

Synergism and antagonism among multiple stressors

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Abstract

This study was designed to test for synergism (increased stress) or antagonism (decreased stress) among multiple environmental stressors using additive, multiplicative, and simple comparative effects models. Model predictions were compared to empirical results of laboratory experiments measuring interactions among thermal stress, toxin exposure, and low food on reproduction and survival of two species of cladoceran zooplankton. Stress was defined operationally as a reduction in reproduction or survival relative to optimal conditions over a 7-d period. These experiments are particularly applicable to episodic stresses such as those associated with short-term heat waves.

Toxin or low food in combination with 30°C temperatures were generally more harmful than high temperature alone. However, most multiple stress effects were antagonistic, in that effects in combination were not as severe as predicted based on the sum or the product of their individual effects. In rare cases, interaction among stressors even diminished effects of the worst single stressor. Optimal conditions for reproduction and survival occurred at 25°C, high food and 0 mg liter⁻¹ toxin (a surfactant, sodium dodecyl sulfate). Suppressive effects of stressors examined individually ranked: high temperature (30°C) > SDS (10 mg liter⁻¹ ≥ low food (~100 μg C liter⁻¹) > low temperature (20°C). *Daphnia pulex* isolated from a pond which experiences high summer temperatures throughout was more tolerant of 30°C conditions than *Daphnia pulicaria* isolated from a lake with a cold-water refuge. Differences were observed in individuals exposed as either adults or as 24-h neonates.

In recent years, the list of chemical, physical, and biological stressors considered to be potentially dangerous to the environment has grown rapidly. Scientific and conservation organizations have urged scientists, managers, and policy makers to consider the ecological effects of stressors for proper regulation and management of natural resources (e.g., Naiman et al. 1995; Arnell et al. 1996). Evaluating systems that are impacted anthropogenically is particularly challenging, because there are many possible stressors eliciting a variety of potential effects. Importantly, effects of environmental stressors are usually tested individually (Vouk et al. 1987), but in nature, organisms often are exposed to several stressors simultaneously (Schindler et al. 1996; Yan et al. 1996). This raises the fundamental question addressed by our study: Are stressors more harmful in combination than alone?

To answer this question we compared three models, com-

parative, additive, and multiplicative, frequently used to identify interactions (i.e., synergism and antagonism) among multiple stressors, and performed an experimental study to evaluate predictions of these models regarding interactive effects of three potentially important stressors (i.e., elevated temperature, a surfactant toxicant, and low food concentrations) on reproduction and survival of two species of zooplankton under representative field conditions. We define a stressor as any environmental factor that reduces population growth via a reduction in survivorship or reproduction relative to optimum conditions (modified from Calow 1989). We chose zooplankton as our focal organisms, in part because plankton respond more rapidly to stress than many other lake taxa (Schindler 1987). Moreover, population-level variables seem more sensitive to effects of stress than ecosystem-level variables (Howarth 1991; Schindler 1987; Ni-yogi et al. 1999). Consequently, population responses of zooplankton may be particularly useful for detecting low-level or episodic environmental stress.

Most experimental investigations of multiple stressors on lake organisms have examined interactions among two natural stressors (e.g., predation and nutrients: Carpenter et al. 1987; Vanni 1987; temperature and natural toxin: Gilbert 1996b) or multiple anthropogenic stressors (e.g., different pesticides: Lichtenstein et al. 1973; Hoagland et al. 1993; acid deposition, climate warming, and ozone depletion: Schindler et al. 1996; Yan et al. 1996; metals: Parker 1979; Borgmann 1980). Few studies have probed how a combination of natural and anthropogenic stressors together influence aquatic organisms, populations, or communities (*but see* Hanazato and Dodson 1995). Results from these few experiments show that effects of stressors are worse in combination than alone (Cooney et al. 1983; Hanazato and Dodson 1995). However, there are several ways of defining “worse than” which affect interpretation of the effects of multiple stressors. We describe three models for comparing

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Example: Yield under stress A alone = 55% of optimal
Yield under stress B alone = 45% of optimal

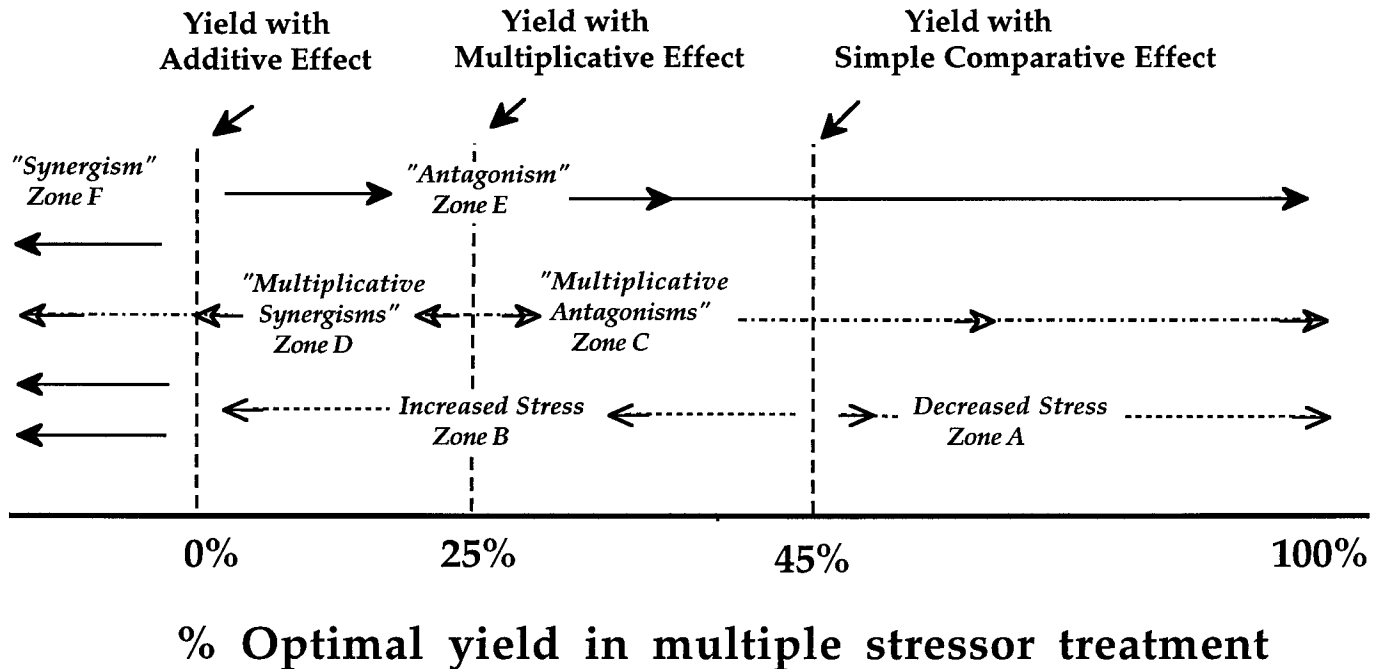


Fig. 1. Graphical model comparing effects of multiple stressors according to three different models (i.e., simple comparative effects, additive, and multiplicative) used to define synergism and antagonism. Dashed vertical lines depict the yield or effect (as percent optimal yield) predicted by each model in a combined stressor treatment (containing both hypothetical stressors A and B, *see text*). Zones A and B are defined by the simple comparative model. Zones C and D are defined by the multiplicative model. Zones E and F are defined by the additive model. The area between the 'yield with additive effect' and 0% optimal yield normally defines Zone F. Note that with this example zone F lies to the left of 0% optimal yield and therefore synergistic effects cannot be measured by the additive model. The area where zones B and C overlap is where all but two of our combined stressor treatments fell. Models and terms explained in text.

multiple stress effects and present the methods and results of our experimental evaluation of these models with our zooplankton system.

Predicting and comparing effects of multiple stressors

A consensus is lacking in the literature regarding the operational definitions of synergism and antagonism among multiple stressors. For example, synergism has been used to describe several types of interactions among multiple stressors with some of these interactions being more severe than others. Distinguishing classes of potential interactions among stressors is important, because it focuses attention on mechanisms likely to produce specific types of effects, and it separates effects based on severity. To this end, we provide a graphical model (Fig. 1) and a classification scheme for defining and comparing effects of multiple stressors. We illustrate several zones of response that characterize the three models and present an example for each. Specific conditions describing synergism and antagonism differ among the models.

Simple comparative effects—The comparative effects model predicts that the effect of stressors in combination is

equal to the effect of the single worst or dominant stressor (Bruland et al. 1991). This is the simplest of the three models and describes situations where a single stressor takes precedence over the other(s) in determining their combined effect. Liebig's law of the minimum describes a somewhat analogous situation for effects of limiting nutrients on growth of organisms. By Liebig's law, only the nutrient that is most limiting in any situation affects growth. Similarly, under the comparative effects model, when the worst stressor is present, lesser stressors have no additional impact.

By the comparative effects model, stressors in combination are synergistic or antagonistic when their combined effects are greater or less than, respectively, the effect of the single worst stressor. To avoid confusion with other models, we suggest that these responses should not be called synergism or antagonism, but rather *increased* or *decreased stress effects* (depicted as zones A and B, Fig. 1). In our examples and discussion, interactive stress effects are measured as the percent yield of a parameter (e.g., biomass, reproduction) in treatments with stressors relative to optimal conditions (no stressors).

Example: Consider an experiment with two natural stressors (e.g., low light and low nitrogen levels) that affect plant

growth. Assuming all other conditions are not limiting, the plants grow to 55% of their optimal yield under the light level treatment (i.e., there is a 45% reduction in yield due to low light) or to 45% of their optimal yield under the nitrogen level treatment (i.e., there is a 55% reduction due to low nitrogen). In this case, low nitrogen is the more severe individual stressor. When both stressors are applied in combination, the simple comparative effects model predicts that plant yield should equal the lowest yield observed for either of the two single stressor treatments (i.e., they should produce 45% of optimal yield). If the plant yield in the combined stressor treatment is <45% of optimal, an *increased stress* effect is said to occur (zone B, Fig. 1). If plant yield is >45% of optimal, there is a *reduced stress* effect (zone A, Fig. 1), and the interaction of the two stressors has diminished the effect of the single worst stressor.

Additive effects—In this model, synergism or antagonism occur when the combined effect of multiple stressors is greater (synergism) or less than (antagonism) the *sum* of effects elicited by individual stressors (Hay et al. 1994; Hay 1996). This is the most common scientific usage of these terms. The additive model has been used frequently in studies of pesticide or metal mixtures (Lichtenstein et al. 1973; Macek 1975; Parker 1979; Hoagland et al. 1993) where effects are thought to accumulate additively. It has also been used to assess competitive interactions (Billick and Case 1994; Hay et al. 1994). The additive model may be particularly appropriate when stressors affect different physiological processes, so that when an organism is exposed to stressors in combination, both processes are affected the same as when they are exposed to either stressor alone. An important limitation of the additive model is that synergism cannot be detected when the sum of the individual effects exceed 100% (Pennings 1996; zone F, Fig. 1). Because high individual effects are common, it is often difficult to detect synergism with the additive model.

Example: Continuing with the plant yield example developed above, the additive effects model predicts that the percent reduction in yield in the combined stressor treatment relative to optimal conditions would equal the sum of the reductions observed for each of the two stressors separately (i.e., there would be a 45% reduction in yield caused by low light plus a 55% reduction caused by low nitrogen). The total percent reduction in yield relative to optimal would equal 100%. There would be no growth in the combined stressor treatment, and it would be impossible to detect synergistic effects.

The additive model provides a conservative way to estimate synergistic effects and is useful when individual effects of stressors are not large. Synergism occurs when combined stress effects are greater than additive (i.e., when values lie in zone F, Fig. 1). Results from combined stressor treatments that fall anywhere to the right of the additive effects value (i.e., throughout zone E, Fig. 1) are antagonistic. This broad categorization of antagonistic effects makes it difficult to distinguish among important additional types of interactions (i.e., those described by zones C and D, Fig. 1). These are more easily separated using the multiplicative effects model.

Multiplicative effects—Multiplicative models have been used to describe a number of competitive interactions (Soluk 1993; Pennings 1996). Mechanistically, a multiplicative model is applicable when stress from one source can be further operated on probabilistically by another source. For ex-

ample, Groves and Williams (1975) found that with competition among plant roots, growth of skeleton weed was only 65% of maximum yield, and with competition among leafy shoots, growth was only 47% of maximum yield. When root and shoot competition both occurred, plant growth was 31% of maximum yield. As predicted by a multiplicative effects model (Begon et al. 1996), the combined competitive effect was approximately equal to the product of the individual effects (i.e., producing a yield of $65\% \times 47\% = 31\%$ of maximum yield).

ANOVAs for interaction generally test the additive model, and a significant interaction term indicates a deviation from an additive expectation (Billick and Case 1994). Such deviations arise from processes such as nonlinear or multiplicative interactions among stressors. If multiplicative interactions are expected a priori, an ANOVA test can be used to determine whether interaction terms are greater than single effects alone (Wootton 1994; Hay 1996).

To avoid confusion regarding the use of the terms synergism and antagonism, we emphasize the need to define these terms with explicit reference to the model (additive or multiplicative) being tested. To be consistent with current usage and prevailing statistical analysis, we suggest using synergism and antagonism to refer to effects detected by means of additive ANOVA models. When a multiplicative model is tested, the zones to the right and left of the multiplicative effect (i.e., values that are greater or less than the *product* of the individual effects) can be termed *multiplicative antagonisms* (zone C, Fig. 1) and *multiplicative synergisms* respectively (zone D, Fig. 1).

Example: Under the multiplicative model, the yield in the multiple stressor trial from the preceding example is predicted to equal the product of the individual effects. Low light reduces yield to 55% of optimal (a 45% reduction) and low nitrogen reduces yield to 45% of optimal (a 55% reduction). Combined stress effects are calculated by multiplying the yields (as percent optimum) expected for each stressor alone. The result is the yield expected in the combined stressor trial (e.g., $0.55 \times 0.45 \approx 0.25$). Here, yield in the multiple stressor trial is predicted to be ~25% of optimal yield.

If yield in the combined stress treatment is lower than the multiplicative prediction (i.e., $\leq 25\%$, Fig. 1), the multiple stressor effect is stronger than the product of individual effects; we call it a “multiplicative synergism.” Similarly resulting yields $\geq 25\%$ are “multiplicative antagonisms.” Yields that fall in the region of Fig. 1 between 25 and 45% are greater than predicted by the multiplicative model yet are lower than yields under the worst individual stressor. If a multiplicative antagonism is more severe than the worst individual stressor, the effects are increased due to the combination of stressors.

An empirical test of model predictions

We conducted a series of short-term life table experiments to compare the separate and interactive effects of environmental stressors on demographic parameters of two *Daphnia* species isolated from lakes with different thermal regimes. We chose three common types of environmental stress that are likely to act in combination in aquatic systems. These are thermal stress, low food levels, and elevated toxicant

levels. We also hoped to elucidate whether any stressor was of overriding importance under typical field conditions.

Daphnia is appropriate for these experiments, because large-bodied species are exceptionally sensitive to a wide range of abiotic and biotic stressors (Moore and Folt 1993; Havens and Hanazato 1993; Stemberger and Lazorchak 1994; Stemberger et al. 1996). Also, knowledge of their response to multiple stressors is important, because they perform a variety of ecological functions in lakes. *Daphnia* can increase water clarity (Edmondson and Litt 1982; Lampert et al. 1986), provide the major diet component for a number of fish species (Post et al. 1992), and increase the efficiency of energy transfer from bacteria and phytoplankton to fish (Havens 1994). Furthermore, regulatory agencies in North America and Europe use *Daphnia* or closely related species in laboratory bioassays to establish water quality criteria for anthropogenic chemicals (Smith et al. 1988; Oris et al. 1991; Soares et al. 1992). However, daphnids kept in culture for long periods or isolated from different environmental conditions may differ in their responses to stressors such as temperature or low food (Carvalho 1987). This may make it difficult to predict responses in natural communities to multiple stressors. Therefore, we extended our study to examine two key factors that may modify cladoceran responses to environmental stress (i.e., age at exposure and exposure history). By exposure history, we mean the source conditions from which the test organisms were isolated.

Specifically, we tested three hypotheses: 1. These stressors in combination will act synergistically. 2. Stressors can be ranked from least to most stressful based on their relative effects on cladoceran reproduction and survival in single and multiple stressor trials. The most severe stressors alter both parameters, and those that are least severe affect reproduction only. 3. Cladoceran responses to these stressors depend upon age at exposure and exposure history.

Methods

Common conditions—Laboratory cultures of *Daphnia pulex* and *Daphnia pulicaria* were established from 30 individuals per species obtained in late July 1992 from water bodies of contrasting thermal regimes located at the Kellogg Biological Station, (Michigan). *D. pulex* was collected from an unstratified, fishless pond (reservoir pond) where temperatures periodically range from 25 to 30°C during summer. *D. pulicaria* was collected from stratified Lawrence Lake where hypolimnetic temperatures remain <15°C. Importantly, *D. pulicaria* resides in the hypolimnion of Lawrence Lake during the day and migrates into the epilimnion at night. Cultures of both *Daphnia* species were probably not clonal, because multiple clones exist in a given season (Carvalho 1987), and cultures were established with more than one individual. Cultures of both species were maintained at 20°C in a 14:10 L/D cycle and fed 300 $\mu\text{g C liter}^{-1}$ of exponentially growing *Cryptomonas erosa* in filtered (1.6 μm) water from Storrs Pond, New Hampshire, to which EDTA (5.9 $\times 10^{-6}$ M Na_2) was added.

We exposed *Daphnia* to each stressor separately and in combinations of two and three stressors. Experiments were

conducted in multiple blocks using a 3-way factorial design including three temperatures (20°, 25°, and 30°C), two food levels [102 and 1,200 $\mu\text{g C liter}^{-1}$, low food (LF) and high food (HF), respectively], and two concentrations of SDS (sodium dodecyl sulfate; 0 and 10 mg liter^{-1}). There were ~5–10 replicates per treatment run in each block with a single individual contained in each replicate. Among the temperature treatments, 30°C is probably above the thermal tolerance limit of many cladocerans (Moore and Folt 1993; Moore et al. 1996). Temperatures of 25° and 30°C, however, are common in summertime in both unstratified and stratified lakes of the northeastern U.S. (Moore et al. 1996; Chen and Folt 1996).

Algal food levels were selected to limit (102 $\mu\text{g C liter}^{-1}$) or enhance (1,200 $\mu\text{g C liter}^{-1}$) reproduction of *Daphnia* based on the population threshold food concentration for *D. pulicaria* (Lampert and Schober 1980). SDS, a surfactant in detergents, was used in these experiments, because it is a relatively benign stressor that is safe for humans to work with in the laboratory. Due to regulation, SDS infrequently occurs at high concentrations in surface waters of the U.S., but it does occur at higher levels elsewhere (Lewis 1991; Anderson et al. 1990). The SDS concentration we used (10 mg liter^{-1}) represents the upper range of effect concentrations measured for invertebrates (0.04–>10.0 mg liter^{-1} , Lewis 1991). SDS concentrations were measured with an Orion surfactant electrode and the Orion 960 autochemistry system. Unless stated, all experiments were conducted for 7 d to mimic effects of episodic exposure to these stressors. Brief, episodic exposure to toxins and low food levels is common in lakes where environmental conditions are dynamic. Furthermore, in the field, elevated water temperatures can persist for 5–7 d during summer heat waves (Chen and Folt 1996).

Pre-experimental procedure—Our pre-experimental procedure controlled for maternal effects and ensured that all individuals used in the experiments were about the same age. Before each experiment, gravid females from laboratory cultures were isolated. Their neonates were harvested and separated into 500-ml beakers and fed ~300 $\mu\text{g C liter}^{-1}$ of *C. erosa* daily. When these neonates became gravid (in 7–10 d), they were randomly assigned to 500-ml beakers with each beaker containing eight gravid females. These adult females, called brood mothers, produced offspring used to initiate all experiments. When the neonates from the second clutch of these brood mothers became reproductively active, they were used to initiate the adult experiments. Similarly, neonates (<24 h old) from the second or third clutch of the brood mothers were used to initiate all juvenile experiments. Individuals from the first clutches were not used because they are known to be small in number and variable in their fitness (Tessier and Consolatti 1989).

Adult experiments—We placed newly gravid *Daphnia* individually in 145 ml of 20°C filtered pond water containing the appropriate SDS and food levels. Water temperature was raised from 20°C during a 10-h period (~1°C h⁻¹) to the appropriate experimental temperature using a water bath. This temperature acclimation process did not alter the de-

mographic parameters of these animals (Chen unpubl. data). Filtered pond water and algal cultures were exposed to each of the experimental temperatures daily, and on the next day, individual animals were transferred into this temperature-adjusted water containing the appropriate levels of algal food and SDS. Neonates and aborted eggs were removed, and survival and reproduction were measured daily. Adult experiments were terminated after 7 d.

Juvenile experiments—Neonates (<24 h old) were placed individually in 40-ml glass vials containing 30 ml of pond water and appropriate SDS and food levels. Once juveniles attained reproductive age (6–12 d), they were transferred to 145-ml jars and reproduction and survival were recorded as described for the adult experiments. Individuals were transferred daily into new containers with temperature-adjusted water and appropriate algal food and SDS concentrations. Juvenile experiments were terminated when all individuals had either died or had lived for 7 d after producing their first clutch. Time to reproductive maturity and daily reproduction of the surviving mature individuals were monitored for a 7-d period (as in the adult experiments).

Survival was analyzed using SAS Lifetest (SAS, statistics version 5). Cumulative reproduction was calculated as the total number of neonates produced per female during the experiment (Chen and Folt 1993). This estimate of reproduction reflects both reproduction and survival, because it measures reproduction over the entire survival period per individual. Temperature, food, and SDS treatments were compared using a GLM three-way ANOVA performed on log-transformed data (SAS, statistics version 5). Block effects were not considered in the ANOVAs because constant conditions were maintained in the cultures throughout the experiments. Any variance due to block would only decrease the chances of significant treatment effects; therefore, our results should be conservative.

Evaluating effects of multiple stressors—To quantify single and multiple effects of the stressors, we calculated observed and predicted values for relative cumulative reproduction in the adult experiments. Cumulative reproduction in all treatments was compared against cumulative reproduction under optimal conditions to determine a relative cumulative reproduction value (RCR) per treatment. Optimal conditions, against which stressor effects were calculated, were defined as the conditions which produced maximum reproduction and survival per species and life stage. For both species, optimal conditions were 25°C, 0 mg liter⁻¹ SDS, and high food (see results).

Observed RCR (RCR_{obs}) values (i.e., individual stressor effects) were calculated by comparing cumulative reproduction under optimal conditions to that observed in each separate stressor treatment (also = percent optimal reproduction). The food effect is the percent reduction of cumulative reproduction in the treatment at 25°C, 0 SDS, LF relative to optimal conditions. The SDS effect is the percent reduction of cumulative reproduction in the treatment at 25°C, 10 SDS, HF relative to optimal conditions. The high temperature effect is the percent reduction of cumulative reproduction in the treatment at 30°C, 0 SDS, HF relative to optimal con-

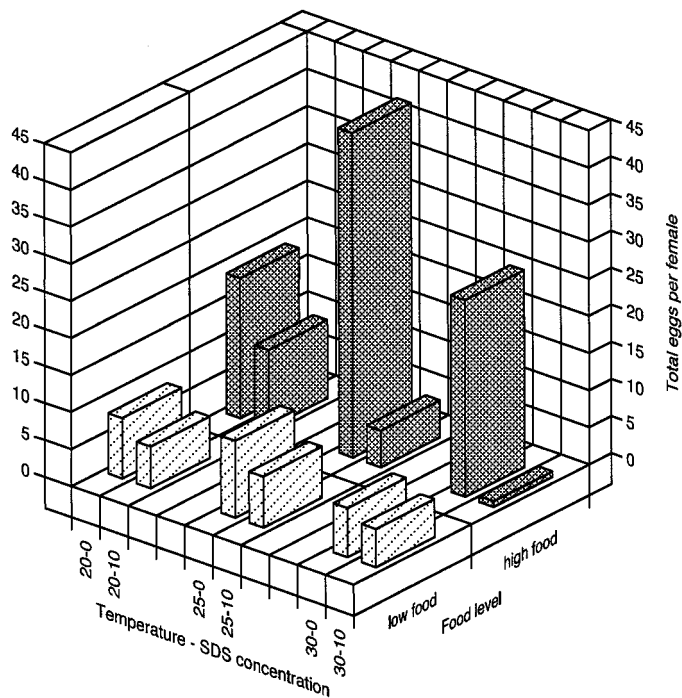


Fig. 2. Mean cumulative egg production of *Daphnia pulex* from adult experiments at three temperatures (20°, 25°, and 30°C), two SDS concentrations (0 and 10 mg liter⁻¹), and high and low food levels (102 and 1,200 μ g C liter⁻¹). Experiment conducted in four blocks (each 7-d duration) in February 1993.

ditions. The low temperature effect is the percent reduction of cumulative reproduction in the treatment at 20°C, 0 SDS, HF relative to optimal conditions.

Predicted RCR (RCR_{pr}) values for multiple stressor treatments were derived differently for each model. For the simple comparative model, RCR_{pr} is the lowest (most stressful) RCR_{obs} among the individual stressors in each multiple stressor treatment. For the additive model, RCR_{pr} is calculated as the sum of the effects of all individual stressors in each multiple stressor treatment. Effects of stressors in this case are reductions in RCR_{obs} relative to optimal for each individual stressor (i.e., 1 - RCR_{obs}). For the multiplicative model, RCR_{pr} is the product of RCR_{obs} for each individual stressor.

Results

Identifying optimal conditions—Our analysis is based on comparisons of results from partial life table experiments under treatment and optimal conditions. We defined optimal conditions as the treatment conditions eliciting greatest reproduction and survival. This was appropriate, because our treatment levels per stressor were chosen to include both optimal and stressful conditions.

For individuals exposed as adults (i.e., adult experiments), cumulative reproduction was greatest at 25°C, 0 mg SDS liter⁻¹, and high food (HF) for both *Daphnia* species (Figs. 2, 3). Survival was also greatest in treatments at 25°C, 0 SDS, and HF, but it did not differ significantly from that at

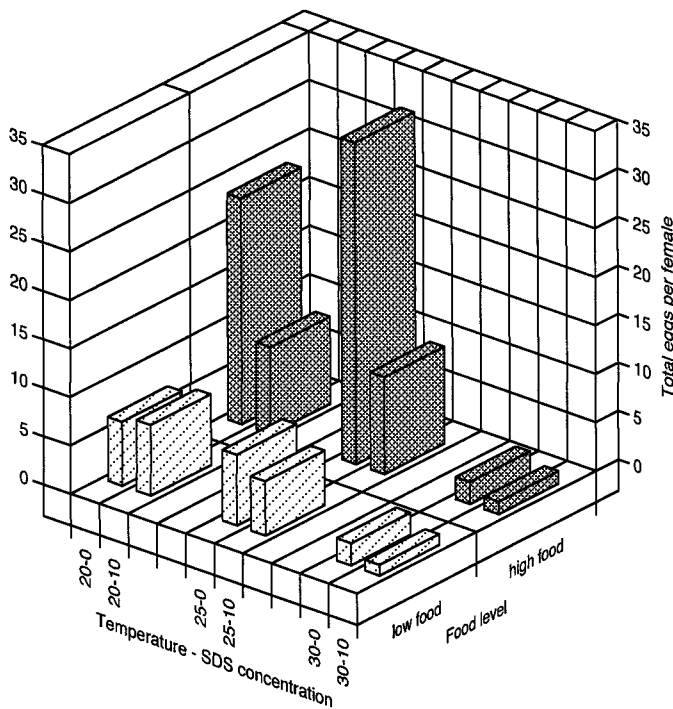


Fig. 3. As Fig. 2, but for *Daphnia pulex* in May 1993.

20° (0 SDS, HF; Figs. 4A, 5A, Table 1). Survival and cumulative reproduction at 30°C (0 SDS, HF) was significantly lower than that at 25°C for both species (Figs. 4A, 5A, Table 1). For *D. pulex* in the juvenile experiments, cumulative reproduction was greatest at 25°C, 0 SDS, and HF (Figs. 6, 7). In contrast, cumulative reproduction of *D. pulex* was similar at 20° and 25°C (0 SDS, HF). Age at first reproduction (AFR) was slightly lower at 25°C than at 20°C for both species (Table 2), whereas survival to maturity was similar at 20° and 25°C, but lower at 30°C (Table 2).

From these results, we concluded that optimal conditions for survival and reproduction were 25°C, 0 SDS, and HF. It should be noted that our definition of optimal is limited to the conditions we tested. It is possible that either species or life stage may achieve greater reproduction and survival under environmental conditions we did not examine.

Effects of individual stressors—We evaluated separate effects of stressors based on reductions of both cumulative reproduction and survival per treatment relative to optimal conditions (e.g., 25°C, 0 SDS, HF). The most severe stressors (30°C and SDS) reduced both reproductive potential and survival, whereas the least severe stressors (20°C and LF) reduced reproductive potential but not survival. Cases where survival was reduced from optimal without affecting reproduction were rarely observed.

There was a significant effect of temperature on cumulative reproduction for both species and age classes (ANOVA, Table 3). This effect was primarily due to lower reproduction in the 30°C treatments (Figs. 2, 3, 6, and 7) which was accompanied by a significant reduction in survival (Figs. 4A, 5A, Table 1). Cumulative reproduction at 20° was also lower relative to that observed at 25°C in adult and juvenile ex-

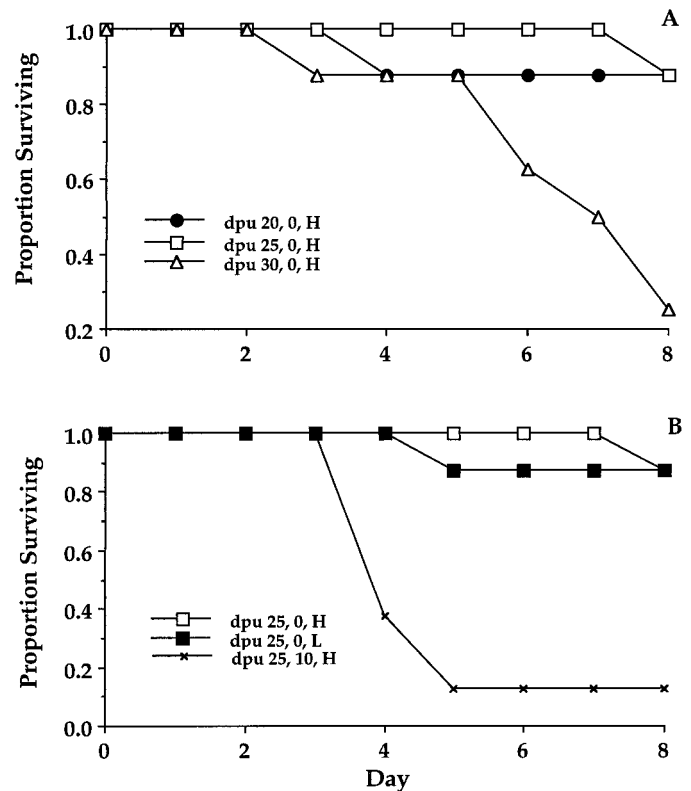


Fig. 4. Survival of *Daphnia pulex* (dpu) from adult experiments in three experimental temperature, SDS, and food treatments. A. Comparison of temperature effects at 0 mg liter⁻¹ SDS and high food. B. Comparison of SDS and food effects at 25°C. Experiment conducted in four blocks (each 7-d duration) in February 1993. In figure key, first number is the experimental temperature (°C), second number is concentration of SDS mg liter⁻¹, and H or L is high or low food. Statistical comparisons are given in Table 1.

periments for both species, but it was not accompanied by a reduction in survival.

Under most conditions, there was a significant effect of SDS on cumulative reproduction (Table 3; Figs. 2, 3, 6, and 7). SDS caused a reduction in reproduction under most conditions for both species and age classes. The only exception to this pattern was the relatively higher cumulative reproduction of *D. pulex* in the juvenile experiments at 25°C and low food (LF) in the presence of 10 SDS vs. that at 0 SDS. Survival was also reduced in a few instances.

In general, low food concentrations had a smaller effect on reproduction and survival of both species and life stages than did either of the other two stressors. Although cumulative reproduction was often significantly lower in the LF than HF treatments (Table 3; Figs. 2, 3, 6, and 7), the food effect was only marginally significant in the *D. pulex* juvenile experiments and not significant for the *D. pulex* adult experiments. Moreover, survival in both adult and juvenile experiments was not reduced in the LF treatments for either species. Age-to-maturity, however, increased slightly at low food levels for both species (Table 2).

Overall, survival was a less sensitive measure of stress than cumulative reproduction for both species and age clas-

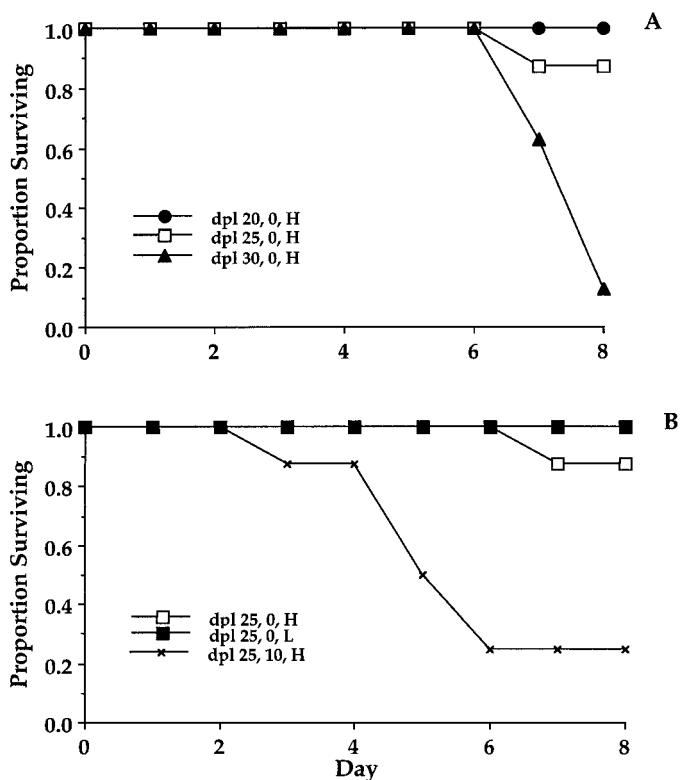


Fig. 5. As Fig. 4, but for *Daphnia pulicaria* (dpl) in May 1993.

ses. In the adult experiments, survival was reduced significantly in the 30°C treatments relative to that at 25°C for all food and SDS concentrations (Figs. 4A, 5A, 8A and B, Table 1), but it was rarely affected by other conditions. The only other significant reduction in survival occurred in the 25°, 10 SDS, HF treatment for both species, suggesting that SDS may be more stressful than low food at the levels used in these experiments. Similarly, juvenile survival to maturity was lowest (often 0%) in all 30°C treatments for both species (Table 2). Survival of *D. pulicaria* was also reduced in LF treatments at 25° and 30°C.

Table 1. *P*-values for statistical comparison of survival treatments reported in text and on figures. Survival was analyzed using SAS Lifetest (SAS, statistics version 5). Under treatment comparisons, the first number is the experimental temperature (°C), second number is concentration of SDS mg liter⁻¹, and the third number (H or L) indicates high or low food, respectively, Details of tests given in the text.

Comparison	<i>Daphnia pulex</i>	<i>Daphnia pulicaria</i>
25°C, 0, H vs. 20°C, 0, H	0.9624	0.3173
25°C, 0, H vs. 30°C, 0, H	0.0022	0.0009
25°C, 0, H vs. 25°C, 0, L	0.9624	0.3173
25°C, 10, H vs. 20°C, 10, H	0.001	0.0073
25°C, 10, H vs. 20°C, 10, L	0.2917	0.9624
25°C, 10, H vs. 30°C, 10, L	0.0002	0.0001
25°C, 10, H vs. 20°C, 10, H	0.0186	0.0436
25°C, 10, H vs. 25°C, 10, L	0.0150	0.0025
25°C, 10, L vs. 20°C, 10, L	0.2202	0.3173

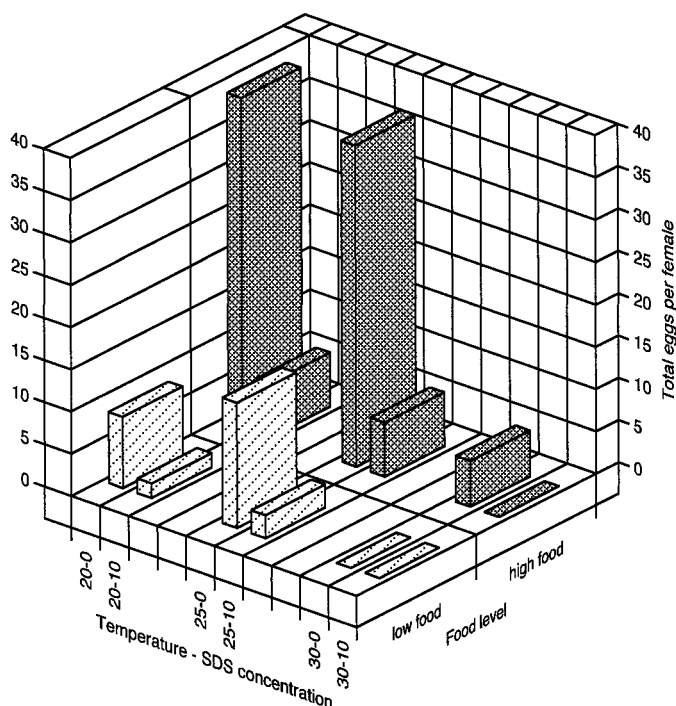


Fig. 6. As Fig. 2, but for *Daphnia pulex* from juvenile experiments in February 1993.

Effects of stressors in combination (ANOVA and survival results)—Cumulative reproduction in treatments with more than one stressor was generally less than or equal to reproduction in single stressor treatments or under optimal con-

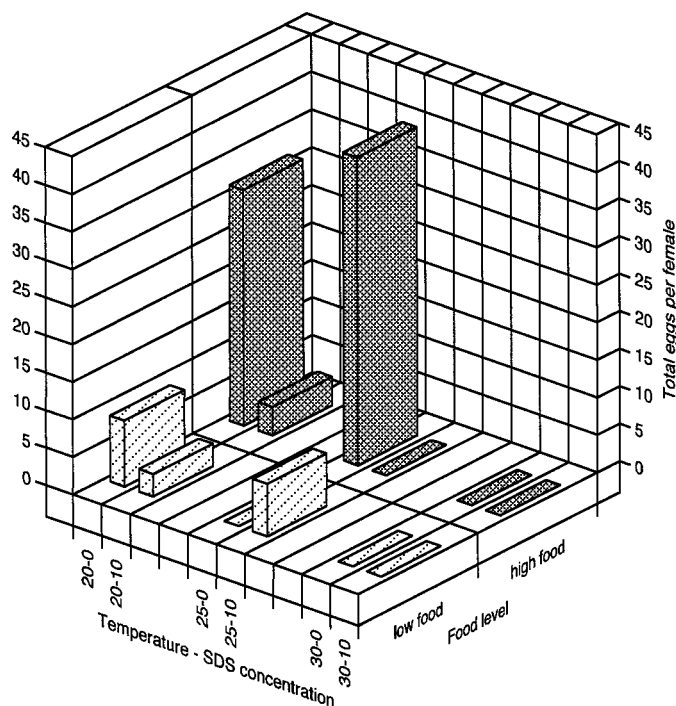


Fig. 7. As Fig. 3, but for *Daphnia pulicaria* from juvenile experiments in May 1993.

Table 2. Age at first reproduction in days (AFR) and percent juveniles surviving to reproductive maturity (in parentheses) for *Daphnia pulex* and *Daphnia pulicaria* in partial life table experiments evaluating effects of three temperatures, two SDS concentrations, and high and low food levels (102 and 1,200 µg C liter⁻¹). Dash—no individuals survived to AFR.

SDS concn (mg liter ⁻¹)	Food level	AFR (% survival)		
		20°C	25°C	30°C
<i>D. pulex</i> (juveniles)				
0	H	7.3 (0.8)	6.1 (0.9)	7.3 (0.3)
0	L	8.3 (0.8)	7.1 (0.8)	—
10	H	10.3 (0.8)	7.1 (0.7)	—
10	L	11.8 (0.5)	8.3 (0.6)	—
<i>D. pulicaria</i> (juveniles)				
0	H	7.3 (0.8)	6.5 (0.8)	—
0	L	8.3 (0.6)	—	—
10	H	8.8 (0.8)	—	—
10	L	12.0 (0.6)	8.7 (0.6)	—

ditions. Two primary types of interactive effects on cumulative reproduction were statistically significant (using the ANOVA model). There was a significant interaction between SDS and food and between SDS, food, and temperature (Table 3). The SDS×food interaction (in all but the *D. pulex* juvenile treatments) seemed to be caused primarily by the greater reduction in reproduction measured in SDS treatments at high food relative to that at LF (Figs. 2, 3, 6, and 7). The SDS×food×temperature interaction was significant in the *D. pulex* adult and *D. pulicaria* juvenile experiments, but was marginal or absent in the *D. pulicaria* adult experiments and *D. pulex* juvenile experiment. For *D. pulex* and *D. pulicaria* adults, this interaction seemed to be largely due to a reduction in the effect of SDS at 20° and LF relative to that at 25°C and to an increase in the effect of SDS at 30°C and HF relative to that observed at LF (Fig. 2).

Survival was affected interactively by temperature and SDS (Fig. 9). For adults of both species, SDS was more stressful at warmer than cooler temperatures. There was a significant reduction in survival in the 25°C and 30°C (10 SDS, HF) treatments, relative to that in at 20°C, 10 SDS, HF (*D. pulex*: $P = 0.0186, 0.0001$; *D. pulicaria*: $P =$

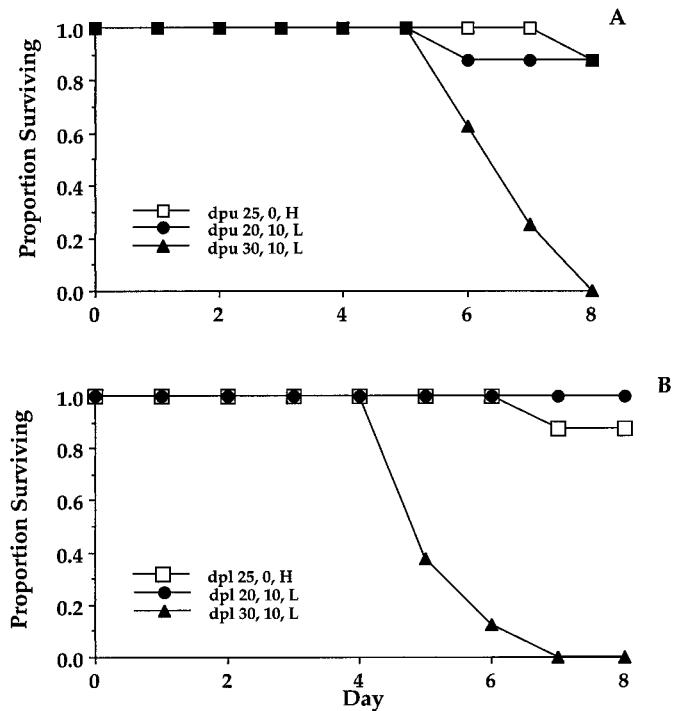


Fig. 8. Survival of *Daphnia* in adult experiments at different temperature, SDS, and food treatments. A. Comparison of optimal conditions vs. combined effects of temperature, SDS, and food for *D. pulex* (dpu). B. As panel A, but for *D. pulicaria* (dpl). Legend to Fig. 4 gives key to numerical and alphabetical symbols and Table 1 shows statistical comparisons.

0.0436, 0.0001; Figs. 8 and 9). There was also an interactive effect on survival between SDS, food, and temperature. SDS was more stressful in the HF than in the LF treatments and at warmer temperatures (Fig. 10). For both species, adult survival was greater at 20°C, 10 SDS, LF than at 20°C, 10 SDS, HF and 25°C, 10 SDS, HF treatments (*D. pulex*: $P = 0.004$; *D. pulicaria*: $P = 0.0073$). Given the small number of juveniles surviving to reproduce, we could not compare survival patterns of mature individuals in the juvenile experiments.

Testing for antagonism and synergism (RCR comparisons)—We tested for synergism and antagonism among the

Table 3. P -values for 3-way ANOVA for effects of food (F), temperature (T), and SDS on cumulative reproduction of *Daphnia pulex* and *Daphnia pulicaria* from the juvenile and adult experiments. Data were log-transformed before analysis.

Age at exposure	F	T	SDS	F × T	SDS × F	SDS × T	SDS × T × F
<i>D. pulex</i>							
Juvenile	0.0018	0.0001	0.00001	0.4670	0.3456	0.1320	0.7711
Adult	0.1196	0.0062	0.001	0.3952	0.0001	0.3395	0.0173
<i>D. pulicaria</i>							
Juvenile*	0.0685	0.0001	0.0331	0.4260	0.0008	0.2870	0.0017
Adult	0.001	0.0001	0.0001	0.1970	0.0075	0.2080	0.2040

* Poor survival in the 30°C treatment resulted in no reproduction and unequal variance in the data.

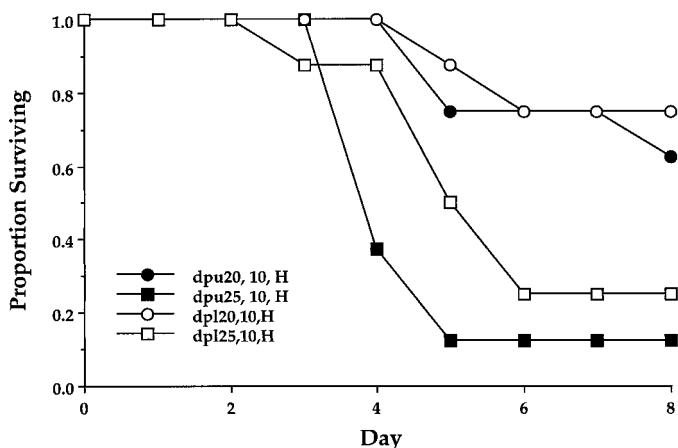


Fig. 9. Survival of *Daphnia pulex* (dpu) and *Daphnia pulicaria* (dpl) from adult experiments in two experimental treatments. Comparison of 20°C and 25°C at 10 mg liter⁻¹ SDS and high food. Legend to Fig. 4 gives key to numerical and alphabetical symbols and Table 1 shows statistical comparisons.

three stressors by qualitatively comparing the RCR_{obs} in the single stressor treatments (Table 4) with the RCR_{pr} by the simple comparative, additive, and multiplicative models. These predictions and comparisons were made using results from the adult experiments only, because few individuals survived to maturity in many of the juvenile experiments.

Individual stressors were qualitatively ranked by compar-

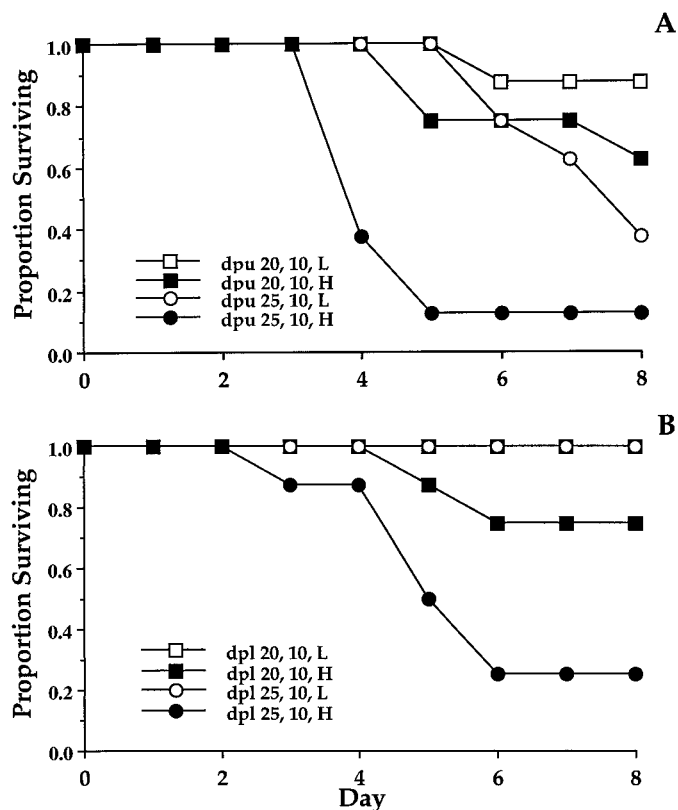


Fig. 10. As Fig. 9, but for high and low food.

Table 4. Predicted effects of multiple stressors from three different models (simple comparative, additive, and multiplicative effects) vs. observed effects of single and multiple stressors on reproduction of *Daphnia pulicaria* and *Daphnia pulex*. Predicted and observed effects expressed as percent of optimal reproduction (see text). Stressors designated as F = low food; SDS = 10 mg liter⁻¹; T (-5°C) = low temperature (20°C); T (+5°C) = elevated temperature (30°C); T = temperature treatments. Determination of optimal cumulative reproduction explained in text.

Type of interaction	Temp (°C)	SCE* (A or B...)	AE†	ME‡	OE§
			(A+B...)	(A×B×...)	
<i>D. pulicaria</i>					
F	25	—	—	—	22.1
SDS	25	—	—	—	30.2
T (-5°C)	20	—	—	—	70.3
T (+5°C)	30	—	—	—	6.9
F × T	20	22.1	0	15.5	19.9
SDS × T	20	30.2	0	21.2	27.2
F × SDS × T	20	22.1	0	4.7	22.1
F × T	30	6.9	0	1.5	6.9
SDS × T	30	6.9	0	2.1	3.9
F × SDS × T	30	6.9	0	0.5	3.9
<i>D. pulex</i>					
F	25	—	—	—	23.7
SDS	25	—	—	—	11.4
T (-5°C)	20	—	—	—	43.1
T (+5°C)	30	—	—	—	60.5
F × T	20	23.7	0	10.2	18.5
SDS × T	20	11.4	0	4.9	24.0
F × SDS × T	20	11.4	0	1.2	12.8
F × T	30	23.7	0	14.3	15.1
SDS × T	30	11.4	0	6.9	1.8
F × SDS × T	30	11.4	0	1.6	11.6

* Simple comparative effect calculated as effect (percent of optimal) of the single worst stressor in the treatment.

† Additive effect calculated as the sum of the effects of all individual stressors in each treatment. Effects in this case are the reductions in cumulative reproduction relative to optimal (e.g. = 1 - %optimal in single stressor trials). In all cases, individuals effects are so large that the sum of effects in multiple stressor trials is >100%.

‡ Multiplicative effect calculated as product of effects of all individual stressors in each treatment (see text).

§ Observed effects are the percent of optimal under each treatment condition.

ing RCR_{obs} from most to least stressful (Table 4, Fig. 11): 30°C > low food ≥ SDS > low temperature for *D. pulicaria*, and SDS > low food > low temperature > high temperature for *D. pulex*. For *D. pulicaria*, RCR_{obs} was greatest relative to optimum in the 20°C, 0 SDS, HF treatments (~70% of optimum, Fig. 11). The greatest stress (lowest RCR_{obs}) occurred at 30°C, 10 SDS, LF (~3% of optimum). In contrast, *D. pulex* RCR_{obs} was greatest relative to optimum in the 30°C, 0 SDS, HF treatments (~60% of optimum). Its greatest stress (lowest RCR_{obs}) occurred at 30°C, 10 SDS, HF (~1% of optimum).

Comparative effects model: In combination, stress effects were generally greater than (zone B, Fig. 1) than the worst individual stressor (Fig. 11). For *D. pulicaria*, RCR_{obs} values

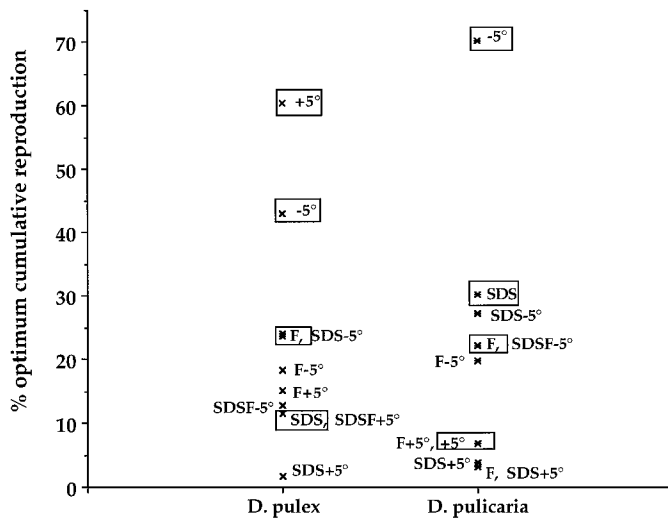


Fig. 11. Rankings of percent optimum cumulative reproduction (= observed relative cumulative reproduction or RCR_{obs}) for single and multiple stressor treatments. Values illustrate gradient of most to least stressful effects (near bottom or top of y-axis, respectively) of single and multiple stressors for *Daphnia pulex* and *Daphnia pulicaria* during a 7-d exposure period. Symbols designate different treatments with F = low food; $-5^{\circ}\text{C} = 20^{\circ}\text{C}$; $+5^{\circ}\text{C} = 30^{\circ}\text{C}$; SDS = 10 mg liter^{-1} SDS. All points (*) represent experimental values and rectangles indicate effects of single stressor treatments for each species. All data from adult experiments (see text).

were \leq the RCR_{pr} by the simple comparative effects model in each treatment (Table 4). In two cases [$F \times T(@30^{\circ}\text{C})$ and $F \times \text{SDS} \times T(@20^{\circ}\text{C})$], the multiple stressor effect was equal to the effect of the stressor that was most severe individually.

For *D. pulex*, RCR_{obs} values were also \leq the RCR_{pr} in most cases. However, in two cases [$\text{SDS} \times T(@20^{\circ}\text{C})$ and $F \times \text{SDS} \times T(@20^{\circ}\text{C})$] effects in the multiple stressor treatments were less than the effect of the worst individual stressor in each treatment. This suggests that some of these stressors, in combination, may interact to decrease the overall level of stress (zone A, Fig. 1).

Additive model: Stress effects in the multiple stressor trials were always antagonistic (less suppressive) as defined by the additive effects model. This means that $RCR_{obs} > RCR_{pr}$ by the additive model in all cases (Table 4). All additive RCR_{pr} values were equal to zero, because the sum of the individual effects was always >1 . In two treatments (Table 4), RCR_{obs} values were so low that they were difficult to distinguish from zero.

Multiplicative effects model: Stress effects in multiple stressor trials also were usually less severe than predicted by the multiplicative effects model. In all cases, except $\text{SDS} \times T$ for *D. pulex*, $RCR_{obs} \geq RCR_{pr}$ by the multiplicative model (Table 4). Eleven of the 12 cases were multiplicative antagonisms—in 50% the RCR_{obs} fell between values predicted by the simple comparative and the multiplicative effects model (where zone B and zone C overlap, Fig. 1), in 25% the RCR_{obs} was equal to the simple comparative effect, and

in the remaining two cases, the RCR_{obs} was greater than the simple comparative effect (zone A, Fig. 1, Table 4).

Comparing species and age at exposure—Thermal responses between the two *Daphnia* species differed with *D. pulex* being more tolerant of elevated temperatures than *D. pulicaria*. The 30°C treatments were less stressful for *D. pulex* than *D. pulicaria* based upon a comparison of the relative difference in reproduction between the optimal and 30°C treatment per species (Figs. 1 and 2). In contrast, the 20°C treatment was less stressful for *D. pulicaria* than *D. pulex* when comparing the relative difference in reproduction between the optimal and 20°C treatment per species (Figs. 2 and 3). A similar response occurred in the juvenile experiments (Figs. 6 and 7, Table 2). We also measured a greater reduction in cumulative reproduction at 30°C by *D. pulicaria* and no survival to maturity of *D. pulicaria* juveniles at 30°C under any conditions (Table 2). In comparison, $\sim 30\%$ of the *D. pulex* juveniles survived to maturity at 30°C , 0 SDS, and HF. Effects of SDS and LF on both species and age classes appeared similar, as discussed above.

Discussion

Under natural conditions, organisms will experience combinations of environmental stressors. We tested for synergism or antagonism among multiple stressors important to lake organisms. We next compare our experimental results with predictions from the simple comparative, additive, and multiplicative stress effects models. Then we discuss the interactive effects among temperature, toxin, and low food on reproduction and survival of zooplankton and their potential importance in the face of projected global climate change.

Evaluating synergism and antagonism among multiple stressors—Although it has been long recognized that stressors often act in combination, we are still in the early stages of evaluating multiple stressor effects on natural communities (Billick and Case 1994; Wootton 1994). Advances in analytical and evaluative methods that increase our ability to generalize multiple stressor effects will further our understanding of these complex phenomena across disciplines and communities (Billick and Case 1994). One of our goals was to compare general models for assessing multiple effects from individual effects and to clarify terminology to distinguish multiple stress effects by mode of action and severity.

The comparative effects model *underpredicted* multiple stress effects in our experiments with two exceptions. This means that multiple stress treatments were usually more stressful than the worst individual stressor (i.e., they fell in zone B, Fig. 1). Exceptions occurred when effects in multiple stressor trials were actually less severe than effects of the worst stressor alone (i.e., decreased stress, zone A, Fig. 1). These are the most extreme cases of antagonism among stressors that we measured and were relatively rare (mechanism described below).

In contrast, both the additive and multiplicative effects models generally *overpredicted* the multiple effects of stressors in our experiments (Table 4). Most of the multiple stress effects fell in the area where zones B and C overlap (Fig.

1). Thus in combination, these stressors are antagonistic (by the additive model) or multiplicatively antagonistic (by the multiplicative model) with respect to cumulative reproduction, even though they increased stress effects beyond the worst stressor alone (by the comparative effects model). Moreover, in these experiments, all individual effects of stressors were great enough that it would have been impossible to detect synergism using an additive model (i.e., the sum of individual effects in all multiple stressor trials exceeded 100%, Table 4).

There is not a single best model. Summing individual effects has been assumed a priori in a number of previous studies and may be applicable to particular types of stress mechanisms. Yet, other stress mechanisms may be better described using a multiplicative model. If the investigator knows in advance the mode of action of the multiple stressors, (s)he can determine which of these models is appropriate to use. This is by far the preferred method to follow. If the mechanism is unknown, as is often the case, all three models can be tested, and mode of action can be inferred or hypothesized from the result.

Effects of thermal, toxin, and low food stressors on Daphnia—Despite growing interest in multiple stressors on natural populations, our study is one of only a few (Cooney et al. 1983; Hanazato and Dodson 1995) to compare effects of single, double, and triple stressors on survival and reproduction of zooplankton. We selected elevated temperature, toxin, and low food because all are known to affect reproduction and survival of *Daphnia* individually (Jones et al. 1991; Lewis and Horning 1991; Moore and Folt 1993; Havens and Hanazato 1993; Gilbert 1996a,b). We were particularly interested in effects of elevated temperature in combination with other stressors because lake temperatures are predicted to increase under most scenarios for climate change (McCormick 1990; DeStasio et al. 1996). Our 7-d experimental design was appropriate for assessing episodes of stress such as those projected to occur with an increase in global warming (Mearns et al. 1992; Kerr 1995). Thermal stress, toxin exposure, and low food stress all occur periodically in natural systems, which makes long-term experiments (i.e., “press” experiments) more difficult to extrapolate to dynamic field conditions.

We hypothesized that these stressors would interact synergistically because we assumed that any factor that reduces vigor is also likely to reduce the ability of an individual to withstand additional stress (e.g., Chandini 1988, 1989; Hanazato and Dodson 1992; Koivisto et al. 1992). As expected, toxin or low food in combination with high temperature was usually more stressful than either one alone. Similar enhancement of effects of toxins at stressful temperatures has been observed in other studies (Lewis and Horning 1991; Gilbert 1996b). For example, Cooney et al. (1983) found that interactions among thermal stress, nutritional state, and a toxin (acridine) reduced survivorship of a copepod additively. However, these stressors were usually less severe in combination than predicted by either additive or multiplicative models. Instead, most of the multiple stress effects we observed are best classified as multiplicative antagonisms (i.e., together they were worse than the strongest individual stress-

or, but not as severe as the expected product or sum of their individual effects).

Moreover, in rare cases (SDS×low temp and the SDS×food×temp interactions) the multiple stressor trials were less stressful than the single worst stressor alone (i.e., a decreased stress effect). There is a plausible mechanism that might explain these cases. The primary exposure pathways for SDS to zooplankton are likely to be via filtering water and consumption of SDS-rich food. Filtering rates of *Daphnia* are increased with temperature (Burns 1969), at least up to a critical threshold. If food levels are low (below the ILL), feeding rates also increase with food concentration. Thus, with either low temperature or low food, we expect to see a reduction in feeding rate, and also exposure, relative to optimum conditions. This could explain the reduction in negative effects on survival (Figs. 8 and 9) and cumulative reproduction (Table 4) in some multiple stressor treatments.

Our second hypothesis was that these stressors could be ranked from least to most stressful. Elevated temperature (30°C) appears most damaging alone and in combination, because of its strong negative effects on both survival and reproduction. Reductions in both survival (e.g., 80%, *D. pulex*, Fig. 4) and reproduction (e.g., ~94%, *D. pulicaria*, Table 4) were observed even under high food and no toxin at 30°C. Survival was especially low for juveniles exposed to 30°C. SDS seems slightly more damaging than low food (at the experimental levels) in that SDS reduced both survival and reproduction in some treatments, whereas low food primarily affected reproduction. Finally, low temperature (20°C) was the least damaging. It suppressed reproduction, but not survival in the short term. In fact, low temperature may have no long-term effect on individual fitness (i.e., if individuals live longer at low temperature).

For both species, the dominant stressor (e.g., the stressor with the greatest individual RCR_{obs}) seemed to drive the magnitude of the stress effects in the multiple stressor trials (Fig. 11). For example, all four of the most stressful treatments for *D. pulicaria* were at 30°C and in magnitude were very close to the effect of 30°C alone. Similarly, for *D. pulex*, all four of the most stressful treatments were at 10 SDS. However, these multiple stressor effects were considerably greater in magnitude than the effect of 10 SDS alone.

Our third hypothesis was that age at exposure and exposure history affects the response of these species to multiple stressors. Responses in the adult and juvenile experiments could not be compared directly. Juveniles were exposed for a longer period than adults to assess both age at first reproduction and subsequent reproduction by individuals that survived juvenile exposure. Generally, effects did not seem to differ qualitatively with age at exposure. However, juveniles were more sensitive to certain stresses (e.g., *D. pulicaria* and *D. pulex* juveniles suffered 100% mortality before reproduction in most 30°C treatments). Further, the effects of these stressors are quantitatively different depending on age at exposure and, as such, may have very different impacts on population dynamics in stressed populations. For example, stress on juveniles may delay age at first reproduction (AFR) and reduce reproduction in adults. A delay in AFR is potentially more suppressive than a decrease in individual repro-

duction by adults because of the overwhelming influence of AFR on population growth.

We also found some support for the importance of exposure history to stress responses. *D. pulex*, isolated from a pond which experiences elevated summer temperatures, was more tolerant of 30°C conditions than *D. pulicaria*, isolated from a lake with a cold-water refuge. These species-specific responses were observed for individuals exposed either as adults or as 24-h neonates. Other investigators have also found considerable genetic diversity among zooplankton genotypes within a population, and this diversity corresponded to seasonal fluctuations in temperature (Carvalho 1987).

Implications for natural populations under climate change—The strong negative effect of thermal stress at 30°C on both juvenile and adult daphnids is likely to be important under global climate change scenarios. Current projections suggest that such temperatures will become more common across the temperate zone (Moore et al. 1997); if so, many cladoceran populations in situ may be suppressed unless temperature-tolerant clones invade rapidly. Further, these effects can be severe even with a 7-d exposure, underscoring the need to consider episodic stress events when examining population dynamics. Additional stresses, such as toxins or low food will probably be exacerbated by thermal stress. However, physiological mechanisms (such as changes in feeding rates with temperature) may produce interactions among stressors that modify their effects in combination. Finally, differences in stress responses in situ may derive in part from exposure histories. Therefore, it will be very difficult to characterize species-specific responses to stress from responses of single clones or laboratory cultures.

Final caveats—A study such as this, relying on laboratory experiments to assess stress, requires some important caveats. First, extrapolating laboratory results to the field is problematic, because our experiments were short term, interspecific interactions (e.g., competition or predation) were absent, and individuals were cultured in high quality conditions prior to exposure to the stressors. However, the nature of the multiple interactions among the stressors (e.g., antagonistic vs. synergistic) and the mechanism underlying these effects are likely to be robust under more natural, whole community conditions. It is also valuable to contrast stress effects on different demographic parameters, because this may be useful in predicting or interpreting population dynamics in stressed populations.

A second caveat is that the level of stress in this and many other experiments determines the ranking of effects among different stressors. Had we selected higher concentrations of SDS, lower food levels, or slightly cooler temperatures, the rankings of individual stressors might have changed. Temperatures and food levels examined in our experiments were well within the range experienced in much of the temperate zone, making effects of these stressors relevant to natural conditions. However, SDS levels were higher than we would expect to see in situ in much of North America. The value of SDS in this study lies in its use for understanding potential exposure pathways (e.g., exposure via feeding) and in investigating higher order interactions.

A third caveat is that we did not examine the ability of these populations to recover from these stressors. In situ, understanding recovery from episodic stress is critical to predicting long-term consequences for populations. The mechanisms underlying each stress response and the severity of the direct and indirect effects are all likely to influence this complex and important process (Niemi et al. 1991).

Our final caveat is that we were unable to expose *Daphnia* to certain levels of stressors without also potentially stressing the food resource. We ran a series of short-term experiments to investigate effects of temperature and SDS on concentrations of *Cryptomonas*. Temperature alone had no effect on food level, so food in all temperature and T × F treatments was what we predicted (i.e., no net loss over a 24-h period). However, SDS reduced algal densities by the end of a 24-h period (relative to 0 SDS). In the HF treatments this was unlikely to be important because we selected food levels to be roughly three times greater than threshold concentrations to allow for some food depletion during a day. Even with depletion, daily food concentrations remained relatively high at 10 mg SDS liter⁻¹ (~dropping to 130 μg C liter⁻¹). This may have been important and could have reduced performance in low food, high SDS treatments. However, we found that at low food SDS was *less* stressful than at high food. Finally, we feel these daily food levels remained high enough to have had only a small effect on survival or reproduction (especially given the low effect of food we observed), because food and water were replenished to original levels daily.

In conclusion, we tested for synergistic interactions among multiple stressors and found that models of multiplicative and additive stress effects overpredicted the effects of these stressors in combination. Our comparison of models describing effects of multiple stressors illustrates the importance of specifying the type of model used to test for synergism and antagonism and the underlying mechanism responsible for multiple effects. Our empirical results highlight the potentially strong direct effects of elevated temperature to important zooplankton taxa and the degree of enhanced stress that is likely when episodes of low food or toxic exposure combine during summer heat spells.

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