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Reproductive toxicity of the endocrine disrupters vinclozolin and bisphenol A in the terrestrial isopod *Porcellio scaber* (Latreille, 1804)

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ABSTRACT

Endocrine Disruptor Compounds (EDCs) have been largely studied concerning their effects on vertebrates. Nevertheless, invertebrates as targets for these chemicals have been neglected and few studies are available. Specifically for edaphic invertebrates, data concerning the effects of EDCs is residual. Influences of EDCs on the reproduction systems of these organisms, with consequences at the population level, are expected but have not been confirmed. This work aimed to study the effects of bisphenol A (BPA) and vinclozolin (Vz) on the reproduction of the terrestrial isopod *Porcellio scaber*. Isopods were coupled and exposed to increasing concentrations of Vz and BPA and the females' reproductive cycle followed for 56 d. Both compounds elicited reproductive toxicity. Vz and BPA decreased female reproductive allocation. Vz reduced pregnancy duration; increased the abortion percentage; decreased the number of pregnancies; and decreased the number of juveniles per female while BPA increased abortions at the lowest and highest test concentrations. The reproductive endpoints presented in here are indicative of the possible impact that this type of compounds might have on isopod population dynamics, which may eventually lead to population decline.

1. Introduction

There are increasing evidences that environmental endocrine disruptor compounds (EDCs) are impacting wildlife populations by interfering with some aspects of endocrine-mediated processes, causing impairments of developmental, reproductive and other hormonally mediated processes (Rodriguez et al., 2007). While for vertebrates significant efforts have been made to study the effects of EDCs, to date there are very few studies on the effects of these compounds on invertebrates, although they constitute the vast majority of organisms of the animal kingdom (deFur et al., 1999). Moreover, chemical signalling systems and their basic mechanisms in the animal kingdom exhibit a considerable degree of conservatism (McLachlan, 2001). Consequently, invertebrate endocrine function should be affected by identical or similar compounds as vertebrates (deFur et al., 1999).

Terrestrial isopods are widespread saprophytic organisms that carry a key role in the organic matter decomposition in soils. These organisms, beside being recommended for assessing the ecotoxicological effects of chemicals in the laboratory (Drobne and Hopkin, 1994), combine the features of continuous growth through a molting regime, sexual reproduction, relative knowledge of endocrinology and a terrestrial mode of living, making them suitable candidates for sentinel species for EDCs in terrestrial environments (Lemos et al., 2009). Moreover, the isopods' female reproductive cycle (ovarian maturation and embryogenesis) is an overlapping/synchronous event along with the molt cycle (Subramoniam, 2000). Therefore, chemicals that interfere with the production or function of molting hormones (ecdysteroids) and interfere with the molt are bound to disrupt reproductive processes (LeBlanc, 2007).

In this study, two hormonally active compounds in isopods (Lemos et al., 2009) were investigated. Vinclozolin (Vz) is a fungicide widely used in agriculture. It is a proven EDC causing anti-androgenic effects due to two of its metabolites (Anway et al., 2005). In vertebrates it is known to inhibit testosterone induced growth of androgen-dependent tissues (Kang et al., 2004). It induces Leydig cell tumors (Kavlock and Cummings, 2005), reduces ejaculated sperm numbers and prostate weight (Monosson et al., 1999), leads to a delayed puberty in exposed rats and demasculinization and feminization of male offspring in rats (Gray et al., 1999). Also, one major concern is that Vz causes transgenerational effects

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in exposed rats (Anway et al., 2006; Anway and Skinner, 2008; Nilsson et al., 2008).

Bisphenol A (BPA) is an industrial compound that has generated concerns due to its high production volume, widespread use in many consumer products, and its proven estrogenicity (Okada et al., 2008). In vertebrate wildlife species, BPA induces alteration of sex determination during gonad organogenesis and alteration of gonad function during and after gonad organogenesis (Crain et al., 2007). It has been shown to be an estrogen receptor agonist (Krishnan et al., 1993) for which anti-androgenic properties were also identified (Sohoni and Sumpter, 1998; Lee et al., 2003).

The aim of this study was to determine the effects of these two EDCs on several reproductive parameters in the terrestrial isopod *Porcellio scaber*, when exposed to contaminated soil.

2. Materials and methods

2.1. Test organism and culture procedures

The isopod, *P. scaber* (Latreille, 1804), was obtained from a laboratory culture with no prior history of contamination and kept for 8 years in clean sand and under the conditions described in Lemos et al. (2009). Gravid females, with fully-formed marsupium, were placed in a secondary culture until the release of manca. Isopods born within the same day were used to start a synchronized culture. Once they attained a weight of 4–5 mg they were reared individually in plastic boxes (Ø60 × 30 mm) perforated on the sides to ensure ventilation and under the conditions described earlier (Lemos et al., 2009).

2.2. Chemicals and preparation of test soils

An agricultural natural soil kept in fallow for at least 5 years was obtained from the lower Mondego valley (Portugal). The soil was oven dried at 60 °C for 48 h.

BPA (Merck Schuchardt, Germany, purity >99%) was dissolved in methanol and mixed in with the soil at 10, 30, 100, 300 and 1000 mg kg^{-1} dry soil. Subsequently, methanol was allowed to evaporate under a fume hood for 12 h and moisture was adjusted to 20% (v/w) with distilled water. A solvent control was prepared in the same way, using the same volume of methanol without BPA (represented as 0^{+}). A control was prepared with water added to dry soil.

Vz (Ronilan® 50% active ingredient; BASF AG, Germany) was mixed with soil at concentrations of 10, 30, 100, 300 and 1000 mg a.i. kg^{-1} dry soil. Afterwards, the soil was moistened to 20% (v/w) with distilled water. A control was prepared with water added to dry soil.

Chemical analysis of Vz and BPA treatments concentration were made and were within ±5% of the nominal concentrations. The results are presented in terms of the nominal values.

2.3. Reproduction testing

Intermolt male and female isopods weighing 20 ± 1 mg were taken from the synchronized individual culture to assure virginity. One male and one female woodlouse were paired per box (Ø100 mm × 50 mm). Ten boxes per concentration were prepared. Each box contained 60 g of agricultural spiked soil, and alder leaf discs were added weekly in order to maintain it in excess and to have an equal number of discs in all boxes. Experiments were performed under the same conditions as the *P. scaber* cultures.

Female reproductive cycle was followed for 56 d after mating and the following parameters were recorded: time to reach pregnancy as the time elapsed between the time they were paired and first signs of pregnancy (assessed by morphological inspection of females, by the same observer in each day of the study); pregnancy duration; percentage of females that successfully reached pregnancy within the 56-d test period; percentage of abortions; number of juveniles per female; individual juvenile weight; percentage of live juvenile after 2 months; juvenile weight increase after 2 months; and reproductive allocation (RA) as the quotient of total juvenile fresh weight and female fresh weight after parturition.

2.4. Statistical analysis

All data were checked for normality and homoscedascity. One way analysis of variance (ANOVA) with Dunnett's multiple comparison of means were employed to determine differences relatively to control treatment. Where applicable, results are presented as mean ± SE. For all statistical tests the significance level was set at $P \leqslant 0.05$. All calculations were performed with SigmaStat software package (Systat Software Inc., 2006).

3. Results

3.1. Lethality of Vz and BPA

The eight weeks LC_{50} (95% CI) for Vz was 824 (512–1135) mg a.i. kg^{-1} dry soil. Exposure to 1000 mg BPA kg^{-1} dry soil led to 30% mortality, thus to a LC_{50} value higher than 1000 mg kg^{-1} dry soil. Since the concentration range of Vz include the LC_{50}, the higher mortality of adults and juveniles at higher Vz concentrations provoked a low number of replicates (e.g. at the highest concentration the reproductive success was zero). This contributed to the impossibility of statistically distinguishing significant differences in several parameters tested, despite the visibility of trends, as in the case time to reach pregnancy, juvenile survival and growth and RA (Figs. 1, 4A and 5A). Moreover, in cases where no data is available due to higher mortality, the concentration is omitted from figures.

3.2. Time to reach pregnancy and pregnancy duration

In the controls, the average time to reach pregnancy was of 23 ± 3 d. This interval was lower for isopods exposed to 1000 mg

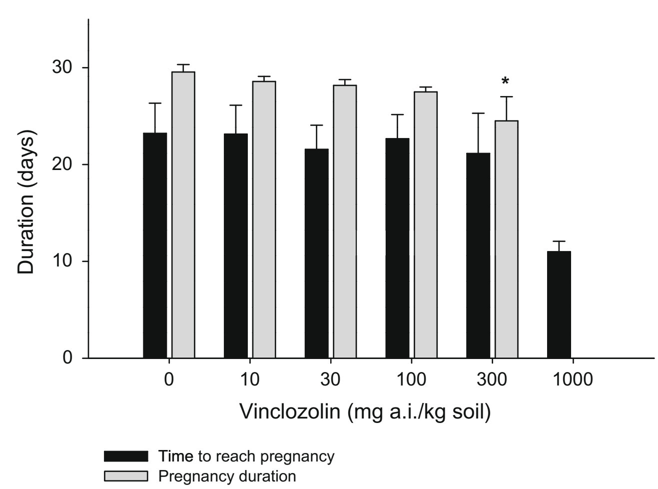

Fig. 1. Number of days until first signs of pregnancy were detected (black bars), and number of days between first signs of pregnancy and release of manca (grey bars) of *Porcellio scaber* exposed to soil treated with vinclozolin. For the time to reach pregnancy *n* ranges from 6 to 9 in all experimental groups except in the group exposed to 1000 mg a.i. kg^{-1} of dry soil, where *n* is 4, due to lower number of pregnancies. Vertical error bars represent the standard error of the mean. An asterisk indicates a significant difference from the control at $P \leqslant 0.05$ (ANOVA, Dunnett's test).

a.i. Vz kg^{-1} dry soil (11 ± 1 d), but this difference was not statistically significant (ANOVA, Dunnett's test, $F_{5.34} = 1.702$; $P = 0.161$) (Fig. 1). This reduced time to achieve pregnancy in the highest concentration is probably due to the fact that only earlier pregnancies were considered while later pregnancies were not found due to the mortality in later periods. Average pregnancy duration was of 30 ± 1 d for the control and decreased with increasing Vz concentrations. This decrease was statistically significant for isopods exposed to 300 mg a.i. Vz kg^{-1} dry soil compared to control (ANOVA, Dunnett's test, $F_{4.23} = 3.226$, $P = 0.031$) (Fig. 1).

BPA did not affected the time to reach pregnancy, which was 21 ± 2 d for the solvent controls, nor the pregnancy cycle duration, of 29 ± 1 d for solvent control (ANOVA, Dunnett's test, $F_{5.36} = 0.141$, $P = 0.982$ and $F_{5.34} = 0.845$, $P = 0.528$, respectively).

3.3. Occurrence of pregnancies and abortions

All control and solvent control females successfully attained gravidity within the test period. Only 40% of the females became pregnant at the highest Vz concentration (Fig. 2A). The percentage of abortions increased with Vz concentration in a dose-dependent way, attaining 100% abortion rate for animals exposed to 1000 mg a.i. Vz kg^{-1} dry soil.

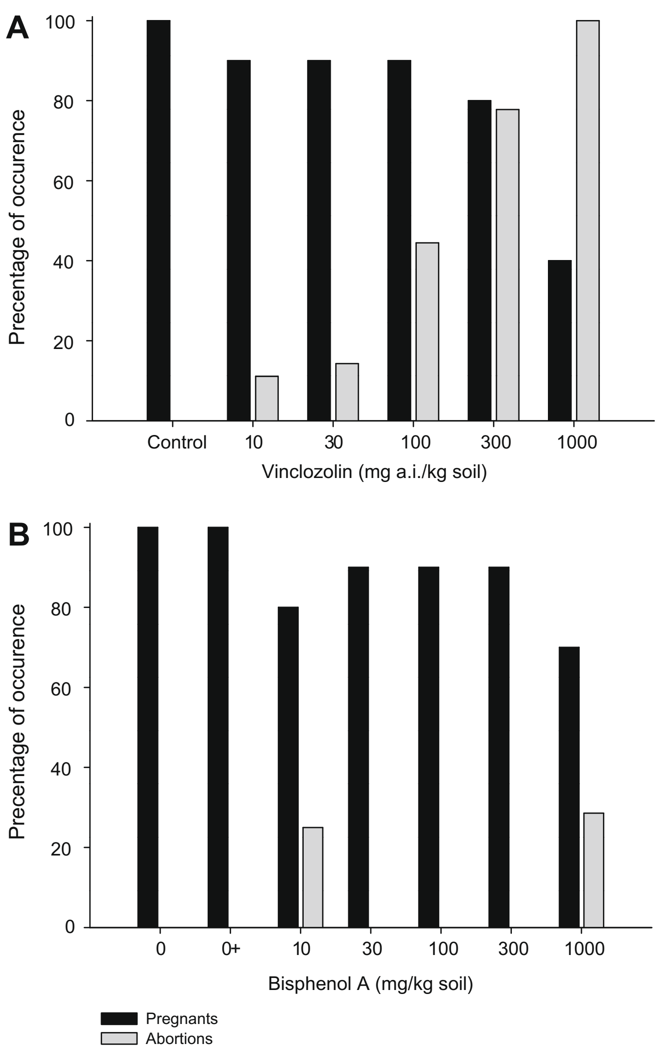

Fig. 2. Percentage of successful females achieving pregnancy (black bars) and percentage of female miscarriages (grey bars) of *Porcellio scaber* exposed to soil treated with (A) vinclozolin and (B) bisphenol A.

The success to achieve pregnancy decreased slightly with increasing BPA concentration, with 30% of the females being unable to become pregnant at 1000 mg BPA kg^{-1} dry soil. In the solvent control, all females carried the pregnancy until the end, but at 10 and 1000 mg BPA kg^{-1} dry soil there were 20% miscarriages (Fig. 2B).

3.4. Number of juveniles per female and their individual weight

The average number of manca delivered per female was of 19 ± 2 in the controls and decreased with increasing concentrations (Fig. 3), with a LOEC of 100 mg a.i. Vz kg^{-1} dry soil (13 ± 1 manca for females exposed to 100 and 300 mg a.i. kg^{-1}) (ANOVA, Dunnett's test, $F_{4.29} = 3.242$, $P = 0.026$). The individual manca weight increased with increasing Vz concentrations (19.7% and 22.0% heavier juveniles at 100 and 300 mg a.i. Vz kg^{-1} dry soil, respectively), although no statistically significant differences were found (ANOVA, Dunnett's test, $F_{4.29} = 1.080$, $P = 0.385$).

The number of born manca was lower in the solvent control compared with the water control (t-test, DF = 14, $t = 2.443$, $P = 0.028$) and was not affected by BPA treatment (ANOVA, Dunnett's test, $F_{5.35} = 0.316$, $P = 0.900$). Weight of individual newborns did not differ between treatments and the solvent control (ANOVA, Dunnett's test, $F_{5.33} = 0.439$, $P = 0.818$).

3.5. Surviving juveniles and their growth after 8 weeks

After 8 weeks exposure to Vz, 71% of the manca survived in the control and only 53% and 25% at 100 and 300 mg a.i. kg^{-1} dry soil, respectively (Fig. 4A), but without statistical significance (ANOVA, Dunnett's test, $F_{4.20} = 1.128$, $P = 0.371$). After that period, control isopods weighed 3.19 ± 0.29 mg with a slightly higher weight gain at lower concentrations and a highly reduced juvenile growth at higher Vz concentrations (1.77 ± 0.30 mg and 1.30 ± 0.91 mg for 300 and 1000 mg a.i. Vz kg^{-1} dry soil, respectively) although this effect was not statistically significant (ANOVA, Dunnett's test, $F_{4.20} = 1.66$, $P = 0.211$).

After 8 weeks exposure to BPA, juvenile survival and growth showed no clear dose–response relationship (Fig. 4B). Significant effects were found on survival (ANOVA, Dunnett's test, $F_{5.31} = 3.270$, $P = 0.017$), but the post hoc test was not able to distin-

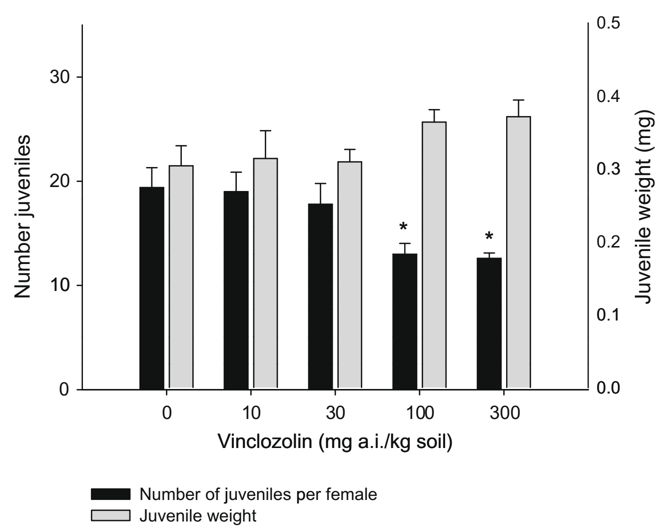

Fig. 3. Number of juveniles hatching per each pregnant female (black bars), and individual manca weight (grey bars) of *Porcellio scaber* exposed to soil treated with vinclozolin. Vertical error bars represent the standard error of the mean. An asterisk indicates a significant difference from the control at $P \leqslant 0.05$ (ANOVA, Dunnett's test).

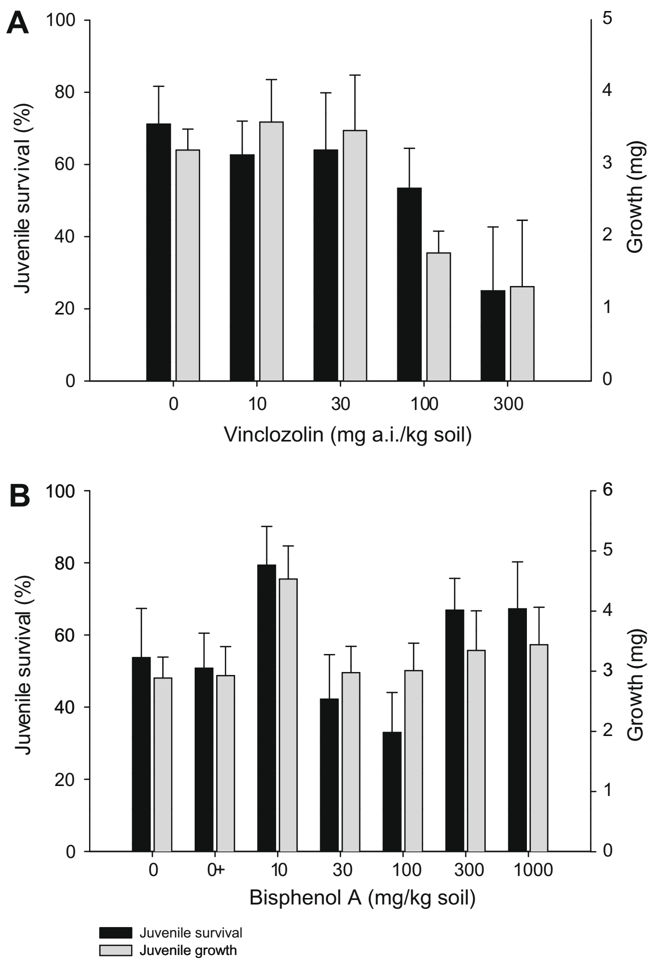

Fig. 4. Percentage of surviving juveniles (black bars), and juvenile increase of weight (grey bars) in *Porcellio scaber* after a 2 months exposure to soil treated with (A) vinclozolin (control group $n = 8$; at 10 mg a.i.kg^{-1} $n = 7$; at 30 mg a.i. kg^{-1} $n = 4$; at 100 and 300 mg a.i. kg^{-1} $n = 3$) and (B) bisphenol A (control group $n = 8$; solvent control $n = 6$; at 10 mg kg^{-1} $n = 4$; at 30 mg kg^{-1} $n = 8$; at 100 mg kg^{-1} $n = 6$; at 300 mg kg^{-1} $n = 8$; and at 1000 mg kg^{-1} $n = 4$). The decrease of replicates along treatments is due to juvenile mortality. Vertical error bars represent the standard error of the mean.

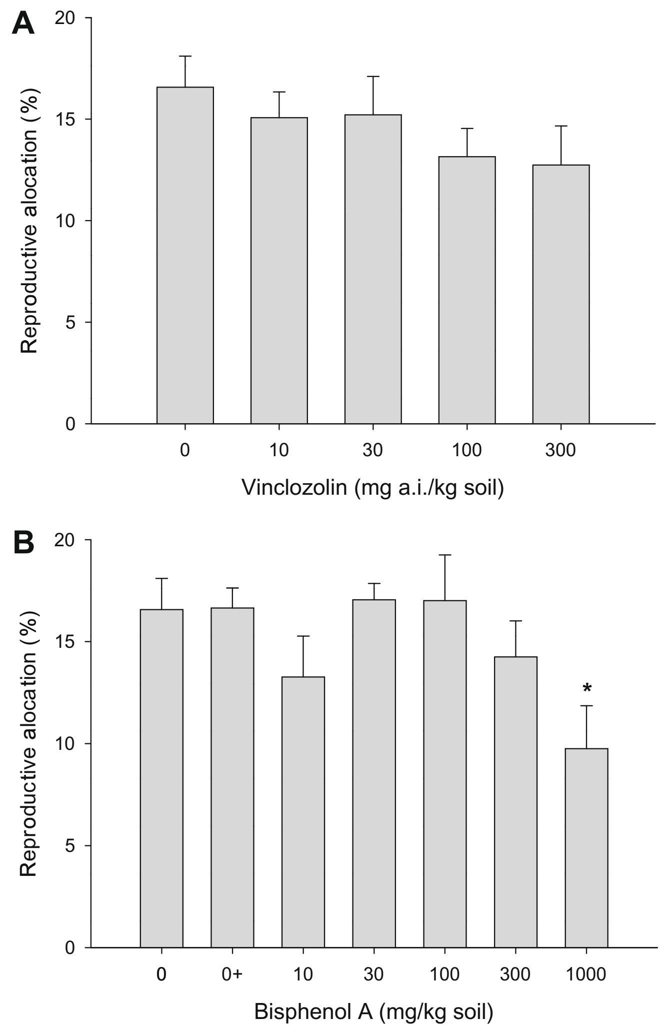

Fig. 5. Reproductive allocation of female *Porcellio scaber* exposed to soil treated with (A) vinclozolin and (B) bisphenol A. Vertical error bars represent the standard error of the mean. An asterisk indicates a significant difference from the control at $P \leqslant 0.05$ (ANOVA, Dunnett's test).

guish between treatments. Survival increased at the lowest concentration. Juvenile growth was stimulated (55%) at 10 mg BPA kg^{-1} dry soil but no significant differences between treatments and solvent control were found (ANOVA, Dunnett's test, $F_{5.31} = 1.317$, $P = 0.284$).

3.6. Reproductive allocation

Reproductive effort, measured as reproductive allocation (RA), decreased with increasing Vz concentrations (23.5% lower at 300 mg a.i. kg^{-1} dry soil), but differences were not significant (ANOVA, Dunnett's test, $F_{4.23} = 0.707$, $P = 0.596$) (Fig. 5A).

The reproductive effort decreased with increasing BPA concentrations. At 1000 mg BPA kg^{-1} dry soil, 41.6% less resources were allocated to reproduction compared to the solvent control (ANOVA, Dunnett's test, $F_{5.33} = 2.621$, $P = 0.042$) (Fig. 5B). An apparent low concentration effect was also observed with 20.4% less RA at 10 mg BPA kg^{-1} dry soil.

4. Discussion

Living organisms allocate their available resources to growth, reproduction, basal metabolism, or to improve survival, in a combination designed to maximize fitness (Sibly and Calow, 1986). Exposure to stressors may give rise to trade-offs in resource allocation as the animal tries to optimize its fitness under the altered environmental conditions, increasing energy expenditure in basal metabolism in order to cope with stress. Ultimately this may lead to growth and reproduction reduction.

It has been shown that the terrestrial isopods *P. scaber* and *Oniscus asellus* from metal polluted sites comprise high reproductive investment suggesting that they were able to redirect resources from other functions, like growth, to meet the physiological costs of detoxification (Jones and Hopkin, 1996). Donker et al. (1993) found that exposure to metals causes increased mortality and slower growth rates in isopod populations, and that this has selected for early reproduction and greater RA. Faber and Heijmans (1995) also noted an increase in RA for *Trachelipus rathkei* exposed to phenanthrene, when these isopods had a history of PAH contamination. Dissimilarly, in this study, exposed *P. scaber* showed a trend to reduce the reproductive effort. Because growth, survivorship and reproduction are targets of selection, life-history theory predicts that habitat disturbances which result in higher adult mortality rates can select for early maturation and increased reproductive effort (Sibly and Calow, 1989). Accordingly, nearly all studies on the breeding phenology of isopods, which have been done using populations with a background of contaminant exposure [e.g. Donker et al. (1993), Jones and Hopkin (1996)], have shown evidence of early reproduction and increased RA. This could be due to changes as result of evolutionary pressures by means of environmental stress drivers (Farkas et al., 1996). In the present study, and because these isopods had no prior toxicant exposure history, the mentioned opposite responses in reproductive effort might be due to the lack of such an evolutionary pressure with results showing a trade-off favouring survival (i.e. more energy allocated to detoxification) rather than growth and/or reproduction.

Of main importance in the crustacean female reproductive cycle is the inextricable link with the molt cycle. The continuous body growth and periodic molting is not deterred by reproduction, except when the eggs are bred in the pleopods, which prevents the onset of the next molting (Subramoniam, 2000). Additionally, it is known that 20-hydroxyecdysone (20E) levels regulate the molting process (Horn et al., 1966). Investigations where crustaceans were injected with 20E showed a precipitation of premolt changes in the cuticle leading to precocious ecdysis (Fingerman et al., 1998; Gunamalai et al., 2004). This may cause the attached embryos to quicken the hatching process and the brood may be lost during the ensuing molt (Gunamalai et al., 2004).

In a previous study (Lemos et al., 2009) we found increased levels of 20E in male isopods exposed to 10 and 1000 mg BPA kg^{-1} dry soil and also to increasing concentrations of Vz. This effect is in agreement with the reduced fecundity and highly increased abortion rate found for both Vz and BPA in this study, at the same exposure concentrations. This may well be the result of the hormonal de-regulation induced by these toxicants.

The reported hyperecdysonism might also be interfering with a key protein of extreme importance in reproduction, vitellogenin (vtg), which is a precursor of the egg yolk proteins (Gohar and Souty, 1984). Increased 20E titres have been correlated with increased yolk protein synthesis and uptake in developing oocytes (Gohar and Souty, 1984), in this way contributing to the disruption of reproductive processes. Induction of vtg production following BPA exposure in fully organized individuals has been reported for fish, amphibians and invertebrates (for a review see Crain et al. (2007)). The stimulatory effect of 20E on vitellogenesis is known to increase the amount of accumulated vitellin in oocytes to be utilized as a source of nutrients during isopod embryonic development (Okumura et al., 1992; Vafopoulou and Steel, 1995). The higher amount of available energy reserves may lead to bigger juveniles even when brooding periods are shorter, as in the case of isopods exposed to Vz concentrations of 100 and 300 mg kg^{-1} dry soil. Another potential reproductive trade-off for stressed females can be the increased number of atretic eggs (Hornung and Warburg, 1994), a strategy resulting in the reuptake of energy for investment in the remaining eggs and juveniles in the marsupium. Added to this, the fact that brood-pouch mortality might be favouring survival of more developed juveniles, may justify both the reduced number of offspring and their increased size. Also noteworthy is that since the isopod marsupium provides only a limited area for egg attachment (Lardies et al., 2004), abnormal egg growth may lead to overcrowding and consequent egg loss or reabsorption, resulting in lower manca numbers (Lardies et al., 2004).

We have shown that vinclozolin induces a decrease of the brood period (43 h earlier at 100 mg a.i. kg^{-1} dry soil and 5 d earlier at 300 mg a.i. kg^{-1} dry soil). A similar observation was seen in female *Armadillidium vulgare* exposed to ants (Castillo and Kight, 2005) and female *Porcellio Laevis* under physical stress (Kight and Nevo, 2004), with stressed females releasing the juveniles almost 48 h earlier than controls. Parental care by female isopods is energetically costly (Lardies et al., 2004). Under stress, this behaviour may represent a trade-off due to the reduction in the amount of metabolic reserves, impairing the successful provision of the juveniles, thereby reducing the length of time a female has to provision for her developing young (Castillo and Kight, 2005).

Smaller freshly released manca of *P. scaber* seem more susceptible to toxicants than more developed juveniles (Fischer et al., 1997). This may be either due to easier absorption of the toxicant through their relatively larger body surface/volume ratio and thin cuticle, or by their lower capacity to metabolize the contaminant. Although being released more developed at higher Vz concentrations, juvenile toxicity due to contamination cannot be excluded. After 2 months, juveniles had grown less at the higher concentrations than at lower concentrations where they were released with lower weights.

Ecdysteroids can bind to vitellin, suggesting that they can be maternally transferred to oocytes (Subramoniam, 2000) and are critical to the normal crustacean embryo development (Subramoniam et al., 1999; Mu and LeBlanc, 2002). Therefore, there is no reason to believe that high 20E titres, reported to have negative effects in adults, will not have similar negative effects in the juveniles, once they are maternally transferred.

Ecdysteroids also have been shown to directly or indirectly regulate some aspects of spermatogenesis emphasizing their importance as reproductive hormones (Gohar and Souty, 1984; Chang, 1997). Additionally, ecdysteroids were also involved in oocyte maturation in the prawn *Palaemon serratus* (Lanot and Cledon, 1989). The first meiotic resumption corresponded to the accumulation of ecdysteroids in oocytes at the premolt stage, and 20E also induced germinal vesicle breakdown *in vitro* (Okumura and Aida, 2000). Therefore, toxicants that interfere with 20E metabolism, as is the case for BPA and Vz (Lemos et al., 2009), will most certainly affect male and female reproduction. Additionally, it has been described (Lemos et al., 2010) that BPA induces the up-regulation of tubulin in isopods' testes. Such a tubulin up-regulation has been shown to lead to aneuploidies in the sea urchin (George et al., 2008) and consequently may lead to fecundity problems and non-viable progeny.

After Vz exposure, we saw a decreasing number of females successfully attaining pregnancy associated with an increased number of miscarriages along with increasing concentrations of the fungicide. These two factors together considerably reduced the total juvenile output. (Fischer et al., 1997) reported a lower reproduction in *P. scaber* exposed to dimethoate, which was not due to a decreased number of manca per female but rather the consequence of a lower proportion of gravid females, suggesting this is the most sensitive reproductive parameter.

The lack of sensitivity of crustacean development and reproduction to estrogens and xeno-estrogens at sublethal concentrations has been reported by several authors (Bechmann, 1999; Hutchinson et al., 1999a,b). Nevertheless, there are numerous examples of xeno-estrogen effects in invertebrate reproductive traits. The xeno-estrogens BPA, 4-tert-octylphenol and 4-nonylphenol (NP) caused significant stimulation of embryo production in the freshwater mudsnail *Potamopyrgus antipodarum* (Duft et al., 2003). BPA also stimulated the reproductive output in *Acartia tonsa* (Andersen et al., 1999). Brown et al. (1999) found both a lower survival and higher fertility in the NP exposed amphipod *Corophium volutator*. A complex "superfemale" syndrome, characterized by massive stimulation of oocyte and spawning mass production, was reported in the BPA exposed gastropods *Marisa cornuarietis* and *Nucella lapillus* (Oehlmann et al., 2000). A structural resemblance of PAHs to estrogens was suggested earlier (Van Brummelen et al., 1996) as an explanation for the stimulatory effect on overall reproduction (increased proportion of gravid females and induction of egg-laying) in *P. scaber*. Albeit the many reports pointing at the stimulatory effects of xeno-estrogens, here we were unable to detect such effect for *P. scaber* exposed to BPA or to Vz. The limited size of the marsupium (Lardies et al., 2004) may restrict the final number of viable offspring a female can deliver (as explained above), making the number of released manca probably not the most suitable parameter to assess an eventual "superfemale" syndrome caused by xeno-estrogens.

The estimated concentration of Vz in the soil, after the maximum recommended application rate of Ronilan®, is of 1 mg a.i. kg^{-1} (Lemos et al., 2009). Also, the only significant route of BPA to the terrestrial environment is through the application of sewage sludge from municipal plants [concentrations of 0.033–36.7 mg kg^{-1} (dw)] (Lee and Peart, 2000) in the land to function as soil improver (Furhacker et al., 2000), indicating that contamination of agricultural soils is a real issue. Whilst the results presented in this work are indicative of the chronic toxicity of these compounds, exposure concentrations at which these effects are elicited were well above the ones expected to be found in the environment for Vz, but some caution should be paid to the ratios at which sludge is mixed in the soil.

5. Conclusions

Reproduction toxicity should be classified as systemic toxicity and not as ED, unless specific parameters such as hormone levels are affected (Barata et al., 2004). In the present study, reproductive impairment has been demonstrated for compounds with proven ecdysteroidal activity. Ecdysteroids are, undoubtedly, the chief hormone factors controlling development and reproduction. Nevertheless, alongside with ED, we cannot exclude that systemic toxicity, such as lethality on the eggs and manca, may play a fundamental role in the reproductive impairment.

On the premise that reproductive success is the ultimate vital population parameter (Hutchinson, 2007), the overall reproductive toxicity is indicative of the possible impact that this type of compounds might have on isopod population dynamics, which may eventually lead to population decline.

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