LABORATORY CULTURE AND LIFE-CYCLE EXPERIMENTS WITH THE BENTHIC AMPHIPOD MELITA PLUMULOSA (ZEIDLER)

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Abstract—In Australia, the collection of estuarine invertebrates from the field for whole-sediment toxicity tests is hindered because of temporal variability in their population densities and distributions. The present study aimed to develop culturing procedures for Melita plumulosa (Zeidler), an epibenthic and intertidal, deposit-feeding amphipod that is native to the southeastern coast of Australia. During a 28-d chronic exposure, the species was tested under a range of salinities (5–35%), temperatures (14–25°C), and sediment particle sizes (sand to silt). Optimal culture conditions with respect to salinity, temperature, sediment particle size, feeding, and light regimes were determined. Compared to survival, amphipod growth and fertility were better predictors of optimal culture conditions. A life-history experiment was undertaken at the initial culture conditions of 22°C and 35% salinity to establish the age at maturity, length of reproductive cycle, and life span for each sex. Under these test conditions, posthatch female M. plumulosa released their first offspring at seven weeks, after which each female produced an average of nine juveniles every 16 d. Male M. plumulosa had an average life span of eight months, whereas females had an average life span of 11 months. Optimal culturing conditions established in the present study have been incorporated into toxicity test procedures with this species and are being used to maximize reproductive output of this species in laboratory cultures to provide a supply of juveniles for routine use in whole-sediment toxicity tests.

Keywords—Amphipod Sediment Laboratory culture Life history Estuarine

INTRODUCTION

The sediments of many estuaries in Australia have high metal and organic contaminant concentrations, derived largely from past industrial discharges and from urban drainage [1–3]. The new Australian and New Zealand Environment Conservation Council/Agriculture and Resource Management Council of Australia and New Zealand Guidelines for Fresh and Marine Water Quality (http://www.ea.gov.au/water/quality/nwqms/index.html#quality) recommend the use of toxicity testing to better understand contaminant bioavailability and to supplement chemical testing in sediment-quality assessments. Dredging of contaminated sediments is a common management option, usually with disposal at sea. It is legally required that the environmental impact of the disposal at sea of dredged material be assessed in chronic toxicity tests (http://www.ea.gov.au/coasts/pollution/dumping). The assessment of contaminated sediments therefore is costly, and decisions need to be based on a sound understanding of contaminant bioavailability and the effects on both target populations and benthic ecological communities [4–6].

Many toxicity tests focus on pore waters as the major route of contaminant uptake. However, sediment ingestion and the effects of contaminants on food sources also can determine if an organism or population will continue to survive in a particular sediment [7,8]. Sediment toxicity tests can provide the cause–effect link between chemical contaminant levels and ecological effects [9]. However, toxicity tests on whole sediments using appropriate sediment-dwelling organisms with different feeding and behavioral characteristics, particularly at higher salinities, need to be developed. The single published and commercially used estuarine whole-sediment invertebrate test species (Corophium colo) in Australia is an infaunal, filter-feeding species found at high densities in the upper reaches of the Hawkesbury River estuary [10,11]. To our knowledge, C. colo has not yet been cultured successfully in the laboratory. Its collection from field populations can be unreliable, and it does not tolerate high salinities [12,13].

In a recent study, the sensitivities of 13 benthic invertebrates, including five amphipods (one being C. colo), from estuaries in the Sydney (Australia) region to metal-contaminated sediments were compared [12–14]. The species tested had varying feeding strategies and habitat preferences and, thus, different routes of exposure to contaminants. Species sensitivity was screened using a 10-d static sediment test with sediment spiked by either copper or zinc. Of the species tested, the epibenthic deposit-feeding amphipod, Melita plumulosa, was one of the most sensitive to both copper- and zinc-spiked sediments [12–14]. In addition, ingestion of sediments has been shown to be an important source of metal uptake in this species [15]. Based on commonly used selection criteria [16,17], including sensitivity, M. plumulosa was recommended for further development as a test species to assess sediment quality in whole-sediment toxicity tests with estuarine and marine sediments [12,13].

The present study was designed to evaluate the optimum culture conditions for the survival, growth, and reproduction of M. plumulosa. This is necessary to establish laboratory
cultures that are capable of providing sufficient numbers of juveniles for use in routine acute and chronic life-cycle toxicity testing with whole sediments.

MATERIALS AND METHODS

Collection of test species from the field

*Melita plumulosa* were collected at low tide on the northern shores of the Hawkesbury River near Brooklyn (Australia; salinity, 27.0–33.8‰). A thin, 1- to 2-cm layer of sediment from the collection site was added to wide-mouthed, 0.5-L polyethylene containers and then covered with seawater from the collection site. *Melita plumulosa* were collected by rinsing the rocks and oyster shells in the water within the collection containers. Alternatively, amphipods could be collected and transferred to containers by hand or with tweezers. A maximum of 100 amphipods were added to each container. Containers were placed in foam boxes to maintain a constant ambient temperature during transportation back to the laboratory.

Gravid females were collected throughout the year, although population density was reduced during the austral winter months (June–August). Other species that co-occurred with *M. plumulosa*, at lower densities in the same habitat included *M. matilda*, *Elasmopus spp.*, *Grandidierella gilesi*, *G. japonica* [4,5], and the recently described *C. colo* [18], which was previously referred to as *Corophium* sp. [10] or *Corophium cf. volutator* [4,5]. We recommend that collectors become familiar with these other species, which except for *M. matilda* can be easily identified in the field, to avoid their collection. Under a dissecting microscope, *M. plumulosa* can be distinguished from *M. matilda* by the posterodorsal spine on urosomite 1 (http://www.crustacea.net/crustace/amphipoda/melitidae/www/plumulo.htm).

On return to the laboratory, amphipods were isolated from field collection containers by pouring the contents through a sieve (mesh size, 500 μm). Using a modified glass or plastic pipette (diameter, 3–5 mm), amphipods were transferred to a watch glass with minimal water. Under a dissecting microscope, male *M. plumulosa* were distinguished from females by the much larger gnathopod 2 [19], and nongravid females were distinguished from gravid females that were carrying eggs.

Holding conditions

Laboratory cultures initially were established using procedures described previously for *Leptocheirus plumulosus* [20] in polypropylene trays (40 × 30 × 10 cm) containing a 1-cm layer of press-sieved (mesh size, 1.1 mm) sediment and 4 to 5 L of filtered seawater (mesh size, 0.45-μm) at a salinity of 30‰. A maximum of 300 adult amphipods sourced from the collection site were added per culture tray. Overlying water of a salinity identical to that measured at the collection site. The gravid females (generally one gravid female for every three or four juveniles required) were held for 7 d to allow release of the juveniles. Females were fed twice during this period using the same food ration as for cultures. After 7 d, juveniles for the tests were collected from the trays containing gravid females by passing the top sediment layer through a sieve (mesh size, 280 μm). All juveniles used in tests therefore were less than 7 d old (posthatch).

Isolation of juveniles for tests

For the particle-size distribution tolerance and cross-breeding experiments, juvenile amphipods were sourced from gravid females in laboratory cultures. For all other experiments, juveniles were sourced from gravid females collected in the field. To obtain juveniles, a maximum of 100 gravid females per tray were added to small polyethylene trays (19 × 13 × 7 cm) containing a 1-cm layer of press-sieved sediment (mesh size, 1.1 mm) from the collection site and 4 cm of overlying seawater of a salinity identical to that measured at the collection site. The gravid females (generally one gravid female for every three or four juveniles required) were held for 7 d to allow release of the juveniles. Females were fed twice during this period using the same food ration as for cultures. After 7 d, juveniles for the tests were collected from the trays containing gravid females by passing the top sediment layer through a sieve (mesh size, 280 μm). All juveniles used in tests therefore were less than 7 d old (posthatch).

General experimental procedures

Sediment used in the life history, preliminary and first food type, first and second food ration, and cross-breeding experiments was collected from Sunshine Bay in the lower Hawkesbury River. Sediment used in the remaining experiments (second food type, salinity, temperature, and particle-size distribution tolerance) was collected from Bonnet Bay in the lower Woronora River, southern Sydney. The catchments of both of these bays are contained primarily in national parks, with isolated urban areas. Sediment (top 2–3 cm) was collected using hand shovels and then press-sieved (mesh size, 1.1 mm) either on site or immediately on return to the laboratory. Sediments were stored in plastic bags at 4°C for a maximum of two months.

Seawater was collected from Rose Bay, Sydney Harbor, or Port Hacking (food type experiment 2 only), southern Sydney, and stored in a tank outside the laboratory until required. Immediately before use, seawater was filtered (mesh size, 5 μm) and ultraviolet-sterilized. Seawater was adjusted to the required salinity by dilution with filtered Sydney mains water, which was delivered via polyvinylchloride pipes and filtered through an activated carbon filter followed by a 5-μm filter. Before the start of experiments, beakers were allowed to equilibrate overnight at the chosen test temperature in a temperature-controlled incubator. The juveniles were allocated randomly to the test beakers to commence the experiments. For the duration of tests, beakers were covered with plastic wrap to minimize evaporation and with a modified acrylic (Perspex) plastic sheet (Allplastics Engineering, Chatswood, New South Wales, Australia), which held shortened glass pipettes that were connected to an air pump to provide gentle aeration to the overlying water in each beaker. Test beakers, except the no-light treatment in the food ration experiment, received a 12:12-h light:dark photoperiod (200–600 lux).

Approximately 80% of the overlying water in replicates was renewed once per week. The physicochemical parameters, temperature, salinity, dissolved oxygen, and pH were measured in the overlying water before and after renewals. In all chronic experiments except the food type and food ration experiments, amphipods in the test beakers were fed twice per week with Sera Micron® (Sera, Heinsberg, Germany) per amphipod, 20 μL of Sera Fishtamins® (Sera) per L of overlying water, and *Phaeodactylum tricornutum* (1 × 10⁵ cells/amphipod). Approximately 80% of the overlying water in cultures was renewed once a week by gentle siphoning. Cultures were renewed every four to six weeks by sieving the contents and isolating the amphipods. Animals of similar size class were then placed in culture trays with fresh sediment and overlying water.

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stirrer as the appropriate amount was transferred by pipette into each test beaker.

At the termination of experiments, surviving adults and their offspring were recovered by sieving (mesh size, <280 \(\mu m\)) the contents of each test beaker. Amphipods were isolated using modified pipettes, fixed in 10% (v/v) neutral phosphate-buffered formalin for 15 min, rinsed three times in deionized water, and then preserved in 70% ethanol. The numbers of surviving adults and juveniles were recorded.

The body lengths of surviving mature amphipods were measured using a digital image-analysis system (Leica DC 100 v 2.41 and Leica Qwin Standard v 2.3 1998; Leica Microsystems Imaging Solutions, Cambridge, UK). The body length (base of rostrum to base of telson) was measured twice and the average taken. The sex of mature amphipods (length, >3.5 mm) and the number of gravid females also were recorded.

**Life history**

Ten immature amphipods (age, 23–30 d) from the holding cultures were added to 250-ml test beakers containing 50 g of press-sieved (mesh size, 400 \(\mu m\)) sediment and overlying water (35‰ salinity) to 250 ml. Each treatment was replicated four times, and the test beakers were incubated at 22°C under a 12:12-h light:dark photoperiod. Test beakers were checked daily until juveniles were observed. The contents of the test beakers were then sieved (mesh size, <280 \(\mu m\)), and the number of surviving adults, their sex, gravidity of females, and number of juveniles in each test beaker were recorded. Juveniles were discarded and adults placed in new test beakers containing fresh sediment and overlying water. This retrieval process was repeated whenever juveniles were observed in test beakers. The mean age at maturity, fertility with age, and life span of each sex were calculated after a 49-week experimental period.

**Salinity tolerance**

Ten juveniles were added to 250-ml test beakers containing 50 g of press-sieved (mesh size, 400 \(\mu m\)) sediment and overlying water to 250 ml. Each treatment was replicated five times. The salinity of the overlying water in all treatments at the start of the experiment was 25‰ but was adjusted for each treatment by 5‰ each day until the desired salinity was reached. The mean salinities of the overlying water in each treatment, measured before and after weekly water renewals, were 5.4, 10.4, 15.3, 20.3, 25.3, 30.2, and 34.9‰ (standard error of the mean [SE], 0.2–0.4‰) for the 28-d experiment.

**Temperature tolerance**

Ten juveniles were added to 250-ml test beakers containing 50 g of press-sieved (mesh size, 400 \(\mu m\)) sediment and overlying water to 250 ml. Beakers were incubated at 25°C under a 12:12-h light:dark photoperiod, with each treatment replicated five times. The experiment was started at 23°C, and the temperature of incubators was adjusted by 2°C per day until the desired water temperature was reached for each treatment. The mean water temperatures for each treatment, measured before weekly water renewals, were 14.0, 17.0, 20.1, 22.9, and 25.1°C (SE, 0.1–0.2°C) for the 28-d experiment.

**Particle-size tolerance**

Five nominal sediment particle-size distributions of the following ratios of sand:silt were tested: 0:100, 25:75, 50:50, 75:25, and 100:0, calculated on a dry-weight basis. The silty sediment (96% of particles < 63 \(\mu m\)) was collected from Bonnet Bay; its physicochemical properties have been described in detail previously [14]. Sediments of increasing sand content were prepared by adding appropriate dry weights of clean Sydney sand (Matcoll, Penrith, Australia). The sand (180 \(\mu m\) > particle size < 1 mm) was sieved (mesh size, 1.1 mm) to remove large aggregates, heated in a muffle furnace at 500°C for 3 h to burn off organic matter, and then washed with seawater. Sediments were prepared 3 d in advance, rolled for 2 h each day on a mechanical rolling machine to aid mixing, and then stored at 4°C. Ten juveniles, from gravid females in laboratory cultures, were added to 250-ml test beakers containing 50 g of the prepared sediment and overlying water to 250 ml. For both a 10-d acute and a 28-d chronic experiment, each treatment was replicated five times, and the test beakers were incubated at 25°C and 25‰ salinity under a 12:12-h light:dark photoperiod. During the 10-d acute test, the amphipods were not fed.

**Food type**

In a preliminary experiment using mature *M. plumulosa*, four food treatments were tested: no food, Sera Micron only, the diatom *P. tricornutum* only, or Sera Micron and *P. tricornutum*. Two chronic experiments (56 and 42 d) using juvenile *M. plumulosa* were performed with different food treatments (Table 1).

All algae were cultured in natural seawater-based F medium (with added sodium nitrate, sodium dihydrogen phosphate, sodium metasilicate, trace metals, and vitamins) [21], with iron and trace metals halved [22], except for *Nitzschia closterium* that was cultured in a natural seawater f medium [21]. Stock and working cultures were maintained according to the method described by Hynes et al. [11]. In the first chronic food experiment, live algae cultures in the growth media were fed directly to test chambers. In the second chronic food experiment, algal cultures were centrifuged (850 g for 7 min) to remove growth media, then rinsed with fresh seawater and recentrifuged to obtain a concentrated algal suspension. Algal suspensions were stored at 4°C for a maximum of four weeks. The cell density of algal suspensions was determined in both experiments using a hemocytometer (Livingstone International, Rosebery, NSW, Australia).

In the preliminary experiment with various food treatments, 10 adults were added to 250-ml test beakers containing 50 g of press-sieved (mesh size, 1 mm) sediment and overlying water to 250 ml. Beakers were incubated at 24°C and 35‰ salinity for four weeks under a 12:12-h light:dark photoperiod. Each treatment was replicated twice, and amphipods in the algal treatments were fed 1 × 10⁵ cells/amphipod twice per week.

In the first chronic experiment, 10 juveniles were added to 250-ml test beakers containing 50 g of press-sieved (mesh size, 400 \(\mu m\)) sediment and overlying water to 250 ml. Beakers were incubated at 21°C and 35‰ salinity for eight weeks under a 12:12-h light:dark photoperiod. Each treatment was replicated three times, and the amphipods were fed 1 × 10⁵ cells/amphipod twice per week.

The sediment used in the second chronic experiment was collected from Bonnet Bay and diluted by 50% on a dry-weight basis with Sydney sand. This reduced the food content of the sediment; consequently, the amphipods were more reliant on food supplements provided for optimal growth. Twenty ju-
juveniles were added to 1-L test beakers containing 100 g of sediment with each treatment replicated four times, and the test beakers were incubated at 25°C and 25% salinity for six weeks under a 12:12-h light:dark photoperiod. The algal treatments were fed once in the first week, then twice per week thereafter, at the rate of 1 × 10^5 cells/L overlying water.

**Food ration and light illumination**

Two 28-d chronic experiments with juvenile *M. plumulosa* were performed with different rations and frequency of feeding of Sera Micron. All treatments were fed the standard rate of Sera Fishtamins (4.4 µL/L overlying water). The first experiment tested the following increasing rations and frequency of feeding per week: 0.5 and 1 mg of Sera Micron per amphipod two or three times per week, and 1 mg of Sera Micron per amphipod three times per week for the first two weeks and then 2 mg of Sera Micron per amphipod three times per week. The second experiment tested the following decreasing rations and frequency of feeding: 0.5 and 0.25 mg of Sera Micron per amphipod once or twice per week.

For each experiment, 10 juveniles were added to 250-ml test beakers containing 50 g of press-sieved (mesh size, 400 µm) sediment and incubated at 25°C and 25% salinity for eight weeks under a 12:12-h light:dark period. The first food ration experiment was replicated four times, and the second food ration experiment was replicated five times. The second food ration experiment also tested the influence of light by including two treatments—no light or light illumination from directly above the test beakers only—that were fed the standard food ration (0.5 mg of Sera Micron twice per week). Test beakers in the treatments exposed to light illumination from directly above were wrapped with aluminum foil around the outside of the beaker to restrict light. Test beakers in the no-light treatment were placed in a temperature-controlled incubator with no lighting.

**Cross-breeding**

To investigate how *M. matilda* came to dominate *M. plumulosa* laboratory cultures after 6 to 12 months, an experiment was conducted to test if both species could coexist in the same test beaker and if the two species were capable of interspecific breeding. Adult *M. plumulosa* and *M. matilda* were isolated from laboratory cultures. Males and females of each species were placed in separate trays for two weeks to ensure that all females were not gravid at the start of the experiment. Trays were established and fed as described previously for gravid females. Five females and five males were placed in each test beaker, and four treatments were tested with the following species and sex (male:female) combinations: *M. plumulosa*: *M. matilda*, *M. matilda*: *M. plumulosa*, *M. plumulosa*: *M. matilda*, and *M. matilda*: *M. plumulosa*. After two weeks, the test was terminated, and the numbers of males and females of each species, gravid females, and juveniles were recorded for each test treatment.

**Statistical analyses**

The percentage survival of adults, fertility (number of juveniles per female), and gravidity (percentage gravid females per total number of females) were calculated from the raw data. Data analyses were performed according to the decision tree for statistical analysis of toxicity test data of the U.S. Environmental Protection Agency [20] except that when no control was included in the experimental design, an analysis of variance post-hoc Tukey’s was performed rather than the Dunnett’s test. Average body lengths of all surviving mature amphipods per replicate were analyzed, as well as all females and males separately, to determine if one sex was more sensitive than the other. If only two or fewer surviving amphipods were available from a replicate, then the average length measurement for that replicate was discarded. All assumption-checking and parametric statistical tests were conducted using the program MINITAB® Version 12.22 (Minitab, State Col-

**Table 1. Survival, body length, and fertility of *Melita plumulosa* following chronic exposure with different food types at 25°C in two experiments**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Survival (%)</th>
<th>Fertility (juveniles/female)</th>
<th>Body length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sera Micron⁺</td>
<td>100 ± 0⁺</td>
<td>3.5 ± 1.1</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Sera Micron + vitamins</td>
<td>100 ± 0</td>
<td>6.3 ± 1.3</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Sera Micron + vitamins + <em>Phaeodactylum tricornutum</em></td>
<td>100 ± 0</td>
<td>4.5 ± 1.5</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Sera Micron + vitamins + <em>Chaetoceros muelleri</em></td>
<td>97 ± 3</td>
<td>2.2 ± 0.4</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Sera Micron + vitamins + <em>Navicula atomus</em></td>
<td>93 ± 7</td>
<td>3.5 ± 0.2</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>Sera Micron + vitamins</td>
<td>94 ± 3</td>
<td>0.9 ± 0.3</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Sera Micron + vitamins + <em>P. tricornutum</em> (diatom)</td>
<td>94 ± 4</td>
<td>1.6 ± 0.5</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Sera Micron + vitamins + <em>Nitzschia closterium</em> (diatom)</td>
<td>95 ± 3</td>
<td>1.2 ± 0.3</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Sera Micron + vitamins + <em>Entomoneis cf. punctulata</em> (diatom)</td>
<td>98 ± 1</td>
<td>0.9 ± 0.3</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Sera Micron + vitamins + <em>Dunaliella tertiolecta</em></td>
<td>98 ± 3</td>
<td>1.3 ± 0.4</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Sera Micron + vitamins + the above three diatoms</td>
<td>100 ± 0</td>
<td>1.5 ± 0.6</td>
<td>5.2 ± 0.2</td>
</tr>
</tbody>
</table>

* Experiment 1 was for 56 d at 35% salinity with sediment containing 96% silt and 4% sand particles.
* Experiment 2 was for 42 d at 25% salinity with sediment containing 50% silt and 50% sand particles.
* Sera, Heinsberg, Germany.
* Mean ± standard error.
leage, PA, USA). Data that failed the assumptions of parametric tests were transformed (e.g., $\log_{10}$ for survival data) and assumptions rechecked. If assumptions were still not met, then nonparametric tests (Steel’s many-one rank test, Wilcoxon rank sum test with Bonferroni’s adjustment, or Kruskal–Wallis test) were performed. All nonparametric tests were conducted using the program ToxStat®3.5 [23]. In all statistical tests, $p = 0.05$ was the level of significance chosen.

RESULTS

General observations of species

*Melita plumulosa* has an irregular distribution through the lower to middle tidal reaches of the intertidal zone in the Hawkesbury River, approximately 40 km north of Sydney. The species was found in areas where the overlying water salinity ranged from 0.9 to 33.8‰ and where the water temperature ranged from 12.7 to 27.5°C. The species typically was found on silty to silt-sandy substrates, inhabiting the undersurface of rocks and oyster-shell grit. Distribution within a suitable habitat varied, with not all rocks being colonized. The population density of amphipods underneath a single rock could vary from a single individual to more than 100 individuals. The microscale factors influencing distribution are unknown. Field-collected amphipods should be identified individually to ensure that *M. matilda* are not introduced into laboratory cultures, because the species was found to outcompete *M. plumulosa* after 6 to 12 months. This phenomenon was investigated further in the present study.

*Melita plumulosa* do not construct burrows. Most juveniles remain buried in the shallow (<5 mm) layers of sediment until approximately four weeks of age and also can be observed on the walls of the test chamber. Mature adults live on the surface of the sediment (epibenthic) and deposit-feet on sediment and detritus. In water-only exposures, juvenile and adult *M. plumulosa* can survive for at least 4 or 10 d, respectively, without the addition of food.

Life history

Under the initially selected culture conditions of 22°C and 35‰ salinity under a 12:12-h light:dark photoperiod, female *Melita plumulosa* released their first juveniles at seven weeks of age (Fig. 1). Their fertility then increased with age up to 21 weeks (Fig. 1). Females 21 to 39 weeks old produced an average of 9 ± 1 (mean ± SE) juveniles every 16 ± 1 d. Female fertility declined after 39 weeks. Male *M. plumulosa* had a mean life span of eight ± 1 month, whereas females had a mean life span of 11 ± 1 month. Subsequent experiments show that these initial culture conditions were not optimal for growth and reproduction of *M. plumulosa*. When cultured at 25°C and 25‰, female *M. plumulosa* released their juveniles at approximately five weeks of age (7-d posthatch juveniles that are cultured for 28 d) (Fig. 2).

Salinity tolerance

Survival and gravidity of *M. plumulosa* were not significantly ($p < 0.05$) different in salinities ranging from 5 to 35‰ in the overlying water following a 28-d chronic exposure, with more than 94% survival in all treatments. Juveniles cultured at 25°C in the varying salinities under a 12:12-h light:dark photoperiod reached maturity after 28 d (Fig. 2). However, fertility and growth, as measured by the increase in body length after 28 d, were significantly reduced at both 30 and 35‰ (Fig. 2).

Temperature tolerance

The survival and growth of juvenile *M. plumulosa* cultured at 25°C and at various temperatures ranging from 14 to 25°C were not significantly ($p < 0.05$) different after 28 d, with more than 92% survival in all treatments. However, although the amphipods cultured at 14 and 17°C had body lengths similar to those at higher temperatures, maturation was delayed, and no fertility was observed after 28 d (Fig. 3).

Particle-size distribution tolerance

The survival and adult *M. plumulosa* after 10 d without feeding was significantly ($p < 0.05$) reduced in sediments consisting of sand only (180 μm > particle size < 1 mm) compared to survival in sediment consisting of more than 96% silt (particle size < 63 μm) (Fig. 4). The growth of

![Fig. 1. Mean fertility of *Melita plumulosa* during its life span cultured at 22°C and 35‰ salinity. Error bars represent the standard error of the mean ($n = 1–4$ replicates) and could not be calculated for time points with only one value.](image)

![Fig. 2. Mean fertility (bars) and body length (○) of juvenile *Melita plumulosa* following a 28-d chronic exposure to overlying waters of various salinities at 25°C. Error bars represent the standard error of the mean. Treatments with the same letter are not significantly different for both fertility and growth ($p > 0.05, n = 5$, analysis of variance post-hoc Tukey’s).](image)
juveniles after 10 d (measured as an increase in body length) also was significantly \( (p < 0.05) \) reduced in sediment consisting of 75:25 sand:silt or in 100\% sand compared to that in sediment consisting of more than 96\% silt (Fig. 4). Survival of juvenile Melita plumulosa was not significantly \( (p > 0.05) \) affected following a 28-d exposure to sediment with particle-size distributions ranging from more than 96\% silt (particle size \(< 63 \mu m\)) to 100\% sand (180 \( \mu m \) > particle size \( < 1 \) mm), with more than 84\% survival in all treatments. However, fertility and growth of Melita plumulosa were significantly \( (p < 0.05) \) reduced in the sand-only treatment compared to that in sediment with a 75:25 sand:silt content or sediment with a lower percentage of sand (Fig. 5).

**Food type**

In the preliminary experiment, mature Melita plumulosa cultured for 28 d in silty sediment without food or with the diatom P. tricornutum had significantly \( (p < 0.05) \) reduced survival of 30\%, compared to the 100\% survival obtained in sediment with Sera Micron only or with Sera Micron and P. tricornutum. In the first chronic experiment (56 d) with juvenile Melita plumulosa, the addition of Sera Fishtamins, P. tricornutum, Chaetoceros muelleri, or Nannochloris atomus did not significantly \( (p > 0.05) \) improve the survival, fertility, gravidity, or growth of Melita plumulosa given a diet of Sera Micron only (Table 1). In the second chronic experiment (42 d) with juvenile Melita plumulosa in sediment with a higher sand content, the addition of P. tricornutum, N. closterium, Entomoneis cf. punctulata, Dunaliella tertiolecta, or all three diatoms together did not significantly \( (p > 0.05) \) improve the survival, fertility, gravidity, or growth of Melita plumulosa given a diet of Sera Micron and Sera Fishtamins only (Table 1).

**Food ration and light illumination**

The survival of Melita plumulosa cultured for 28 d did not change significantly \( (p > 0.05) \) with either increased or decreased food ration or feeding frequency compared to the initially adopted ration of 0.5 mg of Sera Micron per amphipod twice per week. The survival in all treatments ranged from 83 to 100\%. In the first experiment to assess food, rations greater than the initially adopted rations also did not significantly \( (p > 0.05) \) improve the fertility (Fig. 6A), gravidity, or growth of Melita plumulosa, with fertility significantly decreasing in one comparison (Fig. 6A). In the second experiment to assess food rations lower than the adopted ration, the gravidity (data not shown), fertility, and growth (Fig. 6B) of Melita plumulosa were significantly reduced \( (p < 0.05) \) when amphipods were fed only 0.5 mg of Sera Micron per amphipod once per week or 0.25 mg of Sera Micron per amphipod once or twice per week. It was concluded that 0.5 mg of Sera Micron per amphipod twice per week was the optimal food ration for laboratory cultures of Melita plumulosa.

The survival, gravidity, fertility (Fig. 6), and growth of Melita plumulosa were not significantly \( (p > 0.05) \) affected by a 28-d exposure to light illumination from directly above the test beakers only or to no light compared to those same parameters measured for amphipods exposed to the 12:12-h light:dark photoperiod.
**Laboratory culture of the benthic amphipod Melita plumulosa**

**DISCUSSION**

*Melita plumulosa* (family Melitidae) was originally described by Zeidler [19] in freshwater pools at Angourie (New South Wales, Australia). It has since been recorded in most estuaries along the New South Wales coast, from Angourie in the north to Twofold Bay in the south (http://www.crustacea.net). The species has been commonly found in estuarine tidal mudflat areas ranging from silty to sandy sediments in freshwater, estuarine, and marine environments throughout southeastern Australia, and it is an important source of food for higher trophic levels. *Melita plumulosa* is an epibenthic, deposit-feeding amphipod living in close association with sediments; it uses the sediment as both a habitat and a food source. Under laboratory conditions, males grew to an average length of 7 mm and females to an average length of 5 mm.

Laboratory maintenance of amphipods from the genus *Melita* has been described for *M. nitida* and *M. zeylanica* using animals collected from the field [24–26]. However, culture conditions that are optimal for the continuous culture of *Melita* spp. in the laboratory have, to our knowledge, not been investigated and described previously. Preliminary experiments with adult *M. plumulosa* showed that food was required to be added to silty sediment to maintain good survival of the amphipods during a 28-d culture. Environmental factors that have been shown to affect reproduction in amphipods include temperature, salinity, and day length [27,28]. In the present study, the highest overlying salinity that did not significantly reduce the fertility or growth of *M. plumulosa* was 25%<sub>e</sub> at a temperature of 25°C. The fertility or growth of *M. plumulosa* was significantly higher when cultured in estuarine salinities within the range of 10 to 20%<sub>e</sub>, compared to high salinities of 30 to 35%<sub>e</sub>, with a salinity of 25%<sub>e</sub> being intermediate. A salinity of 25%<sub>e</sub> was recommended for culturing and chronic testing using *M. plumulosa*, because it is a typical salinity for the lower reaches of estuaries where contaminated sediments occur [1–3]. Maximum fertility and minimum time to maturation of *M. plumulosa* were achieved at a temperature of 25°C and a salinity of 25%<sub>e</sub>. These two values therefore were selected as the salinity and temperature for further studies undertaken to optimize other culture conditions. The time of oviposition is a species-specific response to day length, with long days ini-

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**Table 2. Recommended culture conditions of Melita plumulosa**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Culturing condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>25%&lt;sub&gt;e&lt;/sub&gt;</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Sediment particle-size range</td>
<td>0:100–75:25 (sand : silt)</td>
</tr>
<tr>
<td>Food type</td>
<td>Fine powdered growth food supplemented with vitamins (Sera Micron and Sera Fishtamin&lt;sup&gt;a&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Food ration</td>
<td>0.5 mg of Sera Micron per amphipod and 4.4 μL of Sera Fishtamin per L of overlying water</td>
</tr>
<tr>
<td>Feeding frequency</td>
<td>Twice per week</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sera, Heinsberg, Germany.
...reproduction in summer ovipositors and short days initiating reproduction in autumn ovipositors [27]. Gravid \textit{M. plumulosa} were collected in the field throughout the year, and the absence of light in the laboratory chronic tests did not affect amphipod growth, fertility, or gravidity. However, because benthic amphipods are phototactic and can burrow to avoid light [10], the laboratory tests with \textit{M. plumulosa} were conducted under a light–dark cycle to increase exposure to sediment-associated contaminants.

For both the 10-d acute test with no added food and the 28-d chronic test with added food, \textit{M. plumulosa} were tolerant to sediments having a range of particle sizes. However, the reduced juvenile growth in the sand-only treatment compared to that in the silt-only treatment in both tests and the reduced survival in the 10-d sand-only treatment suggest that the diet of Sera Micron supplemented with Sera Fishtamins was nutritionally incomplete and that \textit{M. plumulosa} obtained additional nutrients from the silty sediment. As noted previously, juvenile \textit{M. plumulosa} remain buried in the shallow (\textlessthan5 mm) layers of sediment until approximately four weeks of age; consequently, the coarser sand particles in the 100% sand treatment may have hindered burrowing and also may have been a stress factor that contributed to the reduced survival of juveniles. However, because adult \textit{M. plumulosa} live on the sediment surface but their survival also was significantly reduced in the 100% sand treatment, it is more probable that the reduced juvenile and adult survivals were caused by insufficient food reserves in the silty sediment. Organic detritus or living components of ingested sediment, such as bacteria, fungi, and microalgae, are possible food sources in the silty sediment. Structural carbohydrate and dead organic material in sediment generally are used indirectly by microbial decomposers, but bacterial extracellular polysaccharide sediment coatings serve as vectors for the adsorption of metals by benthic amphipods [29,30]. Selective deposit feeders generally feed either by selecting organic detritus or microorganisms within a particle-size range or by ingesting sediment inorganic particles with surfaces that are rich in attached organics [31]. Amphipods, such as \textit{C. volutator}, can only use bacteria adsorbed to clay or silt particles [32]. Mature \textit{M. plumulosa}, cultured for 28 d in silty sediment without feeding or fed with the diatom \textit{P. tricornutum}, had poor survival compared to that of amphipods fed Sera Micron. Moreover, the addition of algae, commonly used in mariculture as food for invertebrates, did not improve the fertility or growth of \textit{M. plumulosa} compared to that achieved from a diet of only Sera Micron and Sera Fishtamins. Further studies are needed to identify the food source associated with the silty sediment that is used by \textit{M. plumulosa}. Bacteria and yeast strains isolated from marine sediment have been shown to have high protein content and to contain other micronutrients [33] that may supplement the Sera Micron diet of \textit{M. plumulosa}.

Interspecific crosses between overlapping and morphologically similar amphipod species of the same genus in estuaries are either sterile or produce sterile offspring [34]. This finding is supported by the present results, because the two co-occurring and morphologically similar \textit{Melita} species, \textit{M. plumulosa}, and \textit{M. matilda}, were unable to interbreed. A potential problem in establishing monocultures of \textit{M. plumulosa} was, however, identified during the present study. Although the population density of \textit{M. matilda} is approximately 10% that of \textit{M. plumulosa} in the field, it completely dominated in laboratory cultures containing both species after 6 to 12 months, and male \textit{M. matilda} were shown to consume female \textit{M. plumulosa}. Extreme care therefore is required when laboratory cultures are initially established using animals from the field, with every animal being identified under the microscope to ensure that monocultures of \textit{M. plumulosa} are established.

**CONCLUSION**

Procedures for culturing and for conducting a chronic whole-sediment toxicity test using juveniles (<7 d posthatch) of the epibenthic, deposit-feeding amphipod \textit{M. plumulosa} (Zeidler) were established. The amphipods reach sexual maturity after 28 to 42 d and produce their first brood. \textit{Melita plumulosa} has been cultured successfully and continuously in the laboratory, with reproduction sufficient to provide a regular supply of juveniles for routine use in whole-sediment toxicity tests.

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