Bull Environ Contam Toxicol (2009) 83:469–473
DOI 10.1007/s00128-009-9835-0

Effects of Temperature on the Sensitivity of *Gammarus aequicauda* (Martynov, 1931) to Cadmium

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Received: 12 November 2007 / Accepted: 9 July 2009 / Published online: 24 July 2009

Abstract *Gammarus aequicauda*, collected at different times of the year was used to assess the mortality rates and the sensitivity to cadmium (96 h LC50), at different laboratory temperatures (10, 15, 20, 25°C). The survival in 10 days sediment control test, was high at all tested temperatures, ranging from 4 ± 1% at 10°C (winter collection) to 13.3 ± 2.1% at 25°C (spring collection). The 96 h-LC50 values recorded, ranged from 1.50 mgCd/L in winter at 10°C to 0.10 mgCd/L in spring and summer at 25°C. The results showed that temperature and season of collection influenced mortality rates and *G. aequicauda* sensitivity to cadmium.

Keywords Temperature · Survival · Sensitivity · *Gammarus aequicauda*

Trace metals (such as cadmium) and temperature are common stressors in many estuarine and coastal areas. Cadmium (Cd) is among the most common metal pollutants in estuaries. It can interfere with various physiological processes in organisms from invertebrates to mammals (Shore and Douben 1994; Roḿeo et al. 2000). Temperature is a critical factor for many marine ectothermic animals inhabiting coastal shallow waters and estuaries because it affects physiological processes in the organism (Kinne 1963) and may alter pollutants bio-availability (Rathore and Khangarot 2002). Generally, as temperature increases, the rate of metabolic processes increases, resulting in enhanced uptake rates of several substances, including toxicants, in marine and estuarine organisms (Bat et al. 2000). In order to generate reproducible and reliable results, most protocols for standardized toxicity tests indicate a range of routine actions such as: temperature salinity, etc., (ASTM 1997; SETAC – Europe 1993; ISO 2005).

The aim of this study was to assess how temperature and season of field sampling influence control mortality rates and the sensitivity to *Gammarus aequicauda* acute cadmium exposure.

The amphipod *G. aequicauda*, a widely spread species along European coastal areas, particularly abundant in the Mar Piccolo estuary (Ionian Sea, Southern Italy), is one of the new studied species to be employed in assessing environmental quality. It can be easily reproduced in laboratory, it is tolerant to a broad range of abiotic factors and sensitive to toxicants (Prato and Biandolino 2005). Notwithstanding, the easy rearing of this species in laboratory (Prato et al. 2006a, b), till now the most common method for obtaining amphipods to conduct sediment testing is field collection. The seasonal variability could influence the response of field collected organisms to toxicant. Therefore it is necessary to evaluate potential seasonal changes in sensitivity. In this way, will be possible to establish a possible range of optimal temperatures that could be used in toxicity tests with this species, in order to improve the bioassay methodology.

Materials and Methods

The experiments were carried out with *G. aequicauda* collected from an unpolluted intertidal area, during winter (January, February), spring (March–May) and summer (June, July) 2007, located in Mar Piccolo basin (Ionian sea, Italy; 40°29′17″N; 17°14′23″E). Collection of

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Table 1 Summary test conditions

	Control tests	Toxicity tests
Seasons	Winter·Spring·Summer	Winter·Spring·Summer
Phases	Sediment	Water
Replicates for each test	3	5
Animals for each replicate	25	20
Size of animals	2–4 mm	2–4 mm
Exposure time	10 days	96 h
Water renewal	None	None
Temperature	10, 15, 20, 25°C	10, 15, 20, 25°C
Salinity	36‰	36‰
Sea-water	0.45 μm filtered	0.45 μm filtered
Aeration	Constant	Constant

G. aequicauda occurred in field by sieving the sediment through 500 μm meshes and rinsing it into polyethylene buckets, with sea-water and native sediment (~3 cm). The native sediment was used as negative sediment control. The test conditions are shown in Table 1.

Prior to evaluating the effect of temperature on cadmium toxicity, temperature influence on survival of test organisms was examined without cadmium addition (negative control sediment). Temperatures (10, 15, 20, 25°C) were selected according to the range of temperatures registered in the locations on the Ionian coast. A gradual adaptation of animals to temperature was performed, varying by 3°C/day from field value to four test temperatures. The test started 48 h after having reached the desired temperature. Amphipods were randomly selected, according to size (2–4 mm total length) and only healthy organisms were used, avoiding mature females and new-born ones. Tests were carried out inside 1 L glass beakers containing about 2–3 cm of native sediment layer and 800 mL of natural filtered seawater. Twenty-five amphipods were introduced into each beaker continuously aerated and maintained to 12:12 h light:dark regime. The exposure time was 10 days with three replicates for each temperature tested. During the test period the amphipods were fed with *Chaetomorpha linum* and *Ulva* sp. ad libitum. At the end of the experiment the mortality was estimated as percentage (±SD) for dead organisms. At the beginning and the end of every test, the water quality parameters, including temperature, pH, salinity and dissolved oxygen, were measured (ASTM 1997).

For evaluating temperature influence on cadmium toxicity, water-only acute toxicity tests were conducted. The five concentrations were made using AAS Cd solutions $Cd(NO_3)_2 \times 4H_2O$ (JT Baker, The Neatherlands) as reference toxicant, plus a control series (ASTM 1997; EPA/USACE 1994). Each series consisted of five replicates with 20 organisms. Preliminary tests were carried out to establish the definitive concentrations of reference toxicant. Cd nominal concentrations were 0.2, 0.4, 0.8, 1.6 and 3.2 mg/L. These concentrations were obtained from a stock solution of 1,000 mgCd/L by serial dilution with filtered natural seawater (GFC Whatman, 0.45 μm). At the beginning of the tests, five water samples were taken for measuring the effective metal concentration. Analyses were carried out using an atomic absorption spectrophotometer with a graphite furnace (Perkin Elmer Zeeman 3030). The detection limit for this procedure was 0.06 μg/L and was calculated on the basis of 20 determinations of the blanks as three times the standard deviation of the blank. The accuracy and precision of the analytical procedures have been checked by analysing a certified reference seawater CRM 403. Analytical results indicate a good agreement between the certified and found value. Cd recovery was equal to 103%. The nominal and effective metal concentrations found are shown in Table 2. All experiments were carried out under 12:12 h light:dark regime. All beakers were continuously aerated, maintaining the dissolved oxygen levels above 70% of air saturation. The amphipods were not fed during the 96 h exposure. The survivors in each beaker at the end of the exposure period were counted. Missing organisms were assumed to be dead and organisms which did not move, were considered dead. At the beginning and the end of every test, the oxygen concentration, salinity and pH were measured, in order to be sure that all replicates were exposed to the same conditions. The average pH was 7.8 ± 0.3, average salinity 35.9 ± 0.2‰, and oxygen saturations were about 80% in all beakers.

These were acceptable conditions for toxicity tests (ISO 2005). The results were tested for conformity to normality (Kolmogorov–Smirnov's test) and variance homogeneity (Bartelett's test). The statistical software package SPSS (version 10.0 for windows) was used to estimate mortality among experiments. Two-way analysis of variance (ANOVA) was used, when the data were normally distributed and the variance was homogeneous; alternatively data were compared with a non-parametric test. Multiple

Table 2 Measured concentration of $CdCl_2$ used in acute toxicity test

Cadmium concentrations (mg/L)	
Nominal concentration	Measured concentration
0.2	0.17 ± 0.009
0.4	0.39 ± 0.020
0.8	0.77 ± 0.039
1.6	1.5 ± 0.087
3.2	3.1 ± 0.169

mean comparisons were made using Tukey's test to estimate differences among experimental conditions. The Trimmed Spearman–Karber method was used to calculate the median lethal concentration (LC50) and 95% confidence intervals (CI) for each test (Hamilton et al. 1977).

Results and Discussion

Temperature has long been recognized as an important environmental factor in aquatic ecosystems in regard to its role over biological activity (growth, reproduction and survival). It is known that a rise in temperature determines an increase in metabolic rate with negative effect on the most physiological and biochemical processes (Wilson and Parker 1996; Bat et al. 2000).

In this study *G. aequicauda*, collected at different seasons of the year, was highly tolerant towards all tested temperatures, showing a high survival rate.

International guidelines (ISO 2005) recommend a control mortality lower than 15% in sediment amphipod toxicity test at 15°C. In this study the mortality of *G. aequicauda* ranged from 4 ± 1% at 10°C for individuals collected in winter to 13.3 ± 2.1% at 25°C for individuals collected in spring. (Fig. 1). These data evidenced that the animals were more tolerant towards low temperature and that the mortality increased as temperature increased. Although, all treatments had little control mortality rates (well below the accepted 15% control mortality for routine toxicity testing), mortality rates were significantly affected by temperature and time collection. Two-way ANOVA also found a significant interaction between these factors in control mortality. Multiple sample comparison revealed significant differences in mortality between individuals collected in winter and spring at all tested temperatures (p-value < 0.05). There were significant differences at 10 and 15°C (p-value < 0.05), between individuals collected in winter and summer, while organisms collected in spring and summer exhibited significant differences at 15–20 and 25°C (Table 3).

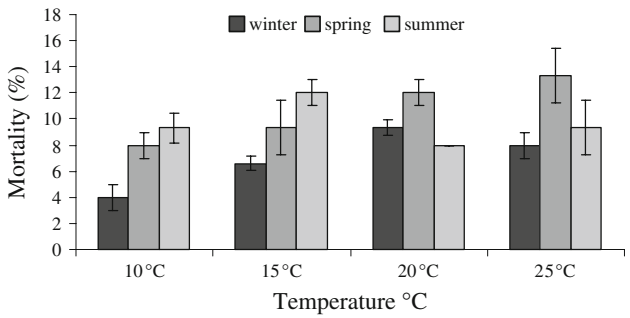

Fig. 1 *Gammarus aequicauda* mortality (%) at different temperatures and sampling periods on a 10 day assay with sediment control

Table 3 Multiple sample comparison of control mortality differences among sampling season at the same temperature

Months	10°C	15°C	20°C	25°C
Winter vs. Spring	*	*	*	*
Winter vs. Summer	*	*	NS	NS
Spring vs. Summer	NS	*	*	*

NS no significant differences

* $p < 0.05$

The statistical analysis indicated that temperature had no significant effect on organisms mortality collected in winter, while significant differences in mortality were detected between 10 and 20–25°C and between 15 and 25°C, in organisms collected in spring. Organisms collected in summer showed a mortality significantly higher at 15°C than at 10–20–25°C (p-value < 0.05) (Table 4).

ANOVA showed that 96 h LC50 was significantly influenced by temperature, season (p-values < 0.05), but not by temperature-season interaction (p-value > 0.05). Tukey's test showed at 10°C significant differences between animals collected in winter and those collected in spring and summer (p-value < 0.05). At 15°C the 96 h LC50 for animals collected in summer was significantly lower compared with the 96 h-LC50 obtained for animals collected in spring (ANOVA, p-value < 0.05).

In the same way, at 20°C the 96 h LC50 value for organisms collected in the summer was significantly lower compared with the 96 h LC50 obtained for those collected in the winter (ANOVA, p-value < 0.05) (Table 5). These data showed that the sensitivity to toxicant increased with temperature rising (Fig. 2). Table 6 summarizes the statistical differences in sensitivity among different temperatures.

Significant differences (ANOVA, p-value < 0.05) were observed for animals collected in winter, with a lower sensitivity at 10°C, compared with those obtained at the remaining temperatures. In spring, the 96 h LC50 at 15°C was significantly different in respect of those at 10, 15 and 20°C (ANOVA, p-value < 0.05). Although the results of

Table 4 Multiple sample comparison of control mortality differences among experimental temperatures at the same sampling season

Temperature (°C)	Winter	Spring	Summer
10 vs. 15	NS	NS	*
10 vs. 20	NS	*	NS
10 vs. 25	NS	*	NS
15 vs. 20	NS	NS	*
15 vs. 25	NS	*	*
20 vs. 25	NS	NS	NS

NS no significant differences

* $p < 0.05$

Table 5 Sensitivity differences to cadmium among sampling seasons at the same temperature

Seasons	10°C	15°C	20°C	25°C
Winter vs. Spring	*	NS	NS	NS
Winter vs. Summer	*	NS	*	NS
Spring vs. Summer	NS	*	NS	NS

NS no significant differences

* $p < 0.05$

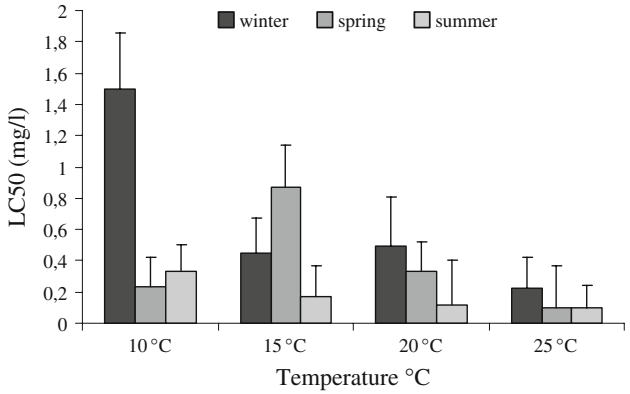

Fig. 2 *Gammarus aequicauda* response (96 h-LC50) to cadmium at four temperatures in the different sampling period (*Errors bars* represent 95% confidence intervals)

Table 6 Sensitivity differences to cadmium among temperatures at the same sampling season

Temperature (°C)	Winter	Spring	Summer
10 vs. 15	*	*	NS
10 vs. 20	*	NS	*
10 vs. 25	*		*
15 vs. 20	NS	*	NS
15 vs. 25	NS	*	NS
20 vs. 25	NS	NS	NS

NS no significant differences

* $p < 0.05$

this study indicated that organisms sampled in the summer were always more sensitive to metal exposure, as it was evidenced by the lower 96 h LC50 values recorded at all temperature treatments, the significant difference has been found only between 10 and 20–25°C (ANOVA, *p*-value < 0.05). Data obtained in this study indicated that *G. aequicauda* was more sensitive to Cd at higher temperatures. This could be the result of increased metabolism which functioning at a higher rate, increases respiration rate (Rathore and Khangarot 2002). These factors determine a metal ion action increase on cellular enzymes and cell membrane, affecting the osmoregulatory mechanisms and indirectly increasing heavy metal toxicity (Yang and Chen 1996; Serra et al. 1999). Trace metals uptake into aquatic organisms generally occurs without the need for specific mechanisms. Simkiss and Taylor (1989) considered that in most cases, uptake occurs through a passive process into the tissues of the organism. But some metals such as Cd may enter by active transport, through calcium ion pumps (Bouché et al. 2000). On the other hand, organisms collected in spring and summer, kept to low temperatures and exposed to cadmium, showed low LC50 values (Fig. 2).

The influence of temperature on metal toxicity is a complex matter, it can be attributed to the relative changes in the rate of metal uptake, elimination, diffusion and biotransformation for an organism. Acute toxicity tests results evidenced that temperature increase reduced the 96 h LC50 values for animals exposed to cadmium. The 96 h LC50 values recorded for different sampling periods and in all temperature treatments ranged from 1.50 mgCd/L (CI 0.81–2.77 mgCd/L) in the winter at 10°C to 0.10 mgCd/L (CI 0.05–0.19 mgCd/L) in spring and 0.10 mgCd/L (CI 0.06–0.17 mgCd/L) in the summer at 25°C (Fig. 2).

In a previous paper *Corophium orientale* collected in winter showed a lower sensitivity to cadmium than *G. aequicauda* with a 96 h LC50 value of 4.48 mgCd/L (CI 2.69–7.12 mgCd/L) at 15°C and a 96 h LC50 value of 1.56 mgCd/L (CI 1.16–2.08 mgCd/L) at 20°C. (Bigongiari et al. 2004). On the other hand *C. insidiosum* showed similar cadmium sensitivity with a 96 h LC50 values of 1.07 mgCd/L (CI 0.84–1.38 mgCd/L) and 0.91 mgCd/L (CI 0.7–1.18 mgCd/L) at 15 and 20°C, respectively (Prato et al. 2007). Acute toxicity tests are an important step in establishing the appropriate water quality criteria. The results of this study on the effects of temperature evidenced the significance of this factor, in determining the toxicity of a given toxicant to organisms. Optimal range selection of temperature is one of the features to take into account for conducting toxicity tests. Therefore, it is necessary, to establish a compromise between the species in ideal conditions and good quality assay. This study highlights some point of interests for developing bioassays with *G. aequicauda*. The mortality percentage has been acceptable for amphipod bioassays, in the negative control sediment, even if it has been significantly influenced by temperatures. Concerning the sensitivity to the reference toxicant, *G. aequicauda* was influenced by temperatures tested, in particular, organisms exposed to the highest temperature (25°C) showed a higher sensitivity in all sampling periods. As the metabolic rate goes up at higher temperatures, heavy metals act more rapidly on the cells and death would occur sooner. Taking into account these results, it will be suitable for bioassay execution not to utilize this temperature. Furthermore *G. aequicauda* appears to exhibit

seasonal variability in Cd sensitivity. Field animals collected in summer were more sensitive to the reference toxicant; this is presumably due to the highest organic matter input in the Mar Piccolo basin, that causes anoxia crises on the bottom (Caroppo and Cardellicchio 1995), making the organisms more stressed. Seasonal changes in sensitivity could be important, because in sediment quality assessment on a routine basis, sediment samples are received throughout the year. Such changes are difficult to eliminate when natural populations are used. Therefore it is necessary to know the seasonal sensitivity to toxicant of test organisms, in order to provide adequate interpretation of sediment bioassay results. The organisms collected in winter exhibited at 10°C as reduced mortality rate, making it less sensitive. Therefore, for this sampling period, the temperature range of 15–20°C for performing toxicity test could be suitable. Amphipods collected in spring showed the highest mortalities rates and high sensitivity to cadmium at temperatures above 20°C.

In the light of the these preliminary results, in order to optimize the methodology to use *G. aequicauda* as a species test in a bioassay, we suggest to adopt a temperature range of 10–20°C for *G. aequicauda* collected in spring and summer, and a temperature range of 15–20°C for organisms collected in winter.

Acknowledgments The authors thank Ms. Maria Filippi for revising the English text.

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