CULTURING AND TOXICITY TESTING WITH THE NEW ZEALAND MYSID *TENAGOMYSIS NOVAE-ZEALANDIAE*, WITH A SUMMARY OF TOXICOLOGICAL RESEARCH IN THIS GROUP

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ABSTRACT

Conditions for laboratory culturing and toxicity testing (acute and short-term chronic) were evaluated for the New Zealand mysid *Tenagomysis novae-zealandiae*. Appropriate laboratory culture and test conditions were established in the absence of toxicants. Mortality and growth were examined as test endpoints. Static culture systems with weekly water renewal were established, and the influence of photoperiod, salinity, density, and feeding regimens was analysed. Mysid sensitivity in acute tests with sewage samples was similar to that of amphipod mortality tests and echinoid embryo development tests, but slightly lower than algae microplate growth tests. The Microtox® system was less sensitive than the mysid acute tests. Survival was a more sensitive endpoint than growth in preliminary short-term chronic tests with zinc and SDS. A brief literature review on the use of mysids in toxicity testing is also presented.

Key words: mysid, culture, toxicity, SDS, zinc

INTRODUCTION

Mysids and penaeid shrimps have generally been found to be the most sensitive estuarine crustaceans in acute toxicity tests (Cripe 1994). The use of mysids in toxicity testing has been reviewed by Nimmo and Hamaker (1982), and a review of papers published since then is presented herein.

Long- and short-term chronic toxicity tests with mysids were developed by several authors, with survival, reproduction and growth as endpoints. The effect of the transference of toxicants through the food chain on mysid survival, reproduction and growth was also analysed (Van Sprang *et al.* 1991). A simple apparatus to be used for continuous flow tests was described and demonstrated in tests with naphthalene using *Neomysis americana* (Smith and Hargreaves 1983), and a more sophisticated system for the preparation of stock solution and conducting flow-through tests with toxicants with low solubility and high volatility was used with *Mysidopsis bahia* and other groups of marine and freshwater organisms (Sousa *et al.* 1995).

Several mysid species have been used for toxicity testing around the world. *M. bahia* is the preferred test species in eastern United States, where it was used in acute toxicity tests with a variety of effluents (Fisher *et al.* 1989; Bleckman *et al.* 1995; Burgess *et al.* 1995), organic compounds such as industrial surfactants (Hall *et al.* 1989) or phthalate esters (Adams *et al.* 1995), sewage sludge (Santoro and Fikslin 1987), and formulations such as drilling muds (Gaetz *et al.* 1986). *Holmesimysis costata* is the most common mysid species in toxicity testing on the North-American west coast, where it has been used for toxicity assessments with effluents, single chemicals and oil dispersants (Martin *et al.* 1989; Singer *et al.* 1990, 1991). Some other mysid species occurring in North America have been shown to be useful and sensitive in acute toxicity tests with kraft pulp mill effluents (*Neomysis integer* - Jacobs and Grant 1974) and with a variety of metals (*Neomysis mercedis* - Brandt *et al.* 1993). *N. integer* was also suggested for use in European estuarine toxicity testing (Emson and Crane 1994), as was *Siriella armata* in tests conducted in France using cadmium

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(Birmelin *et al.* 1995). In South America, *Mysidopsis juniae* and *Mysidium gracile* have been identified as suitable toxicity test species (Nipper *et al.* 1990, 1993; Reynier 1996). In addition, the arctic mysid, *Mysis oculatus*, was shown to be more resistant to lead and zinc than its "relatives" from warmer waters (Chapman 1993).

Metabolic rates were also identified as useful endpoints for toxicity tests with mysids, eg., the effect of naphthalene on oxygen consumption by *N. americana* (Smith and Hargreaves 1984), of benzene on the swimming activity of *M. juniae* (Martinez *et al.* 1992), or of the defoliant DEF (McKenney *et al.* 1991) and of Cd (Carr *et al.* 1985) on the energy metabolism of *M. bahia*.

Gentile *et al.* (1982b) developed a model using *M. bahia* life-cycle tests with mercury and nickel, suggesting the use of life-tables for the assessment of effects of chronic concentrations of pollutants on populations, and defining a quantitative relationship between chronic pollutant exposure and population increase.

The present study had the purpose of identifying a New Zealand mysid species suitable for laboratory culturing and acute and chronic toxicity testing. The influence of several variables on mysid culturing and toxicity testing was assessed. The species selected was *Tenagomysis novae-zealandiae* Thomson 1900, which occurs on both the North and the South Island, New Zealand (Tattersall 1923, Hodge 1964). It has a life cycle of approximately 4 weeks, and remained reproductive year-round in laboratory cultures, allowing its use in toxicity testing at any time.

The reference toxicants zinc and SDS were used for the assessment of the suitability of the species for toxicity tests. Acute toxicity tests with sewage effluents were also conducted to compare the sensitivity of *T. novae-zealandiae* with other marine toxicity test methods.

MATERIALS AND METHODS

Mysids were collected with a dip net by wading during low tide in Raglan Harbour and at the mouth of the Waikato River, on the west coast of the North Island, New Zealand (Fig. 1). Collected organisms were placed into 20 L buckets lined with plastic bags, containing seawater from the sampling site, and transferred to the laboratory inside ice chests to prevent overheating. In the laboratory, mysids were sorted with a wide mouthed Pasteur pipette and placed into 18 L tanks with sand filtered seawater diluted to the salinity of the sampling site with deionised water, and kept at 20 ±4°C and a photoperiod of 16:8 h light:dark. Mysids were initially fed recently hatched brine shrimp nauplii *ad libitum*. If the original salinity was lower than natural seawater, the salinity in the culture tanks was increased by 3 ppt/day until reaching 34 ± 1 ppt. Several species of *Tenagomysis*, a very abundant and widespread genus around the New Zealand coast (Tattersall 1923), were assessed, such as *T. macropsis*, *T. producta*, *T. scotti* and *T. novae-zealandiae*. After a series of preliminary experiments analysing culture conditions and survival in the laboratory, the type species for the genus, *T. novae-zealandiae*, was selected for further testing.

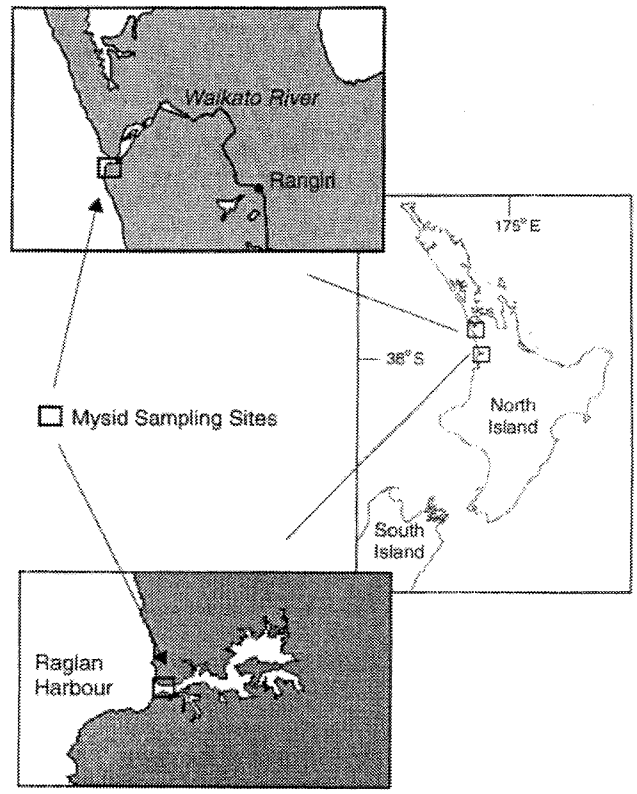

Figure 1. *Mysid sampling locations on the North Island, New Zealand.*

Culture conditions

The influence of the following culture conditions on mysid survival over a two-week period was analysed:

a) Frequency of water renewal (once or twice/week) in semi-static cultures with 20 mysids/L, and in a recirculating system with biological filter.

b) Mysid density (10 or 20 mysids/L), with weekly water renewal.

Experiments were conducted in 18-L clear plastic tanks containing sand-filtered seawater at 34 ± 1 ppt salinity. Prior to initial use, the tanks were soaked in fresh water with daily renewal for at least one week. General experimental conditions were: 20 ± 1°C temperature, gentle aeration through an air-stone, 16:8 h light:dark photoperiod, and light intensity of 0.50 watts/m^2 provided by a shade cloth cover over the tanks. One-day old laboratory born mysids were used, and survival in each tank was assessed weekly, by siphoning most of the water from the tanks and counting the live mysids while gently transferring them with a wide-mouthed Pasteur pipette to tanks containing clean seawater. The siphon had a fine mesh sieve attached to one end to prevent mysids of being siphoned out of the tank. *Tenagomysis novae-zealandiae* in culture experiments were fed daily from Monday through Thursday with 150 recently-hatched brine shrimp nauplii per mysid, and triple the amount of food was added to each tank on Friday.

Testing conditions

Static tests were conducted with juvenile (pre-reproductive) mysids, in 250 mL high-density polyethylene cups with 200 mL dilution water and no aeration. Survival was assessed at 96 hours.

The following experiments were conducted for the assessment of appropriate acute testing conditions.

a) Salinity: mysid survival was analysed in salinities between 5 and 35 ppt, at 5 ppt intervals. Test organisms were fed 100 brine shrimp nauplii per mysid daily.

b) Lighting conditions: the effect of complete darkness vs 16:8 h light:dark photoperiod was analysed in 96-h and 14-day experiments, at 30 ± 1 ppt salinity. With the 16:8 h light:dark photoperiod, two conditions were used: cups with clear cover or covered with a shade-cloth, providing 0.72 and 0.07 watts/m^2 light intensity inside the cups, respectively, during light hours. Test organisms were fed daily with 100 recently-hatched brine shrimp nauplii per mysid.

c) Feeding: mysids were fed 5 diets: recently hatched brine-shrimp nauplii, or 24-hour old brine shrimp nauplii enriched over-night with:

- 7.5×10^5 cells of the microalga *Isochrysis galbana*/mL
- 0.2 mL of cod liver oil
- 0.2 mL of marine fish oil, or
- 0.1 mL of cod liver oil and 0.1 mL of fish oil.

Brine shrimp were enriched by addition of the different enrichment media to a small volume of water, vigorous mixing and subsequent addition to beakers containing 24-h old nauplii. Strong aeration was provided, and nauplii were left in contact with the enriched media overnight. They were then filtered and washed with seawater prior to their addition to the test cups. The test was conducted at 30 ± 1 ppt. Test organisms were fed 100 brine shrimp nauplii per mysid daily.

d) Density: 3, 5, 7 or 10 pre-reproductive mysids were added to test cups with 200 mL dilution water. Test organisms were fed daily with 100 brine shrimp nauplii enriched with cod liver oil per mysid.

Growth

The growth curve of *T. novae-zealandiae* was analysed. Organisms for the growth experiment were obtained by adding 360 adults to 18-L tanks lined with 1 mm mesh netting, and removing the netting with the adults after 24 hours. The juveniles less than 24-h old were able to pass through the netting and remained in the tanks, from which they were transferred to 18-L tanks with clean seawater, at a density of 20 mysids/L. Twelve mysids were preserved daily for measurement and growth curve analysis. This test was conducted under static renewal conditions with weekly water change and feeding. Growth was assessed by length measurements from the tip of the rostrum to the base of the telson, with a digital analyser.

Acute toxicity

Acute 96-hour mortality toxicity tests were conducted in 250 mL cups containing 200 mL of test solution, with 3-5 replicates per treatment. Each replicate contained five organisms, fed daily with 100 recently hatched brine shrimp nauplii per mysid. This food type was selected following international methodology (eg., Martin *et al.* 1989), and some tests were conducted prior to the feeding experiments. Therefore, the same food type and amount was used throughout the acute tests for consistency, although at a later stage it was established that brine shrimp enriched with fish and cod liver oil would be a better food source. The test was conducted at 20 ± 1°C, with 16:8 h light:dark

photoperiod, 0.07 watts/m^2 light intensity, and no aeration. Dissolved oxygen and pH were measured at the beginning and end of the experiments at least in the control and in the lowest toxicant concentration that caused 100% mortality. Dissolved oxygen was between 70 and 100% saturation, and pH was between 7.7 and 8.3 in all measured treatments. Mysids were considered dead when no movement was observed for 30 seconds after gentle prodding with a glass rod.

Static acute toxicity tests with reference toxicants of analytical grade (zinc, as $ZnSO_4.7H_2O$ and sodium dodecyl sulfate - SDS) were conducted at three different salinities: 5, 20 and 34 ppt. The test concentrations were spaced in logarithmic scale, ranging between 0.18 and 1.0 mg/L for zinc, and 10 and 58 mg/L for SDS, except at 5 ppt, where SDS concentrations were between 1 and 18 mg/L.

The acute toxicity of two sewage effluent samples was analysed at 35 ppt salinity and compared to the sensitivity of toxicity test methods performed in our laboratory with other marine species. The original salinity of the effluent was 0 ppt, and it was adjusted to 35 ppt by addition of brine, prepared by freezing seawater and collecting the first thawed fraction. The brine had 80 and 96 ppt salinities in tests 1 and 2, respectively. Further dilutions of the salinised effluent were prepared by addition of full strength seawater. A "brine control", prepared by dilution of brine with deionised water to the highest brine concentration used in the test, showed no toxicity. This suggests that no adverse effects observed in the experiments should be attributed to the brine. Other tests conducted in our laboratory, using the same sewage effluent samples, were: Microtox, (Microbics Corporation 1988), microalgae 48-h growth inhibition test with the diatom *Minutocellus polymorphus* (Walsh *et al.* 1988) using the microplate technique (Environment Canada 1992), 96-h acute mortality test with the indigenous amphipod *Chaetocorophium lucasi* (ASTM 1988), and echinoid embryo development using the indigenous sand-dollar *Fellaster zelandiae* (Nipper *et al.* 1997).

Chronic toxicity

Preliminary 7-day short-term chronic toxicity tests with zinc and SDS were conducted for growth assessment, starting with 7-d old organisms. It was observed by the growth curve that this time frame was sufficient to promote significant growth under control conditions. Static-renewal tests were conducted under the same temperature and lighting conditions of the acute tests, 34 ppt salinity, and no aeration. Test solutions were renewed daily in the SDS test, and after 72 hours in the zinc test. Each test consisted of only two dilutions and a control, with three replicates per treatment. Toxicant concentrations were selected based on acute toxicity test results. Survival and growth were assessed at test termination.

Statistical analysis

Data were analysed for normality and homoscedasticity. These assumptions were met for the growth data and for the chronic toxicity tests. Results were therefore analysed by ANOVA, and significant differences between means ($p<0.05$) were identified by the Tukey's multiple comparisons method. Data from culture and test conditions experiments were not homoscedastic, due to 100% survival in all replicates of some treatments, resulting in zero variance. Therefore non parametric statistics were required, and results were analysed for significant differences between means ($p<0.05$) by the Kruskal Wallis ANOVA by ranks.

Acute toxicity test LC50 values were calculated by the trimmed Spearman Karber method (Hamilton *et al.* 1977) with Abbot's correction. The LC50 values of acute tests were considered significantly different from each other if there was no overlap of their confidence intervals (Reish and Oshida 1987).

RESULTS

Culture conditions

Water renewal frequency and mysid density in culture tanks did not significantly affect survival over 14 days ($p=0.44$), although the tanks with a recirculating system presented the lowest survival rate (Table 1). Therefore, mysids to be used in further tests were cultured in 18-L tanks with 20 mysids/L, weekly water renewal and mild aeration, kept in the dark. Mysids were fed with cod liver oil enriched brine shrimp nauplii. Temperature in the culture room was maintained at 20 ± 3°C.

Testing conditions

Mysid survival in salinities varying from 5 to 35 ppt was not significantly different after 96 hours ($p=0.58$) or 6 days ($p=0.52$) (Table 2), although there was a trend for higher survival at 15 ppt, with a decrease to below 70% after 6 days at salinities below 15 and above 25 ppt.

Lighting conditions did not significantly affect mysid survival either after 96 hours ($p=0.06$) or 14 days ($p=0.15$) (Table 3). After two weeks, however, survival was relatively low (73%) in the light:dark cycle, suggesting that complete darkness could provide better culture conditions.

Table 1. *Survival of* T. novae-zealandiae *over 14 days under different water-renewal conditions and organism densities.*

Culture system	Mysid density (organisms/L)	% survival ± SD
Semi-static: weekly water renewal	10	84 ± 10 (n*=2)
Semi-static: weekly water renewal	20	84 ± 9 (n*=4)
Semi-static: twice weekly renewal	20	83 ± 0 (n*=2)
Recirculating with biological filter	20	74 ± 6 (n*=2)

*n = no of replicates for each treatment

Table 2. *Survival of* T. novae-zealandiae *at different salinities.*

Salinity (ppt)	% survival ± SD (n*=3)	
	96 hours	6 days
5	73 ± 11	67 ± 11
10	87 ± 11	60 ± 20
15	93 ± 11	87 ± 11
20	80 ± 0	73 ± 11
25	87 ± 23	80 ± 20
30	80 ± 0	67 ± 11
35	80 ± 20	60 ± 35

*n = no of replicates for each treatment

Table 5. *Survival of* T. novae-zealandiae *with different organism density per test cup.*

Mysid number/cup	% survival ± SD (n*=3)	
	96 hours	7 days
3	100 ± 0	89 ± 19
5	100 ± 0	87 ± 11
7	90 ± 8	86 ± 14
10	90 ± 10	80 ± 0

*n = no of replicates for each treatment

Table 3. *Survival of* T. novae-zealandiae *under different lighting conditions.*

Lighting conditions	% survival ± SD (n*=6)	
	96 hours	14 days
24-hr dark	100 ± 0	93 ± 10
16:8-hr light:dark (0.72 watts/m^2 light intensity)	93 ± 10	73 ± 21
16:8-hr light:dark (0.07 watts/m^2 light intensity)	87 ± 10	80 ± 22

*n = no of replicates for each treatment

Table 4. *Survival of* T. novae-zealandiae *under different diets.*

Food type	% survival ± SD (n*=3)	
	96 hours	7 days
Recently hatched *Artemia* nauplii	67 ± 58	58 ± 52
Artemia nauplii enriched with *I. galbana*	75 ± 22	42 ± 7
Artemia nauplii enriched with cod liver oil	75 ± 33	62 ± 45
Artemia nauplii enriched with marine fish oil	83 ± 19	58 ± 19
Artemia nauplii enriched with cod liver + fish oil	87 ± 12	67 ± 7

*n = no of replicates for each treatment

Table 6. *Acute 96-hour toxicity tests results with* T. novae-zealandiae *(LC50, with 95% confidence intervals in parentheses), and mortality rates under control conditions.*

Toxicant	**Salinity (ppt)**	**LC50 (CI)** (mg/L chemical; % effl.)	**Control mortality** (%)
Zn (mg/L)	5	0.78 (0.70-0.87)	0
	20	0.88 (0.74-1.05)	24
	34	0.68 (0.53-0.88)	13
SDS (mg/L)	5	20.3 (16.7-29.6)	0
	20	20.6 (18.5-23.0)	6
	34	15.4 (13.2-17.8)	3
Sewage effluent (%)	34	1.85 (1.55-2.25)	5
Sewage effluent (%)	35	1.76 (1.33-2.32)	11.5

Survival of *T. novae-zealandiae* tended to be higher in the treatments where *Artemia* nauplii were enriched with cod liver and marine fish oil, rich in essential fatty acids, but no significant differences in survival were observed with the different food types, either after 96 hours ($p=0.98$) or 7 days ($p=0.67$) (Table 4).

Mysid density in the test cups did not significantly affect survival either after 96 hours ($p=0.15$) or after 7 days ($p=0.85$) (Table 5).

Growth

Tenagomysis novae-zealandiae grew steadily over 33 days. On day 7, length had increased significantly ($p<0.05$) relative to the initial size, and on day 11 it had again increased significantly relative to day 7 (Fig. 2). Based on this result, a duration of 7 days, using 7-day old mysids, was chosen for short-term chronic testing.

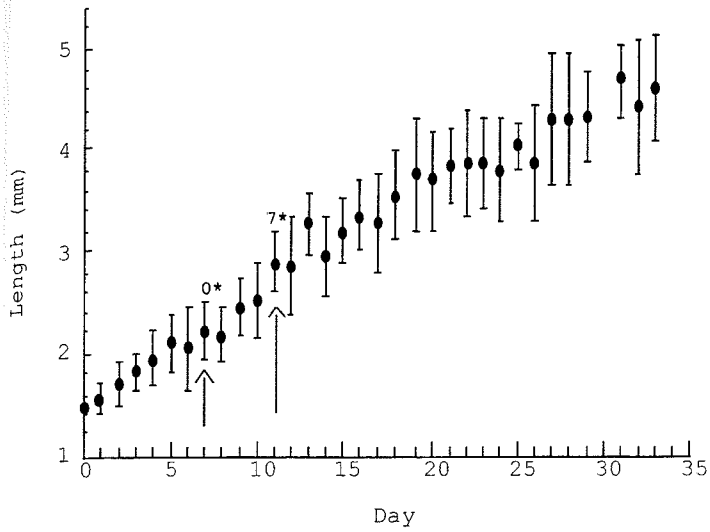

Figure 2. *Growth curve of T. novae-zealandiae, with daily mean length and standard deviation. 0* = significantly different from day 0; 7* = significantly different from day 7.*

Acute toxicity

The LC50 values in acute 96-h mortality tests with zinc and SDS at different salinities were not significantly different, with overlapping confidence intervals (Table 6) (Reish and Oshida 1987).

The mysid test was one of the most sensitive methods to sewage effluent samples, in comparison with a variety of other marine toxicity test methods. Its sensitivity was comparable not only to the acute 96-hour amphipod mortality test with *C. lucasi*, but also to the short-term chronic echinoid embryo development test with the sand dollar *F. zelandiae*. It was only slightly less sensitive than the microalgae growth test with *M. polymorphus*. Microtox® was the least sensitive of the test methods to these effluent samples (Fig. 3).

Chronic toxicity

Survival was a more sensitive endpoint than growth, with increased mortality in concentrations where growth was not significantly affected (Fig. 4).

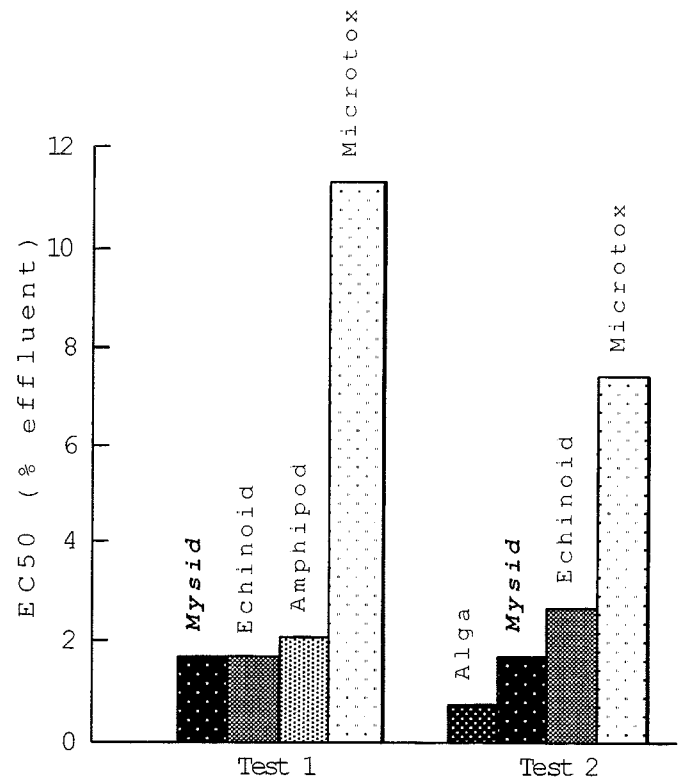

Figure 3. *Comparison of the sensitivity of different marine toxicity tests to sewage effluents.*

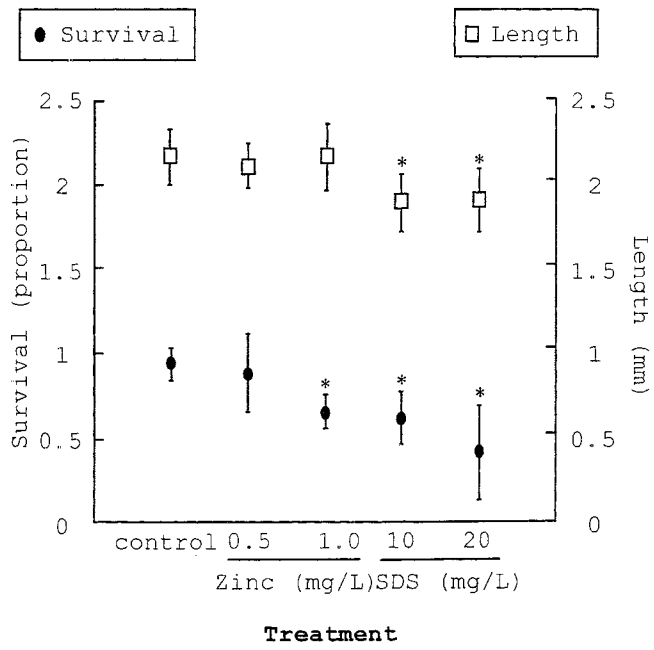

Figure 4. *Mean and standard deviation of growth and survival of* T. novae-zealandiae *in 7-day chronic tests with zinc and SDS.*

DISCUSSION

Static renewal and recirculating systems have been developed for mysid cultures, and the advantages and disadvantages of different systems (Lussier *et al.* 1988; Nimmo *et al.* 1991), as well as the use of artificial (Reitsema and Neff 1980; Leger and Sorgeloos 1982) or natural seawater (Ward 1984) are discussed in the literature. *Tenagomysis novae-zealandiae* cultures presented similar survival under both recirculating and semi-static systems. Therefore, the most cost-effective of the tested culture systems, with a density of 20 mysids/L and weekly water renewal, was adopted for the laboratory routine. Higher survival observed in complete darkness is compatible with the vertical migration habits of mysids, which swim towards the surface at night and spend the day-time near the bottom, in dark or poorly illuminated areas.

Nutritional requirements of mysids and the use of *Artemia* nauplii as the main food source have been extensively studied (Leger and Sorgeloos 1984; Leger *et al.* 1985; Ward 1987; Royan *et al.* 1988). Survival of *T. novae-zelandiae* tended to be slightly enhanced when fed brine shrimp nauplii enriched with products containing high concentrations of some essential fatty acids, which have also improved the survival, growth and biomass production of other mysid species (Leger *et al.* 1985; Royan *et al.* 1988).

The effects of environmental factors such as salinity and temperature, which are directly related to seasonal variations (Bhattacharya 1982; Thompson 1984), as well as of oxygen and ammonia (Kuhlmann 1984) on different mysid species have been described. The tendency for higher survival of *T. novae-zealandiae* at 15 ppt, with decrease to below 70% after 6 days at salinities below 15 and above 25 ppt, suggests that the species should be cultured in a 15-25 ppt salinity range rather than closer to full strength seawater or freshwater salinities. Several mysid species are estuarine, and like *T. novae-zealandiae*, have higher tolerance to intermediate salinities than to full strength seawater (eg. *Mesopodopsis orientalis* and *Neomysis integer* - Bhattacharya 1982; Kuhlmann 1984)

In a comparison of standard acute toxicity tests with rapid-screening toxicity tests, the LC50 of several chemicals to *M. bahia* was generally lower than the LC50/EC50 values of the rapid-screening tests, showing the higher sensitivity of this standard test (Toussaint *et al.* 1995). The sensitivity of *M. bahia* to phthalates was in the same range of that of several other aquatic organisms, including fish, algae, and freshwater cladocerans (Adams *et al.* 1995), whereas it was generally more sensitive than microalgae and marine fish to sewage sludge (Santoro and Fikslin 1987). Similarly, *T. novae-zealandiae* was more sensitive than a variety of marine tests to some sewage effluent samples. *Mysidopsis bahia* was also among the most sensitive crustaceans tested with sediments containing pyrethroids in sufficient amounts to establish lethal concentrations in the overlying water based on sediment/water partitioning (Clark *et al.* 1989). Tests with contaminated field collected sediments showed *M. bahia* to be sensitive to water column toxicity caused by the desorption of contaminants from the sediments (Burgess *et al.* 1993). It also proved to be an appropriate test organism in the first phases of marine Toxicity Identification Evaluation (TIE) procedures, being the most sensitive of five species tested to unidentified toxicants in petroleum refinery wastewater (Bleckman *et al.* 1995). It was used in industrial and municipal effluent TIE procedures (Burgess *et al.* 1995), and presented successful results in analyses of effluent manipulations for TIE procedures, such as the addition of brine to the sample prior to marine tests (Ho *et al.* 1995). Brine addition to effluent samples prior to toxicity testing is a common procedure, and tests with *T. novae-zealandiae* showed its suitability for experiments requiring this treatment.

The influence of a variety of intrinsic and environmental factors, eg., age, food, salinity and temperature, on the acute sensitivity of mysids to toxicants has been assessed. The age of *M. bahia* did not significantly affect their sensitivity to several organic chemicals (malathion, tetrabromobisphenol-A, tributyltin chloride and industrial surfactants) (Goodman *et al.* 1988; Hall *et al.* 1989), whereas the acute toxicity of Cd to *Siriella armata* was higher to juveniles than adults (Birmelin *et al.* 1995). Large reductions in the amount of food tended to increase the sensitivity of mysids to some chemicals (Cripe *et al.* 1989). Both salinity and temperature affected the tolerance of *M. bahia* to cadmium in acute tests (DeLisle and Roberts 1988; Voyer and Modica 1990), whereas in life-cycle tests salinity did not appear to play such an important role on the toxic effects of cadmium (Voyer and McGovern 1991). Salinity and temperature played a fundamental role on the tolerance of the mysid *Praunus flexuosus* to chromium, nickel and zinc, and it was suggested that the increase in sensitivity with salinity and temperature changes would be linked to disruption of normal osmoregulatory patterns (McLusky and Hagerman 1987). Salinity did not significantly affect the sensitivity of *T. novae-zealandiae* in acute toxicity tests with reference toxicants, but a trend for higher sensitivity in full strength seawater (34 ppt) was observed. Effluent tests were conducted in full-strength seawater salinity to simulate a worst case scenario, where effluents would be discharged into fully marine environments.

Life-cycle and short-term chronic tests with *Mysidopsis* sp. presented a diversity of results, apparently depending on both the toxicant tested and the laboratory conducting the study. Neither growth, nor reproduction or survival was consistently the most sensitive endpoint in chronic toxicity tests.

Life-cycle tests with the common solvent-carrier, triethylene glycol, showed no effect on survival or reproduction in the tested concentrations (Montgomery *et al.* 1985). Tests over 32 days with ammonia indicated that survival would be more sensitive than reproduction and possibly than growth (Miller *et al.* 1990). However, tests with silver and the pesticides kepone and endosulfan indicated that growth, and possibly the number of juveniles produced per female, were more sensitive endpoints than survival (Nimmo *et al.* 1977, Breteler *et al.* 1982). The organophosphorus insecticide fenthion promoted reduction in reproduction and growth in lower concentrations than those that affected survival during a life-cycle (McKenney 1986). A hormone analogue used in mosquito control, methoprene, also caused strong reduction in the number of young produced by *M. bahia* in very low sublethal concentrations, also affecting growth and time for the release of the first brood (McKenney and Celestial 1996).

In short-term chronic tests, Jop (1989) concluded that growth was a more sensitive sublethal endpoint to chromium than fecundity or survival. Goodfellow and Rue (1989), however, concluded that chromium did not affect growth (dry weight) and fecundity was the most sensitive endpoint for 7-day chronic tests, while PCP had a stronger effect on growth than on fecundity or survival. Fecundity and sexual maturity were the most sensitive endpoints in a 7-day continuous exposure to chlorine, with growth being depressed to a lesser extent (Fisher *et al.* 1994). Khan *et al.* (1992) developed a 96-h short-term chronic test using sexual maturity as an endpoint, as opposed to fecundity as the endpoint for 7-day tests. Sexual maturity was a sensitive endpoint to cadmium and could be used as an estimator of chronic toxicity. The acute and chronic effects of Cd to *M. bahia* and *M. bigelowi* were practically identical, and could be quantified by three interrelated endpoints: survival, morphological aberrations and reproduction, with aberrations and reproductive rate reduction occurring in concentrations where survival was not significantly affected (Gentile *et al.* 1982a). In long-term exposures, however, Voyer and McGovern (1991) concluded that changes in mortality would represent a reasonable estimate of the chronic effects of Cd on *M. bahia*. Although based on 7-day tests with two reference toxicants and only two concentrations of each, it is suggested that the same would apply to chronic tests with *T. novae-zealandiae*, where survival was more sensitive than growth. However, it is known that responses can differ depending on the toxicant. Chronic responses of *M. bahia* to several heavy metals and to cyanide showed that survival was more sensitive than reproduction (time to sexual maturation, duration of embryonic development and brood size) to cyanide and Cd, while reproduction was the most sensitive response to four other metals, and both endpoints were similar in sensitivity to still four other metals (Lussier *et al.* 1985).

Test method precision is also an important matter and was assessed by various authors. Intra-laboratory test precision of the 7-d chronic test with *M. bahia* was analysed with copper and SDS (Schimmel *et al.* 1989). Intra- and inter-laboratory precision of acute toxicity tests was analysed with effluents (Rue *et al.* 1988), and precision of acute and chronic tests, with single chemicals and effluents (Parkhurst *et al.* 1992). These studies showed that: a) toxicity test precision is generally within the same range of that of chemical analyses of priority pollutants; b) single chemical tests tended to have larger coefficients of variation (C.V.) than effluent tests; c) intra-laboratory studies generally had higher precision than inter-laboratory studies and; d) C.V.s for chronic tests were lower or equal to those for acute tests. Data on test precision are still needed for the methods presented here.

CONCLUSIONS

Tenagomysis novae-zealandiae appears to be a suitable test species for the assessment of potential adverse effects of discharges into marine and estuarine environments. Laboratory cultures tended to present higher survival when kept in 24-hour darkness and it is suggested that culture salinity should be between 15 and 25 ppt. Feeding regime should include *Artemia* nauplii enriched with cod liver oil and/or marine fish oil. Mysid density can be of up 20 organisms/L, with weekly water renewal.

Due to their sensitivity, acute tests may become a useful tool for the identification of environmentally protective limits for discharges. Survival was a more sensitive endpoint than growth in short-term chronic tests with zinc and SDS. More research is necessary for the assessment of acute test precision, and for the development of reliable and sensitive endpoints for chronic toxicity tests *with T. novae-zealandiae*.

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