THE EFFECTS OF TEMPERATURE ON THE SENSITIVITY OF *DAPHNIA PULEX* TO TWO SIMULATED INDUSTRIAL EFFLUENTS

by

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INTRODUCTION

General Background

Diversion of surface waters for industrial process and cooling purposes is increasing. The electric utility industry and the petroleum refining industry are but two examples of industries requiring large amounts of water. Aquatic ecosystems receiving effluents from these industries are impacted by the addition of waste heat and toxic materials.

Use of evaporative cooling towers has increased in order to reduce waste heat additions from thermal power generating facilities and a variety of industries that generate waste heat (Stratton and Lee, 1975). Water requirements for closed cycle evaporative cooling systems may be greater than requirements for once-through cooling systems (Maulbetsch and Zeren, 1976). Thermal additions from closed cycle evaporative cooling water systems (e.g. cooling towers, cooling ponds) may be negligible, but the potential for toxic contaminants to adversely affect aquatic systems is great (Matson, 1977). Concentrations of ionic and particulate matter in the recirculating cooling water increase due to evaporative water loss. Consequently, water is continually added and portions of the recirculating water stream are discharged intermittently, or continuously, as blowdown (Stratton and Lee, 1975; Maulbetsch and Zeren, 1976). In addition to the increased concentration of natural salts, cooling tower blow-

down contains chemical additives to control biological fouling and corrosion in the cooling system. Chlorine is the most common biocide. Chlorophenols, copper sulfate and mercurial compounds have been used in the past (Stratton and Lee, 1975). Biocides are generally added intermittently. Corrosion inhibitors such as zinc and hexavalent chromium are maintained at certain concentrations in the recirculation system and are discharged continuously from cooling towers (Matson, 1977).

Oil refineries may require up to 1000 gallons of water to process one barrel of crude oil (Reid and Streebin, 1972). Water for cooling purposes may account for 80-90% of the water use by petroleum refining plants (Nemerow, 1971). Also, many toxic materials are contained in the total refinery effluent. Predominant forms that may be discharged at levels toxic to aquatic life are: petroleum hydrocarbons, phenol, chromium, sulfides and ammonia (Buikema et al., 1976; EPA, 1977a). The most toxic fractions of the petroleum hydrocarbon component are the water soluble aromatic derivatives (Anderson et al., 1974). Moore and Dwyer (1974) suggest that effluent guidelines for point source discharges of petroleum hydrocarbons should be based on the soluble aromatic content rather than the total hydrocarbon content.

Effluent limitation guidelines have been promulgated by the Environmental Protection Agency for direct and indirect (to publicly owned treatment works) discharges from steam

electric generating facilities (EPA, 1974; EPA, 1977b) and the petroleum refining industry (EPA, 1973; EPA, 1977a). Attempts are currently being made to curtail water usage and total effluent loadings for various categories of petroleum refineries (R. W. Dellinger, pers. comm., Effluent Guidelines Division, Office of Water and Hazardous Materials, U. S. Environmental Protection Agency, Washington, D. C.).

Guidelines, however, do not account for variations in environmental parameters such as temperature. Differential sensitivities of aquatic organisms to these effluents could be evident through seasonal changes in ambient water temperatures or through relatively rapid changes in temperature that may result from accidental releases of waste heat. In addition, guidelines are set separately for each toxic component and may not reflect the impact of the total effluent. Several authors have suggested that risk assessment for toxic substances and mixtures should not be conducted under optimal maintenance conditions but rather should assess the influence of a toxicant upon an organisms capacity to adapt or tolerate normal fluctuations of environmental conditions (Silbergeld, 1973; Laughlin and Neff, 1977).

Purpose

The purpose of this laboratory study was to assess the effect of temperature on the sensitivity of the freshwater cladoceran, Daphnia pulex (Brooks, 1959), to a simulated

cooling tower blowdown and a simulated refinery effluent.

Daphnia have been used to assess toxicity of industrial effluent components (Anderson, 1944) and to screen petroleum refinery effluents (Dorris et al., 1974; Richardson, 1973 and Lee, 1976). Daphnia have been used to assess the acute and chronic toxicities of various metals (Biesinger and Christensen, 1972; Ramger, 1972; Sayrs, 1975 and Winner et al., 1977), insecticides (Sanders and Cope, 1966; Macek et al., 1976; Cardwell et al., 1977) and herbicides (Sanders, 1970; Bunting and Robertson, 1975; Schultz and Kennedy, 1976). Maki and Johnson (1975) and Nebeker and Puglisi (1974) have studied the effects of polychlorinated biphenyls (PCB's) on Daphnia magna.

The Environmental Protection Agency (EPA, 1975) and "Standard Methods" (APHA, 1976) have recommended the use of Daphnia for toxicity tests. Daphnia pulex, used in this study, is a cosmopolitan North American species and is present in both soft and hard waters. D. pulex is a common zooplankter in lakes and may be found in large river systems (Brooks, 1959; Craddock, 1976).

Objectives

Specific objectives of this study were: (1) Evaluate the sensitivity of 10 and 20 C acclimated Daphnia to the two simulated effluents; (2) Determine the influence of short-term thermal additions upon survivorship of Daphnia

pre-exposed to sublethal levels of the two simulated effluents; (3) To assess the compensation capabilities of Daphnia acclimated to various temperatures.

MATERIALS AND METHODS

Maintenance of *Daphnia*

Daphnia pulex, originally obtained from Carolina Biological Supply Co., Burlington, N. C., were cultured in 19 liter all-glass aquaria. Filtered (50 micron mesh size) Blacksburg carbon-dechlorinated tap water served as the culture medium and dilution water for all tests. Water quality characteristics are listed in Table 1. Culture and test containers were kept on a 16L:8D photocycle. Incident light intensity from cool-white flourescent lights was approximately 100 ft-c.

Daphnia were fed daily an ad libitum suspension of the green alga *Chlamydomonas reinhardi* (-, wild strain). Algae were grown in a modified Bold's basal medium (Buikema, 1970). Algal cultures were illuminated by two Naturescent lights (Duro-lite Lamps, Inc., Fairlawn, N. J. 07410) and two cool-white flourescent lights at an incident light intensity of 300-500 ft-c., and a 16L:8D photocycle. Algae were concentrated and washed through a Foerst Centrifuge (Foerst Mechanical Specialties, Chicago, Ill.) and diluted to 250 ml. Feeding was occasionally supplemented with a finely ground suspension of Trout Chow pellets (10 g. pellets mixed in a blender for 5 min. with 250 ml of dechlorinated water. The suspension was passed through a 50 micron nitex filter and the filtrate 95-10 ml) was added to each culture.).

Test populations of Daphnia were maintained at two acclimation temperatures (10 ± 1 C and 20 ± 2 C). Daphnia were gradually acclimated to 10 C by lowering the water temperature 2 C day^{-1} and maintained at that temperature for a minimum of five weeks (ca. 4 molts) before use in any tests.

Composition and formulation of the simulated effluents

The simulated cooling tower blowdown mixture (SBM: Table 2) was a modification of Garton's (1972) mixture. Nitrogenous compounds such as ammonia and morpholine were not included. Only relatively persistent and representative compounds were included; specific biocides such as chlorine were not included due to their intermittent presence in blowdown. Concentrated stock solutions of ingredients were made up every two weeks in distilled water and stored at room temperature in amber polypropylene bottles. The SBM was formulated on each day of use. Chromium concentrations were periodically measured with atomic absorption spectrophotometry for quality control purposes (Table 3).

The artificial refinery mixture (ARM: Table 4) was formulated according to Buikema et al. (1976). The 1X ARM was formulated at nearly the same levels as the 1977 guidelines for effluents from integrated refineries promulgated by the Environmental Protection Agency (EPA, 1973; Lee, 1976). Modifications were: (1) the Gulf No. 2 fuel oil was not added directly to each test solution. Instead, the appropriate

Table 1. Water quality characteristics of carbon-dechlorinated water for January-August, 1977.

PARAMETER[a]	AVERAGE	N	RANGE
Hardness mg l^{-1} $CaCO_3$	39.8	22	31.0 - 44.0
Conductivity umhos cm^{-1} @ 25 C	138.4	14	130 -145
pH	7.27	21	6.21 - 7.90

[a]All parameters assessed according to "Standard Methods" (APHA, 1976).

Table 2. Simulated Cooling Tower Blowdown Mixture (SBM)

Parameter	Concentration as mg l^{-1}	Ingredient
Zn^{++}	2	$ZnSO_4 \cdot 7H_2O$
CrO_4^{--}	15	K_2CrO_4
PO_4^{--}	25	$Na_2HPO_4 \cdot 7H_2O$
SO_4^{--}	824	$Na_2SO_4 \cdot 10H_2O$
B	0.5	H_3BO_3

Table 3. Atomic absorption analysis of total chromium in the SBM

Nominal (mg l^{-1})	Measured (mg l^{-1})		
	Average	± 95% C. I.	N
6.72	7.16	0.97	10

[a]Nominal value of 6.72 mg l^{-1} Cr corresponds to 15 mg l^{-1} CrO_4--.

Table 4. Artificial Refinery Mixture (ARM)

Parameter	Concentration as mg l^{-1}	Ingredient
NH_3-N	10	NH_4Cl
Cr-total	0.25	K_2CrO_4
Oil and grease	10	Gulf No. 2 fuel oil
Phenol	0.1	Phenol
Sulfide	0.17	$Na_2S \cdot 9H_2O$
Total suspended solids	20	Kaolinite*

*Well crystallized kaolinite from the Clay Mineral Society Repository, University of Missouri.

pH was adjusted to 6.8-7.2 with $NaOH/H_2SO_4$ as needed.

amount of oil was added with a Hamilton syringe pipet to the 1X or 0.1X ARM and mixed in a blender for approximately 10 sec. Aliquots of the ARM oil water dispersion were then dispensed rapidly to each test container; (2) the 1X ARM solution was prepared with dechlorinated water rather than distilled water. If a stock solution of the ARM minus oil was not used immediately it was stored at 4 C for a maximum of 48 hours. Atomic absorption analyses of the ARM chromium content were not conducted because Lee (1976) showed that only one third of the nominal chromium was in the aqueous phase. This was accounted for by surface adsorption of chromium by the Kaolinite clay fraction.

Acute toxicity tests

Mixed age adult animals (1.5-2.4 mm) were used for all toxicity tests. Static toxicity tests, without aeration or solution renewal, were conducted in pyrex beakers containing 400 ml of test solution and 10-15 Daphnia. A logarithmic dilution series, consisting of at least six simulated effluent concentrations, and a control were used for all tests. Batches of Daphnia, contained in 10 ml vials, were randomly assigned to each dilution. Containers were covered with saran wrap to retard evaporation and placed randomly in a growth chamber for the test duration. Death was defined as the cessation of movement for all appendages, even after swirling of the test solution. Observations were made with

the aid of a 3X magnifier, or by transferring Daphnia to a depression slide and viewing them with a dissecting microscope.

Median lethal concentrations (LC50s) of the two effluent were estimated with Finney's probit analysis procedure on the Statistical Analysis System (Barr et al., 1976). Analyses were performed on the Log_{10} of the concentration data (Stephan, 1977). In several instances, the LC50 was estimated with the nomographic method of Litchfield and Wilcoxon (1949), due to the lack of partial kills. Several toxicity tests were conducted over a long enough period of time to describe the toxicity curve and to estimate the time for cessation of acutely lethal action (Sprague, 1969). The relationship between acute toxicity and acclimation temperature was determined by comparing the LC50 estimates for 10 and 20 C acclimated daphnids.

Sensitivity to toxicants and toxicant mixtures varies with the stage of the molt cycle and the process of ecdysis per se has been identified as the most sensitive stage (Anderson, 1946; Lee, 1976; APHA, 1976; Schultz and Kennedy, 1976). A toxicity test of at least one instar duration should provide the best estimate of a LC50. The temperature dependency of adult instar duration should be taken into account in order to obtain comparable LC50 estimates for 10 and 20 C acclimated Daphnia. Adults from each population

(N=4 at 20 C, N=5 at 10 C) were maintained in 50 ml portions of filtered water and instar duration was noted over a 2 to 3 instar time period. Feeding and light conditions were as described previously. Containers were checked for the presence of a cast exoskeleton at least twice daily and more frequently as the developmental stage of the young in the brood chamber indicated that ecdysis was imminent (see Lee, 1976, for a description of the developmental stages).

Sublethal tests

Sublethal levels were defined for the purposes of this study as the concentration of simulated effluent causing mortality just detectable above the controls, after the duration of one instar. The predicted LC10 from probit analysis of acute toxicity tests was utilized as the working definition. Tests, involving a log dilution series of test solutions bracketing the predicted LC10 and a control, were conducted to compare observed and predicted mortality. In addition, the incidence of nonviable eggs and young was noted since preliminary results suggested that reproduction was impaired during exposure to acutely sublethal concentrations.

Combined tests: sublethal effluent and thermal stress

Tests were conducted with each population of Daphnia and with each simulated effluent. A random subsample (N~30) of Daphnia was obtained initially to describe the size and

reproductive stage distribution of animals used in a particular test. Tests were initiated by setting up twelve beakers, each containing simulated effluent at the sublethal level and 15 Daphnia, and incubating them for the duration of approximately one instar at acclimation temperature. Three beakers, containing dilution water only and 15 daphnids each, served as controls. All containers were covered to retard evaporation and placed randomly in the growth chamber. At the end of the incubation period the pre-exposed Daphnia were randomly allocated to four treatment groups, each with three replicates. Young produced during the incubation period were removed and counted. Three of these groups were subjected to different thermal shocks of one hour duration. Thermal shocks were: Δt = 12, 8 and 5.4 C above acclimation temperature (groups 12, 8 and 5). Shocks were gradual since it took up to 15 minutes for the test solutions to warm up to the final temperature. Containers were maintained at the final temperature until the hour had elapsed and then returned to the growth chamber. The fourth group (group 0), consisting of animals pre-exposed to the sublethal level, was maintained at acclimation temperature along with the controls (group CO). All groups were monitored for: adult survivorship, and production of viable and nonviable young. Young produced between observation periods were removed and counted.

These static tests, designed to assess short term effects

(e.g. of one to two instar duration), were terminated when it became apparent that test solutions were "conditioned" or capable of supporting the remaining survivors indefinitely.

Temperature acclimation pattern

The temperature acclimation pattern of Daphnia pulex was determined according to Prosser's (1973a) scheme. Metabolic rates, as measured by oxygen consumption rates, of 10 and 20 C acclimated Daphnia were evaluated at 10, 15, 20 and 25 C.

Respiration rates of Daphnia were assessed over a 24 hour interval to integrate any diurnal fluctuations. The respirometers were 60 ml glass stoppered pyrex bottles which were calibrated by weight to the nearest 0.1 ml. Reproductively immature Daphnia (1.1-1.3 mm) were segregated and not fed 12 hours before each test. Daphnids were tested in carbon-dechlorinated water which was filtered through 0.45 micron Metricel filters and asceptically aerated 12 hours before use. Five Daphnia, previously rinsed for several minutes in two successive 50 ml portions of filtered water, were placed in each respirometers containing filtered water at the test temperature. Bottles were stoppered, checked for air bubbles and immersed in beakers containing water at the test temperature. Generally, seven experimental and three control (filtered water only) respirometers were used for each test.

After 24 hours dissolved oxygen concentrations were determined with the azide modification of the Winkler method (APHA, 1976). Daphnia were not removed during dissolved oxygen concentration determinations. Aliquots (25.0 ml) were immediately titrated with a 0.005 N sodium thiosulfate solution delivered from a buret with gradations of 0.02 ml. Daphnia were placed on tared aluminum pans (ca. 10 mg.), dried at 55 C for 36 hours and dessicated for 12 hours. Dry weights were then determined by substitution weighing on a Cahn Electrobalance (Model #4700) with the 2 mg. weight range. Oxygen concentrations of the solutions were corrected for dilution by the precipitating Winkler reagents. Differences between average corrected O_2 concentrations of controls and each experimental respirometer were then corrected to absolute changes in O_2 content.

where: $$\Delta mg\ O_2 = \frac{\text{Corr. } [O_2]_{co} - \text{Corr.}[O_2]exp.}{\text{volume of exp. bottle}}$$

Respiration rates were then expressed as $\mu l\ O_2$/ mg dry weight/ hour.

Statistical treatment

Unless otherwise noted an error rate (α) of 0.05 was utilized for all parametric and nonparametric statistical analyses reported in the results and discussion section.

RESULTS AND DISCUSSION

Instar duration and acute toxicity tests

Temperature dependency of adult instar duration is illustrated in Figure 1. Data from Robertson (1971) are also shown. Her data were obtained with Daphnia pulex maintained in a dilution water of twice the total hardness and fed daily with yeast. Ideally, the duration of an acute toxicity test with adult Daphnia should reflect the duration of one instar, due to the differential response to toxicants with the molt cycle. An acute test with 10 C acclimated Daphnia would have to be approximately 165 hours long to ensure that most animals had molted (Figure 1). Similarly, at 20 C a time period of approximately 70 hours would be required (Figure 1). Starvation-induced stress in static tests will limit realization of these durations.

Starvation mortality of control animals was not common until after 96 hours with 20 C and until after 144 hours with 10 C acclimated Daphnia. However, lack of actual control mortality at 72 or 96 hours with 20 C acclimated organisms does not rule out the likelihood that starvation stress influences acute toxicity results (A. L. Buikema, Jr., pers. comm.). Lemcke and Lampert (1975) found that 2.0-2.4 mm Daphnia pulex withstood starvation conditions better than other size classes. Dry weight losses for this size class, at 20 C, after 1, 2, 3 and 4 days of starvation were 29,

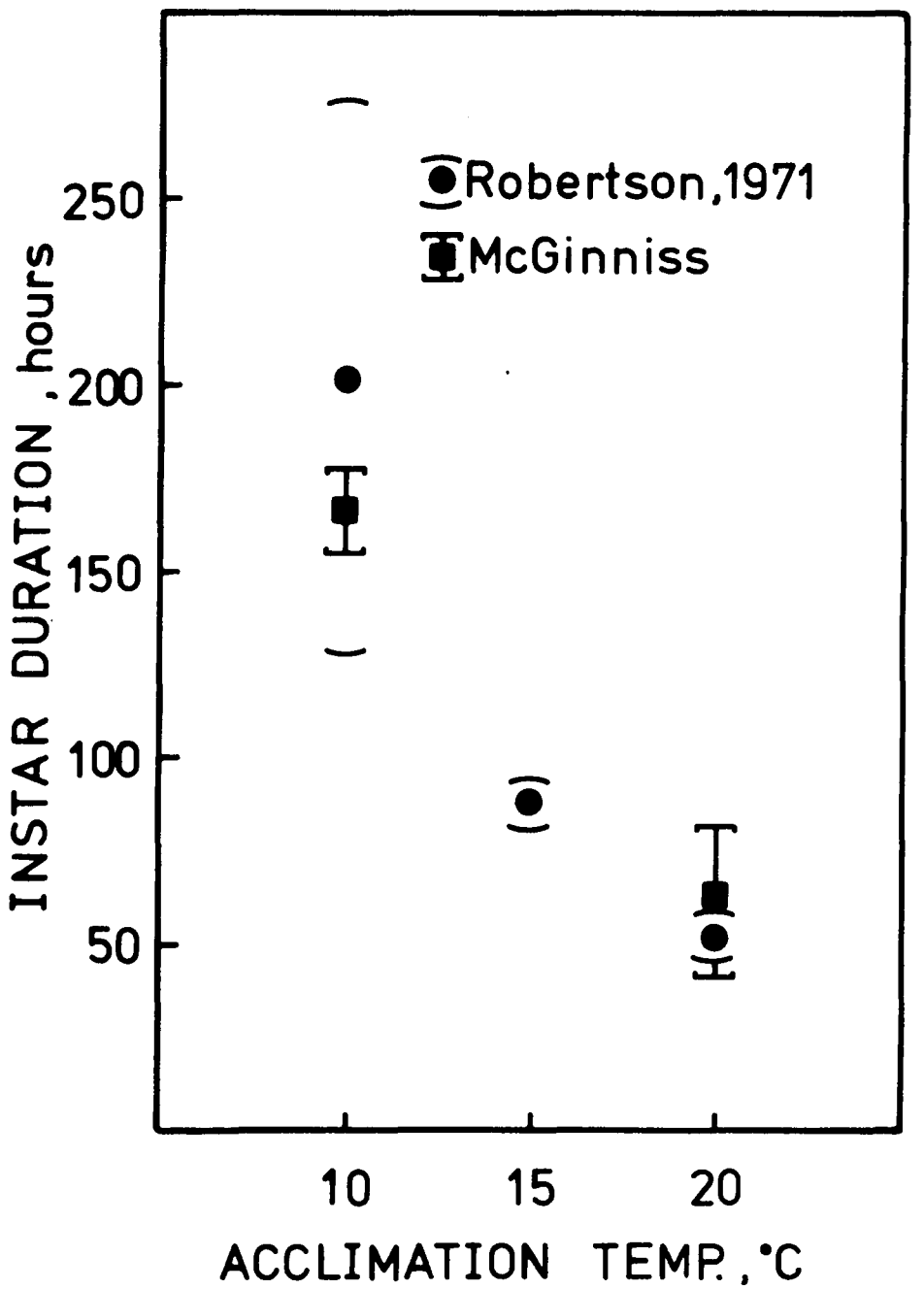

Figure 1. Temperature dependency of adult instar duration. Points represent the average observed duration. Bars indicate 95% confidence limits. Data of Robertson 1971 are also shown.

44, 46 and 51 per cent of initial dry weight, respectively. Starved animals are usually more sensitive to toxicants (Wilson, 1974). The static nature of the tests conducted does allow for proliferation of possible food organisms (e.g. algae, protozoa and bacteria) in the dilution water as the test progresses.

Goodness of fit for the probit model

Chi-square statistics are provided with the probit analysis procedure to give an indication of how well the observed data fit the probit regression line (Barr et al., 1976). A large chi-square value (i.e. $\alpha(\chi^2) \leq 0.05$) indicates a significant deviation of observed responses from the probit regression line. The validity of such a test may be suspect. Inflated chi-square values may be attributed to two factors. Significant deviations might result from a relatively large scatter of observed data around the line. This type of dispersion may indicate that the test organisms were not homogeneous with respect to physiological state. Chi-square values obtained with tests on homogeneous subjects will generally be equal to the number of degrees of freedom (Finney, 1971). Chi-square values obtained in this study were not significantly large, and were generally equal to the degrees of freedom. Only a small percentage of tests (ca. 5-10%) resulted in large chi-square values; these were not reported. Consistent patterns in deviations from the probit regression

line noted in a series of tests may suggest that the probit model is inappropriate for the data. Since no consistent patterns were noted, the probit model is not unreasonable for describing the sensitivity of mixed age adult Daphnia exposed to the two simulated effluents.

SBM acute toxicity and influence of acclimation temperature

Responses of 10 and 20 C acclimated Daphnia to the SBM are presented in Figure 2. Each curve represents one toxicity test with LC50s estimated at successive time intervals. The 95% confidence limits of LC50s generally become smaller through time due, in part, to the larger proportion of test animals that have molted. Several authors have proposed that the most useful parameter for assessing toxicity is the lethal threshold concentration, or incipient LC50 (Sprague, 1969; Wilson, 1974). This is the concentration at which toxicity, for the population tested, is not affected by further exposure. Apparent threshold concentrations of the SBM may be visualized as the inflection point of the toxicity curves (Figure 2). Apparent thresholds correspond to the 72 h and 120 h assessments for 20 and 10 C acclimated Daphnia, respectively. These time periods roughly correspond with the duration of one instar. Static tests of longer duration might only reflect the increasing influence of starvation stress and thus may overestimate toxicity. Purely subjective estimates of the lethal threshold or incipient LC50 are often

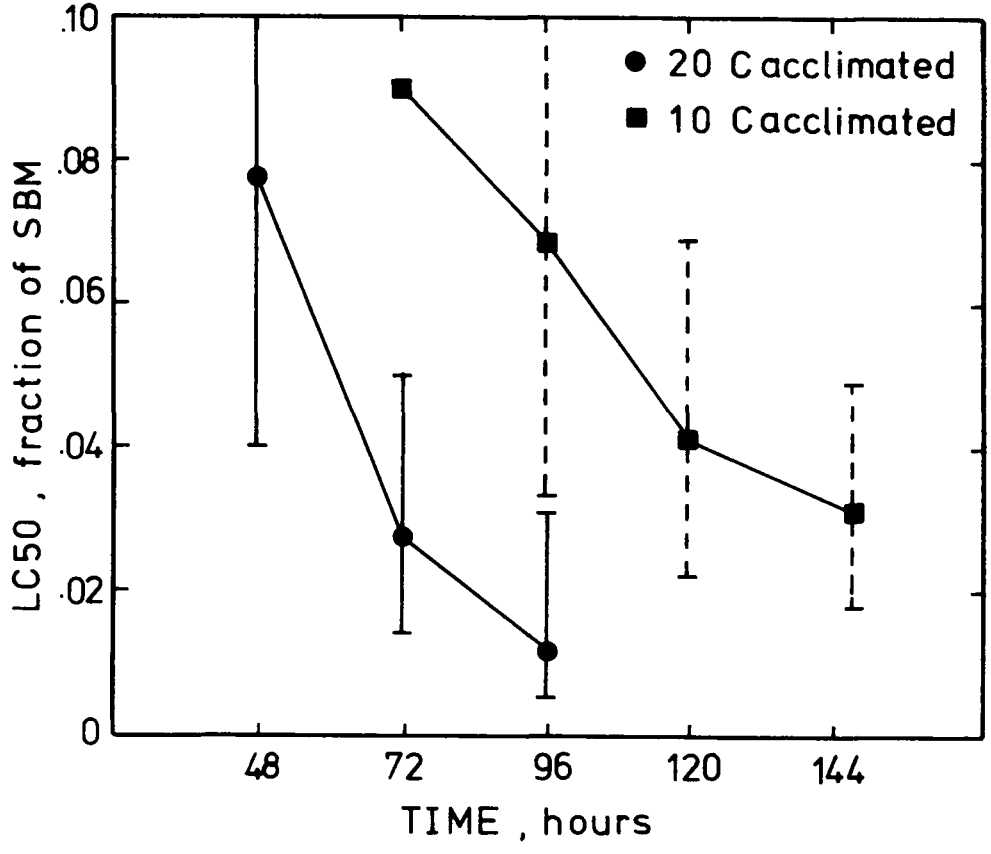

Figure 2. SMB toxicity curves for 10 and 20 C acclimated Daphnia pulex. Each curve represents the results of one static test. Points represent the estimated LC50. Vertical lines indicate the 95% confidence (fiducial) limits.

made (Sprague, 1969). Green (1965) does present an analytic procedure for estimating the LC50 (or any other tolerance parameter e.g. LD50, LT50) for an indefinite exposure period. It was felt that this procedure would not be valid for static tests when organisms are tested under starvation conditions.

Sensitivity of the two populations to the SBM differs at all time periods of assessment as illustrated by the shift or translation in the toxicity curves (Figure 2). Replicate estimates of apparent threshold LC50s are listed in Table 5. Values listed indicate that 10 C acclimated Daphnia are twice as tolerant of the SBM compared to 20 C animals. This trend was significant at the α=0.018 level with the Wilcoxon Rank Sum Test (Hollander and Wolfe, 1973).

Garton (1972) reported that the 96-h LC50 of his simulated cooling tower blowdown mixture to 10 C acclimated juvenile steelhead, in static tests with daily renewal, was 0.068X. This was comparable to the 120-h apparent thresholds for 10 C acclimated Daphnia in static tests (0.032-0.58X, Table 5). He also reported that 0.01X had no apparent effect on growth of the alga Selenastrum capricornutum, at 24 C. A 0.01X solution greatly reduced the growth of this alga.

ARM acute toxicity and influence of acclimation temperature

Preliminary tests, at 20 C, with the ARM were conducted to compare two methods of oil introduction upon the survivorship data obtained. Concurrent tests were conducted with oil

Table 5. Acute toxicity of SBM to 10 and 20 C acclimated Daphnia pulex

10 C 120-h apparent threshold LC50	20 C 72-h apparent threshold LC50
.032[a] (.018-.055)[b]	.022[a] (.013-.036)
.045 (.022-.069)	.023 (.017-.032)
.058 (.030-.127)	.026 (.013-.058)
	.0269 (.014-.050)
	.029 (.017-.083)

[a]LC50 estimated using the method of Litchfield and Wilcoxon (1949)

[b]95% confidence limits

water dispersions and with dilutions of the ARM where oil was added individually. Representative dose-response curves are shown in Figure 3 and 4. Difference in survivorship curves between tests can be attributed to the method of oil introduction (cf Figures 3 and 4). Results with the ARM oil water dispersions appear to be less variable (Figure 4). The greater apparent toxicity of the ARM 0.01X and 0.001X solutions where oil was added directly (Figure 3), may be the result of adding more oil than the minute amount required (0.047 and 0.0047 µl, respectively). This was probably the case since a higher proportion of animals were observed entrained in the surface film at these concentrations than in comparable dilutions of the ARM oil water dispersions. Vanderhorst et al. (1976) reported significant differences in mortality attributable to the method of oil and dilution water introduction in fuel oil bioassay.

The ARM toxicity curves for 10 and 20 C acclimated Daphnia are shown in Figure 5. Again, 95% confidence limits become smaller with time due, in part, to the increasing proportion of test animals that have molted. No definite statements can be made about the true shape of the ARM toxicity curves. Confidence intervals could not be calculated for the LC50 estimates at the longest time period. Continuous flow or static with renewal tests, with feeding, might allow for a complete description of the toxicity curves. Toxicity

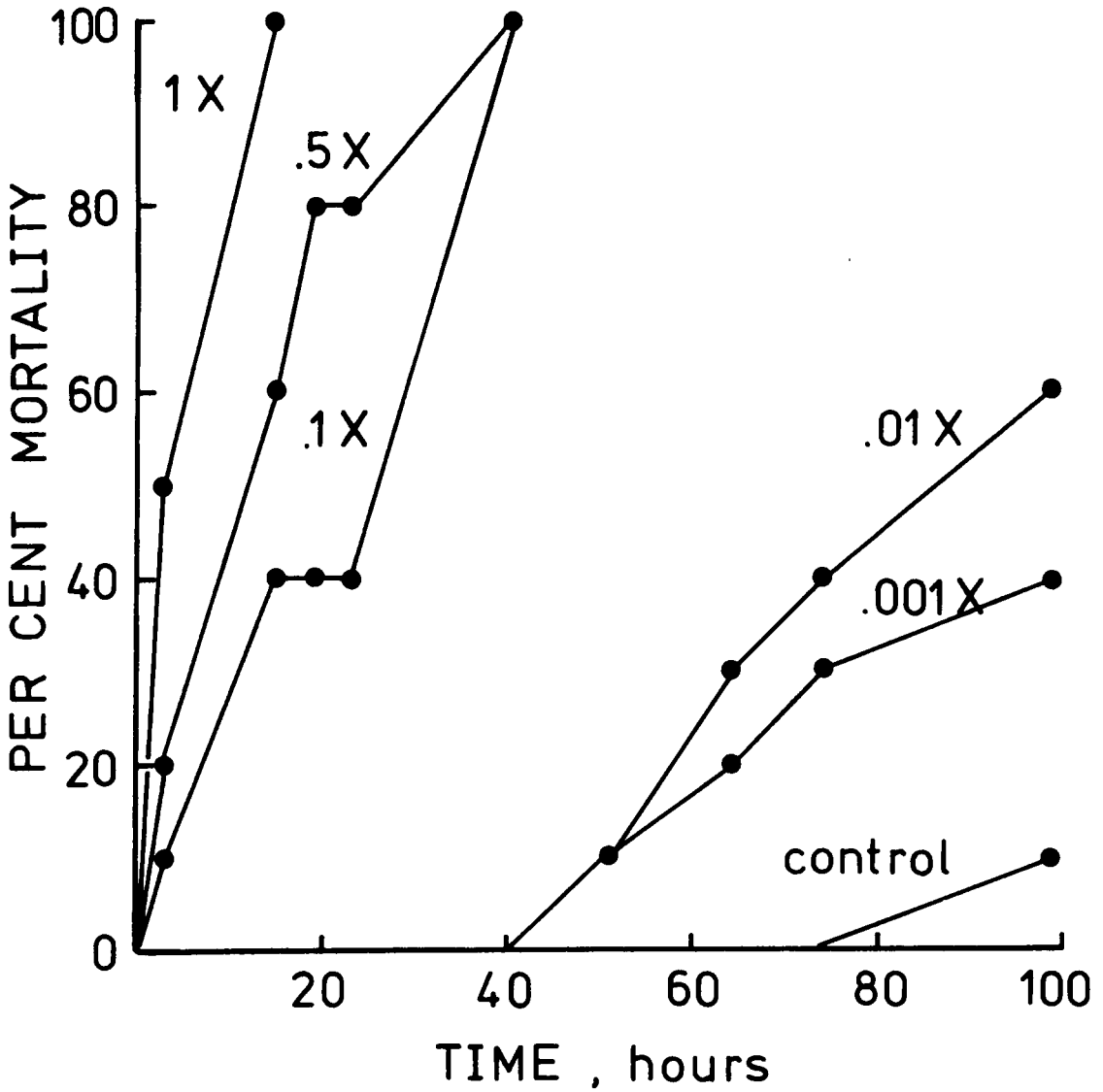

Figure 3. Dose response curves for Daphnia pulex exposed to concentrations of ARM where oil was added individually to each dilution.

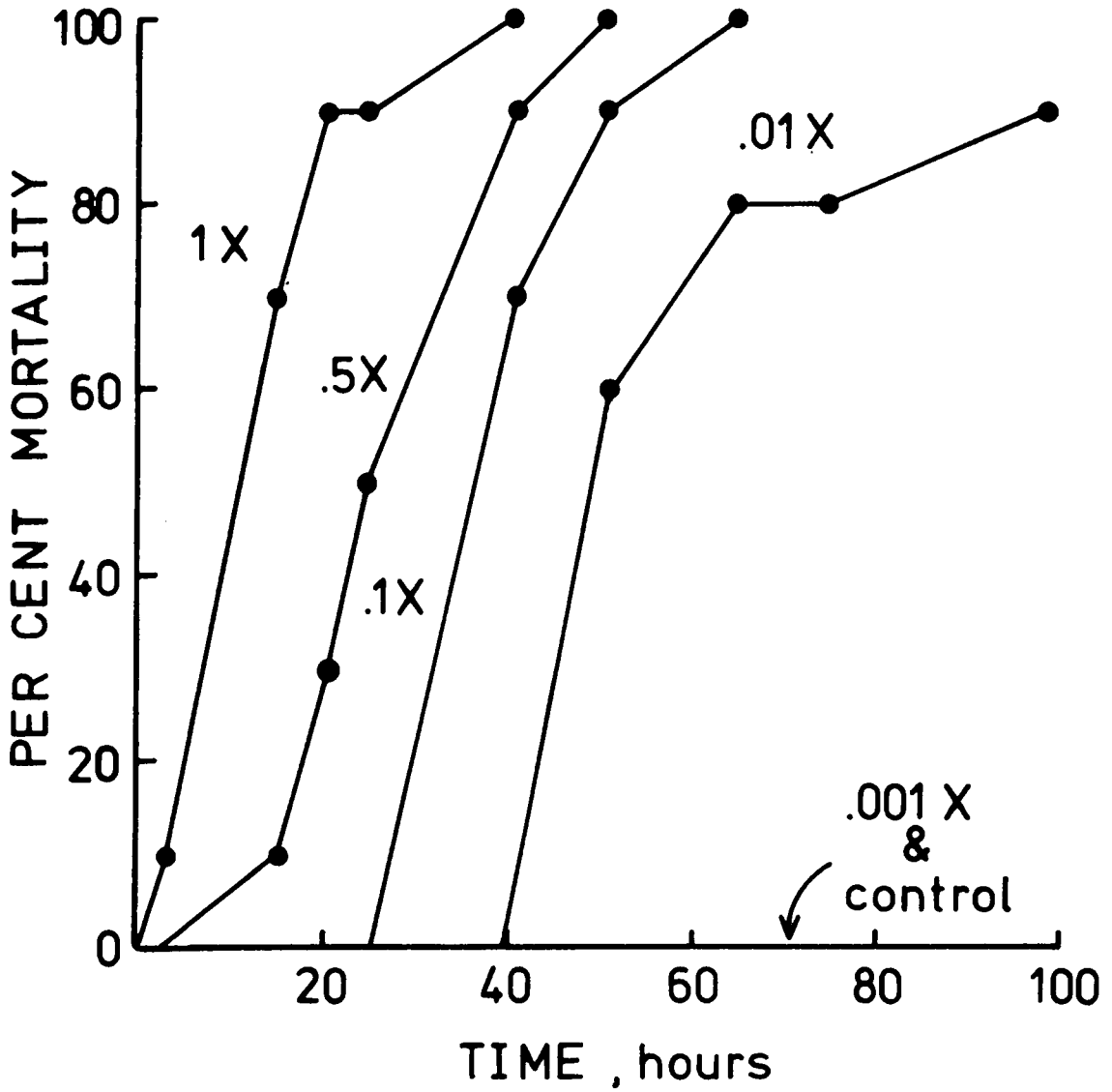

Figure 4. Dose response curves for Daphnia pulex exposed to ARM oil water dispersions.

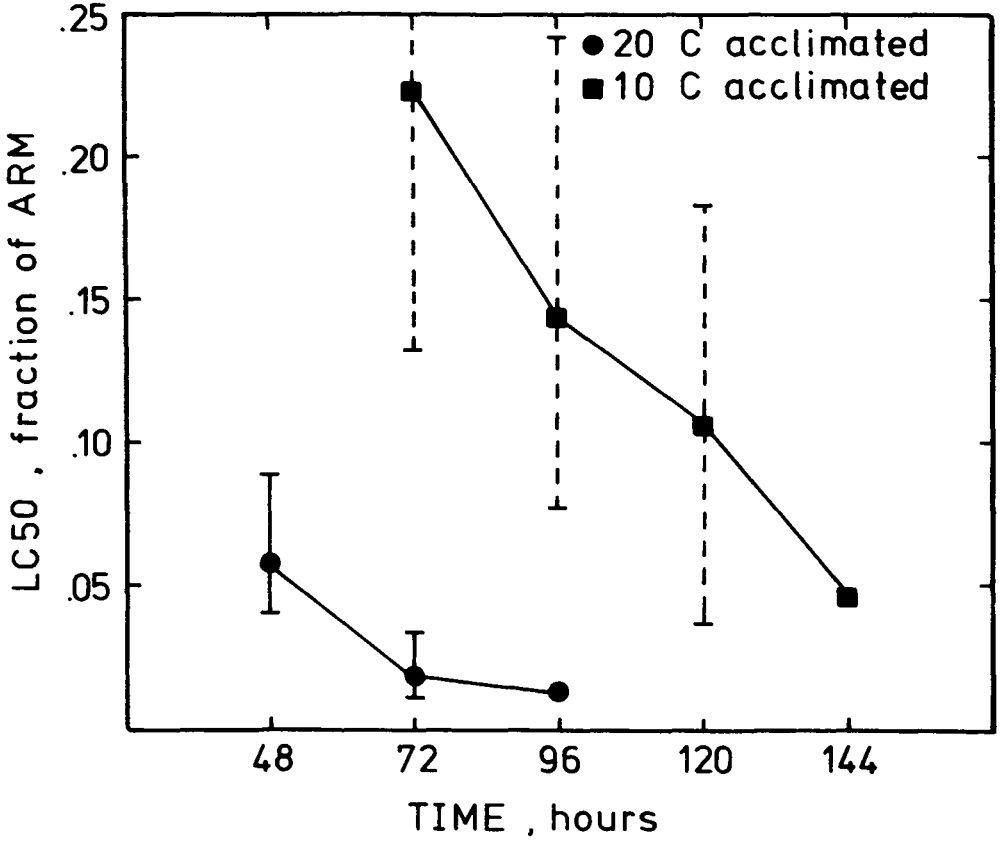

Figure 5. ARM toxicity curves for 10 and 20 C acclimated Daphnia pulex. Curve at 10 C represents one static test. Curve at 20 C is a composite of two tests. Points represent estimated LC50s. Vertical lines indicate 95% confidence (fiducial) limits.

curves obtained from tests with solution renewal may be displaced towards lower threshold values when compared with curves from static tests (Wilson, 1974). Threshold levels may not be evident for the ARM mixture, at least in static tests. This may be due to the increasing influence of starvation with longer exposure periods. Wells and Sprague (1976) reported no obvious threshold in acute lethality with crude oil for lobster larvae (Homarus americanus), in static tests with renewal. Mauck et al. (1976) determined time independent LC50s, or TILC50s, with several species of fish for insecticidal pyrethrins and pryethroids in flow-through tests. They suggested that the TILC50s obtained for a given population were a measure of its detoxification abilities. The lack of obvious thresholds for certain compounds or mixtures, in tests with solution renewal, may be indicative of an organisms inability to metabolize it. Burns (1976) suggested that the sensitivity of fiddler crabs (Uca pugnax) to petroleum hydrocarbons reflects their minimal metabolic capabilities. No induction of the mixed function oxidase enzyme system was noted in crabs obtained from areas polluted by petroleum hydrocarbons.

Replicate estimates of apparent thresholds are listed in Table 6. Threshold concentrations for 10 C acclimated Daphnia were almost an order of magnitude larger than those for 20 C acclimated animals. This trend was significant at

the α=0.029 level with the Wilcoxon Rank Sum test. Toxicity curves are widely separated at all time periods illustrating the influence of temperature on toxicity of the ARM (Figure 5). The influence of temperature upon toxicity of oil containing mixtures will be mediated by both abiotic and biotic factors. Oil water dispersions and water soluble fractions of No. 2 fuel oil may be more toxic at lower temperatures due to the slower rate of loss of the toxic aromatics from the aqueous phase (Rice et al., 1977). Secondly, temperature will have a marked effect on the uptake, metabolite transformation and excretory processes that mediate the biological response to toxic materials. It was assumed that the initial concentrations of oil (both as a surface film and water soluble components) were equivalent at 10 and 20 C since oil water dispersions of the ARM were formulated at room temperature (19-23 C). Dilutions formulated were then allowed to equilibrate at acclimation temperature before test organisms were introduced.

Sublethal toxicity tests

Summaries of the sublethal toxicity tests are presented in Table 7 and 8. The predicted LC10, for both effluents, may have been an underestimate of the true LC10. This may reflect the inapplicability of the probit dose-response model for low dose extrapolation, with these toxicant mixtures. Mortality evident in low levels of the ARM were

Table 6. Acute toxicity of ARM to 10 and 20 C acclimated *Daphnia pulex*

10 C 120-h apparent threshold LC50	20 C 72-h apparent threshold LC50
.088[a] (.057-.133)[b]	.0064 (.002-.017)
.106 (.037-.184)	.0146 (.0076-.063)
.121 (.053-.499)	.018[a] (.0118-.027)
	.0189 (.0112-.031)

[a]LC50 estimated using the method of Litchfield and Wilcoxon (1949)

[b]95% confidence limits

Table 7. Sublethal toxicity tests with the SBM and mixed-age adult Daphnia

Temperature	Elapsed time	Predicted LC10 as fraction of 1X SBM	Predicted LC10 as % of median apparent threshold	Observed effects of LC10 mortality	Observed effects of LC10 production of nonviable young
10	120-h	.020	44	0-10%	noted in all dilutions except controls
20	72-h	.0056	21	0%	noted in all dilutions, including controls; graded response evident

Table 8. Sublethal toxicity tests with the ARM and mixed-age adult Daphnia

Temperature	Elapsed time	Predicted LC10 as fraction of 1X SBM	Predicted LC10 as % of median apparent threshold	Observed effects of LC10 mortality	Observed effects of LC10 aborted eggs
10	120-h	.030	28	0-10%	noted in all dilutions, including controls
20	72-h	.0075	46	0-10%	noted in all dilutions

usually associated with failure to molt and/or surface en- Aborted eggs were noted with the SBM at 20 C (Table 7) and the ARM at 10 C (Table 8). Aborted eggs noted with the control animals may reflect starvation-induced stress during the exposure period. Measures of reproductive impairment (such as the incidence of aborted eggs or the reduction in the production of viable young) will be more informative when assessed with individual Daphnia, where the initial reproductive state is known. Utilizing mixed age adults in batch tests without assessing the initial reproductive state may serve to obscure any patterns such as a graded response in the incidence of aborted eggs.

Schober and Lampert (1977) demonstrated in chronic studies that sublethal levels of the herbicide atrazine impaired the production of young in Daphnia pulex. These data were presented as young produced and no indication of viability was made. The average length of Daphnia after 28 days was inversely proportional to the concentration of atrazine which indicated a marked effect of sublethal levels on growth. Similarly, Canton et al. (1975) reported that sublethal levels of α-hexachlorocyclohexane (α-HCH) reduced the number of young produced by Daphnia magna. Again, no distinction was made between viable and nonviable progeny. Daphnia pulex exposed to water soluble fractions of various petroleum hydrocarbons demonstrated an increased incidence in the pro-

duction of nonviable eggs and young when compared to control animals in life-time chronic studies (J. Geiger; pers. comm.).

A fixed mortality rate approach is more suitable for obtaining sublethal levels of comparable effect between acclimation temperatures than using some arbitrary fraction of the LC50. Dose response curves differ with temperature as illustrated by differences in the LC10/LC50 proportions between acclimation temperature (see Tables 7 and 8). The fixed mortality rate approach takes into account the slope of the dose response curve.

Combined tests: sublethal effluent and thermal stress

Preliminary tests with 20 C acclimated Daphnia indicated that short term (i.e. 1-2 hour) gradual Δt's of 12, 7 and 5 C had no significant effect on survivorship, when compared to controls in static tests.

Mortality curves obtained in tests involving pre-exposure to sublethal levels of a simulated effluent and subsequent short-term thermal shocks were analyzed by Log (X + 1) regression techniques. Empirical curves suggested the following exponential model:

$$(1) \quad M = C_1 \, e^{-c_2 t}$$

Where M = cumulative mortality, t = time and C_1 and C_2 are constants. This model (1) can be linearized by logarithmic transformation and analyzed with parametric regression

techniques (2).

$$(2) \quad 2.3 \text{ Log}(M) = 2.3 \text{ Log}(C_1) - C_2 t$$

$$(\text{i.e.} \quad Y = A + BX)$$

The assumption of statistical independence was not met due to the cumulative presentation of both mortality and time. Thus, the regression analyses were heuristic, or empirical, and served merely as a curve fitting technique (W. R. Pirie, pers. comm.). Regression analyses were performed with the Statistical Analysis System (Barr et al., 1976). Multiple comparison of regression coefficients with the Duncan's New Multiple Range test (Harter, 1960) were informative and served to substantiate the picture supplied by the survivorship curves but, again, were not statistically rigorous. Schober and Lampert (1977) used similar techniques to compare the cumulative production of young by Daphnia pulex exposed to varying concentrations of the herbicide atrazine.

Combined test with the SBM at 10 C

Survivorship curves for 10 C acclimated Daphnia pre-exposed to the LC10 of the SBM (0.02X) for 120 hours and then subjected to short-term thermal shocks are shown in Figure 6. Time zero on the graph pertains to the application of the short-term thermal shocks (Groups 12, 8 and 5). Controls (CO) and Daphnia pre-exposed to the LC10 (Group 0) were not subjected to any temperature alterations. The test was terminated 140 hours after thermal shock because of

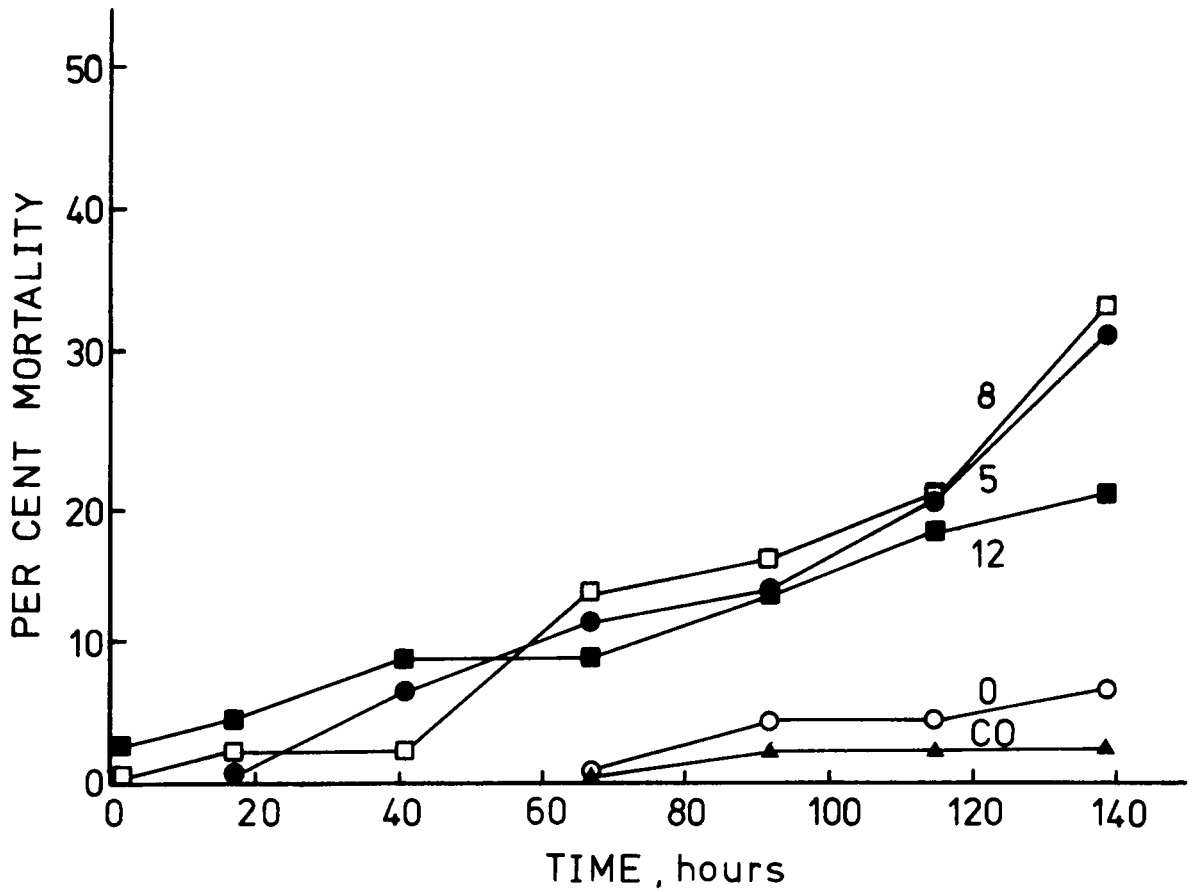

Figure 6. Survivorship curves for groups of 10 C acclimated Daphnia exposed to LC10 of the SBM and then subjected to short-term thermal shocks ($\Delta t's$=+12, +8 and +5.4C). Group O was pre-exposed to the LC10 and maintained at acclimation temperature. Controls (CO) were maintained in dilution water only.

conditioning of the dilution water. Thermal shock groups (12, 8 and 5) demonstrated higher mortality rates than the controls or Groups 0 (Figure 6). Multiple comparisons of the slopes indicated that Groups 12, 8 and 5 were significantly greater than the control or Group 0 slopes (Table 9). Slopes of control and Group 0 were not significantly different. The exponential nature of the curves was not apparent due to the termination of the test at 140 hours. The r^2 values for Groups CO and O were low and indicated that little variation was explained by the model (Table 9). The flat nature of the survivorship curves for these groups probably accounts for this (Figure 6).

The initial and final numbers of viable and nonviable young were analyzed for differences between treatments with the nonparametric Kruskal-Wallis test (Hollander and Wolfe, 1973). If significant differences were found (i.e.$\alpha(H) \leq 0.05$) then a nonparametric multiple comparison of all treatments was performed to determine which treatment differed (Hollander and Wolfe, 1973). The data on production of viable and nonviable young are presented as the final total cumulative number in Table 10 because no significant differences were observed in the initial (i.e. after the one instar incubation period) number of viable and nonviable young. In fact in all tests there were no significant differences in the number of initial viable and nonviable young. The raw data on

Table 9. Summary of regression analyses and multiple comparisons of slopes for survivorship curves obtained with 10 C acclimated Daphnia and the SBM. The α levels and r^2 values are listed for each treatment.

Treatment	slope and multiple comparisons[a]	α(F)	r^2
CO	.00086	.036	.18
0	.00196	.0004	.44
12	.00294	.0013	.38
5	.00474	<.0001	.52
8	.0051	<.0001	.67

[a]Any two values not connected by the same line are significantly different. (α = 0.05, experiment wide)

Table 10. Total cumulative production of viable and nonviable young observed in the test and 10 C acclimated Daphnia and the SBM.

Treatment	Total cumulative number of: Viable young[a]	Nonviable young[a]	nonviable total as a %
12	254	23	8.3
8	222	14	5.9
5	220	18	7.5
0	207	8	3.7
CO	265	9	3.2

[a]No significant difference between treatments in viable or nonviable young ($0.5>\alpha(H)>0.1$, in both cases).

the initial and final numbers of viable and nonviable young for all tests are presented in the appendix (Table A1). This may suggest that the LC10 was below any reproductive impairment level, at least after approximately one instar. Although, no significant differences were noted between treatments regarding the total number of viable and nonviable young certain trends were apparent. The incidence of aborted eggs and young in thermal shock groups was approximately twice that of the controls and Group 0 (Table 10). Controls and Group 0 demonstrated a similarity in the incidence of aborted eggs and young. This may reflect the influence of starvation stress in both groups or that the LC10 of SBM was below a reproductive impairment level. Group 0 did produce the smallest number of viable young, although it was not significantly lower than the control values (Table 10). Apparent increases in the production of total young in the thermal shock groups may reflect the influence of short-term thermal additions on the development of the young in the brood chamber.

Combined tests with the SBM at 20 C

Survivorship curves for 20 C acclimated Daphnia pre-exposed to the LC10 of the SBM (0.0056X) and then subjected to short-term thermal shocks are shown in Figure 7. Because all groups (except 12) would appear to survive indefinitely, in these batch tests, tests were terminated at 180 hours

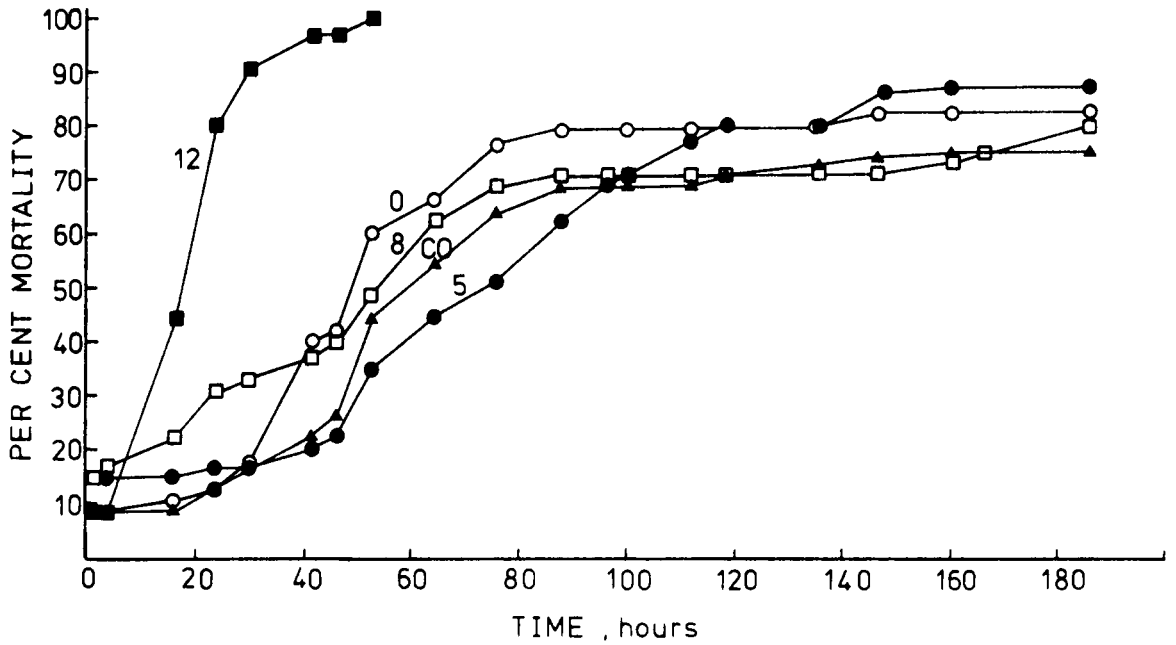

Figure 7. Survivorship curves for groups of 20 C acclimated Daphnia exposed to the LC10 of the SBM and then subjected to short-term thermal shocks (Δt's=+12, +8 and +5.4 C). Group O was pre-exposed to the LC10 and maintained at acclimation temperature. Controls (CO) were maintained in dilution water only.

after thermal shock. The exponential nature of the survivorship curves was more apparent than with the tests on 10 C acclimated organisms. Consequently, the r^2 values, as a measure of goodness of fit to the model, were much higher (Table 11). Mortality at the end of the one instar incubation period was approximately 10% in all groups including the controls. Multiple comparisons of slopes indicated that Group 12 had the highest mortality rate (Table 11). Group 8 showed the smallest mortality rate (slope). However, this was influenced by the relatively high initial mortality (ca. 15%) at time zero. Thus, only a 12 C short-term thermal shock significantly increased mortality rates for Daphnia pre-exposed to the LC10 of the SBM and observed in batch tests under starvation conditions.

No significant differences were noted between all treatment groups regarding the production of viable young (Table 12). Group 12 did produce more nonviable young than any other group. Due to the conservative nature of the nonparametric multiple comparisons, only the number of nonviable young in Group 8 could be shown to be significantly smaller than the Group 12 values (Table 12). The influence of short-term thermal additions and starvation stress, or the combined effects of low levels of SBM starvation stress and short-term thermal additions may have resulted in the high incidence of aborted eggs and young in Group 12. The re-

Table 11. Summary of regression analyses and multiple comparisons of slopes for survivorship curves obtained with 20 C acclimated Daphnia and the SBM. The α levels attained and r^2 values are listed for each treatment.

Treatment	slope and multiple comparisons[a]	α(F)	r^2
8	.0031	<.0001	.73
5	.0043	<.0001	.77
0	.0046	<.0001	.69
CO	.0046	<.0001	.75
12	.0235	<.0001	.68

[a]Any two values not connected by the same line are significantly different (α=0.05, experiment wide).

Table 12. Total cumulative production of viable and nonviable young observed in the test with 20 C acclimated Daphnia and the SBM.

Treatment	Total cumulative number of: viable young[a]	Nonviable young[b]	nonviable total as a %
12	391	36	8.4
8	494	1	0.2
5	302	0	0
0	370	0	0
CO	487	5	1.0

[a]Viable young not significantly different ($0.5>\alpha(H')>0.1$) between treatments.

[b]Incidence of nonviable young between treatments differ ($0.025<\alpha(H')<0.05$). Nonparametric multiple comparisons ($\alpha=0.064$, experiment wide), excluding groups 5 and 0, indicate that group 8 and the controls were not significantly different. However, groups 8 and 12 were.

latively low incidence observed in all other groups may only reflect the influence of starvation stress on reproductive success.

Combined tests with the ARM at 10 C

Survivorship curves for 10 C acclimation Daphnia pre-exposed to the LC10 of the ARM (0.03X) and then subjected to short-term thermal shocks are shown in Figure 8. The test was terminated after 190 hours due to conditioning of the dilution water. All groups exposed to the LC10 (0, 5, 8 and 12) showed significant decreases in survivorship compared to the control group during starvation conditions in these static tests (Figure 8, Table 13). Mortality rates for thermal shock groups (5, 8 and 12) were not significantly higher than the group pre-exposed to the LC10 only (Table 13). Short-term thermal shocks did not appear to decrease survivorship of 10 C acclimated Daphnia pre-exposed to the LC10 of the ARM. The r^2 value for the control group was low (0.38) due to the flatness of this survivorship curve (Figure 8 and Table 13).

No significant differences were observed between all treatment groups regarding the production of viable young (Table 14). An increase in the incidence of aborted eggs and young was evident for groups subjected to short-term thermal shock but this trend was not statistically significant (Table 14).

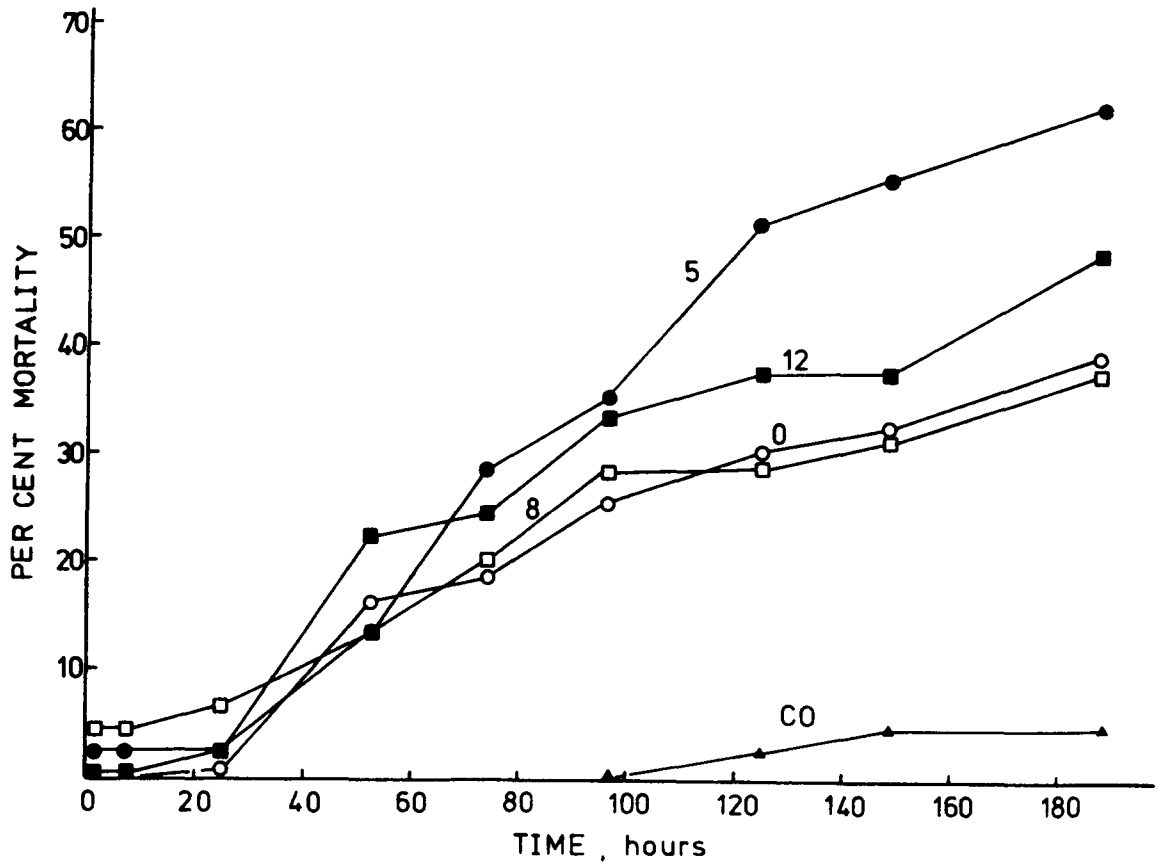

Figure 8. Survivorship curves for groups of 10 C acclimated Daphnia exposed to the LC10 of the ARM and then subjected to short-term thermal shocks (Δt's= +12, +8 and +5.4 C). Group O was pre-exposed to the LC10 and maintained at acclimation temperature. Controls (CO) were maintained in dilution water only.

Table 13. Summary of regression analyses and multiple comparisons of slopes for survivorship curves with 10 C acclimated Daphnia and the ARM. The α levels and r^2 values are listed for each treatment.

Treatment	slopes and multiple comparisons[a]	(F)	r^2
CO	.0011	<.0002	.38
8	.0033	<.0001	.53
0	.0046	<.0001	.66
12	.0053	<.0001	.80
5	.0056	<.0001	.84

[a]Any two values not connected by the same line are significantly different (α=0.05, experiment wide).

Table 14. Total cumulative production of viable and nonviable young observed in the test with 10 C acclimated Daphnia and the ARM

Treatment	Total cumulative number of: viable young[a]	Nonviable young[b]	nonviable total as a %
12	359	22	5.7
8	322	14	4.1
5	357	31	7.9
0	322	7	2.1
CO	399	4	0.9

[a]number of viable young not significantly different ($0.9>\alpha(H)>0.5$) between treatments.

[b]number of nonviable young not significantly different ($0.5>\alpha(H')>0.1$) between treatments.

Combined tests with the ARM at 20 C

Survivorship curves for 20 C acclimated Daphnia pre-exposed to the LC10 of the ARM (0.0075X) and subjected to short-term thermal shocks are shown in Figure 9. The test was terminated ater 120 hours due to conditioning of the dilution water. Multiple comparisons of all treatment regression slopes indicated no significant difference between Group 12 and the controls (Table 15). These two curves were translated, both showing the same slope but quite different intercepts as illustrated by the multiple comparisons of intercepts (Table 15). Thus, the Group 12 survivorship curve was separate from all others. The control group intercept was significantly smaller than all other groups. Control starvation mortality eventually became equivalent to the mortality evident for all other groups, except Group 12. Only a short-term thermal shock of 12 C significantly impaired survivorship of 20 C acclimated Daphnia pre-exposed to sublethal levels of the ARM.

The 20 C acclimated population from which Daphnia were obtained for this test showed a smaller percentage of reproductively active adults (ca. 15%), although the size distribution was comparable with those for all other tests. The relatively small total number of viable young produced by the controls demonstrates this (Table 16). All groups, except Group 12, showed approximately 50% reduction in the

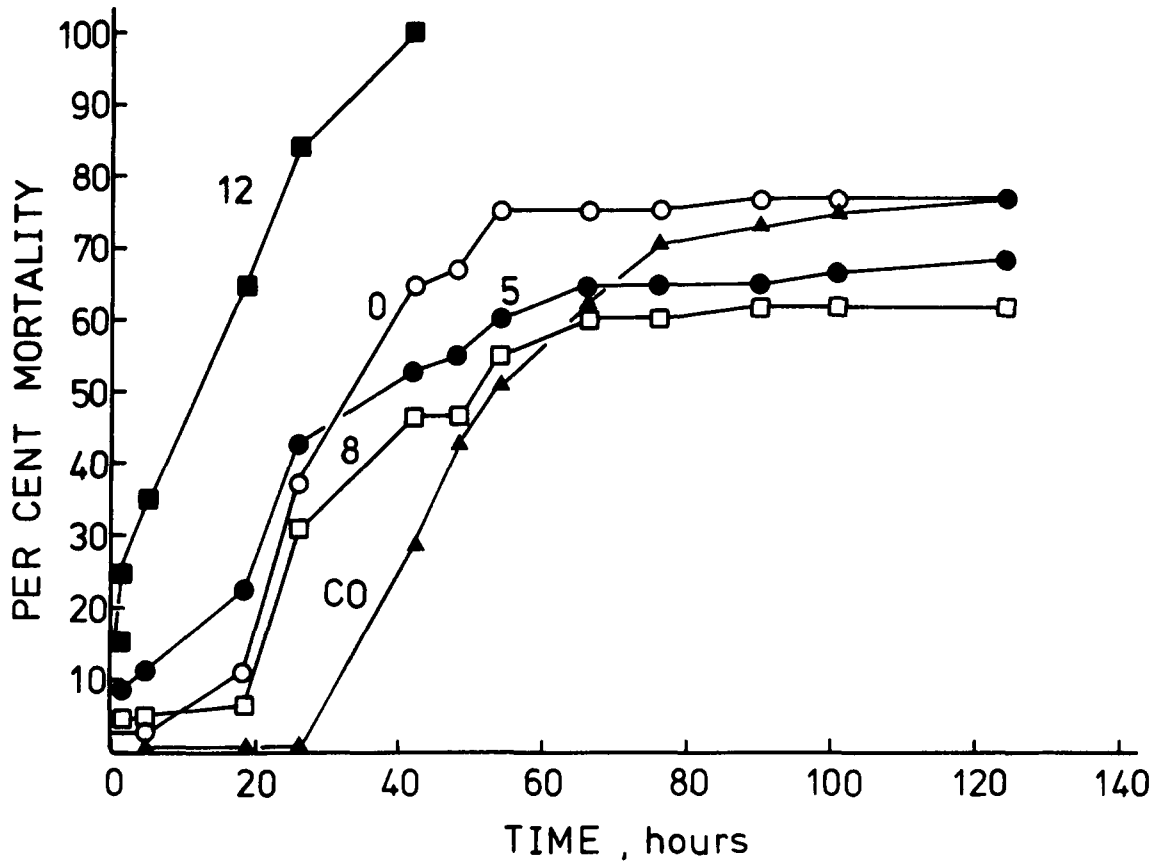

Figure 9. Survivorship curves for groups of 20 C acclimated Daphnia exposed to the LC10 of the ARM and then subjected to short-term thermal shocks (Δt's=+12, +8 and +5.4 C). Group O was pre-exposed to the LC10 and maintained at acclimation temperature. Controls (CO) were maintained in dilution water only.

Table 15. Summary of regression analyses and multiple comparisons of slopes and intercepts for survivorship curves obtained with 20 C acclimated Daphnia and the ARM. The α levels and r^2 values are listed for each treatment

Treatment	slopes and multiple comparison[a]	α(F)	r^2	Treatment	intercepts and multiple comparison[a]
5	.0063	<.0001	.56	CO	.04
8	.0077	<.0001	.68	8	.32
0	.0082	<.0001	.63	0	.39
CO	.0113	<.0001	.78	5	.47
12	.0157	<.0001	.76	12	.63

[a]Any two values not connected by the same line are significantly different (α=0.05, experiment wide).

Table 16. Total cumulative production of viable and nonviable young observed in the test with 20 C acclimated Daphnia and the ARM

Treatment	Total cumulative number of: viable young[a]	Nonviable young[b]	nonviable total as a %
12	9	1	10
8	76	5	6.1
5	30	17	36.1
0	52	1	1.8
CO	124	0	0

[a]Significant differences ($0.025>\alpha(H)>0.01$) in number of viable young between treatments. Nonparametric multiple comparisons ($\alpha=0.06$, experiment wide) revealed that only Group 12 differed from the controls.

[b]Incidence of aborted eggs and young not significantly different ($0.5>\alpha(H)>0.1$) between treatment groups.

number of viable young compared to the control group (Table 16). Group 12 produced fewer than 10% of the control in terms of viable young. The nonparametric multiple comparison procedure indicated that only Group 12 differed significantly from the controls in terms of viable young (Table 16). Group 5 showed the highest incidence of nonviable young but the final number of aborted eggs and young between treatment groups was not significantly different (Table 16).

These results indicate that 10 C acclimated adult Daphnia, pre-exposed to the LC10 of the simulated effluents, withstood short-term thermal additions with little or no apparent reduction in survivorship or impairment of reproduction; at least in static tests. The high incidence of aborted eggs and young noted in groups subjected to sublethal thermal shock and sublethal levels of the effluents may reflect the metabolic costs involved with coping with these conditions. It remains to be seen if this was due to an increased sensitivity of developing young or if it was a direct response by the adults.

Daphnia, acclimated to 20 C, and pre-exposed to sublethal levels of the two simulated effluents withstood thermal shocks of 5 and 8 C with little or no reduction in survivorship. However, 12 C thermal shocks, in both tests, significantly reduced survivorship. An increased incidence of nonviable eggs and young was noted in groups subjected to

short-term thermal additions of 5, 8 and 12 C. Again, this may reflect the high metabolic costs associated with coping with these conditions. Due to the differential sensitivity of 10 and 20 C acclimated Daphnia to both simulated effluents, it might be expected that short-term thermal shocks above ambient would increase the mortality of Daphnia pre-exposed to sublethal levels of the effluents.

Instantaneous short-term thermal shocks (5-60 minutes duration) of up to 10 C above acclimation temperature (24-26 C) had no significant effect on survivorship of Gammarus spp. (Ginn et al., 1976). No reproductive impairment was noted for an 8.3 ΔT (ambient of 26 C), however, a thirty minute instantaneous +11Δt drastically reduced production of young. Survivorship of adults was not affected suggesting the decreased heat tolerance for developing young. Goss and Bunting (1976) indicated that immature Daphnia were not adversely affected by instantaneous temperature changes within their temperature tolerance range. Instantaneous short-term thermal shocks (15 min. duration) of 18 C above an acclimation temperature of 15 C significantly impaired survival and reproduction for adult Daphnia pulex (Craddock, 1976). Survival and reproduction was not affected by short-term exposure to 30 C. Craddock also found that immature Daphnia were more tolerant of thermal alterations than adults. Buikema et al. (1977) demonstrated that short-term instant-

aneous thermal shocks (Δt=+10, acclimation temperature = 20 C) did not significantly impair survivorship of neonate Daphnia pulex compared to those maintained at ambient acclimation temperature when followed in chronic lifetime studies. The thermal shock group did become primiparous one instar earlier than the control group. Burton et al. (1976) studied the effect of a time-temperature regime, simulating once-through entrainment, on the oxygen consumption of a variety of estuarine invertebrates. Organisms were subjected to instantaneous Δt's of +5 C at a variety of acclimation temperatures. No significant differences in pre-and post-thermal shock oxygen consumption rates were observed for several invertebrates, each investigated at several acclimation temperatures. The authors suggested that these estuarine forms might exhibit immediate compensation of rate functions in response to altered temperatures. Several invertebrates (Gammarus sp and Neomysis americana) exhibited overshoots in QO_2 (weight-specific oxygen consumption) fifteen minutes after being subjected to the low Δt regime. Oxygen consumption rates returned to the original pre-thermal shock levels. Overshoots were not considered to be an indication of thermal stress, but rather a normal physiological response.

Relatively few studies have dealt with the effect of sublethal thermal shock upon aquatic organism pre-exposed to sublethal levels of pollutants. Silbergeld (1973) showed that darters (Etheostoma nigrum) pretreated with 2.3 ppb

of the organochlorine insecticde dieldrin demonstrated a higher mortality rate compared to control fish when both were subjected to sublethal increases in temperature (7-9 C above ambient acclimation temperature, applied at a 1 C day^{-1} rate). Stober and Hanson (1974) presented dose-response curves, determined with two species of salmon, suggesting that the tolerance for residual chlorine decreases with increasing magnitude of thermal shock. However, the chlorine levels investigated were acutely lethal not sublethal. Hodson and Sprague (1975), in addition to evaluating zinc toxicity for 3, 11, and 19 C acclimated Atlantic salmon, determined the acute thresholds of zinc for salmon at temperatures other than acclimation temperature. Thresholds obtained depended on the direction and magnitude of thermal shock. Salmon acclimated at 3 C and 11 C were more tolerant of zinc when tested at temperatures 8 C above their respective acclimation temperatures. Conversely, salmon acclimated to 11 and 19 C were less tolerant of zinc when tested at temperatures lower than their respective acclimation temperatures.

Influence of temperature on toxicity: general discussion

Temperature dependence and independence of toxicity has been documented for a wide variety of materials. Earlier literature on the influence of temperature upon the toxicity of pollutants to aquatic organisms has been compiled and reviewed by Middlebrooks et al. (1973) and Cairns et al.

(1975). However, most of the earlier literature does not present complete toxicity curves. Usually toxicity tests of arbitrary duration were reported. Recent examples of acute toxicity-acclimation temperature relationships based on lethal thresholds and/or complete toxicity curves are listed in Table 17. A schematic presentation of the reported toxicity curve-acclimation temperature patterns are shown in Figure 10. Both Table 17 and Figure 10 are meant to portray observed responses and are not meant to apply to all similar compounds. Nor are they meant to suggest that similar species or taxa respond in the same manner.

Temperature independence of acute toxicity has been demonstrated with several toxicants for both vertebrates and invertebrates (Table 17). Toxicity curves are usually separated at least for short-term exposure periods but the lethal thresholds are the same. Acute toxicity-acclimation temperature curves are totated; the axis of rotation being the lethal threshold (Figure 10a). Wilson (1974) indicated temperature independence for toxicity of an oil dispersant to flounder larvae, Pleuronectes platessa, acclimated to 4, 10 and 15 C. However, 20 C acclimated larvae did not appear to exhibit a threshold, at least during the time period studied. Gammarus pseudolimnaeus acclimated to 10 and 15 C showed no difference in lethal thresholds for hydrogen sulfide (Smith et al., 1976). Temperature independence of

lethal thresholds may indicate a temperature independence in the sensitivity of key enzyme systems. Apparent temperature dependencies over short periods of time may only reflect the influence of temperature on uptake mechanisms.

Two general types of temperature dependency were noted, involving instances where warm-acclimated organisms were more or less sensitive than cold-acclimated organisms. Brown (1967) attributed the greated sensitivity of cold-acclimated rainbow trout to phenolics to a lower rate of detoxification compared to the rate of phenolic absorption at lower temperatures. Zinc toxicity curves presented by Hodson and Sprague (1975) determined with 3, 11 and 19 C acclimated Atlantic salmon were somewhat complex. Incipient lethal LC50s for 3 C acclimated salmon were the smallest. Toxicity curves were not simply displaced. The relationship between acute toxicity and acclimation temperature was a combination of translation and rotation of the toxicity curves (Figure 10b). Because of this complexity one could draw three different conclusions about the influence of temperature on the toxicity of zinc (e.g. cold-acclimated are more sensitive; warm-acclimated are more sensitive; and no difference in sensitivity with acclimation temperature) dependent upon the duration of an acute test. Laughlin and Neff (1977) indicated that cold-acclimated eggs and early instar larvae of the horseshoe crab (Limulus polyphemus) were more sensitive to water soluble fractions (WSF) of No. 2 fuel oil than warm-

Table 17. Listing of reported temperature-toxicity patterns for aquatic organisms. Only studies reporting lethal thresholds and/or complete toxicity curves are presented

	Temperature dependence	
Temperature independence	cold-acclimated more sensitive	warm-acclimated more sensitive
Schaefer and Pipes (1973): chromate and arsenate; Philodina	Brown (1967): phenol and phenolics; rainbow trout	Wilson (1974): oil dispersant; 20 C acclimated flounder larvae (Pleuronectes platessa)
Wilson (1974): oil dispersant; 4, 10 and 15 C acclimated amphipods (Gammarus pseudolimnaeus)	Hodson and Sprague (1975): zinc; 3, 11 and 19 C acclimated atlantic salmon	Smith et al. (1976): hydrogen sulfide; 14, 20 and 26 C acclimated goldfish; 8.5-21 C acclimated juvenile brook trout; 14 and 22 C acclimated juvenile and adult crayfish (Procambarus clarkii)
McLeese (1974a); phosphamidon 4 and 12 C acclimated adult lobsters (Homarus americanus)		Present study: simulated cooling tower blowdown and simulated refinery effluent; 10 and 20 C acclimated adult Daphnia pulex
McLeese (1974b): copper; 5 and 13 C acclimated adult lobsters (Homarus americanus)		
Smith et al. (1976): hydrogen sulfide; 10 and 15 C acclimated amphipods (Gammarus pseudolimnaeus)		

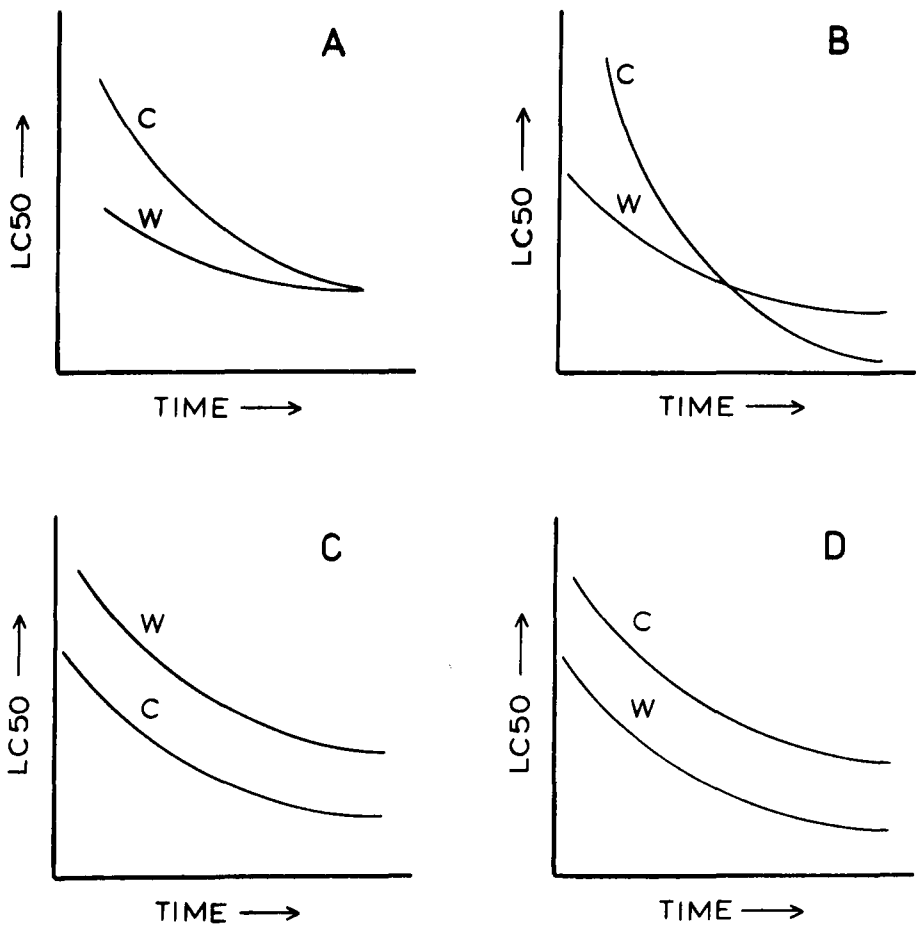

Figure 10. Generalized acute toxicity-acclimation temperature patterns. Patterns shown are only for those materials that demonstrate a threshold effect. C-cold acclimated; W-warm acclimated. See text for discussion.

acclimated ones. This influence of temperature on the toxicity of the WSF was not due to differences in actual exposure concentrations between temperatures. Fucik and Neff (1977) demonstrated a similar pattern with the WSF of a crude oil for a temperate and boreal clam. Both clams showed greater uptake of naphthalenes at lower acclimation temperatures. These authors also showed that the effective concentrations of total naphthalenes was equivalent at all temperatures. Depuration of petroleum hydrocarbons appeared to be temperature independent. However, filtration rate was proportional to acclimation temperature. Thus, uptake rate of total naphthalenes appeared to be inversely proportional to filtering rate. Both of the previous studies did not present toxicity curves. It remains to be seen if certain cold-acclimated estuarine organisms are more sensitive to the WSF's of No. 2 fuel oil and crude oils. Data appear to be lacking on the influence of acclimation temperature on the toxicity of chlorinated hydrocarbons.

Several studies (Peterson, 1973; 1976) have demonstrated cold-induced mortality of fish pre-exposed to DDT analogs. Fish pre-exposed to these compounds will generally select temperatures warmer than acclimation temperature during temperature preference trials.

Koenig et al. (1976) indicated that abrupt decreases from daily mean water temperature correlated with mortality

in DDT contaminated Blue Crabs (Callinectes sapidus). Several mechanisms were proposed to account for this cold-induced mortality. A shift to fat metabolism during cold exposure may have mobilized lipids containing DDT residues. In addition, decreased temperatures may favor the formation of DDT-nerve membrane complexes. Mauck et al. (1976) reported that bluegill sunfish tested in colder waters were more sensitive (based only on 96-h LC50s) to pyrethrins and pyrethroids (Pyrethrum extract, Dimethrin and RU-11679). Perhaps, Figure 10c might describe the acute toxicity-acclimation temperature pattern for certain aquatic organisms and DDT.

Khorram and Knight (1976) showed that the 72-h LC50s of Kelthane, an organochlorine insecticide, for the grass shrimp (Crangon) decreased with increasing acclimation temperature. The authors did not discuss lethal thresholds or present complete toxicity curves. Smith et al. (1976) presented hydrogen sulfide toxicity curves for 14, 20 and 26 C acclimated Goldfish and for juvenile Brook trout acclimated from 8.5 to 21 C. Both species were more sensitive to hydrogen sulfide at higher acclimation temperatures (Figure 10d). Note that the amphipod showed temperature independence for H_2S toxicity while the two fish species were more sensitive at higher acclimation temperatures (Table 17). Temperature dependence of lethal thresholds may reflect changes in the predominance of certain metabolic pathways with acclima-

tion temperature. The complex temperature dependency for zinc toxicity (Hodson and Sprague, 1975) was attributed to the greater sensitivity of isozymes active at lower acclimation temperatures.

Temperature acclimation pattern

Comparison of QO_2 values with other studies

Weight-specific oxygen consumption rates, or QO_2 values (μl O_2/mg dry weight/hour) appeared to be inversely proportional to total dry weight. Strictly speaking, QO_2 refers to volume of oxygen consumed at standard temperature and pressure per unit of dry weight per hour (Prosser, 1973b). Linear regression analyses were employed to describe QO_2 as a function of total dry weight for each set of test conditions. Observed ranges of total dry weight in all tests, except two, included the 0.02 mg value. Comparisons of oxygen consumption rates between tests were facilitated by correcting QO_2 values to 0.02 mg total dry weight. The regression equations describing the relationship between QO_2 and total dry weight at each combination of acclimation and test temperature are presented in the appendix (Table A2).

Corrected QO_2 values for 10 and 20 C acclimated Daphnia assessed at 10, 15 and 20 and 25 C are shown plotted in Figure 11. QO_2 values obtained were higher than the values reported by Richman (1958) and Buikema (1972), for similarly

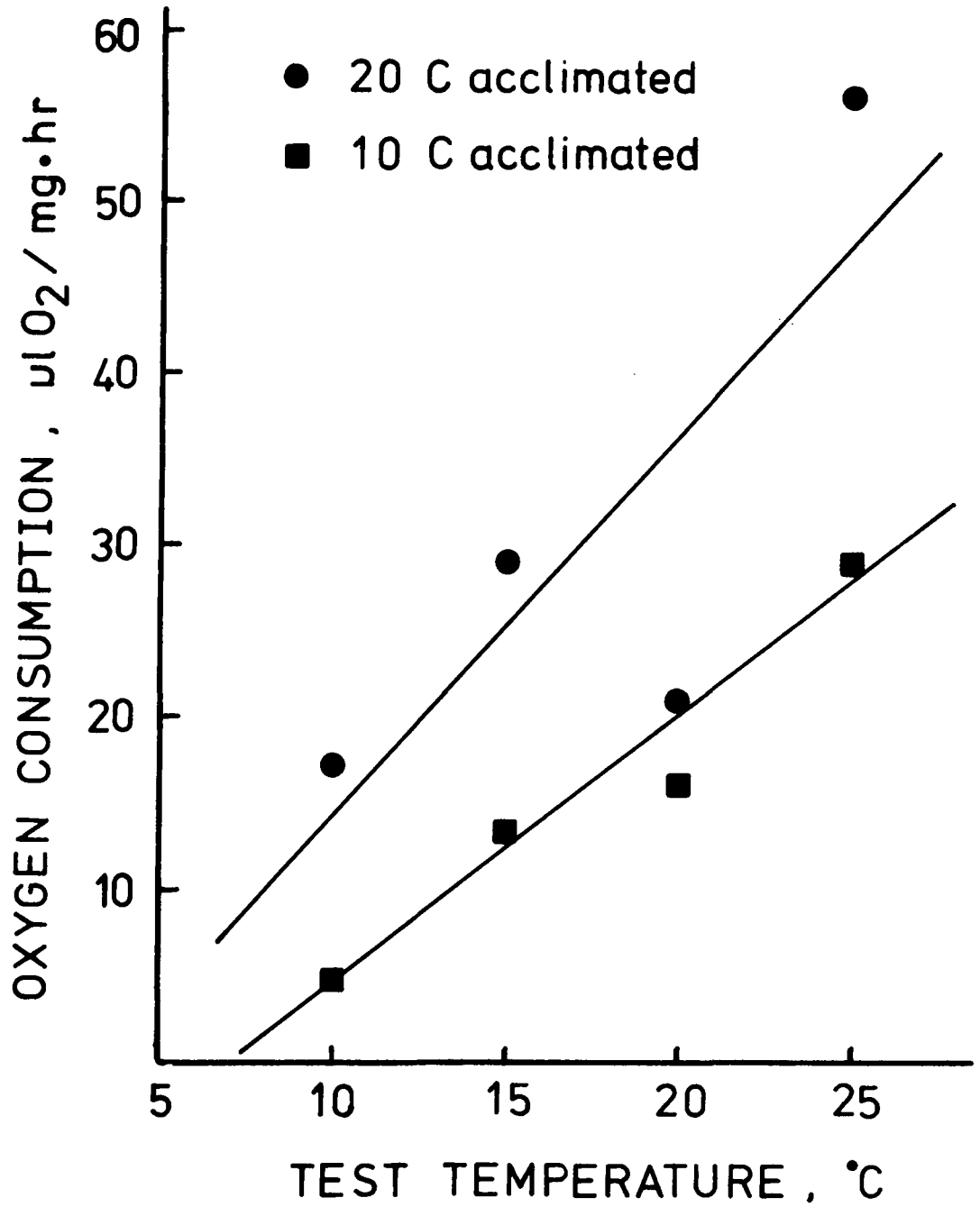

Figure 11. Metabolic rate-temperature curves for 10 and 20 C acclimated Daphnia pulex. Regression equation for 10 C curve was $\overline{QO_2} = 0.00204$ (T C) - 0.0142; $r^2 = 0.93$; $\alpha(F) = 0.035$. Regression equation for 20 C curve: $QO_2 = 0.0028$ (T C) - 0.004; $r^2 = 0.62$; $\alpha(F) = 0.209$.

sized Daphnia pulex. Both authors used the closed bottle technique and assessed rates over a 24 hour time interval. Richman reported a QO_2 value of 5-10 µl O_2/ mg dry weight/ hour for 1.1-1.4 mm Daphnia pulex, acclimated to 20 C and darkness. Buikema indicated that QO_2 values for 1.4 mm D. pulex acclimated to 20 C and a light intensity of 110 ft-c were approximately 11-17 µl O_2/ mg dry weight/hour. Daphnia pulex acclimated to 20 C and a 100 ft-c light intensity demonstrated QO_2 values, corrected to 0.02 mg., of approximately 20 µl O_2/mg dry weight/hour (Figure 11).

Several factors might account for the higher values I obtained. My results were determined over a longer photoperiod (16L:8D) than Buikema's (14L:10D). Very long or short photoperiods appeared to stimulate the parthenogenetic reproduction of a Florida strain of the chydorid, Pleuroxus denticulatus. Parthenogenetic reproduction of a strain from Minnesota was stimulated only by long photoperiods (Shan and Frey, 1968). If late pre-adult instar Daphnia pulex are diverting energy for impending parthenogenetic reproduction and longer photoperiods stimulate these reproductive activities then it is possible that oxygen consumption rates would be higher than those reported for shorter photoperiods.

It is quite possible that physiological race differences may account for the differences in QO_2 values. Differences in the dry weight estimates might account for different QO_2 values. My dry weight values (assuming a dry weight of 0.004

mg for a 1.2 mm D. pulex) underestimate Richman's value for the same size by 60%. Since dry weight determinations were made after an equivalent duration of starvation conditions (36 hours in this study; 48 hours in Richman's study) some other factor should account for the discrepancy. Perhaps, organic constituents (structural and nonstructural) were lost by the Daphnia during exposure to the reagents of the Winkler procedure. Richman titrated a subsample of water that did not contain any Daphnia. Dry weight estimates of D. pulex starved for 48 hours may be 45% lower than the initial well-fed dry weight (Lemcke and Lampert, 1975). Richman (1958) did compare QO_2 values for fed and starved D. pulex over a six day period. His results indicated no significant difference in respiration rates between starved and fed animals even after 6 days of starvation. However, R. Q. values for starved animals steadily decreased suggesting a change in the types of substrates utilized for the production of energy.

Comparisons with other Daphnia studies were not informative due to the wide variety of test conditions and species employed. A recent compilation of respiration rates, for a variety of species, regarding ambient temperature has been presented by Lampert (1977).

The rate versus temperature (R-T) curve for cold-acclimated Daphnia appears to be translated to the right of

the warm-acclimated R-T curve (Figure 11). Thus, the cold-acclimated oxygen consumption rate was lower at all test temperatures. This pattern of acclimation has been described as inverse or paradoxical (Hazel and Prosser, 1970). Linear regressions were fitted to both R-T curves. Due to the displacement from the regression line of the QO_2 value obtained with 20 C acclimated Daphnia tested at 20 C, the lack of fit was apparent (Figure 11). Excluding this value from the analysis the goodness of fit was increased (r^2 = 0.99: $\alpha(F)$ = 0.019). The relationship between QO_2 and test temperature for 10 C acclimated Daphnia was described quite well with linear regression (Figure 11). R-T curves were tested for equality of slopes and intercepts, according to Sokal and Rohlf (1969). Slopes were not shown to be significantly different ($\alpha(F)>0.75$), perhaps implying that the slopes were equal. However, testing the equality of intercepts indicated no significant difference ($\alpha(F)>0.75$). Thus, the two R-T curves could not be shown to be different. The small sample size (N=4) for each curve and the lack of fit for the 20 C curve make it difficult to show differences.

The similarity of QO_2 values obtained with both 10 and 20 C acclimated Daphnia at 20 C (Figure 11) might suggest that 20 C is near the optimal temperature for D. pulex.

Interpretation of noncompensatory acclimation

Many aquatic ectotherms show some degree of metabolic

rate compensation in response to altered temperatures. It is usually evidenced by alterations in metabolic rate functions with temperature that do not follow a Q_{10}, or Arrhenius, relationship. If ambient temperature is increased and certain homeostatic mechanisms are not operative, then a Q_{10} of 2-3 for metabolic rates might be expected (Vernberg and Vernberg, 1970). Classical compensatory acclimations are demonstrated over the major portion of the environmental temperature range by the higher metabolic rate of cold-acclimated organisms compared to warm-acclimated ones (Prosser, 1973a). Physiological activity may be decreased at warmer temperatures to offset the accelerating kinetic effect of warmer temperatures. Conversely, activities may be elevated in the cold to offset the overall decelerating effect of reduced temperatures. This type of whole body metabolic rate compensation has been correlated with compensations in the activities of glycolytic, hexose monophosphate shunt, tricarboxylic acid cycle and electron transport system enzymes (Hazel and Prosser, 1974; Hochachka and Somero, 1973). These metabolic rate compensations are one form of "vectorial homeostasis" (i.e. mediation of both the rates and direction of metabolic reactions during adaptation to altered environmental conditions) (Hochachka and Somero, 1973).

Acclimation patterns will vary seasonally and will depend on nutritional state and photoperiod (Prosser,

1973; Wieser, 1973). In addition, the acclimation pattern obtained with various tissues will not always reflect the whole body acclimation pattern (Prosser, 1973a). The adaptive advantages of noncompensatory acclimations are less readily understood. Wieser (1973) suggested that both compensatory and noncompensatory acclimations may be a part of the homeostatic control of metabolism in ectotherms and that noncompensatory acclimations should not be considered paradoxical.

Hazel and Prosser (1970) presented three explanations that may account for the lack of compensation or for noncompensatory acclimations to temperature. Cold or heat induced torpor may account for a lack of compensation. Daphnia observed in this study appeared equally active in all temperatures. Secondly, oxygen availability and the nutritional state may modify enzymatic activity and override any temperature effect. Ambient dissolved oxygen concentrations will have a marked influence on the respiration rates of Daphnia, but it may vary with the species (Heisey and Porter, 1977). The influence of oxygen availability was assumed to be minimal since dissolved oxygen concentrations in all experimental respirometers were never below 6 mg l^{-1} at the end of a test. The possible influence of nutrition will be discussed later. The last explanation was based mainly on the acclimation patterns observed for various enzymes. As mentioned previously, enzymes showing compensatory patterns are

related to the production of chemical energy. Hazel and Prosser (1970) indicated that enzymes showing no compensation or an inverse acclimation pattern are related to the breakdown and disposition of metabolic intermediates and by products.

McWhinnie and O'Connor (1967) showed that starved premolt crayfish (Orconectes virilis) acclimated at 5 and 18 C demonstrated a lack of compensation or noncompensatory acclimation patterns. Starved intermolt crayfish demonstrated compensatory acclimation patterns. Jungreis and Hooper (1968) reported a combination of noncompensatory translations and rotation for 1, 11, and 21 C acclimated intermolt crayfish (Orconectes virilis). Organisms had been acclimated to these temperatures for 19-24 days. Since feeding was not reported, it was assumed that animals were starved during the acclimation period. R-T curves for 1 and 11 C acclimated crayfish appeared to show inverse translation (i.e. oxygen consumption rates for 11 C acclimated crayfish were greater than rates of 1 C acclimated crayfish at all test temperatures). The 21 C R-T curve showed a significant rotation (i.e. slope for 21 C R-T curve greater than slopes of 1 and 11 C R-T curves). The apparent translation of the 11 C R-T curve, relative to the 1 C R-T curve, might be attributed to a difference in photoperiod. The 11 C group was acclimated to temperature in continuous darkness while the other two groups were acclimated in continuous

light. The authors suggested that noncompensatory acclimation may be adaptive for O. virilis. Significant storage of food reserves may be prevented due to the small size of O. virilis and might indicate they actively forage for food at low ambient temperatures. This explanation assumes that crayfish are equally active at all temperatures. These authors did not take into account the modifying influence of starvation stress on the acclimation pattern.

McCarthy et al. (1976) studied the compensation patterns of 5 and 20 C acclimated premolt and intermolt lobsters (Homarus americanus). The two metabolic rate functions assessed in vitro were RNA and protein synthesis. The R-T curves presented suggested that compensatory acclimation (translation) was evident for both protein synthesis and RNA synthesis in the midgut gland, abdominal muscle and gill tissue of intermolt lobsters. Midgut glands and muscle tissue from premolt lobsters demonstrated a lack of temperature compensation or inverse compensation (i.e. leucine and thymidine incorporation rates for warm-acclimated lobsters were higher at all test temperatures than rates for cold-acclimated lobsters). Protein synthesis in gill tissue showed compensatory acclimation but noncompensatory acclimation was shown for RNA synthesis. They suggested that the lack of compensatory acclimation generally evident in premolt lobsters might be related to the process of molting.

Utilization of stored food reserves through metabolic rate compensation might be maladaptive since the overriding priority in the premolt animal is the completion of molting, a process which utilizes those stored reserves. The authors also suggested that premolt lobsters, at least in temperate latitudes, are rarely subjected to sustained low temperatures, thus obviating the need for compensatory acclimation in response to sustained low ambient temperatures.

R-T curves for 2nd-4th instar, lab reared, mosquito larvae (Culex pipiens pipiens) showed compensatory temperature acclimation (Buffington, 1969). The R-T curves for 15 and 25 C acclimated fourth instar larvae, reared out of doors, revealed noncompensatory acclimation. Buffington suggested the difference was due to induction of genotypic modification by an "uncontrolled environment." Arctic and temperate populations of the zooplankter, Mysis relicta, also showed no metabolic rate compensation over their environmental temperature range (Lasenby and Langford, 1972).

Two general hypotheses might account for the noncompensatory acclimation evident for reproductively immature Daphnia pulex.

It has been suggested that the capacity for compensatory acclimation is lower in organisms that have been exposed to constant temperature regimes (Hazel and Prosser, 1970; Vernberg and Vernberg, 1970). Buffington's results appear to contradict this hypothesis. Daphnia reared in the

laboratory for many generations at relatively constant temperature may have a limited capacity for classical metabolic rate compensation, when compared to populations obtained directly from the field or laboratory populations maintained under a fluctuating temperature regime. Diel vertically migrating populations of Daphnia may traverse temperature gradients up to 12 C, during summer stratification (McNaught and Hasler, 1966; Haney and Hall, 1975).

According to McCarthy et al. (1976) metabolic events such as molting may override compensatory temperature acclimation in premolt lobsters. Reproductively immature Daphnia used in this study were apparently in the last two pre-adult instars. In several tests cast exoskeletons were noted, but ovigerous females were never observed at the end of any test. It would be difficult to determine the influence of molting on the results obtained in the present study. Test durations were relatively short (24 hours) and very few cast exoskeletons were observed at the end of the test period. Metabolic rate of Daphnia does vary with the stage of the molt cycle. Meijering (1960) found that the heart rate, and presumably metabolic rate, of Daphnia magna was highest during ecdysis. The influence of the molt cycle stage upon acclimation pattern could be determined if rates were assessed for very short time period (e.g. 1-2 hours) and measured over successive time periods with known age Daphnia. A readiness

for impending reproductive activities may be the metabolic event that prohibits compensatory acclimation in reproductively immature Daphnia. Another event that may override compensatory acclimation to temperature is starvation stress. Decreases in the amount of stored food during starvation stress may preclude any compensatory increases in metabolic rate during cold acclimation (Vernberg, 1959; McCarthy et al., 1976). Dry weight losses of 30 to 45% may be observed for Daphnia pulex starved for 48 hours (Richman, 1958; Lemcke and Lampert, 1975). Since the crayfish used by Jungreis and Hooper (1968) and McWhinnie and O'Connor (1967) were starved it is quite possible that noncompensatory acclimations are only evident in certain starved ectotherms.

Noncompensatory acclimation to temperature appears to be the pattern for reproductively immature Daphnia pulex reared in the laboratory and assessed under conditions of starvation. Key events such as molting, starvation stress and preparation for impending reproductive activities may preclude compensatory acclimation to temperature. It remains to be seen if this pattern would pertain for field populations or for well fed laboratory specimens.

CONCLUSIONS

1. Acutely lethal thresholds for Daphnia pulex may be estimated with toxicity tests of one instar duration.
2. 20 C acclimated Daphnia were almost twice as sensitive to the SBM compared to 10 C acclimated Daphnia.
3. Survivorship results obtained with oil water dispersions of the ARM were less variable than results obtained with the ARM when oil was added separately to each dilution.
4. 20 C acclimated Daphnia were approximately six times as sensitive to the ARM compared to 10 C acclimated Daphnia.
5. A higher incidence of nonviable eggs and young were noted in groups of 10 and 20 C acclimated Daphnia pre-exposed to the LC10 of both simulated effluents and then subjected to short-term thermal shocks.
6. Survivorship of 20 C acclimated Daphnia pre-exposed to the LC10 of both simulated effluents and then subjected to 12 C short-term thermal shocks was significantly reduced when compared to Daphnia pre-exposed to the LC10 only.
7. Reproductively immature D. pulex showed a noncompensatory temperature acclimation pattern.

LITERATURE CITED

American Public Health Association. 1976. Standard Methods for the Examination of Water and Wastewater. 14th Ed. American Public Health Association, Washington, D. C., 1193 p.

Anderson, B. G. 1944. The toxicity thresholds of various substances found in industrial wastes as determined by the use of Daphnia magna. Sewage Works J. 16(16):1156-1165.

Anderson, B. G. 1946. The toxicity thresholds of various sodium salts determined by the use of Daphnia magna. Sewage Works J. 18:82-87.

Anderson, J. W., J. M. Neff, B. A. Cox, H. E. Tatem and G. M. Hightower. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. (NY), 27:75-88.

Barr, A. J., J. H. Goodnight, J. P. Sall, and J. T. Helwig. 1976. A User's Guide to SAS76. SAS Institute Inc., Raleigh, N. C. 329 p.

Biesinger, K. E. and G. M. Christensen. 1972. Effects of various metals on survival, growth, and reproduction, and metabolism of Daphnia magna. J. Fish. Res. Bd. Can. 29:1691-1700.

Brooks, J. L. 1959. Cladocera. Pages 587-656 in W. T. Edmondson (ed.). Fresh-Water Biology 2nd ed., John Wiley and Sons Inc., New York.

Brown, V. M., D. H. M. Jordan and B. A. Tiller. 1967. The effect of temperature on the acute toxicity of phenol to rainbow trout in hard water. Water Res. 1:587-594.

Buikema, A. L., Jr. 1970. Some Effects of Light on the Biology of the Cladoceran, Daphnia pulex, 1860, emend. Richard, 1896 (in 5 parts). Ph.D. Thesis (Zoology), The University of Kansas, Lawrence, Kansas.

Buikema, A. L., Jr. 1972. Oxygen consumption of the cladoceran, Daphnia pulex, as a function of body size, light and light acclimation. Comp. Biochem. Physiol. 42A:877-888.

Buikema, A. L., Jr., D. R. Lee, and J. Cairns, Jr. 1976. A screening bioassay using Daphnia pulex for refinery wastes discharged into freshwater. J. Testing and Evaluation. 4:119-125.

Buikema, A. L., Jr., S. R. Sherberger, G. W. Knauer, L. A. Newbern, J. T. Reading and J. Cairns, Jr. 1977. Effects of simulated entrainment on the biology of Daphnia pulex (Cladocera). Paper presented at the SREL symposium, Augusta, Georgia Nov. 2-4.

Buffington, J. D. 1969. Temperature acclimation of respiration in Culex pipiens pipiens (Diptera:Culicidae) and the influence of seasonal selection. Comp. Biochem. Physiol. 30:865-878.

Bunting, D. L. and E. B. Robertson, Jr. 1965. Lethal and Sublethal Effects of Herbicides on Zooplankton Species. Research Report No. 43, Water Resources Research Center, The University of Tennessee, Knoxville, Tennessee, 35 p.

Burns, K. A. 1976. Hydrocarbon metabolism in the intertidal fiddler crab Uca pugnax. Mar. Biol. (NY), 36:5-11.

Burton, D. T., L. B. Richardson, S. L. Margrey and P. R. Abell. 1976. Effects of low Δt power plant temperatures on estuarine invertebrates. J. Water Pollut. Control Fed. 48(10):2259-2272.

Cairns, J., Jr., A. G. Heath and B. C. Parker. 1975. The effects of temperature upon the toxicity of chemicals to aquatic organisms. Hydrobiologia 47(1):135-171.

Canton, J. H., P. A. Greve, W. Sloof and G. J. van Esch. 1975. Toxicity, accumulation and elimination studies of α-hexachlorocyclohexane (α-HCH) with freshwater organisms of different trophic levels. Water Res. 9:1163-1169.

Cardwell, R. D., D. G. Foreman, T. R. Payne, and D. J. Wilbur. 1977. Acute and Chronic Toxicity of Chlordane to Fish and Invertebrates. Ecological Research Series, EPA-600/3-77-019, 125 p.

Craddock, D. R. 1976. Effects of increased water temperatures on Daphnia pulex. Fish. Bull. 74(2):403-408.

Dorris, T. C., S. L. Burks and G. R. Waller. 1974. Effects of Residual Toxins in Oil Refinery Effluents on Aquatic Organisms. OWRR Technical Completion Report, OWRR B-025-Okla. Oklahoma Water Research Institute, Oklahoma State University, Stillwater, Oklahoma, 79 p.

Environmental Protection Agency. 1973. Petroleum refining point source category. Effluent limitations guidelines and new source standards. Federal Register, Vol. 38, No. 240, Part II.

Environmental Protection Agency. 1974. Development Document for Proposed Effluent Limitation Guidelines and New Source Performances Standards for the Steam Electric Power Generating Industry. EPA-440/1-74/029-a, 839 p.

Environmental Protection Agency. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecological Research Series, EPA-660/3-75/009, 61 p.

Environmental Protection Agency. 1977a. Supplement for Pretreatment to the Development Document for the Steam Electric Power Generating Point Source Category. EPA-440/1-77/084, 253 p.

Environmental Protection Agency. 1977b. Interim Final Supplement for Pretreatment to the Development Document for the Petroleum Refining Industry Point Source Category. EPA-440/1-77/083a, 115 p.

Finney, D. J. 1971. Probit Analysis. 3rd ed. Cambridge University Press, 333 p.

Fucik, K. W. and J. M. Neff. 1977. Effects of temperature and salinity on naphthalene uptake in the temperature clam, Rangia cuneata and the boreal clam, Protothaca staminea. Pages 305-312 in D. A. Wolfe (ed.). Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Pergamon Press Inc., Elmsford, N. Y.

Garton, R. R. 1972. Biological effects of cooling tower blowdown. Amer. Inst. Chem. Eng. Symp. 129:284-292.

Ginn, T. C., W. T. Waller and G. J. Lauer. 1976. Survival and reproduction of Gammarus spp. (Amphipoda) following short-term exposure to elevated temperatures. Chesapeake Sci. 17(1):8-14.

Goss, L. B. and D. L. Bunting. 1976. Thermal tolerance of zooplankton. Water Res. 10:387-398.

Green, R. H. 1965. Estimation of tolerance over an indefinite time period. Ecology 44:887.

Haney, J. F. and D. J. Hall. 1975. Diel vertical migration and filter-feeding activities of Daphnia. Arch Hydrobiol. 75(4):413-441.

Harter, H. L. 1960. Critical values for Duncan's new multiple range test. Biometrics 16:671-685.

Hazel, J. and C. L. Prosser. 1970. Interpretation of inverse acclimation to temperature. Z. vgl. Physiol. 67:217-228.

Hazel, J. R. and C. L. Prosser. 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiol. rev. 54(3):620-677.

Heisey, D. and K. G. Porter. 1977. The effect of ambient oxygen concentration on filtering and respiration rate of Daphnia galeata mendotae and Daphnia magna. Limnol. Oceanogr. 22(5):839-845.

Hochachka, P. W. and G. N. Somero. 1973. Strategies of Biochemical Adaptation. W. B. Saunders Co., Philadelphia, 358 p.

Hodson, P. V. and J. B. Sprague. 1975. Temperature-induced changes in acute toxicity of zinc to atlantic salmon (Salmo salar). J. Fish. Res. Bd. Can. 32:1-10.

Hollander, M. and D. A. Wolfe. 1973. Nonparametric Statistical Methods. John Wiley and Sons, New York, 503 p.

Jungreis, A. M. and A. B. Hooper. 1968. Physiological properties of cold resistance adaptation in the freshwater crayfish Orconectes virilis. Comp. Biochem. Physiol. 26:91-100.

Khorram, S. and A. W. Knight. 1976. Effects of temperature and kelthane on grass shrimp. J. Env. Engr. Div. (ASCE) 102:1043-1053.

Koenig, C. C., R. J. Livingston and C. R. Cripe. 1976. Blue crab mortality:interaction of temperature and DDT residues. Arch. Environ. Contam. Toxicol. 4:119-128.

Lampert, W. 1977. Studies on the carbon balance of Daphnia pulex De Geer as related to environmental conditions III. Production and production efficiency. Arch. Hydrobiol. Suppl. 48(3/4):336-360.

Lasenby, D. C., and R. R. Langford. 1972. Growth, life history, and respiration of Mysis relicta in an arctic and temperate lake. J. Fish. Res. Bd. Can. 29:1701-1708.

Laughlin, R. B., Jr., and J. M. Neff. 1977. Interactive effects of temperature, salinity shock and chronic exposure to No. 2 fuel oil on survival, development rate and respiration of the horseshoe crab, Limulus polyphemus. Pages 188-191 in D. A. Wolfe (ed.). Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Pergamon Press, New York.

Lee, D. R. 1976. Development of an Invertebrate Bioassay to Screen Petroleum Refinery Effluents Discharged into Freshwater. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 106 p.

Lemcke, H. W. and W. Lampert. 1975. Veranderungen im Gewicht und der Chemischen Zusammensetzung von Daphnia pulex im Hunger. Arch. Hydrobiol. Suppl. 48(1):108-137.

Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96:99-113.

Macek, K. J., M. A. Lindberg, S. Sauter, K. S. Buxton, and P. A. Costa. 1976. Toxicity of Four Pesticides to Water Fleas and Fathead Minnows. Ecological Research Series, EPA-600/3-76/-099, 57 p.

Maki, A. W. and H. E. Johnson. 1975. Effects of PCB (Aroclor 1254) and p,p' DDT on production and survival of Daphnia magna Strauss. Bull. Environ. Contam. Toxicol. 13(4): 412-416.

Matson, J. V. 1977. Treatment of cooling tower blowdown. J. Env. Eng. Div. (ASCE) 103:87-99.

Mauck, W. L., L. E. Olson and L. L. Marking. 1976. Toxicity of natural pyrethrins and five pyrethroids to fish. Arch. Env. Contam. Toxicol. 5:18-29.

Maulbetsch, J. S. and R. W. Zeren. 1976. Technology of power plant cooling. Pages 21-37 in Report of a Workshop on the Impact of Thermal Power Plant Cooling Systems on Aquatic Environments. Electric Power Research Institute, (EPRI), Special Report Vol. II.

McCarthy, J. F., A. N. Sastry and G. C. Tremblay. 1976. Thermal compensation in protein and RNA synthesis during the intermolt cycle of the american lobster, Homarus americanus. Biol. Bull. 151:538-547.

McLeese, D. W. 1974a. Toxicity of phosphamidon to american lobsters (Homarus americanus) held at 4 and 12 C. J. Fish. Res. Bd. Can. 31:1556-1558.

McLeese, D. W. 1974b. Toxicity of copper at two temperatures and three salinities to the american lobster (Homarus americanus). J. Fish. Res. Bd. Can. 31:1949-1952.

McNaught, D. C. and A. D. Hasler. 1966. Photoenvironments of planktonic crustacea in Lake Michigan. Verh. Internat. Verein. Limnol. 16:194-203.

McWhinnie, M. A. and J. D. O'Connor. 1967. Metabolism and low temperature acclimation in the temperature crayfish Orconectes virilis. Comp. Biochem. Physiol. 20:131-145.

Middlebrooks, E. J., M. J. Gaspar, R. D. Gaspar, J. H. Reynolds and D. B. Porcella. 1973. Effects of Temperature on the Toxicity to the Aquatic Biota of Waste Discharges--A Compilation of the Literature. PRWG105-1, Utah Water Research Laboratory, Logan, Utah, 170 p.

Meijering, M. P. D. 1960. Herzfrequenz und Herzschlagzahlen zwischen Hautung und Eiablage bei Cladoceren. Z. wiss. Zool. 164(1/2):127-142.

Moore, S. F. and R. L. Dwyer. 1974. Effects of oil on marine organisms: A critical assessment of published data. Water Res. 8:819-827.

Nebeker, A. V. and F. A. Puglisi. 1974. Effect of polychlorinated biphenyls (PCB's) on survival and reproduction of Daphnia, Gammarus and Tanytarsus. Trans. Amer. Fish. Soc. 103(4):722-728.

Nemerow, W. L. 1971. Liquid Waste of Industry--Theories, Practices and Treatment. Addison-Wesley Publishing Co., Reading, Mass., 584 p.

Peterson, R. H. 1973. Temperature selection of atlantic salmon (Salmo salar) and brook trout (Salvelinus fontinalis) as influenced by various chlorinated hydrocarbons. J. Fish. Res. Bd. Can. 30:1091-1097.

Peterson, R. H. 1976. Temperature selection of juvenile atlantic salmon (Salmo salar) as influenced by various toxic substances. J. Fish. Res. Bd. Can. 33:1722-1730.

Prosser, C. L. 1973a. Temperature. Pages 362-428 in C. L. Prosser (ed.). Comparative Animal Physiology. W. B. Saunders Co., Philadelphia.

Prosser, C. L. 1973b. Oxygen:respiration and metabolism. Pages 165-211 in C. L. Prosser (ed.). Comparative Animal Physiology. W. B. Saunders Co., Philadelphia.

Ramger, R. C. 1972. Laboratory Studies on the Comparison of Instar Duration, Reproduction, and Sensitivity to Cadmium in Lake and Pond Populations of Daphnia laevis. Ph.D. dissertation, The University of Tennessee, Knoxville, Tennessee, 68 p.

Reid, G. W. and L. E. Streebin. 1972. Evaluation of Waste Waters from Petroleum and Coal Processing. Environmental Protection Technology Series, EPA-R2-72-001, 205 p.

Rice, S. D., J. W. Short and J. F. Karinen. 1977. Comparative oil toxicity and comparative animal sensitivity. Pages 78-94 in D. A. Wolfe (ed.). Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Pergamon Press, New York.

Richardson, D. 1973. Evaluation and Application of a Cell Culture System to Detect Toxicity in Oil-Refinery Effluents. Ph.D. Dissertation, Oklahoma State University, Stillwater, Oklahoma, 88 p.

Richman, S. 1958. The transformation of energy by Daphnia pulex. Ecol. Monogr. 28(3):273-291.

Robertson, S. D. 1971. The Effects of Temperature on Instar Duration and Egg Development of Daphnia pulex Leydig. M.S. Thesis, University of Tennessee, Knoxville, Tennessee, 55 p.

Sanders, H. O. and O. B. Cope. 1966. Toxicities of several pesticides to two species of cladocerans. Trans. Amer. Fish. Soc. 95(2):165-169.

Sanders, H. O. 1970. Toxicities of some herbicides to six species of freshwater crustaceans. J. Water Pollut. Control Fed. 42(8):1544-1550.

Sayrs, R. L., Jr. 1975. Lethal and Sublethal Effects of Cadmium on Daphnia (Crustacea:Cladocera). M.S. Thesis, The University of Tennessee, Knoxville, Tennessee, 54 p.

Schaefer, E. D. and W. O. Pipes. 1973. Temperature and the toxicity of chromate and arsenate to the rotifer, Philodina roseola. Water Res. 7:1781-1790,

Schober, U. and W. Lampert. 1977. Effects of sublethal concentrations of the herbicide atrazin on growth and reproduction of Daphnia pulex. Bull. Environ. Contam. Toxicol. 17(3):269-277.

Schultz, T. W. and J. R. Kennedy. 1976. Cytotoxic effects of the herbicide 3-amino-1,2,4-triazole on Daphnia pulex (Crustacea:Cladocera). Biol. Bull. 151:370-385.

Shan, K. R. and D. G. Frey. 1968. Induced interbreeding between two stocks of a chydorid cladoceran. BioScience 18(3):203-205.

Silbergeld, E. K. 1973. Dieldrin. Effects of chronic sublethal exposure on adaptation to thermal stress in freshwater fish. Environ. Sci. Technol. 7(9):846-849.

Smith, L. L., Jr., D. M. Oseid, I. R. Adelman and S. J. Broderius. 1976. Effect of Hydrogen Sulfide on Fish and Invertebrates Part I-Acute and Chronic Toxicity Studies. Ecological Research Series, EPA-600/3-76/062a, 286 p.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco, 776 p.

Sprague, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3:793-821.

Stephan, C. E. 1977. Methods for calculating an LC50. Pages 65-84 in F. L. Mayer and J. L. Hamelink, (eds.). Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. American Society for Testing and Materials, Philadelphia.

Stober, Q. J. and C. H. Hanson. 1974. Toxicity of chlorine and heat to pink (Oncorhynchus gorbuscha) and chinook salmon (O. tshawytscha). Trans. Amer. Fish. Soc. 103 (3):569-576.

Stratton, C. L. and G. F. Lee. 1975. Cooling towers and water quality. J. Water Pollut. Control Fed. 47(7): 1901-1912.

Vanderhorst, J. R., C. I. Gibson and L. J. Moore. 1976. The role of dispersion in fuel oil bioassay. Bull. Environ. Contam. Toxicol. 15(1):93-100.

Vernberg, F. J. 1959. Studies on the physiological variation between trophical and temperate zone fiddler crabs of the genus Uca. III. The influence of temperature acclimation on oxygen consumption of whole organisms. Biol. Bull. 117:582-593.

Vernberg, F. J. and W. B. Vernberg. 1970. Aquatic invertebrates. Pages 1-14 in G. C. Whittow (ed.). Comparative Physiology of Thermoregulation Vol. I. Invertebrates and Nonmammalian Vertebrates. Academic Press, New York.

Wells, P. G. and J. B. Sprague. 1976. Effects of crude oil on american lobster (Homarus americanus) larvae in the laboratory. J. Fish. Res. Bd. Can. 33:1604-1614.

Wilson, K. W. 1974. Toxicity testing for ranking oils and oil dispersants. Pages 11-22 in L. R. Beynon and E. B. Cowell (eds.). Ecological Aspects of Toxicity Testing of Oils and Dispersants. Halstead Press, New York.

Winner, R. W., T. Keeling, R. Yeager and M. P. Farrell. 1977. Effect of food type on the acute and chronic toxicity of copper to Daphnia magna. Freshwater Biol. 7:343-349.

Wieser, W. 1973. Temperature relations of ectotherms: A speculative review. Pages 1-23 in W. Wieser (ed.). Effects of Temperature on Ectothermic Organisms Ecological Implications and Mechanisms of Compensation. Springer-Verlag, New York.

APPENDIX

Table A1. Initial and final numbers of viable and nonviable young in combined tests

	SBM 10 C acclimation				
	TREATMENT				
Initial:	12	8	5	0	CO
viable	42, 58, 33	25, 50, 25	33, 44, 48	23, 43, 60	38, 37, 42
nonviable (after 120 h)	3, 0, 5	5, 2, 0	2, 0, 0	0, 0, 0	0, 0, 2
Final: viable	96, 86, 72	90, 71, 61	66, 56, 98	51, 78, 78	84, 79,102
nonviable	10, 1, 12	6, 7, 1	6, 6, 6	2, 6, 0	0, 3, 6

	SBM 20 C acclimation				
	TREATMENT				
Initial:	12	8	5	0	CO
viable	156, 60, 69	61,160, 94	49, 22,132	50, 41,103	97, 79,162
nonviable (after 72 h)	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
Final: viable	194,101, 96	105,235,154	83, 38,181	121, 95,154	139,145,203
nonviable	14, 7, 15	0, 0, 1	0, 0, 0	0, 0, 0	4, 1, 0

Table A1. - continued

ARM 10 C acclimation

Initial: (after 120 h)	TREATMENT 12	8	5	0	CO
viable	34, 45, 41	22, 29, 56	16, 59, 46	41, 13, 22	32, 26, 26
nonviable	0, 0, 0	5, 0, 0	0, 3, 1	0, 0, 0	0, 0, 0
Final: viable	99,137,123	87, 84,151	71,178,108	188, 58, 76	149,173, 77
nonviable	4, 7, 11	9, 1, 4	2, 17, 12	4, 1, 2	2, 1, 1

ARM 20 C acclimation

Initial: (after 72 h)	TREATMENT 12	8	5	0	CO
viable	1, 6, 0	24, 11, 5	11, 3, 0	15, 5, 6	20, 15, 10
nonviable	0, 0, 0	2, 0, 0	1, 4, 0	0, 0, 1	0, 0, 0
Final: viable	3, 6, 0	30, 28, 18	16, 14, 0	27, 8, 17	56, 40, 28
nonviable	1, 0, 0	3, 2, 0	11, 6, 0	0, 0, 1	0, 0, 0

Table A2. Relationship between QO_2 and total dry weight observed for each combination of acclimation and test temperature

Temperature		Range of dry weights(mg)[a]	LINEAR REGRESSION				QO_2--corrected to 0.02 mg. dry weight[c]
Acclimation	Test		Equation	N	$\alpha(F)$[b]	r^2	
10	10	0.03 -.07	QO_2=-66.25(DW)+6.049	4	.0294	.942	4.72
10	15	0.012-.02	QO_2=-2465.1(DW)+63.04	6	.0008	.953	13.74
10	20	0.01 -.03	QO_2=-1253.38(DW)+41.14	4	.066	.728	16.07
10	25	0.003-.017	QO_2=-609.55(DW)+40.16	4	.385	.377	27.97
20	10	0.009-.03	QO_2=-1748.2(DW)+52.3	5	.118	.610	17.33
20	15	0.013-.058	QO_2=-713.38(DW)+43.68	5	.0148	.895	29.41
20	20	0.022-.039	QO_2=-639.35(DW)+33.49	6	.0394	.694	20.70
20	25	0.008-.039	QO_2=-3100.8(DW)+118.72	6	.0275	.742	56.70

[a] dry weight -- total for 5 *Daphnia*

[b] alpha level attained for the regression (F-test)

[c] QO_2-- as µl O_2/ mg dry weight 5 *Daphnia*/hour

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THE EFFECTS OF TEMPERATURE ON THE SENSITIVITY OF *Daphnia pulex* TO TWO SIMULATED INDUSTRIAL EFFLUENTS

by

Matthew Jerome McGinniss

ABSTRACT

The purpose of this study was to assess the effect of temperature on the sensitivity of mixed age adult *Daphnia pulex* to a simulated cooling tower blowdown and a simulated refinery effluent. In addition, the metabolic rate compensation capability of *Daphnia* exposed to temperature was determined.

An artificial refinery mixture (ARM) simulated the 1977 guidelines promulgated by EPA. Components of the ARM in mg ℓ^{-1} were: ammonia (10), chromium (0.25), oil (10), phenol (0.1), sulfide (0.17) and suspended solids (20). Components of the simulated blowdown mixture (SBM) in mg ℓ^{-1} were: zinc (2), phosphate (25), chromate (15), sulfate (824) and boron (0.5).

After 72 hours at 20 C and 120 hours at 10 C, the median apparent threshold LC50s of the SBM were 0.045 at 10 C and 0.026 at 20 C. Similarly, threshold LC50s of the ARM were 0.106 at 10 C and 0.016 at 20 C.

Mixed age adult *Daphnia* were pre-exposed to sublethal levels (LC10) of the SBM and the ARM at both 10 and 20 C and then subjected to Δt's above ambient of 5.4, 8 and 12

C for one hour. One other group received sublethal exposure only and the controls were maintained only in dilution water. After exposure survival and the number of viable and nonviable young were assessed. Daphnia pre-exposed to both simulated effluents and short-term thermal shock had significantly more nonviable eggs and young. Survival of 20 C Daphnia pre-exposed to both simulated effluents and subjected to a 12 C thermal shock was significantly reduced.

Reproductively immature Daphnia showed a noncompensatory temperature acclimation pattern. Several hypotheses were presented to explain the adaptive nature of the noncompensatory pattern observed.