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SOME PRACTICAL CONSIDERATIONS IN THE MEASUREMENT OF POLLUTION EFFECTS ON BIVALVE MOLLUSCS, AND SOME POSSIBLE ECOLOGICAL CONSEQUENCES

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A consideration of some physiological (rates of oxygen consumption, the scope for growth) and cellular (the cytochemical latency of a lysosomal enzyme) processes in bivalve molluscs suggests that animal size and seasonal changes related to the gametogenic cycle are important sources of natural variability. Correcting for size using regression techniques, and limiting measurements to one part of the gametogenic cycle, reduces observed natural variability considerably. Differences between populations are then still apparent, but the results of laboratory experiments with hydrocarbons from crude oil suggest that it should be possible to detect sub-lethal effects due to pollution (the 'signal') in the presence of the remaining natural variability (the 'noise'). Statistical considerations, taken together with results from current studies on *Mytilus edulis* and *Scobicularia plana*, indicate that sample sizes of 10–15 individuals should suffice for the detection of possible pollution effects. The physiological effects to be expected in the presence of sub-lethal levels of polluting hydrocarbons are on a scale that can cause significant ecological damage to a population through a reduction in fecundity and the residual reproductive value of the individuals.

Key words: bivalve molluscs, stress responses, natural variability, hydrocarbons, sampling strategy, ecological effects

INTRODUCTION

In measuring the biological effects of pollutants, three questions arise. (1) Can the effect be detected in the environment, amongst the natural variability to be expected between animals in nature? (2) If an effect is evident, what significance has this for the animal's fitness? (3) Can the effect be ascribed to a particular pollutant? Many papers in this Symposium bear on the third of these questions. We wish to consider questions of detection and, more briefly, of ecological significance. We use data obtained by the Stress and Pollution team at I.M.E.R. (see Acknowledgements), working with the common mussel, *Mytilus edulis* L. and the clam *Scrobicularia plana* (da Costa). The various methods employed are described in detail in the original papers referred to in the text.

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In considering the detection of a 'pollution effect', two major, natural causes of variance must be considered, viz. the size of the animal and seasonal variability which is linked to the stage of the gametogenic cycle. Recognizing, and removing these sources of variance can greatly increase the chance of detecting an effect (physiological or biochemical) due to a pollutant. We discuss some examples that illustrate dose–response relationships from experiments with hydrocarbons. Studies such as these in turn determine the magnitude of difference that must be detected among the natural variance (or 'noise') if a pollution effect is to be established; we illustrate from data the sample sizes that are necessary in such studies. Research into the ecological relevance of such effects is still at an early stage, but some suggestions can be made on the basis of our results.

RESULTS

Two natural causes of variance

Size If individual animals of very similar size (= weight) can be chosen for measurement, the precision in determinations of rates of oxygen consumption (V_{O_2}) and rates of feeding (= clearance rates, CR) can be high. In Table I (line A) V_{O_2} and CR measurements are quoted as means ±2 SE, together with the coefficient of variation, for a group of 12 *Mytilus* which were selected to be closely matched in shell length and flesh weight. More realistically, a sample with a greater range of individual sizes is normally the best that is available (Table I, line B); less precision is then possible, with coefficients of variation of 20–30% to be expected.

An allometric model of the form:

$$\text{rate} = a \cdot \text{weight}^{b},$$

where a and b are fitted parameters, is normally used to describe the relationship between a physiological rate and the weight of the animal. In the statistical treat-

TABLE I

Measurements of the rates of oxygen consumption (V_{O_2}; ml $O_2 \cdot h^{-1}$) and of clearance rate (CR; litres $\cdot h^{-1}$) in *Mytilus* of mean dry weight 1.66 g. Values are means ±2 SE, with the coefficient of variation [(SD/mean) × 100] in parentheses. A: animals chosen to be very similar in size; B: animals selected with less attention to similarity in size.

	Dry weight	V_{O_2}	CR
A	1.65 ± 0.016 (1.6%)	0.431 ± 0.022 (8.2%)	2.55 ± 0.34 (21.3%)
B	1.68 ± 0.193 (18.2%)	0.400 ± 0.050 (20.0%)	2.56 ± 0.49 (30.4%)

TABLE II

...analysis of covariance in 14 data sets re... ...ody size (dry flesh weight). The F value

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...erences between slopes	14
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...erences between means	14
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Such a procedure would be mo... ...logical process were constant or ... ...shows the results of regression an... ...consumption by *Scrobicularia pl*... ...between May 1977 and June 19... ...Worrall). In the analysis of cova... ...values for b emerged, whereas d... ...equation) were highly significan... ...Bayne, 1980). In these circumst... ...data sets and used to reduce the ...

$$V_{O_2} \cdot W^{-b}.$$

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CR
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TABLE II

An analysis of covariance in 14 data sets relating the rate of oxygen consumption by *Scrobicularia plana* to body size (dry flesh weight). The F value for differences between means is significant at the 1% level.

Source of variance	d.f.	SS	MS	F
Total residual variance	238	6.86	0.029	
Differences between slopes	14	0.45	0.032	1.12
Sub-total residual variance	252	7.31	0.029	
Differences between means	14	10.17	0.719	24.8
Totals	266	17.38		

ment of such data the allometric equation is usually fitted (on $\log_{10}$ scales) by least-squares regression analysis; it is then possible to 'correct' the physiological determinations for variation in body size by dividing by weight taken to the power b i.e. rate·weight^{-b}.

Such a procedure would be most useful if values for b for any particular physiological process were constant or at least reasonably similar, over time. Table II shows the results of regression and co-variance analyses of 14 data sets for oxygen consumption by *Scrobicularia plana* measured at seasonally ambient temperatures between May 1977 and June 1979 (data by courtesy of J. Widdows and C.M. Worrall). In the analysis of covariance no significant differences between the fitted values for b emerged, whereas differences between intercepts ($=a$ in the allometric equation) were highly significant (see also Bayne and Widdows, 1978; Newell and Bayne, 1980). In these circumstances a common value for b can be derived for all data sets and used to reduce the variance due to body size as

$$V_{O_2} \cdot W^{-b}.$$

This treatment ascribes no particular biological relevance to the fitted value for b; it is, rather, a statistical convenience to reduce the physiological rate data to a

TABLE III

Measurements of the rates of oxygen consumption (V_{O_2}; ml $O_2 \cdot h^{-1}$) and of clearance rate (CR: litres·h^{-1}) in *Mytilus* of mean dry weight 1.66 g, corrected for variability in body size by use of the fitted parameter b from expressions relating V_{O_2} and CR to dry flesh weight W (see text). A: animals chosen to be very similar in size; B: animals selected with less attention to similarity in size (cf. Table I).

	Dry weight	$V_{O_2} \cdot W^{-0.69}$	$CR \cdot W^{-0.41}$
A	1.65 ± 0.016 (1.6%)	0.304 ± 0.016 (8.2%)	2.09 ± 0.28 (21.5%)
B	1.68 ± 0.193 (18.2%)	0.279 ± 0.023 (12.9%)	2.08 ± 0.30 (22.9%)

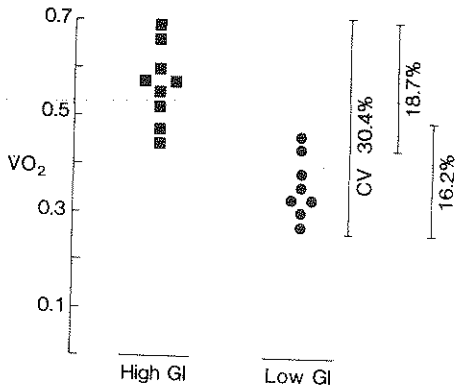

Fig. 1. Rates of oxygen consumption (V_{O_2}, ml $O_2 \cdot h^{-1}$ by *Mytilus edulis* related to the state of gamet genesis. High GI, gametogenesis is in an advanced stage. Low GI, gametogenesis at an early stage Ranking according to gametogenic index reduces the coefficient of variation (CV) from 30.4% t 16.2–18.7%.

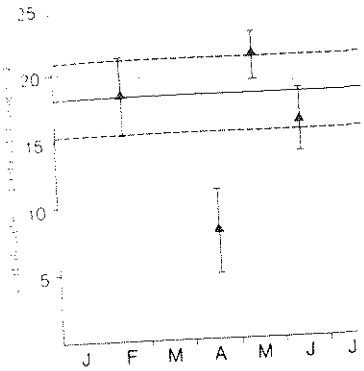

Fig. 2. The labilization period (min) of *dulis*, measured under standard cond and the dashed lines indicate the 95% October samples.

common body size. In the experiments referred to in Table I, b values of 0.69 (for V_{O_2}) and 0.41 (CR) were recorded over a wide size-range of animals. When these values were used to correct for variation in body size, no improvement in precision resulted for the sample carefully selected for size similarity (Table III, line A) but a significant improvement was achieved for the more randomly chosen sample (Table III, line B).

The gametogenic cycle In Fig. 1, 17 values for oxygen consumption rate by *Mytilus* are plotted; after each measurement, the animal was examined by a stereological procedure (Lowe et al., 1981) to determine the stage of gametogenic development. Over the entire data set the coefficient of variation was 30%; when two groups were distinguished, one of individuals late in the gametogenic cycle (high gamete index, GI) and the other of individuals early in the cycle (low GI), the coefficient of variation was reduced to 19% and 16% respectively. This effect was independent of any size differences between the two groups.

The effects of the gametogenic stage on variability in the biological response are most apparent, of course, when measurements are made at intervals over a year. The latency of the lysosomal enzyme *N*-acetyl-β-hexosaminidase provides a sensitive general index of the stress response in *Mytilus* (Moore, 1976, 1980; Bayne et al., 1979). Measurements made on a single population over 1 yr (Fig. 2) showed two periods of reduced labilization period (= latency), in April and in October. These periods coincide with spawning in this population (Lowe et al., 1981) and experimental evidence (Bayne et al., 1978) has confirmed that mussels immediately after spawning, at a time of enhanced autolysis in the tissues, show reduced latency of lysosomal enzymes. However, measurements made at all other times of the year

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Larger seasonal variability oxygen consumption rate. T temperature, salinity, the con tension. However, here alsc (Fig. 1). Bayne and Widdow ment explained 58% of the field-ambient conditions. W not feasible, therefore, popu year, accepting the increase gametogenic condition. Two

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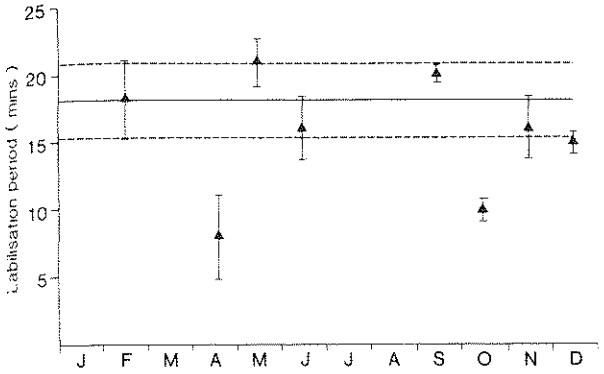

Fig. 2. The labilization period (min) of lysosomal *N*-acetyl-β-hexosaminidase in digestive cells of *Mytilus edulis*, measured under standard conditions over 1 yr. Values are means ±95% confidence limits (CL) and the dashed lines indicate the 95% CL of the mean value for the population, excluding the April and October samples.

(Fig. 2) did not differ significantly from a mean of 18 min ±0.4 (SE); a total of 40 values in this study had a coefficient of variation of 34%.

Larger seasonal variability is apparent in some physiological processes, such as oxygen consumption rate. These processes tend to be responsive to changes in temperature, salinity, the concentration of suspended particulate matter and oxygen tension. However, here also there is a correlation with the gametogenic stage (Fig. 1). Bayne and Widdows (1978) found that the stage of gametogenic development explained 58% of the variance in measures of V_{O_2} in *Mytilus*, made under field-ambient conditions. Where a full characterization of these seasonal cycles is not feasible, therefore, population comparisons must be made at the same time of year, accepting the increased variance that will result from slight differences in gametogenic condition. Two examples of such a study are briefly considered below.

Variability in the scope for growth and in lysosomal latency

During October, 1978 ten mussels from each of five sites in the Shetland Islands were measured for the suite of physiological processes necessary to the calculation of the scope for growth viz. rate of oxygen consumption, clearance rate, ammonia excretion rate and absorption efficiency. The mussels were chosen to be of similar size and the physiological rates all converted to a weight-specific rate, equivalent to an animal of 1 g dry flesh weight, using exponents fitted by least squares to measurements over a wider weight range made in May, 1978 (Widdows et al., 1981). The results of the scope for growth calculations are shown in Fig. 3. In spite of a wide scatter of points within each population, an analysis of variance indicated significant differences between populations and an *S-N-K* test (Sokal and Rolf, 1969)

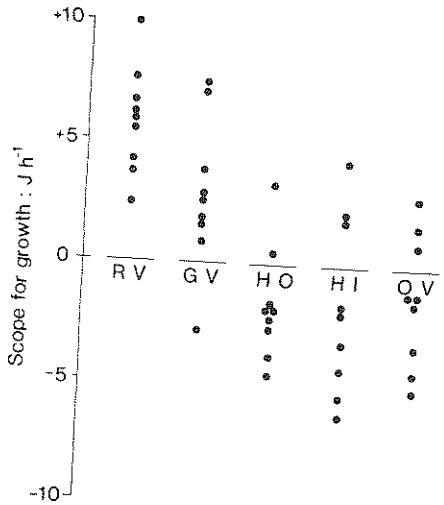

Fig. 3. The scope for growth as $J \cdot h^{-1}$ for *Mytilus edulis* from five populations in the Shetland Islands. RV, Ronas Voe; GV, Gluss Voe; HO, Outer Houb of Scatsta; HI, Inner Houb of Scatsta, OV, Orca Voe. Values are single determinations for $n = 9$.

resolved the following ranking:

Ronas Voe > Gluss Voe > Outer Houb = Inner Houb = Orca Voe.

These sites were all relatively unpolluted at the time of the survey. The gametogenic condition of the animals was similar, with the exception of a slightly higher proportion of ripe gametes in individuals from Gluss Voe (Widdows et al., 1981). Judging from these results, therefore, differences between means of $\times 1.5$ to $\times 2.0$ need to be detectable across populations, when measured at the same time of year, if the 'signal' from a potential pollutant is to be detectable.

In a related study on the same five populations of mussels, samples were taken in May 1980 for the assessment of lysosomal latency (Table IV). Measurements were made on five individuals from each site. In the analysis of these data (Table IV) animals from Orca Voe show a slight but insignificant reduction in latency. In order to detect a pollution signal above the natural variability, differences between means of $\times 1.1$ to $\times 1.2$ should suffice.

The effects of hydrocarbons

Bayne et al. (1979), Moore et al. (1980) and Widdows et al. (1981) have reported results from laboratory experiments in which mussels were exposed to low levels of the water-accommodated fraction (WAF) of North Sea crude oil for long periods

BLE IV

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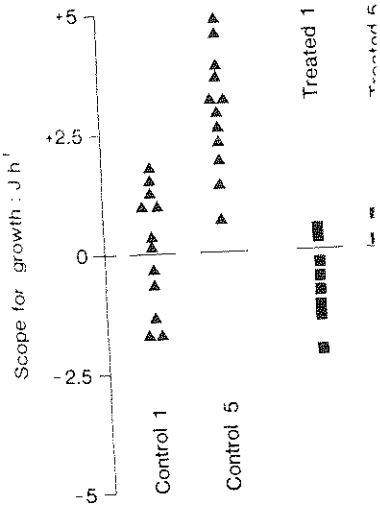

Fig. 4. The scope for growth as J·h
accommodated fraction of North Sea C
water. Values are single determination

TABLE IV

The labilization period of β-*N*-acetylhexosaminidase in digestive cells of *Mytilus*, measured on five populations from the Shetland Islands, U.K.

	Population				
	Ronas Voe	Gluss Voe	Inner Houb	Outer Houb	Orca Voe
Labilization period (min: mean ± SD for $n = 5$)	24.0 ± 5.2	25.2 ± 2.7	22.8 ± 2.7	22.8 ± 4.0	19.8 ± 1.6
Analysis of variance Source of variance	d.f.	SS	MS	F	
Between populations	4	80.6	20.2	1.67	n.s.
Within populations	20	241.2	12.1		
Total	24	321.8	–		

and various physiological, cytochemical and biochemical effects observed. In one such experiment (not reported in the papers cited) the scope for growth in control mussels (water without oil) and in mussels exposed to 20–35 μg WAF·l^{-1} was measured after 1 and again after 5 wk; the sample size was 12, and the mussels were carefully selected to be of similar size at approximately 1 g dry flesh weight. During this experiment the ration conditions improved, resulting in higher values for the scope for growth after 5 wk in both conditions (Fig. 4). Nevertheless, a significant depression of the scope for growth in the WAF-exposed mussels was apparent even

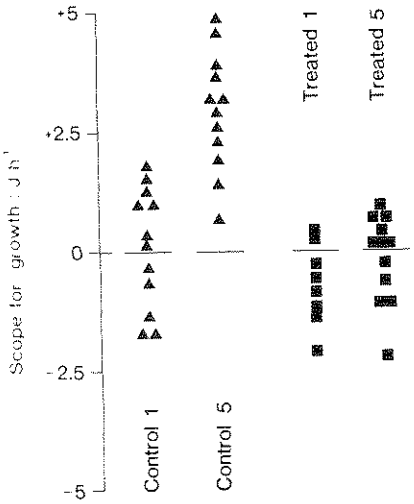

Fig. 4. The scope for growth as J·h^{-1} for *Mytilus edulis* exposed to 20–35 μg·l^{-1} of the water-accommodated fraction of North Sea Crude oil over 1 and 5 wk, compared with controls held in clean sea water. Values are single determinations for $n = 12$.

after 1 wk. As with the results in Fig. 3, the individual scope for growth measurements were variable (low precision) within any one condition, but they showed high sensitivity to the oil. At these low and entirely sub-lethal concentrations WAF, the 'signal' (i.e. a depression of the scope for growth) was >100%.

The latency of lysosomal hexosaminidase has similarly proved to be sensitive WAF (Widdows et al., 1981). At a concentration of 7.7 μg WAF·l^{-1}, the latization period was reduced from 21.2 ± 3.5 min to 12.5 ± 4.6 min.

These experiments suggest that the effects of low levels of hydrocarbons *Mytilus* should be detectable in field surveys where the main causes of natural variability i.e. differences in animal size and in gametogenic development, have been controlled or accounted for. The question follows: Is there a reliable dose-response relationship between the biological effect and the pollutant?

The dose-response relationship

Sub-lethal, physiological response studies of the type discussed have not examined the dose-response relationship in detail for any single class of pollutant. The relationship need not be linear. Widdows (1978) determined the scope for growth in *Mytilus* at different combinations of temperature and ration level and described the results by means of multiple regression equations and response surface diagrams; a polynomial expression with 17 terms explained 98% of the variance in 125 data points.

Less is known of the form of relationship to be expected between scope for growth (or lysosomal latency) and the concentration of a pollutant. Some data exist for hydrocarbon effects on bivalves, however; Gillfillan et al. (1976) observed an

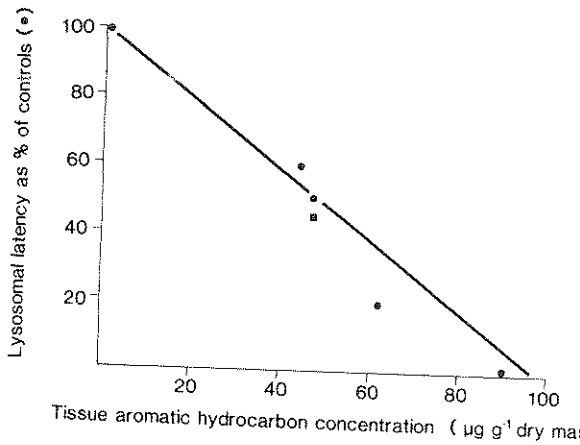

Fig. 5. The relationship between the latency of the lysosomal enzyme *N*-acetyl-β-hexosaminidase (expressed as a % of values in control animals not exposed to hydrocarbons) and the hydrocarbon concentrations in the tissues of *Mytilus edulis*. The data are taken from Widdows et al. (1981).

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would then follow.
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inverse correlation between carbon flux (=scope for growth) and tissue aromatic hydrocarbon concentration in *Mya arenaria* from sediments polluted by petroleum. Widdows et al. (1981) have confirmed an inverse correlation between the scope for growth of *Mytilus* and the concentration of aromatic hydrocarbons in the WAF to which the animals were exposed in the laboratory. Fig. 5 shows results for lysosomal latency (for hexosaminidase) related to aromatic hydrocarbon concentration in the body tissue of *Mytilus*, with latency expressed as a percentage of the control.

Sample size

Measurements of physiological and cytochemical responses by animals in their natural habitats and in laboratory experiments indicate the differences between populations that must be detected in any programme of 'effects monitoring'. On the other hand, knowledge of the natural variance in these responses, due to size and to seasonal effects, identifies the overall variability within which potential pollutant effects must be discriminated. These two properties of inherent variance and the magnitude of the pollution 'signal' are, in turn, the criteria that determine the required size of sample.

Suppose that data are to be collected from two populations, or from a single population at two separate times, in order to test for a difference in the mean of a rate measurement y (transformed by taking $\log_{10}$). Three cases are considered:

(a) Either y is not a function of animal size, or animals all of the same size are to be selected. Comparison of the populations is by a simple '2-sample' t-test.

(b) y is linearly related to a covariate x, for example $\log_{10}$ weight. The slope β of this relation is to be estimated from the data, assuming it is the same for both populations. A t-test (equivalently, an F-test) of the equality of intercepts would then follow.

(c) The regression lines $y = \alpha_1 + \beta_1 x$ and $y = \alpha_2 + \beta_2 x$ for the two populations are not assumed to have common slope. All four parameters will be estimated from the data and equality of mean y values examined at a specified size x_0. This involves a t-test of the null hypothesis $(\alpha_2 + \beta_2 x_0) - (\alpha_1 + \beta_1 x_0) = 0$ (see Appendix).

A further case is as in (b) except that β is not estimated from the data; a known value (β_0) from previous experiments is used. 'Corrected' measurements $y' = y - \beta_0 x$ can be defined (see the earlier discussion), and this case is then exactly equivalent to (a).

If any of the above tests is carried out at a fixed significance level $P = 0.05$ or 0.01, the number (n) of observations of y that should be taken from each population is determined by the power of the test. This is defined as the probability of rejecting the null hypothesis (of no difference between the population rates) when it is false, that is, when the true difference between the mean y values (or intercepts) is $\Delta\alpha$. Power is a function of n, P, $\Delta\alpha$, σ and k, where σ^2 is the usual 'error' variance about the mean y values (or regression lines), assumed constant over all observations

from both populations. The factor k reflects expected differences between the animal sizes in the two samples. For the 3 cases above:

(a) $k = 1$.

(b) $k^2 = 1 + (\bar{x}_1 - \bar{x}_2)^2/[2V(x_1) + 2V(x_2)]$, where $\bar{x}_1$ and $V(x_1)$ denote the mean and variance of the x values in the 1st sample, etc. Thus k will often be close to 1, rising only to $\sqrt{2}$ if the mean of one set of sizes is expected to coincide with an extreme of the other set, and to $\sqrt{5}$ if the two sets are (just) disjoint.

(c) $k^2 = 1 + [(x_0 - \bar{x}_1)^2/2V(x_1)] + [(x_0 - \bar{x}_2)^2/2V(x_2)]$. Note that k has roughly the same value here as in (b) if the comparison between the mean y values is made near the point $x_0 = (\bar{x}_1 + \bar{x}_2)/2$; otherwise k will be larger.

Data from previous laboratory or field experiments are used to supply approximate values for σ (and k). It should be emphasized that such 'guessed' estimates are not used in the analysis ultimately carried out but only in design of the experiment.

Fig. 6 is a set of power curves for n = 5(1) 10(2) 20(5) 50(10) 100(50) 250 and $P = 0.05$. (See Appendix for construction details.) Continuous, dashed and dotted lines correspond to cases (a), (b) and (c) respectively. After the first few n values

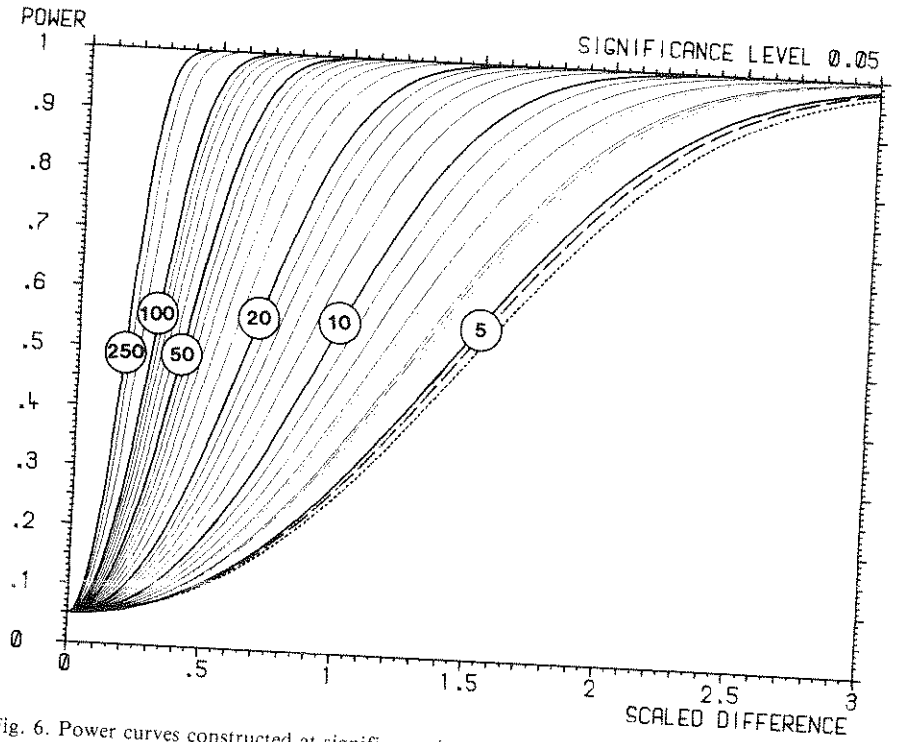

Fig. 6. Power curves constructed at significance level $P = 0.05$ for determining the required sample size for statistical tests described in the text. The circled numbers indicate the sample size ($n = 5$ ... 250) for three cases discussed in the text: a, solid lines; b, dashed lines; c, dotted lines. At values higher than $n = 7$, the power curves for all three cases are coincident.

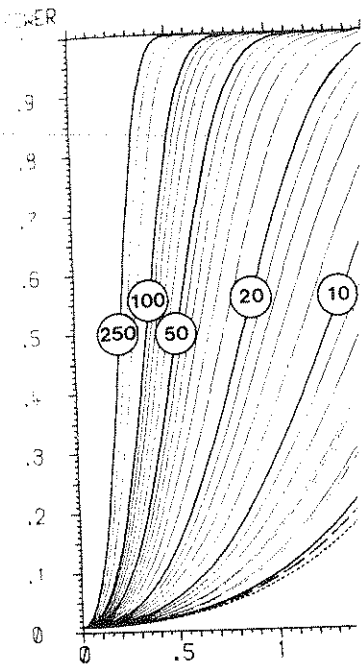

Fig. 7. As for Fig. 6, but with $P = 0.0$

these lines are coincident and
specified mean difference Δ
$(\Delta\alpha)/(k\sigma)$, and for this abscissa
proposed tests for various n.
advisable to use a more strin
samples are to be compared b

As an example of this proc
oxygen consumption (e.g. *Scr*
population at two seasons of
$\log_{10}$ (oxygen consumption) =
ence in mean $\log_{10}$ (oxygen
animal weight of 200 mg (x_0 =
the residual mean square in t
σ therefore equals 0.1. Also f
ence in mean weights ($\log_{10}$)
0.150. No assumption is mac
in the two data sets, so that
sample size at a power ≥ 0.9

differences between the

;) denote the mean and
will often be close to 1,
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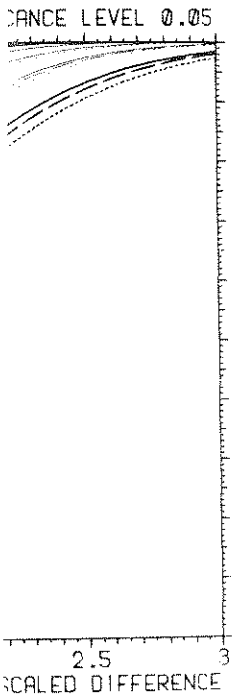

ermining the required sample
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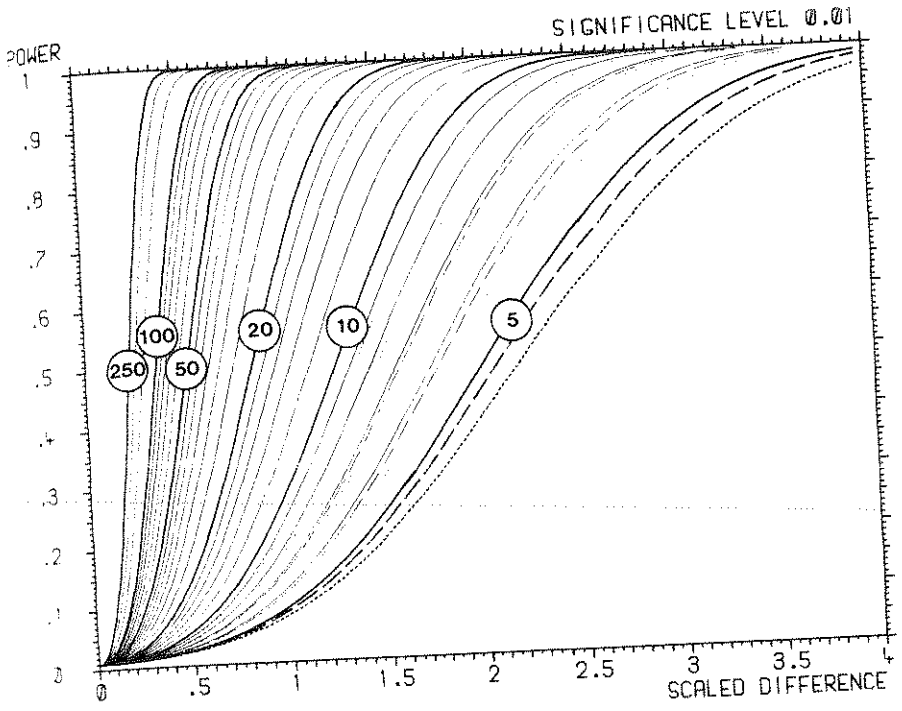

. As for Fig. 6, but with $P = 0.01$.

ese lines are coincident and the continuous lines apply for all 3 cases. For a ecified mean difference $\Delta\alpha$ of interest, first calculate the scaled difference $\alpha)/(k\sigma)$, and for this abscissa read off ordinates which are the powers of the posed tests for various n. Fig. 7 provides a similar graph for $P = 0.01$. (It is isable to use a more stringent significance level, like $P = 0.01$, if a number of uples are to be compared by t-tests carried out on all possible pairings.)

As an example of this procedure, we consider a programme to measure rates of gen consumption (e.g. *Scrobicularia plana*, see Table II) by individuals from one pulation at two seasons of the year (winter and summer). In the regressions of (oxygen consumption) $= \alpha_1 + \beta_1 \log_{10}$ (dry weight), we wish to detect a differ- e in mean $\log_{10}$ (oxygen consumption) values of 0.2, at $P = 0.01$, for a mean nal weight of 200 mg ($x_0 = 2.30$). From previous experience we accept a value for residual mean square in the regression analysis (log $\dot{V}_{O_2}$ against log W) of 0.01; erefore equals 0.1. Also from previous experience we are able to predict a differ- e in mean weights ($\log_{10}$) of 0.200 with a variance in the weight measurements of 50. No assumption is made as to equality of slopes (β) in the regression analysis the two data sets, so that the case (c) (above) is used to determine the required mple size at a power ≥ 0.9.

Using the equations given: $\Delta\alpha = 0.200$; $\sigma = 0.100$; $k = 1.03$. The scaled differe... $[(\Delta\alpha)/(k\sigma)] = 1.94$ and (from Fig. 7) a sample size of $n = 10$ would provide a te... with power $= 0.9$.

ECOLOGICAL CONSEQUENCE

We posed the question as to the ecological significance of an observed response ... a pollutant, implying that unless the response has a damaging ecological conse... quence pollution cannot truly be said to occur. With the kinds of data discussed ... this paper in mind, the question is more succinctly put: What is the ecological sign... ficance of a reduction in the scope for growth?

A decline in the scope for growth, under conditions of constant ration, signifie... an impaired growth efficiency, and an inevitable result will be the smaller size o... individuals. *Mytilus*, in common with most bivalves, increases egg production wit... increase in size (and concomitant advance in age). Thompson (1979) fitted allometri... equations to data relating weight loss on spawning (an index of fecundity) to dr... flesh weight of *Mytilus* from three populations in North America; in all cases the ... weight exponent was >1.0. Bayne and Worrall (1980) recorded weight exponents o... 1.40 and 1.29 for spawning weight losses in two populations in the U.K. These ... values >1.0 signify an acceleration in the allocation of resources to gametes as the ... mussels grow in size. Reduced growth efficiency, therefore, lowers the fitness of the ... individual by indirectly reducing fecundity.

Evidence from laboratory experiments suggests that environmental stress may ... also affect fecundity directly. In experiments reported by Bayne et al. (1975, 1978), ... mussels were held at temperatures and rations designed to force a reduction in the ... scope for growth. The mussels were then induced to spawn and the eggs released ... were counted (Fig. 8A); the eggs were also analysed for protein, lipid and carbo... hydrate content and the organic weight calculated as the sum of these components ... (Fig. 8B). There was a linear relationship between the scope for growth and ... fecundity. The weight of the eggs, however, did not reduce linearly with scope for ... growth. Over the range from $+5$ to -5 mg·day^{-1} in growth potential the weight of ... the eggs was similar at 73 ± 8.6 (SD)$\cdot 10^{-3}$ μg. Under more extreme stress the eggs ... were smaller.

The morphological processes that occurred in the gonads of mussels that were ... stressed during gametogenesis were complex. Resorption of morphologically ripe ... gametes occurred by autolysis (increased activity of lysosomal hydrolases within the ... oocytes) and by haemocytic infiltration of the gonad followed by phagocytosis. ... However, these processes occurred heterogeneously within the gonad tissue and, in ... addition, some gametocytes continued to develop within some follicles. At the end ... of 8 wk all individuals had some (albeit few) ripe gametes present in small follicles. ... The net effect was a reduction in fecundity under stress but a maintenance of the ... organic weight of the eggs, at least until the stress was considerable.

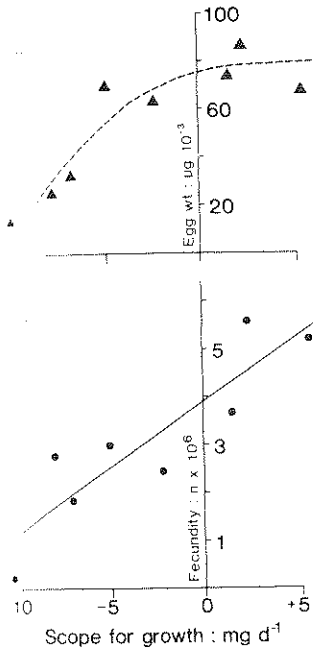

Fig. 8. A: numbers of eggs released by *Myi* ... weight of eggs from the experiments show

Fig. 9. The residual reproductive value (RI ... to age. △, Lynher population; ○, Cattew

In order to relate these findings ... studied a population of mussels ... tures, combined with poor ratio... the winter and early spring (Bayr... As expected, fecundity was m... fecundity per spawning was 2.8 ... 14.2×10^5 eggs·g^{-1} in a nearby ... both populations (Worrall and I... data for fecundity and mortality ... culated as a fundamental compo... ised to a value of 1.0 for the high... Age-related RRV is much lowe... addition, the age of maximum ... further disadvantage, since thes... the breeding population ($<10\%$ ... the Lynher ($>30\%$). Sub-lethal

= 1.03. The scaled difference
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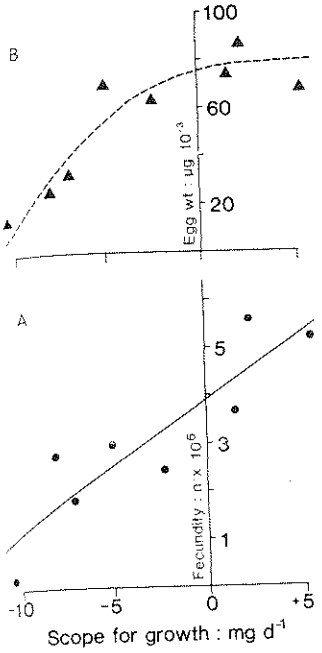

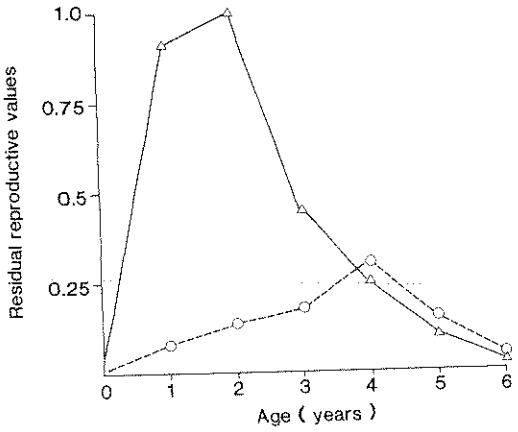

Fig. 8. A: numbers of eggs released by *Mytilus edulis* related to the scope for growth as mg · day^{-1}; B: the weight of eggs from the experiments shown in Fig. A.

Fig. 9. The residual reproductive value (RRV) of individual *Mytilus edulis* from two populations, related to age. △, Lynher population; ○, Cattewater population (see Bayne and Worrall, 1980).

In order to relate these findings to conditions in the natural environment, we have studied a population of mussels in which unseasonally high winter water temperatures, combined with poor ration conditions, caused negative scope for growth in the winter and early spring (Bayne and Widdows, 1978; Bayne and Worrall, 1980). As expected, fecundity was much reduced in this population; estimated mean fecundity per spawning was 2.8×10^5 eggs per gram dry weight, compared with 14.2×10^5 eggs · g^{-1} in a nearby population. We have also estimated mortality in both populations (Worrall and Bayne, unpubl. data). From age (= weight) related data for fecundity and mortality the residual reproductive value (RRV) can be calculated as a fundamental component of fitness (Fisher, 1930). The results, normalised to a value of 1.0 for the highest RRV in either population, are plotted in Fig. 9. Age-related RRV is much lower in the stressed population (the Cattewater). In addition, the age of maximum RRV increases in this population and this incurs further disadvantage, since these older individuals comprise a small proportion of the breeding population (< 10%) compared with the age class of maximum RRV in the Lynher (> 30%). Sub-lethal environmental stresses that reduce the individuals'

scope for growth clearly can have profound ecological consequences for t
population.

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Department of the Environment.

APPENDIX

Let y_{ij} denote the (log) rate from animal j in sample i, and x_{ij} its (log) weight, where $i = 1, 2$ and $j = 1, 2, \ldots, n$. The three cases, and the null (H_0) and alternative (H_1) hypothesis examined are:

(a) $y_{ij} = \alpha_i +$ 'error', H_0: $\alpha_2 = \alpha_1$, H_1: $\alpha_2 - \alpha_1 = \Delta\alpha$.
(b) $y_{ij} = \alpha_i + \beta x_{ij} +$ 'error', H_0: $\alpha_2 = \alpha_1$, H_1: $\alpha_2 - \alpha_1 = \Delta\alpha$.
(c) $y_{ij} = \alpha_i + \beta_i x_{ij} +$ 'error', H_0: $\alpha_2 + \beta_2 x_0 = \alpha_1 + \beta_1 x_0$, H_1: $(\alpha_2 + \beta_2 x_0) - (\alpha_1 + \beta_1 x_0) = \Delta\alpha$.

Here the 'error' is assumed normally distributed with constant variance σ^2.

The test statistics T are functions of

$$\bar{y}_i = \sum_j y_{ij}/n,\ V(y_i) = \sum_j (y_{ij} - \bar{y}_i)^2/n,\ C(x_i, y_i) = \sum_j (x_{ij} - \bar{x}_i)(y_{ij} - \bar{y}_i)/n,$$

etc., namely:

(a) $T = (\bar{y}_2 - \bar{y}_1)/(2s^2/n)^{\frac{1}{2}}$, where $s^2 = [n/(2n-2)][\sum_i V(y_i)]$.
(b) $T = (\hat{\alpha}_2 - \hat{\alpha}_1)/(2k^2 s^2/n)^{\frac{1}{2}}$ where $\hat{\alpha}_i = \bar{y}_i - \hat{\beta}\bar{x}_i$ $(i = 1, 2)$, $\hat{\beta} = [\sum_i C(x_i, y_i)]/[\sum_i V(x_i)]$ and $s^2 = [n/(2n-3)][\sum_i V(y_i) - \hat{\beta}^2 \sum_i V(x_i)]$, (for k see text).
(c) $T = [(\hat{\alpha}_2 + \hat{\beta}_2 x_0) - (\hat{\alpha}_1 + \hat{\beta}_1 x_0)]/(2k^2 s^2/n)^{\frac{1}{2}}$ where $\hat{\alpha}_i = \bar{y}_i - \hat{\beta}_i \bar{x}_i$, $\hat{\beta}_i = C(x_i, y_i)/V(x_i)$ $(i = 1, 2)$ and $s^2 = [n/(2n-4)][\sum_i (V(y_i) - \hat{\beta}_i^2 V(x_i))]$, (for k see text).

Defining $t_\nu(P/2)$ to be the upper 100 $(P/2)$% point of the t distribution on ν d.f., H_0 is rejected by a 2-tailed test of significance level P if

$$T > t_\nu(P/2) \text{ or } T < -t_\nu(P/2)$$

here (a) $\nu = 2n-2$, (b) $\nu = 2n-3$, (c
bles of the F distribution on $(1, \nu)$
The power of such a test, against
ity that

$$> t_\nu(P/2) \text{ or } T'_{\nu,\delta} < -t_\nu(P/2),$$

here $T'_{\nu,\delta}$ has a non-central t distr
$(n/2)^{\frac{1}{2}}(\Delta\alpha)/(k\sigma)$. (See Scheffe (
entral t distribution.) Note that
rough the 'scaled difference' $\Delta\alpha$
etermines the power for all experi
The non-central t distribution d
mputation are not available on
ations are required as part of a co
alues of P and n not covered by F
s suggested:

$$\text{Power} \simeq 1 - \Phi(z(P/2) - c) + \Phi(-z$$

where $z(P/2)$ is the upper 100 $(P/$
$P = 0.05)$, $\Phi(x)$ is the $N(0, 1)$ distri

$$c = \delta\left[1 - (z(P/2))^2\left(\frac{1}{2\nu} + \frac{1}{8\nu^2}\right)\right]^{\frac{1}{2}}.$$

The approximation relies only on
adequate for $\nu \geq 8$ and very accur

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Mya arenaria caused by a spill of No.

where (a) $\nu = 2n-2$, (b) $\nu = 2n-3$, (c) $\nu = 2n-4$. Equivalently, T^2 can be referred to tables of the F distribution on $(1, \nu)$ d.f.

The power of such a test, against alternative H_1, can be shown to be the probability that

$$T_{\nu,\delta} > t_\nu(P/2) \text{ or } T'_{\nu,\delta} < -t_\nu(P/2),$$

where $T'_{\nu,\delta}$ has a non-central t distribution on ν d.f., with non-centrality parameter $\delta = (n/2)^{\frac{1}{2}}(\Delta\alpha)/(k\sigma)$. (See Scheffe (1959), Appendix IV, for a definition of the non-central t distribution.) Note that the power is a function of $\Delta\alpha$, k and σ^2 only through the 'scaled difference' $\Delta\alpha/(k\sigma)$; thus, for given P a single set of curves determines the power for all experimental conditions.

The non-central t distribution does not have a closed form and routines for its computation are not available on most computing systems. Thus if power calculations are required as part of a computer programme (or if curves are needed for values of P and n not covered by Figs. 6 and 7) the following simple approximation is suggested:

$$\text{Power} \doteq 1 - \Phi(z(P/2) - c) + \Phi(-z(P/2) - c),$$

where $z(P/2)$ is the upper $100\,(P/2)\%$ point of the $N(0,1)$ distribution (e.g. 1.96 if $P = 0.05$), $\Phi(x)$ is the $N(0,1)$ distribution function, and

$$c = \delta\left[1 - (z(P/2))^2\left(\frac{1}{2\nu} + \frac{1}{8\nu^2}\right)\right]^{\frac{1}{2}}.$$

The approximation relies only on widely available routines (or tables) for $\Phi(\cdot)$; it is adequate for $\nu \geq 8$ and very accurate for $\nu \geq 15$.

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...HYDROMINERAL BALANCE ...ROUT *SALMO GAIRDNERI*, ...S AFFECTED BY PETROLEU...

... INGELHARDT[1], M.P. WONG an...
...rtment of Biology, University of O...

...ived 1 June 1981; accepted 27 Aug...

...Rainbow trout, *Salmo gairdneri*, whi... ...d to petroleum by a number of mod... ...for their effect on ion balance, osm... ...using micron-size particulate emuls... ...able component of the oils only, or ...sion exposure, evidenced by epithel... ...y lamellae. Other treatments had few ...$^{+}$, Na^{+}, K^{+}) were depressed in fres... ...ved from control sea-water acclim... ...eatments. Osmolality decreased in fr... ...dromineral imbalances were conclud... ...ortisol imbalance and perhaps ATPas...

Key words: trout; salinity; petroleum;

INTRODUCTION

Much of the petroleum spill... emulsion, or particulate form ... action or the aerosol effect of ...sants to counteract oil spills al... oil since emulsion formation is ... has been carried out to assess ... concentrations, little definitiv... the effects of oil-in-water em...

A specific and direct site of ... ting a number of physiologic...

[1] Present address: Department of In...