Reproduction of *Cubaris murina* (Crustacea: Isopoda) under laboratory conditions and its use in ecotoxicity tests

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(With 3 figures)

Abstract

Reproduction of the isopod *Cubaris murina* was studied in the laboratory in order to observe the offspring number per brood, the number and duration of the incubation periods, and the influence of repeated mating on number of young per brood. An experiment was conducted with two different groups: female/male pairs and females that were isolated after mating. No significant difference was found between the two reproductive female groups for the mean number of young per brood (x = 25) and the duration of the incubation periods (x = 17 days). One mating was enough for at least five broods, with no reduction in brood size. There was no significant difference between the number of adult males and females. Low-density cultures had a significantly higher growth rate compared to high-density cultures. Furthermore, the experimental results for optimization isopod culture conditions for terrestrial ecotoxicity testing are reported.

Keywords: isopods, *Cubaris murina*, laboratory, reproduction, ecotoxicology.

Reprodução de *Cubaris murina* (Crustacea: Isopoda) em condições de laboratório e seu uso em testes de ecotoxicidade

Resumo

A reprodução do isópodo *Cubaris murina* foi investigada em laboratório para observar: o número de filhotes por ninhada, número e duração dos períodos de incubação, e se repetidas cópulas influenciam o número de juvenis por ninhada. Um experimento foi conduzido com dois diferentes grupos: pares com uma fêmea e um macho e fêmeas mantidas individualmente depois da cópula. Não foram encontradas diferenças estatisticamente significantes entre os dois grupos de fêmeas em relação ao número de filhotes por ninhada (x = 25) e a duração dos períodos de incubação (x = 17 dias). Uma cópula foi suficiente para ao menos cinco ninhadas, sem redução em seu tamanho. A proporção sexual dos adultos de *C. murina* em laboratório não apresentou diferenças significativas entre a proporção de machos e fêmeas nos cultivos. A densidade influencia o crescimento dos isópodos de forma significativa: culturas com baixa densidade têm maior taxa de crescimento do que culturas com alta densidade. Os resultados experimentais sobre a eficiência dos cultivos em laboratório para ensaios ecotoxicológicos terrestres são apresentados.

Palavras-chave: isópodos, *Cubaris murina*, laboratório, reprodução, ecotoxicologia.

1. Introduction

Interest in soil ecotoxicity tests has grown considerably in recent years (Van Straalen and Van Rijn, 1998). Aside from earthworms (oligochaetes), terrestrial isopods are one of the most frequently used terrestrial invertebrates for testing the effects of chemicals (Drobne, 1997). These organisms play an important role in the recycling of organic matter, because changes in terrestrial isopod feeding rate affect decomposition processes and subsequent matter and energy flow through ecosystems (Drobne, 1997).

Although there is not yet an international standard protocol to use terrestrial isopods in ecotoxicity tests, many studies with several endpoints have been conducted with these organisms. Examples of such studies include the effects of chemicals on survival and body size

(Jones and Hopkin, 1998), adult mortality and number of juveniles (Jänsch et al., 2005), biomass (Niemeyer et al., 2006), acetyl-cholinesterase activity (Ribeiro et al., 1999), energy budget (Khalil et al., 1995), bioaccumulation (Odendaal and Reinecke, 2004), exposure routes and toxicokinetics (Vink et al., 1995; Sousa et al., 2000; Loureiro et al., 2002), locomotor behavior (Sorensen et al., 1997) and avoidance behavior (Odendaal and Reinecke, 1999a; 1999b; Niemeyer et al., 2006; Loureiro et al., 2005). Most of these tests have been developed using species from temperate regions, and therefore, were not able to provide an appropriate answer to the tropical contamination problem, due to the lack of an ecological perspective. One of the first conditions in establishing a standard protocol to use certain organisms in an ecotoxicity test is their cultivation under laboratory conditions, as Caseiro et al. (2000) did for *Porcellio dilatatus* Brandt, 1833, species from temperate climate.

Information regarding reproductive aspects such as sex-ratio, that is important for understanding mating relations and reproductive potential of dioecius organisms (Nair, 1984), the offspring number per brood, the number and duration of the ovigerous periods, and whether repeated mating influence the offspring number per brood, is required to assess the sustainability of any organism to be used in ecotoxicity tests. These types of tests require test organisms that reproduce rapidly and generate a sufficient offspring number. Furthermore, these organisms should be readily available in the field and well adapted to laboratory manipulation.

The present study aimed to assess a population of the pantropical terrestrial isopod *Cubaris murina* Brandt, 1833, under laboratory conditions. Aspects related to *C. murina* reproduction were determined and conditions necessary to provide continuous offspring for ecotoxicity tests were also evaluated.

2. Materials and Methods

C. murina is a pantropical species, widely distributed in Brazil, after its introduction and found mainly in synanthropic habitats (Lemos de Castro, 1971). The species is registered also for several other zones throughout the world (Leistikow and Wägele, 1999).

The isopods used in these experiments were collected once on a farm in Simões Filho (BA, Brazil), identified and cultured under laboratory conditions, at 27 ± 2 °C. Organisms were kept in closed plastic boxes (±300 cm²) on a 2 cm layer of natural soil (pH 6.5 and 3.8% of organic matter, sieved in 2-mm mesh), collected in Cruz das Almas (BA, Brazil), and small holes were made on the box cover to ensure ventilation.

In all experiments, isopods were fed with commercial fish food (AlconBasic®) and tiny slices of potatoes (Carolina, 2004). Periodic spraying of distilled water maintained an adequate moisture level to avoid either desiccation or fungal growth, and moldy food items were immediately removed.

2.1. Reproduction experiment and sex ratio

Ovigerous females at different ages were collected from cultures and transferred individually or with a male to plastic boxes (6 x 6 cm, 5 cm height), containing 2 cm of soil, similar to the culture boxes. As a whole 22 boxes with a single ovigerous female (Group A) and 10 boxes with a female/male pair (Group B) were observed daily over four months. For each female, the number and duration of ovigerous periods, the offspring number per brood, and the molt frequency were recorded. Neonates were removed from the box at the moment of counting.

The ovigerous period was considered from the parturial molt, because the passage of the fertilized eggs to marsupium coincides with this molt (Heeley, 1941), until birth of the young. The results of reproduction of solitary females (Group A) was compared to that of females with a male (Group B), using one-way ANOVA ($\alpha = 0.05$). The successive broods were compared for the mean offspring number using Tukey-Kramer multiple comparison tests (Zar, 1996).

The *C. murina* adult's sex-ratio was analyzed in 30 culture boxes from neonate organisms kept in the laboratory. Adult males can be identified externally by the modifications of the first 2 pairs of pleopods, which form the external genitalia used in sperm transfer (Sutton, 1980). The number of females versus males was compared using a Student *t*-test (Zar, 1996).

2.2. Density experiment

Five different densities were established: 6, 12, 18, 24 and 30 animals per culture box (260 cm²), employing the previous cultivation procedure. For each treatment, 3 replicates were used with animal ages ranging from 5 to 8 days. Animals were not weighed at the beginning of the experiment to avoid additional stress, but were distributed at random to avoid bias. The weight of a separate randomly chosen sample of 75 young individuals, with age ranging from 5 to 8 days, was averaged as initial weight. Specimens were weighed at the 40th, 77th and 110th day of life and fed ad libitum. Dead organisms were continuously removed and not replaced. The parameters measured were survival, growth and reproductive performance (number of young /female, and the time of the first reproduction). The weight from individuals was compared in the different time periods using one-way ANOVA, after testing the data for ANOVA requirements (Zar, 1996). In the event of significant differences, a Tukey-Kramer multiple comparison test was conducted. The offspring number/female among different densities was compared using nonparametric Dunn's multiple comparison test (Zar, 1996).

3. Results

3.1. Reproduction experiment and sex ratio

The offspring number from 111 broods was recorded and analyzed, together with the time from 90 ovigerous periods. The results of 3 successive broods are shown in Table 1.

Table 1. Reproduction statistics for female isopods kept individually after mating (group A) or kept with a male continuously (group B), standard deviation (SD).

	Group A		Group B	
	Mean	**SD**	**Mean**	**SD**
First brood				
Duration of ovigerous period (days)	15.08	1.74	16.17	1.80
Offspring number	23.67	12.34	23.90	9.72
Second brood				
Duration of ovigerous period (days)	16.00	1.17	16.10	1.70
Offspring number	25.11	10.04	28.50	7.53
Third brood				
Duration of ovigerous period (days)	16.38	1.43	16.71	1.63
Offspring number	27.83	9.62	30.47	7.77

The mean number of young per brood was 25 (confidence limit 95% = 23.3 to 26.8), with a minimum of 7, and a maximum of 54 young/brood.

No significant difference was found between group A and group B concerning offspring number and duration of gravid periods ($p < 0.05$). No statistical differences were found among successive broods either ($p < 0.05$).

One mating was enough to obtain at least five broods. After 4 months, 100% of the females generated at least three broods. Although in group A mating could occur only before the experiment beginning, the females became just as frequently gravid as the females from group B, where mating could occur more frequently.

Most specimens consumed their own exuviae, as observed by Nair (1984) in a population of *Porcellio laevis* (Latreille, 1804). Moreover, three cases of cannibalism were observed, where the female ate the male during its molting. Concurrently, some isopods had difficulties during molting and died, because they could not remove their own exuviae.

A sex-ratio of 1:1.2, male to female, was found, with no significant differences between the number of males and females.

3.2. Density experiment

In the first weighing (40th day), the weight from organisms at different densities (18, 24 and 30), showed a significant difference ($p < 0.05$), when compared to the lowest density (6 animals/box) (Figure 1). However, at the end of the experiment (110th day), there was no significant difference in weight between the different densities. It is possible that the higher level of mortality occurring in the higher density groups led to this result, hence lower densities showed far more favorable conditions to survival (Figure 2).

The first individuals were born at the 67th day, except for the highest density (30 animals/box), which took a few more days to generate the first neonate. All offspring were counted at the 110th day. The total number in each replicate was divided by the number of females, and then, the mean offspring number/female in each density was obtained (Figure 3).

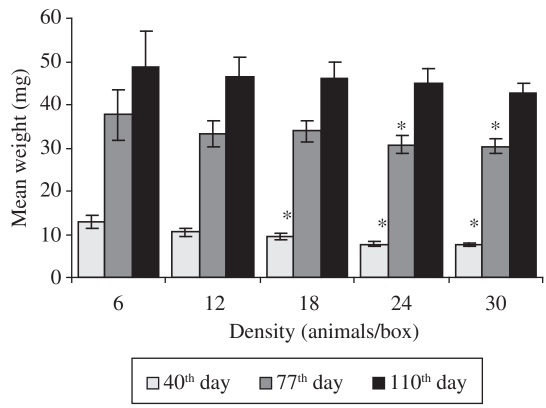

Figure 1. Mean weight of isopods (±SD) in the different density groups. Asterisks indicate significant differences ($p < 0.05$) in relation to the lowest density group.

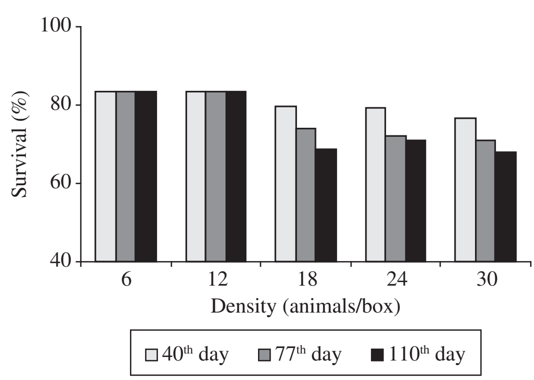

Figure 2. Percentage survival in the different density groups of *C. murina*.

The females in the lowest densities generated more offspring, when compared to higher densities. However, the nonparametric Dunn's multiple comparison tests did not reveal any statistical difference among treatments.

4. Discussion

4.1. Reproduction

Females of *C. murina* were able to store sperm and use it for at least 5 broods without a reduction in brood size. Continuous reproduction under laboratory conditions for at least 4 broods was found by Vink and Kurniawati (1996) for *Porcellionides pruinosus* (Brandt, 1833), and for at least 6 broods by Mocquard et al. (1989) for *Armadillidium vulgare* (Latreille, 1804). Vink and Kurniawati (1996) found no reduction in the size of successive broods after one mating for a population of *P. pruinosus.* The results of the present study are in good agreement with these previous findings and confirm the ability of isopods to reproduce without continuous mating.

The absence of a statistical difference between the number of males and females born in the laboratory indicates a potential equal effort on male and female reproductive tactics. Whether under natural conditions males die earlier than females as in *Armadillo officinalis* Duméril, 1816 (Warburg and Cohen, 1992), the fact that *C. murina* females were able to reproduce continuously under laboratory conditions without copulation, could indicate compensation to the lower number of males in the population.

C. murina appears to be suitable for use in ecotoxicological studies, because supplying a sufficient number of animals for testing can be obtained in a short period. A comparison between *C. murina* and other isopods are provided in Table 2.

4.2. Density

The density of individuals in the maintenance boxes plays an important role in the survival of the organisms, because the lowest densities (6 and 12 animals per box) provided higher growth rate and reproductive output (but not in a significant way). These results are in good agreement with Caseiro et al. (2000), who observed that density affected survival, growth and reproductive output of *P. dilatatus* in laboratory cultures.

The decreased growth rate of individuals in high density boxes had a direct effect on reproduction, because there was a positive relationship between female size and fecundity (Nair, 1984; Hassal and Dangerfield, 1990). Therefore, intraindividual competition at high densities affected the individual body size, which then influenced survival and reproduction success.

Considering that the isopods were fed ad libitum, the negative effects on survival, growth and reproduction were not related to lack of food. Dangerfield (1989) found intraspecific density affected growth, survival and reproduction in a laboratory population of *Armadillidium vulgare*, when food was not a limiting resource. Thus, these effects were due to competition between individuals rather than resource exploitation, confirming the results generated in this study. Ganter (1984) suggested that the nature of intraindividual competition seemed to be related to pheromonal responses of isopods, which, according to Hassall et al. (2005), should be the subject of further investigation.

The fact that reproduction was not affected by density, as no statistical difference was found between the results, might be due to the great variability observed in the data. This variability might be associated with several conditions not addressed here, such as female size, randomness of the experiment (individuals were distributed without any previous sex determination) and the inherent variability of the species.

Cannibalism is common in laboratory isopod cultures (Sutton, 1980) and was observed throughout the present experiments. Molting distress was also a conspicuous feature during the experiments; however, no clear cause could be determined for this. Araújo et al. (2004), working with *Atlantoscia floridana* (van Name, 1940), recorded several cases of mortality during molting, especially in the final stage, when the exuvia collapses with the body wall and the animal cannot remove it.

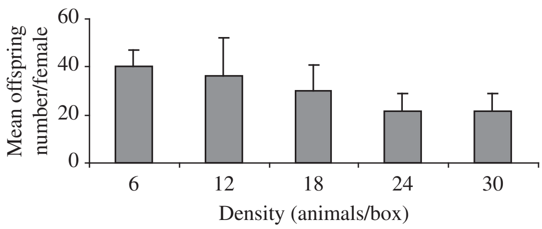

Figure 3. Mean offspring numbers per female (±SD) of *C. murina,* in the different density groups, under laboratory conditions, at the 110th day. No statistically significant differences were found among the densities.

Table 2. Mean offspring number per brood and duration of ovigerous period of *C. murina* and other terrestrial isopod species.

Species	Mean of offspring number per brood	Duration of ovigerous period (days)
Cubaris murina	25.0	17.0
*Porcellionides pruinosus***	28.0	16.5
*Trichoniscus pusillus** Brandt, 1833	5.5	41.5
*Porcellio dilatatus**	17.5	35.5
*P. scaber** Latreille, 1804	24.0	41.0
*Oniscus asellus** Linnaeus, 1758	30.0	39.5
*Philoscia muscorum** (Scopoli, 1763)	13.0	24.5
*Armadillidium vulgare**	113.0	41.0

*Heeley (1941); and **Vink and Kurniawati (1996).

Animals have been easily grown under laboratory conditions, and the use of a standardized commercial fish food, as seen for other crustaceans (Nelson and Brunson, 1995; Maranhão and Marques, 2003), supplemented with a carbohydrate source (slices of potatoes), employed also by other authors (Brody and Lawlor, 1984; Carefoot, 1984; Caseiro et al., 2000), provided the nutritional requirements for their growth and reproduction.

5. Conclusion

Our results indicate that *C. murina* could be cultivated under laboratory conditions and be used as a test organism in ecotoxicology. This species provides great offspring numbers in a relatively short time and is also easily manipulated and maintained in laboratory cultures. Furthermore, *C. murina* is well adapted to tropical conditions and may provide an exceptional tool to assess environmental impact in terrestrial ecosystems in the tropics. Finally, the importance of density in the maintenance boxes was also confirmed and such conditions are to be followed in future cultivation protocols.

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References

ARAUJO, PB., AUGUSTO, MM and BOND-BUCKUP, G., 2004. Postmarsupial development of *Atlantoscia floridana* (van Name, 1940) (Crustacea, Isopoda. Oniscidea): the manca stages. *J. Nat. Hist.,* vol. 20, p. 951-965.

BRODY, MS. and LAWLOR, LR., 1984. Adaptive variation in offspring size in the terrestrial isopod, *Armadillidium vulgare*. *Oecologia*, vol. 61, no. 1, p. 55-59.

CAREFOOT, TH., 1984. Nutrition and growth of *Ligia pallasii*. *Symp. Zool. Soc. Lond.*, no. 53, p. 455-467

CAROLINA BIOLOGICAL SUPPLY COMPANY, 2004. Living Materials Care and Handling Guide For Organisms Used in STC®, STC/MS™, and Other Inquiry-Based Units. Report. 56 p. Available from: http://www.carolina.com/text/pdf/care_guides/Livingmatcareguide.pdf

CASEIRO, I., SANTOS, S., SOUSA, JP., NOGUEIRA, AJA. and SOARES AMVM., 2000. Optimization of culture conditions of *Porcellio dilatatus* (Crustacea: Isopoda) for laboratory test development. *Ecotoxicol. Environ. Saf.*, vol. 47, no. 3, p. 285-291.

DANGERFIELD, JM., 1989. Competition and the effects of density on terrestrial isopods. *Monitore Zool. Ital.*, vol. 4, p. 411-423.

DROBNE, D., 1997. Terrestrial isopods – a good choice for toxicity testing of pollutants in the terrestrial environment. *Environ. Toxicol. Chem.*, vol. 16, no. 6, p. 1159-1164.

GANTER, PF., 1984. The effects of crowding on terrestrial isopods. *Ecology*, vol. 65, no. 2, p. 438-445.

HASSALL, M. and DANGERFIELD, JM., 1989. Inter-specific competition and the relative abundance of grassland isopods. *Monitore Zool. Ital.*, vol. 4, p. 379-397.

-, 1990. Density-dependent processes in the population dynamics of *Armadillidium vulgare* (Isopoda: Oniscidea). *J. Anim. Ecol.*, vol. 59, p. 941-958.

HASSALL, M., ZIMMER, M. and LOUREIRO, S., 2005. Questions and possible new directions for research into the biology of terrestrial isopods. *Eur. J. Soil Biol.*, vol. 41, no. 3-4, p. 57-61.

HEELEY, W., 1941. Observations on the life-histories of some terrestrial isopods. *Proc. Zool. Soc. Lond.*, vol. 111B, p. 79-119.

JÄNSCH, S., GARCIA, M. and RÖMBKE, J., 2005. Acute and chronic isopod testing using tropical *Porcellionides pruinosus* and three model pesticides. *Eur. J. Soil Biol.*, vol. 41, no. 3-4, p. 143-152.

JONES, DT. and HOPKIN, SP., 1998. Reduced survival and body size in the terrestrial isopod *Porcellio scaber* from a metal-polluted environment. *Environ. Pollut.*, vol. 99, no. 2, p. 215-223.

KHALIL, MA., DONKER, MH. and VAN STRAALEN, NM., 1995. Long-term and short-term changes in the energy budget of *Porcellio scaber* Latreille (Crustacea) exposed to cadmium polluted food. *Eur. J. Soil Biol.*, vol. 31, p. 163-172.

LEISTIKOW, A. and WÄGELE, JW., 1999. Checklist of the terrestrial isopods of the new world (Crustacea, Isopoda, Oniscidea). *Rev. Bras. Zool.*, vol. 16, no. 1, p. 1-72.

LEMOS DE CASTRO, A., 1971. Isópodos terrestres introduzidos no Brasil (Isopoda Oniscoidea). *Bolm. Mus. Nac. R. de J.*, vol. 282, p. 1-14.

LOUREIRO, S., SOUSA, JP., NOGUEIRA, AJA. and SOARES, AMVM., 2002. Assimilation efficiency and toxicokinetics of ^{14}C-lindane in the terrestrial isopod *Porcellionides pruinosus*: the role of isopods in degradation of persistent soil pollutants. *Ecotoxicology*, vol. 11, no. 6, p. 481-490.

LOUREIRO, S., SOARES, AMVM. and NOGUEIRA, AJA., 2005. Terrestrial avoidance behavior tests as screening tool to assess soil contamination. *Environ. Pollut.*, vol. 138, no. 1, p. 121-131.

MARANHÃO, P. and MARQUES, JC. 2003. The influence of temperature and salinity on the duration of embryonic development, fecundity and growth of the amphipod *Echinogammarus marinus* Leach (Gammaridae). *Acta Oec.*, vol. 24, no. 1, p. 5-13

MORCQUARD, JP., JUCHAULT, P. and SOUTY-GROSSET, C, 1989. The role of environmental factors (temperature and photoperiod) in the reproduction of the terrestrial isopod *Armadillidium vulgare* (Latreille, 1804). *Monitore Zool. Ital.* (N.S.) Monogr., vol. 4, p. 455-475.

NAIR, GA., 1984. Breeding and population biology of the terrestrial isopod, *Porcellio laevis* (Latreille), in the Delhi Region. *Symp. Zool. Soc. Lond.*, no. 53, p. 315-337.

NELSON, MK. and BRUNSON, EL. 1995. Postembryonic growth and development of *Hyalella azteca* in laboratory cultures and contaminated sediments. *Chemosphere*, vol. 31, no. 4, p. 3129-3140.

NIEMEYER, JC., VILAÇA, D. and DA SILVA, EM., 2006. Efeitos na biomassa de *Cubaris murina* Brandt (Crustacea: Isopoda) expostos ao solo com glifosato em laboratório. *J. Braz. Soc. Ecotoxicol*., vol. 1, no. 1, p. 17-20.

ODENDAAL, JP. and REINECKE, AJ., 1999a. Short-term toxicological effects of cadmium on the woodlouse, *Porcellio laevis* (Crustacea, Isopoda). *Ecotoxicol. Environ. Saf.*, vol. 43, p. 30-34.

-, 1999b. The toxicity of sublethal lead concentrations for the woodlouse, *Porcellio laevis* (Crustacea, Isopoda). *Biol. Fertil. Soils*, vol. 29, p. 146-151.

ODENDAAL, JP. and REINECKE, AJ., 2004. Evidence of metal interaction in the bioaccumulation of cadmium and zinc in *Porcellio laevis* (Isopoda) after exposure to individual and mixed metals. *Water Air Soil Pollut.*, vol. 156, p. 145-161.

RIBEIRO, S., GUILHERMINO, L., SOUSA, JP. and SOARES, AMVM., 1999. Novel bioassay base don acetylcholinesterase and lactate dehydrogenase activities to evaluate the toxicity of chemicals to soil isopods. *Ecotoxicol. Environ. Saf.*, vol. 44, no. 3, p. 287-293.

SORENSEN, FF., WEEKS, JM. and BAATRUP, E., 1997. Altered locomotory behavior in woodlice (*Oniscus asellus* (L.)) collected at a polluted site. *Environ. Toxicol. Chem*., vol. 16, no. 4, p. 685-690.

SOUSA, JP., LOUREIRO, S., PIEPER, S., FROST, M., KRATZ, W., NOGUEIRA, AJA. and SOARES, AMVM., 2000. Soil and plant diet exposure routes and toxicokinetics of lindane in a terrestrial isopod. *Environ. Toxicol. Chem*., vol. 19, no. 10, p. 2557-2563.

SUTTON, SL., 1980. *Woodlice.* Oxford: Pergamon Press. 144p.

VAN STRAALEN, NM. and VAN RIJN, JP., 1998. Ecotoxicological risk assessment of soil fauna recovery from pesticide application. *Rev. Environ. Contam. Toxicol*., vol. 154, p. 83-141.

VINK, K., DEWI, L., BEDAUX, J. and TOMPOT, A., 1995. The importance of the exposure route when testing the toxicity of pesticides to saprotrophic isopods. *Environ. Toxicol. Chem*., vol. 14, no. 7, p. 1225-1232.

VINK, K. and KURNIAWATI, TA., 1996. Iteroparous reproduction, body growth and energy reserves in a tropical population of the isopod, *Porcellionides pruinosus*. *Pedobiologia*, vol. 40, p. 467-476.

WARBURG, MR. and COHEN, N., 1993. Breeding patterns in two terrestrial isopods from the Mediterranean region of Israel. *Biologia Gallo-Hellenica*, vol. 20, p. 55-68.

ZAR, JH., 1996. *Biostatistical Analysis*. 3 ed. London: Prentice-Hall. 662 p.