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Influence of Temperature on the Mortality and Sensitivity of *Corophium orientale*

N. Bigongiari,[1] T. Braida,[2] F. Carretti,[1] D. Pellegrini[2]

[1] CIBM, Interuniversity Center of Marine Biology and Applied Ecology, Viale N. Sauro, 4-57127 Livorno, Italy
[2] ICRAM, Central Institute for Research Applied to the Sea, Via di Casalotti, 300-00166 Rome, Italy

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Local and abundant species with an ecological relevance are recommended for marine sediment toxicity tests (Chapmann 1988; ASTM 1993). Amphipods are known to be among the most sensitive organisms of benthic species as shown in a multispecies whole-sediment test (Swartz 1997).

Amphipods, like the other ectotherm organisms, are not able to control their body temperature, but reflect the temperature where they live. For this reason, the environmental temperature becomes one of the determining factors for animal survival (Mills and Fish 1980). This factor can also influence vital cycle, number of generations during the year (McLusky 1968; Birklung 1977; Steel et al. 1977; Nair and Anger 1979; Hastings 1981; Gratto et al. 1983; Donn and Croker 1986), growing (Greze 1977; Peer et al. 1986; Ciarelli et al. 1997), reproduction cycle and egg production (Sheader 1978; Fish and Mills 1979; Wilson and Parker 1996).

In this study, we investigated the influence of temperature on some biological responses of the amphipod *Corophium orientale* (Schellenberg 1928), an endemic Mediterranean species (Ruffo 1982). It is frequently present with high density, up to 20,000 specimens per m^2, in some periods of the year (Carretti 1998) in brackish inshore areas, lagoons and estuaries. *C. orientale* is a tube-building organism living in small burrows both in mud and muddy sand bottoms that behaves as a deposit filter-feeder. However, there is very little information in the scientific literature about this species. Some aspects of biology and ecology regarding a population living at the mouth of the Magra River (North Italy), with particular attention to the life cycle, has been described by Carretti (1998).

The abiotic effects on these organisms must be carefully evaluated both in natural environmental and laboratory conditions since there is a crescent employment of this species in bioassays for the evaluation of marine and brackish sediment toxicity with an usual field collection (Pellegrini et al. 1996; Bigongiari et al. 1998; Onorati et al. 1999). Recently, in fact, *C. orientale* has been introduced as standard species in the ISO (2001) protocol draft.

The aim of the present study was to offer an improvement in bioassay methodology

Correspondence to: N. Bigongiari

by studying the influence of temperature on this specie and the possible effect on its sensitivity with respect to a toxic substance. The most appropriate temperatures are suggested for executing bioassay and toxicity test with *C. orientale*.

MATERIALS AND METHODS.

Amphipods were collected during August - September 1998 and January 1999 from a relatively clean area of the Magra River (Liguria, Italy) where previous analyses indicated the absence of significant contamination (Pellegrini *unpublished data*). The organisms were collected by sieving the sediment through a 500 μm mesh, introduced into polyethylene buckets with native sieved sediment and field water and immediately transported to the laboratory. The amphipods were selected according to size and life stage (large immature or young mature specimens) corresponding to 2-4 mm body length. They were gradually acclimated at the same salinity (35 ‰) and light conditions (500-1000 lux) used for the experiments. Native sediment, sieved through a 350 μm mesh was stored at 4°C in appropriate containers for no longer than two weeks before the experiments. During acclimation, temperature was varied by 3°C/d from field value to five different test temperatures (10°C, 15°C, 20°C, 25°C and 30°C) and organisms were maintained at that value for 48 hr before the experiments. This procedure was repeated at each sampling period and the effect of temperature lower than 10°C and higher than 30°C respectively were not investigated since field values do not exceed these limits.

For the assessment of basal survival rate, the organisms (25 specimens for 4 replicates for each temperature) were transferred in 1 L glass beakers containing a 2 cm layer of sediment (≈200 cc) and approximately 750 ml of natural filtered and continuously aerated seawater. Mortality (expressed as percentage ± SD of dead organisms) was calculated after 10 d which is the standard time in amphipod bioassays for acute toxicity assessment (Hill 1993). Chemico-physical parameters of water (temperature, dissolved oxygen, pH, NH_4^+ and salinity) were monitored at the beginning and end of the test. A regime of continuous light was maintained throughout the experimental periods.
Normality and homogeneity of variances were analysed using Shapiro Wilk's test for small sample sizes and Hartley's F-test, respectively (USEPA 1994a; USEPA 1994b). Mortality values between experiments were compared by nonparametric Wilcoxon's Rank Sum Test when the data were not normally distributed or the variances among treatments were not homogeneous; alternatively the Student T-test ($p < 0.05$) was used.
The influence of temperature on the sensitivity to a reference toxicant was investigated by calculating the variations of LC50 at 10°C, 15°C, 20°C, 25°C and 30°C. For each temperature, the amphipods (20 specimens x 2 replicates) were exposed for 96 hr to increasing concentrations of $CdCl_2$ solutions (control, 0.5, 1.0, 2.0, 4.0, 8.0, mg l^{-1}). The LC50 values and associated 95 % confidence limits were estimated by the Litchfield-Wilcoxon method (Litchfield and Wilcoxon 1949). Table 1 summarizes the experimental conditions.

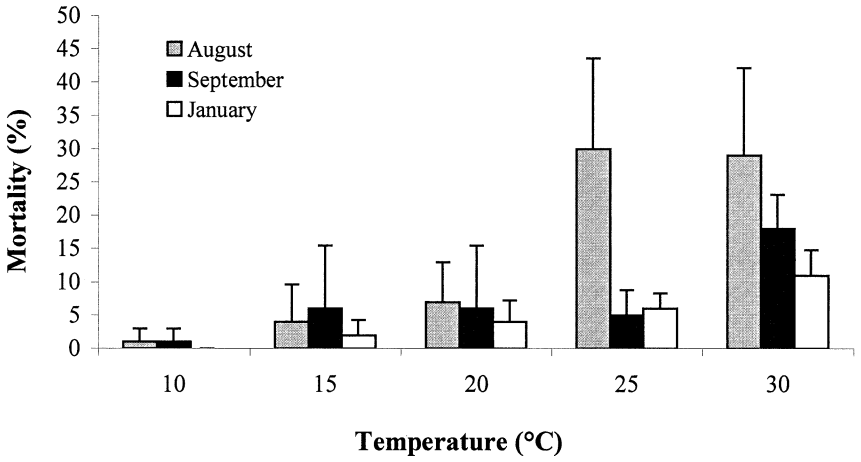

Figure 1. *Corophium orientale* mortality percentages (mean ± standard deviation) at different experimental temperatures.

Table 1. Experimental design.

Objective	Month	Temperature (°C)	Matrix	Replicate	N° organisms	Duration
Mortality	Aug Sep Jan	10-15-20 25-30	sediment	4	25	10 d
Sensitivity to chemicals	Aug Sep Jan	10-15-20 25-30	water	2	20	96 hr

RESULTS AND DISCUSSION.

This study demonstrated that the estuarine amphipod *C. orientale* is very sensitive to variations of temperature. Mortality progressively increased at higher temperatures (Figure 1) despite survival values changing in organisms from different sampling periods.

International agencies (ASTM 1993; ISO 2001) define as acceptable for amphipod bioassays a mortality value lower than 10 % -15 % in control sediment at 15 °C. Based on this rule, organisms maintained at 30°C exhibited high mortality with values of 29 ± 13.22, 18 ± 5.16, 11 ± 3.83 respectively in amphipods sampled in August, September and January. At 25°C only the organisms sampled in August appeared sensitive while below this temperature mortality was always lower than 10 % in all experimental conditions.

Table 2 summarizes the significant differences ($p < 0.05$) obtained in mortality at various temperatures, confirming the main differences between organisms

maintained at temperatures higher or lower than 20°C. Therefore, a statistical difference was not found between 15°C and 20°C.

Table 2. Mortality differences by temperature for each month.

Temperature (°C)	**August**	**September**	**January**
10 vs. 15	N.S.	N.S.	N.S.
10 vs. 20	N.S.	N.S.	N.S.
10 vs. 25	*Significant*	N.S.	*Significant*
10 vs. 30	*Significant*	*Significant*	*Significant*
15 vs. 20	N.S.	N.S.	N.S.
15 vs. 25	*Significant*	N.S.	*Significant*
15 vs. 30	*Significant*	N.S.	*Significant*
20 vs. 25	*Significant*	N.S.	N.S.
20 vs. 30	*Significant*	N.S.	*Significant*
25 vs. 30	N.S.	*Significant*	N.S.

N.S. = Not Significant

Table 3. Mortality differences by months at the same temperature.

Month	**10 °C**	**15 °C**	**20 °C**	**25 °C**	**30 °C**
Aug vs. Jan	N.S.	N.S.	N.S.	*Significant*	*Significant*
Aug vs. Sept	N.S.	N.S.	N.S.	*Significant*	N.S.
Sept vs. Jan	N.S.	N.S.	N.S.	N.S.	N.S.

By comparing responses of organisms from different periods (Table 3), the main difference obtained was for amphipods sampled in August, which were significantly more sensitive at the higher temperatures (25°C and 30°C).

Exposures to cadmium clearly indicated that LC50 was also influenced by temperature, which increased sensitivity of the organisms to the effect of the toxicant (Figure 2). Such a relationship was not evident in organism sampled in August which were always more sensitive to metal exposure as indicated by the constant and low LC50 values obtained at all experimental temperatures.
Organisms sampled during the winter period have a low metabolic activity (Carretti 1998) which could reasonably reduce their sensitivity to toxicants; in fact, when exposed to cadmium at low temperatures very high LC50 values were obtained. On the other hand, elevated metabolic activity would enhance sensitivity of summer amphipods; these amphipods appear close to their limits, showing high mortality at temperatures above 20°C and elevated sensitivity to metal exposure. Therefore, both mortality and LC50 values could be correlated to different metabolic activity in field conditions.
The present study aimed to optimise the methodology for use of *C. orientale* in a bioassay. Talking into account that it would be opportune to execute the bioassay with organisms having constant sensitivity and low mortality in control sediment (< 15 %) the following conclusions can be drawn:

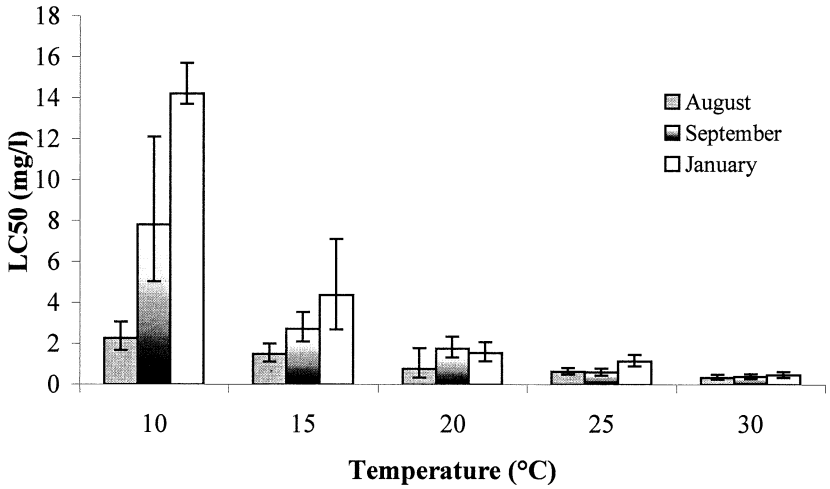

Figure 2. Amphipods response (LC50 calculated at 96 hr) to $CdCl_2$ exposition in the three months considered at the different experimental temperatures. Error bars represent 95 % confidence limits.

1. The temperature at 10°C reduces mortality remarkably but it makes the organisms less sensitive; on the contrary, temperatures at 25°C and 30°C reduce the LC50 values but increase mortality.
2. The optimal temperature for the bioassay appears to be between 15°C and 20°C. It yields, in control sediment, acceptable values concerning organism sensitivity and mortality.
3. Responses in mortality in the temperature range 15°C - 20°C do not significantly differ between organisms sampled in different periods. This aspect is of fundamental importance since summer and winter organisms showed very different responses at the lowest and highest temperatures investigated in the present study.
4. Concerning the sensitivity, the temperature range 15°C - 20°C should be suitable for performing test. In January, anyway, at 15°C the LC50 value is 4.38 mg/l (2.69 - 7.12, range of variation), that is slightly high; consequently, in winter, it would be better to adopt the temperature of 20°C because the LC50 value is 1.56 mg/l (1.16 - 2.08).

For these reasons and taking into account that *C. orientale* generations have a different sensitivity during the year, the adoption of the temperature range 15°C - 20°C for bioassay execution could be suitable. The preliminary results obtained in this study suggest that bioassays with *C. orientale* should be executed at 20 °C in cold months and at 15°C in the other seasons. The adoption of these two different temperatures in the bioassay execution provides constant levels of both control mortality and sensitivity during the year.

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