

Metabolic costs incurred by crayfish (*Procambarus acutus*) in a trace element-polluted habitat: further evidence of similar responses among diverse taxonomic groups[☆]

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Abstract

Recent studies of several vertebrates and an invertebrate have shown elevated standard metabolic rate (SMR) following chronic exposure to a mixture of trace elements in a contaminated habitat. In this study, we examined whether another invertebrate, a crayfish (*Procambarus acutus*), also experienced elevated SMR in response to the same contaminants. We compared SMR of individuals inhabiting the contaminated site with SMR of individuals from uncontaminated reference sites. We also examined SMR of individuals collected from the reference areas and exposed in the laboratory for 50 days to sediment and food derived from the contaminated site. Individuals collected from the contaminated site had elevated SMR compared to individuals collected from the unpolluted areas (25.1 vs. 19.2 J g⁻¹ day⁻¹). Individuals exposed to contaminated sediment and food in the laboratory experienced elevations in SMR compared to controls after 27 days of exposure (35.2 vs. 29.4 J g⁻¹ day⁻¹), but after 50 days of exposure, metabolic rate no longer differed between treatments. Growth of contaminant-exposed individuals was lower than growth of reference animals throughout the laboratory study. Elevated SMR associated with contaminant exposure may reflect energy-demanding mechanisms required to combat deleterious effects of contaminants. Our results support the prediction that increases in energy expenditure in the contaminated habitat would negatively influence production processes, such as growth. Results from this study in conjunction with observations from other species suggest that increased SMR is a common response among several taxa to the mixture of contaminants in the study site. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

Chronic exposure to low levels of environmental contaminants can negatively impact the overall performance of individuals without being directly lethal. As interest in sublethal effects of environmental contaminants has grown, more emphasis has been placed on examining connections between individuals and effects on ecological systems (see Calow, 1994; Clements and Kiffney, 1994). Thus, in studies of environmental contamination, there is growing need for applications of physiologically based measurements which have strong demonstrated or theoretical relationships to ecological processes.

Examining animal energetics in contaminated systems allows investigators to examine endpoints that integrate multiple physiological processes, which may in turn influence phenomena at higher levels of organization. One useful approach is to examine the ways in which energy allocation to the competing processes of maintenance and production are altered by chronic exposure to environmental stress (e.g. Calow and Sibly, 1990; Calow, 1991; Widdows and Donkin, 1991).

Assimilated energy derived from food is allocated to support two distinct pathways, maintenance (a catabolic pathway) and production (an anabolic pathway). The maintenance pathway includes standard maintenance (standard metabolic rate, 'SMR'; supporting basic costs of survival), activity (active metabolic rate), and specific dynamic action (post-prandial metabolic rate; supporting costs associated with food digestion). The production pathway supports tissue formation (growth, replacement of shed or lost tissues, energy storage, reproductive products). Whereas standard maintenance is an obligatory cost, other processes, such as activity and production, can be suppressed temporarily without compromising survival (Lucas, 1996). Standard maintenance requirements must therefore be satisfied before energy is allocated to other processes, and, all else being equal, an increase in SMR will detract from the amount of energy available for production. Moreover, because the maintenance portion of a total energy budget can be the largest single compartment (see Congdon et al., 1982), a relatively small elevation in SMR that increases the total maintenance budget can result in a proportionally larger reduction in allocation to production. Thus, in the absence of compensatory

processes, stressful environmental conditions that significantly increase an individual's SMR would be expected to negatively impact growth, energy storage, and/or reproduction.

Theoretical and empirical studies suggest that sublethal exposure of organisms to some pollutants can result in elevated energy expenditures (Bayne et al., 1979; Moore et al., 1987; Sibly and Calow, 1989; Calow and Sibly, 1990; Calow, 1991; Widdows and Donkin, 1991; Weber and Spieler, 1994; Rowe, 1998; Rowe et al., 1998a; Hopkins et al., 1999). The mechanisms by which contaminants can lead to increased energy expenditures are not specifically known. However, contaminant-related processes, such as excretion of toxicants and elevated protein synthesis and turnover rates would be expected to have measurable effects on an individual's energy requirements (Hawkins et al., 1986; Hawkins, 1991; Koehn and Bayne, 1989; Sibly and Calow, 1989).

We examined whether chronic exposure of crayfish (*Procambarus acutus*) to contaminated sediments and food had measurable effects on SMR and growth. The contaminated study site was a drainage swamp associated with a coal ash disposal system. This site, which is characterized by high concentrations of trace elements (including As, Cd, Cr, Cu and Se) in sediments and tissues of resident organisms, has been the subject of recent studies of animal energetics in response to contaminants (Rowe, 1998; Rowe et al., 1998a; Hopkins et al., 1999). We compared SMR of crayfish inhabiting the contaminated site to SMR of crayfish from nearby uncontaminated sites. We also tested whether: (1) metabolic differences could be induced by laboratory exposure to sediments and food collected from the contaminated site; and (2) changes in SMR were related to growth rates of crayfish used in the laboratory study. We present our findings in light of previous results which, when viewed as a whole, suggest that an increase in energy expenditure may be a common response by several, distantly related species when chronically exposed to conditions in the contaminated site.

2. Materials and methods

2.1. Study sites

D-Area Swamp receives contaminated outflow

from a series of settling basins used for removal of particulate matter from slurried coal ash derived from the D-Area power facility on the Savannah River Site, Aiken, SC, USA. The swamp and settling basins have been the subject of intensive research examining transport and uptake of trace elements by animals and resulting lethal and sublethal effects (Guthrie and Cherry, 1979; Rowe et al., 1996, 1998a,b; Rowe, 1998; Hopkins et al., 1997, 1998, 1999, 2000a,b; Raimondo et al., 1998). Reference sites used in this study were a man-made pond (Fire Pond) and three sloughs and swamps (Risher Pond wetland system), all located within approximately 10 km of the contaminated site, and having no known history of contamination.

2.2. Protocol

Adult crayfish were collected from D-Area swamp and reference sites during June and July using minnow traps. Crayfish were removed daily from traps, taken to the laboratory, and acclimated for 5 days in dechlorinated tap water. Animals from reference sites were pooled as a single reference group. During the acclimation period, crayfish were unfed, and the temperature of the holding room was maintained at 25°C. Individuals that appeared unhealthy (e.g. displayed trapping-related damage or were unresponsive to prodding) were removed and excluded from the study. A total of 23 and 19 individuals from the contaminated and uncontaminated sites, respectively, were subsequently used for measurement of standard metabolic rate (described below). Four additional crayfish from each site were used for determination of whole-body concentrations of coal ash-related trace elements by inductively coupled plasma-mass spectrometry.

We also conducted a laboratory experiment in which adult crayfish from uncontaminated reference sites were exposed to sediments and food collected from: (1) D-Area Swamp; or (2) an uncontaminated reference site. Crayfish were held individually in 24 20-l aquaria containing 3 cm of contaminated or uncontaminated sediments from the respective study sites (12 aquaria per site) and filled with dechlorinated tap water. Visible pieces of detritus were removed from sediments prior to the study. Feeding was ad lib by daily addition of food (largemouth bass filet portions) previously collected from the contaminated or uncontami-

nated study sites. At 24-h intervals, uneaten food was removed and replaced with fresh food. The laboratory remained at 23–24°C during the study. After 27 and 50 days, SMR was measured for all individuals following a period of 5 days, during which they were unfed.

Standard metabolic rates (hourly rates of oxygen consumption by post-absorptive individuals at rest) were obtained using a computer-controlled micro-respirometer (Micro OxyMax, Columbus Instruments, Columbus, OH). Respirometry chambers were 220-ml vessels containing 100 ml of dechlorinated tap water. One individual was placed in a chamber that was randomly assigned to one of 20 independent channels of the respirometer. Oxygen consumption was determined from the headspace of air overlying the water in each respirometry chamber at approximately 2-h intervals over 24 h. Chambers were housed in a dark, 25°C incubator during measurement of oxygen consumption. Because trapping success in the study sites varied over the study period, four separate 24-h trials were conducted. Each trial included at least four individuals collected from both sites. Following each trial, individual crayfish were towel-dried and weighed to the nearest 0.001 g.

The highest 50% of measurements were excluded from analyses to avoid overestimating SMR due to unobserved periods of activity (Rowe et al., 1998a). Prior to truncating the data set, there frequently was an irregular saw-tooth pattern in the relationship between oxygen consumption and time, indicating hyperactivity during some sampling intervals, yet there was no clear evidence of a diurnal pattern. The truncated data set resulted in a fairly smooth and horizontal relationship between oxygen consumption and time. Absence of a decreasing trend in oxygen consumption with time suggested that the crayfish were post-absorptive following the pre-measurement fasting period. Allometric relationships between mass and oxygen consumption produce a scaling factor not equal to 1 (Packard and Boardman, 1987; Beaupre and Dunham, 1995). Therefore, we obtained residuals from a regression of oxygen consumption versus body mass, and analyzed for treatment effects on residuals using one-tailed *t*-tests. Because previous studies reported increases in oxygen consumption in the contaminated site (Rowe, 1998; Rowe et al., 1998a; Hopkins et al., 1999), we used one-tailed *t*-tests to specifically examine the

Table 1

Trace element concentrations in crayfish (whole bodies) collected from the coal ash-polluted site (ASH) or unpolluted reference sites (REF)

Site	N	As	Cd	Cr	Cu	Pb	Se
(A) Crayfish bodies (ppm)							
ASH	4	3.99 ± 0.28	4.88 ± 0.69	1.37 ± 0.50	223.72 ± 20.62	DNM	14.70 ± 0.49
REF	4	0.95 ± 0.14	0.29 ± 0.05	0.66 ± 0.11	46.99 ± 4.61	DNM	2.41 ± 0.22
(B) Sediments (ppm)							
ASH	3	39.64 ± 2.70	0.25 ± 0.01	10.87 ± 0.82	18.39 ± 1.31	6.46 ± 0.50	4.38 ± 0.19
REF	3	0.34 ± 0.01	0.03 ± 0.01	7.02 ± 0.19	4.04 ± 0.10	4.22 ± 0.08	0.10 ± 0.01
(C) Water (ppb)							
ASH	3	17.17 ± 0.52	0.11 ± 0.02	0.44 ± 0.01	2.53 ± 0.11	0.08 ± 0.03	7.00 ± 1.50
REF	3	0.35 ± 0.03	0.04 ± 0.01	0.07 ± 0.01	1.04 ± 0.06	0.03 ± 0.01	< 1.00

Included also are trace element concentrations from sediment and water in the polluted site and one of the reference sites (from Rowe, 1998; Rowe et al., 1998a). Values are means ± 1 × S.E. (ppm dry mass for tissue and sediment, ppb for water). DNM, did not measure.

hypothesis of elevation of consumption rates following exposure, rather than a two-tailed test for differences, regardless of the direction (such as ANOVA; Neter et al., 1990). Even with the use of directional hypothesis tests, statistical power remained fairly low, especially in the laboratory study (power = 0.51), indicating that only large differences among means would be judged statistically significant. Oxygen consumption was translated into units of energy expenditure using the relationship 1 ml O₂ consumed = 20.08 J.

We also measured growth of crayfish in the laboratory study. Like many other invertebrates

and some vertebrates, crayfish growth is indeterminate, continuing for the duration of the life cycle (Heino and Kaitala, 1999). Measurements of growth allowed us to examine a portion of the production budget that we predicted would be negatively influenced by elevated SMR. Instantaneous growth rates (k ; Brody 1945) were calculated as $k = (\ln M_2 - \ln M_1)/t$, where M_1 and M_2 were wet masses at the beginning and end of the experimental interval, respectively, and t was the interval (0–27 or 27–50 days). Instantaneous growth rates provided an estimate of the change in mass within an experimental interval. Growth

Table 2

Oxygen consumption at rest in crayfish collected from the coal ash-polluted site (ASH) or unpolluted reference sites (REF), and for crayfish experimentally exposed to food and sediments from the site in the laboratory for 27 and 50 days

Site	N	Wet mass (g)	O ₂ consumed		
			(ml h ⁻¹ individual ⁻¹)	(ml h ⁻¹ g ⁻¹)	
(A) Field-collected					
ASH	23	7.699 ± 0.136	0.401 ± 0.022	0.052 ± 0.003	
REF	19	7.669 ± 0.147	0.308 ± 0.015	0.040 ± 0.002	<i>P</i> < 0.001
(B) Laboratory-exposed for 27 days					
ASH	8	6.594 ± 0.530	0.482 ± 0.059	0.073 ± 0.008	
REF	11	6.716 ± 0.410	0.411 ± 0.033	0.061 ± 0.003	<i>P</i> = 0.037
(C) Laboratory-exposed for 50 days					
ASH	7	7.301 ± 0.619	0.555 ± 0.036	0.078 ± 0.005	
REF	8	7.889 ± 0.538	0.619 ± 0.074	0.078 ± 0.007	<i>P</i> = 0.704

Analyses were conducted at 25°C. Values are means ± 1 S.E. Statistical results are those obtained by directional hypothesis tests on residuals (see text).

was also examined as relative change in mass, $(M_2 - M_1)/M_1$, to provide a direct comparison of sizes at the beginning and end of an interval. Measures of growth were analyzed by *t*-tests, to examine the hypothesis that growth would be reduced due to contaminant exposure. Survival during the laboratory experiment was analyzed by χ^2 . The strong bimodal distribution of tissue trace element concentrations between the contaminated and reference study sites precluded statistical comparison of body burdens between sites.

3. Results

Whole-body trace element concentrations were 2–16-fold higher in crayfish collected from the contaminated site than the reference sites (Table 1). Relative differences in whole body trace element accumulation between contaminated and uncontaminated areas ranked as: Cd > Se > Cu > As > Cr.

Mean SMR was significantly higher for crayfish collected from the contaminated site than for those collected in the uncontaminated reference site (Table 2; Fig. 1). Exposure to contaminated sediments and food for 27 days in the laboratory also led to an increase in SMR, but the difference between treatments was not retained through the remainder of the experiment (Table 2; Fig. 1).

Instantaneous growth rates and relative changes in mass in the laboratory reflected trace element exposure. During the first interval of the study (days 0–27), contaminant-exposed individuals experienced significantly lower instantaneous growth rates ($P = 0.007$; Fig. 1) and relative change in mass (9 vs. 21% change for contaminated and reference, respectively; $P = 0.007$; Fig. 1). During the remaining portion of the experiment (days 28–50), average instantaneous growth rates remained lower for contaminant-exposed individuals ($P = 0.046$, Fig. 1). During this interval, relative change in mass also remained lower for contaminant-exposed individuals compared to individuals in the reference treatment (11 vs. 27%, respectively; $P = 0.039$; Fig. 1).

There was no difference in mortality between treatments at either sampling time (day 27 or 50). By day 27, four of 12 crayfish in the contaminated treatment and one of 12 crayfish in the reference treatment died ($\chi^2 = 2.27$, $P = 0.132$). By day 50, five and four crayfish of the original 12 had died

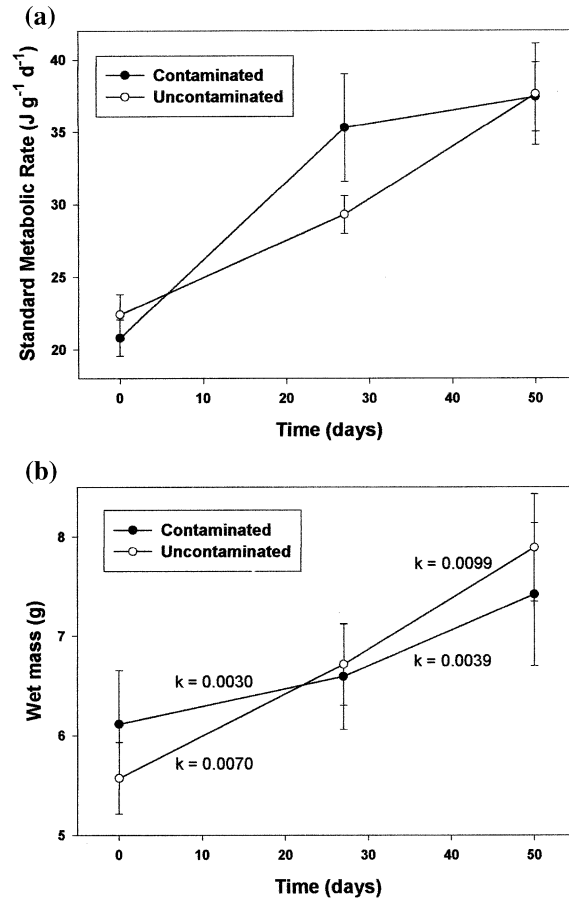


Fig. 1. Temporal trends in daily standard metabolic rate (panel A) and rates of growth (panel B) during the laboratory study. k = instantaneous growth rate during the corresponding interval. Values are means \pm 1 S.E.

in the contaminated and reference treatments, respectively ($\chi^2 = 0.178$, $P = 0.673$).

4. Discussion

Crayfish collected from the contaminated habitat had higher body burdens of some trace elements and approximately 30% higher SMR than did individuals from uncontaminated areas (Tables 1 and 2). The relationship between contaminant accumulation and SMR supports the prediction that combating deleterious effects of contaminants is energetically costly (see Calow, 1991). Metabolic rates for individuals from the reference populations ($0.83 \text{ J g}^{-1} \text{ h}^{-1}$ at 25°C) are similar to those reported by Kossakowski (1974) for the crayfish *Orconectes limosus* of the

same approximate size, but lower temperature ($0.61 \text{ J g}^{-1} \text{ h}^{-1}$ at 18°C), indicating that our metabolic measurements for *P. acutus* are comparable to some other crayfish. We are aware of no previous reports of SMR for *P. acutus* to which we can directly compare our measurements.

The results from the first 27 days of the laboratory experiment seemed to support the prediction that an increase in SMR would be associated with a decrease in growth. When crayfish from reference sites were placed in contact with contaminated sediments and fed contaminated food for 27 days, SMR increased, and growth declined (Fig. 1). However, following continued exposure of the individuals for another 23 days, the differences in SMR were no longer present, but differences in growth remained (Fig. 1). If we assume that growth rate was directly influenced by

SMR, the results from the final portion of the study seem to suggest that SMR of contaminant-exposed individuals remained elevated for some period of time following day 27, having an overall negative effect on growth rate over the final period of the study. Because measurements in the laboratory experiment were not continuous, but rather were made at distinct intervals (days 0, 27, 50), we were unable to identify how rapidly SMR diverged prior to day 27 between the treatments, or for how long the differences may have lasted prior to converging by day 50.

In light of the results from the field-collected crayfish, the convergence of metabolic rates of the two treatment groups by the end of the laboratory experiment following an initial divergence is enigmatic. The field-collected individuals almost certainly had been exposed to conditions

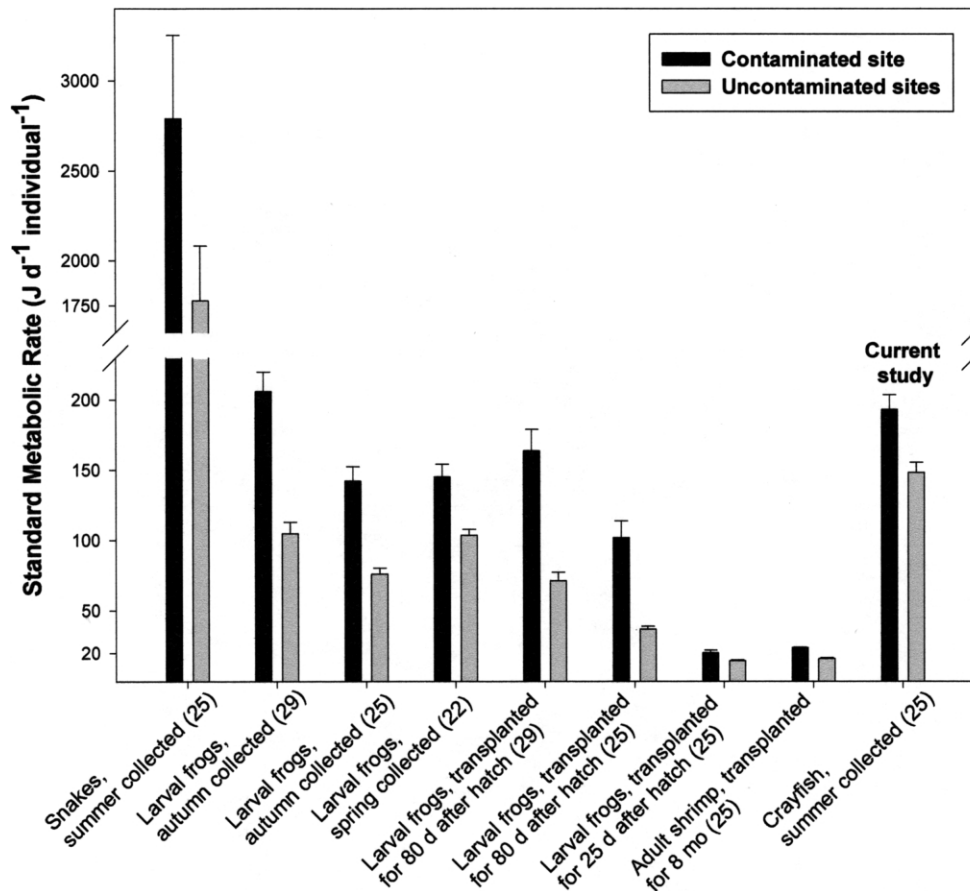


Fig. 2. Estimates of daily standard metabolic energy expenditures per individual for crayfish and three other species collected from or transplanted into the contaminated and reference sites. Numbers in parentheses following study designations are the temperatures at which metabolic expenditures were measured. Data for snakes, larval frogs, and shrimp are taken from Hopkins et al. (1999), Rowe et al. (1998a), and Rowe (1998), respectively.

in their respective field sites for their entire lifetimes, suggesting that high SMR was a sustained response by individuals in the contaminated site. Yet in the laboratory, the longest exposure regime produced no differences in SMR, despite the initial increase in SMR for contaminant-exposed individuals. A longer exposure period in the laboratory, coupled with more frequent measurements of SMR, and possibly a more natural diversity in food items, would be required to determine whether laboratory exposures can be used to adequately model exposure regimes and physiological responses that occur in natural systems receiving contaminants.

In three previous studies, SMR was examined for another crustacean (the grass shrimp, *Palaemonetes paludosus*), an amphibian (larvae of the bullfrog, *Rana catesbeiana*), and a reptile (the banded water snake, *Nerodia fasciata*) inhabiting the contaminated site, or transferred into the contaminated site from uncontaminated areas (Rowe, 1998; Rowe et al., 1998a; Hopkins et al., 1999). All three species had significantly elevated SMR following chronic exposure to conditions in the contaminated site (Fig. 2). Shrimp from a single population transferred into the contaminated site for 8 months experienced a 52% elevation in energy expenditures compared to those held in a reference site (Rowe, 1998). Field-captured and transplanted larval bullfrogs had energetic expenditures 40 to 100% higher than reference animals (Rowe et al., 1998a). Larval bullfrogs experienced the elevations after as little as 25 days of exposure (Rowe et al., 1998a). Finally, water snakes captured from the contaminated site had 32% greater metabolic expenditures than those captured from uncontaminated sites (Hopkins et al., 1999). As in the current study, elevated metabolic activity was associated with elevations in whole-body or hepatic trace element concentrations in exposed animals, suggesting a relationship between coal ash-derived trace elements and metabolic function of the individuals.

The species found to have increased metabolic rates in the contaminated system are taxonomically and ecologically diverse. Crayfish, shrimp, and larval bullfrogs characteristically forage in the polluted surface sediments and other coal ash-covered substrates for algal matter and detritus (all three species), as well as microinvertebrates (shrimp) and carrion (shrimp and crayfish). On the other hand, water snakes directly ingest

little sediment while foraging; instead they feed primarily on large prey items, such as larval and adult amphibians and fish, which accumulate high levels of trace elements in the coal ash-polluted area (Hopkins et al., 1999). Also, snakes are less likely to experience epithelial uptake of trace elements than the other, gill-breathing species studied. Yet, despite differences in trophic levels, feeding regimes and physiology, all species examined in the above studies accumulated trace elements from the polluted habitat and experienced elevated metabolic rates. An exception to the pattern of increased metabolic rates following chronic exposure to coal ash is the mosquitofish (*Gambusia holbrooki*); individuals collected from the ash-contaminated system did not have metabolic rates different from those collected from an uncontaminated site (Staub, 2000). Further work is needed with other species to determine how common the metabolic response is to conditions in the contaminated habitat.

Natural selection should favor energy allocation strategies that optimize fitness of individuals under the range of environmental conditions normally experienced. However, if environmental conditions diverge significantly from the historical regime, the new conditions may impose constraints upon, or require changes to, optimal energy allocation patterns of individuals. Introduction of novel compounds or complex mixtures of pollutants into natural systems may confront individuals with such physiological challenges. All else being equal, elevated metabolic expenditures as a result of contaminant exposure will reduce energy available for growth, energy storage, and/or reproduction. Such changes in energy use by individuals, in the absence of compensatory mechanisms, could subsequently lead to reduced fitness, and perhaps population-level changes.

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