WATER QUALITY AND STRESS INDICATORS IN MARINE AND FRESHWATER ECOSYSTEMS: LINKING LEVELS OF ORGANISATION (INDIVIDUALS, POPULATIONS, COMMUNITIES)

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The ecological impact of different mechanisms of chronic sub-lethal toxicity on feeding and respiratory physiology

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Sub-lethal toxicity tests, such as the scope-for-growth test, reveal simple relationships between measures of contaminant concentration and effect on respiratory and feeding physiology. Simple models are presented to investigate the potential impact of different mechanisms of chronic sub-lethal toxicity on these physiological processes. Since environmental quality is variable, even in unimpacted environments, toxicants may have differentially greater impacts in poor compared to higher quality environments. The models illustrate the implications of different degrees and mechanisms of toxicity in response to variability in the quality of the feeding environment, and variability in standard metabolic rate. The models suggest that the relationships between measured degrees of toxic stress, and the maintenance ration required to maintain zero scope-for-growth, may be highly nonlinear. In addition it may be possible to define critical levels of sub-lethal toxic effect above which no environment is of sufficient quality to permit prolonged survival.

Introduction

Current ecological risk assessment models frequently utilise the widely available data on the acute toxicities of xenobiotics, obtained from laboratory-based single-species toxicity assays performed under standard conditions. These tests (e.g. Anon 1975) provide an adequate basis for comparing the acute, lethal toxicities of various stressors, but were not designed for the evaluation of ecological risk. An approach often taken by risk assessment models is to use data on the median acute lethal dose to define, by the application of somewhat arbitrary safety factors, no-effect concentrations for toxicants (e.g. O'Neill *et al.* 1982; Bartell *et al.* 1992; Van Leeuwen *et al.* 1992). Irrespective of the sophistication of these approaches, reservations must exist over the use of LD_{50} and LC_{50} data as the basis of extrapolation to long-term physiological, population and higher community level effects (Cairns 1980). This is especially so for organisms whose generation times are greatly in excess of the duration recommended by many acute and chronic test protocols.

Physiological measures of toxic impact have many advantages over the more traditional assays based on levels of lethal toxicity. Physiological processes integrate toxicant-induced changes at many levels of functional organisation and provide a means of assessing toxic effects at levels below those causing acute distress (Widdows & Donkin 1991). Physiological responses include direct toxicant-induced physiological and biochemical dysfunction, the metabolic consequences of cellular repair mechanisms, the metabolic consequences of physiological adaptation, and compensation to toxic stress, including mechanisms of toxicant-induced metabolism and depuration (Winston & Di Giullio 1991).

Scope-for-growth (SfG) is widely used as an energy-based measure of the sub-lethal effect of stressors on the physiological performance of animals. The technique involves the determination of the major components of the energy budget, *viz.* food intake A (the assimilated ration) and respiration R, over an appropriately small period of time:

$$SfG = dA/dt - dR/dt \qquad (1)$$

and then comparing values for control and stress-affected experimental groups. Scope-for-growth then represents the rate at which energy is made available for growth and reproduction. The loss of energy through the elimination of nitrogenous waste products is small compared to A and R, and is usually neglected. The technique has been successfully applied in both field and laboratory studies, and provides a sensitive measure of sub-lethal toxic stress (Widdows 1985; Widdows & Johnson 1988).

Animals suffering toxic stress manifest reduced SfG resulting from either a lowered food intake rate or increased rate of respiration. Both physiological functions may be affected simultaneously, dependent on the specific mechanisms of toxicity induced. The SfG measure makes no distinction between the effects of stressors which act differentially on feeding or respiration. Here I consider the ecological implications of three distinct mechanisms of sub-lethal toxic impact which are known to act differentially on feeding and respiratory physiology. The mechanisms are (1) non-specific narcosis, (2) inhibition of oxidative metabolism and (3) uncoupling of oxidative metabolism.

Mechanisms of toxicity

Narcosis

Two distinct forms of narcosis, defined as a pharmacologically induced and reversible disruption of neural pathways, are recognised. The two forms are distinguished on the basis of the acute physiological response of vertebrates to narcotic intoxication. Non-specific narcosis is considered to be the least damaging and can be induced by a wide range of organic chemicals, of which aliphatic and aromatic hydrocarbons, and chlorinated hydrocarbons, are particularly important in the environment. Alcohols, esters, ketones, aldehydes, weak organic acids and bases also induce non-specific narcosis (review by Bradbury *et al.* 1989; see also Roth 1980; Veith *et al.* 1983; Lipnick 1990). A more acute form of narcosis, termed polar narcosis, is induced by certain esters, phenols and anilines (Bradbury *et al.* 1989).

Metabolic inhibition

A range of organic compounds are known to inhibit ATP synthesis. These act in a variety of specific ways, but many bind with particular membrane sites, disrupting the function of specific enzymes involved in the oxidation of respiratory substrates via the respiratory pathway and electron transport chains. The inhibition of ATP synthesis is accompanied by increased concentrations of various intermediate respiratory-chain substrates, dependent on the action of the metabolic inhibitor (Terada 1990; Snoeij *et al.* 1987). The physiological effects of metabolic inhibition can be mimicked by exposure to hypoxic and anoxic conditions which limit the supply of oxygen and hence prevent complete respiratory capacity from being realised (Widdows *et al.* 1989). Examples of compounds which inhibit oxidative metabolism are the respiratory poison cyanide and organotins, including dibutyltin.

Metabolic uncoupling

A wide variety of compounds are known to disrupt the protonophoric properties of the H^+-impermeable mitochondrial membrane. As a result of this action the phosphorylation of ADP by ATP-synthase is short-circuited from the redox reactions which attempt to maintain the cross-membrane gradient in hydrogen ion concentration (Terada 1990). In effect the efficiency of ATP synthesis is reduced. Many weak organic acids act in this manner. The antifouling paint constituent tributyltin also acts as a metabolic uncoupler by binding to specific membrane-bound proteins and disrupting the function of ATP-ases (Snoeij *et al.*, 1987).

Modes of toxicity and scope-for-growth (SfG)

The effects of three selected contaminants on the components of SfG, as exemplified by the marine mussel *Mytilus edulis*, are shown in Figure 1. In the case of each contaminant the relationship between the proportionate reduction in feeding (ε_a) or increase in respiration (ε_r), or both, can be described as a linear function of the Log exposure or Log tissue concentration of the contaminant. Hydrocarbons with a narcotic mode of action act primarily by reducing the particle clearance rate of mussels, so reducing net assimilation A. Widdows *et al.* (1988) reported that napthalene acts to reduce clearance rate in mussels by reducing the beating rate of cilia upon the gill. In an experiment in which mussels were dosed with crude oil (Widdows *et al.* 1987) the reduction in SfG was primarily due to a reduction in feeding rate, with no significant effect on the rate of respiration. Figure 1a shows the effect of toluene on the clearance rate of mussels (Donkin *et al.* 1989).

The action of a non-specific metabolic uncoupler is illustrated by pentachlorophenol (Fig. 1b) (Widdows & Donkin 1991). Above a common no-effect concentration (about 2 mg kg^{-1} wet tissue weight) both feeding and respiration rate are affected. The effect of pentachlorophenol is to increase respiration rate until it is approximately double the non-impacted rate at a tissue concentration of 25 mg kg^{-1} wet wt, above which respiration is rapidly depressed. Feeding rate is suppressed with increasing tissue concentrations until almost zero at 35 mg kg^{-1} wet wt.

Finally, in an experiment in which mussels were exposed to sea-water containing tributyltin (TBT) (Widdows & Page 1993) both respiration and feeding were affected, but the threshold concentration of contaminant for an effect on respiration rate was approximately one order of magnitude less than the threshold effect on feeding rate. Maximum increased respiration and decreased feeding were recorded at about the same concentration of TBT (15 µg g^{-1} dry tissue weight). However, TBT is unstable in aquatic solution, and a proportion of dibutyltin (DBT) is also always present. Since TBT (an uncoupler of respiratory metabolism) and DBT (thought to be an inhibitor of metabolism) have different modes of toxic action the interpretation of the results shown in Figure 1c is not straightforward. Widdows & Page (1993) have interpreted the increased respiration rate as due to the action of TBT, while the suppression of feeding rate may be due to the action of DBT.

An important conclusion of these studies (Widdows *et al.* 1987, 1990; Donkin *et al.* 1989) is that the SfG measure of sub-lethal toxic effect is, by the application of a carefully controlled experimental protocol, apparently independent of environmental influences known to have large impacts on these physiological variables. For example, the data shown in Figure 1c are the combined results of three experiments undertaken at different times of year, and yet the proportionate toxic response is apparently independent of seasonal effects on feeding and respiration rate.

Models of sub-lethal toxicity on SfG components

Firstly note that the effect of a contaminant, as measured by the change in physiological function (decreased dA/dt or increased dR/dt), is proportional to the Log concentration of contaminant between a lower threshold concentration and some upper concentration limit (Fig. 1). Therefore I define two toxic coefficients, ε_a and ε_r ($0 \leq \varepsilon \leq 1$), to define the effect of the Log contaminant concentration on assimilation rate and respiration rate respectively between these two limits. When $\varepsilon = 1$ the impact of the toxicant is maximal; when $\varepsilon = 0$ there is no effect.

In order to define the effect of toxicants on respiratory metabolism it is first necessary to discriminate respiration into its constituent parts. I shall sub-divide respiration using
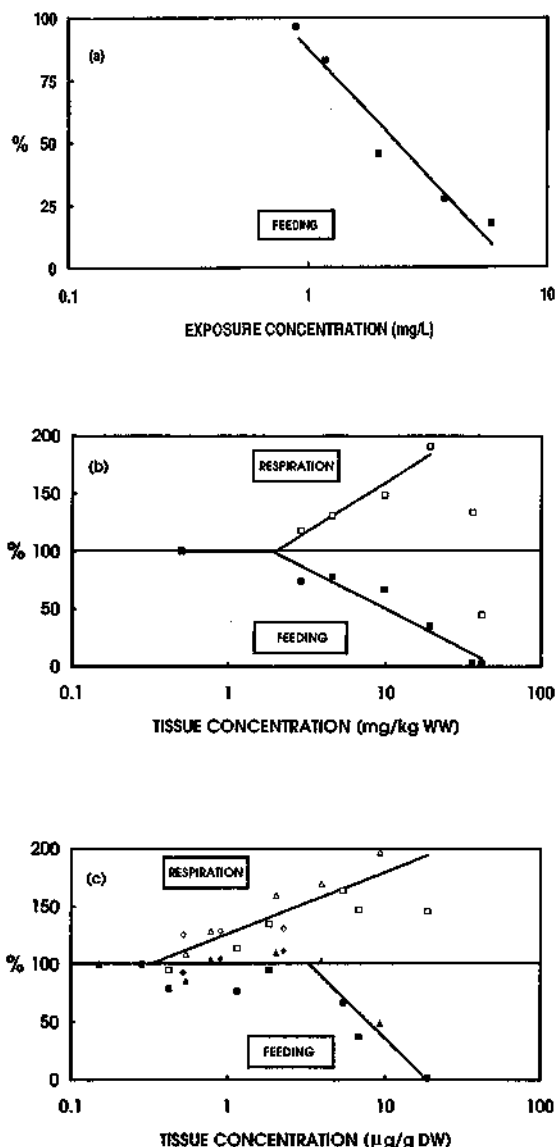
Figure 1. Examples of the action of chemicals with different modes of toxic action on the respiration rate (measured as oxygen consumption) and feeding rate (measured as particle clearance rate) of the mussel *Mytilus edulis*. In each figure the feeding rate and respiration rate are shown as the percentage change from control values. (a) Aromatic hydrocarbons, such as toluene, reduce feeding rate through a non-specific narcotic mode of action affecting gill cilia (figure redrawn from Donkin *et al.* 1989). (b) Non-specific metabolic uncoupling illustrated by pentachlorophenol which acts both on respiration rate and feeding, with a common no-effect concentration. Note that respiration rate almost doubles before, at higher tissue concentrations, respiration is suppressed (data redrawn from Widdows & Donkin 1991). (c) When exposed to the antifouling paint constituent tributyltin (TBT), respiration rate increases and feeding rate decreases, but they do so at different threshold concentrations, and at different rates. TBT is believed to uncouple respiratory pathways, whereas dibutyltin (DBT) may act as a respiratory inhibitor (figure redrawn from Widdows & Page 1993; symbols represent the results from three different experiments). The value of the sub-lethal toxic stress parameter, ε, can be determined from the text-figure, for any level of contaminant of known mode of toxic action.

conventional concepts derived from whole organism ecological physiology. I shall use the term standard metabolic rate (SMR) to represent the level of metabolic demand required by an individual in a particular unstressed environment. This is the sum of basal metabolic rate (BMR) together with the metabolic costs associated with the rates of other activities, such as feeding, movement, growth, etc., in that environment. I shall also assume that there exists some maximum level of sustainable metabolism (SMR_{max}), representing the complete induction and utilisation of respiratory capacity. Although these terms reflect the current empirical level of understanding they are, however, barely adequate for the interpretation of toxic mechanisms affecting underlying physiology.

I assume that the following may represent the three different mechanisms of toxic action:

Narcosis: $SfG = dA/dt \, (1 - \varepsilon_a) - SMR$ (2)

Metabolic Inhibition: $SfG = \{dA/dt - (SMR - BMR)\} \, (1 - \varepsilon_r) - BMR$ (3)

Metabolic Uncoupling: $SfG = dA/dt - SMR / (1 - q\varepsilon_r)$ (4a)

where: $q = 1 - SMR/SMR_{max}$ (4b)

For a narcotic mode of action the model of toxic effect is relatively straightforward: the reduction in the rate of assimilation at concentrations above the toxic threshold is assumed to be proportional to ε_a, the measured effect on the feeding rate. The narcotic is assumed to have no impact on the standard rate of metabolism, a hypothesis supported by empirical evidence of the effect of hydrocarbons on *Mytilus edulis* (Widdows *et al.* 1987; Donkin *et al.*, 1989). Although a reduced rate of feeding and digestion may entail some reduction in active metabolic rate, these costs are believed to be a relatively small component of overall respiration rate (Widdows & Hawkins 1989; Willows 1992), and do not appear to be discriminated in practical tests of toxic impact.

For toxicants that have a direct impact on respiratory metabolism the situation is less clear, and it is necessary to make some assumptions concerning the impact of toxicants upon components of metabolism. For metabolic inhibition, I assume that basal metabolic rate is conserved and that it is the active components of metabolism that are affected (eqn 3). Assimilation rate is assumed to be reduced in parallel since it is dependent on active metabolism. The effect of ε_r on active metabolism (i.e. SMR – BMR) is scaled such that active metabolism becomes zero as the impact of the toxicant is maximal ($\varepsilon_r = 1$), and dR/dt = BMR. I assume that the organism is able to sustain a minimum level of metabolism equal to basal metabolic rate in the presence of a metabolic inhibitor, at least in the medium term, through non-oxidative metabolic pathways. This assumption arises from the observation that the action of metabolic inhibitors can be mimicked by hypoxic conditions in *Mytilus edulis* (Widdows *et al.* 1989). It should be noted, however, that the ability to maintain prolonged periods of anoxic respiration is not universal to all species.

For contaminants that increase metabolic rate by uncoupling oxidative metabolism, it is necessary to specify the maximum rate of metabolism (SMR_{max}) that can be sustained. In equation 4 this maximum rate is determined by the value of parameter q. SMR_{max} is generally believed to lie within the range of 2–3 times SMR, although the SMR is itself known to be variable, dependent on both physiological state and environmental conditions. For example, in the experiment in which *Mytilus edulis* was exposed to organotins, Widdows & Page (1993) found that total respiration rate almost doubled from control values until, at higher concentrations, there was evidence of complete respiratory collapse (Fig. 1c).

In order to consider the ecological implications of these different mechanisms of chronic toxicity, it is first necessary to define the relationship between assimilation rate and the availability of food in the environment. For simplicity I assume an Ivlev-type functional

response between assimilation rate and the available food level F, with the maximum rate given by A_{max}:

$$dA/dt = A_{max} (1 - exp^{-kF}) \tag{5}$$

The ecological impact of a toxicant on SfG, then, depends on the particular mechanism of toxic action, together with the values for A_{max}, BMR, SMR and SMR_{max} which will be dependent on both environmental and physiological states. In order to reduce the number of variables, it is convenient to scale all variables by the BMR. Hence dimensionless SfG' = SfG/BMR, and other variables are redefined relative to BMR, so that:

$$\omega = A_{max} / BMR$$

$$v = SMR/BMR$$

$$v_{max} = SMR_{max} / BMR$$

Therefore,

Narcosis: $\qquad$ $SfG' = \omega (1 - exp^{-kF}) (1 - \varepsilon_a) - v$ $\qquad$ (6)

Metabolic Inhibition: $\qquad$ $SfG' = \{\omega (1 - exp^{-kF}) - (v - 1)\} (1 - \varepsilon_r) - 1$ $\qquad$ (7)

Metabolic Uncoupling: $\qquad$ $SfG' = \omega (1 - exp^{-kF}) - v/(1 - q\varepsilon_r)$ $\qquad$ (8a)

where: $\qquad$ $q = 1 - v/v_{max}$ $\qquad$ (8b)

Analysis of models

Note that when SfG = 0 the energy assimilated from the food equals that utilised by total respiratory metabolism. Hence equations (6) to (8) can be solved for the availability of food required to maintain energetic balance as a function of the mechanism and degree of toxic action ε. The level of food necessary to maintain SfG = 0 is termed the required maintenance ration RMR. Relative to BMR, RMR' increases with the degree of toxic impact (Fig. 2, eqns 9–11), with the rate of increase dependent on the mechanism of toxic action.

Narcosis: $\qquad$ $RMR' = - \log_e [1 - v/\{\omega (1 - \varepsilon_a)\}] / k$ $\qquad$ (9)

Metabolic Inhibition: $\qquad$ $RMR' = - \log_e [1 - \{v - 1 + 1/(1 - \varepsilon_r)\}/\omega] / k$ $\qquad$ (10)

Metabolic Uncoupling: $\qquad$ $RMR' = - \log_e [1 - v/\{\omega (1 - q\varepsilon_r)\}] / k$ $\qquad$ (11)
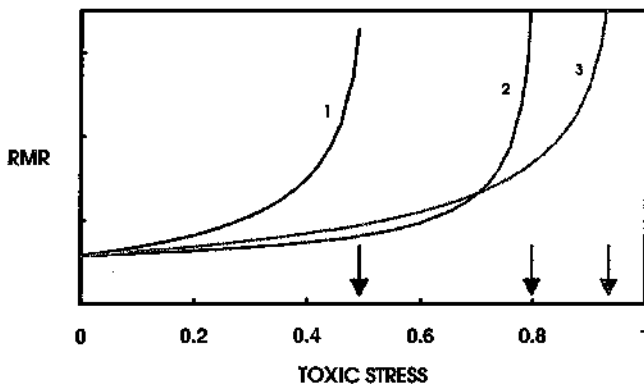

Figure 2. Form of the relationships between the value of the sub-lethal toxic stress paramater ε and RMR, the maintenance ration required for scope-for-growth to be zero (see eqns 9–11). The curves represent RMR for three modes of toxic action; (1) neurotoxicity, (2) metabolic inhibition and (3) metabolic uncoupling. Arrows indicate the corresponding values of ε_{crit}.
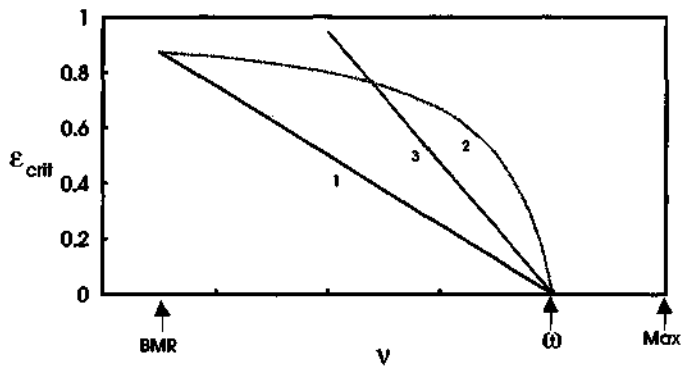
Figure 3. The effect of variation in standard metabolic rate (SMR), represented relative to basal metabolic rate (v), on the critical value of toxic stress ε_{crit} (see the text for definitions of symbols). At values of sublethal toxic stress ε greater than ε_{crit} positive scope-for-growth is impossible even in the best feeding environments (see eqns 12–14). Lines represent toxic stress operating via (1) neurotoxicity, (2) metabolic inhibition and (3) metabolic uncoupling.

The maximum SfG that can be achieved, at a given metabolic rate, occurs when food is abundant and assimilatory capacity is saturated, i.e. when $dA/dt = A_{max}$ (or, relative to BMR, ω). Under these conditions it is possible to define, for each toxic mechanism, a critical level of toxic stress at which no amount of food availability can support positive SfG (see Fig. 2). For the narcotic and inhibitory modes of toxic action the critical level of toxic impact (ε_{crit}) depends on the relative magnitudes of the maximum assimilatory capacity and the standard metabolic rate (eqns 12 and 13). For metabolic uncoupling the critical level of toxicant impact also depends on the factor q, representing the extent to which the toxicant can act to increase metabolic rate up to its physiological limit (eqn 14).

Narcosis: $\varepsilon_{crit} = 1 - v/\omega$ (12)

Metabolic inhibition: $\varepsilon_{crit} = 1 - 1/(1 + \omega - v)$ (13)

Metabolic uncoupling: $\varepsilon_{crit} = (1 - v/\omega)/q$ (14)

where $v < \omega$ and $q < 1 - v/\omega$.

Just as the severity of toxic effect is dependent on the availability of food, it may also depend on the underlying metabolic rate. The level of the non-stressed metabolic rate is variable, dependent on the organism's physiological state and the effect of current environmental conditions. A stressor at a particular concentration may have a greater or lesser impact dependent on the underlying metabolic rate. This is illustrated in Figure 3. As the unimpacted value of the standard metabolic rate relative to basal metabolic rate v varies, so too does the sensitivity of both the required maintenance ration as a function of ε, and the value of ε_{crit}.

Discussion

The scope-for-growth (SfG) test has been widely deployed for both field and laboratory assessments of environmental quality and sub-lethal toxicity. Recently we are beginning to gain more detailed understanding of the biochemical, cellular and physiological basis for the toxicity-based changes in feeding and respiration rates which together explain the greater part of changes in SfG. It is important to understand whether contaminants, having the same effect as measured by SfG (i.e. as the proportionate change from the non-impacted or control state), but with different modes of toxic action, have the same impact on the organism.
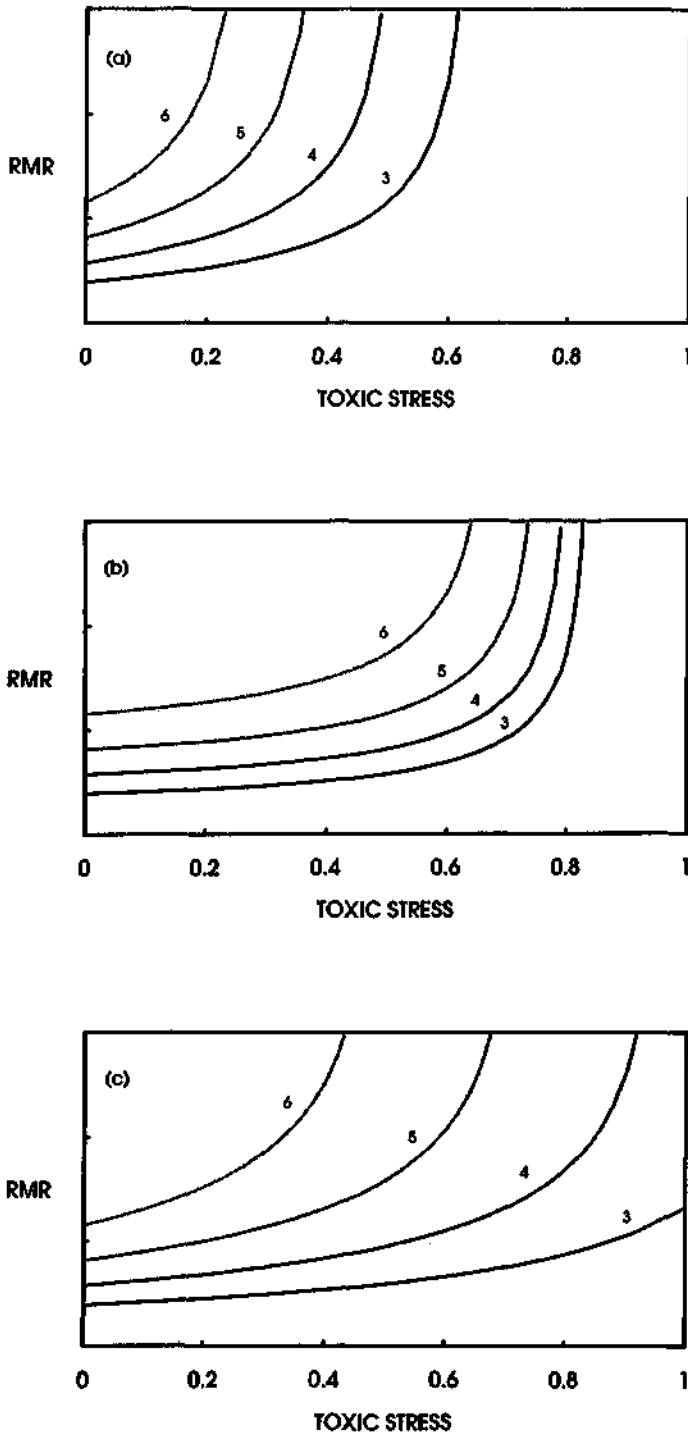
Figure 4. Effect of variation in the level of sub-lethal toxic stress ε and the unimpacted value of standard metabolic rate (SMR) on the required maintenance ration (RMR) for each of three modes of toxic action: (a) neurotoxicity, (b) metabolic inhibition and (c) metabolic uncoupling. SMR values (3–6) against the curves indicate SMR 3 to 6 times the basal metabolic rate.

The development of sub-lethal toxicity tests has shown that organisms show stress responses to low, chronic levels of environmental contaminants. It is frequently difficult to know how important these measured effects are to the health of the organisms concerned since these tests fail to recognise that natural environments are frequently of variable quality, and that the effect of a contaminant, particularly at sub-lethal levels, may be dependent both on the intrinsic state of the organism and on the quality of the environment. The use of SfG as a measure of toxic impact is particularly useful in this respect since we can use energetics to relate the toxic effect to aspects of the organism's physiological state, to its environment, and ultimately to aspects of its life history.

These simple models are not meant to predict the exact nature of the relationship between short-term measures of toxic effect, mode of toxic action, and toxic impact. Rather, they fulfil a number of roles. Firstly, by using generalised descriptions of physiological relationships, such as the relationship between food energy intake rate and food availability, and the observed relationships between physiological effects and concentrations of substances with recognised modes of toxic action, they enable us to gain an understanding of the possible consequences of different levels of sub-lethal toxic stress and different modes of toxic action in different environments. Secondly, they point out areas where we lack the necessary understanding, and the difficulties of making specific predictions, without detailed knowledge of the component processes involved.

The case of metabolic uncoupling is particularly troublesome. It is currently not possible to establish with any confidence a generalised relationship between tissue concentration of toxicant, ε, standard metabolic rate (SMR) and the maximum rate of sustainable metabolism (SMR_{max}). SMR is not a constant, indeed it is known to vary greatly, dependent on both environmental and internal physiological states, which are likely to be correlated. A value for SMR_{max} is even more difficult to establish, a difficulty compounded by the usual practice of expressing it in terms of a multiple of the variable SMR. Within the model of metabolic uncoupling presented here I have let the toxic effect ε be scaled by the factor q such that the maximum rate of metabolism is always realised when $\varepsilon = 1$. It is not clear whether this is the most appropriate representation.

These models do suggest that, given a generalised functional response between feeding rate and food availability, the relationship between the measured sub-lethal toxic effect and the ability of an organism to meet its basic metabolic requirements (i.e. the required maintenance ration RMR) may be extremely non-linear. This suggests that, at a given level of food availability, low or moderate levels of toxic stress may have no or little impact on the animal in the environment, but small additional burdens may have disproportionately large impacts (Fig. 4). Likewise, levels of toxic effect that can be sustained at one food level may be unsustainable if the quality of the feeding environment is reduced.

In addition the models suggest that critical levels of sub-lethal stress may exist. Although the measured impact of the stressor may be sub-lethal, it is possible to define levels of stressor which would prevent organisms from achieving positive energetic balance, and hence there are levels of impact which cannot be sustained even in the best-quality habitats.

The mode of toxic action is clearly indicated as an important factor in determining the effect of the contaminant on an organism. Put simply, organisms with increased energetic demands, due to the presence of a contaminant, may be able to meet those demands by increased rates of energy intake in favourable environments. However, if feeding is suppressed by a contaminant, and the organism is unable to reduce energy expenditure (as these simple models assume), then the effect of the toxicant is likely to be more severe.

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