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Monocorophium insidiosum (Crustacea, Amphipoda) as a Candidate Species in Sediment Toxicity Testing

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Selection of test organisms is one of the critical point for acute bioassays. A good sediment toxicity test species is sensitive to sediment contaminants and tolerant to natural environmental variables such as temperature and salinity, has a short life cycle, a close association to sediment, is amenable to experimental investigation (Costa and Costa 2000), is locally abundant and ecologically relevant (ASTM 1993).

Infaunal amphipods are recommended and commonly used as sediments toxicity test species. Standardized sediment toxicity tests have been developed for burrowing amphipods, including *Rhepoxynius abronius, Leptocheirus plumulosus* (ASTM 1993; EPA 1994), and tube-dwelling species, including *Ampelisca abdida* (ASTM 1993; EPA 1994). The SETAC – Europe guide (1993) particularly recommends the use of *Corophium volutator* or other locally available amphipods. This species is not available in the Ionian coastal area, but there are other endemic species as *Monocorophium insidiosum.*

M. insidiosum is a tube-dwelling amphipod that lives in the brackish and estuarine water of the infralittoral zone and feeds both on sediment and suspended particulate matter. It is the numerically dominant amphipod in the Mar Piccolo basin (Ionian Sea, Italy).

In this paper we report the results of a preliminary study to determine the suitability of *M. insidiosum* (Crawford 1937) as a sediment toxicity test species.

The first objective of the present research was to determine the sensitivity of *M. insidiosum* to non-contaminant variables. Since mortality can often be caused by environmental factors and can lead to inaccurate conclusions (Costa et al. 1996), we have determined the relationship between mortality and natural environmental variables.

The tolerance of a test species to variations in sediment characteristics such as particle size distribution, organic enrichment, water salinity and temperature should be established before responses can be ascribed to contaminant effects.

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MATERIALS AND METHODS.

M. insidiosum was collected from a clean intertidal area, located in Mar Piccolo basin (Ionian sea, Italy; 40° 29' 17"; 17° 14' 23") where the level of toxicants in the sediments is low (Buccolieri et al. 2004). The native sediment characteristics are summarised in Table 1.

Table 1. Physical and chemical data of sampling site.

Native Sediment characteristics	
Organic matter (%)	0.8
Sand :> 63 mm (%)	85.8
Silt : 3- 63 mm (%)	12.9
Clay :< 3 mm (%)	1.2
Cd (μg/g d.wt.)	ND
Hg (μg/g d.wt.)	ND
Pb (μg/g d.wt.)	9.7 ± 0.1
Cu (μg/g d.wt.)	4.2 ± 0.1
Ni (μg/g d.wt.)	18 ± 0.1
Overlying water-quality parameters	
Temperature	16°C
Salinity	36 - 37%
pH	8.3 (7.9 - 8.6)
Dissolved oxygen (mg/litre)	8.3 (8.0 - 8.5)

For metal determinations (Hg, Cd, Cu, Pb and Ni) subsamples of dried sediment were submitted to acid digestion (1:4 HNO_3/HCl and HF) in a teflon vessel (EPA 1996). Mercury was determined by cold vapour atomic absorption spectrometry (Weltz and Schubert-Jacobs 1991). Other metals (Cd, Cu, Pb, Ni) were determined by atomic absorption spectrophotometry with a graphite furnace using a Perkin Elmer Zeeman 3030 spectrophotometer. Sediment samples were analyzed in triplicate. The detection limits were 0.06 μg g^{-1} d.w. for Cd, 0.09 μg g^{-1} d.w. for Hg, 0.11 μg g^{-1} d.w. for Pb, 0.2 μg g^{-1} d.w. for Cu and 0.18 μg g^{-1} d.w. for Ni.

Sediment samples were sieved through a battery of screens of decreasing mesh size (1000–700-500-150μm), the amphipods were rinsed into polyethylene buckets containing seawater and immediately carried to the laboratory where they were maintained in glass aquaria with filtered natural seawater (0.45 μm and 36g/kg) under constant aeration with their native sediment. Experimental organisms were acclimated for 3-4 days before the beginning of the tests. Amphipods retained by a 500 μm mesh sieve, were randomly selected, only active and healthy organisms were used. At the beginning and the end of every test the overlying water quality parameters, including temperature, pH, salinity and dissolved oxygen, were measured to ensure the acceptability of the tests.

The experimental procedure for tests of sensitivity to non - contaminant variables are shown in Table 2.

Table 2. General design for tests of sensitivity to non - contaminant variables.

	T° and Salinity	Grain size and TOM	Type of Diets	Organism density
N°replicates	3	5	3	5
N° animals	20	20	20	10-20-40
Size of animals	2-4mm	2-4mm	2-4mm	2-4mm
Exposure time	10days	10days	28 days	28 days
Water renewal	none	none	biweekly	5 days
Temperature	16 ± 2°C	16 ± 2°C	16 ± 2°C	16 ± 2°C
Salinity	36 ‰	36 ‰	36 ‰	36 ‰
Filtred Seawater	0.45 μm	0.45 μm	0.45 μm	0.45 μm
Aeration	Constant	Constant	Constant	Constant

The effects of four different temperatures (10, 15, 18, 20°C) and five salinities levels (36, 25, 15, 3, 0 g/kg) on survival were examined. Saline solutions were prepared by diluting the water from the reference site (36 g/kg) with distilled water. No gradual adaptation of the animals to salinity was performed, they were exposed directly from 100% of the initial salinity to different treatments. Tests were carried out in 1-litre glass beakers containing an about 2 cm sediment layer and 800ml seawater.

The effects of organism density on survival and growth of *M. insidiosum* were determined. The densities tested were 10, 20 and 40 animals per beaker (about 2.5, 5 and 10 individuals/dm^2). The exposures were carried out with a sediment layer about 2 cm. During assays the amphipods were feed *ad libitum* with dried diet. Amphipods from each treatment were pooled and collectively weighed at the beginning and at the end of the 28-d exposure period. Production was calculated as the proportional increase in the biomass (Final biomass - Initial biomass/ Initial Biomass).

The survival of *M. insidiosum* to different sediment particle sizes and organic matter was examined. The sediments were prepared by mixing the required proportion of FF (fine fraction) of a clean organically enriched sediment with the control sediment. Sediment samples were taken prior to testing and were analysed for particle grain size and organic matter content. Total organic matter (TOM %) was estimated as percentage of weight lost after ignition of dry sediment at 550°C for 4 hours. The natural sediment which was used in this experiment had 0.82% TOM and was enriched with the fine fraction in different proportions: 25, 50, 75 to 100% fine fraction corresponding to 1.91, 2.04, 3.49, and 4.21% of TOM, respectively. The results obtained were tested for conformity to normality (Kolmogorov-Smirnov's test) and variance homogeneity (Bartelett's test). The statistical software package SPSS was used to estimate one and two-way analysis of variance (ANOVA).

Water-only acute toxicity tests were conducted using five concentrations plus a control series of $CdCl_2$, $CuCl_2$ and $HgCl_2$ (ASTM 1997; EPA 1994). Each series consisted of four replicates with 20 organisms.

Preliminary tests were carried out to set the definitive concentrations of each substance. The nominal concentrations of Cd and Cu (added as $CdCl_2$ and $CuCl_2$) were 0.4, 0.8, 1.6, 3.2 and 6.4 mg L^{-1}, the concentrations of Hg (added as $HgCl_2$) were 0.00625, 0.0125, 0.025, 0.05 and 0.1mg Hg L^{-1}. The water used in the experiments consisted of natural seawater (36‰) and filtered through a GFC Whatman (0.45µm) filter. All experiments were carried out at 16±2°C, under 12:12 hour light: dark regime with continuous aeration. The amphipods were not provided with food during the 96h exposure. The number of survivors in each chamber examined at the end of the exposure period was counted and missing animals and organisms which did not move even after gentle stimulation were considered dead.

Differences in the sensitivity to $CdCl_2$ between different size classes have been also examined. The animals were grouped in four different size classes: 1000, 700, 500 and 150µm, adults, sub-adults, juveniles and newborns respectively according to the mesh size. Tests were conducted as described above for water-only toxicity tests. The one-way analysis of variance (ANOVA) was used to compare the mortality rate of the different experimental classes. The cadmium, copper and mercury LC_{50} values, was determined by the Trimmed Spearman–Karber method (Hamilton et al. 1977).

RESULTS AND DISCUSSION.

The results revealed that survival was highest (100%) at 18°C and 15-25-36 g/Kg salinity. In general the survival was high (≥ 90%) at 36 g/kg and at any temperature tested (10-15-18 and 20°C). However, for the salinities of 0 and 3 g/kg, survival decreased to 67-55% at 20°C respectively, but was still high at 18°C and 3 g/kg. ANOVA demonstrated that the survival of *M. insidiosum* was affected by salinity, temperature and salinity-temperature interaction ($p < 0.05$) (Tables 3-4).

The Tukey test carried out *a posteriori* showed that survival at 0 and 3 g/kg was significantly different from survival at 15-25-36 g/kg ($p<0.05$), while at 10-15-20°C it was significantly different from survival at 18°C ($p<0.05$). Therefore we can confirm this species is euryhaline, such as other species of corophiidae that show different tolerance limits (McClusky 1970) preferring a range of 18 °C and 15 - 25- 36 g/kg .

Table 3. *M. insidiosum* survival for different temperatures and salinities.

Water Salinity(g/kg)	Amphipod survival (% ± SD)			
	10°C	15°C	18°C	20°C
0	75±0.8	75±0.8	70±1.6	55±0.9
3	70±1.6	60±1.6	93±0.6	67±1.3
15	80±1.6	78±1.7	100	79±1.3
25	80±1.4	88±1.3	100	100
36	90±1.4	93±1.7	100	98±0.6

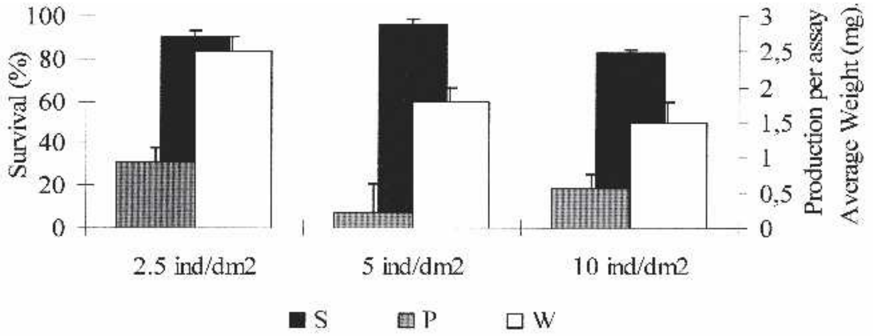

Figure 1. Survival (S), mean weight increase (W) and production (P) of *M. insidiosum* for three organism densities.

Table 4. Two-factor ANOVA of effects of salinity and temperature on survival.

Source	d.f.	MS	F	P
S(g/kg)	4	84.344	63.656	0.000
T°C	3	35.877	27.069	0.000
S xT	12	11.294	8.524	0.000
Residual	79.5	1.325		

At the end of 28-d the survival at different organism densities ranged between 82.5 -95 %, the highest values were found at 5 ind/dm^2. The one-way ANOVA indicated that density affected survival (df = 11, $p < 0.05$), while the average weight increase and the production effects were not significant (df = 11, $p > 0.05$). Hence the appropriate organism density of this species should be about 5 ind./dm^2 (Figure 1).

Amphipod survival was generally high in all of the laboratory manipulated sediments. The variation of sediment particle size and the inherent alteration in the organic content did not affect survival of this species (p>0.1 df=9) (Table 5).

Table 5. Survival of *M. insidiosum* after 10 days of exposure to different sediment particle sizes and organic matter.

Fine Fraction	(%) TOM	Survival (%±SD)
0	0.37	92±1.14
25	2.47	100
50	3.03	88±1
75	3.39	95±1
100	3.57	95±2.08

M. insidiosum showed differing sensitivity to cadmium and copper: the 96h nominal LC_{50} of cadmium, in the aqueous phase was 1.68 mgCd/L (95% confidence intervals: 0.94-2.40 mg/L) and for copper the LC_{50} was 5.74 mgCu/L (95% confidence intervals: 3.02 – 10.91 mg/L). The lowest concentration at which

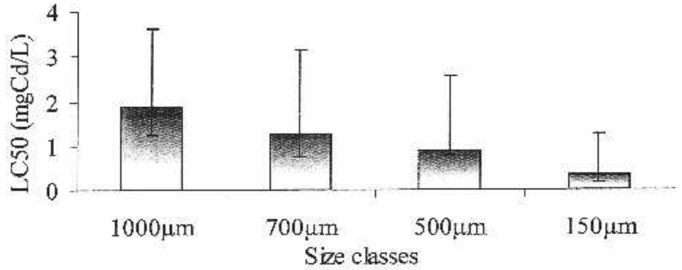

Figure 2. Sensitivity to $CdCl_2$ between different size classes.

survival differed from the control (LOEC) was 0.00625 mgHg/L and the acute toxicity of mercury in sea water at 96h LC_{50} was 0.07mg/L (95% confidence intervals: 0.08 – 0.06 mg/L). Mean percentage of survival in the controls of all experiments was high (97.1%; SD = ± 0.81) (Table 6).

The sensitivity to $CdCl_2$ showed that newborn and juveniles were significantly more sensitive than adults and sub-adults ($p<0.05$) (Figure 2).

Table 6. LC_{50} values for *M. insidiosum* exposed to metals in seawater.

Toxicity test	LC50 (95% confidence interval)
96 h cadmium chloride in seawater	1.68 mg/L (0.94-2.40)
96 h copper chloride in seawater	5.74 mg/L (3.02-10.91)
96 h mercury chloride in seawater	0.07mg/L (0.06- 0.08)

Several standard methods have been developed for assessing the toxicity of contaminants using amphipods species from Atlantic and Pacific coasts (ASTM 1993), however few tests have been developed with European species, more specifically with Mediterranean species (Cesar et al. 2002). This study shows that *M. insidiosum* is potentially suitable as a Mediterranean test organism.

Over a 10-day period, the survival of this species was detected in response to salinity/temperature, the survival rate was in general still high although at low salinities (0-3g/kg) and at 20°C the survival was lowest. This is due, probably, to no gradual adaptation of the amphipods to different salinities/temperatures. McClusky (1970) reported that adult estuarine *C. volutator* survived in salinities of 2 to 59 mg/kg but preferred a range of 10 to 30mg/kg.

M. insidiosum showed good qualities of survival within the tested range of particle size (97%; SD 4.4). Nevertheless, further reference controls are necessary when the tested sediments are outside that range. Schlekat et al. (1992) reported a survival for *Leptocheirus plumulosus* of 97% after 10-d for the sediment ranging from sand to clay; survival of *Eohaustorius estuarius* was 92.4% for sediments with ≥80% silt-clay content (DeWitt et al. 1989).

To select the appropriate organism density to conduct the test it is necessary to establish a compromise between the species optimum conditions and assay viability in term of statistical analysis of the results. In our case, the density of organisms affects survival, however, in the toxicity test no food is provided. In our experiments the amphipods were fed and that will affect survival.

Water only toxicity test, conducted to determine the sensitivity of *M. insidiosum* to reference toxicants, showed that this amphipod was sensitive to metals. The sensitivity of *C. insidiosum* for cadmium seems to be lower than that from Reish (1993). Onorati et al. (1999) reported for *C. orientale* a range from 2.91 to 4.28 mgCd/L and Ciarelli (1994) for *C. volutator* an LC50 range from 1.85 to 5.30 mgCd/L. The sensitivity of *C. insidiosum* towards copper is higher than that reported by Reish (1993). Comparison of the LC50 values for mercury are not possible considering the lack of studies.

Robinson et al. (1988) reported differences in sensitivity (to cadmium chloride) in relation to various life-stages for *R. abronius*, and found that the early life stage was less sensitive than the adults. Ciarelli et al. (1997) for *C. volutator*, reported no significant differences in sensitivity between organisms of different size classes.

Previous studies have shown that *M. insidiosum* is less sensitive than other amphipods such as *R. abronius* (Tay et al. 1992). Even if *R. abronius* has some restrictions (sensitive to salinities less than 25‰, extensive effort to collect, does not perform well in fine-grained sediment, restricted geographical distribution) it is employed in sediment toxicity tests (De Witt et al.1989).

The SETAC- Europe guide (1993) recommends the use of indigenous amphipods, because, although standardized test species are extremely important as screening tools to provide contaminant effects information, these species do not always answer the question of local relevance.

Because this species is ecologically relevant in the Mar Piccolo of Taranto, and fulfill most criteria required for suitable sediment toxicity tests, *M. insidiosum* may be an alternative or complementary species for ecotoxicological studies in Mediterranean ecosystems. Clearly, there is a need to carry out further experiments with specific contaminants on this species to assess suitability for detecting toxicity.

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