Marine Biology 94, 241–249 (1987)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Effects of exposure to petroleum hydrocarbons on the gill functions and ciliary activities of a marine bivalve

V. Axiak [1] and J. J. George [2]

[1] **Mathematics and Science Department, University of Malta; Msida, Malta**
[2] **The Polytechnic of Central London; 115 New Cavendish Street, London W1M 8JS, England**

Abstract

The effects of long-term exposure to low levels of water-accommodated fractions of Kuwait Crude oil, or to short-term exposure to chemically dispersed oil, on the gill performance of the clam *Venus verrucosa* were investigated. Reduced pumping activities of the lateral cilia as well as interference with the normal beating activities of the eulaterofrontal cirri led to reduced clearance rates and retention efficiencies of food particles less than 6 μm in diameter. On the other hand, frontal ciliary activities were significantly accelerated, while any retained oil droplets were conducted to the mouth region as food particles. The activities of terminal and sensory cilia were also enhanced and mucus production increased. The significance of these responses to the clam's energy budget is discussed.

Introduction

The gills (ctenidia) of lamellibranch bivalves play a dominant role in controlling the interaction between the individual and its environment. A great deal of literature is available on the mechanisms of food particle retention, as well as on the nature and activities of the various ciliary systems of such organs (recently reviewed by Morton, 1983). However, much less is known about the responses of these bivalve systems to environmental stressors, including chemical pollutants.

In the present study, the effects of exposure to petroleum hydrocarbons (PHC) on the efficiency of gill performance as well as on the activities of its various ciliary systems in the marine bivalve *Venus verrucosa* were investigated. PHC represent a major class of coastal contaminants, especially in the Mediterranean which, though representing only about 0.8% of the earth's oceanic surface, may receive as much as 10 to 60% of the annual global input of PHC in the marine environment (GESAMP, 1982). *V. verrucosa* is a typical infralittoral filter feeder locally found in shallow semi-enclosed inshore areas. Such sites include harbours which are more liable to be exposed to oil pollution and whose hydrodynamic characteristics are such that they minimize the dispersal and dilution of any contaminating PHC. Moreover, the application of oil dispersants to small but frequent oil slicks close to the shore is a common regional practice and it may be expected that such benthic infralittoral species may be exposed both to chemically dispersed PHC as well as to water-accommodated fractions (WAF) of oil.

Materials and methods

Venus verrucosa, 35 to 45 mm in shell length, were collected from unpolluted sites on the north-eastern coastline of Malta, and held in a system of recirculating seawater at 37.5‰ S, 20 °C and a pH range of 7.2 to 7.8, under artificial overhead illumination of 50 to 150 Lux (as measured at the water surface), switched on for 12 h daily. During acclimation, the clams were allowed to burrow in natural sediments in all-glass aquaria and were periodically fed on Liquifry Marine, which is a commercial filter feeder-food, obtained from Liquifry Co. Ltd., Dorking, England (batch code number: 0308). All clams behaved normally during the 8-week acclimation period. All investigations were carried out under the same conditions as those of acclimation.

Experiments on particle retention

Individual bivalves were allowed to burrow, each within approximately 20 g of natural sediments in small plastic cups. Each cup was then placed in a 1-liter cylindrical chamber with 900 ml of natural unfiltered seawater flowing at 60 ml min^{-1} through it, with an inflow at the bottom and an outflow at the top of the chamber. A suspension of Liquifry Marine was injected into the flow so as to have an

approximate concentration of 3 to 4×10^6 particles ($>4\,\mu m$ in diameter) per liter. Clams were allowed to settle for a minimum of 1 h in this flow-through system. The decrease in the concentration of particles (ranging from 2.3 to 9 μm in diameter) due to retention by the clams was then calculated by comparing their frequency distribution in the outflow of the clam chambers with that in the control (no clam) as analysed by the Coulter Counter (Model ZB Industrial) fitted with a 140-μm orifice tube. Particle counts were taken in quadruples within a maximum of 10 min of collection. Moreover, all samples were maintained at a constant temperature of 20 °C, by keeping them immersed in a water bath, before being analysed. Preliminary experiments indicated that temperature fluctuations may cause appreciable changes in the particle counts in samples left standing for more than a few minutes. The retention efficiency for each size range of particles was calculated from the percentage reduction in their concentration. Particle sizes were expressed in diameters of the equivalent or 'effective' spheric volumes recorded by the Coulter Counter.

Investigations on ciliary activities

These were carried out both on 'half clams' and on isolated gill fragments. In the former case, the left valve and mantle were removed and the clam was then allowed to recover for at least 2 h. It was then placed in an observation chamber (Fig. 1) with its exposed half upwards. The observation chamber was constructed of perspex and contained within a cylindrical water jacket with an internal diameter of 12 cm and a height of 6.5 cm. Seawater at 20 °C ± 0.5 C° circulated through this jacket. Seawater flowed through the central chamber at a similar temperature of 20 °C and a salinity of 37.5 to 37.8‰ S. This preparation was observed under a Bausch and Lomb binocular microscope at magnifications up to 75×. When the clam was fully recovered from the operation, as indicated by the cessation of mucus production by the gills, and by the relaxation and opening up of the gill's plicae, Latex particles (Coulter Counter calibrating Latex spheres) of 8.06 μm diameter, were introduced into the water flow. The activity of frontal cilia was investigated by measuring the rates of transport of such spheres along the plical grooves of the inner demibranch by means of an eyepiece micrometer and a stopwatch. The mean rate of transport as measured in mm s^{-1} was calculated from a minimum of ten observations. For any one particular experiment, all observations and measurements were made on the same area of gill surface. In all cases, only particles unbound to mucus had their rates of transport measured. In some cases, the rate of transport of Latex particles along the ventral marginal groove of the inner demibranch was also calculated.

Ciliary activities were also investigated by observing isolated gill fragments. Such fragments were always prepared from the inner demibranch, including the marginal groove on one side, and measured approximately

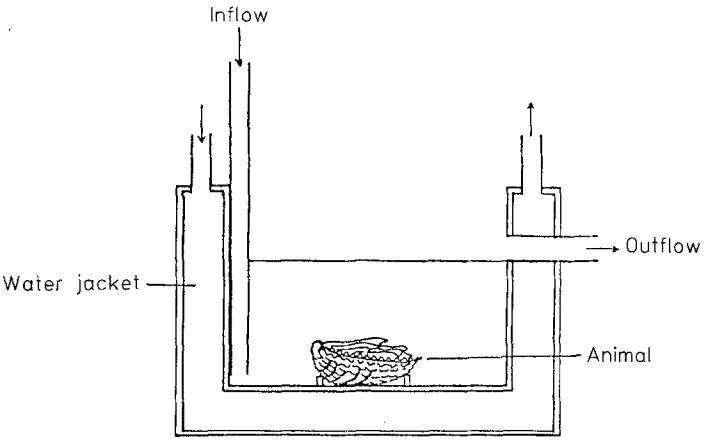

Fig. 1. *Venus verrucosa.* Observation chamber used for studies on the ciliary activities of 'half clams'

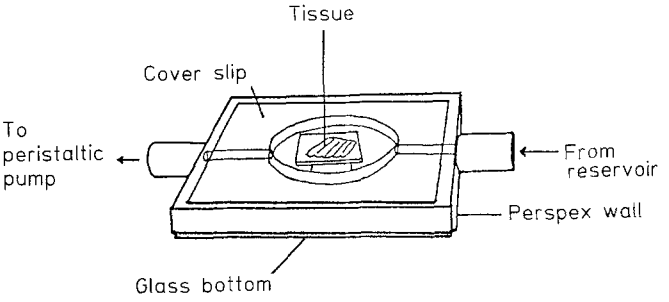

Fig. 2. *Venus verrucosa.* Observation chamber used for the studies on the ciliary activities of gill fragments

10 × 10 mm. After incision, they were allowed to relax and recover in flowing seawater for a minimum of 1 h. Then such a gill fragment was gently placed into an observation chamber (Fig. 2). This chamber was constructed of perspex with a glass bottom, and measured 50 by 50 mm. The central disc-shaped chamber had a diameter of 30 mm and was 8 mm high. Seawater flowed through the chamber at a constant rate of 3.5 ml min^{-1} by means of a peristaltic pump. This renewal of seawater ensured that the observed ciliary activities were not affected by any decrease in oxygen tensions, as it is known that such cilia are highly sensitive to any lack of oxygen (Jørgensen, 1975). This flow also ensured no increase in temperature due to the microscope lighting system. The gill fragment itself was raised over a small glass block so that it could be focused under a high power oil immersion objective lens. Observations were made with a Bausch and Lomb Balplan microscope (fitted with a 35-mm, C-35 photographic camera) at magnifications up to 1000×. Transport rates of Latex particles (8.06 μm diameter) along the frontal surface of the ctenidia, as well as along their ventral marginal groove, were calculated by means of an eyepiece micrometer and stopwatch.

Frontal cilia activities were also investigated by measuring the crawling rates of gill fragments. Most bivalve gill pieces crawl along a glass surface, while immersed, due to the beating of the frontal cilia (Malanga, 1974), though water currents created by the lateral cilia

may also be involved (Gosselin and O'Hara, 1961). In the present study, gill fragments from the inner demibranch, measuring approximately 10×10 mm and including an intact ventral margin, were placed in a Petri dish and covered with seawater to a depth of 3 to 5 mm. Such preparations were then observed under a microscope at a magnification of 400×. Crawling speeds were measured by means of an eyepiece micrometer and stopwatch, and means of at least three observations were recorded.

The structure and organisation of the various ciliary systems were also studied on scanning electron micrographs of gill fragments fixed in 2% osmium tetroxide, and followed by critical point drying. All reported dimensions of cilia were calculated from observations of fresh material with Nomansky interference microscopy.

Exposure to PHC

In the case of particle retention experiments, clams were exposed to approximately 100 $\mu g\, l^{-1}$ of WAF of Kuwait Crude oil for 140 d. Clams were allowed to burrow in natural sediments which covered the bottom of an exposure chamber measuring 30×60 cm and 30 cm high, containing 30 l of natural seawater flowing in a closed circuit at a rate of 1 liter min^{-1}. Details of the oil dosing mechanism is given elsewhere (Axiak, in press). Particle retention efficiences were measured 4 d before, and then 84 and 140 d after beginning of exposure.

Half clams were exposed to either WAF of Kuwait crude oil (initial concentrations: 1 020 and 4 300 $\mu g\, l^{-1}$) prepared by the 'slow stirring method' of Anderson *et al.* (1974) or PHC chemically dispersed with FINASOL OSR 7 (initial concentrations: 1 200 and 10 200 $\mu g\, l^{-1}$). Ciliary observations were carried out for at least 2 h before exposure, then test mixtures were made to flow over the preparations for 5 h, during which frequent observations were made.

Two exposure experiments were carried out using gill fragments. In the first, the ciliary activities of gill fragments were observed for 2 h under running clean seawater (20 °C, 37.5‰ S). Then seawater containing WAF of Kuwait crude oil, at a concentration of 300 $\mu g\, l^{-1}$, and the same temperature and salinity, was made to flow through the chamber for 3 h, while any reactions by the ciliary activities of the gill fragments were continuously monitored. In the second experiment, gill fragments (10×10 mm) were placed in covered 100-ml glass beakers containing 50 ml of different concentrations of WAF of oil (initial concentrations: 360, 180, and 30 $\mu g\, l^{-1}$) for 48 h. During this exposure period, test mixtures were left unaerated. Control runs were also carried out on gill fragments in clean sea water. At 24 and 48 h of exposure, gill fragments were mounted on the observation chamber as described above, and their ciliary activities observed under the microscope, with seawater containing WAF at the same concentration of exposure flowing through the chamber.

In all exposure experiments, the levels of PHC in the test mixtures were periodically analysed by extraction into dichloromethane by means of shaking. The PHC were then measured in terms of Kuwait Crude oil equivalents by spectrofluorometry at an excitation wavelength of 310 nm and emission at 360 nm (IOC/WHO/UNEP, 1977). Details of the aromatic composition of the WAF used in these investigations as analysed by high pressure liquid chromatography are given elsewhere (Axiak, in press).

Results

Efficiency of particle retention

Fig. 3 shows the particle size distribution of a typical sample of Liquifry Marine suspension used for particle retention experiments. It indicates that, in terms of ppm by volume, particles in the size range between 5.2 and 4.1 μm in diameter had the highest frequency of occurrence.

Several experiments on particle retention in control *Venus verrucosa* indicated that, while particles above 7 to 8 μm in diameter were almost invariably retained with relatively high efficiencies, significant inter-experiment

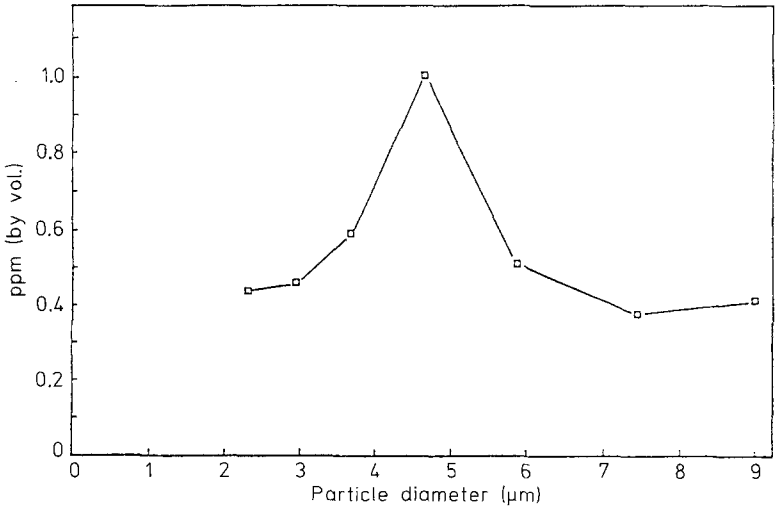

Fig. 3. *Venus verrucosa.* Size distribution of a typical sample of Liquifry Marine suspension used in the retention efficiency experiments

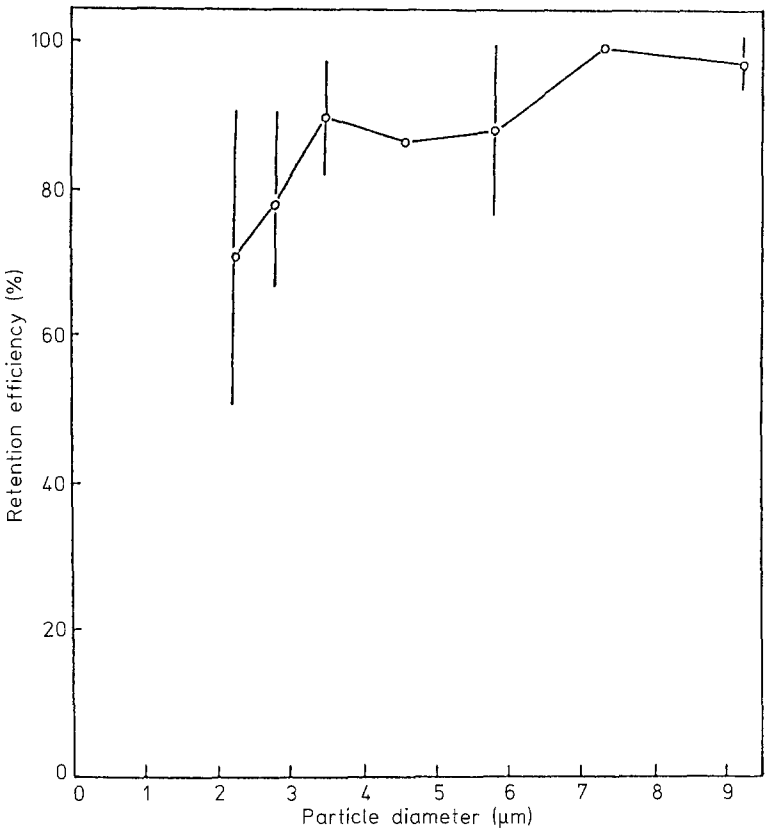

Fig. 4. *Venus verrucosa.* Retention efficiency as a function of particle size. Means of 6 replicates with 95% confidence limits. First control experiment

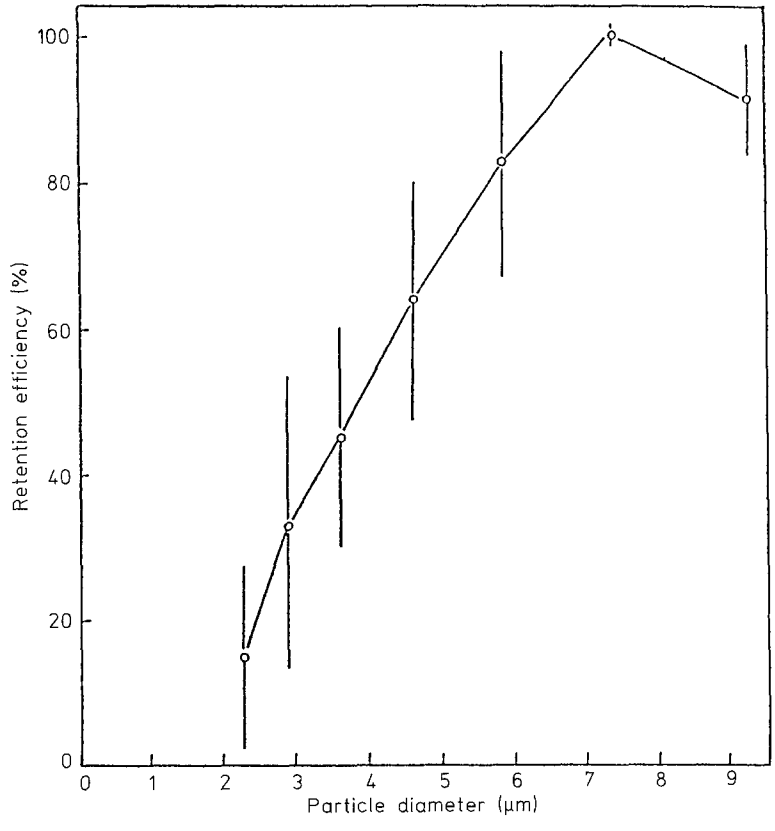

Fig. 5. *Venus verrucosa.* Retention efficiency as a function of particle size. Means of 6 replicates with 95% confidence limits. Second control experiment

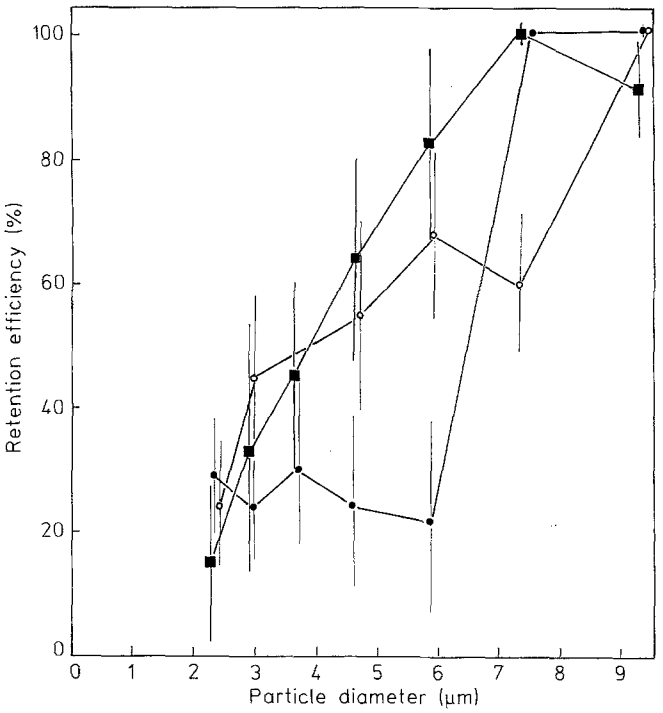

Fig. 6. *Venus verrucosa.* Effects of long-term exposure to 100 μg l^{-1} of WAF of oil on particle retention by gills. Black squares: pre-exposure; white circles: after 84 d of exposure; black circles: after 140 d of exposure. Means of 6 replicates with 95% confidence limits

variations were evident in the retention of particles with a diameter less than 6 μm. This is illustrated by Figs. 4 and 5 which show the results of two such experiments, showing retention efficiency as a function of particle sizes (mean values of 6 clams in each case). The percentage retention efficiency of particles with a mean diameter of 2.3 μm (range: 2.6 to 2.1 μm) varied from less than 20 to over 70%, while that of particles with a mean diameter of 7.5 μm ranged from 90 to 100%. In Fig. 5 (where the same 6 clams used for the exposure experiment were utilized), the mean retention efficiency increased from 15 to 83% from 2.3 to 5.9 μm. A 50% retention efficiency was recorded at a particle size of 4.14 μm.

Fig. 6 illustrates the effects of long-term exposure to 100 μg l^{-1} of WAF on the retention efficiency of the gills of exposed clams. After 84 d of exposure, the retention efficiencies of particles less than 8 μm in diameter were appreciably lower than those before exposure. Such differences in particle retention were, however, not statistically significant as analysed by Student's t-test ($P = 0.05$), probably due to the high variability of such values below 6 μm in the pre-exposure period. After 140 d of exposure, efficiency did not show the gradual increase, with an increase in particle size from 2 to 6 μm as shown in pre-exposure clams. Instead, particles from 2.3 to 6 μm were retained with approximately equal and lower efficiency. In this case, the retention efficiencies at 4.6 and 5.9 μm were significantly lower than at pre-exposure as analysed by Student's t-test ($P < 0.001$).

Ciliary activities in control half clams and gill fragments

The structure and ciliary activities of gills of Veneridae in general have been described by Atkins (1937) and Ansell (1961). In *Venus verrucosa,* both outer and inner demibranchs are broadly plicated and slightly heterorhabdic, with a well developed ventral marginal groove on the latter (Fig. 7).

Eulaterofrontal cirri project out of the lateral sides of the gill filaments (Fig. 8) spanning the interfilamentous space and presumably form the major filtering mechanism of the bivalve gill. Each cirrus which was approximately 22.8-μm-long ($N = 38$, SD = $\pm$ 0.25 μm) was found to be composed of two parallel rows of cilia, with each individual cilium bending to one side or the other of the main axis of the cirrus, thus forming regularly spaced side branches with a mean repeat distance of 0.89 μm ($N = 53$, SD = $\pm$ 0.09). The mean gap between such side branches was calculated to be approximately 0.6 μm, assuming a thickness of 0.3 μm for each side branch. The beating of the eulaterofrontal cirri, as seen in gill fragments under flowing seawater, was similar to that described for other bivalves (Dral, 1967) with adjacent cirri being 180° out of phase in most cases. At the beginning of their effective stroke, such cirri lie across the interfilamentous space, so that opposite cirri arising from adjacent gill filaments nearly overlap at their tips. At the termination of their effective stroke, approximately 2/3 of their distal part is bent through an angle of 90° to lie in a plane at right angles to the gill's frontal surface. In most cases, eulaterofrontal cirri remained active on excised gills for at least 5 to 8 h in flowing seawater.

The dense lateral cilia (Fig. 8), which propel water through the interfilamentous gill spaces, project to a distance of approximately 13 μm. They beat in a well defined metachronal rhythm, with metachronal waves flowing in opposite directions on the opposite lateral surfaces of a single gill filament at an approximate velocity of 60 to 70 μm s^{-1}. Such normal activity of the lateral cilia on gill fragments persisted for at least 48 to 72 h, provided they had an adequate supply of oxygen, i.e. in the flow-through gill chamber.

Latex spheres (8.06 μm in diameter) were easily retained by the gills and moved along the frontal surfaces of the gill under the action of the frontal cilia, at a mean velocity of approximately 0.18 mm s^{-1} at 20 °C. Such latex spheres, as well as other particulate food retained on the gill's surface, were normally found to travel while freely suspended in the water currents and unbound to any mucus. Mucus bands were produced along the gill's frontal surfaces and along the marginal grooves only when the gills were disturbed, e.g. just after incision, or on mechanical stimulation. When such particles reached the marginal grooves of the inner demibranch, they were propelled oralwards at a mean velocity of approximately 0.2 mm s^{-1}. Often particles could also be seen moving oralwards, along the lateral raised folds of the marginal groove, presumably

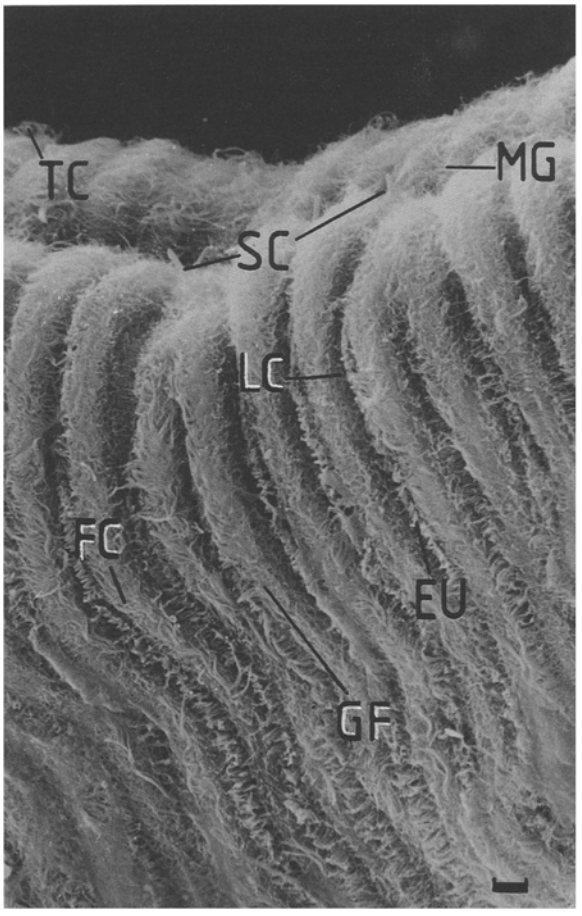

Fig. 7. *Venus verrucosa.* Scanning electron micrograph of inner gill demibranch. FC: frontal cilia; EU: eulaterofrontal cirri; GF: gill filament; MG: marginal groove; LC: lateral cilia; SC: sensory cilia; TC: terminal cilia. (Scale bar = 40 μm)

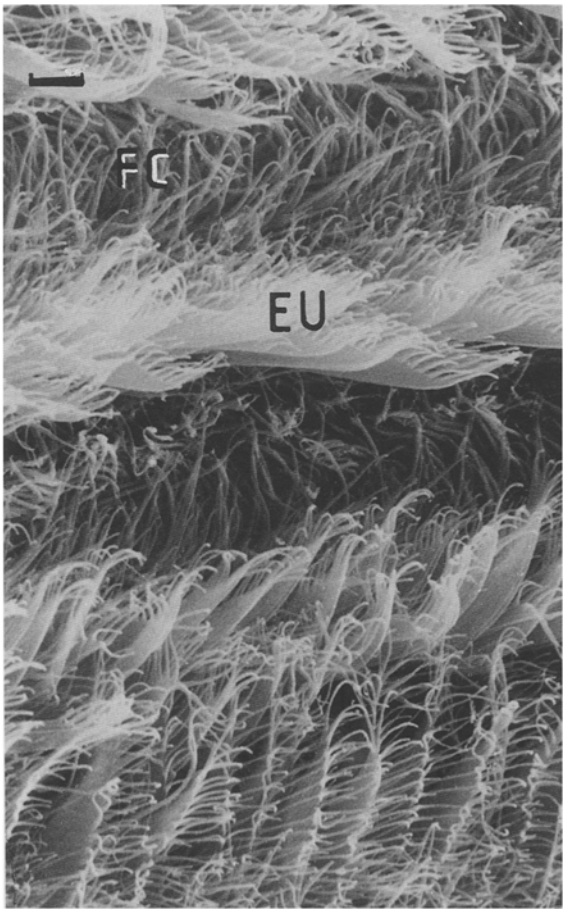

Fig. 8. *Venus verrucosa.* Scanning electron micrograph of inner demibranch showing the fine structure of the eulaterofrontal cirri, with component cilia coming out of the main cirrus thus forming side branches. FC: frontal cilia; EU: eulaterofrontal cirri. (Scale bar = 5 μm)

under the action of the coarse terminal cilia at a mean velocity of approximately 0.1 mm s^{-1}.

Effects of exposure to PHC on ciliary activities

Effects of exposure to oil upon frontal ciliary activities as measured by rates of transport of particles along the frontal gill surface in half-clam preparations are illustrated in Fig. 9. On exposure to chemically dispersed oil, no initial response was detected, but within 1.5 to 8.5 h of exposure, there was an increase in frontal particle velocities. There was a similar increase in such velocities on exposure to 1.0 mg l^{-1} of WAF of oil which persisted for at least 2 h of exposure, while an appreciable drop in frontal particle velocities was evident on exposure to 4.3 mg l^{-1} of WAF. No significant fluctuations in frontal ciliary activities were shown over at least 6 h of experiment in the case of control gill preparations.

Observations of the marginal groove in exposed clams indicated that it always remained open throughout such exposures, and, except when it was full of mucus, the rates of particle transport along its floor remained approximately constant (within a range of 0.19 to 0.25 mm s^{-1}). On exposure to the higher concentrations of WAF of oil and of chemically dispersed oil, there was generally a conspicuous increase in mucus production in the form of strands of mucus flowing down the gill's frontal surface and along the ventral marginal groove. Such mucus strands incorporated large amounts of latex spheres, but in the case of frontal particle velocity measurements, only particles unbound to mucus were investigated so that any change in such particle velocities on exposure to oil may not be attributed to the presence or otherwise of mucus.

When the clams were exposed to chemically dispersed crude oil, oil droplets ranging from 4 to 10 μm in diameter were observed to be efficiently retained by the ctenidial filaments and subsequently carried in the frontal water currents, as were other particles. Such oil droplets often reached the ventral marginal groove of the gill from where (as happens to food particles) they were conducted oralwards. These observations indicate that the gill's ciliary systems acted upon the oil droplets in the same manner as on food particles. When mucus production was enhanced, many of the oil droplets were found to be rapidly incorporated within the strands of mucus.

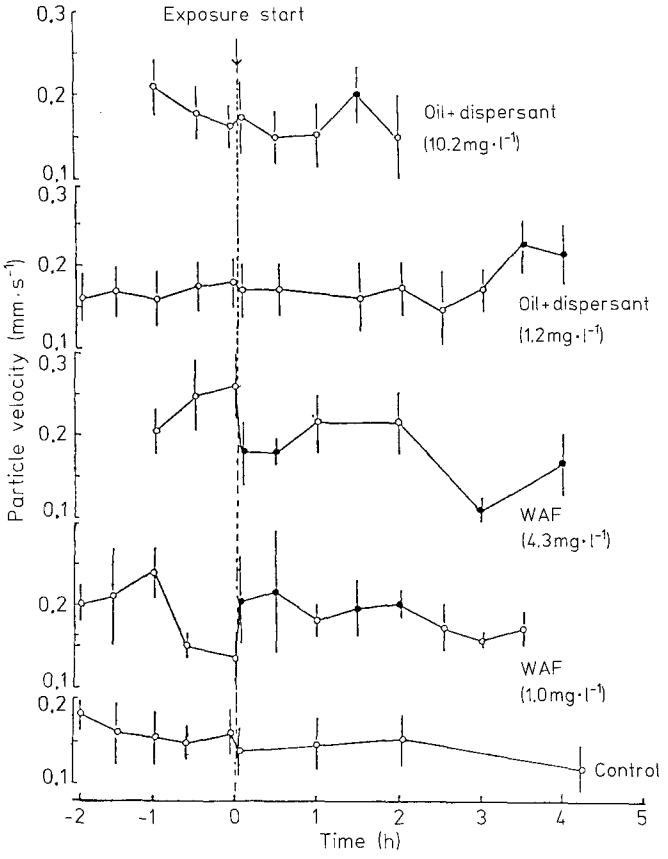

Fig. 9. *Venus verrucosa.* Effects of exposure to different forms of petroleum hydrocarbons on particle transport along frontal gill surfaces. Means of five clam replicates and 95% confidence limits. Mean particle velocities which are significantly different from those immediately prior exposure as analysed by Student's *t*-test ($P < 0.05$) are presented by black circles

Ciliary activities could be directly investigated in greater detail in isolated gill fragments mounted in a flow-through observation chamber. When such gill preparations were exposed to 300 μg l^{-1} of WAF of crude oil, the activities of frontal cilia remained apparently undisturbed. On the other hand, groups of eulaterofrontal cirri, as well as terminal cilia, appeared to become rather sluggish with less rapid effective strokes. Moreover, the angle of beat of eulaterofrontal cirri in many cases were decreased to less than 90°. This effect was only rarely observed throughout the whole gill piece, but more frequently occurred in isolated groups of cirri which apparently occurred at random.

Exposure to 300 μg l^{-1} of WAF of oil had a marked effect on the 'sensory' cilia. These long and coarse cilia were found either isolated or in groups of two at the ventral margins of both demibranchs (Fig. 7). They were found to be usually immobile, but could flex intermittently at their base through 90° in the oralward direction. On exposure to 300 μg l^{-1} of WAF, such intermittent beating of these cilia became more frequent; 3 or 4 successive beats were followed by periods of rest of irregular duration. Their mean beat frequency in this 'excited' state was found to be approximately 9 beats min^{-1}.

Table 1. *Venus verrucosa.* Effects of exposure to WAF of crude oil on the velocity of particle transport along the frontal surface of excised gill fragments

Conc. of WAF (μg l^{-1})		Mean velocity (mm s^{-1})	±SEM	% of control	*N*	Significance level *P*
Control	24 h	0.13	0.005		14	
	48 h	0.12	0.014		7	
30	24 h	0.11	0.022	90	11	NS
	48 h	0.19	0.019	158	10	0.05
180	24 h	0.16	0.012	123	8	0.001
360	24 h	0.22	0.010	169	4	0.001

SEM = Standard error of the mean
N = Number of gill replicates
P = Student's *t*-test between control mean and experimental mean

Gill fragments were also exposed to different concentrations of WAF in static conditions and ciliary systems were observed immediately before and then after 24 and 48 h of exposure. Results of effects on frontal particle velocities are presented in Table 1. These indicate that statistically significant increases in frontal ciliary activities were recorded after 24-h exposure to 180 and 360 μg l^{-1} of WAF. At these concentrations, no reliable measurements of frontal particle velocities could be made after 48 h of exposure due to the crawling activities of the gill fragments during microscopic observations. Exposure of 48 h to 30 μg l^{-1} of WAF also significantly increased such frontal ciliary activities.

Frontal ciliary activities were also monitored by measuring crawling rates of gill fragments (Table 2). The results indicate that, after 24 h of exposure, crawling rates were significantly increased at all test concentrations, with the effect being concentration-dependent. This effect was sustained after 48 h of exposure at test concentrations of 180 and 360 μg l^{-1}. When exposed gill fragments, exhibiting this hyper-crawling activity, were covered by a cover slip in static seawater, as usual, most of the eulaterofrontal cirri became inactive within a few minutes. However, crawling continued well after this inactivation of such cirri, confirming that frontal cilia, rather than eulaterofrontal cirri, are mostly responsible for gill crawling. In some gill fragment preparations, crawling rates were difficult to monitor due to either rotational movement or to a highly oblique direction of movement.

Results of effects of 24-h exposure on particle transport rates along the ventral margin groove are presented in Table 3. In most cases of exposed gills, such particles were bound to mucus strands, and as such these rates represent the rate of flow of mucus along the groove. Only in the case of exposure to 180 μg l^{-1} of WAF was there any significant effect, with the rates being approximately twice those in control preparations.

Results of effects of 24-h exposure on velocities of metachronal waves of lateral cilia are presented in Table 4. While at 360 μg l^{-1}, a statistically significant decrease was

Table 2. *Venus verrucosa.* Effects of exposure to WAF of crude oil on the crawling rates of excised gill fragments

Conc. of WAF ($\mu g\ l^{-1}$)		Mean velocity ($mm\ s^{-1}$)	±SEM	% of control	N	Significance level P
Control	24 h	0.008	0.0006		5	
	48 h	0.005	0.0007		6	
30	24 h	0.019	0.0040	238	8	0.05
	48 h	0.006	0.0092	120	9	NS
180	24 h	0.048	0.0053	600	7	0.001
	48 h	0.074	0.0135	1 480	3	0.001
360	24 h	0.065	0.0133	806	8	0.001
	48 h	0.060	0.0210	1 200	3	0.001

SEM = Standard error of the mean
N = Number of gill replicates
P = Student's t-test between control mean and experimental mean

Table 3. *Venus verrucosa.* Effects of 24-h exposure to WAF on particle transport along ventral marginal groove of the gill

Conc. of WAF ($\mu g\ l^{-1}$)	Mean velocity ($mm\ s^{-1}$)	±SEM	% of control	N	Significance level P
Control	0.127	0.0147		4	
30	0.195	0.0260	154	5	NS
180	0.271	0.0217	213	4	0.05
360	0.142	0.0248	112	5	NS

SEM = Standard error of the mean
N = Number of gill replicates
P = Student's t-test between control mean and experimental mean

Table 4. *Venus verrucosa.* Effects of 24-h exposure to WAF on velocity of metachronal waves of lateral cilia

Conc. of WAF ($\mu g\ l^{-1}$)	Mean velocity ($mm\ s^{-1}$)	±SEM	% of control	N	Significance level P
Control	0.080	0.0025		6	
30	0.082	0.0028	103	4	NS
180	0.131	0.0057	167	7	0.05
360	0.064	0.0025	80	6	0.05

SEM = Standard error of the mean
N = Number of gill replicates
P = Student's t-test between control mean and experimental mean

recorded relative to controls, at $180\ \mu g\ l^{-1}$ such velocities increased to 164% of those in controls. This indicates that exposure to oil exerts a different effect on the activities of lateral cilia depending on the concentration of exposure.

Observations on other ciliary systems indicated that after 24-h exposure, terminal cilia became more active, especially at higher concentrations. The same applies to the stiff 'sensory' cirri, where, on exposure both to 30 and to $360\ \mu g\ l^{-1}$, most of them became highly active, beating at an irregular frequency of approximately 9 beats min^{-1}.

Observations on the activities of the eulaterofrontal cirri in such gill fragments were rather difficult to make, and were only qualitative. In many cases, on exposure to WAF the angle of beat of such cirri was reduced, and their normal coordinated rhythm was apparently lost, at least in several groups of cirri distributed at random over the exposed gill's surface.

Discussion and conclusions

The various models of particle retention by bivalve gills and the role played by the eulaterofrontal cirri have been recently reviewed by Morton (1983). In most cases, it is suggested that such structures act as the primary sieve and that both their dimensions as well as their activities determine the particle retention efficiencies of the gills. The mean distance between the side branches on the eulaterofrontal cirri (presumably the mesh size of the gill filter) in *Venus verrucosa* were found to be approximately 0.6 to 0.8 μm. The results of the present study, however, indicate that there is no well defined critical particle size for efficient retention. Retention efficiencies in unexposed clams gradually declined with decreasing particle size starting at 7 to 8 μm diameter size. Moreover, below this upper size range, particle retentions varied significantly, even for the same particle size, in different pre-exposure experiments. This suggests that particle retention in *V. verrucosa* at the lower particle size range is determined not only by the structure of its eulaterofrontal cirri, but also by the degree of coordination of their activities, as well as by the way they may interact with the activities of the other frontal and lateral cilia, with such coordination being easily interfered with by experimental conditions.

Similar observations were made by Jørgensen (1975) for *Mytilus edulis.* He stated that disturbance of mussels due to experimental conditions easily produces leaky gills and that gills *in vitro,* despite apparently normally beating cilia, only inefficiently retain particles as small as those that are completely retained in the undisturbed feeding *M. edulis.* However, the present study indicates that long-term exposure to low concentrations of WAF of oil may lead to such significant reductions in retention efficiencies of particles less than 6 to 8 μm in diameter, which may not be simply explained by experimental disturbances. This effect was found to be dependent on the period of exposure. This result may be considered of significant ecological importance since it implies that exposed individuals will be able to feed less on the normally abundant, naturally occurring food particles below this size range. Direct visual observations of the effects of WAF exposure on the activities of eulaterofrontal cirri, in gill fragments, suggested that this reduced particle retention is due to the resultant loss in the coordination of such ciliary activities, as well as in their reduced angle of beat.

Metachronal rhythms of beating lateral cilia were still evident in exposed gills. However, the reduced velocities of such metachronal waves at 360 $\mu g\,l^{-1}$ probably indicate reduced frequency of beating with a resultant decrease in pumping rates in the exposed *Venus verrucosa*. The reduced clearance rates in bivalves exposed to PHC reported elsewere (Axiak, in press) confirm these observations.

On the other hand, the activities of frontal cilia (as well as the terminal cilia) were generally enhanced on exposure both to WAF of oil, as well as to low concentrations of chemically dispersed oil, as evident from increased rates of particle transport across the gill's frontal surface and increased rates of crawling of gill fragments. Kittredge *et al.* (1974) reported similar observations for *Crassostrea virginica*. Moreover, on exposure to chemically dispersed oil, the frontal and terminal ciliary activities of *Venus verrucosa* gills handled the retained oil droplets as if they were food particles, leading them to the ventral marginal grooves, and from here oralwards until they reached the labial palps. Stainken (1975) likewise indicated that *Mya arenaria* retained oil droplets and its gill ciliary systems led them from the gill's frontal surfaces, to the mouth where they were then ingested. The role of the labial palps in the rejection or acceptance of such retained oil droplets has not been investigated in the present study. However, when mucus production is increased, much of the large mucus masses are brought to the free edge of the labial palp for rejection (Bernard, 1974). This implies that, on exposure to relatively high concentrations of PHC, many of the oil droplets which may become incorporated in mucus strands are actually rejected.

There may be several physiological implications of increased frontal ciliary activity. It may lead to an increase in the metabolic energy demand, as well as an increase in the production of waste products. Moreover, any altered ciliary activities may imply a direct or indirect effect of oil exposure on either nervous control and/or on some other aspect of the physiological and/or biochemical processes linked with ciliary movement (e.g. on membrane-bound factors responsible for ciliary membrane polarization, ATP production, etc.).

The significance of increased activity of the 'sensory' cilia at the ventral margins of gills on exposure to even low concentrations of WAF cannot be identified at present. While this effect may reflect the general enhanced activities of other ciliary systems such as the frontal and terminal cilia, it may also represent some chemotactic response of the gill to the presence of PHC. This possibility is at present being investigated in further laboratory experiments.

To conclude, it has been shown in the present study that exposure to PHC contaminants leads to important effects on the ciliary activities of a bivalve's gills, which may reduce the feeding rates of the bivalve as well as the retention efficiencies of food particles less than 6 μm in diameter. At the same time, mucus proudction by the gill filaments is greatly enhanced, while the activities of other ciliary systems are significantly increased, thereby increasing the energy demand of the exposed clam. All such responses may lead to a reduced or negative energy balance by the clam. In fact, reduced 'scope for growth' in *Venus verrucosa* exposed to similar levels of PHC has been reported (Axiak, in press).

Acknowledgements. This work forms part of a Ph.D thesis carried out under the supervision of Dr. M. N. Moore, Institute for Marine Environmental Research, Plymouth, England and the second author of this paper. We wish to thank Dr. M. Avian, University of Trieste, Italy for performing the scanning electron microscopy and Mr. C. Galea, Mr. J. Debono, and Mr. M. Pace for their technical assistance.

Literature cited

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

Ansell, A. D.: The functional morphology of the British species of Veneracea (Eulamelli-branchia). J. mar. biol. Ass. U.K. *41*, 489–515 (1961)

Atkins, D.: On the ciliary mechanisms and interrelationships of lamellibranchs, Part III: Types of lamellibranch gills and their food currents. Q. J. microsc. Sci. *79*, 375–421 (1937)

Axiak, V.: Physiological responses of a marine bivalve (*Venus verrucosa*) to pollution by petroleum hydrocarbons. Mar. envir. Res. (In press)

Bernard, F. R.: Particle sorting and labial palp function in the Pacific oyster *Crassostrea gigas*. Biol. Bull. mar. biol. Lab., Woods Hole *146*, 1–10 (1974)

Dral, A. D. G.: The movements of the latero frontal cilia and the mechanism of particle retention in the mussel, (*Mytilus edulis* L.). Neth. J. Sea. Res. *3*, 391–422 (1967)

GESAMP: The health of the oceans. UNEP regional seas reports and studies No. 16. 108 pp., Geneva: UNEP 1982

Gosselin, R. E. and G. O'Hara: An unsuspected source of error in studies of particle transport by lamellibranch gill cilia. J. Cell comp. Physiol. *58*, 1–9 (1961)

IOC/WHO/UNEP: Manual for monitoring of oil and petroleum hydrocarbons in marine waters and on beaches. Supplement to manuals and guides 7. 20 pp. Paris: UNESCO 1977

Jørgenson, C. B.: On gill function in the mussel *Mytilus edulis* L. Ophelia *13*, 187–232 (1975)

Kittredge, J. S., F. T. Takahashi, F. T. and S. O. Sarinana: Bioassays indicative of some sublethal effects of oil pollution. *In:* Proc. mar. technol. Soc., pp 891–897. Washington D.C. 1974

Malanga, C. J.: Effects of dopamine on anaerobic metabolism and ciliary activity in bivalve gills. Comp. gen. Pharmac. *5*, 51–59 (1974)

Morton, B.: Feeding and digestion in bivalvia. *In:* The mollusca, vol. 5, Physiology, Part 2, pp 65–187. Ed. by A. S. M. Saleuddin and K. Wilbur. London: Academic Press 1983

Stainken, D. M.: Preliminary observation on the mode of accumulation of No. 2 fuel oil by the soft shell clam, *Mya arenaria*. *In:* Proc. Conf. Prevention and Control of Oil Spills, pp 463–468. New York: Petroleum Institute 1975

Date of final manuscript acceptance: October 30, 1986.
Communicated by O. Kinne, Oldendorf/Luhe