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Effect of Three Aromatic Hydrocarbons on Respiration and Heart Rates of the Mussel, *Mytilus californianus*

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The effect of petroleum derivatives on morality of marine invertebrates is now well established. Aromatics are considered to be the most toxic of all oil fractions (ANDERSON et al. 1974). The toxicity of these compounds is related not only to their physical behavior in seawater, but to the physiology, life history and habitat of the organisms involved. The effect of sublethal exposures, on the other hand, is poorly understood. Results indicate a disparity of physiological responses to petroleum hydrocarbons generally occurs.

Recent studies of marine invertebrates have focused mainly upon respiration, locomotion, and growth. Special attempts have been made by various researchers to utilize specific invertebrates in measuring changes in certain variables as an index of oil pollution (FOSSATO 1975, DISALVO 1975, GOLDBERG et al. 1978). The filter feeding marine bivalves have received primary attention due to their proximity to coastal oil spills, importance in human food consumption and possible role in petroleum hydrocarbon bioconcentration. The papers cited above deal primarily with measuring levels of hydrocarbon in bivalve tissues. If these organisms are to be used as monitors in the future, then it is important to learn something of how quickly the bivalve responds metabolically to changes in ambient levels of petroleum hydrocarbons.

Studies on respiratory responses adequately fit criteria for answering such questions. In addition, cardiac activity may serve to indicate changes as the relatively constant lamellibranch heart rate may be the least variable of all metabolic processes (EARLL 1975). Thus, respiration and heart activity reveal a great deal of information concerning the physiological state of the bivalve. We report measurements of these two variables in the mussel, Mytilus californianus (Conrad), under conditions of exposure to, and recovery from, three aromatic hydrocarbons.

MATERIALS AND METHODS

Animals. Mussels were collected from the rocky intertidal zone at Pillar Point, Princeton, CA, USA. They were transported immediately to a laboratory at California State University, Hayward, for heart recordings or flown directly to Louisiana State University at Baton Rouge for measurement of respiration rates. The mussels were maintained in the laboratory for 5 days in instant ocean (Aquarium Systems, Inc.) at 11 $\pm$ 1°C and 32 $\pm$ 2 o/ooS prior to experimentation. All reported procedures were conducted at this

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temperature and salinity. Mussels were not fed during the investigation (reported rates should therefore be considered standard), and neither control respiration nor heart rate declined significantly during any experimental period. Size of the mussels ranged from 0.4 to 1.2 g dry wt of soft tissues.

Heart recordings. For the determination of the effect of aromatic hydrocarbons upon the heart rate of M. californianus silver electrodes were implanted in acclimated mussels using a modification of TRUEMAN et al. (1973). Small holes were drilled through each valve of a mussel and fine silver wire loops (30 ga.) soldered to brass connectors were placed in each hole. Care was taken to ensure that wires did not penetrate mantle tissues. If an imaginary line was drawn between the two electrodes, it would pass directly through the heart. A mixture of Eastman 910 glue and finely ground mussel shell was used to seal each hole and secure the connector to the shell. Recorder leads could then be connected and removed at anytime during experimentation. The leads passed to an impedance converter (Biocom, Inc.) and a polygraph pen recorder (Grass Instr.) was utilized to monitor heart rate.

For each investigation a group of electrode-bearing mussels was placed in an aquarium containing 8 L of artificial seawater. After a short surgery acclimation period of one to two days, each individual mussel's heart rate was recorded as a pre-exposure control (CONTROL). A prepared aliquot of either benzene, toluene, or benzo(a)pyrene (BP) was then added to the seawater by a method similar to that described by ANDERSON et al. (1974) for preparation of water soluble fractions. The aquarium was covered to minimize evaporation. Mussels were exposed to each hydrocarbon for a period of 24 h.

TABLE 1. Nominal and actual seawater concentrations at the start and end of the 24 h exposure period.

Compound	Nominal	Initial[1]	Final
Benzene	10 ppm	8.5 ppm	5.7 ppm
	50	58.7	43.4
Toluene	10 ppm	7.8 ppm	5.7 ppm
	100	88.3	63.5
BP[2]	1 ppm	-	-
	10	-	-

[1]Measurements were made under conditons simulating actual experiments by uv spectrophotometry, not during experimentation.

[2]Actual concentration of BP was not determined. Nominal concentration for BP is mg/L added to seawater, thus actual seawater concentration was much lower.

Heart rates were recorded between hours 2 and 12 of the 24 h exposure (EXPOSED), immediately after replacement of exposure water with fresh artificial seawater (PE-1) and at a later time interval (PE-n) until it was determined that control rates were reestablished or the hydrocarbon no longer had an effect. Water was changed daily to avoid refiltration of depurated hydrocarbon.

Oxygen consumption. The protocol for respiration experiments was similar to that used for heart rate measurements. In this case eight mussels were placed in an aquarium containing 12 L of artificial seawater for each of the concentrations of aromatic hydrocarbon. A flow through respiration system was used to continuously monitor oxygen uptake of animals under control, exposed, and post-exposed conditions. The flow rate of water past each mussel was 10-12 mL/min and the water was maintained at full oxygen saturation (158 $\pm$ 2 torr) at all times. Oxygen levels were measured with an International Biophysics Corp. differential oxygen analyzer. As in heart recordings, each mussel was used as its own control. Before each measurement a period of 20 min was determined essential for acclimation to the respiration chamber, so recording began after this time period had elapsed. Each mussel's oxygen uptake rate was determined over the course of approximately 1 h for reported values. Following final recordings mussels were dried in an oven to constant weight at 90°C and the dry wt of soft tissues was recorded.

Statistics. Weight-rate regressions were run for both heart rate and oxygen uptake rate controls, but only the respiration rate-weight relationship was significant for the size range used. The regression line for the mussels O_2 uptake rate is described by the equation, $VO_2 = 0.341W^{0.652}(n = 48)$. Since individual mussels were used for all phases of experimentation, tests of significance were performed via paired Student t-tests. Neither normalized heart rate nor respiration rate differed statistically between hours 2 and 12 within any group during hydrocarbon exposure. Therefore, means reported of EXPOSED mussels are indicative of the consistent response between hours 2 and 12 of an exposure period.

RESULTS

Table 2 lists the mean heart rates (beats $\cdot$ min^{-1}) recorded prior to, during and following each experimental exposure period. Heart rates during exposure to 10 ppm BP, 50 ppm benzene and 100 ppm toluene were significantly below controls. The greatest decline, 55%, occurred in the toluene exposure. The lower concentration assayed for each hydrocarbon did not effect a significant rate change at the $P < 0.05$ level. Figure 1 is an example of recordings made before, during and after exposure to 10 ppm BP. This was the general trend observed for each concentration high enough to produce significant bradycardia. The somewhat erratic heart activity elicited during the exposure period occurred in virtually all experiments.

Mean values of all heart rates were higher for PE-1 versus CONTROL mussels with the exception of the 10 ppm toluene data. Statistical analysis, however, shows that none of these changes (PE-1 vs CONTROL) is significant at the 0.05 level. These data indicate that cardiac rates return to normal shortly after replacement of hydrocarbon-ladened seawater with clean seawater.

Mortality was only observed with a 100 ppm initial concentration of toluene. The death can most likely be atributed to the high concentration of hydrocarbon for two reasons. First, the collection and acclimation procedures were well regimented

TABLE 2. Heart rates (beats · min^{-1} ± 1 s.e.m.) and tests of significance between CONTROL AND EXPOSED, and between EXPOSED AND POST-EXPOSED (PE-1), mussels. NS = not significant at the $P < 0.05$ level.

Hydrocarbon (parts per million)	BP (1)	BP (10)	Benzene (10)	Benzene (50)	Toluene (10)	Toluene (100)
CONTROL	16.88±0.74 ($1\bar{2}$)*	14.75±0.06 ($1\bar{2}$)	13.77±0.62 ($1\bar{1}$)	16.68±0.73 ($1\bar{1}$)	17.32±1.14 ($1\bar{1}$)	14.55±1.65 ($1\bar{0}$)
EXPOSED (day 0)	15.79±0.68 ($1\bar{2}$)	12.13±0.31 ($1\bar{2}$)	14.59±0.52 ($1\bar{1}$)	14.77±0.46 ($1\bar{1}$)	18.32±0.87 ($1\bar{1}$)	6.15±0.33 ($1\bar{0}$)
PE-1 (day 1)	18.25±0.53 ($1\bar{2}$)	15.13±0.31 ($1\bar{2}$)	14.45±0.52 ($1\bar{1}$)	18.27±0.46 ($1\bar{1}$)	15.77±1.37 ($1\bar{1}$)	17.11±1.34 ($\bar{9}$)
PE-2 (day 2)	17.13±0.52 ($1\bar{2}$)		14.64±0.56 ($1\bar{1}$)			15.00±1.13 ($\bar{7}$)
PE-4 (day 4)	17.00±0.40 ($1\bar{2}$)	14.25±0.35 ($1\bar{2}$)		16.82±0.60 ($1\bar{1}$)	15.77±1.30 ($1\bar{1}$)	
PE-11 (day 11)						15.90±1.33 ($\bar{5}$)
CONTROL vs EXPOSED	NS	$P<0.001$	NS	$P<0.05$	NS	$P<0.001$
EXPOSED vs PE-1	NS	$P<0.01$	NS	$P<0.001$	$P<0.05$ decrease	$P<0.001$

* sample size is located within parentheses.

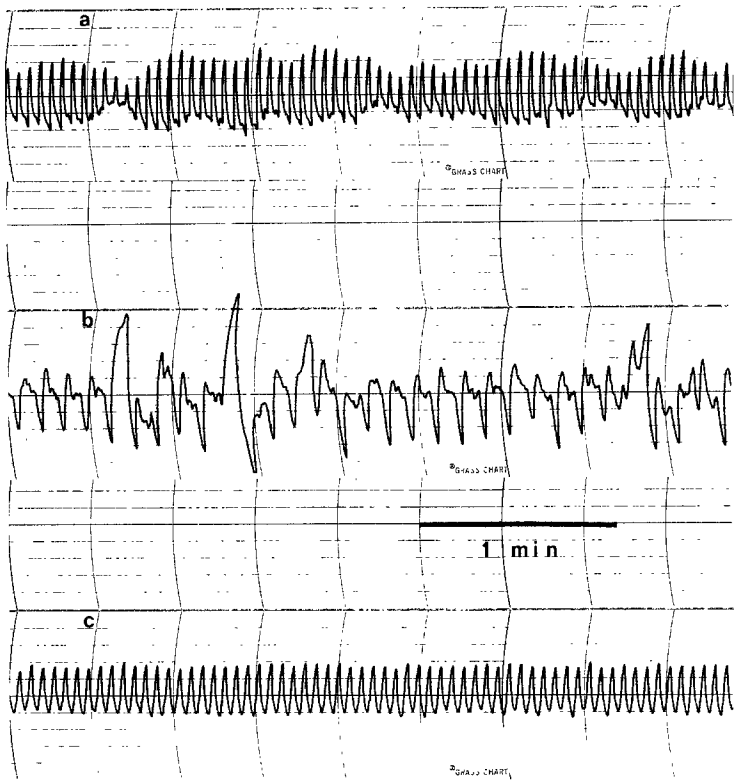

Fig. 1. Polygraph pen recordings showing the heart rate of a single M. californianus (a) before, (b) during, and (c) after exposure to 10 ppm BP.

during all phases of the study to maintain equivalent nutritive and metabolic states of experimental animals, and, secondly, no mortality was observed in mussels collected and maintained simultaneously as non-experimental controls. Therefore, all exposure concentrations in this study can be considered sublethal (well below the TL_m's for each aromatic).

The data indicate that the critical concentration effecting bradycardia for M. californianus lies somewhere between the two concentrations used for each aromatic hydrocarbon. Subsequent determination of the precise initial quantity of each hydrocarbon required to produce significant alteration of cardiac rate was not attempted.

Respiration rates recorded under the various experimental conditions are listed in Table 3. The degree of variability in these results is evidenced by the significance tests. Significant declines in the rate of oxygen consumption occurred for 50 ppm benzene, 10 and 100 ppm toluene and 1 ppm BP. Replacement of exposure seawater with clean seawater generally resulted in increased respiration rates. Following three days of depuration, only the oxygen uptake rate of mussels exposed to 10 ppm BP differed from the recorded controls (PE-3 higher than CONTROL).

Table 3. Respiration rates ($\mu L\ O_2 \cdot g\ dry\ wt^{-1} \cdot h^{-1}$) and tests of significance between CONTROL and EXPOSED, and between EXPOSED and POST-EXPOSED (PE-1), mussels. NS = not significant at the P<0.05 level. n = 8 for all values.

Hydrocarbon (ppm)	BP (1)	BP (10)	Benzene (10)	Benzene (50)	Toluene (10)	Toluene (100)
CONTROL	233±13	211±21	205±20	210±10	234±30	239±15
EXPOSED (day 0)	186±23	189±27	155±27	176±6	119±16	161±24
PE-1 (day 1)	172±17	305±20	207±25	268±21	235±26	219±24
PE-3 (day 3)	247±27	286±24	169±22	236±15	212±15	249±19
CONT vs EXPOSED	P<0.05	NS	NS	P<0.05	P<0.05	P<0.05
EXPOSED vs PE-1	NS	P<0.05	NS	P<0.01	P<0.05	P<0.05

DISCUSSION

The heart rate of M. californianus from the Puget Sound region was determined by BAYNE et al. (1976) to be approximately 20 beats $\cdot$ min^{-1} at 13°C. In addition, they found that oxygen uptake rates were described by the equation $VO_2 = 0.542W^{0.648}$ for fed mussels and $VO_2 = 0.233W^{0.648}$ for starved mussels. Our results agree with these values as mean control heart rates at 11°C ranged from 13 to 18 beats $\cdot$ min^{-1} and the fitted weight regression for oxygen consumption was strikingly similar to their report.

Several investigators have demonstrated that alterations of physical environmental factors will profoundly affect heart rates of bivalve molluscs (DEFUR & MANGUM 1979). Long term recordings of Mytilus edulis by TRUEMAN et al. (1973) and of Mya arenaria by EARLL (1975) along with those of M. californianus in this study indicate that, during continual immersion, there is little short term fluctuation in heart rates. Thus, any significant changes observed in cardiac rates can likely be attributed to the environmental factor being altered.

Nominal concentrations of 10 ppm BP, 50 ppm benzene and 100 ppm toluene effected significant decreases in heart activity for M. californianus. This effect is similar to that recorded during air exposure for M. edulis both in the lab and the field (HELM & TRUEMAN 1967). COLEMAN (1974) emphasized that bradycardia occurs as a natural response of bivalves to aerial exposure and water of lowering oxygen content. Apparently the heart response of M. californianus in water of increasing hydrocarbon content parallels that of a mussel incurring normal environmental stress. Preliminary experiments with the freshwater clam, Anodonta, by TRUEMAN et al. (1973) indicated that suppression of heart rate also occurs when freshwater is chlorinated. Contraction of the cardiac muscle may be dependent upon the availability of oxygen and the presence of normal respiratory functions.

Benzene and toluene appear to have similar critical initial concentrations eliciting bradycardia in M. californianus. BP, on the other hand, has a critical concentration lying between 1 and 10 ppm whole aromatic. Considering the relative solubility properties (benzene >toluene>>BP; NATIONAL ACADEMY OF SCIENCES 1975) of these three aromatics, the polycyclic compound exerts the more potent sublethal effect.

When fresh artificial seawater replaced exposure seawater, heart rates immediately returned to normal. This response is similar to that which occurs when a natural environmental stress is removed. COLEMAN (1974) describes an overshoot of the heart rate, exceeding control values, when the specific stress (eg., emersion) is removed. This can probably be attributed to an oxygen debt built up during stress. The excess in heartbeat normally lasts for about ten minutes (HELM & TRUMAN 1967). One might expect a similar overshoot of extended length in the present experiment, due to the 24 h exposure period. An overshoot of the mean control value was found to occur, but was not significant at the 0.05 level.

A decline in the respiratory rate during exposure to aromatic compounds would be expected if a similar response occurs when mussels are stressed by either aromatic hydrocarbons or normal environmental changes. BAYNE (1971) has shown that M. edulis lowers its oxygen utilization when experiencing lowered oxygen tensions. Intertidal organisms facing environmental stress may generally respond by reducing activity, followed by a corresponding decrease in oxygen uptake, where metabolic rate falls from routine to standard levels. In the present study, lowered VO_2 may be a valuable adaptation commonly used during stress by the mussels until favorable conditions return.

The results of this study contradict those of GILFILLAN (1975), who reported that small quantities of crude oil extracts reduced respiratory activity, while large amounts increased the oxygen consumption of M. edulis and Modiolus demissus. AVOLIZI & NUWAYHID (1974), on the other hand, observed decreased oxygen utilization during crude oil exposure with the bivalves Donax trunculus and Brachidontes variablis. It is evident that respiratory responses of lamellibranchs to petroleum hydrocarbons is variable and, as was found for heart activity, a product of complex physiological and behavioral interactions.

Mussels are normally exposed to stressful changes in natural conditions for brief periods. The present investigation was conducted with only a 24 h exposure period. Chronic exposures, such as those occurring in harbors and after widespread oil accidents, might slow heart and respiration rates permanently and, subsequently, affect the important life processes of growth and reproduction. It would be beneficial to follow up on field studies such as DISALVO et al. (1975) and FOSSATO (1975) and record metabolic activity in mussels transported from clean to oil-contaminated waters. BAYNE & WIDDOWS (1978) have shown that stressful environmental factors elicit differing effects on the growth energetics of two populations of M. edulis.

Changes in heart and respiration rates during the present investigation followed the same general trends. Oxygen uptake

seems to be the least predictable of the two variables. The presence of an aromatic hydrocarbon could have affected normal gas exchange in the mussels, even though water was fully saturated with air throughout the experimentation. This hypothesis is supported by observations of high levels of ^{14}C-labelled aromatic hydrocarbons found in gill epithelia of exposed M. edulis (LEE et al. 1972) and M. californianus (SABOURIN 1977).

Of the two metabolic variables, the heart rate change appeared to be the more consistent under the aromatic hydrocarbon stress. After four days of depuration the VO_2 did not return to control level in either the 50 ppm benzene or the 10 ppm BP experiments. These results indicate that properly controlled measures of metabolic rates may be useful in future determinations of the sublethal effects of man-induced and natural stresses on marine mussels. The response to such stresses appears to be rapid and reversible.

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