PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Some Ecological Consequences of the Physiological and Biochemical Effects of Petroleum Compounds on Marine Molluscs [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1982 **297**, 219-239
doi: 10.1098/rstb.1982.0039

Phil. Trans. R. Soc. Lond. B **297**, 219–239 (1982)
Printed in Great Britain

Some ecological consequences of the physiological and biochemical effects of petroleum compounds on marine molluscs

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The problems of relating the results of experiments in the laboratory to events in nature are twofold: to equate the response to a single variable (hydrocarbons) with the natural variability in the biological material in a multivariate environment, and to consider whether the response established experimentally has any relevance to the animal's chances of survival and reproduction (i.e. its fitness) in the natural population. Recent studies of the effects of petroleum hydrocarbons on marine invertebrates are reviewed, with an emphasis on the physiological and cytochemical responses by bivalve molluscs. The dose–response relations that emerge suggest the intensity of the 'signal' that must be detected in nature if the chronic, sublethal effects of petroleum pollution are to be measured.

The natural variability in these physiological and cytochemical processes are then reviewed and the main causes of variability in natural populations, both endogenous and exogenous, discussed. These results indicate the extent of the 'noise' above which the signal from possible pollution effects must be detected. The results from recent field studies on the common mussel, *Mytilus edulis*, are discussed. The results are as complex as expected, but it proves possible to reduce the variance in the measured responses so that pollution effects, including those due to hydrocarbons, can be detected.

The ecological consequences of the observed effects of petroleum hydrocarbons are then discussed in terms of reproductive effort and reproductive value. Considerable variation between populations exists here also and this can be used to help in the interpretation of the extent of the impact of the environment on the ecology of the population. The result is to place the findings of the laboratory experiments in an ecological context of natural variability and of the physiological costs of adaptation.

Introduction

There have been many studies in the laboratory on the effects of oil and various oil-derived compounds on the physiology and biochemistry of marine animals. Much of the earlier work, however, was carried out at high and environmentally unrealistic concentrations and under experimental conditions (e.g. short exposures in tanks of static seawater) far removed from ecological realism. More recently, the use of flow-through aquarium systems for exposing animals to low levels of the water-accommodated fraction of crude and refined oils for long periods has brought the experimental investigations closer to conditions of chronic exposure in nature. Problems remain in comparing results from different studies, owing to procedural differences in the preparation of the oil for experimental use and in the frequent lack of precise analytical data on the types and the amounts of hydrocarbons to which the animals have been exposed. There remains also the difficulty of matching laboratory exposures qualitatively to the mixtures experienced by animals in the field; the effects on the oil of photo-oxidation, microbial activity and general 'weathering' are difficult to simulate in the laboratory. Nevertheless, experimental opportunities are available for the study of the effects of hydrocarbons under environmentally realistic conditions and the question arises: what do such studies tell us about the ecological impact in chronically polluted situations in nature?

The problems of relating laboratory studies on individual animals to events in the natural environment are twofold. Firstly, the responses observed in the laboratory in experiments that are designed to vary one or a very few factors must be shown to be detectable in the multifactorial natural situation with all the attendant variability in the biological material. This problem is compounded by the fact that most chronically polluted situations are subjected to multicontaminant inputs, and seldom is the potential polluting factor simple in chemical terms. Secondly, even if the experimental response is detectable in spite of the natural variability, the problem arises as to its ecological significance, i.e. its significance in terms of growth, reproduction and survival of the individual and in terms of the persistence of the population and its role in the community.

In a recent report (Gesamp 1980) three sets of criteria were recognized in considering the utility of experimentally observed pollutant effects for biological monitoring. One category referred to the 'practical value' of the measured effect, i.e. its quantitative relationship with the cause, its sensitivity and the ease of detection above the background of natural variability. A second set of criteria concerned more fundamental scientific aspects such as the relation of the effect to the physiological and ecological fitness of the individual, its relevance to other potential effects and whether it is reversible when the cause is removed. The third group of criteria concerned problems of cost and application.

In this paper we examine the results of some recent studies on the effects of petroleum hydrocarbons on marine molluscs with a particular exphasis on statistical properties and the problems of ecological relevance. The full ecological significance of many of the observed sublethal effects of hydrocarbons have not, however, been established by experiment. Instead, we argue from first principles that some of the effects of hydrocarbon exposure on components of energy balance in the individual can reasonably be expected to impair the ecological fitness of individuals and therefore to be significant for population wellbeing and survival.

Some physiological and cytochemical effects of hydrocarbons on molluscs

The literature on the effects of petroleum hydrocarbons on aquatic animals has recently been reviewed by Connell & Miller (1981 *a*, *b*). Comparisons are difficult for the reasons discussed earlier, but considering only the studies based on low concentrations of oils (and the water-accommodated fractions, or w.a.f., of these) and on the responses of marine molluscs, some general conclusions are possible.

Feeding

Sublethal concentrations of petroleum hydrocarbons depress the rate of feeding in bivalves and in gastropods. This has been documented for *Crassostrea virginica* (reduction in the rate of production of faeces and pseudofaeces) (Stegeman & Teal 1973); *Mytilus edulis* (decline in the rate of clearance of food particles from suspension) (Gilfillan 1975; Widdows *et al.* 1982) (see figure 1); *Mercenaria mercenaria* (decline in clearance rate) (Keck *et al.* 1978); *Macoma balthica* (reduction in feeding activity) (Stekoll *et al.* 1980) and in *Nassarius obsoletus* (impaired feeding behaviour) (Atema 1976). There is probably a direct inhibition by hydrocarbons of the cilia involved in bivalve feeding (Johnson 1977), which may be mediated by the inhibition of membrane-bound respiratory enzymes (Stekoll *et al.* 1980). In *Nassarius* low concentrations of specific fractions of oil interfere with chemosensory detection of food (Atema 1976).

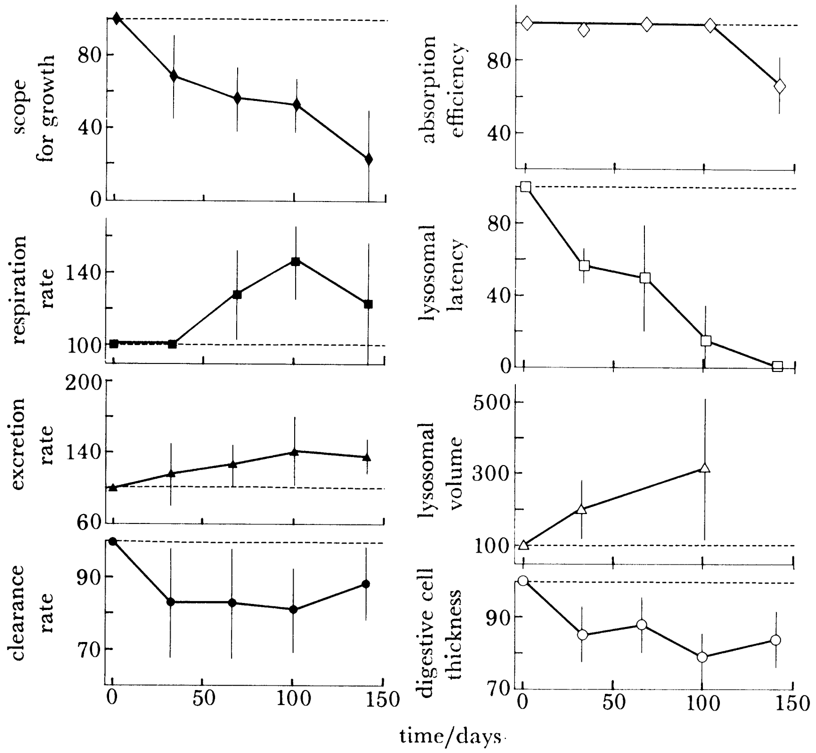

FIGURE 1. Effect of long-term exposure to 30 μg hydrocarbon per litre on the physiological and cellular responses of *Mytilus edulis*. Results are expressed in terms of experimental mean as a percentage of control mean with 95 % confidence limits, taking into account the variability in both controls and experimentals by using a Taylor series approximation to the variance.

There is some evidence that absorption efficiency may also be depressed at relatively low levels of hydrocarbons (Gilfillan *et al.* 1977; Keck *et al.* 1978; Widdows *et al.* 1982). However, absorption efficiency is also reponsive to the numbers and mass of particles ingested, and the interactions between food and hydrocarbons may prove complex. Widdows *et al.* (1982) observed reduced absorption efficiency in mussels exposed to 30 μg total hydrocarbons per litre at a ration level of 0.35–0.70 mg dry mass of algal cells per litre; at a similar hydrocarbon concentration but with a smaller ration of *ca.* 0.20 mg l^{-1}, absorption efficiency was less severely adversely affected.

Cytological examination of the digestive gland suggests possible histopathological correlates of impaired food absorption. Stainken (1976) reported an increased vacuolization and size reduction of the digestive cells of *Mya arenaria* exposed to sublethal concentrations of no. 2 fuel oil. Wolfe *et al.* (1981) observed accumulations of lipid and of lysosomal residual bodies in digestive cells of mussels exposed to oil residues from the *Amoco Cadiz*. In our experiments (figure 1) we observed a reduction in the height of the digestive cells of mussels exposed for 140 days to 30 μg total hydrocarbons per litre, together with a loss of synchrony between digestive tubules (Lowe *et al.* 1981). Simultaneously, there was an increase in volume (and a reduction in number) of the lysosomes in the digestive cells and much reduced values for lysosomal stability (Widdows *et al.* 1982). Viarengo & Moore (1982) recorded a 42% increase in the activity of the lysosomal enzyme β-hexosaminidase in mussels exposed for 17 weeks to w.a.f. of North Sea crude, together with significantly reduced levels of RNA polymerases. The effects of these changes, if any, on absorption efficiency were not apparent until 100–140 days of exposure, possibly as a result of the low ration level (Widdows *et al.* 1982). However, the main consequences of these structural and cytochemical changes in the digestive cells of *Mytilus* may be an increased mobilization of stored energy reserves (mainly glycogen), rather than impaired digestion (personal observations).

Respiration and excretion

At low concentrations of petroleum hydrocarbons, rates of oxygen consumption are increased in bivalves (*Mya arenaria* (Fong 1976; Gilfillan *et al.* 1976, 1977; Stainken 1978; Gilfillan & Vandermeulen 1978), *Mytilus edulis* (Widdows *et al.* 1982) (see figure 1) and *Macoma balthica* (Stekoll *et al.* 1980)) and in gastropods (*Littorina littorea* (Hargrave & Newcombe 1973)). At higher concentrations, respiration rates may be reduced (Dunning & Major 1974; Gilfillan 1975; Stainken 1978; Sabourin & Tullis 1981) possibily as a result of valve closure or narcotization of ciliary surfaces, or both. Stainken (1978) suggested that the impaired feeding (and ventilation) rates of *Mya* exposed to high concentrations of w.a.f. (of no. 2 fuel oil) resulted in less accumulation of hydrocarbons than clams at low and intermediate concentrations. Therefore, in evaluating the dose–response relations for physiological effects it is necessary to consider the tissue concentrations of the contaminant as well as its concentrations in the water.

The fundamental causes of these increased respiration rates remain unknown. Respiration is normally dependent in part upon the level of activity of the animal, and depressed rates at higher concentrations of hydrocarbons may reflect suppressed activity (Percy 1977) or valve closure in bivalves. However, Hargrave & Newcombe (1973) concluded that crawling behaviour and respiration rate of *Littorina* were 'not quantitatively related'; extracts of Bunker C crude oil directly affected respiration. Stekoll *et al.* (1980) also concluded that increased respiration rates in *Macoma* represented direct metabolic effects not mediated through changes in behaviour. Hydrocarbons may cause uncoupling of oxidative phosphorylation, resulting in increased metabolic rate (Stainken 1978) or increased flux through the glycolytic pathway (Widdows *et al.* 1982) (monitored as increased activity of the enzyme phosphofructokinase). Increased activity in the cytochrome P_{450}-linked mixed function oxygenase system may increase oxygen demand, but this has not been quantified.

Whatever the ultimate cause of accelerated metabolic rates, the result signifies increased energy expenditure, which, together with depressed feeding rates, results in less energy available for growth and reproduction. This point is discussed later. The other possibilities for energy loss include mucous secretion (known to increase on exposure to hydrocarbons (Stainken 1976, and personal observation)) and the loss of excretory products. Widdows *et al.* (1982) recorded an increase in ammonia production by *Mytilus edulis* at 30–40 μg total hydrocarbons per litre (figure 1). The energy equivalents of these losses are small relative to heat (= respiratory) losses, but they signify increased basal metabolism and an enhanced utilization of proteins as substrate for energy production.

Growth

The observed effects of petroleum hydrocarbons on feeding rate and on respiration, if realized in an environment with no compensating increase in the quality or the quantity of food available, will result in less energy available for growth or reproduction or both. In a series of observations on populations of *Mya arenaria* subjected to oil spills (the *Tamano* spill in Maine in 1972 and the *Arrow* spill in Nova Scotia in 1970), Gilfillan *et al.* (1976, 1977) and Gilfillan & Vandermeulen (1978) have demonstrated a reduction in carbon flux (i.e. carbon available for growth) and in tissue growth due to reduced feeding and assimilation rates and increased respiratory losses. Keck *et al.* (1978) report reduced growth by juvenile *Mercenaria mercenaria* exposed to 60 μg total hydrocarbons per litre (w.a.f. from Nigerian crude oil) due also to depressed feeding and assimilation. In our experiments with *Mytilus edulis*, hydrocarbon concentrations (w.a.f. from North Sea crude oil) of 30–40 μg l^{-1} depressed the scope for growth (Widdows *et al.* 1982) (see figure 1). Bayne & Worrall (1980) have illustrated the close correlation between the scope for growth (determined physiologically) and measured tissue growth in two populations of *M. edulis*. Stekoll *et al.* (1980) observed many symptoms of the effects of stress, including losses in dry mass, decreased shell growth, resorption of gametes and decrease in carbohydrate content, in *Macoma balthica* exposed for 6 months to 300 μg hydrocarbons per litre; at 30 μg hydrocarbons per litre, shell growth was also depressed.

Behaviour

Low levels of petroleum hydrocarbons also affect the behaviour of molluscs. The production of the byssus by juvenile and adult mussels may be reduced, leading to weakened attachment to the substrate; Lindén (1977) reports an effect on byssus production by *Mytilus* at 130 μg hydrocarbons per litre. The burrowing behaviour of infaunal bivalves such as *Macoma balthica* may be impaired. The rate of burrowing by this species is reduced at 70 μg hydrocarbons per litre (Lindén 1977), 230 μg l^{-1} (Taylor & Karinen 1977) and at 300 μg l^{-1} (Stekoll *et al.* 1980). Clams may also be stimulated to leave contaminated sediments (Taylor & Karinen 1977; Stekoll *et al.* 1980). The ecological consequences of such effects may be difficult to quantify but are intuitively apparent.

Hargrave & Newcombe (1973) observed increased rates of crawling by *Littorina littorea* at high concentrations (750 μg l^{-1}) of Bunker C oil. Lindén (1977) recorded decreased crawling rates by *Theodoxus fluviatilis* at a lower concentration (140 μg l^{-1}). Atema (1976) summarized results from behaviour experiments with *Nassarius obsoletus* exposed to low concentrations of hydrocarbons. The w.a.f. of no. 2 fuel oil caused reduced feeding, aggression and increased alarm responses; other hydrocarbon fractions enhanced the attractiveness of standard feeding stimuli. Atema points out that biological factors such as hunger and the strength of the feeding stimulus may modify the effects of the hydrocarbons.

Dicks (1973) has argued the importance of behavioural effects, suggesting that the ecological impacts of pollutants are likely to be realized by disturbance of normal behaviour patterns, with implications for fitness and survival. Another observation commonly made is that individuals may survive chronic exposures in the laboratory, where no other environmental factors are set at extreme or stressful levels, whereas in the field where biotic and abiotic factors are likely to add to the general stress imposed on the individual, survival may be more difficult (Kinne 1980).

This brief review draws attention to some of the effects experimentally established in molluscs exposed to low levels of petroleum hydrocarbons. Table 1 summarizes some of the available data for levels of hydrocarbons in the water column and in sediments from various regions, and table 2 shows some of the data published for levels in the tissues of mussels. The experimental studies that we have chosen to review were carried out at contaminant concentrations towards the lower end of the observed environmental and body burden levels, which are more typical of chronically polluted estuarine and coastal habitats than of the open sea. Few of these studies have sought to quantify the dose–response relation. However, Stegeman & Teal (1973) related biodeposition (i.e. faecal and pseudofaecal production) by *C. virginica* to hydrocarbon concentration; Stainken (1978) recorded dose–response values for

TABLE 1. HYDROCARBON CONCENTRATIONS IN SEAWATER AND SURFACE SEDIMENTS FROM ESTUARINE AND COASTAL ENVIRONMENTS

(Data on hydrocarbon contamination resulting from major oil spills are not included.)

location	concentration in seawater µg l^{-1}	concentration in sediment mg kg^{-1}	reference
English Channel (western)	1.1–1.7	1.1–2.0	Law (1981)
Irish Sea	2.1–3.5	6–16	
Bristol Channel (outer)	4.9–10	34	
Thames Estuary (outer)	43	—	
Tees Estuary (outer)	60	—	
Mersey Estuary (outer)	74	340	
Copenhagen Sound	—	46–1800	Jensen (1981)
Danish coast (industrialized)	—	5–390	
Narragansett Bay (north)	—	500–5700	Farrington & Quinn (1973)
Narragansett Bay (central)	—	130–440	
Narragansett Bay (south)	—	50–120	

Analytical procedures: Law (1981), u.v. fluorescence calibrated with Ekofisk crude oil; Jensen (1981), gas chromatography of alkanes and aromatics, calibrated with oil; Farrington & Quinn (1973), combined mass of alkane and aromatic fractions.

TABLE 2. HYDROCARBON CONCENTRATIONS IN THE TISSUES OF MUSSELS COLLECTED FROM ESTUARINE AND COASTAL MARINE ENVIRONMENTS

hydrocarbon concentration in mussel tissues µg g^{-1} dry mass	location	reference
0–23	clean sites in Westernport Bay, Australia	Burns & Smith (1977)
21	northeast Gulf of Alaska	Wise *et al.* (1980)
92	oil seep, Coal Oil Point, Santa Barbara	Wise *et al.* (1980)
46–77	North Sea oil production platform	Rowland & Volkman (1982)
3–298	various U.S. mussel watch stations	Farrington *et al.* (1980)
7–26†	clean sites, Scottish coast	Mackie *et al.* (1980)
19–71†	industrial areas, Scottish coast	Mackie *et al.* (1980)
100–200	sites affected by boating activity, Westernport Bay	Burns & Smith (1977)
600–1200	sites influenced by refinery outfall, Westernport Bay	Burns & Smith (1977)
1900	oil seep, Goleta Point, south California	Risebrough *et al.* (1980)
800–3500‡	Copenhagen Sound	Jensen (1981)

Analytical procedures: all results derived from g.l.c. analyses of alkanes, except for the g.l.c. data of Burns & Smith (1977) and Jensen (1981), which also include aromatic hydrocarbons.

† Concentrations in digestive gland; results calculated from wet mass data, assuming 80% water.

‡ Calculated from micrograms per gram of lipid, assuming 8% lipid in body tissues.

the respiration rate of *M. arenaria*; Gilfillan *et al.* (1977) have presented dose–response data for carbon flux in the same species; and Widdows *et al.* (1982) and Bayne *et al.* (1981) plotted dose–response curves for the scope for growth and lysosomal latency in *M. edulis*. For reasons discussed earlier, the dose–reponse relation should be constructed for concentrations of contaminants in the tissues of the animal, as well as in the environment. The impression gained from the few data available is that hydrocarbon levels typical of chronically polluted sites do have significant and measurable effects on the physiology and cytochemistry of some molluscan species. Some results from our experiments with *M. edulis* exposed to w.a.f. of North Sea crude oil are plotted as dose–response curves in figure 2. These data compound the effects of time on the accumulation of hydrocarbons in the tissues (Widdows *et al.* 1982) and the exact nature of the curves is expected to vary with effects related to size and season. Nevertheless the general magnitude of the response to hydrocarbons, against which measurements on animals from chronically exposed situations must be posed, is apparent.

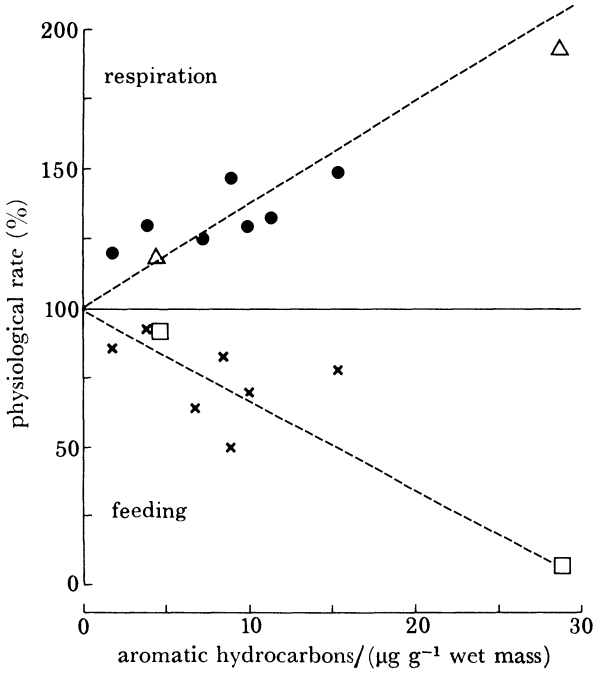

FIGURE 2. Dose–response relation between the concentration of aromatic hydrocarbons in body tissues of *Mytilus edulis* and two physiological responses, respiration and feeding rate. ●, ×, determined during long-term (140 days) exposure to 30 μg l^{-1}; △, □, determined after short-term (14 days) exposure to 50 and 600 μg l^{-1}. Results expressed as percentages of control.

NATURAL VARIABILITY IN PHYSIOLOGICAL PROCESSES

The main causes of natural variability in such processes as feeding, respiration and growth in *Mytilus* and other bivalves are body size, seasonal changes associated with the annual reproductive cycle and exogenous factors such as temperature and ration level. Sing & Zouros (1978) and Zouros *et al.* (1980) have recorded genetic components in the growth of the American oyster *Crassostrea virginica* that suggest another important source of variability, but similar analyses have not yet been carried out for other species.

Body size

Rates of feeding and of oxygen consumption may be related to the mass of body tissue by the allometric model

$$y = a X^b,$$

where y represents the physiological rate, X is body mass and a and b are fitted parameters. Figure 3*a* and *b* shows the results of feeding (as clearance rate, litres per hour) and oxygen consumption (V_{O_2}, millilitres per hour) measurements made on mussels from a population in Whitsand Bay, Cornwall in March 1981. The sample size was 30 individuals. An analysis of variance indicated that 53 % of the variability associated with clearance rate ($r = 0.73$) and 69 % of the variability in oxygen consumption ($r = 0.83$) may be explained by the regression against body mass. This source of variability can therefore be removed from the data by applying the standard correction for body size:

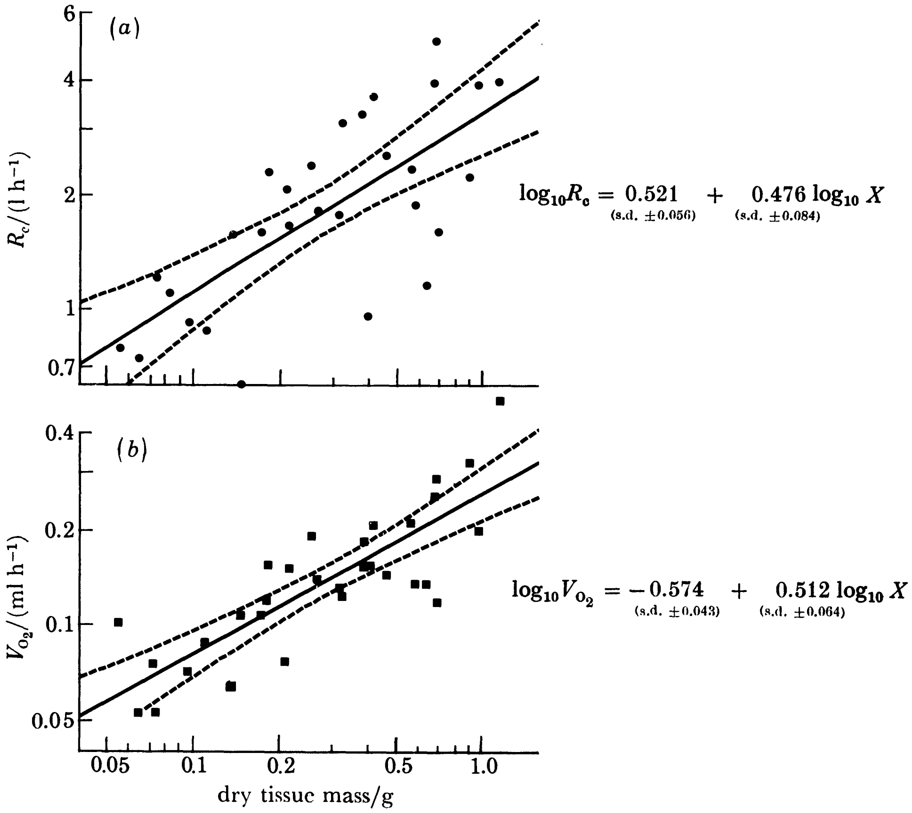

FIGURE 3. Relation between dry tissue mass and (*a*) clearance rate (R_c) and (*b*) oxygen consumption rates (V_{O_2}) in *Mytilus edulis* (Whitsand population). The fitted regression lines are shown, together with 95 % confidence limits (as broken lines).

$$y' = y X^{-b},$$

where y' is the mass-corrected value for the physiological rate. When this is done for the results in figure 3 the improvement in the precision of the measurements (represented by the coefficient of variation (c.v.), which is 100 × s.d./mean) is shown in table 3.

When the results have been corrected for variability due to differences in body size, the resulting variance represents residual population variability on one occasion and may be taken to describe the 'noise' above which any 'signal' due to the effects of hydrocarbons must be detected. The likelihood of detecting such effects (e.g. a 20–30% change in feeding or respiration rates) is posed in the question: what sample size would be required, given the measured residual natural variability, to detect a change of the predicted magnitude in the physiological rates?

Table 3

	mean	s.d.	c.v. (%)
clearance rate:			
(i) the raw data	2.07	1.14	55.1
(ii) the data corrected for body size	3.53	1.32	37.4
oxygen consumption:			
(i) the raw data	0.152	0.070	46.0
(ii) the data corrected for body size	0.268	0.064	23.9

The one-sample case

This refers to the hypothetical situation in which data are available for a single population before exposure to hydrocarbons; these data provide reliable estimates of the population mean (μ_0) and variance (σ_0^2) for each of clearance rate and respiration rate. We wish to determine the sample size necessary to test the hypothesis that, after exposure to hydrocarbons, a new population mean is established (μ) where $\mu = \alpha\mu_0$, and α is a known value less than unity representing the proportional difference between the altered physiological index and the 'control' or baseline value. The associated null hypothesis is that $\mu = \mu_0$ and a sample size is required that will ensure the rejection of this null hypothesis, if the alternative hypothesis is true, with 90% probability (i.e. the power (P) of the test is to be 0.9) at the 5% significance level:

$$P = \Phi[\{\mu_0(1-\alpha)\,n^{\frac{1}{2}}\}/\sigma_0 - t_{0.05,\,\infty\,\mathrm{d.f.}}],$$

where Φ is the cumulative (0, 1) normal distribution function, n is the sample size and $t_{0.05,\,\infty\,\mathrm{d.f.}}$ is the value of the t-statistic (one-sided) at $p = 0.05$ and with infinite degrees of freedom.

Values for P at various values of n are plotted in figure 4 for clearance and respiration rates at variance values calculated earlier; α represents a 20 or 30% (clearance rate) or 15 or 20% (respiration rate) change in the mean value for the respective physiological functions. It is clear from the curves that for $P = 0.9$ a sample size $n = 15$–20 is required to detect a 30% or greater change in clearance rate and a 20% or greater change in oxygen consumption.

The two-sample case

This refers to the more complex situation in which measurements are to be made on two populations, one of which is thought to be affected by hydrocarbons whereas the other is unaffected. What sample sizes might now be required to detect population differences in physiological responses of the magnitude predicted by laboratory experiments? This situation was analysed by Bayne *et al.* (1981), who distinguished three alternatives: (i) all animals from both populations are of the same size; (ii) animals of different sizes are measured for y (a physiological process), which is linearly related to body size (X) such that the two regression lines $y_1 = a_1 + \beta X$ and $y_2 = a_2 + \beta X$ have a common slope β; (iii) the two regression lines are not assumed to have a common slope. Let us take the second alternative and consider the data for mussels from Whitsand (figure 4): a sample size of 25 would be required for a test with power $P = 0.9$ to detect a difference in clearance rate of *ca.* 30 % and a sample of 10 to detect a 15 % difference in respiration rate. As with the simpler one-sample case, these are realistic sample sizes, suggesting that the predicted signal due to hydrocarbon effects should be detectable in comparisons of two populations.

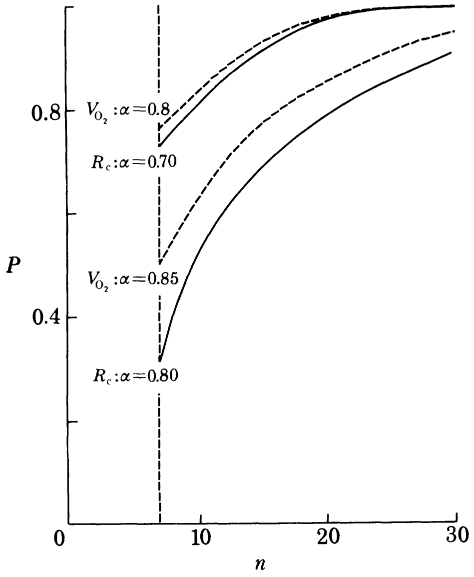

FIGURE 4. Effect of sample size (n) on the probability (P) of detecting significant differences between two groups of animals showing a 20 or 30 % change in mean clearance rate and 15 or 20 % change in mean rate of oxygen consumption (see text, the 'one sample case').

Season

The argument developed above applies to samples on a single occasion, for which seasonal components of the variability have been excluded. Seasonal factors, due in part to metabolic correlates of the gametogenic cycle and in part to changes in temperature and ration, affect the mean values for physiological measurements such as feeding, respiration and growth, as well as cytochemical measurements such as the latency of lysosomal enzymes. These seasonal differences include more than a doubling between minima and maxima for rates of oxygen consumption and feeding (Bayne & Widdows 1979; Worrall *et al.* 1982). Clearly, comparisons within and between populations should be made at comparable stages in the annual gametogenic and temperature cycles.

Present indications are, however, that the value for b in the allometric equations relating physiological rates to body size in bivalve molluscs does not vary significantly with season (Bayne & Widdows 1979; Newell & Bayne 1980; Vahl 1981; Bayne *et al.* 1981; Worrall *et al.* 1982). That is, variability due to body size can reasonably be excluded from the results of measurements at different seasons of the year by use of a single, carefully determined rate – body size relation.

Seasonal changes in the variance associated with these physiological processes must also be considered. In a study of two populations of *Mytilus*, Bayne & Widdows (1979) recorded variance estimates for clearance rate and the rate of oxygen consumption that showed no seasonal trend. Overall means (±s.d.) for the coefficients of variation (c.v.) were ($n = 25$): clearance rate, 16.0±5.7; rate of oxygen consumption, 9.0±3.4. Similarly, in a study of populations of *Scrobicularia plana* over 2 years Worrall *et al.* (1982) recorded no seasonal changes in c.v., although the mean values were considerably higher than for *Mytilus* ($n = 15$): clearance rate, 52.8±17.3; rate of oxygen consumption, 40.9±9.9.

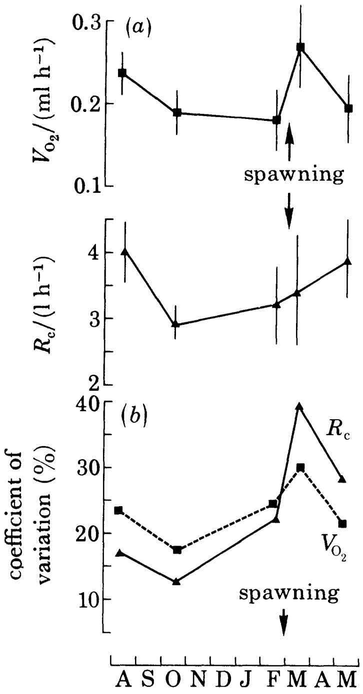

FIGURE 5. Seasonal changes in (*a*) mean oxygen consumption and mean clearance rate, and (*b*) their associated variances (as coefficient of variation), determined for mussels from the Whitsand population.

In spite of this relative constancy, there are occasions when the residual variance, after correction for mass, is uncommonly high, possibly as a result of asynchronism between individuals in the gametogenic cycle. Figure 5*a* and *b* shows values for mean oxygen consumption, mean clearance rate, and their associated variances, measured in mussels from a population at Whitsand, Cornwall. There was an unusually high variance in March, immediately after many individuals in the population had spawned. It is of interest to determine what effect this high level of variance has on the sample size requirement discussed earlier (the one-sample case). For $\mu_0 = 3.4$, $\sigma_0 = 1.615$ (c.v. = 47.5%), $\alpha = 0.7$ and $t = 1.65$, a sample size of 22 individuals suffices for a test of the required power ($P = 0.90$).

Results from reciprocal transplant experiments

The discussion so far has been concerned with animals sampled from their natural population and with the results of measurements interpreted in terms of environmental conditions pertinent to those populations. In these circumstances, an unknown proportion of the residual variability in the physiological measurements may be due to genetic differences between populations (Koehn 1978; Freeman & Dickie 1979). By transplanting individuals between populations it becomes possible to examine the phenotypic plasticity (Alderdice 1972) of the physiological and other processes and to determine the rate at which these processes may change (acclimatize) in the natural environment.

Figure 6*a* and *b* shows the results of measurements of clearance rate and of the scope for growth of *M. edulis* transplanted reciprocally between Kings Dock (Swansea, south Wales) and

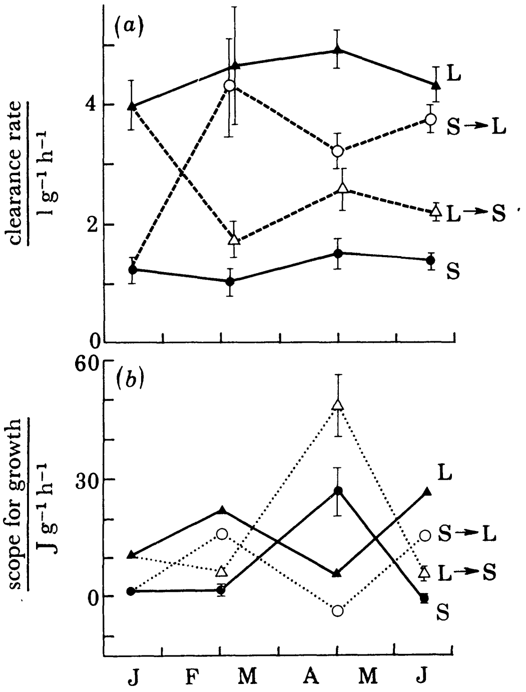

FIGURE 6. Effect of a reciprocal transplant of mussels (*Mytilus edulis*) between the Lynher estuary (southwest England) and Swansea docks (south Wales) on (*a*) the clearance rate and (*b*) the scope for growth (means ± s.e.). L, Lynher natives; S, Swansea natives; L → S, Lynher mussels transplanted to Swansea; S → L, Swansea mussels transplanted to Lynher.

the Lynher estuary (Cornwall, southwest England). The results of these experiments will be described in detail elsewhere; they illustrate that all the physiological, cytological and cytochemical processes assessed, adjusted to values typical of the adopted (= transplanted) site within a period 2–3 months, suggest a plasticity of response similar to observations in the laboratory. It is also of interest to establish how the variability in the responses might be affected by transplantation (figure 7). In the first few weeks of such transplants the coefficient of variability increased considerably, although it decreased, both for clearance rate and for the rate of oxygen consumption, to normal values within a few months.

These results suggest that, for these particular populations at least, mean values for physiological and biochemical processes are sensitive to the prevailing environmental conditions in a way that lends confidence to their use as measures of the effects of environmental stresses including pollution. There are other cases, however (R. K. Koehn & R. I. E. Newell, personal communication), where physiological plasticity is less evident after transplantation; the topic requires more study both from genetic and physiological points of view.

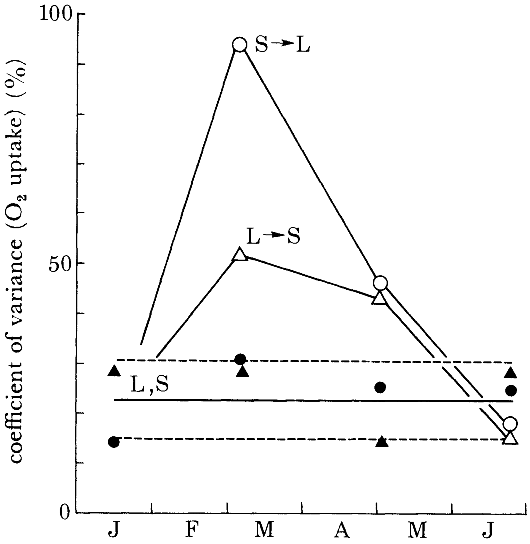

FIGURE 7. Effect of transplantation on the coefficient of variation for the rate of oxygen uptake by *Mytilus edulis*. L, Lynher natives; S, Swansea natives; L → S, Lynher mussels transplanted to Swansea; S → L, Swansea mussels transplanted to Lynher. The mean (thick line) and 95 % confidence limits (broken lines) are given for the Lynher and Swansea natives ($n = 8$).

ECOLOGICAL CONSEQUENCES OF PHYSIOLOGICAL RESPONSES TO STRESS

There is a lack of pertinent research into the consequences for populations of the cytochemical and physiological responses of individuals that have been observed in the laboratory. The view is commonly expressed that disruption to and interference with physiological and behavioural processes at low concentrations of hydrocarbons are likely also to damage reproductive potential and the ability to colonize habitats, to increase susceptibility to predation and to parasitism and to 'lead to changes in populations of individual species...[and]...result in shifts in species composition and diversity' (GESAMP 1980) (see also Hyland & Schneider 1976; Anderson 1977; Michael 1977; Connell & Miller 1981 *a*, *b*). On *a priori* grounds ecological consequences may be expected to result from most of the laboratory effects discussed earlier. Disruption of gastropod olfaction and foraging behaviour and reduced suspension feeding by bivalves at low concentrations of hydrocarbons are likely to result in sub-optimal feeding whereas less subtle effects, such as the suppression of growth, may be expected to impair ecological efficiency, reduce competitive ability and, in some cases, increase susceptibility to predators. Studies by Gillfillan and his colleagues (Gillfillan *et al.* 1977; Gilfillan & Vandermeulen 1978) have implicated petroleum hydrocarbons (specifically the aromatic fractions) in observed increased mortality and reduced recruitment in *Mya arenaria* populations some years after the original oil spill. More combined laboratory and field studies, conducted in chronically exposed sites as well as in situations after oil spills, are urgently needed (Sanders *et al.* 1980). There is a particular need for information on the extent to which individuals already affected by hydrocarbons are thereby made more vulnerable to environmental extremes that are normally tolerated.

In conducting field studies, disturbance to reproductive processes should be emphasized. From observations on bivalve molluscs, a number of direct and indirect effects on reproductive capacity, and therefore on population fitness, are to be expected (Bayne *et al.* 1981).

Hydrocarbons may disturb the normal rhythm of the annual gametogenic cycle, leading to asynchronous spawning among individuals (personal observation) and possibly to the release from the adults of gametes that are not optimally ripe; Lannan (1980*a*, *b*, *c*) has recently demonstrated reduced growth and survival of oyster larvae that developed from the sub-optimal spawning of adults. Bayne *et al.* (1978, 1981) reported experiments in which the fecundity, the mass of individual eggs and the rates of growth of larvae were measured after adult *Mytilus* had been exposed to various degrees of stress (by high temperature or low ration or both). The results are summarized in figure 8, in which each variable is related to the scope for growth of the adult and normalized to a value of unity for the control condition. (These controls showed a scope for growth of 5.5 mg dry flesh per day, a fecundity of 5.3×10^6 eggs released per individual, egg masses of 80 ng per egg and a rate of growth of the larvae for 5 days after fertilization of 5.5 µm shell length per day.) Fecundity declined linearly with reduced scope for growth. Egg mass, however, was maintained close to control values over a 30–40 % reduction in the scope for growth, then declined sharply. Similarly, larval growth rates, which were dependent upon the mass (and the lipid content) of the eggs showed a sigmoidal relation with the scope for growth.

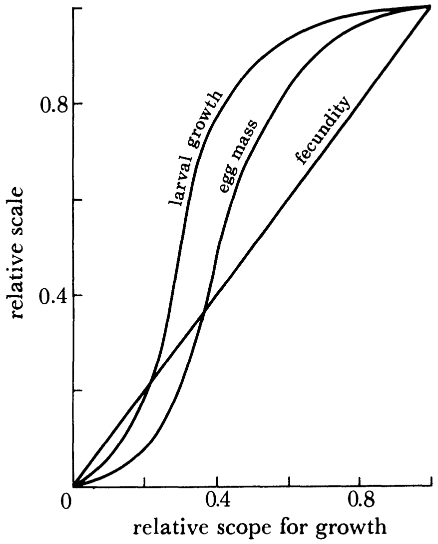

FIGURE 8. Effect of reduced relative scope for growth in adult *Mytilus* on relative fecundity, egg mass and larval growth rate.

These results are from short-term laboratory experiments. A more realistic experimental simulation of natural events for this species would require experiments lasting at least a year, since the normal gametogenic cycle in *Mytilus* is annual. Nevertheless, the results suggest that aspects of reproductive function are vulnerable to environmental stresses that decrease the energy available for growth. This is consistent with theoretical expectations for an iteroparous breeder, with control of egg development dependent on the growth of the parent (Calow 1973). When growth is impaired, reproductive output is reduced by egg resorption (Bayne *et al.* 1978) and possibly by the diversion of nutrients from storage sites in the tissues to maintenance metabolism (Thompson *et al.* 1974, and personal observation). Experiments of this kind have not yet been attempted with hydrocarbons, but we assume that reductions in the scope for growth, however caused, will have similar effects on reproductive capacity.

We have studied three populations of *Mytilus edulis* near Plymouth (Bayne & Widdows 1979; Bayne & Worrall 1980; Worrall & Bayne, unpublished) to relate some of these findings to population processes. Annual production by individuals of similar mass was greatest in the Lynher and least in the Cattewater, and annual fecundity ranked in the same order. However, when temperature differences at the sites were taken into account by referring production rates to day degrees, somatic production (P_g) at the three sites was similar but gamete production (P_r) was much reduced in the Cattewater. In figure 9 the reproductive effort of individuals from each population has been calculated as P_r as a proportion of total production (reproductive effort = $P_r/(P_r+P_g)$, and related to units of age from which differences in rates of growth have been removed (Hughes & Roberts 1980). Reproductive effort by individuals at Mothecombe and the Lynher was similar, but was much reduced in the Cattewater.

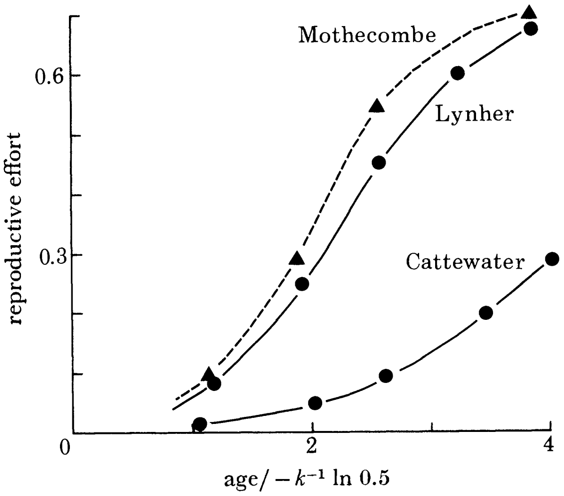

FIGURE 9. Reproductive effort by individuals of *Mytilus edulis* of different ages in three populations, plotted against an index of age, where k is an estimate of the growth constant in the Bertalanffy growth equation. Reproductive effort is calculated as $P_r/(P_r+P_g)$.

Calculations of the population (as opposed to the individual) reproductive efforts are shown in table 4. The Mothecombe and Lynher populations showed similar reproductive efforts, whereas the Cattewater population had a much reduced effort. Bayne *et al.* (1981) have also shown that the reproductive value of individual year classes in the Cattewater population was reduced below those of the Lynher and that the maximum reproductive value in the Cattewater population occurred in older year classes. These all suggest a major reduction in population fitness in the Cattewater, which is consistent with reduced scope for growth in individuals compared with values for the Lynher (Bayne & Widdows 1978). An important implication of the results, however, is that the decreased energy available to individuals in the Cattewater is used preferentially for somatic growth; reproductive processes are disproportionately affected.

Calow (1979) proposed an alternative index of reproductive effort (C) that considers the allocation of energy derived from the ration to reproduction relative to the energy demand of maintenance metabolism:

$$C = 1-\{(A-P_r)/R'\},$$

where A is the absorbed ration, P_r is the energy equivalent of gamete production and R' is the

Table 4. Biomass and production estimates for three populations of *Mytilus edulis*, with calculated population reproductive effort

(From Worrall and Bayne, unpublished results.)

year class	biomass (dry mass) g m^{-2}	annual dry matter production/(g m^{-2}) P_g†	P_r	reproductive effort
		Lynher		
2	0.55	0.95	0.09	8.6
3	3.18	1.8	1.0	36.8
4	11.93	11.8	6.7	36.2
5	37.56	23.9	33.3	58.2
6	48.82	12.1	48.1	79.9
7	63.83	1.5	70.0	97.9
8	33.84	0.8	28.8	97.3
9	17.97	0.3	11.9	97.5
		Mothecombe		
2	3.27	6.6	1.1	14.8
3	19.19	23.7	16.7	41.3
4	70.14	36.8	118.3	76.3
5	146.23	59.0	358.7	85.9
6	46.24	4.3	55.8	92.8
		Cattewater		
2	0.81	0.6	0.02	2.6
3	19.10	33.6	0.9	2.7
4	119.34	21.2	10.0	32.1
5	323.60	2.3	36.3	94.0

† P_g is calculated as tissue growth and mortality and does not include estimates of shell production.

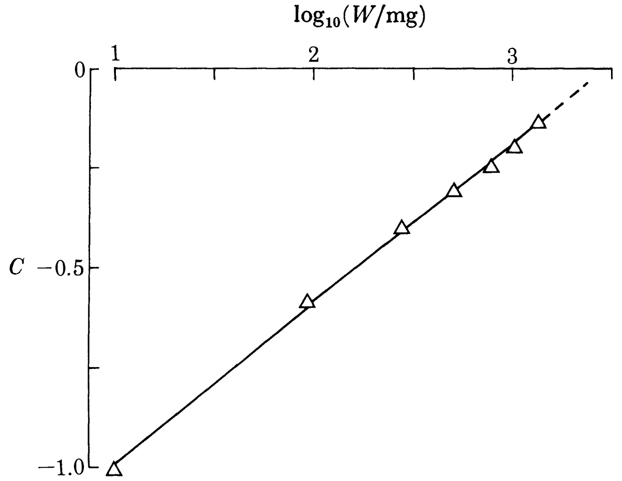

Figure 10. Reproductive effort (C) of *Mytilus* of different dry flesh mass, W. The index of reproductive effort is calculated according to Calow (1978).

maintenance energy demand. Values of C less than zero signify that the energy allocation to reproduction does not erode the energy needed for maintenance; such a strategy is 'restrained'. Values of C greater than zero signify that gamete production proceeds at the expense of the maintenance demand and is designated by Calow as 'reckless'. For *Mytilus*, as expected of an iteroparous, long-lived species, values for C are normally negative, although the degree of restraint is less for larger (older) individuals (figure 10).

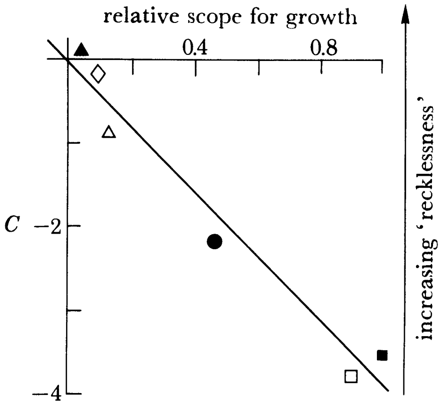

FIGURE 11. Reproductive effort (C) of *Mytilus* of 1 g dry flesh mass from six populations, related to relative scope for growth. The index of reproductive effort is calculated according to Calow (1979).

For figure 11 we have calculated C for five populations of *Mytilus* to illustrate the considerable differences observed; reduction in the scope for growth increases reproductive effort as measured by this index. A reasonable working hypothesis suggests that these increases in reproductive effort, effected through an altered balance in the allocation of available energy to growth and to reproduction and at the expense of the maintenance demand, render populations less fit. Experiments in the field under various conditions of stress, including hydrocarbons, are needed to test this hypothesis.

CONCLUSION

Available information on the levels of oil-derived hydrocarbons in some environments and the associated concentrations within the tissues of molluscs, when assessed together with the results of experiments on exposures of bivalves and gastropods to low levels of water-accommodated hydrocarbons in the laboratory, suggest that sublethal effects are to be expected in some chronically affected environments. Statistical and other considerations suggest that these effects should be measurable as disturbed physiological and cytochemical responses by individual animals, in spite of considerable natural variability. More research is needed to quantify the ecological relevance of these disturbed states to the natural populations. However, we should not expect simple relations between physiological disturbance and ecological events. Evidence from studies on bivalve molluscs suggests that fecundity and reproductive processes in general may be disproportionately affected by environmental stresses; individuals may continue to survive and to grow in conditions of chronic pollution exposure but be rendered effectively sterile by the allocation of all available energy to growth and maintenance. We lack the information to predict the time lags, or the necessary spatial scale, within these effects would result in population collapse; this is especially so for species that reproduce and recruit by means of a planktotrophic larval stage. Nevertheless, the implication is that we should heed the physiological information in the first place and not rely on detecting delayed population damage which, when it occurs, may be rapid and catastrophic.

This work has been made possible through collaboration with many colleagues at I.M.E.R., particularly D. M. Lowe, S. Moore, M. Day and S. Evans. We are particularly grateful to Dr K. R. Clarke for statistical advice. This work forms part of the experimental ecology programme of the Institute for Marine Environmental Research, a component of the Natural Environmental Reseach Council. The research was funded in part by the Department of the Environment through contract no. DGR 480/683.

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Discussion

G. M. Dunnet (*Zoology Department, University of Aberdeen, U.K.*) The authors' experiments were conducted, over several months, at constant pollution concentrations or environmental stresses, and the best indicator of a stressful environment was breeding output. In many situations in the field pollutant concentrations vary, and may be present for short periods. Have the authors done any experiments in the significance of the timing and duration of environmental stress in relation to the reproduction of mussels which is, after all, a highly seasonal annual event, involving a sequence of metabolic activities?

B. L. Bayne. We have done experiments of the kind suggested but so far we have conflicting results. Present indications are that whereas general stress causes overall energy limitation and reduces reproductive output in the way discussed in our paper, the specific effects of hydrocarbons are likely to be more complex, possibly affecting reproduction at specific times in the reproductive cycle only. Nevertheless, although reproduction is seasonal, as Professor Dunnet says, there are many points in the cycle at which stressors can act, such as initiation of energy storage preceding initiation of gametogenesis right through to the final act in the cycle, namely spawning.

D. J. Crisp, F.R.S. (*Marine Science Laboratories, Menai Bridge, Gwynedd, U.K.*). Dr Bayne showed some biological effects on the ordinate related to 'scope for growth' on the abscissa. Were these biological effects caused by oil pollution, and if so, could 'scope for growth' be replaced by oil concentration?

Some of the effects produced by dissolved oil, such as the enhancement of respiration and excretion and the reduced latency of lysosomes, suggest modification or derangement of membranes through incorporation of oil into membrane lipids. Oils belong to the class of acute physical toxicants recognized by Ferguson in 1939 and characterized by lipid solubility and non-involvement in metabolic processes. Ferguson showed that if as the measure of concentration the thermodynamic activity were used, rather than concentration, all physical toxicants fitted into a narrow range of toxicity, limited only by a solubility cut-off. This approach would greatly simplify an attempt to generate a dose–response curve, as also would the substitution of defined compounds in the place of the complex mixture of hydrocarbons in crude oil.

B. L. Bayne. Some of the experiments that I illustrated were not conducted with hydrocarbons; they were designed to explore the effects of environmental disturbances in general on the reproductive processes. Nevertheless, we also showed good dose–response correlations between aromatic hydrocarbons in the tissues and scope for growth. I am grateful to Professor Crisp for drawing Ferguson's work to our attention.

J. S. Gray (*University of Oslo, Department of Marine Biology and Limnology, Oslo, Norway*). Bradshaw's work on genetic adaptation of grasses to heavy metal pollution shows that adaptation occurs rapidly. It is therefore perhaps surprising that Dr Bayne's data from polluted sites, such as the Cattewater population, do not show adaptation. Does Dr Bayne think that this may be due to only intermittent pollution not allowing sufficient time between pulses to give adaptation or that, owing to larval movements, the population is derived from outside the polluted area?

B. L. Bayne. *Mytilus*, as you know, breeds by means of a planktotrophic larval stage; I therefore believe that a local, heavily stressed population such as the Cattewater may survive through recruitment from other populations that are not disturbed. However, mussel populations have been shown to be genetically very variable and I agree that we must in future take account of this variability, not only in pollution studies, but in general attempts to understand physiological adaptation.