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EFFECTS OF WATER HARDNESS AND TEMPERATURE ON TOXICITY OF DETERGENTS TO THE FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII* DE MAN

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ABSTRACT

The toxicity of detergents to giant freshwater prawns varied considerably according to the hardness of the water in which the prawns were treated. The 96-h LC_{50} (96-hour median lethal concentration) values of soft detergent were, respectively, 21.9, 18.2 and 18.1 mg/l in soft, moderate and hard waters, whereas the 96-h LC_{50} values of hard detergent were, respectively, 61.7, 54.7 and 54.4 mg/l in soft, moderate and hard waters. The detergent was more toxic in hard water than in soft water. Increasing water temperature increased considerably the toxicity of detergent to prawns. At 23°, 28° and 33°C, the 96-h LC_{50} values of soft detergent were, respectively, 20.9, 18.2 and 11.8 mg/l, and of hard detergent were, respectively, 59.5, 54.7 and 41.3 mg/l.

INTRODUCTION

Detergents are common pollutants found in natural waterways because of the surfactants' slow biodegradation. The slow breakdown allows relatively high surfactant concentration to be discharged in many effuents, which results in unsightly foam that frequently develops and persists on waterways. Residues of surfactants in the environment may have biological effects and create problems, especially causing toxicity to aquatic animals. Their potential hazard to aquatic life depends on biodegradability and waste treatment (HERBERT et al., 1957; SWISHER, 1970), type of surfactant (DOOLEY & CAVIL, 1964), species (THATCHER, 1966) and numerous environmental and biological factors (MARCHETTI, 1965). The most important environmental factors related to lethal threshold concentrations of surfactants are dissolved oxygen, water hardness, acclimation to surfactants and temperature (HOKANSON & SMITH, 1971). Water hardness is an important factor in the toxicity of many poisons, and the toxicity of detergents has been variously reported to increase, decrease or be unaffected by increasing the level of water hardness (HENDERSON et al., 1959; CAIRNS & SCHEIER, 1962; WURTZ & BRIDGES, 1961).

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This studv reports on the effects of water hardness and temperature on the toxicity of anionic detergents to the giant freshwater prawn, *Macrobrachium rosenbergii.*

MATERIALS AND METHODS

Juvenile giant freshwater prawns. *M. rosenbergii,* with a range of 1.5 – 2.0 cm in body length (edge of orbital to tip of telson), were obtained from Bang Pakong Fishery Station, Chachoengsao Province. The prawns were held in 41×77×42-cm glass aquaria containing continuously aerated tap water. The qualities of the tap water were as follows: total hardness as $CaCO_3$ 95.2 ± 4.2 mg/l, methyl orange alkalinity 84.2 ± 4.1 mg/l (measured by sulphuric acid titrimetric method) (APHA, 1980), dissolved oxygen 7.1 ± 0.46 mg/l (measured by azide modification of the Winkler method) (APHA, 1980), pH 8.2 ± 0.14, temperature 27.2° ± 1.1°C and surfactant 0 mg/l.

Water hardness was found by measuring the total concentration of bivalent cations using the the ethylene-diaminetetraacetic acid method (EDTA) (APHA, 1980). Three different tvpes of water were used: soft, moderately hard and hard water. Soft water (54.9±3.9 mg/l) consisted of 50 parts of distilled water and 50 parts of tap water, moderately haru water (95.2 ± 4.2 mg/l) was just tap water, and hard water (171.4 ± 3.5 mg/l) was obtained by adding 192.0 mg/l $NaHCO_3$, 120.0 mg/l $CaCO_4 2H_2O$, 120.0 mg/l $MgSO_4$ and 8.0 mg/l KCl to tap water (EPA, 1975).

Three water temperatures, 23°, 28° and 33°C, were selected and were maintained by means of a constant temperature control waterbath (Forma Scientific Model 2095) in which the test containers were immersed (WEISS, 1955).

The detergents used were household powder detergents of alkylbenzene sulphonate (ABS) and linear alkylbenzene sulphonate (LAS) types. The approximate surfactant levels of ABS and LAS detergents were 30.6% and 23.6%, respectively, as measured by the methylene blue active substance method (MBAS) (APHA, 1980).

The prawns were acclimatized in glass aquaria in the laboratory for at least 14 days (TOVELL et al., 1974). They were fed daily with a commercial fish food. Excess food and waste products were removed from the aquaria every day using a siphon. Water in stock aquaria was changed twice a week to remove waste, bacterial and fungal contamination. The prawns were not fed 2 days prior to exposure and during experimentation.

Five prawns at the intermolt stage from the acclimatization aquaria were placed in each 1.5 – l experimental glass container using a stratified randomization method (SPRAGUE, 1969). Each experiment consisted of 5 concentrations and a control and was replicated 3 times. Since the prawns were aggressive and tended to be cannibalistic, each was isolated in a cylindrical steel mesh cage in the aquaria. The exposure time was 96 h. The test solutions were replenished 48 h after exposure by siphoning.

Analyses for dissolved oxygen, pH and alkalinity were determined for each test initially and at the end of the test period. The 96-h LC_{50}, 95% confidence intervals and slopes of probit lines were determined for each experiment by the method described by FINNEY (1970). The median lethal concentration (LC_{50}) was defined as the concentration at which 50% of the experimental prawns died during the exposure time.

RESULTS

No prawns died in the control treatments in any experiment. In every detergent concentration tested, the prawns began to die 8 h after exposure. The rates of mortality increased with increased concentration and exposure time. Behavioural reactions in the order of their appearance were: decreasing activity, stopping swimming, clinging to the mesh separaters, trying to stay on the water surface for respiration, sinking to the bottom, and death.

The acute toxicity of detergents on *M. rosenbergii* at 3 different levels of water hardness is shown in Table 1, and the mortality rates of *M. rosenbergii* exposed to various concentrations of detergents at 3 different water hardnesses for 96 h are shown in Figs. 1 and 2. The 96-h LC_{50} values of soft detergent in soft, moderate and hard waters were, respectively, 21.9, 18.2 and 18.1 mg/l MBAS, and of hard detergent in soft, moderate and hard waters were, respectively, 61.7, 54.7 and 54.4 mg/l MBAS. The detergent was more toxic in hard water than in soft water.

The acute toxicity of detergents on *M. rosenbergii* at 3 different water temperatures in shown in Table 2, and the mortality rates of *M. rosenbergii* exposed to various concentrations of detergents at 3 different water temperatures for 96 h are shown in Figs. 3 and 4. At 23°, 28° and 33° C, the 96-h LC_{50} values of soft detergent were, respectively, 20.9, 18.2 and 11.8 mg/l MBAS, and of hard detergent, respectively, 59.5, 54.7 and 41.3 mg/l MBAS. The toxicity of detergent increased with increasing water temperature.

The toxicity curves of soft and hard detergents on *M. rosenbergii* at 3 different water hardnesses and 4 different exposure times are shown in Figs. 5 and 6. Toxicities increased with increasing exposure time. For hard detergent, the toxicity to prawns exposed in hard water increased more sharply than others with increasing exposure time (Fig. 6).

The toxicity curves of soft and hard detergents on *M. rosenbergii* at 3 different water temperatures and 4 different exposure times are shown in Figs. 7 and 8. Toxicities increased with increasing temperature and exposure time. For soft detergent, the toxicity of prawns exposed at 33°C increased more sharply with increasing exposure time than others (Fig. 7).

For soft detergent at different water hardnesses, the slopes of the LC_{50} values versus time regression lines did not differ significantly from each other ($F_{2,6}$ = 1.43), but slopes for different water temperatures did differ significantly ($F_{2,6}$ = 33.63). This suggests that water hardness has a constant effect of the LC_{50} value over time,

Table 1. Acute toxicity of detergents on survivorship of *M. rosenbergii* at 3 different water hardnesses.

Detergent	Water hardness level	96-h LC_{50} (mg/1)	95% confidence intervals	Slopes of probit lines
Soft detergent	Soft	21.9	20.39 – 23;47	12.09
	Moderate	18.2	16.88 – 19.64	9.85
	Hard	18.1	16.40 – 19.91	7.55
Hard detergent	Soft	61.7	59.01 – 64.60	20.44
	Moderate	54.7	50.84 – 58.80	15.24
	Hard	54.4	50.22 – 58.93	11.21

Table 2. Acute toxicity of detergents on survivorship of *M. rosenbergii* at 3 different water temperatures.

Detergent	Water temperature (C°)	96-h LC_{50} (mg/1)	95% confidence intervals	Slopes of probit lines
Soft detergent	23	20.9	19.73 – 22.23	12.22
	28	18.2	16.88 – 19.64	9.85
	33	11.8	10.30 – 13.63	6.05
Hard detergent	23	59.5	57.11 – 62.30	17.92
	28	54.7	50.84 – 58.80	15.24
	33	41.3	39.82 – 42.74	23.82

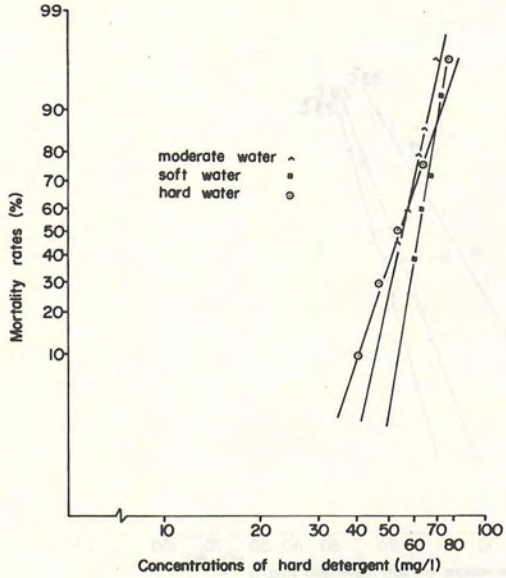

Figure 1. Mortality rates of *M. rosenbergii* exposed to various concentrations of soft detergent at 3 different water hardness levels for 95 hours.

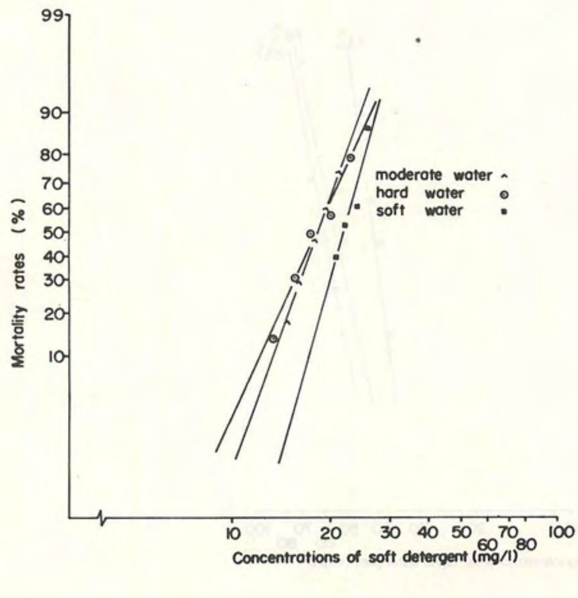

Figure 2. Mortality rates of *M. rosenbergii* exposed to various concentrations of hard detergent at 3 different water hardness levels for 96 hours.

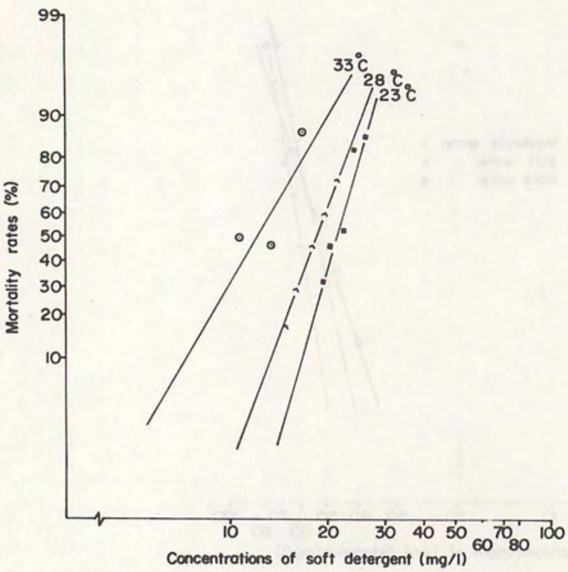

Figure 3. Mortality rates of *M. rosenbergii* exposed to various concentrations of soft detergent at 3 different water temperatures for 96 hours.

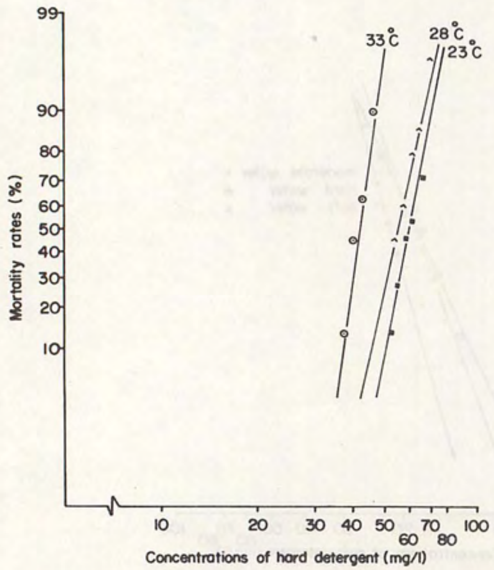

Figure 4. Mortality rates of *M. rosenbergii* exposed to various concentrations of hard detergent at 3 different water temperatures for 96 hours.

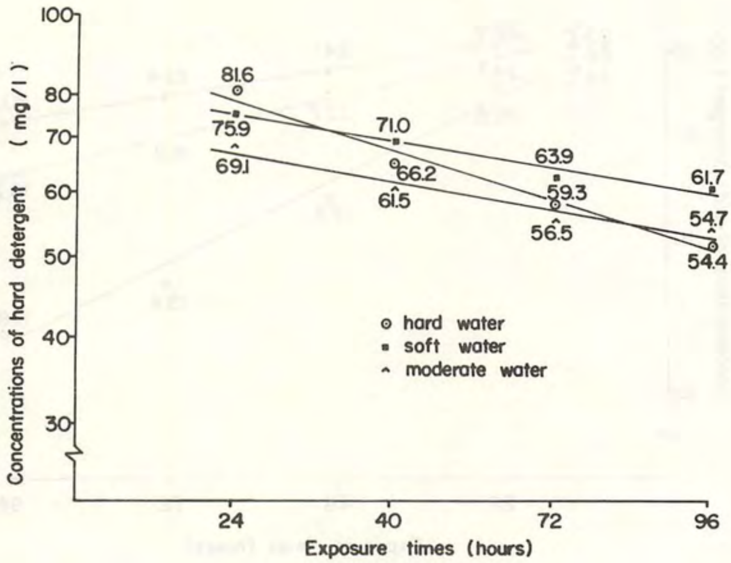

Figure 5. Toxicity curves of soft detergent on *M. rosenbergii* at 3 different levels of water hardness and 4 different exposure times (28°C).

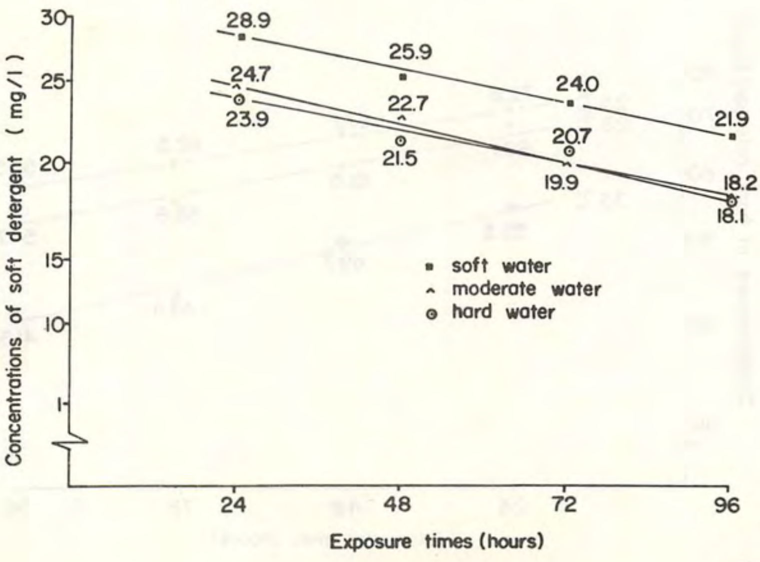

Figure 6. Toxicity curves of hard detergent on *M. rosenbergii* at 3 different levels of water hardness and 4 different exposure times (28°C).

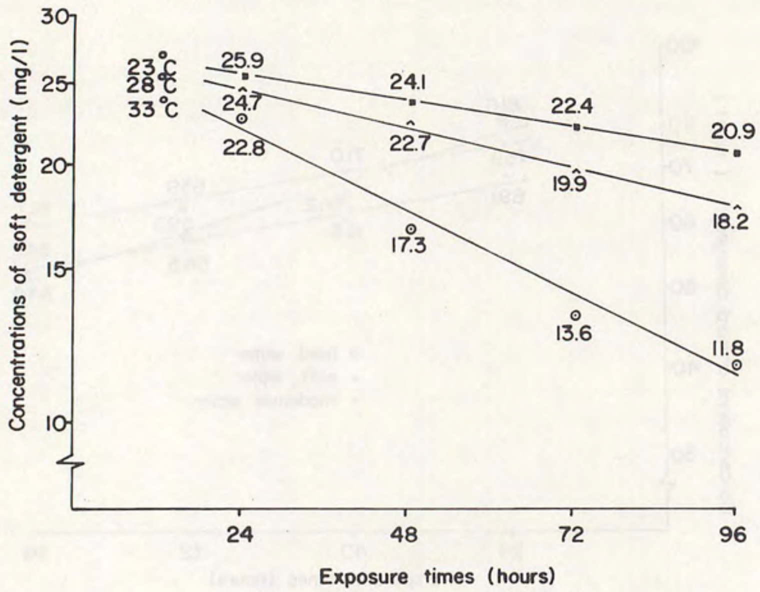

Figure 7. Toxicity curves of soft detergent on *M. rosenbergii* at 3 different water temperatures and 4 different exposure times (moderate water hardness).

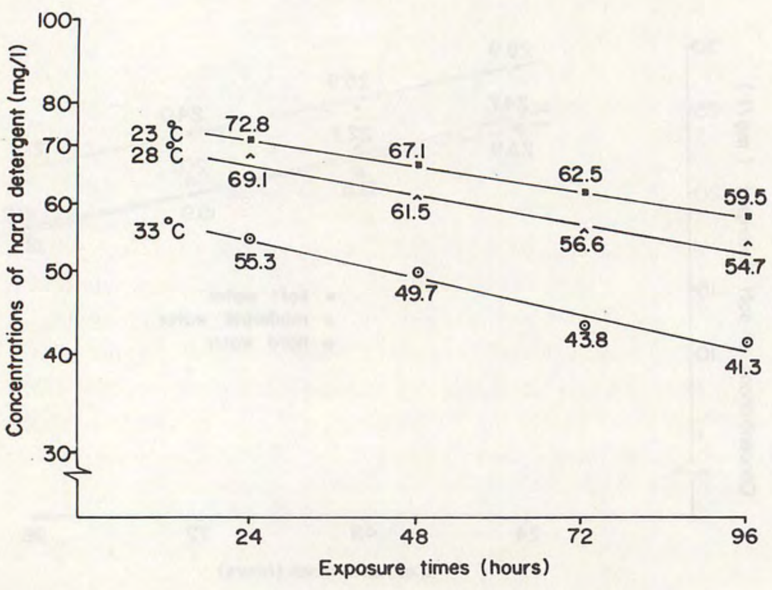

Figure 8. Toxicity curves of hard detergent on *M. rosenbergii* at 3 different water temperatures and 4 different exposure times (moderate water hardness).

only the initial concentrations differing, but that water temperature does not have such a constant effect.

For hard detergent at different water hardnesses and temperatures, the slopes of the LC_{50} values versus time did not differ significantly from each other ($F_2=2.17$ for water hardness, $F_{2,6}=1.54$ for water temperature, $F_{4,10}=5.46$ for both). This suggests that water hardness and temperature have a constant effect over time on the LC_{50} values, only the initial concentrations differing.

Comparisons of toxicity curves between soft and hard detergents using 3 different water hardnesses at 23°C showed that the slopes of the LC_{50} values of both detergents did not differ significantly from one another ($F_{1,4}=3.07$ for moderate water hardness, $F_{1,4}=0.59$ for soft water, $F_{1,4}=0.91$ for hard water, $F_{1,4}=0.06$ for 23°C), but at 33°C the slopes of the LC_{50} values of both detergents differed significantly from one another ($F_{1,4}=23.95$).

DISCUSSION

This study has shown that the toxicity of detergents to freshwater prawns was affected by water hardness, and was more pronounced in hard water than in soft water. HENDERSON et al. (1959) reported that ABS was more toxic to fathead minnows in hard water, sodium alkly sulphate was more toxic in soft water, and that toxicity of a nonionic ethoxylate was unaffected by water hardness. HOKANSON & SMITH (1971) found that LAS was significantly less toxic to bluegill in soft water than in hard water. ABEL (1974) showed that ABS and LAS were more toxic to carp in hard water than in soft water. TOVELL et al. (1974) recently reported that the anionic detergent sodium lauryl sulphate (SLS) was more acutely toxic to rainbow trout and goldfish in hard water than in soft water. The authors explained that in hard water, fish absorbed more anionic detergent due to an acute change in the permeability of the gill, or to a change in the availablility of detergent for absorption due to some interaction with dissolved Ca^{2+}. The presence of Ca^{2+} or other divalent cations did not affect the toxic response to detergent by action on the diffusion membranes of the gill; instead the toxic response relied on an interaction between the ions in the solution and the detergent which in turn modified the solubility and/or diffusion characteristic of the detergent itself. MARCHETTI (1965) stated that the increased toxicity of ABS in the presence of Ca^{2+} or other divalent cations could be due to the formation of ABS-Ca-ABS by replacement of sodium from Na-ABS. Although these findings concerned fish, a similar phenomenon might possibly be occurring with prawns.

This study has also shown that the toxicity of detergents to freshwater prawns increases with increasing water temperature. MARCHETTI (1965) reported that a significant change in the threshold concentration occurred with increasing water temperature. High water temperature causes a pollutant to be more toxic to animals

(SPRAGUE, 1970), and thereby their resistance to diseases becomes lower (JONES, 1964). The sensitivity of the prawns to detergents increases at high water temperature, basically owing to decrease in oxygen concentration. According to MILLER (1977), more water must be passed over the gills and, therefore, more toxic substance passes over the permeable membrane. The immediate cause of death from acute detergent poisoning where extensive gill damage occurs is likely to be either asphyxiation or loss of osmotic or ionic stability (ABEL & SKIDMORE, 1975). GARRETT (1972) explained that the capacity of the skin to resist the action of surfactants depends on the structure of the surfactant molecule, its concentration, the time of contact, the concentration of additional electrolytes, and the temperature, and that increase of temperature markedly increases the rate of adsorption (interaction between surfactant and skin proteins).

The solubility of detergents increases with increasing water temperature, and LAS is generally more soluble than ABS, especially at or below room temperature. Consequently, the solubility and rate of adsorption of detergents by epithelial cells of gills and the surface body of the prawns increases with increasing water temperature, thus rendering them more sensitive to detergents.

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