Effect of Atrazine on Growth, Photosynthesis, and Between-Strain Variability in *Scenedesmus subspicatus* (Chlorophyceae)

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Abstract. The magnitude of between-strain differences in the sensitivity of algal species to toxicants is not well known. Yet, informations obtained with a single strain are used for ecotoxicological assessment and for interspecific comparisons. Using 12 strains, we determined whether intraspecific variability occurs in the green alga *Scenedesmus subspicatus*. We examined growth, photosynthetic activity, and short-term sensitivity thereof to the herbicide atrazine ($500 \mu\text{g} \cdot \text{L}^{-1}$) as well as how these responses are affected by chronic exposure to low atrazine concentrations (0, 1, 5, and $20 \mu\text{g} \cdot \text{L}^{-1}$). Independent of chronic exposure to atrazine, the strains differed in growth rate and photosynthesis rate. Yet the short-term sensitivity to atrazine was affected by the chronic treatments. All but one strain became more tolerant, their short-term sensitivity being inversely related to the applied atrazine concentration and dependent on the duration of exposure. Differences in response to increments in atrazine concentration resulted in differences in rank order of sensitivity of strains. Moreover, between-strain variability was markedly higher in the atrazine treatments than in the controls. These results can be explained by the significant role of genotype versus environment interactions in determining intraspecific differences in adaptive physiological responses of *S. subspicatus* to chronic exposure to atrazine.

The prediction of the effects of chemicals on phytoplankton relies mainly on relatively simple ecotoxicological tests with a small number of algal species. Tests on single strains are often used to measure the sensitivity of a species and to derive water quality standards aimed at protecting the natural populations of (at least) that species.

Yet, genetic diversity in important ecological traits has been shown to occur in natural populations of phytoplankton species (as reviewed in Wood and Leatham 1992). In fact, between-strain and interclonal variability has been detected for most of the physiological and biochemical characters that have been investigated. Because sensitivity to pollutants is influenced by the molecular make-up of the target cells, some degree of intraspecific variability may be expected to occur in the

tolerance to toxicants. Indeed, differences in sensitivity to toxicants were found in some species between strains differing in geographic origin (Menzel *et al.* 1970; Fisher *et al.* 1973; Murphy and Belastock 1980). Broad intraspecific differences in tolerance to various metals were found between various pristine sites and between pristine and polluted sites (Foster 1982). This points to the role of the exposure history of individuals as another factor in causing intraspecific differences in tolerance to pollutants.

Thus, between-strain differences in the response to exposure to chemicals can be partitioned into genetic variability (g), environmental variability (e), and the interaction of these two ($g \times e$) (Hoffmann and Parsons 1991). The genetic component shows in the different response of two or more strains in one environment and the environmental component in the different response of one strain to different environments. The $g \times e$ interaction shows in the differential effect of an increasing exposure concentrations on different strains. Moreover, some strains may be better adapted to the conditions prevailing before exposure, whereas other strains may, through the acquisition of tolerance, fare better under the new conditions. Therefore, the ranking of sensitivity among strains will not necessarily be the same before and after exposure.

Few studies have examined the variability in sensitivity to toxicants among strains, clones, or individuals of aquatic species and the effect of exposure history on sensitivity changes (Baird *et al.* 1991; Münzinger and Monicelli 1991; Soares *et al.* 1992; Forbes *et al.* 1995). Even less is known on intraspecific sensitivity differences in algae (Foster 1982; Hersh and Crumpton 1989), though such knowledge bears on the relevance of ecotoxicological testing on single strains as representative of a species. Taking this variability into account may contribute to a more realistic prediction of safe concentrations for natural populations than using data on single strains (Forbes and Forbes 1994).

The purpose of this study was to examine whether intraspecific variability occurs among 12 strains of the green alga *Scenedesmus subspicatus*. The traits that we examined were growth, photosynthetic activity, and short-term tolerance to an inhibitor of photosynthesis, the herbicide atrazine. Moreover, we investigated whether growth and photosynthetic responses are affected differently by a chronic exposure to a low atrazine level. Differences between strains were analyzed in terms of the sources of variability, that is, g, e, and $g \times e$.

Materials and Methods

Strains and Culture Conditions

We used 12 clones of *S. subspicatus* Chod. 1926 (Chlorophyceae) that differed in their geographic origin (Table 1) and morphology. The strains were kindly supplied by E. Hegewald and U. G. Schlösser (culture collections of Jülich and Göttingen, Germany). They were held separately as axenic batch cultures in a medium at pH 7.2, containing 75 mg · L⁻¹ MgSO₄ · 7H₂O, 500 mg · L⁻¹ NaNO₃, 330 mg · L⁻¹ NH₄NO₃, 39.6 mg · L⁻¹ K₂HPO₄, 35 mg · L⁻¹ CaCl₂ · 2H₂O, 43.6 mg · L⁻¹ Na₂CO₃, 6 mg · L⁻¹ citric acid, 2.9 mg · L⁻¹ H₃BO₃, 1.81 mg · L⁻¹ MnCl₂, 0.11 mg · L⁻¹ ZnCl₂, 0.08 mg · L⁻¹ CuSO₄ · 4H₂O, 0.018 mg · L⁻¹ (NH₄)₆Mo₇O₂₄ · 4H₂O, 2.5 mg · L⁻¹ FeCl₃ · 6H₂O, 1 mg · L⁻¹ Na₂ EDTA · 2H₂O. The clonal cultures were maintained at 21°C under a continuous illumination of 30 µE · m⁻² · s⁻¹ provided by cool-white fluorescent lamps. The strains were acclimated to these culture conditions, as described by Brand and Guillard (1981). We sequentially established new batch cultures by transferring an inoculum from an early exponentially growing batch culture before the culture populations depleted the nutrients in the medium. Acclimation was considered to be complete when the exponential growth rate of successive cultures were the same. For the experiments described hereafter, an aliquot (0.05–1 ml) from each acclimated test culture in early exponential growth phase was added to 80 ml of medium in a 200-ml Erlenmeyer flask to yield an initial cell density of 10⁵ cells · ml⁻¹.

Experimental Procedures

Twelve strains were cultured for 60 days and examined for their growth rates, photosynthetic activity, and short-term sensitivity thereof to an exposure of 30 min to 500 µg · L⁻¹ atrazine (6-chloro-N-ethyl-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine). To investigate the effect of chronic exposure to atrazine, each strain also was cultured in medium with 5 µg · L⁻¹ atrazine for the same time period. Additionally, five strains (1, 2, 5, 6, and 12) were exposed to 1 µg · L⁻¹ and 20 µg · L⁻¹ atrazine.

Population growth was measured daily as the temporal change in optical density (OD) at 680 nm (Uvikon 930, Kontron Instruments). The linear function of OD₆₈₀ versus cell density was derived initially by direct microscope counts of algal cells at 100× magnification in a Neubauer cell. To minimize self-shading, which was noticeable at OD₆₈₀ > 0.6, samples were diluted when exceeding that value. Growth rates during the exponential growth phase were calculated by linear regression of ln(OD₆₈₀) against time (Stein 1973).

Photosynthetic activity was expressed as the net oxygen evolution. To measure oxygen evolution and its short-term sensitivity to atrazine, aliquots of the algal culture were washed three times with culture medium and then resuspended in medium to obtain a cell density with an OD₆₈₀ between 0.15 and 0.40. The algae were incubated for 20 min in the dark in the absence or presence of 500 µg · L⁻¹ of atrazine (see below). Preliminary experiments showed that at this atrazine concentration an incubation in the dark of about 10 min was sufficient to induce maximal inhibition of oxygen evolution. After that time, 2 ml of the algal suspension were placed in a glass incubation chamber equipped with a Clark oxygen electrode and a magnetic stirrer (Rank Brothers, Cambridge, UK; Delieu and Walker 1972). The temperature was maintained at 21°C. To measure the rate of oxygen consumption by respiration, the algae were kept in the dark for another 10 min. Then, the algae were provided with an illumination of 120 µE · m⁻² · s⁻¹ from a 35-W halogen lamp, and net oxygen evolution was recorded during 10 min. Rates of oxygen consumption or evolution were calculated from the linear part of the curves. For comparisons between strains, the net oxygen evolution was related to the OD₆₈₀ of the algal suspensions

Table 1. Designation and geographic origin of the strains of *Scenedesmus subspicatus* examined

Strain	Origin
1 Hegewald 1971-67	Hungary
2 Hegewald 1973-374	Peru
3 Hegewald 1975-272	Germany
4 Hegewald 1980-24	Germany
5 Hegewald 1981-23	Indonesia
6 Hegewald 1981-42	Singapore
7 Hegewald 1988-30	New Zealand
8 Hegewald 1988-54	New Zealand
9 Payer 1971-124	Thailand
10 Bai 1971-41	Hungary
11 Felföldy 5640	Hungary
12 SAG 86.81	Germany

and expressed as µM O₂ · min⁻¹ · OD⁻¹. The inhibition of oxygen evolution by atrazine was calculated by dividing the rates before addition of atrazine by the corresponding rates after addition, and expressed as the percent difference between the control and the treated sample. The variability between measurements required that each measurement be done twice, as indicated by a one-tailed *t* test on untreated samples (Gad and Weil 1989).

Technical grade atrazine (purity > 99%) was obtained from Riedel-de Haen. Aqueous solutions were used. Atrazine concentrations were determined by HPLC chromatography with a Vidac C18 column (25 cm × 4.6 mm) and methanol as a solvent. Samples were prepared for analysis as described by Davoli *et al.* (1987). The atrazine was detected spectrophotometrically at 221 nm. In that way we determined the atrazine concentration of the stock solution (28.9 mg · L⁻¹) and in the chronically exposed cultures of strain 6 after 38 days of culturing. At that time the effective concentrations in cultures exposed to nominal concentrations 1, 5, and 20 µg · L⁻¹ were 0.80, 3.42, and 14.14 µg · L⁻¹, respectively.

Data Analysis

Data analyses were done with Excel (Microsoft Corp.), StatView (Feldman *et al.* 1987), and SAS (SAS Institute, Cary, NC). The sources of variability between strains in their physiological traits and in their response to atrazine were tested as follows. A multivariate ANOVA was performed on oxygen evolution and short-term sensitivity to atrazine with a test of the effects of strain (*i.e.*, “genotype”), of atrazine concentration in the chronic exposure (*i.e.*, “environment”), and of the interaction (*i.e.*, “genotype × environment”). The assumption of normality was tested by two-tailed tests on skewness and kurtosis; the assumption of homogeneity of variances was tested by F tests (Sokal and Rohlf 1981). Log-transformed data met those assumptions, except for comparisons of growth rates between strains. The latter comparisons were made on untransformed data by the nonparametric Kruskal-Wallis one-way analysis of variance, with a test of the effect of chronic exposure concentration and of strain.

Results

Variability in Growth Rate, Photosynthetic Activity, and Short-Term Response to Atrazine

The 12 strains differed in their growth, as indicated by the time needed for the control cultures to reach the stationary growth

phase: Strains 3, 4, and 5 did so by day 25; strain 7 by day 30; and strains 8, 9, 12, and 13 by day 60; whereas strains 1, 2, 6, and 10 were still in exponential growth at day 60 (not shown). The highest and lowest growth rates differed by a factor of ~ 13 , with values ranging from 0.028 to 0.360 OD $\cdot$ day $^{-1}$. However, except for strains 3 ($\mu = 0.36$) and 4 ($\mu = 0.136$), growth rates were similar (Table 2). Attempts to relate differences in growth rates to the morphological characteristics of the strains were negative.

The 12 strains also differed in their photosynthetic activity, which varied from 2.32 to 12.76 $\mu\text{M O}_2 \cdot \text{min}^{-1} \cdot \text{OD}^{-1}$ during early exponential growth (Table 3). The photosynthetic activity of each strain, as shown for five strains in Figure 1, changed during growth, with maximal activities generally found during the early exponential phase, followed by a decline during the transition to the stationary phase. This was due only in part to a higher respiration which, depending on the strain, contributed 1.0–33.3% during early exponential growth and 3.5–34.2% later in growth to the observed decrease in net photosynthesis (not shown). Rather, it was mainly due to a decrease in the gross photosynthesis of each strain.

Finally, the 12 strains also differed in the short-term response of photosynthetic activity to 500 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine. Inhibition ranged from 36.4% to 105.6% during early exponential growth (Table 3). For most strains, sensitivity to atrazine declined during growth, as shown for five strains in Figure 2, and was not correlated to the growth rate or the photosynthetic activity of each strain.

Effect of Chronic Exposure to Atrazine on Growth Rate, Photosynthetic Activity, and Short-Term Response to Atrazine

Exposure of strains to 1, 5, or 20 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine did not significantly affect growth rate (Table 2). Yet, as measured for strain 6, the effective atrazine concentrations were still high at day 38 (0.80, 3.42, and 14.14 $\mu\text{g} \cdot \text{L}^{-1}$, respectively). A test for the effect of chronic exposure concentration indicated that the inhibition observed in the atrazine treatments was similar and not different from the control ($H = 1.08$, $p > 0.78$, $df = 3$, $n = 34$). Rather, strain was a significant cause for the differences in growth rates ($H = 30.3$, $p < 0.005$, $df = 11$, $n = 34$).

Exposure of strains to 1, 5, or 20 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine did not significantly affect photosynthetic activity (Table 3, Figure 1). As for the control cultures, oxygen evolution of the strains decreased with time in culture in all treatments (Figure 1). The strains exposed only to 5 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine behaved in a similar manner (not shown). Analysis of the photosynthetic data obtained with the control and with the treated cultures indicates that differences in oxygen evolution depend on the strains, but not on the exposure to atrazine (Table 4).

In contrast to the photosynthetic rate, the short-term sensitivity of the photosynthetic activity to 500 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine was affected by the chronic exposure to atrazine (Table 3, Figure 2). Generally, during the first days of exposure, sensitivity increased relative to the controls. Within 15 to 20 days, however, the tolerance (except for strain 12) increased at a rate proportional to the exposure concentration. Analysis of the data confirmed that the sensitivity of oxygen evolution to short-term atrazine exposure depends on the atrazine concentration in the

Table 2. Growth rates of various strains of *Scenedesmus subspicatus* treated with 0, 1, 5, and 20 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine

Strain	Growth rate (OD $\cdot$ day $^{-1}$)			
	0 ($\mu\text{g} \cdot \text{L}^{-1}$)	1 ($\mu\text{g} \cdot \text{L}^{-1}$)	5 ($\mu\text{g} \cdot \text{L}^{-1}$)	20 ($\mu\text{g} \cdot \text{L}^{-1}$)
1	0.051	0.060	0.051	0.047
2	0.045	0.044	0.040	0.044
3	0.360	—	0.259	—
4	0.136	—	0.310	—
5	0.035	0.025	0.023	0.021
6	0.046	0.040	0.040	0.038
7	0.028	—	0.025	—
8	0.038	—	0.028	—
9	0.036	—	0.033	—
10	0.044	—	0.052	—
11	0.035	—	0.034	—
12	0.033	0.029	0.030	0.033

chronic exposure and on the strain (Table 4). The interaction between strain and atrazine concentration was also significant, which indicates that the strains were affected differently by the chronic exposure (Table 4).

Response Variability Among Strains

The growth rate, photosynthetic activity, and short-term sensitivity data obtained for all 12 strains in the control and in the 5 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine treatments were analyzed in terms of the range of response. The variability between strains in the control treatments was highest for the growth rate data, whereas the short-term sensitivity data varied less. Growth and photosynthesis in the chronic treatment ranged within values similar to those of the controls (Tables 2 and 3). However, the response range of the short-term sensitivity in the 5 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine treatments was wider than in the controls and the absolute values were lower (Table 3), indicating an increase in tolerance. This can also be seen in the breadth of the envelopes in the set of inhibition curves, which is widest at the lowest atrazine concentration and decreases with increasing exposure concentrations, a consequence of differences between strains in the rates of tolerance increase (Figure 2). Accordingly, the ranking of the short-term sensitivity also was different in the two treatments (Table 3).

Discussion

Physiological Characteristics and Short-Term Sensitivity to Atrazine

The 12 strains of *S. subspicatus* examined in this study differed in their geographic origin and morphology. They were assumed to be different genotypes. Under the given experimental conditions, differences occurred in the growth rates and in net oxygen evolution rates of acclimated cultures. Moreover, differences in the duration of the exponential growth phase were found even between strains that had similar exponential growth rates. This is probably due to differences in the acclimation rate of individual cells to the changing chemical conditions in the

Table 3. Oxygen evolution and inhibition thereof by 500 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine during exponential growth of strains treated with 0 (control) and 5 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine (values are means $\pm$ SE, $n = 2-3$)

Strain	Control		Atrazine	
	Oxygen Evolution ($\mu\text{M O}_2 \cdot \text{min}^{-1} \cdot \text{OD}^{-1}$)	Inhibition of Oxygen Evolution (% of control)	Oxygen Evolution ($\mu\text{M O}_2 \cdot \text{min}^{-1} \cdot \text{OD}^{-1}$)	Inhibition of Oxygen Evolution (% of control)
1	4.32 $\pm$ 1.10	48.0	5.51 $\pm$ 0.90	30.4
2	2.32 $\pm$ 0.01	105.6	4.93 $\pm$ 0.08	24.6
3	4.36 $\pm$ 0.68	55.6	5.68 $\pm$ 0.62	35.1
4	6.61 $\pm$ 1.33	38.5	6.40 $\pm$ 1.05	29.8
5	6.27 $\pm$ 0.01	95.2	5.40 $\pm$ 0.25	72.1
6	11.04 $\pm$ 1.41	72.0	7.85 $\pm$ 0.16	51.7
7	12.76 $\pm$ 1.39	65.3	4.85 $\pm$ 1.19	52.0
8	6.21 $\pm$ 1.60	74.5	4.15 $\pm$ 0.58	4.5
9	5.43 $\pm$ 0.23	36.4	5.20 $\pm$ 0.45	28.6
10	9.36 $\pm$ 1.41	51.4	8.86 $\pm$ 1.28	34.2
11	10.74 $\pm$ 3.53	41.2	6.83 $\pm$ 1.42	21.0
12	8.90 $\pm$ 0.74	56.6	7.33 $\pm$ 0.17	72.9

medium. Differences between strains were large in terms of growth rates, but smaller for photosynthetic activity. Comparative data are scarce, but are generally indicative of intraspecific variability for various biochemical and physiological traits, such as nutrient requirement for growth, nutrient metabolism, temperature-dependence of growth, or photosynthesis (Wood and Leatham 1992).

The short-term response of photosynthetic activity is a parameter commonly used to test the toxicity of herbicides to algae. The concentration of atrazine used in our experiments (500 $\mu\text{g} \cdot \text{L}^{-1}$) was chosen so that all strains would be affected to some extent after short-term exposure. At this concentration, the short-term response of photosynthetic activity varied between strains by a factor of only three. Concentrations of atrazine similar to that applied in this study have been reported as being effective in the short-term inhibition of photosynthesis in various algal species (Hollister and Walsh 1973; Stratton 1984; Gonzalez *et al.* 1985; Larsen *et al.* 1986; Hersh and Crumpton 1989; Solomon *et al.* 1996) and phytoplankton assemblages (Gustavson and Wangberg 1995). It is remarkable that although this type of result has been obtained with different methods and conditions, atrazine concentrations that affect short-term photosynthetic activity in various species vary within a small range (Solomon *et al.* 1996). This may be related to the specificity of the primary effects of atrazine on photosynthesis, which are due to the intrinsic chemical properties of atrazine and to its binding to an evolutionary conserved site in photosystem II (Moreland 1980; Fedtke and Trebst 1987).

That between-strain differences in traits were still found after acclimation to the culture medium suggests that these differences have a genetic basis. This was confirmed by the significant influence of the factor "strain" on growth rate, net oxygen evolution, and inhibition thereof by atrazine.

Effects of Chronic Exposure to Atrazine

The concentrations of atrazine used in the chronic exposure (1, 5, and 20 $\mu\text{g} \cdot \text{L}^{-1}$) are commonly found in many agricultural watersheds (Huber 1993; Detenbeck *et al.* 1996; Solomon *et al.* 1996). These concentrations did not significantly affect the

Fig. 1. Temporal change of oxygen evolution in the presence of different concentrations of atrazine. Strains: □ 1, ♦ 2, ○ 5, ◇ 6, ■ 12

Fig. 2. Temporal change of inhibition of oxygen evolution in the presence of different concentrations of atrazine. Strains: □ 1, ◆ 2, ○ 5, ◇ 6, ■ 12

Table 4. Test by ANOVA of the effects of strain (genotype), atrazine concentration (environment), and genotype-environment interaction on oxygen evolution and inhibition thereof by 500 $\mu\text{g} \cdot \text{L}^{-1}$ atrazine ($n = 123$)

Factor	df	O ₂ Evolution		Inhibition of O ₂ Evolution	
		F	α	F	α
Strain	11	4.64	0.014	5.34	0.009
Concentration	3	2.14	0.17	7.00	0.01
Strain $\times$ concentration	19	1.16	0.42	5.48	0.0064

growth rate or the net photosynthetic activity of the various strains. In algae there is, however, a high interspecific variability in sensitivity to atrazine (Huber 1993): Minimal concentrations of atrazine reported to affect photosynthesis and growth

rate of phytoplankton range from as low as $1 \mu\text{g} \cdot \text{L}^{-1}$ for communities (De Noyelles *et al.* 1982; Lillelund *et al.* 1987; Lampert *et al.* 1989) to $1,000 \mu\text{g} \cdot \text{L}^{-1}$ for single species (Hollister and Walsh 1973; Butler 1977; Larsen *et al.* 1986; Hersh and Crumpton 1987). Most of the studies reporting high effective atrazine concentrations were done on pure algal cultures under short exposure times. Other studies conducted on the chronic impact of low atrazine concentrations have reported successional effects (De Noyelles *et al.* 1982; Gustavson and Wangberg 1995). On the basis of toxicity data, including laboratory bioassays and microcosm, mesocosm, and field studies, atrazine concentrations up to $20 \mu\text{g} \cdot \text{L}^{-1}$ are not considered to represent a problem for aquatic ecosystems (Huber 1993; Solomon *et al.* 1996).

In this study, chronic exposure to low atrazine levels did strongly affect the short-term photosynthetic response to $500 \mu\text{g} \cdot \text{L}^{-1}$ atrazine. All strains except one, when treated with 1, 5, or $20 \mu\text{g} \cdot \text{L}^{-1}$ atrazine, were less sensitive than control cultures. An increase in tolerance was obvious after a few days in culture, and it increased during the first 15 days. The rate of tolerance increase was proportional to the atrazine concentration in the chronic exposure. Such a tolerance increase, as well as its temporal dynamics, may result from a homeostatic mechanism triggered by the exposure to atrazine. Low atrazine concentrations have been shown to induce a reorganization of the photosynthetic apparatus of algae similar to the adaptive reorganization induced by low light intensities (Hatfield *et al.* 1989; König 1990). Responses such as the synthesis of thylakoid components are considered to be a general adaptation response to situations in which electron transport rate is limited for photosynthesis (Melis *et al.* 1985).

The ecotoxicological interpretation of the increase in tolerance of short-term photosynthetic response is not obvious. This increase may reflect a cellular perturbation or just a reaction that allows to sustain growth and photosynthesis under altered cellular conditions. The answer remains elusive even though we know that the photosynthetic activity of the various strains treated with atrazine was similar to that of the control strains. In fact, it is conceivable that a combination of stress factors that limit electron transport would demand an energy allocation for mechanistic compensation too high to maintain photosynthesis or growth.

The rank order of short-term photosynthetic sensitivity of strains was different for the various chronic treatments. There was a significant genotype $\times$ environment interaction, which reflected a difference in response to increments in atrazine concentration in the chronic exposure. Along with the change in rank order of strain response under increasing atrazine exposure levels a change occurred in the variability of response between strains. The range of response covered by the strains cultured at $5 \mu\text{g} \cdot \text{L}^{-1}$ was markedly higher (4.5–72.9%) than that in the strains cultured in the absence of atrazine (36.4–105.6%). This agrees with observations that moderate chemical stress can lead to an increase in the variability of biological traits at various levels of organization (Forbes and Depledge 1996).

Few studies have tested the influence of genotype and of genotype $\times$ environment interactions on the response of aquatic organisms to toxic stress (Baird *et al.* 1991; Münzinger and Monicelli 1991; Soares *et al.* 1992; Forbes *et al.* 1995). Yet, these studies point to the significance of intraspecific phenotypic variability and its bearing on the design of ecotoxicologi-

cal studies. When the goal is to compare results and protocols between laboratories, the designation of the strain or clone and the description of the culture medium used are essential. On the other hand, when the goal is to assess the impact of a chemical on a species, intraspecific variability should be regarded as a key parameter affecting the potential for evolution of tolerance to environmental stress.

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