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Influence of Life-History Adaptations on the Fidelity of Laboratory Bioassays for the Impact of Heavy Metals (Co^{2+} and Cr^{6+}) on Tolerance and Population Dynamics of *Tisbe holothuriae*

H. MILIOU*, G. VERRIOPOULOS, D. MAROULIS, D. BOULOUKOS and M. MORAITOU-APOSTOLOPOULOU
Section of Zoology – Marine Biology, University of Athens, Athens 15784, Greece

To investigate the influence of life-history adaptations on the fidelity of laboratory bioassays, copepods *Tisbe holothuriae* were collected from two areas (polluted and non-polluted) and maintained in the laboratory under temperatures similar to their wild habitat. The sensitivity of *Tisbe* increased with increasing temperature. The wild animals from the polluted area were more tolerant to Co^{2+} and Cr^{6+} than those from the non-polluted area. Laboratory animals (40 generations after sampling) from the polluted area, were still more tolerant than those from the non-polluted area. These animals also exhibited a similar or increased tolerance, whereas those of the first generation showed a higher sensitivity, compared to the wild ones from the same area. Demography also varied in successive generations after sampling. Laboratory bioassays using *T. holothuriae* can predict most of its responses in the field, but attention should be paid to adaptations to temperature, pollution and the laboratory environment. © **2000 Elsevier Science Ltd. All rights reserved.**

Chapman (1983) questioned 'Do organisms in laboratory toxicity tests respond like organisms in nature' and answered that the selection of test species can greatly influence the applicability of the data in different situations. He also suggested that when appropriate test parameters are chosen, the responses of laboratory organisms correspond to that of naturally occurring organisms.

The harpacticoid copepod *Tisbe holothuriae* Humes has been used as a test organism in many toxicological studies (e.g. Hoppenheit, 1977; Moraitou-Apostolopoulou *et al.*, 1983; Brand *et al.*, 1986; Verriopoulos and Moraitou-Apostolopoulou, 1989). This fact reflects its tolerance to environmental fluctuations and laboratory conditions, its substantial reproductive capacity and its short life cycle. Furthermore, *T. holothuriae* is widespread, abundant in littoral regions throughout the year, can live under highly different ecological conditions and although it is mostly epibenthic, it also can be found in the neritic plankton (Volkmann-Rocco, 1971). Thus, it seems to be a suitable representative organism for studying the impact of pollutants on marine near-shore invertebrate fauna.

Chapman (1983) identified, by summarizing existing data, the factors that can influence the variable responses of laboratory and wild organisms to toxic chemicals. From the laboratory-to-field comparisons into one species, Chapman (1983) concluded that acclimation/selection is responsible for the greater magnitude of response range. Where essentially continuous exposure occurs in nature, the opportunity exists for the development of a population weakened by debilitation, or toughened by acclimation or selective survival. These factors may influence the laboratory-to-field comparison of acute toxic episodes. By reviewing the genetic adaptation to heavy metals in aquatic organisms, Klerks and Weis (1987) concluded that most populations in polluted areas are subject to selective pressures for an increased resistance to toxicants. This can result in the evolution of resistance, which may have important implications for decisions regarding safe ambient toxicant levels.

Most laboratory toxicity tests employ continuous exposure to essentially constant levels of test chemicals, whereas the exposure of organisms in nature usually is dynamic. This difference in the exposure pattern also may produce apparent inconsistencies between the responses of organisms in the laboratory and those in the field. A related question is whether natural selection, in the absence of toxic stresses, results in a population of organisms, whose response to toxic substances is appreciably biased.

*Corresponding author.

The purpose of the present study is to evaluate the fidelity of toxicity bioassays using the copepod *T. holothuriae* as a test animal. The influence of adaptations, induced during life history, to temperature, pollution and the laboratory environment on tolerance and population dynamics was examined. Furthermore, the present study aims to contribute to the determination of appropriate test parameters for improving the accuracy in predicting responses of wild organisms in the laboratory and the repeatability of laboratory data.

Materials and Methods

Tisbe holothuriae Humes were collected from two areas in the Saronicos Gulf (Gulf of Athens): Skaramagas near the coast of Elefsis Bay (polluted, eutrophic) and Lagonissi (non-polluted, oligotrophic) 25 km southwards of the former. These copepods were found free-living among the alga *Ulva* sp.

In the laboratory about 200 ovigerous females were retained. These 'brood stock' females were kept under conditions (temperature and salinity) similar to those of their wild habitat. Their offspring were used for producing either stock cultures or cohorts of specific generation numbers (G1–G7) under temperature levels identical to those during each sampling period.

Breeding conditions were the most favourable for rearing the Greek strain of *T. holothuriae* collected from Lagonissi area (Miliou and Moraitou-Apostolopoulou, 1991a,b; Miliou, 1992). Artificial seawater (Ultramarine-Waterlife) was used, with a salinity of 38‰. The cultures were kept in constant temperature rooms (±0.5°C), with a photoperiod of LD 12:12. Animals were fed on fronds of non-polluted *Ulva* and a liquid Fryfood (Waterlife) offered in excess.

Stock cultures were placed into bowls containing 250 ml of seawater and were slightly aerated. They were exploited weekly at a 50% rate after an initial period of 18 days according to Gaudy and Guérin (1982). The water was renewed twice per week at a rate of 100%. Every month, new cultures were initiated with 20 ovigerous females selected from different previous cultures.

In order to control the generations of laboratory animals, separate cohorts of specific generation number (G1–G7) were maintained by the following procedures. A restricted number of females (F1) carrying an egg sac were isolated from the 'brood stock'. Their offspring were observed daily until the appearance of fertilized females (F2). When the F2 females were carrying their first egg sac, they were put individually in 50 ml bowls. Ten to twenty females were used for each experiment. After the release of one egg sac the females were transferred to a new bowl until the naulpii of the next egg sac were hatched. The nauplii from the previous egg sac were left in the original container and gave the F3. From the F3 offspring of one generation the F2 females of the next were derived.

Table 1 presents the 'process-flow' of the experimental design. We evaluated the acute toxicity of two heavy metals, cobalt in the form of $CoCl_2.6H_2O$ (experiment 1) and chromium in the form of $Na_2CrO_4.4H_2O$ (experiment 2) to *T. holothuriae*. Median lethal concentrations LC_{50}(48 h) were estimated by the Bliss method (1938). In these static bioassays only females carrying their first or second egg sac were used, which were put individually in 50 ml bowls (10 for each toxicant dose). All treatments were carried out under the same temperature as that of sampling. Wild animals came from 'brood stock' four days after sampling, and laboratory animals either from stock cultures after at least 40 generations or cohorts of a specific generation number. Controls were also kept for all treatments and their mortality was negligible.

In the third experiment, *T. holothuriae* population dynamics were estimated in cohorts from the first (G1) to the seventh (G7) generation after sampling for assessing adaptations to the laboratory environment. In addition, sublethal effects of Cr^{6+}on *T. holothuriae* population dynamics were estimated in cohorts from stock cultures for evaluating adaptations to pollution in the laboratory. Twenty females were used for each generation and put individually in 50 ml bowls. During the experiment specimens (females or offspring) were removed daily to containers with new solution and fed after transfer. The following life cycle parameters for each generation were measured: (1) Times from hatching of F2 females to the appearance and release of their successive broods, (2) Number of nauplii at the time of hatching, (3) Number of egg sacs per female, (4) Total number of offspring per female, (5) Longevity, (6) Survival (%) to adulthood and (7) Sex ratio. Measurement of these biological characteristics enables determination of the following demographic variables: mean generation time (T), net reproductive rate (R_0) and intrinsic rate of natural increase (r_m) (Gaudy and Guérin, 1977).

In the fourth experiment, chronic toxicity of Co^{2+} to *T. holothuriae* females was investigated during continuous or cyclic exposure (every 48 h) to two sublethal concentrations. A 100 females were placed in pairs in 50 ml bowls and fed after the change of the solution every 48 h. From the daily mortality, the median lethal time LT_{50} was calculated as the time (days) needed for death of 50% of the test organisms (UNEP/FAO/IAEA, 1987).

To compare LC_{50} and LT_{50} values between the groups of animals the Wilcoxon's signed-ranks test was used. To compare mean values of biological characteristics among the groups whose population dynamics was estimated, one-way analysis of variance was performed (Newman–Keuls multiple range test). Differences were considered significant at $p < 0.05$.

Results

The LC_{50} values, their 95% confidence limits (UCL, LCL) and the respective linear regression equations of

TABLE 1

'Process-flow' of the experimental design.

Experiment 1: Acute toxicity of Co^{2+} to *T. holothuriae*
nine experimental conditions – 79 counts

Polluted area	*Non-polluted area*
A. Wild animals	
(1) 19°C	(2) 19°C
B. Laboratory animals	
B1. Cohorts of first generation after sampling (G1)	
(3) 19°C	
B2. Stock cultures	
(4) 16°C	(7) 16°C
(5) 19°C	(8) 19°C
(6) 22°C	(9) 22°C

Experiment 2: Acute toxicity of Cr^{6+} to *T. holothuriae* (19°C)
five experimental conditions – 26 counts

A. Wild animals
(1) Polluted area
(2) Non-polluted area
B. Laboratory animals (non-polluted area)
(3) Cohorts of first generation after sampling (G1)
(4) Stock cultures untreated
(5) Stock cultures treated to 1/100 LC_{50}(48 h) of Cr^{6+}
(F2 females of second generation of treatment)

Experiment 3: Population dynamics of *T. holothuriae* (non-polluted area, 19°C)

A. Effects of laboratory cohorts generation number (G1–G7) after sampling
F1 wild females
↓offspring→F2 laboratory females (G1)
↓offspring→F2 females (G2)
↓offspring→F2 females (G3)
↓offspring→F2 females (G4)
↓offspring→F2 females (G5)
↓offspring→F2 females (G6)
↓offspring→F2 females (G7)

B. Sublethal effects of Cr^{6+}
(1) F1 females from stock cultures
↓ offspring treated to 1/100 LC_{50}(48) of Cr^{6+} (first generation)
↓ F2 females → offspring treated to 1/100 LC_{50}(48) of Cr^{6+} (second generation)
(2) F1 females from stock cultures
↓ offspring untreated (first generation)
↓ F2 females → offspring untreated (second generation)

Experiment 4: Chronic toxicity of Co^{2+} to *T. holothuriae*
(stock cultures, non-polluted area, 19°C)
four experimental conditions – 48 counts

(1) Continuous exposure (12 days) to 1/100 LC_{50}(48 h) of Co^{2+}
(2) Continuous exposure (12 days) to 1/50 LC_{50}(48 h) of Co^{2+}
(3) Cyclic exposure (12 days), every 48 h, to 1/100 and 1/50 of LC_{50}(48 h) of Co^{2+}
(4) Controls

doses on (%) mortality of *T. holothuriae* are given in Table 2 for Co^{2+} and in Table 3 for Cr^{6+}. The observed values of (%) mortality for the tested concentrations and the estimated regression lines are given in Fig. 1 for Co^{2+} and in Fig. 2 for Cr^{6+}.

Laboratory animals at 16°C were more tolerant than those at 19°C, giving significantly higher LC_{50} values for Co^{2+}. On the contrary, laboratory animals at 22°C were more sensitive than those cultured at 19°C, giving significantly lower LC_{50} values for Co^{2+}.

The LC_{50} values of the wild animals collected from the polluted area were significantly higher than those of the wild animals collected from the non-polluted area for Co^{2+} and Cr^{6+}. It is noticeable that cultured animals after 40 generations derived from the polluted area still exhibited significantly higher LC_{50} values for Co^{2+} than those from the non-polluted area at all temperatures tested.

Laboratory animals from cohorts of first generation after sampling (G1) gave significantly lower LC_{50} values

TABLE 2

LC_{50} (48 h) values (mg/l) and their 95% confidence limits (LCL, UCL) calculated by the respective linear regression equations of Co^{2+} doses (x) on (%) mortality (Y) of *T. holothuriae* wild and laboratory animals from cohorts of first generation (G1) or stock cultures collected from a non-polluted or polluted area at three different temperatures (R: correlation coefficient).*

Experimental conditions	LC_{50} (48 h)	LCL	UCL	$Y = a + bx$	R
1. Polluted area wild 19°C	3.500^{d}	2.401	4.693	$Y = -13.286 + 18.080x$	0.904
2. Non-polluted area wild 19°C	2.516^{ab}	1.887	3.245	$Y = 9.183 + 16.225x$	0.948
3. Polluted area G1 19°C	2.453^{a}	2.135	2.785	$Y = -9.703 + 24.337x$	0.984
4. Polluted area stock cultures 16°C	6.093^{f}	4.631	8.373	$Y = -23.933 + 12.133x$	0.920
5. Polluted area stock cultures 19°C	3.560^{d}	2.542	5.104	$Y = -33.378 + 23.423x$	0.891
6. Polluted area stock cultures 22°C	3.064^{c}	2.572	3.662	$Y = -15.242 + 21.290x$	0.974
7. Non-polluted area stock cultures 16°C	5.097^{e}	3.726	6.892	$Y = -27.526 + 15.210x$	0.894
8. Non-polluted area stock cultures 19°C	3.050^{bc}	2.575	4.001	$Y = -34.855 + 27.826x$	0.902
9. Non-polluted area stock cultures 22°C	2.351^{a}	1.679	3.082	$Y = -1.034 + 21.709x$	0.955

* LC_{50} values having different letter in superscript are significantly different ($p < 0.05$).

TABLE 3

LC_{50} (48 h) values (mg/l) and their 95% confidence limits calculated by the respective linear regression equations of Cr^{6+} doses (x) on (%) mortality (Y) of *T. holothuriae* wild and laboratory animals from cohorts of first generation (G1) or cultures treated to 1/100 LC_{50} of Cr^{6+} and untreated, derived from a non-polluted or polluted area at 19°C.*

Experimental conditions	LC_{50} (48 h)	LCL	UCL	$Y = a + bx$	R
1. Polluted area wild untreated	4.933^{c}	2.821	7.010	$Y = -7.697 + 11.697x$	0.978
2. Non-polluted area wild untreated	2.837^{b}	1.070	4.224	$Y = 16.251 + 11.894x$	0.991
3. Non-polluted area G1 untreated	1.699^{a}	0.072	2.886	$Y = 7.263 + 25.161x$	0.949
4. Non-polluted area stock cultures untreated	7.327^{d}	3.010	12.490	$Y = -0.120 + 6.840x$	0.942
5. Non-polluted area stock cultures treated	9.600^{e}	7.047	13.497	$Y = -46.000 + 10.000x$	0.945

* LC_{50} values having different letter in superscript are significantly different ($p < 0.05$).

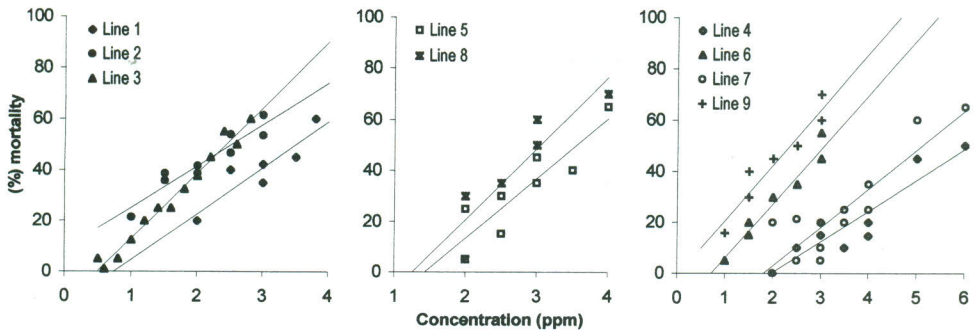

Fig. 1 Observed values of *Tisbe* (%) mortality (48 h) for the tested nominal concentrations of Co^{2+}. The order of the regression lines corresponds to that of the experimental conditions in Table 2.

for Co^{2+} and Cr^{6+} compared to those of the wild animals collected from the same area.

Short longevity and high mortality of their offspring (Table 4) characterized first generation (G1) females. Furthermore, they showed high offspring production per egg sac, but relatively low total offspring production due to a lower number of sacs. Second generation (G2) females had a higher number of egg sacs per female and of total offspring per female, and consequently higher R_0 (43.80) and r_m (0.343) values. In the following generations (G3–G7), the number of egg sacs, offspring per egg sac and total offspring per female gradually decreased, whereas development time (8–9 days) and the time of hatching of nauplii in successive egg sacs increased. Sex ratio decreased from the first to the seventh generation, whereas survival and longevity increased up to the third generation and then slowly decreased. These procedures led to identical T levels and depressed R_0 and r_m values from the second generation onwards. However, from the second to fourth generation, total offspring production and sex ratio values did not differ significantly, and R_0 (>30) and r_m (>0.300) values stabilized at high levels. F2 females from stock cultures of at least 40 generations, showed no statistically different levels of longevity, survival, sex ratio, number of egg sacs, number of offspring per egg sac, number of total offspring per female and R_0 compared to those from cohorts of the sixth generation. Higher T and lower r_m values were mainly due to the longer development period (12 days).

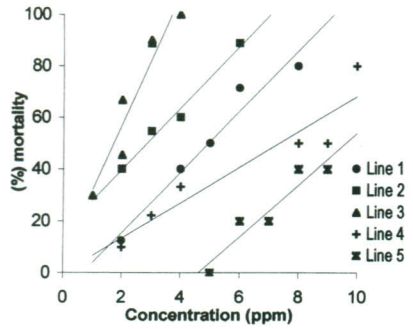

Fig. 2 Observed values of *Tisbe* (%) mortality (48 h) for the tested nominal concentrations of Cr^{6+}. The order of the regression lines corresponds to that of the experimental conditions in Table 3.

F2 females from stock cultures, treated to 1/100 LC_{50} of Cr^{6+} (0.007 mg/l) exhibited a significantly lower survival of offspring compared to those of the untreated animals (Table 4). However, the offspring survival of F2 females was (38.5%) higher than that of F1 females (20.3%). Furthermore, these treated females showed significantly higher LC_{50} values for Cr^{6+} than the untreated ones (Table 3).

The LT_{50} values, their confidence limits (LCL, UCL) and the respective linear regression equations determined for *T. holothuriae* exposed continuously or periodically to two sublethal concentrations of Co^{2+} are given in Table 5. The observed values of (%) mortality for each day and the estimated regression lines are given in Fig. 3. The continuous exposure to 1/50 LC_{50} caused LT_{50} values significantly lower than those to 1/100 LC_{50}. The cyclic exposure to two sublethal concentrations resulted in LT_{50} values not significantly different from those caused by the continuous exposure to 1/100 LC_{50}.

Discussion

It is well established that temperature can influence the tolerance of aquatic animals to toxicant (Cairns *et al.*, 1975). In the present study, a twofold increase in the sensitivity to Co^{2+} of *T. holothuriae* was observed by an increase of temperature from 16°C to 22°C. The sensitivity of the planktonic copepod *Acartia clausi* to Cd^{2+} and Cr^{6+} was also increased by a factor of two, when temperature was raised from 14 to 22°C (Moraitou-Apostolopoulou *et al.*, 1979; Moraitou-Apostolopoulou and Verriopoulos, 1982a). Our results confirm that toxicity bioassays should include the range of environmental conditions over which a toxicant acts.

The tolerance of wild *Tisbe* to Co^{2+} and Cr^{6+} was higher for animals collected from a polluted area than from a non-polluted area. Furthermore, laboratory-cultured *Tisbe* in a sublethal concentration of Cr^{6+} (1/100 LC_{50}) exhibited a higher tolerance than untreated animals. These results indicate increased resistance of *T. holothuriae* due to acclimation or/and selective survival. Bryan (1976) noted that in some species increased

TABLE 4

Mean values (±S.E.) of biological characteristics and demographic variables of *T. holothuriae* from cohorts of first (G1) to seventh (G7) generation after sampling and stock cultures treated to 1/100 CL_{50} of Cr^{6+} for second generation and untreated.*

	No. of offspring of first egg sac	No. of offspring of second egg sac	Total offspring per female F2	Number of egg sacs per female	Longevity of females F2 (days)	Survival of F3 offspring (%)	Sex ratio of F3 offspring (%)	T	R_0	r_m
Cohorts										
G1	58.2 ± 2.63^{a}	32.1 ± 3.05^{ab}	83.4 ± 7.17^{b}	1.9 ± 0.14^{b}	18.3 ± 0.54^{cd}	39.8 ± 0.11^{d}	70.3 ± 0.27^{a}	11.950	23.32	0.264
G2	52.6 ± 2.48^{ab}	38.8 ± 2.87^{a}	144.6 ± 9.70^{a}	5.1 ± 0.21^{a}	30.7 ± 1.44^{ab}	46.0 ± 0.10^{c}	65.8 ± 0.23^{b}	11.145	43.80	0.343
G3	49.0 ± 3.00^{ab}	26.0 ± 2.00^{ab}	105.0 ± 5.00^{ab}	4.5 ± 0.50^{a}	32.5 ± 0.50^{a}	55.3 ± 0.73^{a}	65.5 ± 1.19^{b}	11.060	38.00	0.329
G4	45.4 ± 2.25^{abc}	24.0 ± 2.00^{ab}	95.0 ± 5.00^{ab}	3.0 ± 0.32^{b}	28.3 ± 1.45^{b}	50.4 ± 1.56^{b}	64.0 ± 0.37^{b}	11.054	31.00	0.311
G5	41.5 ± 2.72^{abc}	26.5 ± 3.50^{ab}	78.5 ± 6.50^{b}	2.9 ± 0.19^{b}	26.7 ± 0.90^{ab}	51.0 ± 0.40^{b}	60.0 ± 0.51^{c}	11.170	24.00	0.285
G6	36.0 ± 3.21^{abc}	17.7 ± 1.76^{ab}	61.7 ± 6.12^{b}	3.0 ± 0.00^{b}	24.3 ± 0.85^{bc}	49.8 ± 0.23^{b}	53.3 ± 0.31^{d}	11.072	16.33	0.252
G7	23.0 ± 2.31^{bc}	11.3 ± 0.88^{b}	36.7 ± 3.71^{b}	2.0 ± 0.00^{b}	17.6 ± 0.60^{d}	51.0 ± 1.04^{b}	52.0 ± 0.98^{d}	11.074	9.67	0.205
Stock cultures										
Treated	25.4 ± 3.48^{bc}	13.2 ± 3.76^{b}	42.8 ± 9.22^{b}	2.4 ± 0.22^{b}	24.8 ± 1.11^{bc}	38.5 ± 0.47^{d}	52.0 ± 0.79^{d}	15.630	8.65	0.138
Untreated	38.7 ± 2.96^{abc}	17.3 ± 2.19^{b}	57.7 ± 5.17^{b}	2.5 ± 0.38^{b}	24.6 ± 1.97^{bc}	52.0 ± 0.41^{b}	52.5 ± 0.74^{d}	16.870	15.80	0.163

*Mean values having different letter in superscript are significantly different ($p < 0.05$).

TABLE 5

LT_{50} values (days) and their 95% confidence limits calculated by the respective linear regression equations of time (x) of exposure to Co^{2+}, continuously (1/100 LC_{50} and 1/50 LC_{50}) and periodically (1/100-1/50 LC_{50} every 48 h), on (%) mortality (Y) of *T. holothuriae*.*

Experimental conditions	LT_{50}	LCL	UCL	$Y = a + bx$	R
1/100 LC_{50}	9.545[b]	8.497	10.635	$Y = -12.419 + 6.539x$	0.993
1/50 LC_{50}	7.977[a]	7.212	8.750	$Y = -6.368 + 7.067x$	0.996
1/100-1/50 LC_{50}	9.376[b]	8.144	10.660	$Y = -12.087 + 6.622x$	0.990
Control	11.353[c]	10.220	12.518	$Y = -9.638 + 5.253x$	0.992

* LT_{50} values having different letter in superscript are significantly different ($p < 0.05$).

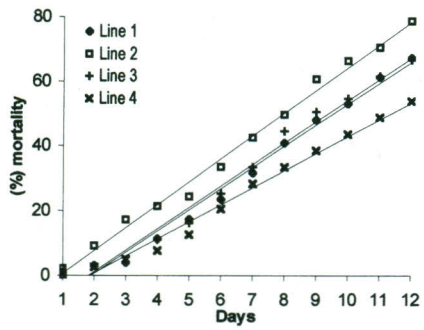

Fig. 3 Observed values of *Tisbe* (%) mortality (48 h) for the tested days of exposure to Co^{2+}. The order of the regression lines corresponds to that of the experimental conditions in Table 5.

tolerance to toxic metals can be acquired by previous exposure to sublethal concentrations under laboratory conditions, as well as by metal-resistant strains of organisms collected from contaminated areas. A pollution-adapted population of *A. clausi* was more resistant to sublethal copper stress (Moraitou-Apostolopoulou and Verriopoulos, 1979). From the literature, it can be concluded that most populations in polluted areas do have an increased resistance (Klerks and Weis, 1987). But usually it cannot be determined if such an increased resistance has a genetic basis. Organisms may have acquired a degree of tolerance by physiological acclimation during exposure to sublethal concentrations at some prior period of their lives. On the other hand, populations may have evolved genetically based resistance, through the action of natural selection. Selection for tolerance of *T. holothuriae* to Co^{2+} was apparent, since laboratory animals cultured for several generations (at least 40) from the polluted area were still more tolerant than those from a non-polluted area.

Apart from the tolerance of copepods, which usually increases after exposure to toxicants, other biological characteristics may also be influenced. The impact of Cr^{6+} on *T. holothuriae* F2 females resulted in a decrease of the longevity, the number of egg sacs, the number and the survival of offspring (9.25% at 0.5 mg/l) and an increase in the percentage of abortions of egg sacs with increasing the dose (0.5–2 mg/l, 14°C) (Verriopoulos and Moraitou-Apostolopoulou, 1981). In the present study, *T. holothuriae* F2 females of the second generation of treatment to 0.07 mg/l (1/100 LC_{50}, 19°C) of Cr^{6+} exhibited the same levels of longevity, development time, number of egg sacs per female and sex ratio as the controls. Also, abortions of egg sacs were not observed and the survival of their offspring was improved in comparison to that of the first generation exposed. However, the offspring survival of treated F2 females still remained lower than in controls, although their tolerance has already been increased. Verriopoulos and Hardouvelis (1988) found that the survival of *T. holothuriae*, especially of nauplii, reached the levels of the controls in the third generation of acclimation to 0.007 ppm Zn (1/100 LC_{50}). Moreover in the present study, the counting of newly hatched nauplii per egg sac of females of the second generation of acclimation revealed a number 26% lower, but not significantly different, than that of the untreated females. Moraitou-Apostolopoulou et al. (1983) found that the development time of *T. holothuriae* matched the control level in the second generation of acclimation to the copper, with sublethal concentrations equal to or higher than 0.004 mg/l (1/100 LC_{50}), while tolerance increased proportionally to these copper concentrations. Furthermore, LC_{50} values were higher for the fourth generation than for the second one and the delay in maturation time was less pronounced. These procedures indicate that selection is actually going on instead of just acclimation in *T. holothuriae* populations.

Concentrations of heavy metals lower or equal to 1/50 of LC_{50} lead *T. holothuriae* populations to disappearance in laboratory experiments, as was indicated by the present study and literature data (Verriopoulos and Moraitou-Apostolopoulou, 1981; Moraitou-Apostolopoulou *et al.*, 1983; Verriopoulos and Hardouvelis, 1988). The proportion 1/100 LC_{50} of heavy metals leads *Tisbe* populations to acclimation/selection phenomena, which however were not detectable at lower concentration (Moraitou-Apostolopoulou *et al.*, 1983). Furthermore, the exposure to toxicants in nature usually fluctuates and their increased resistance is related to the degree of prior exposure, time and concentration. In the present study, *Tisbe* adults during a cyclic exposure to two sublethal concentrations of Co^{2+}, 1/50 and 1/100 LC_{50}, exhibited a mortality rate similar to that achieved during a continuous exposure to 1/100 LC_{50}, indicating an increased tolerance. These results reinforce the view that life-history adaptations of *Tisbe* populations to pollution should be taken into consideration when performing laboratory bioassays.

Laboratory *Tisbe* females from cohorts of first generation after sampling exceeded in sensitivity to Co^{2+}

and Cr^{6+} the wild ones. In addition, these females exhibited a restricted longevity and offspring survival. Doyle and Hunte (1981) mentioned that the amphipod *Gammarus lawrencianus* Bousfield, which was maintained for 26 generations in a laboratory environment, showed increased survival and fertility. These heritable changes in life-history traits were called 'domestication' and interpreted in terms of selection for Darwinian fitness in a controlled environment.

In the present study, domestication of *T. holothuriae* to the laboratory environment began in the second generation by increasing longevity, survival as well as fertility. Demography of *T. holothuriae* varied in successive generations cultured in the laboratory. In the first generation, fecundity was early and intense, but total offspring production was low. In following generations late reproduction and low offspring production per egg sac, but high total offspring production appeared. This combination of iteroparity and low reproductive effort per time unit usually is taken to reflect a risk-spreading mechanism associated with uncertain juvenile survival (Stearns and Crandall, 1981). However, Bergmans (1984) claimed that higher R_0 should be selected for in equilibrium populations in *T. furcata* (35 generations), and concluded that natural selection almost certainly underlies the differences in fecundity between culture and wild animals. Indeed, *T. holothuriae* populations from the second to fourth generations showed high R_0 and r_m values. However, in subsequent generations inbreeding effects, mainly by depressing fecundity and sex ratio, caused a gradual decrement of the demographic variables R_0 and r_m. In the experiment with cohorts of successive generations inbreeding effects appeared earlier than in stock cultures, as it was initiated from a restricted number of ancestors.

The fluctuations of demographic variables related to the life history of laboratory-cultured *Tisbe* populations result in uncertainty regarding predictions of toxicant impacts on natural populations. In relative toxicity bioassays, specimens must be collected from cohorts with the same life history in order to compare the results of population dynamics. Laboratory *Tisbe* from stock cultures were proved capable to predict with accuracy the tolerance to Co^{2+} of the wild animals living in both polluted and non-polluted areas. However, they were proved more tolerant to Cr^{6+} than the wild animals. Thus, adaptations of test organisms to the laboratory environment may affect the fidelity of laboratory bioassays for predicting the impact of toxic metals on the organisms in nature.

The LC_{50} values of laboratory-cultured *T. holthuriae* estimated for different toxicants, by different researchers and for two strains showed a great repeatability taking into account differentiation due to temperature fluctuations (Verriopoulos, 1980; Moraitou-Apostolopoulou and Verriopoulos, 1982b; Verriopoulos and Moraitou-Apostolopoulou, 1982; Moraitou-Apostolopoulou *et al.*, 1983; Verriopoulos and Dimas, 1988; Verriopoulos and Moraitou-Apostolopoulou, 1989). This repeatability may be attributed to the stability of longevity and survival to adulthood of *T. holothuriae* collected from different non-polluted areas and reared for several generations, under identical conditions of temperature and salinity, as revealed by the present and literature data (Gaudy *et al.*, 1982; Miliou and Moraitou-Apostolopoulou, 1991a).

Conclusively, *T. holothuriae* can be used as a test organism in toxicity bioassays, but for a better fidelity of the results attention must be paid to life-history adaptations to temperature, pollution and the laboratory environment. Furthermore, it is necessary to standardize the parameters for conducting these toxicity bioassays in order for a better repeatability of the results of inter-laboratory tests to be achieved.

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