

Physiological responses of a clam *Gafrarium divaricatum* (Gmelin) to xylene, benzene and gear oil-WSF

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The intertidal clams, *Gafrarium divaricatum* were exposed to sublethal concentrations of xylene, benzene and water soluble fractions of gear oil up to one month. Alterations in oxygen consumption and filtration rate of the *G. divaricatum* were noted after 96 h and one month exposure period. Pollutant stress was found to suppress the oxygen consumption and filtration rate of the clams when exposed for a long period.

At present marine ecosystem is under immense pressure of anthropogenic activities. Since, exploration and transportation of petroleum products is connected to marine ecosystem directly, petroleum products are major pollutants in the marine environment. Oil is a complex mixture of aliphatic, alicyclic and aromatic hydrocarbons. Among these hydrocarbons the aromatic fraction is responsible for toxicity of the oil to marine organisms^{1,2}. Therefore, there is an increasing interest in determining the toxicity of individual aromatic hydrocarbons to marine organisms.

Intertidal bivalves have been used in pollution studies^{3,4}. However, there is meager data regarding effects of oil on the intertidal clams which form an important part of shell-fish fishery. Therefore, the present investigation on physiological responses of an intertidal clam *Gafrarium divaricatum* to the petroleum hydrocarbons xylene, benzene and water soluble fraction of gear oil was carried out.

Samples of *Gafrarium divaricatum* (Gmelin) were collected during low tide from Nariman Point area of Bombay coast and after cleaning with seawater were brought to the laboratory as a single stock. The clams were acclimatized for 24 h in a aquarium (60×30×30 cm) containing seawater 30 to 32×10⁻³, temperature 27 to 29°C and pH 7.7 to 8. Same conditions were maintained throughout the experimental period. No food was given to the clams as these animals thrived well under laboratory conditions. Only those sets of stock animals which did not show any mortality during

acclimatization period were used for the experimentation. The active clams (with protruding siphon and foot) were selected for experimentation. The clams were kept in cleaned glass aquaria (20×15×15 cm) each containing one litter of ambient seawater and 10 clams. The aquarium water was replaced by freshly collected seawater at every 24 h with addition of fresh toxicant.

Standard solutions of the hydrocarbons (xylene and benzene) were prepared in acetone (1:1 ratio of acetone and hydrocarbons). Aliquots of these stock hydrocarbon solutions were added to the experimental aquaria water to obtain desired concentrations of xylene (4.25 and 8.5 mg/l) and benzene (4.35 and 8.7 mg/l), two sets of clams were kept as controls under the same experimental conditions except that no toxicant was added to the control aquaria. Only acetone was added in equal quantities used for the aliquots.

The water soluble fractions of gear oil was prepared by mixing 500 ml of gear oil and 500 ml of seawater. The mixture was stirred in a close container for 24 h at 2000 rpm. After mixing the oil and water layers were allowed to separate for 6 h and then the water phase at the bottom was siphoned off and utilized immediately for the experimentation. The water soluble fractions obtained as 100% water soluble fraction and was added to test tanks to get desired concentrations of WSF (1% to 2%). The control set of animals kept for gear oil WSF experiments were without addition of any chemical.

The above mentioned sub lethal concentrations (4.25 and 8.5 mg/l xylene, 4.35 and 8.7 mg/l benzene, 1% and 2% gear oil-WSF) were selected on the basis of acute toxicity studies carried out earlier⁵. The 96h LC50 values are 720 mg/l of xylene, 960 mg/l of benzene and 25% WSF of gear oil. Different sets of clams were used for 96 h and one month exposure to pollutants.

Wide mouthed amber bottles (one liter)) were used as respirometers. Each bottle contained 2 clams filled with clean aerated seawater and the pollutant was added (4.25 and 8.5 mg/l xylene, 4.35 and 8.7 mg/l benzene and 1% and 2% gear oil-WSF). The bottles were made air tight. Before the experiment, the initial oxygen content of the test water was determined. A blank bottle without clam was also maintained just to check if the level of oxygen in test water varies (which did not show variation). Simultaneously control respirometers with unexposed clams and normal seawater were also set up. All the experimental as well as control bottles were left undisturbed for 2 h and after that the water from the respirometers was gently siphoned out to find out it's oxygen content. The oxygen content was measured by the azide modification of Winkler's method⁶. Oxygen consumption rates (O_2 /h/wet wt.) were determined from difference between the pre-experimental and post-experimental oxygen values. At the end of experiment, the clams were dissected and the flesh was recorded.

At the end of exposure period (i.e. 4 and 30 days) clams were used for filtration rate estimation. Two clams were placed in one liter beaker containing 500 ml of seawater to recover from handling for 15 min. Then 1.5 ml of freshly prepared 1% neutral red dye (BDH Ltd.) was added to each test beaker and gently mixed by stirring and 10 ml of sample was immediately extracted from each beaker after every 1 h for 3 h. Dye concentration in the samples was determined spectrophotometrically (Spectronic 20). Filtration rate is given by:

$$m = M/nt \log co/ct$$

where m = filtration rate in ml/ min volume of test in ml, M = volume of test solution in ml, co = dye concentration in samples at the beginning of the experiment, ct = dye concentration in samples at the end of experiment (i.e. after 1 h, 2h, 3h) and

t=time between dye samples in minutes.

An elevation in the rates of oxygen consumption was observed in clams after exposure to xylene, benzene and gear oil WSF for 96 hrs, where as significant depletion in the rate of oxygen consumption was noticed after 30 days exposure to these pollutants (Table 1, Fig. 1).

The clams exposed for 96 h to petroleum hydrocarbons and gear oil WSF showed significant elevation in filtration rates. Elevation was more pronounced at 1h. However 1 %WSF of gear oil brought slight elevation in filtration rates of the clams. Exposure of clams to all the pollutants for 30 d showed depletion with marked depletion in clams exposed to 8.5 mg/l xylene (Table 2, Fig. 2).

Total oxygen consumption which is the sum of basal metabolic rate and the rate due to activity, increases in many organisms including clams when exposed to petroleum products. Increased oxygen demand in *Gafrarium divaricatum* after 96 h exposure to xylene, benzene and gear oil WSF,

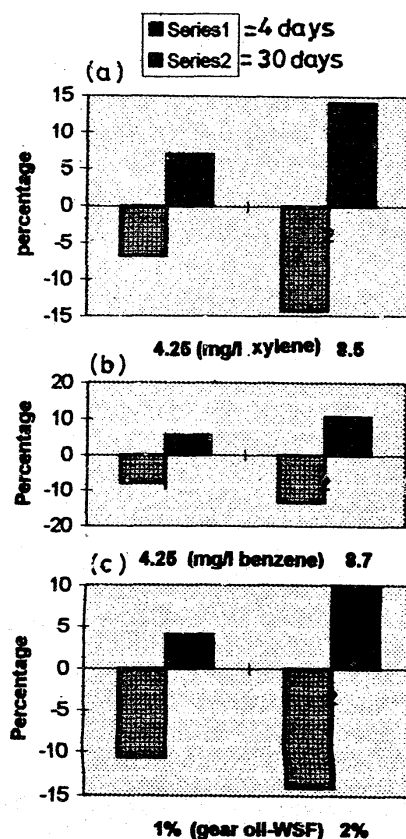


Fig. 1—Percent variation in oxygen consumption in *G. divaricatum*

Table 1—Oxygen consumption (ml O₂/h/gm body wt.) in *G. divaricatum* exposed to xylene, benzene and gear oil
(Values are mean $\pm$ SD of 5 determinations)

Exposure period (days)	Control	Xylene(mg/l)	
		4.25	8.5
4	0.399 $\pm$ 0.010	0.467** $\pm$ 0.019	0.542** $\pm$ 0.023
30	0.388 $\pm$ 0.026	0.318** $\pm$ 0.008	0.247** $\pm$ 0.014
		Benzene(mg/l)	
		4.35	8.7
4	0.375 $\pm$ 0.071	0.455* $\pm$ 0.014	0.509** $\pm$ 0.014
30	0.387 $\pm$ 0.021	0.332** $\pm$ 0.007	0.280** $\pm$ 0.008
		Gear oil (WSF)	
		1%	2%
4	0.336 $\pm$ 0.051	0.443** $\pm$ 0.024	0.477** $\pm$ 0.010
30	0.389 $\pm$ 0.037	0.349* $\pm$ 0.003	0.290** $\pm$ 0.019

*P < 0.05; **P < 0.01

Table 2—Filtration rate (ml O₂/h/gm body wt.) in *G. divaricatum* exposed to xylene, benzene and gear oil
(Values are mean $\pm$ SD of 5 determinations)

Exposure period (days)	Hours	Control	Xylene (mg/l)	
			4.25	8.5
4	1	3.82 $\pm$ 0.08	4.28** $\pm$ 0.12	4.51** $\pm$ 0.09
	2	3.43 $\pm$ 0.05	3.68** $\pm$ 0.04	3.92** $\pm$ 0.04
	3	3.48 $\pm$ 0.05	3.61** $\pm$ 0.04	3.76** $\pm$ 0.04
30	1	4.22 $\pm$ 0.58	4.04 $\pm$ 0.16	3.60* $\pm$ 0.12
	2	3.59 $\pm$ 0.33	3.63 $\pm$ 0.26	3.20* $\pm$ 0.13
	3	3.68 $\pm$ 0.11	3.43* $\pm$ 0.19	3.03** $\pm$ 0.29
			Benzene(mg/l)	
			4.35	8.7
4	1	3.71 $\pm$ 0.11	3.76 $\pm$ 0.07	4.22** $\pm$ 0.33
	2	3.45 $\pm$ 0.04	3.61** $\pm$ 0.04	3.68** $\pm$ 0.07
	3	3.53 $\pm$ 0.08	3.65* $\pm$ 0.06	3.70** $\pm$ 0.07
30	1	4.24 $\pm$ 0.92	4.48 $\pm$ 0.70	3.71 $\pm$ 0.11
	2	3.47 $\pm$ 0.35	3.77** $\pm$ 0.21	3.31 $\pm$ 0.09
	3	3.63 $\pm$ 0.14	3.63 $\pm$ 0.14	3.09 $\pm$ 0.29
			Gear oil(WSF)	
			1%	2%
4	1	3.71 $\pm$ 0.11	3.76 $\pm$ 0.07	4.35** $\pm$ 0.07
	2	3.45 $\pm$ 0.04	3.58** $\pm$ 0.03	3.71** $\pm$ 0.07
	3	3.53 $\pm$ 0.08	3.53 $\pm$ 0.05	3.60 $\pm$ 0.12
30	1	4.17 $\pm$ 0.62	4.46 $\pm$ 0.41	3.84 $\pm$ 0.18
	2	3.49 $\pm$ 0.30	3.73 $\pm$ 0.19	3.38 $\pm$ 0.18
	3	3.71 $\pm$ 0.11	3.54 $\pm$ 0.35	3.35 $\pm$ 0.37

*P < 0.05

**P < 0.01

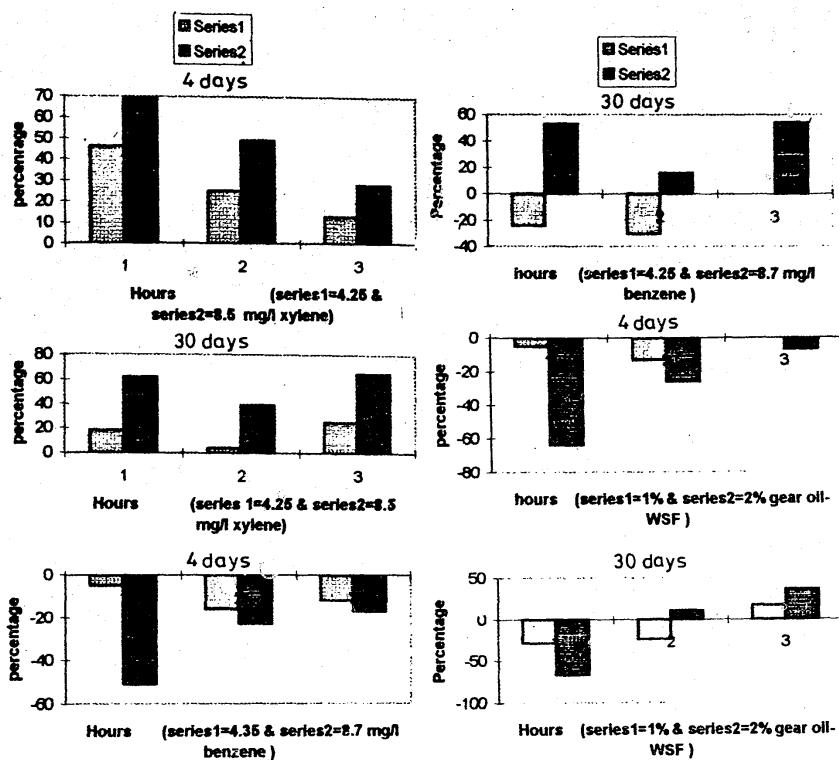


Fig. 2—Percent variation in filtration rate of *G. divaricatum*.

could be the result of more water intake essential for removal of oil and petroleum hydrocarbons in the gills. Gills in the bivalves are one of the organs which accumulate petroleum hydrocarbons.

Stekoll *et al*³ noticed increased respiratory rates in bivalves *Macoma balthica* exposed to oil. Similarly, enhanced oxygen consumption in *Venus verrucosa* was observed when exposed to petroleum hydrocarbon⁷. In the present investigation the increased pumping activity in the experimental clams is indicated by enhanced filtration rates up to 96 h exposure period, which supports the enhanced respiratory rates in the experimental clams.

Depletion of oxygen consumption and filtration rates in the clams after one month exposure to xylene, benzene and WSF of gear oil might be due to the histopathological damage to gills, which resulted in malfunctioning of the gills. Such damage to gills of *G. divaricatum* was observed when exposed to petroleum hydrocarbons and copper⁵. Therefore, the depleted rate of filtration and oxygen consumption in experimental clams might not be sufficient to meet the metabolic

demands of clams which might force the animal to switch over to anaerobic processes of metabolism. Such a switch over to anaerobic metabolism in clams under stressful conditions is well known^{8,9}.

References

- 1 Anderson J W, Neff J M, Con B A, Taten H E & Hightower G M, in *Pollution and physiology of marine organisms*, edited by F J Vernberg and W B Vernberg (Academic Press, New York), 1974, 285.
- 2 Kulkarni B.G. & Masarekar V.B, *Geobios*, 10 (1983), 63.
- 3 Stekoll M S, Clement L E & Shaw D G, *Mar. Biol*, 57 (1980) 51.
- 4 Philip M & Menon N.R, *Bull Environ Contam Toxicol*, 48 (1992) 185.
- 5 Tendulkar S P, *The effects of oil and some petroleum hydrocarbons on the intertidal clam Gafrarium divaricatum* (Gmelin), Ph.D. thesis, University of Bombay, 1996.
- 6 Barnes H, in *Apparatus and methods of oceanography Part-I Chemical*, (Allen and Unwin Ltd, London) 1959, 341.
- 7 Axiak V & George J J, *Mar. Biol*, 94 (1987) 241.
- 8 Roy S S, *Effects of copper on eco-physiological aspects of the clam Gafrarium divaricatum*, M.Sc. thesis, University of Bombay, 1994.
- 9 DeZwaan A & Zandee D T, *Comp. Biochem. Physiol*, 43B, (1971), 47.