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Direct and indirect effects of metal stress on physiology and life history variation in field populations of a lycosid spider

Debbie Eraly [a,*], Frederik Hendrickx [a,b], Thierry Backeljau [b,c], Lieven Bervoets [c], Luc Lens [a]

[a] Terrestrial Ecology Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium
[b] Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium
[c] Ecophysiology, Biochemistry and Toxicology Group, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

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ABSTRACT

1. Under stress, life history theory predicts reduced growth rates and adult sizes, reduced reproductive allocation, production of larger offspring and postponed reproduction. Both direct and indirect effects of metals can explain these trends, mainly linked to energetic constraints. Metallothionein-like proteins (MTLP's) are believed to be an important defense mechanism against the adverse effects of metals and other stressors.
2. We tested these predictions comparing six field populations of the wolf spider *Pardosa saltans*, three of which were on sites that are historically polluted with heavy metals.
3. As expected for life histories evolving under energetic constraints, adult size and condition correlated negatively and egg mass positively with Cd concentrations for a subset of four populations. In the population that showed the highest cadmium and zinc body burdens, reproductive output and allocation were lowest and reproduction was postponed.
4. Contrary to our expectation, for all six study populations MTLP concentrations did not increase in exposed populations, indicating that this defense mechanism cannot explain the observed variation in life histories.
5. We conclude that indirect and synergistic effects of metal pollution may be more important than physiological defense mechanisms in shaping life history traits in field populations.

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1. Introduction

Even in (semi-)natural habitats, organisms are often exposed to variable concentrations of pollutants that primarily originate from human activities. Among these, metals comprise one of the most persistent components, with strong direct and indirect impacts on both plant and animal populations (Hunter et al., 1987a,b; MacNair, 1998; Klerks and Levinton, 1993; Posthuma and Van Straalen, 1993; Das et al., 1997; Shaw 1999; Bertin and Averbeck, 2006; Morgan et al., 2007). Direct physiological effects may occur when metals bind with essential molecules and alter their structure or function, which can lead to the inhibition of various enzymatic pathways and antioxidant activity. Indirect physiological effects may occur when reactive oxygen species are produced that disturb lipid structure, cause lipid peroxidation or apoptosis (Viarengo and Nott, 1993; Lukkari et al., 2004; Wilczek, 2005; Bertin and Averbeck, 2006). While less well understood, metal pollution may also impact natural populations through cascading ecological effects. For instance, pollution-driven changes in vegetation structure or reduction in prey availability may result in a reduction in fitness due to increased intra- and interspecific competition for critical resources and changes in predation risk, or in a reduction in reproductive output or survival due to a lower energy budget (Van Hook and Yates, 1975; Hunter et al., 1987b; Posthuma and Van Straalen, 1993; Lock et al., 2003). Population-level changes may, in turn, cause effects at community level, especially if highly sensitive species are keystone members, and may result in alterations in critical ecosystem functions (Coughtrey et al., 1979; Hunter et al., 1987a; Tyler et al., 1989; Read et al., 1998; Nahmani and Lavelle, 2002; Creamer et al., 2008; Clements and Rohr, 2009).

However, organisms dispose of a variety of defense mechanisms against adverse effects of metals, such as intake avoidance, decreased accumulation, increased excretion, detoxification or sequestration in a non-toxic form (Bengtsson et al., 1992; Posthuma and Van Straalen, 1993; Viarengo and Nott, 1993;

* Corresponding author. Fax: +32 9 264 87 94.
E-mail addresses: debbieraly@gmail.com, debbie.eraly@ugent.be (D. Eraly), frederik.hendrickx@naturalsciences.be (F. Hendrickx), Thierry.backeljau@naturalsciences.be (T. Backeljau), lieven.bervoets@ua.ac.be (L. Bervoets), luc.lens@ugent.be (L. Lens).

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Wilczek et al., 2004; Xie and Klerks, 2004; Bahrndorff et al., 2006; Morgan et al., 2007). Metallothionein-like proteins (MTLPs) are believed to play an important physiological role in defense against metal stress. MTLPs are non-enzymatic proteins with a low molecular mass and high affinity for metals, mainly Cu, Zn and Cd, due to their high cysteine content consisting of sulphydryl-groups that directly bind metals in insoluble fractions (Viarengo and Nott, 1993; Dallinger, 1996; Roesijadi, 1996; Nordberg, 1998; Park et al., 2001; Amiard et al., 2006; Janssens et al., 2009). While MTLPs are constitutively expressed, their synthesis can also be induced upon exposure to metals, which may render them suitable biomarkers for metal exposure (Hopkin, 1989; Dallinger and Rainbow, 1993; Viarengo and Nott, 1993; Roesijadi, 1996; Viarengo et al., 1999). Induction levels have been shown to vary among individuals, species, metals, and biotic and abiotic conditions (Amiard et al., 2006).

Correlative as well as experimental studies on invertebrate populations from polluted sites have revealed multiple and often complex effects of metals on life-history traits such as development time, survival, body size, age at reproduction, fecundity and egg size (Bengtsson et al., 1992; Posthuma and Van Straalen, 1993; Posthuma et al., 1993; Shirley and Sibly, 1999; Hendrickx et al., 2003b; Lagisz and Laskowski, 2008). Metal-induced changes in life-history traits can be considered adaptive if they result in fitness increases in individuals originating from polluted sites relative to individuals from non-polluted sites ('life history adaptation' *sensu* Posthuma and Van Straalen, 1993; Klerks and Weis, 1987; Brandon, 1991; Bengtsson et al., 1992; Mouneyrac et al., 2002; Kawecki and Ebert, 2004). However, life-history changes may also reflect non-adaptive trade-offs with traits associated with tolerance mechanisms against pollution. Because these mechanisms are believed to be energetically costly, life history theory predicts resource demanding traits, such as growth and fecundity, to be reduced under metal stress (Southwood, 1988; Sibly and Calow, 1989; Calow, 1991; Donker et al., 1993a; Posthuma et al., 1993; Zera and Harshman, 2001). Body condition (i.e. body mass relative to body size) is considered a sensitive individual-based measure of stress effects (Danielson-Francois et al., 2002; Aisenberg, 2009). While body size is fixed after maturation (Hagstrum, 1971; Hendrickx and Maelfait, 2003), body mass varies with nutritional status and energetic balance and is often positively correlated with fecundity (Jones and Hopkin, 1998; Danielson-Francois et al., 2002). When energetically stressed, females can be expected to invest in less but larger offspring relative to their own size since the latter generally mature earlier, develop faster and survive better (Van Noordwijk and Dejong, 1986; Stearns, 1992; Fox and Czesak, 2000; Tamate and Maekawa, 2000; Hendrickx et al., 2003b). In metal-exposed populations, individuals can also be expected to mature earlier to counter the adverse effects of metals that accumulate during lifetime (Sibly and Calow, 1986, 1989; Posthuma and Van Straalen, 1993; Tranvik et al., 1993; Donker et al., 1993a,b). However, energetic constraints in polluted environments may also cause non-adaptive delays in reproduction (Colvin and Gatehouse, 1993; Winterer and Weis, 2004). Such stress-induced shifts in reproductive timing in response to stress have been widely studied in plants but less so in animals, despite their presumed effects on population dynamics and, together with changes in body size, on reproductive isolation between stressed and reference populations (Fox, 2003; Winterer and Weiss, 2004; Weis et al., 2005; Eraly et al., 2009).

Here we quantify effects of metal stress on MTLP concentrations and life-history traits in a ground-dwelling lycosid spider *Pardosa saltans* (Töpfer-Hofmann et al., 2000). Spiders play a key role in ecosystem functioning and constitute macro-concentrators of metals as predators and polyphages (Dallinger and Rainbow, 1993; Maelfait and Hendrickx, 1998; Heikens et al., 2001; Tojal et al., 2002; Hendrickx et al., 2003a; Jung et al., 2008b). As opposed to insects, which mainly excrete metals (Van Straalen et al., 1987; Janssen et al., 1991; Lindqvist and Block, 1994, 1995; Kramarz, 1999), detoxification in spiders is predominantly based on storage of metals in intracellular granules (Breymeyer and Odum, 1969; Van Hook and Yates, 1975; Brown, 1982; Hopkin and Martin, 1985; Janssen et al., 1991; Kramarz, 2000; Hendrickx et al., 2003a). Higher concentrations of metals are measured in the midgut, or hepatopancreas, that has a low metabolic rate and may serve as a protective barrier for other sensitive organs such as the gonads (Ludwig and Alberti, 1988; Hopkin, 1989; Posthuma and Van Straalen, 1993; Foelix, 1996; Wilczek and Babczynska, 2000). While spiders have higher internal metal concentrations compared to most other soil-dwelling invertebrates (e.g. Van Hook and Yates, 1975; Hunter et al., 1987a; Larsen et al., 1994; Rabitsch, 1995; Maelfait, 1996; Wilczek and Migula, 1996; Maelfait and Hendrickx, 1998; Stone et al., 2001), they do show considerable between-species variation related to lifestyle. For instance, lycosid spiders generally have higher Cd, Zn, Pb and Cu burdens than web-building spiders, likely as a result of their more direct contact with polluted soil (Van Hook and Yates, 1975; Nyffeler and Benz, 1981; Salo et al., 1991; Marczyk et al., 1993; Larsen et al., 1994; Wilczek and Migula, 1996; Hendrickx et al., 2004; Wilczek et al., 2008; Jung et al., 2008a).

Here we assess if, and to what extent, life history and physiological traits of *P. saltans* are affected by metal pollution under field conditions. Contrary to most laboratory experiments, indirect effects of metal pollution are also taken into account. We tested the following predictions: (i) internal concentrations of Zn, Cd and Cu and MTLP are higher in individuals from polluted than from reference sites; (ii) individuals from polluted sites have smaller adult body sizes and lower body conditions, and produce fewer but larger offspring than individuals from reference sites; and (iii) individuals from polluted sites are expected to differ in maturation time from individuals from reference sites.

2. Material and methods

2.1. Study species

Pardosa saltans is a lycosid spider of open forests, forest fringes and clearings, and is widespread across Western Europe (Edgar, 1970; Alderweirt and Maelfait, 1990; De Bakker et al., 2000; Roberts, 1998; Töpfer-Hofmann et al., 2000; Hendrickx et al., 2001). In Belgium, the species occurs in both polluted and unpolluted habitats in the south and northwest (Hendrickx et al., 2001). It is closely related to *P. lugubris*, from which it was taxonomically separated only recently, together with other species of the Pardosa lugubris s.l. complex, based on male courtship display behavior (Töpfer-Hofmann et al., 2000). Like other lycosid species, *P. saltans* forages on ground-dwelling prey, and the species may attain high local densities (Edgar, 1970; Hendrickx et al., 2001). Because females carry their eggs in a cocoon attached to their spinnerets (Roberts, 1998), female reproductive output (cocoon mass, number of eggs and egg mass) can accurately be assessed and related to female characteristics.

2.2. Field sampling

Study populations were located in the province of Liège (southeast Belgium). The region is known for the presence of natural metal-rich outcrops of zinc and lead that were industrially extracted since the 13th century. Extraction peaked during the 19th century and ended in 1970 (Duvigneaud and Jortay, 1987). Polluted sites were initially located based on a study on the distribution of *Viola calaminaria* (Gingins), a typical metalliferous plant species that grows on soils with average soil metal concentrations of 11.886 mg/kg Zn, 24.3 mg/kg Cd and 9.342 mg/kg Pb (Bizoux et al., 2004). Three areas with high densities of *P. saltans* were selected as study sites. La Rochette à Prayon Pond (CH-P1, 50°35′N, 5°40′E) and Bois les Dames (CH-P2, 50°35′N, 5°39′E), both in Chaudfontaine, are located at the east bank of the Vesder river and contaminated due to atmospheric pollution with metal dust (Graitson et al., 2005). Schmalgraf, La Calamine (CM-P, 50°42′N,

6°00′E) is a very small (1.5 ha) site in the Hohnbach alluvial plain, close to the river Gueule, polluted through mining activities in nearby sites with natural ore deposits (Duvigneaud et al., 1979). All three sites are sparsely vegetated with a metal-adapted calamine flora (Bizoux et al., 2004; Graitson et al., 2005). Within these sites, *P. saltans* preferred patches of isolated *Quercus robur* trees. For each of these historically polluted sites, a nearby non-polluted (reference) site was selected consisting of recent clearings that were dominated by beech and lacked a metalliferous flora: Louveigné (CH-R1, 50°33′N, 5°42′E) and Fraipont (CH-R2, 50°33′N, 5°44′E), both in Chaudfontaine, and Kelmis, La Calamine (CM-R, 50°42′N, 6°01′E). Distances between the study populations within the municipalities of Chaudfontaine and La Calamine ranged between 1 and 4 km, while both municipalities were circa 20 km apart. Since sample areas were small (100 m^2 maximum) and interspersed with unsuitable habitat for our study species (i.e. grasslands, arable fields and urban areas), we expect gene flow among the populations to be partially reduced. However, few studies showed that even under high levels of gene flow, genetic divergence in ecologically relevant traits is possible (Dhuyvetter et al., 2007; Nosil, 2008).

Starting on April 3rd 2007, individuals were counted, sexed and aged (by developmental stage) on a weekly base during a period of 8 weeks in sites CH-R1 (51–210 per period), CH-R2 (30–102 individuals per period), CH-P1 (60–72 per period) and CH-P2 (49–92 per period). Due to the large amount of work to monitor these populations on a weekly basis, only four out of six populations could be used for life history assessments. Adult males and females with egg cocoons were randomly collected alive by hand picking and transported individually in plastic containers to prevent cannibalism and avoid mixing of cocoons and mothers. Upon arrival in the lab, individuals were frozen alive at −20 °C for subsequent measurements. In 2008, ten females carrying a cocoon were sampled at random in all six populations to determine metal body burdens and MTLP concentrations. We restricted our analyses to females because former studies showed differences in metal burdens between sexes (Wilczek et al., 2004, 2008).

2.3. Metal and metallothionein-like protein (MTLP) analyses

Estimating the degree of metal exposure of each population was based on the metal body burdens of the spiders rather than soil concentrations as physico-chemical properties of the soil and prey composition hamper the translation of soil concentrations to exposure levels (Dallinger et al., 1992; Hopkin, 1993; Maelfait, 1996; Van Straalen et al., 2001; Du Laing et al., 2002; Tojal et al., 2002; Hendrickx et al., 2004). Moreover, as the excretion of ingested metals in spiders is virtually absent, their internal metal concentration is the most accurate reflection of their lifetime exposure (Hendrickx et al., 2003a, 2003b).

After recording the fresh weight (Mettler Toledo, AT261, Deltarange), we added 350 μl of buffer A (10 mM tris HCl and 85 mM NaCL, pH 7.4, Sigma-Aldrich, USA) and homogenized each individual for 1 min on ice in new polypropylene vials with a Tissue Ruptor (Qiagen). After extracting 100 μl homogenate for MTLP measurement, samples were retained for measuring the metal concentration. To determine internal metal burdens, remaining sample was first dried at 60 °C for 48 h, and a nitric acid (70%, Merck, Pro Analysis, Germany)/hydrogen peroxide (30%, Merck, Pro Analysis) microwave digestion (Blust et al., 1988) was performed. All samples were diluted with Milli-Q water (Millipore, USA) and weighed again. Cd, Zn and Cu concentrations were measured using ICP-MS (Varian Ultra Mass 700, Australia). Certified mussel reference material (CRM 278) of the EU Community Bureau of Reference was used as a quality control. Recoveries were within 10% of the certified values.

To measure MTLP concentrations, 100 μl samples were centrifuged (20 min, 13,200*g*, 4 °C; Eppendorf Centrifuge 5804 R, Germany), 50 μl of the supernatants was separated and stored at −80 °C until analyzed. A cadmium saturation thiomolybdate assay (Klein et al., 1994) was applied. During this process, oxidized MT is converted to native MT by 2-mercaptoethanol and a metal donor Zn^{2+}, followed by saturation with the radioactive Cd^{109} isotope. Cd^{109}- concentrations were then quantified with a Minaxi-Autogamma 5530 counter (Canberra Packard, USA). To calculate Cd-MTLP concentrations (expressed as nmol/g wet weight), we assumed total saturation of MT with the metal ions at a ratio of 7 mol Cd per mol MT, as has been demonstrated for vertebrates and most invertebrates (Kito et al., 1982; Viarengo and Nott, 1993; Hensbergen et al., 2000; Dabrio et al., 2002). Metal concentrations were both expressed in μg (for comparison with other studies) and nmol per g wet weight (better insight in biological effects and for comparison with MTLP concentrations).

To assess the extent to which Cd-, Zn- and Cu-molecules were bound by MTLPs, we also estimated the concentration of free metals by assuming a metal stoichiometry of 7 mol Cd, 12 mol Cu and 7 mol Zn per mol MTLP (Kito et al., 1982; Viarengo and Nott, 1993; Hensbergen et al., 2000; Hollis et al., 2001; Dabrio et al., 2002). Since MTLP's have the highest affinity for Cd, which is the most toxic metal, we also calculated this measure for Cd alone.

2.4. Life history traits

Adult males and females (restricted to females with cocoon) mass was measured in frozen state to the nearest 0.01 mg on a Galaxy™ 110 Ohaus balance after which individuals were stored in a 100% ethanol solution (Merck, Pro Analysis, Germany). Maximum cephalothorax width (Hagstrum, 1971) of all adult spiders on ethanol was measured with a Wild M3 stereomicroscope with eyepiece graticule (Heerburg, Switserland; measurement error = 0.06 mm; male and female cephalothorax width). Body condition was determined by regressing spider mass against cephalothorax width. To quantify reproductive output and investment, we measured cocoon mass in frozen state, reproductive allocation by regressing cocoon mass against cephalothorax width, fecundity expressed as the number of eggs or young within the cocoon, and average egg mass, determined as the weight of the cocoon divided by the number of eggs. The weight of the silk surrounding the cocoon was not detectable by our balance (< 0.01 mg) and does therefore not affect our estimates of cocoon weight and egg size. To study phenological differences between the four CH study populations, we compared the relative proportions of sub-adult spiders, adult males and females, and females with cocoon, captured during a period of 8 weeks (spring 2007).

2.5. Statistical analysis

We applied general linear models and post-hoc Tukey tests to compare metal burdens, MTLP-concentrations and life history traits among populations (proc glm, SAS 9.1, SAS Institute Inc©, 2004). To model co-variation between female size and values of the reproductive traits, female cephalothorax width was included as co-variable. To scale the dimensions of the various traits, cube root values of body mass were used. To model trade-offs between egg mass and fecundity, fecundity estimates were regressed against egg mass (cube rooted), while including female cephalothorax width as a covariate. When residuals did not fulfill the assumptions of normality (Shapiro–Wilk test statistic) and homoscedasticity (Levene's test), dependent variables were $\log_e$-transformed.

To analyze the significance and direction of the relationships between Cd concentration and life history traits, ordered heterogeneity tests were performed per trait (Rice and Gaines, 1994). The statistic of this test constitutes the product $r_s P_c$, with r_s being the Spearman rank correlation coefficient of average metal concentration against average life history trait value per population, and P_c the complement of the *p*-value of the respective factor testing variation in life history traits among populations (ANOVA).

We used general linear models to test for relationships between MTLP concentrations and Cd, Cu and Zn concentrations (both in nmol/g ww) and to compare the direction and strength of these relationships among populations. Estimated free metal concentrations were compared between populations with an Anova test with post-hoc Tukey comparison (proc glm, SAS 9.1, SAS Institute Inc©, 2004). We used polynomial models to compare non-linear temporal trends in proportion of adult males, adult females, and females with cocoon among populations by including a quadratic factor (period∗period) and the two-factor interaction with population (proc genmod with binomial distribution, SAS 9.1, SAS Institute Inc©, 2004). Error variance components were corrected for overdispersion.

3. Results

3.1. Metal body burden

Internal Cd-burdens significantly differed among populations ($F_{5,57}=47.54$; $P<0.0001$), with the highest values measured in CH-P1 and CH-P2. Populations CM-P, CH-R1 and CH-R2 did not mutually differ in mean Cd-burdens but the latter two showed significantly higher values than CM-R and lower ones than CH-P2 and CH-P1 (Fig. 1(A)). Zn-burdens also significantly differed among populations ($F_{5,57}=4.55$, $P=0.0016$), with lower values in CH-R1 compared to the three polluted sites (Fig. 1(B)). Cu concentrations also significantly differed among populations ($F_{5,57}=13.27$; $P<0.0001$); however, the overall pattern was inversed compared to Cd and Zn, i.e. values in CH-R1 were significantly higher compared to the three polluted populations and CH-R2, while values in CM-P were lower than in CH-R2 and CM-R and values in CM-R were higher than in CH-P2 (Fig. 1(C)).

3.2. MTLP concentrations

MTLP concentrations did not significantly differ among the studied populations ($F_{5,57}=1.31$, $P=0.27$; Fig. 2). However, estimated concentrations of free metals were always larger than zero and differed significantly among populations ($F_{5,57}=3.32$, $P=0.011$), with the highest concentration in CH-P1 and CH-P2

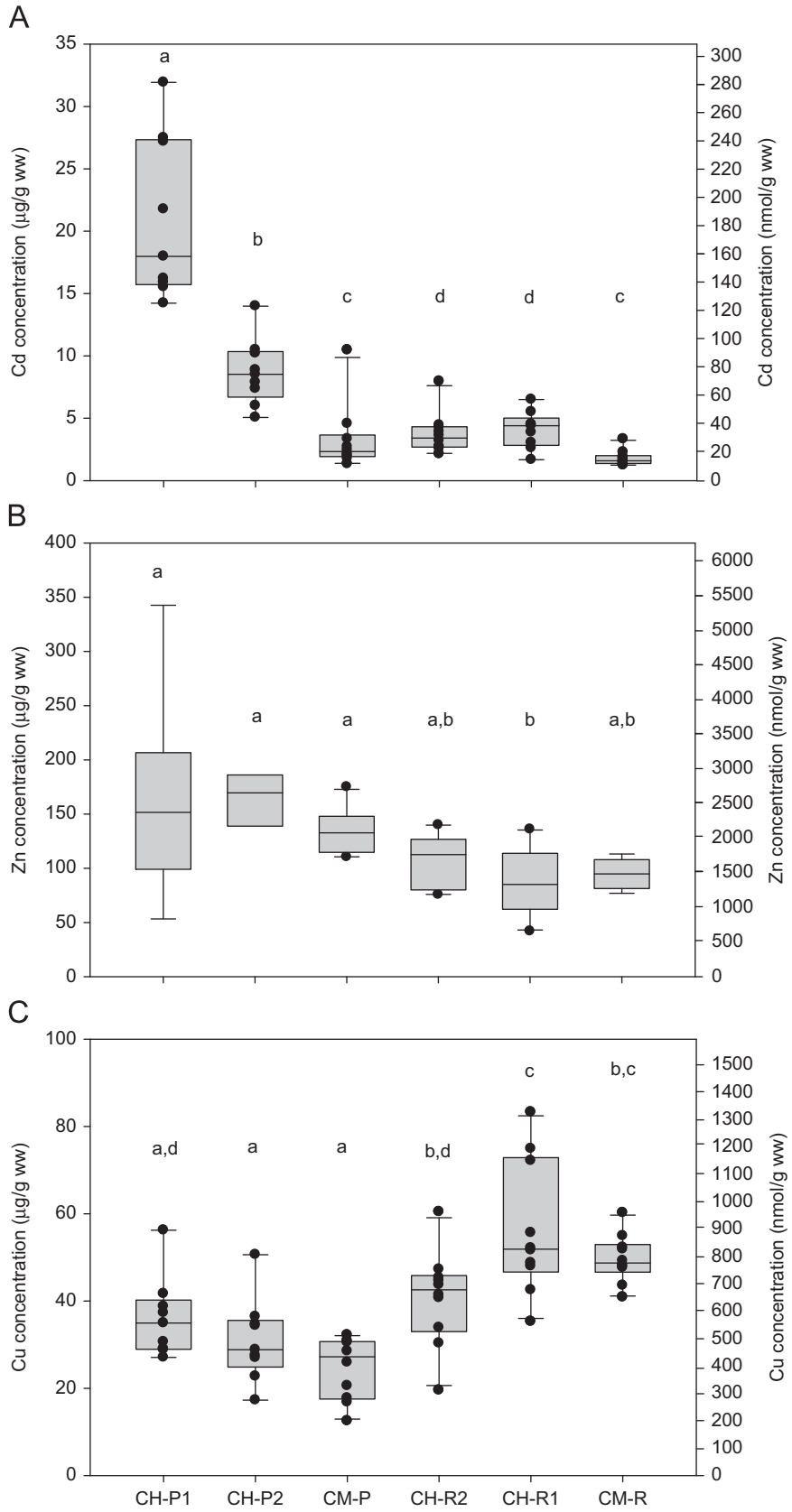

Fig. 1. Boxplot diagrams of Cd (A), Zn (B) and Cu (C) concentrations (expressed both as µg and nmol per g wet weight) in three polluted (-P) and three reference (-R) populations of *P. saltans*. Horizontal lines represent average values; boxes represent upper and lower quartiles, whiskers represent 90th and 10th percentiles; dots represent extreme values. Populations with the same superscript letters do not significantly differ.

and the lowest concentration in CH-R1. When only considering Cd-binding potential, among-population differences were even stronger ($F_{5,57}=31.74$, $P<0.0001$), with higher concentrations in CH-P1 and CH-P2 than in CH-R1, CH-R2, CM-R and CM-P (all without free Cd molecules. Fig. 2). MTLP concentrations were not significantly correlated with internal body burdens of Cd, Cu or Zn, neither for the entire dataset, nor within populations (all P between 0.15 and 0.95).

3.3. Life history traits

Male cephalothorax width, body mass and condition significantly differed among populations (cephalothorax width: $F_{3,158}=21.77$; $P<0.0001$; body mass: $F_{3,145}=7.3$; $P=0.0001$; Fig. 3(A), (B); condition: $F_{3,145}=3.03$; $P=0.032$) and were negatively correlated with Cd-burdens (cephalothorax width: $r_sP_c=-0.8$; $P=0.01$; body mass: $r_sP_c=-0.6$; $P=0.05$). Female cephalothorax width, body mass and condition significantly differed among populations (cephalothorax width: $F_{3,205}=17.6$; $P<0.0001$; body mass: $F_{3,228}=26.84$; $P<0.0001$; Fig. 3(C), (D); condition: $F_{3,205}=6.11$; $P=0.0005$). At population level, Cd burden was inversely related to female mass ($r_sP_c=-0.8$; $P=0.01$), but not to cephalothorax width ($r_sP_c=-0.4$; $P=0.1$) although females from the most polluted (CH-P1) and least polluted (CH-R1) population were smallest and largest, respectively. Body condition of CH-R1 females was significantly larger than of CH-P1 and CH-P2 females.

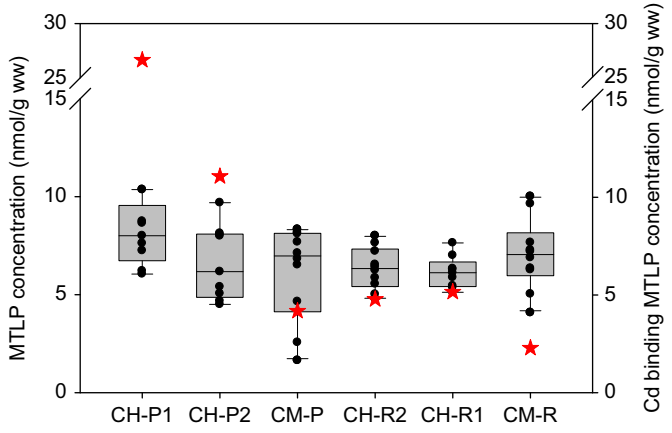

Fig. 2. Boxplot diagram of MTLP concentrations in three polluted (-P) and three reference (-R) populations of *P. saltans*. Horizontal lines represent average values; boxes represent upper and lower quartiles, whiskers represent 90th and 10th percentiles; dots represent extreme values. Stars indicate average MTLP concentrations required for binding all Cd.

Cocoon mass significantly differed among the studied populations ($F_{3,227}=30.99$, $P<0.0001$; Fig. 4(A)) but was not significantly correlated with Cd-burden ($r_sP_c=-0.4$; $P=0.1$). Cocoon mass was lowest in CH-P1, intermediate in CH-R2 and highest in CH-P2 and CH-R1. Cocoon mass increased with increasing cephalothorax width (slope=0.81; $F_{1,204}=127.48$, $P<0.0001$) and this relationship did not significantly differ among populations ($F_{3,204}=1.44$; $P=0.23$). Reproductive allocation also differed among populations ($F_{3,204}=12.12$; $P<0.0001$) and was highest in CH-P2 and CH-R1 and lowest in CH-P1 and CH-R1.

The number of eggs in a cocoon significantly differed among populations ($F_{3,202}=29.14$, $P<0.0001$; Fig. 4(B)) but was not significantly correlated with Cd burden ($r_sP_c=-0.4$; $P=0.1$). Females of CH-R1 and CH-P2 produced significantly more eggs than those of CH-R2, while females from the most heavily polluted population (CH-P1) produced significantly less eggs than those from CH-R1, CH-P2 and CH-R1. The fecundity was positively correlated with cephalothorax width ($b=29.11$; $F_{1,179}=100$, $P<0.0001$), and this relationship did not differ significantly among populations ($F_{3,179}=0.77$, $P=0.51$). When taking variation in female cephalothorax width into account, the difference in fecundity remained significant among populations ($F_{3,179}=5.63$,

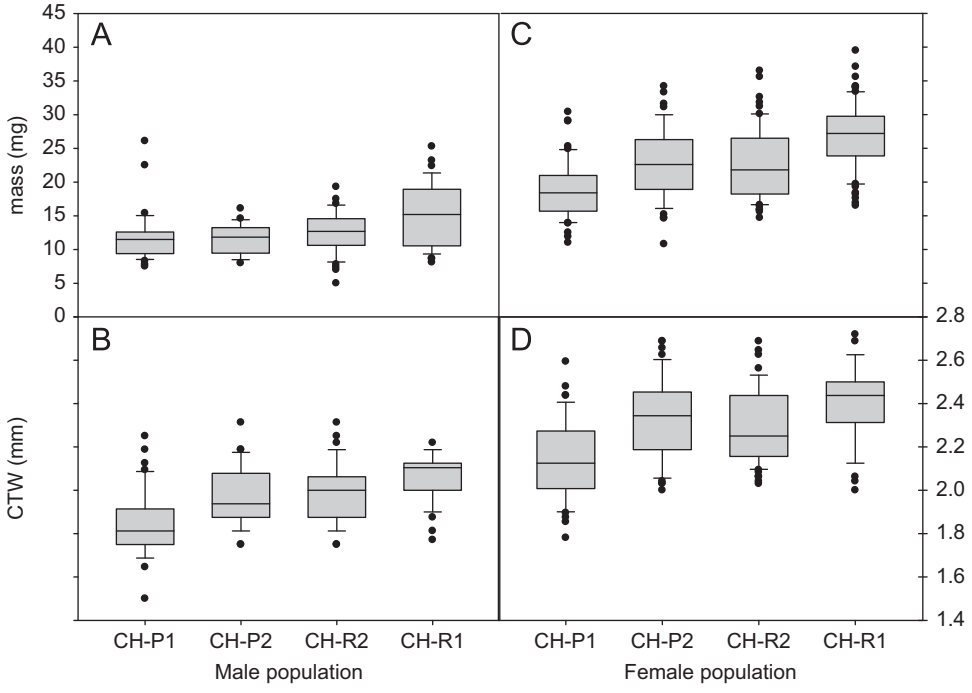

Fig. 3. Boxplot diagrams of male and female body mass (A, C), and cephalothorax width (CTW; B, D) in three polluted (-P) and three reference (-R) populations of *P. saltans*. Horizontal lines represent average values; boxes represent upper and lower quartiles, whiskers represent 90th and 10th percentiles; dots represent extreme values.

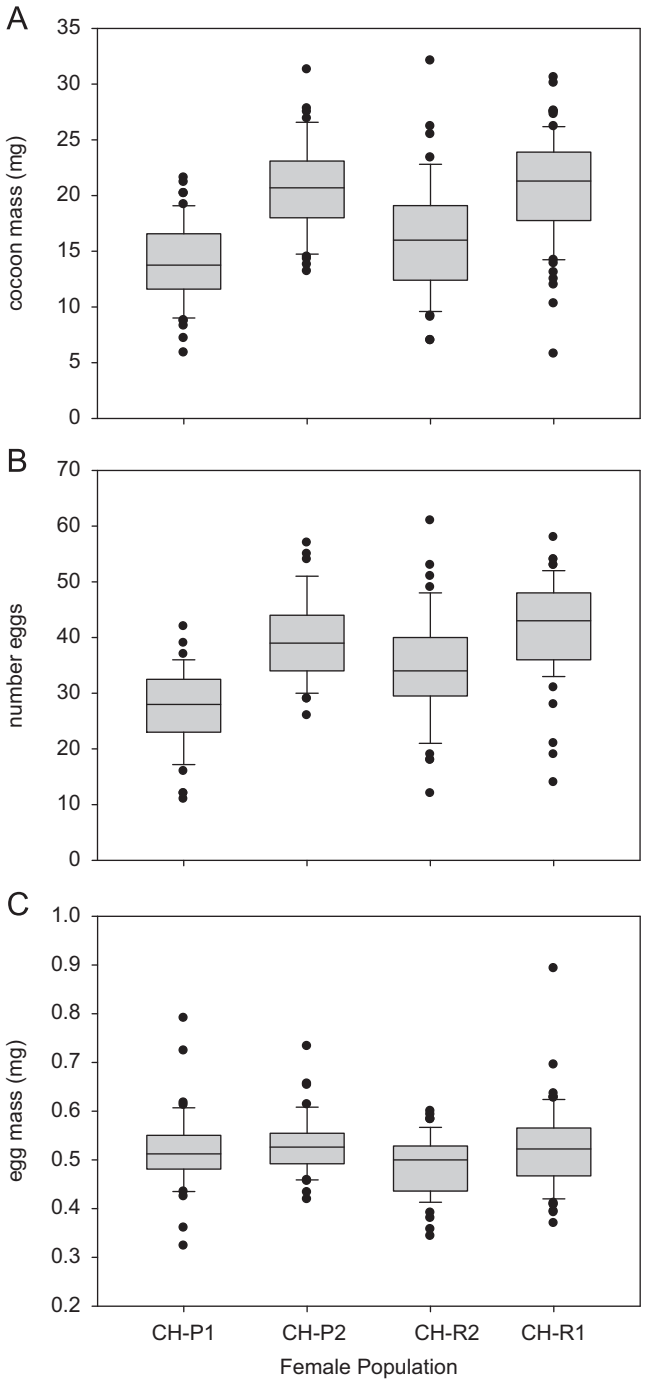

Fig. 4. Boxplot diagrams of cocoon mass (A), egg number (B) and egg mass (C) in three polluted (-P) and three reference (-R) populations of *P. saltans*. Horizontal lines represent average values; boxes represent upper and lower quartiles, whiskers represent 90th and 10th percentiles; dots represent extreme values.

$P=0.0011$) and females of the most heavily polluted population CH-P1 still produced a lower number than those from CH-R1, CH-P2 and CH-R2.

Egg mass significantly differed among populations ($F_{3,200}=3.07$, $P=0.029$; Fig. 4(C)), and females from populations with a higher average Cd-burden produced larger eggs ($r_sP_c=0.78$; $P=0.015$). Egg mass was not significantly correlated with maternal cephalothorax width ($F_{1,178}=0.6$, $P=0.44$) and was negatively correlated with fecundity ($b=-0.0026$; $F_{1,177}=36.31$, $P<0.0001$). This relationship did not differ among populations ($F_{3,177}=2.42$, $P=0.07$).

Table 1
Generalized linear model testing temporal shifts in sex distribution in two polluted and two reference populations of *P. saltans* during an eight week sampling period. Significant effects are indicated in bold.

	Proportion adult males		Proportion adult females		Proportion Females with cocoon	
	χ^2	P	χ^2	P	χ^2	P
Site	2.53	0.47	3.37	0.34	9.42	**0.024**
Period	19.8	**<0.0001**	39.85	**<0.0001**	71.71	**<0.0001**
Period*period	27.52	**<0.0001**	45.25	**<0.0001**	44.48	**<0.0001**
Site*period	8.03	**0.046**	14.05	**0.0028**	12.89	**0.0049**
Site*period*period	3.73	0.29	0.57	0.9	15.5	**0.0014**

3.4. Phenology

Adult males and females were present from the first and second week of April onwards, respectively. Females with cocoons were present from the end of April onwards, while all adult males had disappeared by the first week of June. Whereas temporal shifts in the proportion of adult males and females significantly differed among sites (Table 1; Fig. 5), none of the pairwise comparisons remained significant after correction for multiple testing. Females from the most polluted population (CH-P1) that carried a cocoon showed a slower increase in numbers with time and peaked later during the season compared to conspecifics from the least polluted population (CH-R1) (Table 1).

4. Discussion

As expected from life history theory, adult female *P. saltans* were smaller and in poorer body condition, delayed their reproductive period and produced heavier eggs under increased cadmium stress. Given the positive relationship between female size and reproductive output, cocoon masses and egg numbers were reduced in the most polluted sites. This decrease in fecundity was further increased by a lower relative amount of energy devoted to reproduction. In the least Cd and Zn polluted site, adult males and females were largest and females carried the heaviest cocoons and showed the highest fecundity. Populations from intermediately polluted sites showed life history traits that resembled those from unpolluted reference sites. The observed shifts in life history traits could induce population differentiation, as for example a shift in reproductive period (Winterer and Weis, 2004) and size assortative mating (Eraly et al., 2009), may ultimately induce reproductive isolation between populations.

Life history responses of exposed populations exhibited a rather large amount of variation and effects often were only present in the most polluted population, suggesting that metal concentrations in natural populations are to exceed threshold values before resulting in measurable effects. Life history alterations in response to metal pollution have been shown in other invertebrate groups such as lumbricids (Rozen, 2006), isopods (Donker et al., 1993a,b) and collembolans (Posthuma et al., 1993; Tranvik et al., 1993). However, reported directions of these changes were not consistent. For instance, isopods from polluted areas tended to mature earlier, rather than later, than in unpolluted sites and also showed higher, not lower, levels of reproductive output in the former (Donker et al., 1993a,b). Patterns opposite to those expected from life history theory can result from the fact that metal body burdens in species such as isopods affect their life histories only when they approach lethal concentrations after long-term exposure. This induces higher mortality at later ages, which is expected to select for earlier maturation.

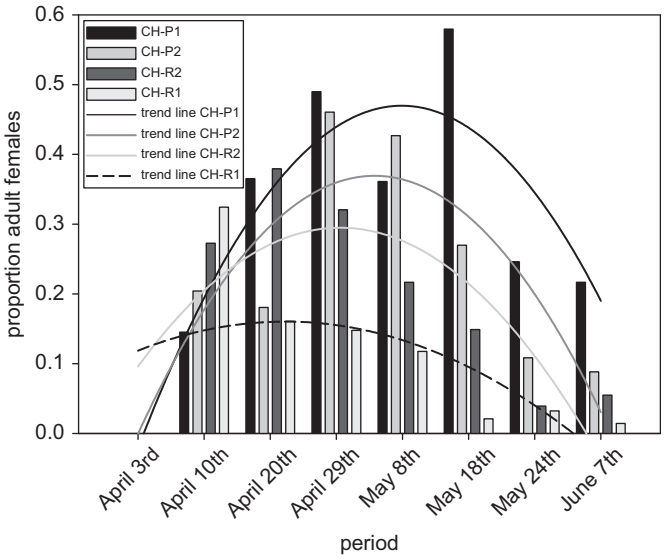

Fig. 5. Temporal shifts in adult female distribution in two polluted (-P) and two reference (-R) populations of *P. saltans* during an eight week sampling period. Trend lines represent the quadratic functions for each population.

For our model organism, Cd body burden in the most exposed population is still far below the lethal body concentration, estimated as 1050 mg Cd/kg DW for the congeneric species *Pardosa astrigera* (Jung et al., 2007).

Contrary to our expectations, MTLP concentrations were not significantly higher in populations from polluted sites, despite their higher cadmium and zinc concentrations. This implies larger concentrations of free, potentially toxic, metals in more polluted populations. Results of this study therefore do not support induced expression (acclimation) or increased constitutive production (adaptation) of MTLP. Nevertheless, peak Cd concentrations in our specimens were relatively high and in the same order of magnitude compared to other ecotoxicological studies in spiders, taken into account an average fresh to dry weight ratio of 4 in this species (Hunter et al., 1987b; Rabbitsch, 1995; Wilczek and Migula, 1996; Wilczek and Babczynska, 2000; Heikens et al., 2001; Hendrickx et al., 2003a; Jung et al., 2007; Wilczek et al., 2004).

Lack of an increase in MTLP concentration under increased metal stress in this study contrasts with results obtained from an experimental study on the same populations that showed elevated MTLP concentrations when spiders were fed with cadmium spiked fruit flies (Eraly et al., 2010). Hence, while *P. saltans* appears physiologically able to induce increased MTLP expression in response to metal exposure, MTLP levels observed under polluted field conditions were comparable to those measured in individuals from unpolluted reference populations and from control laboratory treatments. It therefore seems reasonable to assume that field concentrations of metals are often too low to trigger increased MTLP concentrations, even though this implies free molecules of Cd and other metals. However, the role of MTLP's is broader than metal defense since they are also involved in (i) homeostasis of essential metals, (ii) non-toxic metal donorship to metalloproteins, (iii) antioxidant defense, and (iv) activities of gene regulatory compartments (Stegeman et al., 1992; Roesijadi, 1996; Amiard et al., 2006). Moreover, other parameters that differ between natural and laboratory conditions, such as temperature, hormones, food availability and pesticides may affect MTLP concentrations as well, thereby masking direct effects of metals (Viarengo et al., 1999; Wilczek, 2005; Amiard et al., 2006). Results from this and other studies (Roesijadi, 1996; Mouneyrac et al., 2002; Amiard et al., 2006; Forbes et al., 2006; Morgan et al., 2007; Santiago-Rivas et al., 2007) hence plead for a cautious use of MTLP as universal biomarker, as previously advocated by Hopkin (1989), Dallinger and Rainbow (1993) and Viarengo et al. (1999). Besides, other metal protection mechanisms, like storage in metal-rich granules (Mason and Jenkins, 1995), glutathione (Viarengo and Nott, 1993; Wilczek et al., 2008) and esterases (Wilczek, 2005) are known to be important in spiders and their concentrations differ depending on the degree of pollution. The detoxification pathway of MRGs' is not independent of MTLP's since evidence is present for lysosomal products of MTLP to breakdown remaining as insoluble residual bodies (Viarengo and Nott, 1993; Amiard et al., 2006).

Absence of measurable effects on individual growth and survival in a laboratory study (Eraly et al., 2010), despite increased MTLP production and internal Cd concentrations that were three times higher than those measured in the most polluted field population (this study), suggests that indirect and/or synergistic effects of metal pollution may be more important in shaping life history variation in natural populations (Clements and Rohr, 2009). For instance, changes in prey species composition (Klerks, 2002), reduced prey availability or decreased energetic value of prey due to the toxicity of metals to less tolerant species (Clements and Rohr, 2009; Tranvik et al., 1993; Jung et al., 2008b) and changes in vegetation (Donker et al., 1993b; Posthuma et al., 1993; Graitson et al., 2005; Jung et al., 2008b) may all result in reduced resource availability. In support of this, most field studies reported a lower diversity of soil and ground-running macro-invertebrates on more polluted sites (Bengtsson and Rundgren, 1984; Read et al., 1998; Lock et al., 2003; Creamer et al., 2008). Discrepancies between laboratory and field studies, such as reported here, hence plead for rigorous and long term field testing in order to reveal population effects of toxicants (Lock et al., 2003; Amiard et al., 2006; Clements and Rohr, 2009).

5. Conclusion

From this study on 4 field populations of *Pardosa saltans* we can conclude life history and physiological traits of *P. saltans* are affected by metal pollution under field conditions, however not always in a straightforward manner: (i) internal concentrations of Zn and Cd are higher in individuals from sites containing a metalliferous flora than those without, except for CM-P; Cu concentration however showed a reversed trend; contrary to the expectations MTLP levels were not significantly higher in individuals from contaminated sites; (ii) males are lighter, smaller and have a lower body conditions when Cd concentrations are higher, while in females only body mass is negatively influenced. Reproductive values (cocoon mass, fecundity and egg mass) only were affected in the population with highest internal Cd (CH-P1); and (iii) the number of females carrying a cocoon peaked later in the season in the most polluted site.

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