

Naphthalene induced activities on growth, respiratory metabolism and biochemical composition in juveniles of *Metapenaeus affinis* (H.Milne Edward, 1837)

Z A Ansari*, Parveen Farshchi** & Moosa Faniband

National Institute of Oceanography, Dona Paula, Goa

** Islamic Azad University, Hussarak- Poonak, Tehran

*[E-mail: zakir@nio.org]

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Toxicity of naphthalene was carried out on *Metapenaeus affinis* (H. Milne Edward, 1837) to investigate its effects on growth, metabolic index and biochemical constituents. Growth rate in terms of weight gain was 32.13% in control, 12.12% in 0.125 ppm and only 5.22% in 0.25 ppm. Metabolic index assessed in terms of oxygen consumption showed a reduction in respiratory rate up to 44.4% in prawns exposed to naphthalene. Activity increased initially but reduced subsequently. Protein content and organic carbon of the body tissue of juveniles showed reduction on exposure to naphthalene. Inhibitory effect was dose dependent and duration of exposure. However, the lipid content increased after exposure to naphthalene. Changes in the growth and respiratory rate and other biochemical constituents resulting from exposure to naphthalene indicate some adaptive response and recovery during detoxification in the prawns exposed to pollutant. Study revealed that the physiological and biochemical parameters could be used as indicator of stress condition in aquatic organisms and the use of naphthalene should be regulated because of its toxic nature.

[Keywords: Naphthalene toxicity, Physiology, Biochemistry, *M. affinis*.]

Introduction

Naphthalene is one of the major constituents of produced water soluble fractions of petrogenic oils¹. Naphthalene is among the most toxic fractions of oil to marine organisms and has most narcotic effects among all polycyclic aromatic hydrocarbon². Potential impact of naphthalene to environment and biota has been reviewed^{3,4}. Sublethal concentration of PAH often affect the physiology and biochemistry of the aquatic organisms⁵. Principal mechanism behind PAH toxicity is that they bind to the hydrophobic sites of macromolecules of the cell resulting in molecular and cellular damage and thereby causing disturbances in normal functioning of the physiology⁶.

Experimental approaches have long been used to determine the acute and sub-lethal effects of pollutants on physiological processes⁷. Many PAH are known to modify physiological activities in marine organisms, whereby altering the reproductive output of such organisms⁸. Studies on changes in physiological processes in organisms exposed to sub lethal concentrations of hydrocarbons are of great

help in identifying the fitness of the organisms. In the present study we have determined the toxicity of naphthalene and its effect on the physiological activities such as growth, oxygen uptake and biochemical constituents of a commercially important prawn *Metapenaeus affinis* under laboratory condition.

Materials and Methods

Juveniles of *Metapenaeus affinis* were collected from Mandovi estuary and transported to the laboratory. Extreme care was taken to avoid any injury to the specimen while handling. Prawns were acclimatized to the laboratory condition in glass aquaria for 96 hours prior to the toxicological experiments. During this period they were fed with commercial diet. Naphthalene has low solubility in water, and hence analar grade acetone was used as solvent. Stock solution having Nap concentration of 1.5mg Nap ml⁻¹ acetone was prepared⁹. From the stock solution, required concentrations of Naphthalene were prepared and added to the seawater (25 psu) in each tank. Concentration for the sub-lethal

toxic effect evaluation was selected after evaluating the 96 hours LC₅₀.

A static bioassay technique was followed to evaluate the toxic effect of the naphthalene. Following successful acclimatization, test animals of uniform size (40 ± 0.5mm TL) were selected for experimental purpose. Ten healthy juveniles were introduced into the aquaria containing seawater and Nap concentration. The total length (TL) and wet weight of test organism was taken individually prior to the inoculation. Salinity of seawater was maintained same as to the natural habitat of the test organisms (i.e.25 psu). All bioassay tests were carried out in duplicate and a control was maintained. Six test concentrations of Naphthalene (0.125, 0.25, 0.5, 1.0, 1.5, 2.0 ppm) were prepared from the stock solution in six aquaria. Feeble aeration was provided in each tank to keep the optimum dissolved oxygen level in the tank. Acute toxicity test were carried out according to the method given in APHA¹¹ and acute toxicity was determined by experimenting with increasing narrower ranges of Nap concentration. The number of dead prawn was recorded in each aquarium at 24, 48, 72 and 96 hours interval. The experiments were carried out at room temperature (30±1°C) with filtered seawater having 25 psu salinity, dissolved oxygen 4.5±0.5 ml l⁻¹ and pH 7.5 and free ammonia 0.95-µ mol l⁻¹ (Table 1). The LC₅₀ values were calculated by probit analysis¹⁰.

For the study of growth two sublethal concentrations (0.125 and 0.25 ppm) of naphthalene were selected, to get a wider range effect. Concentrations were based on 96 hrs LC 50 value. In each concentration 10 juveniles of uniform size (40±2 mm) were selected and introduced. Initial average length (mm) and weight (mg) were noted down prior to introduction in the tank. Total length was measured in mm and wet weight taken after 30 days exposure. During the experiments static renewal bioassay was followed in which experimental medium was changed

every alternate day. During the experiment the juveniles were fed with inert diet and slow aeration was provided.

For the oxygen consumption a 6 L capacity rectangular glass jar was used. Sublethal concentration chosen for the oxygen consumption was 0.25 ppm, based on 96 hr LC₅₀ value. Two tanks were maintained, one as control and other with naphthalene. Tanks were covered with black carbon. In each tank one animal was introduced in 5 L water. Tops of the tanks were sealed with liquid paraffin to avoid any contact with atmosphere. Oxygen uptake was measured at hourly interval for 6 hours by siphoning required amount of water and measuring oxygen concentration by Winkler's method.

Test juveniles were exposed to wide range of sublethal concentrations (0.125, 0.150, 0.20, 0.25, 0.35 and 0.45 ppm) for 4 weeks to see the effect of graded naphthalene on biochemical constituents. A control was maintained. At the end of the experiment all the prawns were collected from each aquaria for the analysis of biochemical constituents. Samples were dried in an oven at 60°C to get constant weight. The dried samples were finally powdered and stored in a desiccator until analysis.

Total carbon and nitrogen were determined with a CHN Analyzer (Perkin-Elmers-PE 2400) using 2-mg dry powdered sample. Protein was calculated by multiplying the Nitrogen values with a factor of 6.25. Lipid was estimated by the method of Parson *et al*¹².

Results and Discussion

Acute Toxicity

The percent survival of *M. affinis* in various concentrations of naphthalene is given in Table 2. The LC₅₀ values of naphthalene were determined by

Table 1—Physico-chemical parameters of the test medium

Environmental parameters	Value
Temperature (°C)	30.0±1.0
pH	7.5±0.6
Dissolved oxygen (ml l ⁻¹)	4.5±0.8
Salinity (psu)	25.0±1.8
Ammonia (µM)	9.55±0.16

Table 2—Survival rate of *M. affinis* on exposure to naphthalene

Concentration of naphthalene (ppm)	% survival rate (hr)			
	24	48	72	96
Control	100	100	100	100
0.125	100	100	100	100
0.25	100	100	100	100
0.50	100	80	80	80
1.0	100	80	60	40
1.5	80	60	50	40
2.0	50	40	20	20

plotting the cumulative mortality percentage after 96 hours against the varying concentrations.

The LC₅₀ values for 24 hr, 48 hr, 72 hr and 96 hrs was 1.98, 1.74, 1.56 and 0.98 ppm, respectively (Table 3). The 96 hrs LC₅₀ value was taken as basis for evaluating the physiological effect of pollutant¹³. The LC₅₀ values decreased with an increase in the exposure time. This implies that the tolerance of *M. affinis* to naphthalene decreased with increase in experimental time. It has been reported that medium to low boiling point aromatics such as naphthalene are the most toxic petroleum fractions to marine organisms⁹ and the majority of effect concentrations ranged from 0.4 to 5 mg l⁻¹. There are very limited data on the chronic naphthalene exposure⁴.

The increase in total length (mm) and wet weight (mg) of the juveniles were recorded after 30 days exposure (Table 4). From the initial average length of 40mm and 778 mg weight, the prawns achieved the growth of 63 mm in length and 1028 mg in weight at the end of the 30th day in control. Under the treatment of naphthalene (0.125 mg/l) the prawn grew from an initial average length of 41 mm and weight of 775 mg to 57 mm and 870 mg, respectively. In the higher

concentration of 0.25 mg l⁻¹ the growth in length was from 41 mm to 53 mm and in weight 765 mg to 805 mg, respectively. The growth increment in terms of weight gained was 32.13 in control, 12.25% in 0.125 ppm and 5.22% in 0.25 ppm, respectively. The differences were significant (p<0.05). The prawns exposed to sublethal concentration of naphthalene weighed significantly lower than control (p<0.05). Adam and Rice¹⁴ reported slower growth rate in juveniles of pink salmon exposed to water-soluble oil and naphthalene.

The growth of the prawn mainly depends on the physiological moulting process. Naphthalene is reported to inhibit moulting in speckled prawn, *Metapenaeus monoceros*¹⁵. It has been suggested that uptake rate of naphthalene depends on their concentration in water¹⁶. The results obtained from exposure of prawn to different concentration corroborate the earlier findings.

The measurement of oxygen consumption provides an index for sublethal stress condition and for biomonitoring the potentially toxic chemicals that elicit this response. Effect of naphthalene on oxygen consumption is given in Table 5. The respiratory rate increased initially in the chambers. This could be attributed to the oxidative stress induced by naphthalene⁷ and increased movement activity due to excitability of the prawn in the new medium¹⁷. When the animal is under stress and in motion, it consumes more oxygen to sustain its life. The percent respiratory rate dropped significantly (p<0.05). The decrease was more rapid in the tanks having naphthalene than in control and the changes were more pronounced after 6 hours. The percent reduction in respiratory rate was up to 44.4% in naphthalene. Since the oxygen consumption is an index of respiratory metabolism; the fluctuation in the activity of respiratory enzymes will serve as an early marker to assess the extent of pollution in aquatic animals¹⁸. On an average the oxygen consumption by the prawn in control was found to be 0.35-mg g⁻¹ body weight/hour and by the exposed prawn 0.24 mg g⁻¹

Table 3—LC₅₀ values of different exposure time in *Metapenaeus affinis*

Exposure time (hr)	LC ₅₀ value (ppm)
24	1.98
48	1.74
72	1.56
96	0.98

Table 4—Effect of naphthalene on the growth of *M. affinis* after 30 days exposure

Concentration in ppm	Initial length in mm	Initial weight in mg	Final length in mm	Final weight in mg
control	40±1.9	778±15	63±3.6	1028±17
0.125	41±2.2	775±16	57±1.8	870±16
0.25	41±1.6	765±14	53±1.9	795±12

Table 5—Effect of naphthalene on the oxygen consumption of *M. affinis*

Concentration (ppm)	Dissolved oxygen consumption (mg g ⁻¹ body weight hr ⁻¹)					
	1 st hour	2 nd hour	3 rd hour	4 th hour	5 th hour	6 th hour
Control	0.54±0.016	0.58±0.009	0.34±0.012	0.18±0.005	0.11±0.003	0.08±0.002
0.25	0.18±0.005	0.43±0.007	0.35±0.009	0.23±0.007	0.18±0.006	0.108±0.007

body weight/hour. An initial increase in oxygen consumption of *Etroplus suratensis* exposed to hydrocarbons, with a subsequent decrease was observed¹⁹. After prolonged exposure the decrease in oxygen consumption suggest a narcotic effect of naphthalene. The fish possess a variety of cellular mechanisms which protect against the deleterious effects of naphthalene during the metabolic process²⁰.

During the process of oxygen uptake through aquatic medium, the entry of naphthalene would have taken place into the body of prawn. The xenobiotic chemicals affect the respiratory system by causing respiratory and circulatory failure or by specific toxic action²¹. In other studies reduction in the oxygen consumption in crustacea on exposure to fuel oil has been reported^{22,23}. Bates *et al.*⁴ reported that the naphthalene contaminated invertebrates when exposed to uncontaminated water; the naphthalene gets depurated rapidly within few days.

The values of biochemical constituents of juvenile *M. affinis* on exposure to naphthalene is given in Table 6. All the constituents except lipid decreased with increasing concentration of naphthalene. The effect was dose and duration dependent. Carbon content decreased with increasing concentration of naphthalene as compared to the values in control. Changes in hematological constituents are reported on exposure to naphthalene in crabs⁶.

Extent of decrease was 3.5% in the lowest concentration of 0.125 ppm, and 12.9% in the highest concentration of 0.45 ppm. Effect was dose dependent. Similar effect was seen in the case of nitrogen and protein. Average value of protein in the prawns of control tank was 43.33. On exposure to naphthalene, the protein content reduced significantly

($p < 0.05$) under all concentrations. Small degree of protein damage at cellular level has been reported in small crustaceans¹ after exposure to low levels of naphthalene.

The reduction was to the extent of 2.53% in 0.125 ppm, 13.86% in 0.15 ppm, and 51.0% 0.45 ppm. Effect was inversely related to naphthalene concentration. Slower growth rate and decreased caloric content in pink salmon exposed to naphthalene (<0.8 mg/l) have been reported¹⁴. Reduction was to meet the extra energy required by the animal for metabolic alteration caused due to naphthalene stress.

The average lipid content was 10.13% in control. On exposure to naphthalene the concentration increased. Increase was to the extent of 17.18% in 0.25 ppm, 33.94% in 1.0 ppm, 35.46% in 1.5 ppm and 44.29% in 2 ppm. The effect was directly related to dose concentration and was contrary to the reducing effect on carbon and protein. Our findings corroborate the observation made earlier on exposure to petroleum hydrocarbon²⁴.

The trace contaminants like PAH in aquatic ecosystem appears to pose environmental hazard because of their great toxicity or persistence²⁵. The hydrophobic chemicals like naphthalene appears to get accumulated to the greatest extent and is retained by marine animals for a long period²⁶. Present study revealed that the aquatic organisms loose the equilibrium due to alteration in the normal physiological activities such as growth, metabolic index and biochemical changes after exposure to pollutants. Under environmental stress, the organisms have to adjust their energy budget to compensate for the effects so as to maintain their vital functions⁸. Should the available energy be insufficient to maintain the standard metabolism and detoxification processes, the organism will face severe physiological problems and some times die. Present study revealed the influence of naphthalene in altering the normal physiological and biochemical metabolism resulting in breakdown and utilization of important energy reserves to overcome the stress caused by the pollutant¹⁰. Changes are manifested by causing functional failure of different body organs⁷. Toxic effect was directly related to the exposure concentration.

Physiological and biochemical parameters are good indicator of stress condition with reference to PAH.

Table 6—Elemental composition of *M. affinis* on exposure to naphthalene

Concentration (ppm)	Carbon % dry wt	Nitrogen % dry wt	Protein % dry wt	Lipid % dry wt
Control	37.50±3.25	6.95±0.76	43.33±2.78	10.13±2.46
0.125	36.20±5.12	6.82±0.66	42.26±4.19	12.33±1.78
0.150	35.12±3.86	6.10±0.92	38.12±4.62	14.34±1.56
0.20	34.30±4.52	5.22±0.58	32.62±5.11	18.17±2.18
0.25	34.20±3.94	5.11±0.74	31.93±3.86	18.18±2.52
0.35	34.00±3.38	5.02±0.82	31.37±3.15	16.23±1.96
0.45	33.23±4.26	4.59±0.75	28.68±2.78	17.23±2.04

Addition of low levels of naphthalene in the environment may cause stress and mortality in marine biota. It is apparent that mechanistic studies evaluating metabolism and disposition of naphthalene should continue to get a better understanding of its bioavailability for the recreational and commercial fishery and marine food chain.

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