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국내산저서단각류 *Mandibulophoxus mai*와 *Monocorophium acherusicum*의 생존, 성장 및 민감도에 대한 온도의 영향

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Influence of Temperature on the Survival, Growth and Sensitivity of Benthic Amphipods, *Mandibulophoxus mai* and *Monocorophium acherusicum*

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요 약

본 연구는 최근 한국에서 퇴적물 독성 실험생물로 개발되고 있는 저서 단각류 *Mandibulophoxus mai*와 *Monocorophium acherusicum*의 생존과 성장 및 민감도(sensitivity)에 대한 온도의 영향를 평가하기 위한 일련의 실험으로 수행되었다. 본 연구에 사용된 각 실험 단각류의 생물학적 영향으로 각기 다른 수온 조건에서의 생존과 성장률을 통해 결정하였다. 표준 독성물질인 카드뮴(Cd)을 평가하는데 있어서 독성 민감도에 대한 온도의 영향은 각기 다른 온도에서 카드뮴이 첨가되지 않은 해수와 카드뮴이 첨가된 해수에 노출된 단각류의 생존률을 비교하여 결정하였다. 연구결과, 온도는 두 실험 단각류 모두의 생존, 성장 및 카드뮴 민감도에 영향을 미치는 것으로 평가되었다($p<0.05$). 두 실험종 모두 80%이상의 생존율을 보인 온도범위는 13-22°C인 것으로 확인되었다. 두 실험종의 일일 성장률은 *M. mai*의 경우 20°C, *M. acherusicum*의 경우 25°C에서 다른 온도 조건에 비해 상대적으로 높게 나타났다. 반면, Cd 민감도에 대한 영향은 *M. mai*는 20°C, *M. acherusicum*은 15°C가 다른 온도 조건에 비해 상대적으로 큰 것으로 평가되었다. 이상의 연구 결과에 근거할 때, 온도는 단각류를 이용한 치사 및 반치사 생물 검정 연구에 있어 매우 중요한 변수인 것으로 판단되었다. 따라서, 독성평가에 활용되는 생물종에 대한 적절한 실험 온도 범위를 결정하는 것은 표준 독성시험법을 개발하는데 우선적으로 고려되어야 할 사항인 것으로 사료된다.

Abstract – A series of experiments was conducted to evaluate the effects of temperature on the survival, growth and sensitivity of the benthic amphipods, *Mandibulophoxus mai* and *Monocorophium acherusicum*, which have been recently developed as new sediment toxicity testing species in Korea. The biological performance for each amphipod species was determined by the survival and growth rates at different water temperatures. The influence of temperature on the sensitivity to reference toxicant, Cd, was determined by the comparison of survival rates of amphipods exposed to control and Cd-spiked seawater at different temperatures. Temperature significantly influenced on the survival, growth and Cd sensitivity of both amphipods. Tolerable ranges of temperature for the >80% individuals of both *M. mai* and *M. acherusicum* with sediment substrates were mostly overlapped (13 to 22°C). The daily growth rates of *M. mai* and *M. acherusicum* increased proportionally with temperature up to 20°C and 25°C, respectively. Similarly, the sensitivities of *M. mai* and *M. acherusicum* to Cd increased

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with temperature up to 20°C and 15°C, respectively. Overall results showed that temperature is a substantially important factor potentially influencing the results of lethal and sublethal bioassays using the amphipods. Therefore, defining the adequate ranges of experimental temperature for the toxicity testing species is the pre-requisite for the development of standardized bioassay protocols.

Keywords: Amphipods(단각류), Temperature(온도), Survival(생존률), Growth(성장), Bioassay(생물검정법)

1. INTRODUCTION

Sediment toxicity tests using amphipods are the very essential part of the environmental quality assessment of aquatic systems and it is typically used to decide whether the tested sediment is toxic or non-toxic by comparing biological responses such as survival, growth and reproduction rates of test organisms exposed to target sediments with references. Various experimental factors (e.g. water temperature, salinity, pH and dissolved oxygen concentrations) can influence the bioassay results (Luoma[1995]; Andres *et al.* [1998]). Among those factors, temperature controls the rates of numerous physiological functions such as respiration, water ventilation and enzyme activity and also significantly influences on the sensitivity of test organisms exposed to pollutants because the uptake and elimination kinetics were closely related to the surrounding temperature (Tessier *et al.*[1993]; Langston and Spence [1995]; Wang and Fisher[1997]; Hutchins *et al.*[1998]; Brockington and Clarke[2001]). Therefore, when developing a new bioassay protocol, adequate experimental conditions such as temperature should be defined in advance (Burton[1992]).

The benthic amphipods, *Mandibulophoxus mai* and *Monocorophium acherusicum* were found along the Western and Southern coast of Korea (Jo[1989]; Kim[1991]). Free burrowing amphipods, *M. mai* were usually found in sand bottoms and have been successfully employed to assess the toxicity of contaminated sediments (Song[2001]; Lee *et al.*[2004]). Tube-building amphipods, *M. acherusicum* were found in various substrates from rocky shores to fine mud sediments. *M. acherusicum* have been developed as sediment toxicity testing species and was applied in sediment bioassays in a recent research (Lee *et al.*[2004]). The applicability of these amphipods as toxicity testing species should be further evaluated and the adequate experimental conditions including temperature should be determined in various ecotoxicological studies.

The objectives of the present study were to evaluate the influence of temperature condition on the survival and growth of benthic amphipods, *M. mai* and *M. acherusicum* and also on the sensitivity of these amphipods to dissolved Cd, the reference toxicant. The results obtained will assist in determining the adequate temperatures to increase the sensitivity of test species to pollutants without influencing the biological performance in controls.

2. MATERIALS AND METHODS

2.1 Test animals

Amphipods *M. mai* were collected from Manripo beach (36°48'N; 126°11'E) and *M. acherusicum* from a mudflat in Daebudo (37°17'N; 126°34'E), located in the Western coast of Korea. Upon returning to the laboratory, the collected amphipods were acclimated gradually to the experimental conditions (temperature, salinity, etc.) for ~2 weeks. During the acclimation period, amphipods were reared in the 40-L containers with clean sediment and overlying water with continuous aeration, and they were fed on diatom *Phaeodactylum tricornutum* and ground fish meal (TetraMin®). Test individuals of *M. mai* and *M. acherusicum* were sorted by sieving. The individuals of *M. mai* with 6±1 mm of body length passing through 2-mm sieve, but remaining at 1-mm sieve were used and those of *M. acherusicum* with 4±1 mm of body length remaining between 1-mm and 0.5-mm sieve were used for all the experiments.

2.2 General experimental preparations

Experimental temperatures were maintained using the temperature-controlled incubators and water baths. Temperatures of tested waters were checked everyday and the mean measured tempera-

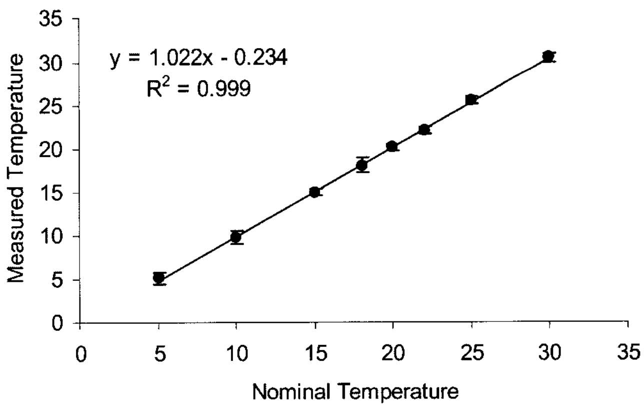

Fig. 1. Comparison of the mean measured temperatures (°C) to the nominal experimental temperatures for *Monocorophium acherusicum*. Error bars indicate standard deviations (N=10).

tures were not significantly different from the nominal temperatures (Fig. 1). Filtered (GF/F) seawater provided by Incheon Fisheries Research Institute in Yeongheung-do was used as test media for all experiments. Water quality of test seawater was checked periodically. Salinity was maintained at 30±1 psu and dissolved oxygen was maintained over 80%. Test seawaters were exchanged twice a week for the survival and growth tests with sediment substrates or exchanged everyday for Cd-spiked water-only tests. Negative control was always added to each experiment to confirm the acceptability of test animals condition and the experimental procedure.

2.3 Survival and growth tests

Amphipods *M. mai* and *M. acherusicum* were introduced into 1-l beakers each of which had 800 ml of seawater and 200 ml of sediment to determine the survival and growth rates of each species under different temperature conditions. Individuals of *M. mai* were incubated at 5 different temperatures (13, 17, 20, 22 and 25°C) and *M. acherusicum* at 8 temperatures (5, 10, 15, 18, 20, 22, 25 and 30°C) for 10 days. Twenty individuals of amphipod species were introduced into each replicate beaker and four replicates were employed for each temperature treatment. Overlying seawater was continuously aerated and an individual of both *M. mai* and *M. acherusicum* was fed on 0.1 mg of TetraMin® every day.

To determine the survival rates, the remaining live individuals were recovered from sediments in test beakers using 0.5-mm sieve at the end of the incubation. Survival rate (%) was calculated by dividing the number of survived animals by the initial number of introduced animals and multiplied by 100. The wet weights of the survived individuals of *M. mai* and *M. acherusicum* were determined at the beginning and end of the incubation. The initial body weight of each species was measured using randomly selected individuals, which were collected using the same sieving methods for the introduced ones. The mean weight of *M. mai* at the beginning of the incubation was 2.5±0.5 mg (n=3 of 5 ind.) and that of *M. acherusicum* was 0.10±0.02 mg (n=3 of 30 ind.).

2.4 Reference toxicant tests

Amphipods *M. mai* were exposed to control and Cd-spiked seawater (0.5 mg/L) for 15 d at 5 different temperatures (13, 17, 20, 22 and 25°C) and *M. acherusicum* were exposed to control and Cd-spiked seawater (1.0 mg/L) for 4 d at 8 temperatures (5, 10, 15, 18, 20, 22, 25 and 30°C). Dead individuals were counted and removed every day to compare the survival rates of each species between control and Cd-spiked water during the exposure period.

Preparation of Cd-spiked seawater was followed by established protocols (ASTM[1999]). The concentration levels of the spiked Cd were decided by referring the previous test results (Lee *et al.*[*in press*]). Ten individuals of each amphipod species were introduced into each 1-l replicate beaker and three replicates were used for control and Cd-spiked water at each temperature treatment.

2.5 Data analysis

Student t-tests and ANOVA tests were conducted using SPSS® to compare means of survival and growth rate data among treatments. The median lethal time values (LT50) were estimated for *M. mai* in control and Cd-spiked water during the water-only test period using Probit method (USEPA[1994]).

3. RESULTS

3.1 Influence of temperature on the survival of amphipods

The survival rates of *M. mai* were not significantly different from 13 to 25°C and those of *M. acherusicum* from 10 to 30°C (Fig. 2). The ranges of temperature for the >80% survival of both amphipods were very similar (13 to 22°C for *M. mai* and 10 to 22°C for *M. acherusicum*) (Fig. 2). All individuals of *M. mai* survived at 13, 17 and 20°C but the survival rates (mean±SD) of *M. mai* over 20°C decreased with increasing temperature up to 77% at 25°C during the 10-d incubation period. The survival rate of *M. acherusicum* was peaked at 18°C and >90% in the range of 15 to 20°M. About 80% of individuals of *M. acherusicum* were dead at the lowest temperature (5°C), but the considerable number of individuals (83%) could survive at the highest temperature (30°C) during the 10-d incubation period (Fig. 2).

3.2 Influence of temperature on the growth of amphipods

The individual weight of *M. mai* (mean±SD) proportionally increased from 2.4±0.3 to 3.5±0.4 mg with increasing temperature from 13 to 20°C, but did not significantly vary from 20 to

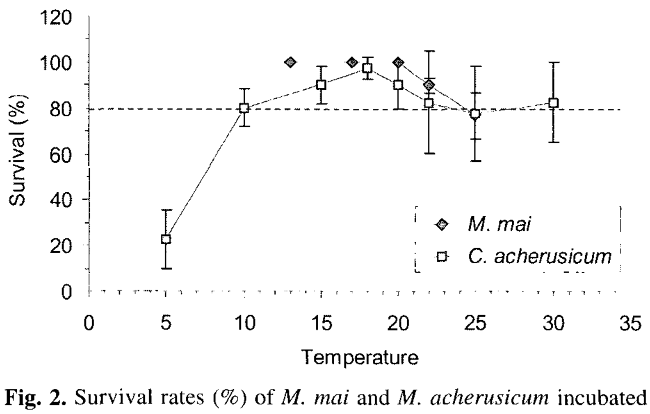

Fig. 2. Survival rates (%) of *M. mai* and *M. acherusicum* incubated for 10 d in clean sediments under various temperature conditions (°C). Error bars indicate standard deviations (N=5).

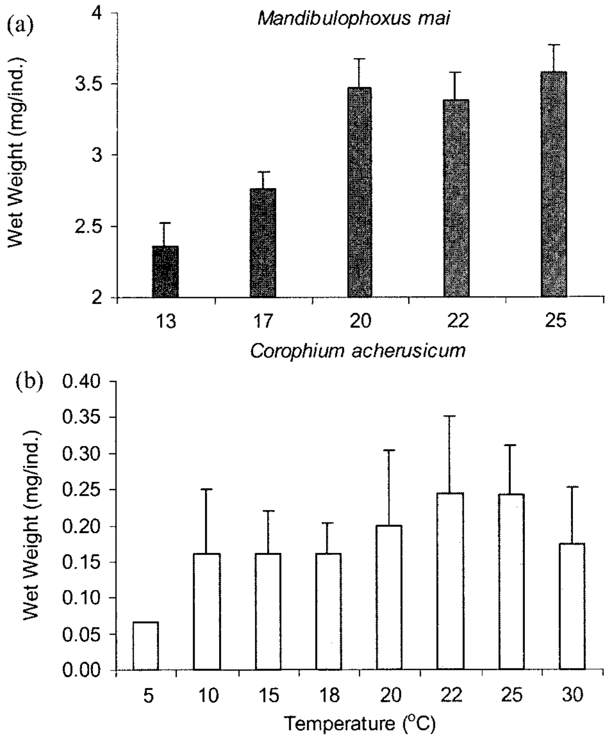

Fig. 3. Mean individual wet weights (mg/ind.) of (a) *M. mai* and (b) *M. acherusicum* at the end of the 10-d sediment incubation at different temperatures (13, 17, 20, 22 and 25°C for *M. mai* and 5, 10, 15, 18, 20, 22, 25 and 30°C for *M. acherusicum*). Each species was fed 0.1 mg/day/ind. of supplementary food. Error bars indicate standard deviations (N=3).

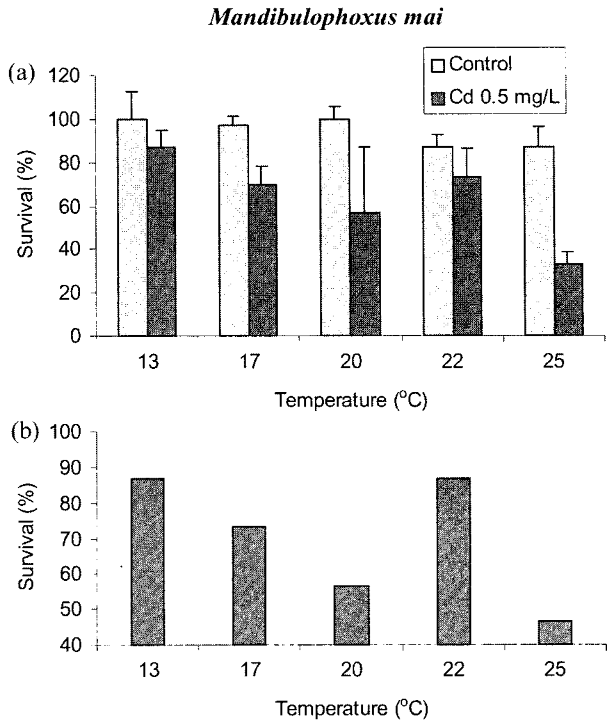

Fig. 4. (a) Survival rates (%) of *M. mai* after the incubation in control (light bar) and Cd-spiked water (dark bar; 0.5 mg/L) for 4 days under 5 different temperature conditions (13, 17, 20, 22 and 25°C) and (b) normalized survival rates of *M. mai* exposed to Cd to control mortality at each temperature. Error bars indicate standard deviations (N=3).

25°C (Fig. 3a). Similarly, the individual weight of *M. acherusicum* increased from 0.07±0.00 to 0.24±0.11 mg with increasing temperature from 5 to 22°C (Fig. 3b). The weight of *M. acherusicum* did not increase at more than 22°C and even decreased to 0.17±0.08 mg at 30°M.

3.3 Influence of temperature on Cd sensitivities of amphipods

The survival rates of *M. mai* and *M. acherusicum* decreased with the increase of temperature both in control and Cd-spiked seawater during the 4-d incubation period. The difference between the survival rates of *M. mai* at the lowest and highest temperature was considerably higher for the Cd-spiked treatments compared to controls. The survival rates (%) of *M. mai* in control seawaters were near 100% in the range of 13 to 20°C, and those were decreased to 87% at 22 and 25°C in the water-only experiment after the 4-d incubation period, while those of *M. mai* exposed to Cd-spiked seawater (0.5 mg/L) decreased from 87 to 33% with increasing temperature from 13 to 25°C (Fig. 4a). The survival rates of *M. acherusicum* also decreased with temperature both in control and Cd-spiked seawater after the 4-d incubation period. The survival rates (%) of *M. acherusicum* in control did not vary from 5 to 15°C and mostly >95%, and decreased to 40% with increasing temperature to 25°C after the 4-d incubation period, however, those of *M. acherusicum* exposed to Cd-spiked seawater (1.0 mg/L) decreased proportionally depending on temperature across the whole temperature range (5 to 30°C).

The survival rates of test organisms exposed to Cd-spiked water were normalized to control mortality to evaluate the influence of temperature on the sensitivity to the reference toxicant This normalization can eliminate any other cause of mortality such as thermal stress than Cd toxicity (Fig. 4 & 5b). The normalized survival rates in Cd-spiked waters decreased proportionally with the increase of temperature from 13 to 20°C for *M. mai* (Fig. 4b) and 5 to 15°C for *M. acherusicum* (Fig. 5b), and remained relatively constant at the higher temperatures.

The survival rates of *M. mai* in controls and Cd-spiked seawaters were compared for 15 days at 5 different temperatures. The more than 80% of individuals survived in controls at lower temperatures (13, 17 and 20°C), while only 53 and 30% of individuals survived at 22 and 25°C, respectively, after the 15-d incubation period (Fig. 6). The lethal time of *M. mai* exposed to Cd decreased

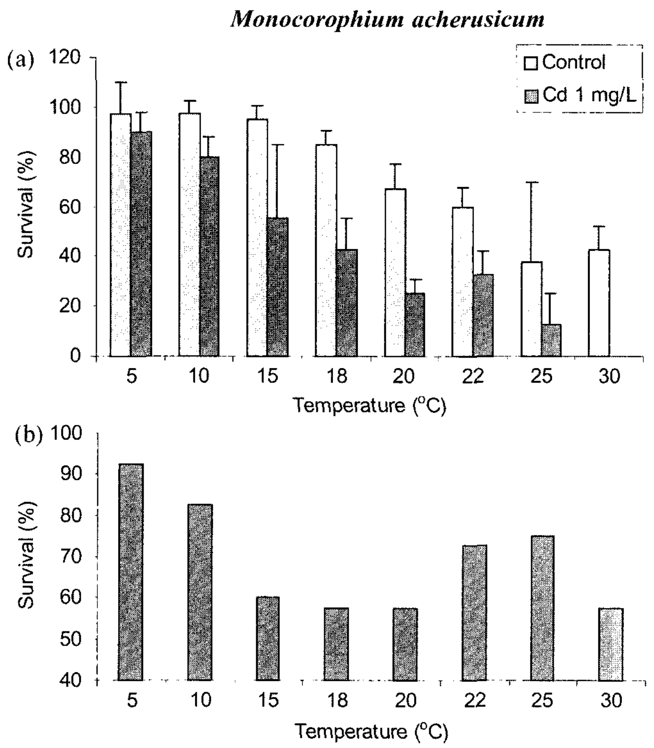

Fig. 5. (a) Survival rates (%) of *M. acherusicum* after the incubation in control (light bar) and Cd-spiked water (dark bar; 1.0 mg/L) for 4 days under 8 different temperature conditions (5, 10, 15, 18, 20, 22, 25 and 30°C) and (b) normalized survival rates of *M. acherusicum* exposed to Cd by control mortality at each temperature. Error bars indicate standard deviations (N=3).

with water temperature. All the individuals of *M. mai* exposed to Cd-spiked waters at 13, 17, 20, 22 and 25°C were dead at 15, 12, 10, 10 and 7 days after the beginning of the exposure (Fig. 6). The LT50s of *M. mai* in controls at lower temperatures (13, 17 and 20°C) were more than hundreds of days, while those at 22 and 25°C were only 34 and 14 days, respectively. The LT50s of *M. mai* exposed to Cd was considerably lower than those in controls at a given temperature. The LT50s from Cd treatments significantly decreased with increasing temperature from 13 to 25°C, however, the substantial change of LT50 was not observed in the range of 20 to 25°M.

4. DISCUSSION

4.1 Influence of temperature on natural populations

Water temperatures at the collecting sites of *M. mai* and *M. acherusicum* may be varied widely among seasons. Previously, Lie *et al.* (1986) showed that mean temperatures of 16 years (1967-1982) near the collection sites of *M. mai* and *M. acherusicum* varied monthly from 4°C (February) to 24°C (August). Therefore, these amphipods can tolerate more than this range of water temperature, because they were found most of the year and the daily variation of temperature will be greater than the monthly variation. Furthermore, since these amphipods spend most of their

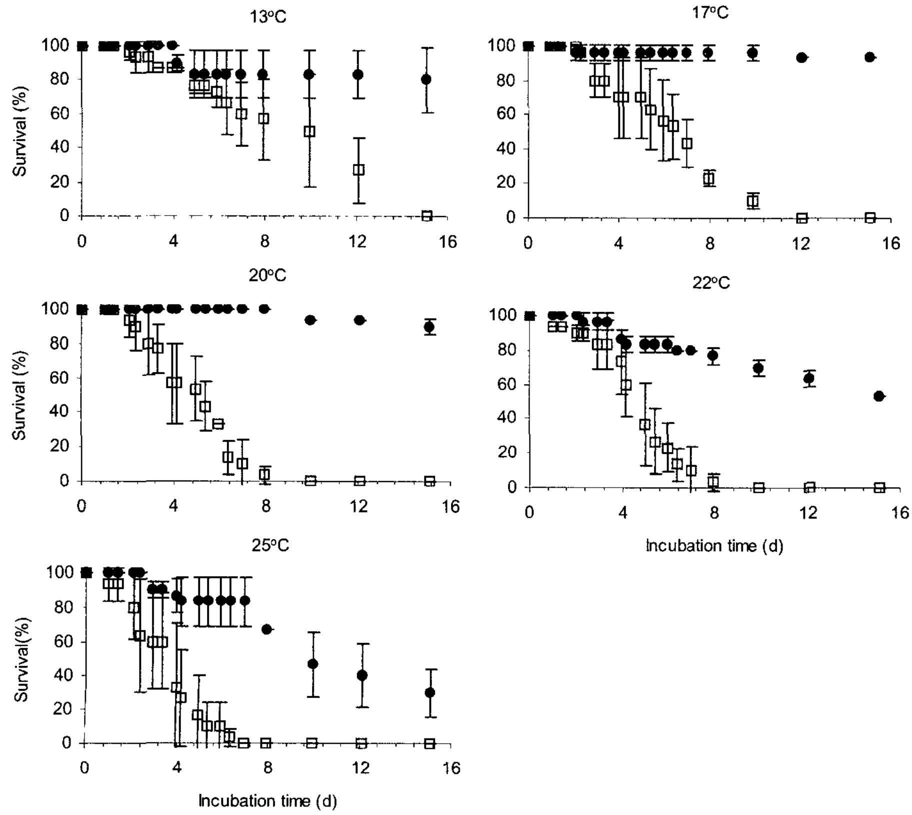

Fig. 6. Temporal variations of survival rates of *M. mai* incubated in control (dark circle) and Cd-spiked water (open square; 0.5 mg/L) for 15 days under 5 different temperature conditions (13, 17, 20, 22 and 25°C). Error bars indicate standard deviations (N=3).

Table 1. Median lethal times (LT50; day) of *M. mai* incubated in control and Cd-spiked water (0.5 mg/L) for 15 days under 5 different temperature conditions (13, 17, 20, 22 and 25°C).

		Temperature (°C)				
		13	17	20	22	25
LT50 (day)	Cd - 0.5 mg/L	8.8	5.6	4.2	4.1	3.0
	Control	381.0	>500	>500	33.6	14.4

life spans in the intertidal zones, the surrounding temperatures would be changed more severely during the low tide.

The ecological information for either *M. mai* or *M. acherusicum* was barely available from literatures. The density of *M. mai* at the collection site was changed seasonally, which was relatively high from September to November (autumn) and usually sparse from December to March (winter-early spring). Sexually matured and neonate-brooding females of *M. mai* were often found from June to October, therefore, most of dense population found in autumn might be newly-born individuals during the spawning period (personal observation). The life span of *M. mai* seems to be longer than 1 year, and it is not certain whether the female of *M. mai* can brood once or multiple times per year. No density data was available for *M. acherusicum* at the collection site or elsewhere. We usually found the dense population of *M. acherusicum* at the most sampling times including February, April and October, 2003, indicating that the reproduction of *M. acherusicum* might be less influenced by season. The *Monocorophium volutator*, the sibling species of *M. acherusicum*, had one or two generations per year depending on water temperature (Wilson and Parker[1996]). Temperature may control the population growth of *M. mai* and *M. acherusicum* in natural environments by influencing the growth and reproduction of the amphipod individuals.

4.2 Defining adequate temperatures for amphipod bioassays

Most standardized bioassay protocols recommended the appropriate experimental conditions including temperature, salinity and so on, and the bioassay results are supposed to be confident and consistent when conducted under the recommended conditions (ASTM[1999]). The recommended temperature conditions for the amphipod toxicity tests varied from 4°C (*Diporeia* sp.), 15°C (*Rhepoxynius abronius*), 20°C (*Ampelisca abdita*) to 25°C (*Leptocheirus plumulosus*) (U.S. EPA[1994]; ASTM[1999]). In fact, these recommended temperatures might be related to the mean temperatures at the amphipod collection sites and there were not many systematic studies trying to decide more adequate experimental temperature condition for each amphipod species.

The adequate temperature condition for test species should be decided by considering both the control performance and sensitivity to pollutants as well as the ecological relevance (ASTM[1999]). The natural variation of water temperatures, which *M. mai* and *M. acherusicum* have experienced, would be very wide. Therefore, the wide ranges of temperature covering the natural variation were used in the present study to determine the adequate temperature conditions for bioassays using both amphipods.

Control performances of each amphipod species in terms of survival and growth were compared among various temperatures for 10 days with clean sediment substrates in the present study. There were no general criteria for control performance in survival or growth. Usually, more than 90% survival in control was used as the criteria for test acceptability when using amphipod in the 10-d sediment toxicity test. More than 90% of *M. mai* survived in the range of 13 to 22°C and that of *M. acherusicum* in the range of 15 to 20°C in the present study. Therefore, we could assume that there would be only negligible stress caused by water temperature within these temperature ranges.

The sensitivity to dissolved Cd of *M. mai* and *M. acherusicum* for more than 4 days in the present study. Ambient temperature is known to influence on the toxicokinetics of pollutants in water and sediment for aquatic animals including amphipods (Mishima and Odum[1963]; van Wezel and Jonker[1998]; Hutchins *et al.*[1998]), and consequently the toxic responses of test animals to pollutants can be also influenced by temperature. Results in the present study showed that the sensitivities of *M. mai* and *M. acherusicum* to aqueous Cd varied significantly among different water temperatures. The survival rates after 96-h exposure to spiked Cd significantly decreased from 13 to 20°C for *M. mai* and from 5 to 15°C for *M. acherusicum*, probably due to the increased Cd uptake by test organisms or due to the increased vulnerability of animals to Cd toxicity at higher temperatures. Even though the sensitivity of *M. mai* to Cd was greatest at 25°C, the most adequate temperature for toxicity tests in terms of sensitivity would be 20°C because the control survival at 25°C in the sediment test was <80% due to the thermal stress. The LT50 results of *M. mai* exposed to control and Cd-spiked water clearly showed which temperature was most adequate for toxicity tests in terms of sensitivity and also control performance. At 20°C, *M. mai* performed in control seawater as well as those at the lower temperatures and also response to Cd as quickly as at the higher temperatures. The corrected survival rates of *M. acherusicum* exposed to dissolved Cd

Table 2. Summary of the recommended temperatures for sediment bioassays using *M. mai* and *M. acherusicum* based on the natural variation of temperatures at the collection sites, control performances in survival and sensitivities to Cd tested in the present study. Recommended temperatures were suggested by considering all the above criteria.

Category	*M. mai*	*M. acherusicum*
Natural variation at collection sites	4-24°C	4-24°C
Control performance (Survival >90%)	13-22°C	15-20°C
Sensitivity to Cd	20°C	15-20°C
Recommended temperature	20°C	15-20°C

were mostly similar from 15 to 30°C except two anomalies (22 and 25°C). Therefore, it seems to be reasonable to conduct toxicity tests using *M. acherusicum* at temperatures from 15 to 20°C, which was also the temperature range for >90% survival in control with sediment substrates. The appropriate temperatures recommend for sediment toxicity tests using *M. mai* and *M. acherusicum* were summarized in Table 2.

Temperature can also influence on the sublethal endpoints such as growth, as shown in the present study, consistently with previous studies (Brockington and Clarke[2001]; Maranhão and Marques [2003]). In the present study, growths of *M. acherusicum* were determined only for 10 days, but the final weights were 3-5 times higher than the initial weights except 5°C, indicating this species can be employed in the longer-term sublethal bioassays. Similarly, the significant increase of individual weight of *M. mai* at 20-25°C during the incubation period also implied that this species would be applicable in the sublethal tests. The growth rates of *M. acherusicum* and *M. mai* were not significantly different in the range of 10-30°C and 20-25°C, respectively. However, further evaluation adopting longer-term incubation period would be needed to determine which temperature would be most appropriate for growth test using these amphipod species. Contaminated sediments usually contain the mixture of various pollutants. Therefore, the influence of temperature on the sensitivity of amphipods to various pollutants should be evaluated further to find out more adequate test conditions for sediment bioassays using the indigenous amphipods, *M. mai* and *M. acherusicum*.

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