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Variable Tolerance to Copper in Two Species from San Francisco Bay

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ABSTRACT

In static toxicity experiments, tolerance to soluble Cu of the bivalve, Macoma balthica, *and the copepod,* Acartia clausi, *varied substantially among populations sampled within San Francisco Bay. Intraspecific tolerance differed ten-fold or more for both species over relatively small distances, suggesting geographical isolation of populations is not a prerequisite for the development of intraspecific differences in tolerance by aquatic organisms.*

INTRODUCTION

Many of the methods used to detect and study metal stress in nature imply the response of organisms to toxins is species specific and constant. For instance, it is assumed that bioassays establish an LC_{50} characteristic of all populations of a species. Studies that transplant organisms from one environment to another (e.g. Eganhouse & Young, 1978) assume that the response to metal stress in the transplanted population is similar to that of native animals. The use of indicator organisms or community indices to

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show stress also includes implicit assumptions about the homogeneity of stress responses among environments. These assumptions are being challenged by a growing body of literature that shows intraspecific differences in response to environmental stress. Differences in tolerance to trace metal toxicity among populations of a single species have been observed in a number of aquatic organisms, including polychaetes (Bryan & Hummerstone, 1971), isopods (Brown, 1976), phytoplankton (Murphy & Belastock, 1980), and zooplankton (Browne, 1979; Moraitou-Apostolopoulou, 1978; Moraitou-Apostolopoulou *et al.*, 1979).

All studies of trace metal tolerance in aquatic organisms have compared populations from distinctly different geographical locations. Anthropogenic trace metal enrichment, however, is often localized in the area of specific discharges (Popham *et al.*, 1980). For example, metal concentrations in sediments and indicator organisms show that metals in San Francisco Bay are spatially and temporally heterogeneous (Luoma & Cloern, 1982). Several major rivers and a number of small streams carry large loads of trace metals into the estuary from urban and agricultural runoff. More than 200 permits have been granted for point discharges of domestic and industrial wastes into San Francisco Bay, and at least one accidental spill of a hazardous substance occurs in the Bay Area each day (Luoma & Cloern, 1982). An environment such as this, affected by a number of locally restricted discharges, becomes highly patchy, with metal enrichment conditions persisting in many small areas (Luoma & Cloern, 1982).

Localized genetic or ontogenetic adaptations to enrichment would increase the fitness of an organism in a heterogeneously stressed system. Selective mortality along steep gradients of metal concentration could restrict gene flow between populations (Levinton, 1980). Survival in stressed habitats would be limited to tolerant genomes. Alternatively ontogenetic mechanisms of detoxification, latent in most populations, could be induced in stressed habitats (e.g. Pascoe & Beattie, 1979). In either case, populations which do not appear to be geographically isolated could display quite different responses to metal exposure.

In this paper we study the toxicity of Cu to two organisms common in San Francisco Bay, the burrowing bivalve, *Macoma balthica*, and the plankton *Acartia clausi*. We observe distinct differences in response to Cu among some populations of these species, suggesting that Cu tolerance can be very heterogeneous in at least some types of species over small geographical distances within a single estuary.

METHODS AND MATERIALS

Organisms and study sites

Three populations of *M. balthica* were studied: one from North Bay (Station 2) and two from South Bay (Stations 5 and 6, Fig. 1). Station 2 is located south of Pinole Point, Station 5 is at the north-east corner of the San Mateo Bridge, and Station 6 is at Sand Point. North Bay and South Bay populations may be geographically isolated because of poor mixing of the two segments of the Bay during most of the year, but intermixing of the pelagic larvae of *M. balthica* probably occurs between Stations 5 and 6 in South Bay.

A. clausi are entirely pelagic and currents tend to mix populations

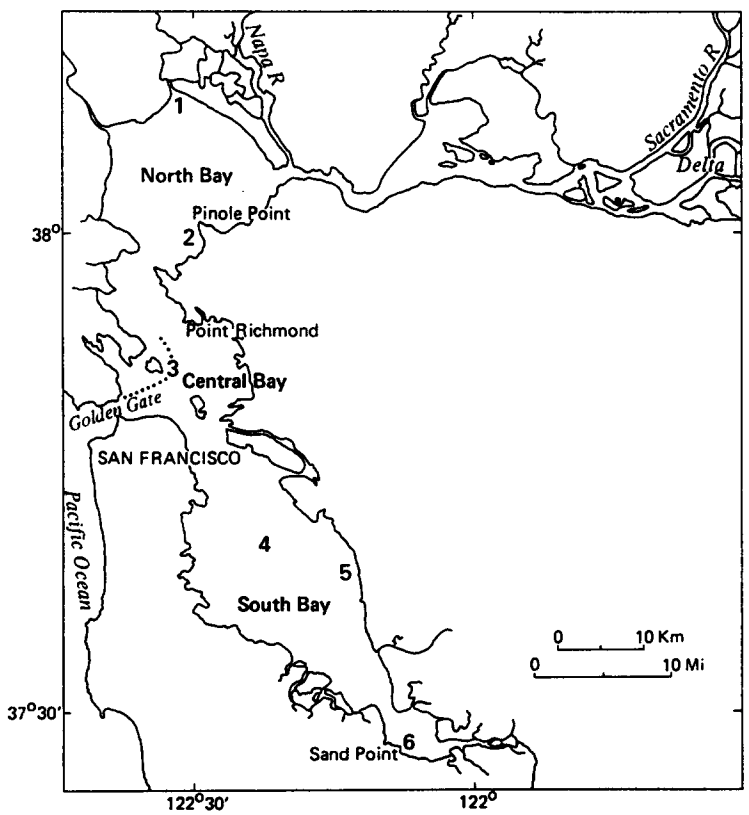

Fig. 1. Collection stations in San Francisco Bay.

within the Bay. Thus, copepod populations are probably not as discrete as clam populations. Localized responses to stress would be difficult to detect in *A. clausi* if currents significantly mix animals from two localities. Such mixing is more likely to occur between Stations 1 and 3 than between Stations 3 and 4.

Toxicity tests

M. balthica were collected from intertidal mudflats at low tide between February and October 1979. Each sample consisted of approximately 100 individuals ranging in size (shell length) from 1·3–3·5 cm. In preliminary studies, differences in susceptibility to Cu were not evident among different-sized organisms within a particular population.

Following collection, the clams were acclimated to experimental conditions of salinity and temperature (25‰ and 12 °C) for 3–4 days in sediment from the collection site, and then depurated for 2 days in filtered seawater prior to testing. The clams were separated into size classes and an equal number of individuals in each size class were employed in the experiment.

All experiments were run for 11 days in seawater obtained from San Francisco's Steinhart Aquarium. The seawater was diluted to 25‰ with distilled water and adjusted to pH 7·9 ± 0·2 as needed with NaOH or HCl. Between 15 and 20 animals were immersed in 2 liters of water to which $CuSO_4$ was added to give nominal Cu concentrations ranging from 10 μg liter^{-1} to 1900 μg liter^{-1}. The condition of the animals was checked once or twice daily. Dead (gaping) animals were removed immediately from the aquaria. Water was moderately aerated and replaced daily.

Experiments with Station 5 animals were conducted in February and October 1980. The experiments with animals from Stations 2 and 6 were conducted in March and July 1980, respectively.

Acartia clausi were collected by filtration onto a 64 μm mesh net from three areas in San Francisco Bay. We collected on 16 October 1980, 17 December 1980 and 10 February 1981 from mean depths of 14 m in Central Bay between Golden Gate Bridge and Point Richmond (Station 3); on 9 February 1981 from 10 m depth in South Bay (Station 4); and on 17 December 1980 and 10 February 1981 from shallow water (1 m) over the shoals of North Bay (Station 1).

The *Acartia* were stored for 24–48 hours on shipboard and acclimated for 24–48 hours in the laboratory. During the acclimation period the

animals were fed a culture of the green algae *Cryptomonas* sp. Experiments were conducted in Steinhart seawater for 20–24 hours in continuous light at 12 °C. Salinity was controlled between 26‰ and 28‰ and pH between 7·73 and 7·78. Only large active animals with food in their digestive tract were selected for the experiment. The animals were placed in experimental tanks with 2 liters of seawater. Nominal Cu concentrations ranged from 10–500 μg liter^{-1}. At the end of the allotted time, the animals were sieved through a 105 μm teflon screen, counted and observed for mortality. Animals were considered dead when there was no visible response to probing.

LC_{50}'s were determined using methods described in Standard Methods (1975), and statistical comparisons among tests were conducted using two-way analysis of variance.

Percent mortality at each concentration was corrected for control mortality using the equation:

$$\text{Net mortality} = \frac{x - y}{x} \times 100$$

where x = percent alive in the control and y = percent alive in the treatments.

TABLE 1
Temperature and Salinity at Stations in San Francisco Bay where *Acartia clausi* and *Macoma balthica* were Collected

Station	*Location*	*Temp., °C*	*Salinity, ‰*
	North Bay		
1	17 Dec. 1980	9·5	18·8
	10 Feb. 1981	11·6	12·9
2	9 Mar. 1979		11·0
	Central Bay		
3	16 Oct. 1980	14·6	31·5
	17 Dec. 1980	11·5	30·8
	10 Feb. 1981	11·9	21·4
	South Bay		
4	9 Feb. 1981	11·8	25·8
5	18 Feb. 1979		27·0
	10 Oct. 1979		30·0
6	15 July 1979		25·0

Mortality in unexposed zooplankton ranged from 12 to 20%, showing no trends among stations or through time. The similarities in control mortalities indicated the different populations tested were all similarly affected by the storage and acclimation procedures. No mortalities occurred in clam controls. Salinity was determined with a refractometer on water collected from the sediment surface and from the mantle cavity of the clams; or from the same station as the zooplankton samples were collected (Table 1).

RESULTS

Temperature and salinity at times of collection

Table 1 shows the temperature and salinity of Bay waters when the zooplankton and clam samples were collected. The lower salinity at Stations 1 and 2 reflects high discharge from the Sacramento–San Joaquin rivers. Neither temperature nor salinity of North Bay or Central Bay exceeded the range which would normally be stressful to *A. clausi* or *M. balthica* (Hutchinson, US Geological Survey, unpublished data). The temperature and salinity established during the experiments was approximately the median of the range of field conditions.

Temporal and spatial differences in tolerance

Distinct differences were observed among the responses of clam populations to Cu. The 10 day LC_{50} of the population at Station 6 was one-and-a-half- to two-fold greater than that at Station 5, and six-fold greater than at Station 2 (Table 2, Fig. 2). Exposure to 1000 μg liter^{-1} Cu, killed nearly all clams from Station 2, but only 25% of those from Station 6. The response of animals from Station 2 was also significantly different to that of the animals from Station 5 in October ($p < 0{\cdot}02$), but was not significantly different in February ($p > 0{\cdot}05$). The replicates at Station 5 were not significantly different from one another ($p > 0{\cdot}10$).

Table 3 shows the percent mortality and LC_{50} values for *Acartia clausi*. Both spatial and temporal differences were observed in the response of the zooplankton to Cu (Table 3, Fig. 3). The response of *A. clausi* from Central Bay in October was significantly different to the responses in February or December ($p < 0{\cdot}01$). The LC_{50} was approximately five-fold higher in December and February than in October.

TABLE 2

Percent Mortality of Three Populations of *Macoma balthica* after Ten Days Exposure to Copper

Nominal concentration of Cu in seawater ($\mu g\ liter^{-1}$)	*Station number*			
	6 (*July*)	*5* (*Feb.*)	*5* (*Oct.*)	*2* (*Mar.*)
10	ND	0	0	0
50	0	0	0	29
100	0	32	5	23
500	0	54	30	77
1 000	25	50	55	92
1 900	86	ND	ND	ND
LC_{50}	1 290	680	880	210

ND = not determined.

TABLE 3

Percent Mortality of *Acartia* after 20–24 h Exposure to Copper
(Values are corrected for mortality in controls. Number of individuals are in parentheses. In data sets with different superscripts mortality is significantly different as determined by ANOVA paired comparison sets ($p < 0{\cdot}001$).)

Station	*Location*	*Nominal copper concentration* ($\mu g\ liter^{-1}$)					LC_{50}
		10	*25*	*50*	*250*	*500*	
1	*North Bay* (*shoals*)						
	17 Dec. 1980	0 (45)	0 (37)	0 (65)	0 (41)	ND	>250
	10 Feb. 1981	ND	ND	ND	9 (31)	60 (20)	450
3	*Central Bay*						
	16 Oct. 1980[a]	0 (29)	9 (34)	52 (49)	100 (37)	ND	48
	17 Dec. 1980[b]	0 (37)	2 (29)	5 (24)	40 (34)	79 (49)	280
	10 Feb. 1981[b]	0 (48)	12 (34)	10 (29)	39 (41)	100 (46)	220
4	*South Bay*						
	9 Feb. 1981[b]	0 (35)	4 (36)	13 (51)	40 (32)	85 (41)	260

ND = not determined.

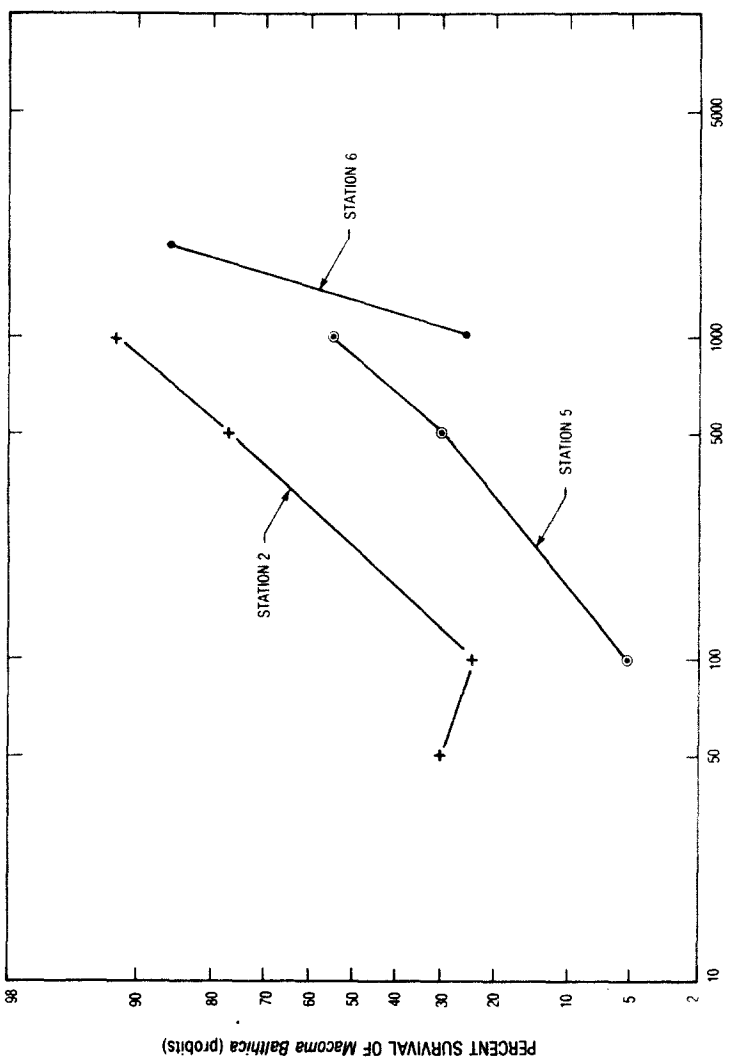

Fig. 2. Response to Cu exposure of *Macoma balthica* from three populations in San Francisco Bay

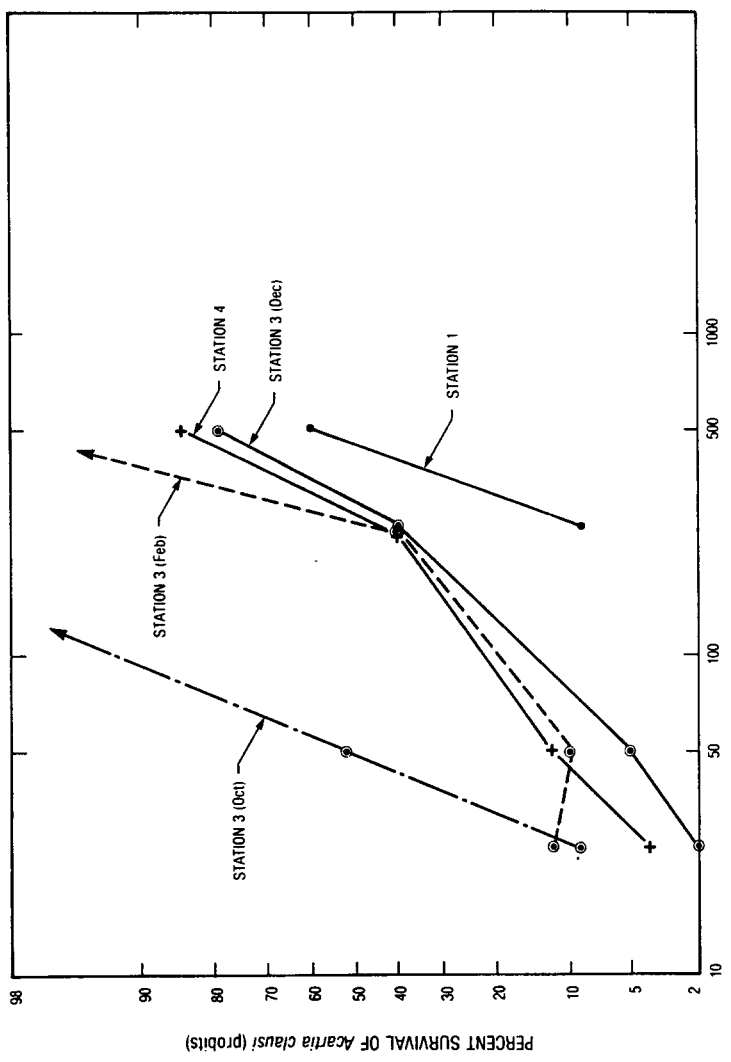

Fig. 3. Response to Cu exposure of three populations of *Arcartia clausi* from San Francisco Bay. Animals were collected from Station 3 in October 1980, December 1980 and February 1981. Animals collected in December 1980 from Station 1 showed no mortality up to exposures of 250 μg litre^{-1} and are not shown here.

A. clausi collected in February from Station 4 responded similarly to Cu exposure to animals collected in December and February from Station 3 ($p > 0{\cdot}05$). However, the responses of animals collected in two samplings from the shoals of North Bay (Station 1) were distinctly different from other stations (Table 3). Little or no mortality was observed below 500 μg liter^{-1} in the shoal population, so statistical comparisons with other stations were not possible. However, the LC_{50}, where it could be determined, was 450 μg liter^{-1}, and animals exposed to 250 μg liter^{-1} Cu were active and vigorous. In contrast, all animals collected from Station 3 in October died upon exposure to 250 μg liter^{-1} Cu, and the LC_{50} was 48 μg liter^{-1}. Approximately one-half of the *A. clausi* collected from Stations 3 and 4 died upon exposure to 250 μg liter^{-1} Cu and those which survived were sluggish and obviously stressed.

Relationship of tolerance to Cu exposure

The elevated Cu tolerance in *M. balthica* at Station 6 coincided with exposures to high concentrations of biologically available Cu, as reflected by Cu concentrations in the tissues of the population. We collected Cu data monthly for seven years from Station 6 and for two years from Stations 2 and 5 (Luoma & Cain, 1979; Strong & Luoma, 1981; Luoma & Cloern, 1982). Concentrations in animal tissues change seasonally at all stations, but are consistently two- to ten-fold higher at Station 6 than at the other stations. Concentrations of Cu in *M. balthica* at Station 6 occasionally reach levels higher than reported anywhere else in the world for this family of bivalves (Luoma & Cain, 1979), and were as much as twenty-fold higher than found in *M. balthica* from the least contaminated parts of the Bay (30–50 μg liter^{-1}). Concentrations at Stations 2 and 5 differ little from one another during most of the year, although a short-term (one- to two-month) increase in Cu has been observed at Station 2 in winter. The differences among animals' tissues also are reflected by differences in Cu concentrations in sediments, but not with as much sensitivity (Table 4; Thomson *et al.*, US Geological Survey, unpublished data).

No direct measure of exposures to biologically available Cu for *A. clausi* in San Francisco Bay are available. Measurements of Cu in solution and suspended particulates indicate that higher Cu concentrations occur in North Bay than in Central or South Bay (Table 4; Girvin *et al.*, 1977, 1978; Gordon, 1980). Trace metal enrichment in the Napa

TABLE 4

Copper Concentrations in Soft Tissues of *Macoma balthica*, Sediments and Water Collected from San Francisco Bay

(*Macoma balthica* and sediments samples were collected over a 7-year period at station 6 and a 2-year period at stations 2 and 5)

Station	*Cu in* Macoma balthica $\mu g\ g^{-1}$		*Total Cu in sediment* $\mu g\ g^{-1}$		*Solute Cu,* $\mu g\ liter^{-1}$ ($\bar{x} \pm S.D.$)	
	Summer minimum	*Winter maximum*	$\bar{X}$	*Range*	*Dissolved*	*Particulate*
2	46	242	51	41–77		
5	38	77	21	16–26		
6	103	505	88	60–150		
1[a]					2·3 ± 0·6	6·7 ± 6·8
3[a]					1·5 ± 0·6	1·8 ± 1·2
4[b]					2·3 ± 0·6	2·2 ± 0·9

[a] Gordon, 1980. Samples collected in North and Central Bay shipping channels, March 1979 and 1980.

[b] Girvin *et al.*, 1977; 1978. Samples collected in South Bay shipping channel, March 1976 and 1977.

River, which enters the Bay near Station 1, also has been documented (Anderlini *et al.*, 1975). However, neither of these observations conclusively proves that the elevated tolerance to Cu observed in *A. clausi* from Station 1 is a response to the exposure to enriched concentrations of biologically available Cu.

DISCUSSION

Different responses to Cu exposures among populations of the same species were observed within San Francisco Bay, despite the lack of geographical isolation of the populations. The observation of statistically significant tolerance differences within *A. clausi* collected on the same date proved that spatial differences in Cu tolerance occurred within this species on at least two dates. Although the same conclusion appears to apply to *M. balthica* our inability to conduct the bioassays simultaneously limits the conclusiveness of the evidence.

The LC_{50}'s for Cu observed in *A. clausi* at Stations 2 and 4 are

within the range of 100–300 μg liter^{-1} observed in other studies with calanoid copepods (Arnott & Ahsanullah, 1979; Moraitou-Apostolopoulou, 1978; Moraitou-Apostolopoulou *et al.*, 1979). However, the LC_{50}'s from Station 1 were at least two-fold higher than observed in such studies. Although, conclusive association of elevated Cu tolerance in *A. clausi* at Station 1 with elevated Cu exposure was not possible, the distinct occurrence of elevated Cu tolerance in *M. balthica* was associated with a well-documented instance of extreme Cu enrichment.

The statistically significant temporal differences in the Cu response of *A. clausi* could reflect changes in the general hardiness of this species with season; or could result from the mixing of zooplankton populations of different tolerances. The onset of elevated Cu tolerance in December at Station 3 coincided with a sharp increase in river flow from 3000–5000 cfs (cubic feet per second) in Oct.–Nov. to 10 000–20 000 cfs in Dec.–Feb. (Bureau of Reclamation, unpublished data). Thus the enhanced tolerance response in winter could have reflected the downstream transport of some individuals from the more tolerant populations upstream near Station 1.

We can only speculate on the mechanism causing the heterogeneity of Cu responses within *A. clausi* and *M. balthica*. For example, Cu stress could be exerting selective pressure on *M. balthica* at Station 6 (Luoma, 1977) and/or inducing a physiological adaptation to stress. Within an estuary, selective larval mortality along sharp environmental gradients created by localized pollutant inputs could limit survival to tolerant genomes (Levinton, 1980). Population success would be dependent upon a sufficient abundance of these genotypes in the pool of pelagic larvae to maintain the population. Levinton (1980) suggested a large, genetically varied gene pool may be a common characteristic of estuarine opportunistic species. Some evidence also suggests Cu tolerance within *Macoma balthica* could be enhanced by a physiological detoxification mechanism. A metallothionein-like Cu binding protein has been isolated in *M. balthica* from Station 6 (Johansson & Luoma, 1981), which could provide a means for individuals to rapidly adapt physiologically to chronic metal stress (Pascoe & Beattie, 1979). Of course, genetic and physiological adaptation mechanisms need not be mutually exclusive.

Whatever the mechanisms, intraspecific heterogeneity in response to environmental contaminants illustrates the equivocacy of relying on tools such as species-specific LC_{50} values for making decisions regarding potential pollutant impacts on biota. A species' survival depends more

upon the range of its adaptive capacity than upon a single value identifying a lethal or sublethal toxicant concentration. Understanding the mechanisms and adaptive strategies behind heterogeneous stress responses within species may be essential to defining the limits to survival under stress.

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