Ecotoxicology 8, 133–141, 1999

Life-History Patterns in Reference and Metal-Exposed Earthworm Populations

DAVID J. SPURGEON[1]* AND STEPHEN P. HOPKIN[2]
[1]*Institute of Terrestrial Ecology, Monks Wood, Abbotts Ripton, Huntingdon, Cambs, PE17, 2LS, UK. Email: d.spurgeon@ite.ac.uk*
[2]*Division of Zoology, School of Animal and Microbial Sciences, University of Reading, PO Box 228, Reading, RG6 6AJ, UK*

Received 11 November 1998; Accepted 22 December 1998; Revised 7 February 1999

Abstract. The growth and development of F1 *Lumbricus rubellus* bred from reference and one metal-exposed (smelter) populations were assessed in soils from both sites. In the clean soil, faster growth (as reflected by mean weight) and maturation were found at selected time intervals for the smelter worms. This result is in agreement with predictions from life-cycle models, which indicate that polluted-site populations will be adapted for faster growth, earlier maturation and increased reproductive effort. Life-history adaptation is not however the only explanation for the differences in maturation rates found. During the exposures, mortality of smelter worms was higher than for the reference strain. This resulted in a reduction in the density of the smelter strain. In earthworms, both growth rate and maturation time are known to be density dependent. Thus it is probable that density-mediated responses of growth and development, rather than adaptation, are primarily responsible for the observed life-history.

Exposure of the two strains to the smelter site soil was carried out to quantify differences in performance indicative of physiological resistance in the smelter worms. In fact, no consistent differences in growth or maturation were found. Thus it is unlikely that growth and development responses of *Lumbricus rubellus* are adapted to the metals present in soils at the smelter site.

Keywords: earthworm, *Lumbricus rubellus*, life-history adaptation, resistance, density dependence

Introduction

Spurgeon and Hopkin (1995, 1996a) found effects on survival probability, length of juvenile period and adult reproduction rate for worms maintained in cadmium, copper, lead and zinc contaminated field soils, indicating that metals affect earthworm life-cycles and hence may impact individual fitness. Despite the effects found on fitness, earthworm populations persist at the field sites from which the metal-contaminated soils were collected (Spurgeon and Hopkin, 1996b, 1999). The presence of earthworms in soils at which concentrations of one or more metals exceed NOEC concentrations determined in laboratory tests, suggest that populations are subject to metal-related selection pressures at these sites. Furthermore, the long-term nature of the contamination (the field sites are polluted by emissions from a smelting works that has been in operation for 70 years) means that selection may have led to the evolution of a metal adapted population.

Despite the circumstantial evidence suggesting the presence of adapted earthworm populations at metal-contaminated sites, studies designed to demonstrate physiological tolerance have so far failed to find clear effects. Spurgeon and Hopkin

*To whom correspondence should be addressed.

(in press) compared zinc sensitivity in a reference and two contaminated sites (Avonmouth smelter and Shipham lead/zinc mine) populations of *Lumbricus rubellus*. No significant inter-population differences in sensitivity for effects on survival or weight change were found. For reproduction, marginal increases in tolerance were found in polluted-site populations. However these were unlikely to be of major ecological significance in determining population fitness. Further studies by Bengtsson and Rundgren (1992), Corp and Morgan (1991) and Marino and Morgan (in press) have also failed to find significant inter-population differences in sensitivity for the effects of metals on uptake, protein elution patterns or survival time.

Physiological resistance is not the only means by which animals inhabiting contaminated locations adapt to pollutants (Posthuma and Van Straalen, 1993). Selection of life-history characteristics can also occur when populations are exposed to factors (such as a pollutant) that reduce survival and lower reproductive effort (Charlesworth, 1980). Specifically, animals are selected for early maturation and increased reproductive effort (Posthuma *et al*., 1993). Divergence of life-history patterns as a result of selection by pollutants occurs most frequently when populations are exposed to non-degradable chemicals such as metals. Under these conditions, selection can occur over a number of generations.

Materials and methods

Origin of selected populations

Adaptation in life-histories of populations inhabiting metal-contaminated sites has been found for a number of soil invertebrate groups (Posthuma and Van Straalen, 1993). However, to date earthworms are not included. To determine if exposure of earthworms to persistent pollutants results in selection of life-history traits, the development of F1 animals from a reference and a single (originally two were intended) polluted site strains of the epigeic (litter dwelling) earthworm *L. rubellus* was assessed in a clean and a polluted soil. Comparisons in clean soil were used to determine if there was any evidence of population divergences in life-history traits, while comparisons in the polluted soils were used to determine if there was any evidence for physiological adaptation in the polluted-site strain.

Parent reference strain *L. rubellus* were collected in early spring 1995 from the campus of the University of Reading (Ordnance Survey Grid Reference SU 737714). Previous work at this site has indicated that metal levels are within the range found in uncontaminated soils (Spurgeon and Hopkin, 1995, 1996b). Smelter strain parent worms were collected from a location (Hallen Hill) (Ordnance Survey Grid Reference ST 554802) approximately three kilometers from a cadmium/lead/zinc smelter located at Avonmouth in south-west England. Soils at this site have been subject to aerial deposition of metals for the past 70 years and contain cadmium, copper, lead and zinc levels at least an order of magnitude higher than clean soil values (Martin and Bullock, 1994; Vale and Harrison, 1994; Spurgeon and Hopkin, 1996b). At each sites, 60 adult *L. rubellus* were collected by digging and handsorting. Worms were returned to the laboratory on own site soil where they were used for production of the F1 juveniles.

Prior to the start of the experiment it was intended to measure life-history patterns in a second polluted-site population obtained from soils around an ancient lead/zinc mine located at Shipham in south-west England (Ordnance Survey Grid Reference ST 451573). However, due to high mortality and low reproduction of worms from this population (both in culture and during trial exposures) this strain had to be excluded from the study. The absence of life-history measurements for a second polluted-site population is a major factor restricting the interpretation of data from the study, since any differences between the two measured strains may be due to genetic variations between populations or factors other than metal exposure. That said, the extent of the metal contamination in the smelter soil means that metal levels are likely to be an important selection pressure for worms at the smelter site.

Culturing of F1 animals

To breed sufficient F1 juvenile *L. rubellus* for the exposure phase of the study, adults were cultured in a medium consisting of a commercially available loam topsoil mixed with 20% by weight of ground *Sphagnum* peat. This topsoil/peat mix has been found to be suitable for the long-term maintenance of *L. rubellus*. For each culture, eight worms were incubated in 2 kg of moistened soil at 15 ± 2°C in constant light. An excess of suitable food (finely ground fresh horse manure, dried and rewetted to 75% water content) was supplied. After six weeks, soil cultures were sieved and the cocoons present collected and transferred to moist filter paper maintained in darkness at 15 ± 2°C for hatching. The cocoons were examined twice weekly and the newly emerged F1 juveniles removed and transferred to a moist soil kept at 3°C. Incubation at this low temperature prevented the development of worms prior to introduction to the test soils.

Collection and preparation of exposure phase soils

Exposure phase soils were collected from the same sites from which the parent earthworms were collected. Soils were taken from the top 2cm layer (after removal of surface vegetation and litter). On return to the laboratory, soils were dried in a fan oven at 40°C, passed through a 2mm mesh and one kilogram placed into each of four replicate containers (plastic boxes with dimensions 175 mm × 120 mm × 60 mm) for each soil/worm combination (total of 16 containers). Distilled water was added, to give moisture content of approximately 65% of field capacity and the soils left to stabilise for one week. Finally, eight juvenile *L. rubellus* were weighed and placed into each container.

Physiochemical analysis of site soils

To determine metal levels, a sub-sample of soil was collected for the determination of cadmium, copper, lead and zinc concentrations by flame atomic absorption spectrometry of nitric acid digests (see Hopkin, 1989). For the analysis, approximately 1 g dry weight of soil was placed into a conical flask with 10 ml of concentrated nitric acid. Flasks were heated until all organic matter had been digested and the digests were diluted to 100 ml with double distilled water. Solutions were analysed by flame atomic absorption spectrometry (Varian Spectra 30 AAS). During soil analysis, standard reference materials (tomato leaf and bovine liver from the National Bureau of Standards, Washington, lobster hepatopancreas from the National Research Council, Canada, and calcareous loam soil from the Community Bureau of Reference, Brussels) were used as recommended by Hopkin (1989). In all cases measured concentrations were within ten percent of certified values. Percentage loss on ignition (organic matter content) and soil (H_2O) pH were also measured. Results of all soil analysis are given in Table 1.

Measurement of life-history characteristics

To measure life-histories characteristics, the F1s were first examined 28 days after the start of the exposure phase. At this time, all worms were sorted from the soil and survival, individual weights and sexual development recorded. Sexual development was assessed according to the scheme of Van Gestel *et al*. (1991). Worms with a full clitellum were recorded as adult, those with a full tubercle pubertatis but no clitellum as subadult, and individuals without either of these reproductive structures as juveniles. After sampling, the worms were returned to the same test soil. All measurements were repeated a further 15 times at 28 day intervals throughout the study giving a total duration for exposures of 448 days.

Table 1. Soil pH, percentage loss on ignition (% LOI) and metal concentrations in soils sampled from sites from which adult *Lumbricus rubellus* were collected. All values are expressed on a dry weight basis and are means (± SE) of six replicates.

	pH (median)	% LOI	Cadmium $\mu gCdg^{-1}$	Copper $\mu gCug^{-1}$	Lead $\mu gPbg^{-1}$	Zinc $\mu gZng^{-1}$
Clean (Campus)	6.02	21.6 ± 2.7	0.1 ± 0.02	17.5 ± 1.02	8.3 ± 1.82	58.3 ± 2.28
Smelter (Hallen Hill)	7.25	22.7 ± 1.9	13.2 ± 0.85	74.8 ± 7.05	449 ± 52.2	710 ± 116

To ensure growth and maturation during the experiment, uncontaminated horse manure dried and rewetted to 80% water holding capacity and mixed with an equal volume of the relevant soil was added as a source of food (Holmstrup *et al.*, 1991). Due to the small size of the worms, food supply was limited at the beginning of the experiment to ensure there was not an excess of uneaten material into which the worms could escape. Thus at day 0, 4 g (dry weight) of manure was supplied per container, at day 28 and day 56, 8 g were supplied, while at all subsequent sample intervals, 10g of food was added. In all cases, the soil food mix was spread on the soil surface. Any excess was removed during the next sampling and a new quantity of food mix added. This amount was calculated from the number of worms alive in the container to ensure an equal supply of food throughout the experiment.

Data analysis

Data for weights of exposed worms in the reference and smelter soils were analysed by ANOVA and post-hoc comparisons for separate time intervals. To determine if the proportion of adults is independent of population, the percentage of adults present at each time interval was compared using a G test.

Results

Mortality

Different patterns of mortality were found depending on the soil to which the two populations were exposed. For worms exposed to the uncontaminated soil, low levels of mortality were found in both the reference and smelter-site strains (Fig. 1). Thus in the reference strain, survival was at 100% until day 392 and remained at 96% for the rest of the study, while for the smelter worms, survival was 97% after 196 days, reducing to 84% by the end of the experiment. For worms exposed to the smelter soil, survival was lower than in the clean soil. Thus for the reference population,

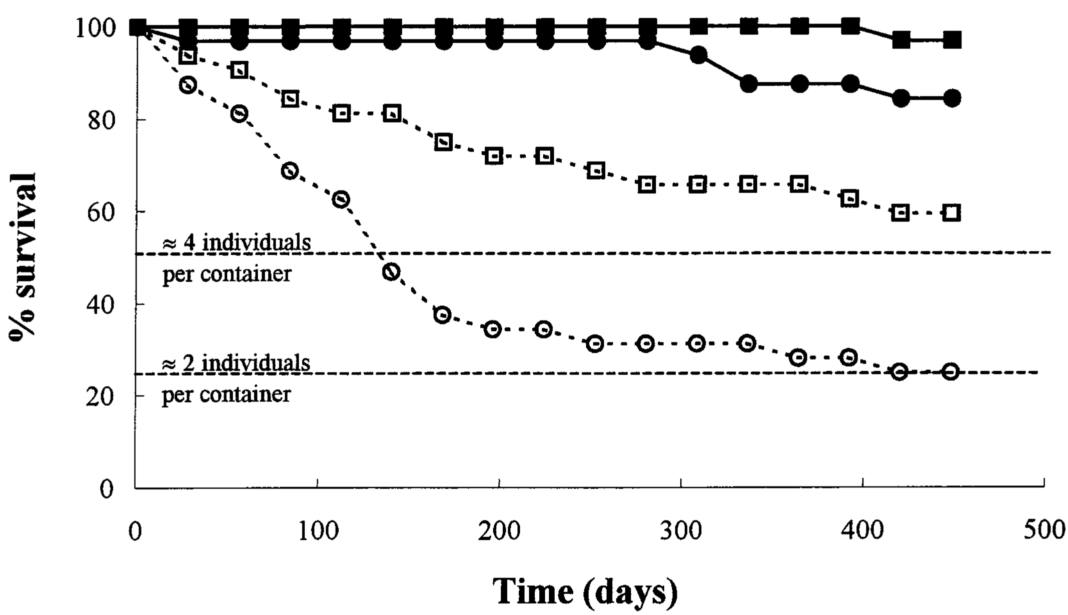

Figure 1. Mortality patterns for first generation *Lumbricus rubellus* from a reference (squares) and a polluted (circles) site. Solid symbols and lines indicate mortality patterns for animals maintained in reference site soil. Open symbols and dashed lines indicate patterns for animals maintained in soil from the polluted site.

survival was 71% after 196 days, reducing to 65% at day 448, while for the smelter strain, survival was 34% after 196 days and 25% at the end of the experiment. Comparisons of the number of earthworms alive after 448 days by t-test indicated that survival of the smelter strain was significantly lower than the reference population ($P < 0.03$) in both the reference and smelter soils. Comparisons of the survival of the smelter strain when maintained in the two different soils indicated significantly lower ($P < 0.05$) survival in the smelter soil. No significant differences were found between soils in the survival of the reference worms.

Large variations in growth rates were found between individual worms (Fig. 2). Thus at the

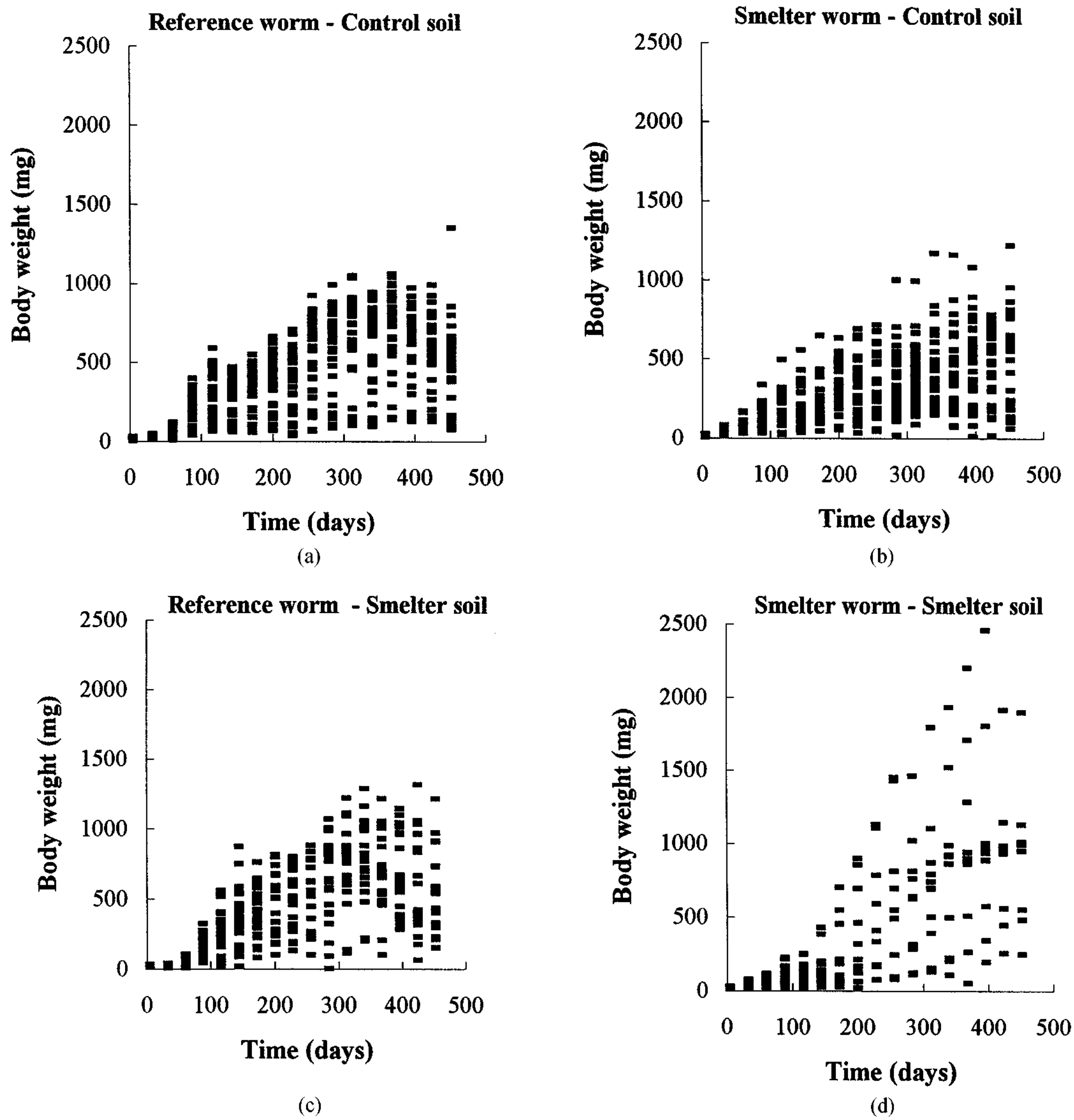

Figure 2. Four weekly fresh (unstarved) weight measurement (mg) for reference strain *Lumbricus rubellus* F1 juvenile worms exposed to (a) control and (c) smelter contaminated soil and smelter strain *Lumbricus rubellus* F1 juveniles exposed to (b) control and (d) smelter contaminated soil.

end of the experiment, the weight of the fastest growing individual exceeded that for the slowest growing by factors of 17.8 and 19.9 for the reference and smelter strains in the control soil and 8.9 and 7.7 for the reference and smelter strains in the smelter soil. Subtle differences in growth patterns were found for the two strains maintained in the reference soil. In the early phase of the experiment, reference worms initially grew faster than the smelter population. Indeed comparisons of individual weights of the two strains using ANOVA indicated a higher mean weight for worms from the reference population at day 28 and 56. However subsequently, to this time, worms from the smelter strain ‘caught up’ and ‘overtook’ the reference population so that by day 140 mean weights of smelter juveniles were significantly higher than for the reference population (Fig. 3a). Mean weight of the smelter population then remained above that for the reference strain for the remainder of the exposure, although differences were not significant due primarily to the high variability of individual weights.

Growth patterns for the two strains incubated in the smelter soil were similar to those found in the reference medium. Thus the reference population initially grew faster than the smelter strain, but was overtaken at day 196 and mean weight remained below the polluted-site worms for the remainder of the experiment (Fig. 3b). Comparisons of mean individual weights by ANOVA indicated that the mean weight of reference strain worms was significantly higher than for the smelter strain at day 56, 84 and 112, while at day 252 and all subsequent sample dates, the mean weight of the smelter strain worms was significantly higher than for the reference population.

In addition to determining mortality and growth patterns for the four soil/worm combinations, sexual development of the exposed worms was also assessed. For worms maintained in the control soil, comparisons of the relative proportion of adults present indicated earlier maturation for the smelter strain. A significantly higher fraction of adults was found in the smelter population at 224 and 252 days when compared to the reference population (G-test, $P < 0.05$). For the worms incubated in the smelter soils, no significant differences in the proportion of adults was found for any time interval indicating similar rates of maturation in the two populations.

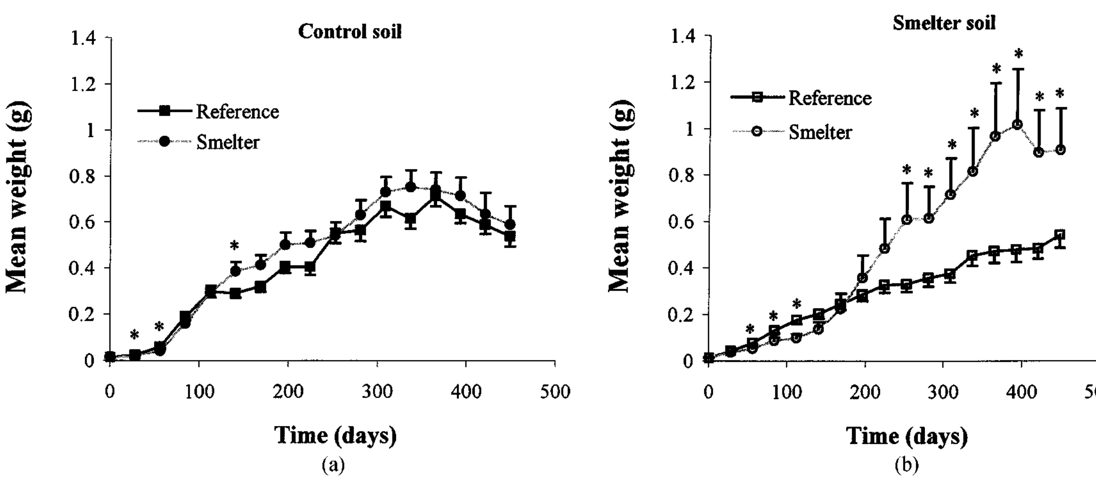

Figure 3. Mean weights (±SE) of first generation *Lumbricus rubellus* from a reference (solid line) and a smelter (dashed line) population maintained in (a) reference and (b) smelter contaminated soil. Asterisks indicate sample dates for which the mean weights of populations are significantly different (ANOVA: $P < 0.05$).

Discussion

Comparisons of survival patterns in the two tested populations maintained in clean soil indicated significantly higher mortality after 448 days for the smelter strain. This suggests a possible cost of tolerance for this population. Cost of tolerance, where polluted-site animals are outperformed by clean-site individuals when both are maintained in uncontaminated soil, has been demonstrated in isopods captured near a zinc smelter (Van Capelleveen, 1987). In this study, smelter individuals did badly when maintained on a reference diet, but performance of the isopods improved when the food was supplemented with cadmium and zinc. This suggests that the smelter isopods were adapted to the high metal concentrations present in the polluted soil. For the work detailed in this paper, physiological deficiency of micronutrients is unlikely to be the primary cause of the lower survival of smelter worms in clean soil, since survival of this strain in the polluted soil was even lower than for the uncontaminated medium. Instead other factors, such as an increase in energy demand to maintain genetically evolved tolerance mechanisms may account for the observed differences.

Survival of the two strains after 448 days in the polluted soil was lower than in the reference soil, although these differences were significant only for the smelter population. A comparison of the survival of the two strains in the smelter soil indicated that the number of worms alive at day 448 was significantly higher in the reference strain. This did not fit with our expectations. Prior to the experiment it was anticipated that lower mortality would occur in the smelter worms. That this was not the case suggests that physiological adaptation, as it effects juvenile mortality patterns, is unlikely to be of major importance in determining survival probabilities for populations in contaminated regions, although further studies with polluted-site strains are required to confirm this conclusion.

Comparisons of growth and development in the control soils were intended to quantify differences in life-history patterns. A number of studies have found variations in the life-cycles and population structure for soil invertebrates inhabiting polluted sites. Jones and Hopkin (1998), in a study with *Porcellio scaber* from eight sites at different distances from the Avonmouth factory, found that the maximum size of individuals declined significantly from the least to the most polluted site. Donker *et al*. (1993) found higher growth rates and earlier maturation in metal-exposed populations of the isopod *Porcellio scaber*. Tranvik *et al*. (1993) found increased juvenile growth, reduction of the juvenile period and increased reproductive effort in a population of the springtail *Onychiurus armatus* from a contaminated site. In addition, Posthuma *et al*. (1992, 1993) found evolutionary changes including higher juvenile growth, lower age at first reproduction and increased numbers of clutches per female in an exposed population of the collembolan *Orchesella cincta*.

The studies outlined above indicate that changes in life-history patterns can occur for species exposed to metals. Specifically, polluted-site populations are selected for faster growth, earlier maturation and increased reproductive effort (Charlesworth, 1980; Sibly and Calow, 1989). An initial examination of the data from the current study also suggests adaptation to life-history patterns for the polluted-site population. For growth as indicated by mean weight, significant and time-dependent inter-population differences were found. Thus in both soils, although reference worms initially grew fastest, highest mean weight at the end of the experiment was found for the smelter strain (Fig. 3a, b). For sexual development, a significantly increased proportion of adults at selected time intervals was found in the smelter strain worms when incubated in the control soil. This suggests that smelter-site worms maturate faster, due possibly to selection resulting from the high metal levels present at this site. However, a more detailed scrutiny of the data indicates there may be another explanation for the variations in growth and maturation found in the two strains. Namely, differences could result from density-dependent effects.

In this study, eight worms were maintained in 1 kg of experimental soil. This number is much higher than found for *L. rubellus* in the field. Density is known to be an important factor limiting earthworm growth and development and any changes during an experiment would be expected to have marked effects. For example, a reduction in the number of worms present in culture has been found to favour increased growth, rapid maturation and higher reproduction (Dominguez and Edwards, 1997; Kaushal *et al.*, 1995; Reinecke and Viljoen, 1990). As detailed earlier, higher mortality was found for the smelter worms when the two strains were maintained in clean soil. This increased mortality resulted in a reduction in the mean numbers of worms in the smelter strain. Thus it is likely that a reduction in earthworm density rather than adaptation of life-history characteristics, particularly in the latter stages of the exposures when weight increases were highest, is responsible for the accelerated growth and maturation found for the smelter worms. The problems resulting from changes in density during experiments can be avoided if tests are conducted with only one worm in each container.

Measurements of growth and maturation in the polluted soil were used to investigate the presence of physiological tolerance in the smelter worms. The smelter population has been exposed to elevated concentrations of metals for the 70 years over which the factory has been operational. Furthermore comparisons of survival in the clean and polluted soils, as well as previous work studying cocoon production rate and length of the juvenile period, have indicated a selection pressure for worms inhabiting this site (Spurgeon and Hopkin, 1995, 1996b). Despite the presence of a clear selection pressure in the smelter-site soil over many tens of generations, no clear evidence for the presence of physiological tolerance was found. For example, comparison of the performance of the two strains in the smelter soil indicated highest survival for the control-site animals (Fig. 1) and no significant differences in the proportion of adults on any sample date (Fig. 4). Higher mean weights in the latter phase of the experiment were found for the smelter strain worms. However, these can be attributed to the lower survival and hence density of this strain at the end of the experiment.

An absence of clearly developed tolerance traits has also been found in studies of adult survival, weight change and cocoon production in two polluted-site populations of *L. rubellus* (Spurgeon and Hopkin, in press). In this work, direct toxic effects of zinc on polluted-site populations were compared to those for a reference strain. Prior to exposure, it was anticipated that worms from the contaminated sites would show greater tolerance to zinc. This was not the case for all measured parameters. Indeed, no differences in survival or weight change were found, while for

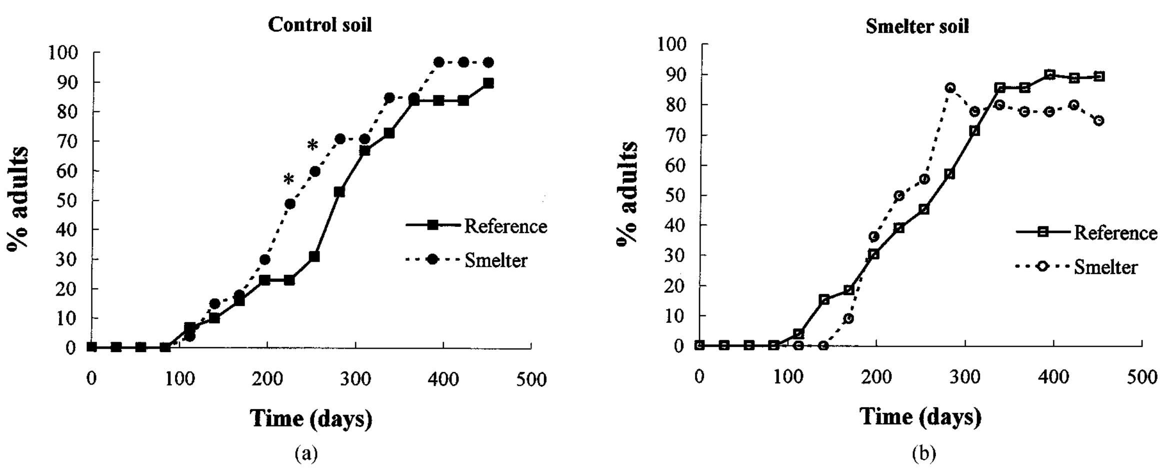

Figure 4. Proportion of adults of first generation *Lumbricus rubellus* from a reference (solid line) and a smelter (dashed line) population maintained in (a) reference and (b) smelter contaminated soil. Asterisks indicates significant difference in fraction of adults between populations (G-test: $P < 0.05$).

cocoon production, smelter and mine-site worms were marginally more tolerant than the reference population. Thus it appears that physiological tolerance, which limits the effects of zinc on important life-cycle traits, is unlikely to be a major factor influencing the distribution of *L. rubellus* in contaminated regions.

Acknowledgements

Thanks go to Mrs V. Rimmer for help with the initial culturing of the earthworms and to Dr Martin Holmstrup for his comments on the manuscript. This work was conducted while DJS was employed at Reading University on a research grant from the Leverhulme Trust. DJS currently holds a NERC Advanced Fellowship.

References

Bengtsson, G., Ek, H. and Rundgren, S. (1992). Evolutionary response of earthworms to long term metal exposure. *Oikos* **63**, 289–297.

Charlesworth, B. (1980). *Evolution in Age-Structured Populations*. Cambridge: Cambridge University Press.

Corp, N. and Morgan, A.J. (1991). Accumulation of heavy metals from polluted soils by the earthworm *Lumbricus rubellus*: can laboratory exposure of 'control' worms reduce biomonitoring problems? *Environmental Pollution* **74**, 39–52.

Dominguez, J. and Edwards, C.A. (1997). Effects of stocking rate and moisture content on the growth and maturation of *Eisenia andrei* (Oligochaeta) in pig manure. *Soil Biology and Biochemistry* **29**, 743–746.

Donker, M.H., Zonneveld, C. and Van Straalen, N.M. (1993). Early reproduction and reproductive allocation in metal adapted populations of the terrestrial isopod *Porcellio scaber*. *Oecologia* **96**, 316–323.

Holmstrup, M., Østergaard, I.K., Nielsen, A. and Hansen, B.T. (1991). The relationship between temperature and cocoon incubation time for some Lumbricid earthworm species. *Pedobiologia* **35**, 179–183.

Hopkin, S. P. (1989). *Ecophysiology of Metals in Terrestrial Invertebrates*. London: Elsevier Applied Science.

Jones, D.T. and Hopkin, S.P. (1998). Reduced survival and body size in the terrestrial isopod *Porcellio scaber* from a metal-polluted environment. *Environmental Pollution* **99**, 215–223.

Kaushal, B.R., Kalia, S. and Bisht, S.P.S. (1995). Growth and cocoon production by the earthworm *Drawida-Nepalensis* (Oligochaeta, Moniligastridae) in oak and pine litter. *Pedobiologia* **39**, 417–422.

Marino, F. and Morgan, A.J. (in press). The time-course of metal (Ca, Cd, Cu, Pb, Zn) accumulation from a contaminated soil by three populations of the earthworm *Lumbricus rubellus*. *Environmental Pollution*.

Martin, M.H. and Bullock, R.J. (1994). The impact and fate of heavy metals in an oak woodland ecosystem. In S. M. Ross (ed.) *Toxic Metals in Soil-Plant Systems*, pp. 327–365. Chichester: John Wiley.

Posthuma, L. and Van Straalen, N.M. (1993). Heavy-metal adaptation in terrestrial invertebrates: a review of occurrence, genetics, physiology and ecological consequences. *Comparative Biochemistry and Physiology* **106c**, 11–38.

Posthuma, L., Hogervorst, R.F. and Van Straalen, N.M. (1992). Adaptation to soil pollution by cadmium excretion in natural populations of *Orchesella cincta* (L.) (Collembola). *Archives of Environmental Contamination and Toxicology* **22**, 146–156.

Posthuma, L., Hogervorst, R.F., Joosse, E.N.G. and Van Straalen, N.M. (1993). Genetic variation and covariation for characteristics associated with cadmium tolerance in natural populations of the springtail *Orchesella cincta* (L.). *Evolution* **47**, 619–631.

Reinecke, A.J. and Viljoen, S.A. (1990). The influence of worm density on growth and cocoon production of the compost worm *Eisenia fetida* (Oligochaeta). *Revue D'Écologie et de Biologie du Sol* **27**, 221–230.

Sibly, R.M. and Calow, P. (1989). A life-cycle theory of responses to stress. *Biological Journal of the Linnean Society* **37**, 101–116.

Spurgeon, D. J. and Hopkin, S.P. (1995). Extrapolation of the laboratory-based OECD earthworm test to metal-contaminated field sites. *Ecotoxicology* **4**, 190–205.

Spurgeon, D.J. and Hopkin, S.P. (1996a). The effects of metal contamination on earthworm populations around a smelting works - quantifying species effects. *Applied Soil Ecology* **4**, 147–160.

Spurgeon, D.J. and Hopkin, S.P. (1996b). Effects of metal contaminated soils on the growth, sexual development and early cocoon production of *Eisenia fetida*, with particular reference to zinc. *Ecotoxicology and Environmental Safety* **35**, 86–95.

Spurgeon, D.J. and Hopkin, S.P. (1999). Comparisons of metal accumulation and excretion kinetics in earthworms (*Eisenia fetida*) exposed to contaminated field and laboratory soils. *Applied Soil Ecology* **11**, 227–243.

Spurgeon, D.J. and Hopkin, S.P. (in press). Tolerance to zinc in populations of the earthworm *Lumbricus rubellus* from uncontaminated and metal contaminated ecosystems. *Archives of Environmental Contamination and Toxicology*.

Tranvik, L., Bengtsson, G. and Rundgren, S. (1993). Relative abundance and resistance traits of two Collembola species under metal stress. *Journal of Applied Ecology* **30**, 43–52.

Vale, J.A. and Harrison, J. (1994). Aerial inputs of pollutants to the Severn Estuary. *Biological Journal of the Linnean Society* **51**, 45–54.

Van Capelleveen, H.E. (1987). Ecotoxicity of metals for terrestrial isopods. PhD Thesis, Vrije Universiteit, Amsterdam.

Van Gestel, C.A.M., Van Dis, W.A., Dirven-Van Breemen, E.M., Sparenburg, P.M. and Baerselman, R. (1991). Influence of cadmium, copper and pentachlorophenol on growth and sexual development of *Eisenia andrei* (Oligochaeta: Annelida). *Biology and Fertility of Soils* **12**, 117–121.