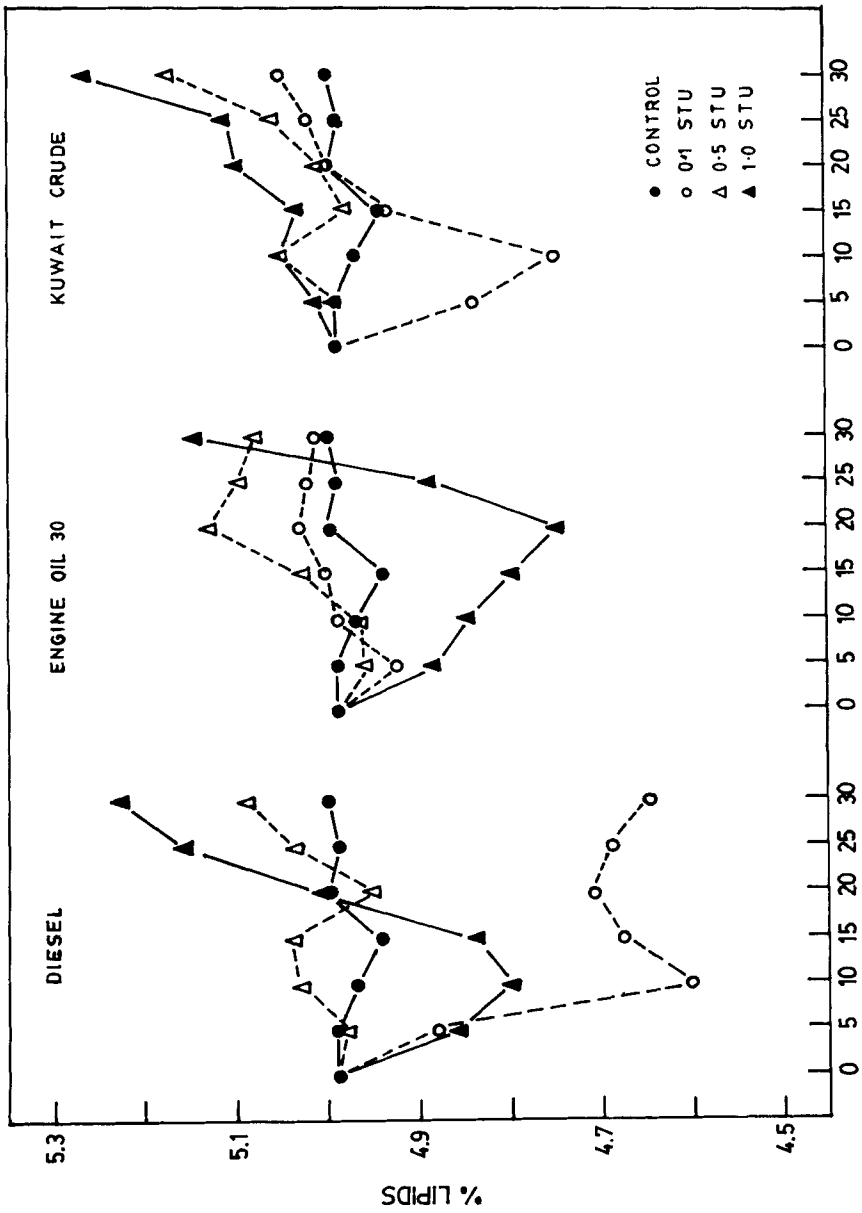

Figure 6 Lipid content of *Meretrix casta* exposed to WSF of the test oils.

release of hydrocarbons by marine organisms and in the present study could have influenced the discharge of PHC from the contaminated tissues, making it more rapid; higher temperatures are usually known to increase the rates of depuration (Laughlin *et al.*, 1979).

CONCLUSION

Thus many abiotic and biotic factors influence the rates and patterns of PHC uptake, retention and release by marine organisms. Since some cannot be controlled in the laboratory and none in the field, it gives rise to variation in reports of hydrocarbon uptake and depuration in marine organisms. Further research at the microcosmic level would shed better light on these aspects of oil pollution. In our study, laboratory results give a good picture of the uptake and depuration of PHC by *Meretrix casta* exposed to WSF of the three oils. In comparison, in the field study (Vellar estuary) ambiguity prevails, revealed by the fluctuations in the depuration status. The sensitivity of the clams to these chronic inputs is brought out clearly, stressing the need for added caution in the disposal of oily wastes and other effluents into such highly productive environments that often serve as fin and shellfish resources.

Acknowledgement:

The authors are grateful to Mr R. Law, Ministry of Agriculture, Food & Fisheries, Burnham-on-Crouch, U.K., for his analytical assistance. This study was supported by the University Grants Commission, New Delhi.

References

Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E. & Hightower, G.M. (1974) Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Marine Biology*, **27**, 75–88.

Balouet, G., Berthou, F., Bodennec, G. & Marchand, M. (1985). Effects de la pollution de l'Amoco Cadiz sur l'ostreiculture (1978–1984). IFREMER — Centre de Brest. 95 pp.

Bieri, R.H. & Stamoudis, V.C. (1977). The fate of petroleum hydrocarbons from a No. 2 fuel oil spill in a seminatural estuarine environment. In: *Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms* (D.A. Wolfe, ed.), pp. 332–344. Pergamon Press, New York.

Blumer, M., Souza, G. & Sass, J. (1970). Hydrocarbon pollution of edible shellfish by an oil spill (Buzzards Bay, Mass.). *Marine Biology* **5**, 195–202.

Boehm, P.D., Fiest, D.L., Mackay, D. & Paterson, S. (1982). Physical-chemical weathering of petroleum hydrocarbons from the Ixtoc I blow-out: Chemical measurements and a weathering model. *Environmental Science and Technology*, **16**, 498–505.

Boehm, P.D. & Quinn, J.G. (1977). The persistence of chronically accumulated hydrocarbons in the hard-shell clam, *Mercenaria mercenaria*. *Marine Biology*, **44**, 227–233.

CBPCWP (1986). Monitoring of water quality along Tamil Nadu coast. Annual Report of research project sponsored by the Central Board for the prevention and control of water pollution, New Delhi.

Clarke, A. & Law, R. (1981). Aliphatic and aromatic hydrocarbons in benthic invertebrates from two sites in Antarctica. *Marine Pollution Bulletin*, **12**, 10–14.

Clement, L.E., Stekoll, M.S. & Shaw, D.G. (1980) Accumulation, fractionation and release of oil by the intertidal clam, *Macoma balthica*. *Marine Biology*, **51**, 41–50.

Cox, B.A., Anderson, J.W. & Parker, J.C. (1975) An experimental oil spill: the distribution of aromatic hydrocarbons in the water, sediment and animal tissue within a shrimp pond. In *Proceedings of a Joint Conference on Prevention and Control of Oil Spills, San Francisco, California*, 1975, pp. 607–612. American Petroleum Institute, Washington, D.C.

DiSalvo, L.H., Guard, H.E. & Hunter, L. (1975) Tissue hydrocarbon burden of mussels as potential monitor of environmental hydrocarbon insult. *Environmental Science and Technology*, **9**, 247–251.

Erhardt, M. (1972). Petroleum hydrocarbons in oysters from Galveston Bay. *Environmental Pollution*, **3**, 257–271.

Farrington, J.W., Albaiges, J., Burns, K.A., Dunn, B.P., Eaton, P., Laster, J.L., Porter, P.L. & Wise, S. (1980). Fossil Fuels. In: *The International Mussel Watch*, pp 7–77. National Academy of Science, Washington, D.C.

Farrington, J.W., Davis, A.C., Frew, N.M. & Robin, K.S. (1982). No. 2 fuel oil compounds in *Mytilus edulis*. Retention and release after an oil spill. *Marine Biology*, **66**, 15–26.

Fossato, U.V. (1975). Elimination of hydrocarbon by mussels. *Marine Pollution Bulletin*, **6**, 7–10.

Fossato, U.V. & Canzonier, W.J. (1976). Hydrocarbon uptake and loss by mussel (*Mytilus edulis*). *Marine Biology*, **36**, 243–250.

Fossato, U.V., Nasei, C. & Dolei, F. (1979). 3, 4-Benzopyrene and perylene in mussels, *Mytilus sp.* from the Laguna Veneta, northeast Italy. *Marine Environmental Research*, **2**, 47–53.

Laughlin, R.B., Linden, O. & Neff, J.M. (1979). A study on the effect of salinity and temperature on the disappearance of aromatic hydrocarbons from the water-soluble fraction of No. 2 Fuel oil. *Chemosphere*, **10**, 741–749.

Lee, R.F., Sauerheber, R. & Benson, A.A. (1972). Petroleum hydrocarbons: uptake and discharge by the marine mussel, *Mytilus edulis*. *Science*, **177**, 344–346.

Mix, M.C., Riley, R.T., King, K.I., Trenholm, S.R. & Schaffer, R.L. (1977). Chemical carcinogens in the marine environment. Benzo (a) pyrene in economically-important bivalve molluscs from Oregon estuaries. In: *Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems* (D.A. Wolfe, ed.) pp 421–431. Pergamon Press, New York.

Neff, J.M. (1979). Polycyclic aromatic hydrocarbons in the aquatic environment. Sources, fates and biological effects pp 262. Applied Science Publishers, London.

Neff, J.M. & Anderson, J.W. (1975). An ultraviolet spectrophotometric method for the determination of naphthalene and alkylnaphthalenes in the tissues of oil contaminated marine animals. *Bulletin of Environmental Contamination and Toxicology*, **14**, 122–128.

Neff, J.M., Cox, B.A., Dixit, D. & Anderson, J.W. (1976). Accumulation and release of petroleum derived aromatic hydrocarbons by four species of marine animals. *Marine Biology*, **38**, 279–2j89.

Nunes, P. & Benville, P.E. (1979). Uptake and depuration of petroleum hydrocarbons in the Manila clam, *Tapes semidecussata* Reeve. *Bulletin of Environmental Contamination and Toxicology*, **21**, 719–726.

Rajan, E.J.T. (1986). The toxic effects of WSF of diesel on *Anadara rhombea* (BORN). M.Phil, thesis, pp 48. C.A.S. in Marine Biology, Annamalai University, Porto Novo.

Rice, S.D., Moles, A., Taylor, T.L. & Karinen, J.F. (1979). Sensitivity of 39 Alaskan marine species to Cook Inlet Crude oil and No. 2 fuel oil. In: *Proceedings 1979 Oil Spill Conference (Prevention, Behaviour, Control Cleanup), Los Angeles, California*, 1979. American Petroleum Institute Publ., Washington D.C.

Sophia, A.J.A. (1987). The effects of petroleum hydrocarbons on the backwater clam, *Meretrix casta* Chemnitz: Some characteristics relevant to hydrocarbon monitoring. Ph.D. Thesis, pp 194. C.A.S. in Marine Biology, Annamalai University, Porto Novo.

Stegeman, J.J. (1974). Hydrocarbons in shellfish chronically exposed to low levels of fuel oil. In: *Pollution and Physiology of marine organisms*. (F.J. Vernberg & W.B. Vernberg, eds.) pp 329–347. Academic Press, New York.

Stegeman, J.J. & Teal, M. (1973). Accumulation, release and retention of petroleum hydrocarbons by the oyster, *Crassostrea virginica*. *Marine Biology*, **22**, 37–44.

Widdows, J., Bakke, T., Bayne, B.L., Donkin, P., Livingstone, D.R., Lowe, D.M., Moore, M.N., Evans, S.V. & Moore, S.L. (1982). Responses of *Mytilus edulis* on exposure to the water — accommodated fraction of North Sea oil. *Marine Biology*, **67**, 57–81.