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ORIGINAL PAPER

The impact of the 'New Zealand flatworm', *Arthurdendyus triangulatus*, on earthworm populations in the field

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Abstract The 'New Zealand flatworm', *Arthurdendyus triangulatus*, is a native of the South Island of New Zealand, which has established in the UK, Ireland and the Faroe Islands. In its introduced range, it is a predator of lumbricid earthworms. To assess the impact of *A. triangulatus* on earthworm species, flatworm distributions were manipulated into 'high', control and 'low' densities within a replicated field experiment. Earthworm biomass in the 'high' flatworm density treatment was significantly lower than the control or 'low' treatments. This was due to a reduction in the anecic species *Lumbricus terrestris* and, to a lesser extent, *Aporrectodea longa*. There was little evidence of negative effects on other earthworm species, with even a weakly positive relationship between flatworm density and epigeic biomass. Principal components analysis showed a clear separation of anecic species from *A. triangulatus*, but the epigeic species *Lumbricus festivus* and *Lumbricus rubellus* grouped with *A. triangulatus*, suggesting that they could be benefitting from reduced intraguild competition. Flatworm densities of 0.8 per m^2, comparable to natural infestations in grassland, were predicted to give a reduction in total earthworm biomass of c. 20 %. The bulk of this was comprised of a reduction in anecic species biomass. In particular, it is considered that *A. triangulatus* poses a serious risk to *L. terrestris* populations, with implications for soil functioning and indigenous earthworm-feeding wildlife.

Keywords Terrestrial flatworm · Ecological impact · Earthworms · Manipulative experiment · *Lumbricus terrestris*

Introduction

The 'New Zealand flatworm' *Arthurdendyus triangulatus* (Dendy) (Terricola: Tricladida) is an invasive alien species that preys on earthworms. The flatworm is native to the South Island of New Zealand (Johns et al. 1998; Johns and Boag 2003) and was first found outside its native habitat in Belfast, Northern Ireland in 1963 (Anonymous 1964; Willis and Edwards 1977). The most likely mode of transportation is thought to have been the horticultural trade and genetic analyses of flatworm populations suggests several introductions over time (Dynes et al. 2001). *A. triangulatus* has established throughout Northern Ireland (Moore et al. 1998), the Republic of Ireland, Scotland, northern England and the Faroe Islands (for reviews see Blackshaw and Stewart 1992; Cannon et al. 1999; Boag and Yeates 2001). Despite being well established in the British Isles and the Faroe Islands, including offshore islands such as the Scottish outer and inner Hebrides, the Orkney and Shetland Islands (Boag et al. 1994) and the Isle of Man (Boag et al. 1995;

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Jones and Boag 1996), *A. triangulatus* has not been recorded from continental Europe or elsewhere, with the exception of a single record, probably transient, from Iceland (Bloch 1992).

Assessing the impact of invasive alien species on native fauna is crucial, in order to provide the evidence whereby policy-makers and legislators can approve action to manage these species (Murchie 2010). Quantifying the damage caused by *A. triangulatus* is a fundamental requirement of pest risk analyses. The impact of *A. triangulatus* predation on earthworm communities is contentious. Some workers consider that the flatworm has the potential to seriously deplete earthworm numbers with severe consequences for soil fertility and earthworm-feeding wildlife (Alford 1998); whereas, others have suggested that it will attain an equilibrium with earthworm populations (Gibson et al. 1997), with perhaps a cyclical predator/prey dynamic (Blackshaw 1995). Although at what level this equilibrium may settle remains unknown. The situation is further complicated because there are at least 26 species of native Lumbricidae recorded from the British Isles (Sims and Gerard 1999; Sherlock and Carpenter 2009), with six species common in grassland (c.f. Boag et al. 1997), each with its own particular niche within the soil, environmental preferences and reproductive strategies. As such, each species is likely to respond to flatworm predation in a different way.

The initial study which drew attention to the problems posed by *A. triangulatus*, concerned a reduction in earthworms in plots treated with a calcareous seaweed product (Blackshaw 1989). This showed a marked decline to undetectable levels of the earthworm population over a 4 year, 8 month period. Revisiting these data, Blackshaw and Stewart (1992) considered that species active on the surface such as *Lumbricus rubellus* and *Lumbricus terrestris* seemed to be more vulnerable to *A. triangulatus* predation, whereas deep-dwelling species such as *Octolasion cyaneum* were less vulnerable. Using data derived from the tube vermaria studies of Blackshaw (1990), Blackshaw and Stewart (1992) further suggested that *Aporrectodea caliginosa* was less affected by predation as it lived deeper in the soil. Blackshaw and Stewart (1992) therefore considered that prey availability was the driving factor determining differential predation, rather than any intrinsic prey preference. Similarly, Lillico et al. (1996) found that earthworm burrow width (wide) and depth (shallow), increased *A. triangulatus* predation in vermaria studies. They suggested a graduation of earthworm vulnerability from *L. rubellus* and *L. terrestris* most vulnerable to *O. cyaneum* and *Aporrectodea rosea* least, based on niche characteristics rather than specific prey preference. Similarly, Blackshaw and Stewart (1992) contended that *L. terrestris* were vulnerable because of the large vertical burrows that allow the flatworm to follow them deep into the soil. Earthworm choice experiments in Petri dishes would seem to support the contention that prey availability rather than prey choice was most important (Stewart 1993). However, Stewart (1993) pointed out that it was difficult to make a true comparison as the sizes of the earthworms were so different; nevertheless concluding that *A. triangulatus* was, as with many other terrestrial flatworms, an opportunistic predator.

In a long-term monitoring study, Blackshaw (1995) compared fluctuations in earthworm and flatworm numbers over 8 years at two sites in Belfast. At both sites, the number of earthworm species declined from 6–7 to two. *L. terrestris*, *Ap. caliginosa* and *Allolobophora chlorotica* were not recovered in the later samples, with *O. cyaneum* and *Aporrectodea limicola* the remaining species. Blackshaw (1995) considered that *O. cyaneum* may be less vulnerable to flatworm predation because it is deep-dwelling and can reproduce parthenogenetically. A different approach was used by Jones et al. (2001) who utilised a variety of univariate and multivariate analyses, to provide evidence that differences in species composition between two pasture fields infested with *A. triangulatus* and 48 comparable non-infested fields in western Scotland were partly due to a reduction in anecic species in the infested fields. In particular, they used the non-parametric Kruskall-Wallis median test to demonstrate that the two infested fields were atypical in terms of a range of earthworm species, with the most marked difference found in *Aporrectodea longa*. However, the results from this study are suggestive rather than absolute as the comparison was of two fields on the same farm with mean values derived from 48 disparate fields. So the sample size is not sufficient to draw conclusions about *A. triangulatus* infestation per se, although it forms part of an increasing body of evidence in the literature showing the impact of *A. triangulatus* on anecic earthworms.

Extremely valuable as the above studies are, they have their limitations: chronological studies that lack controls are difficult to interpret as many extraneous factors can impact on earthworm populations; whereas, container-based studies do not deal with a naturally-established earthworm fauna and are inherently limited by the size of the container, which would, for example restrict earthworm movement. One possible method to overcome these problems would be to inoculate virgin sites with *A. triangulatus* and monitor any deleterious effects. However, it is an offence under the UK Wildlife and Countryside Act 1981 to knowingly release *A. triangulatus* into the wild (Alford 1998). An alternative approach, as pursued in this study, is to choose an infested site and manipulate flatworm densities therein, according to a replicated experimental design. There are three sequential objectives of this study: (1) to determine the impact of *A. triangulatus* on overall earthworm densities and biomass; (2) the impact on individual earthworm species biomass; (3) based on the results from objective 2, the effects of *A. triangulatus* on earthworm community structure are also evaluated, along with the implications for soil productivity and earthworm-feeding wildlife.

Materials and methods

Experimental design

A flatworm-infested grassland field at Newforge Lane, Belfast, Northern Ireland (World Geodetic System 54°33′24″N, 5°56′47″W; Irish Grid J330698) was selected. This was close to the site studied by Blackshaw (1995) and the field was known to be infested with flatworms for at least 15 years prior to the current study. The field had been in permanent grass since 1993 and has a slight slope of approximately 5° from SW to NE. The soil is sandy loam topsoil over sandy loam/sandy clay loam subsoil (eutri-stagnic cambisol).

The field was divided into nine blocks, with each block consisting of three treatment plots, each measuring 8 m by 8 m and separated from each other by a 1 m path. Plot boundaries were treated with creosote twice a year, as a marker but also as a potential means of limiting *A. triangulatus*/earthworm surface movement. Plots were mown regularly during the summer to keep the grass short. Within blocks, treatments of 'high' or 'low' flatworm density or control were randomly allocated to plots.

Sampling and manipulation of flatworm densities

Flatworm numbers were assessed on a weekly basis by examining underneath nine shelter traps placed on the soil surface within each plot. The traps were similar to those used by Blackshaw et al. (1996) and consisted of heavy-duty black polythene sacks measuring 360 × 240 mm and filled with 5 kg pea gravel and sand at a 4:1 ratio. The nine traps were positioned in a 3 by 3 grid inside the plot, with 2.3 m between traps. The outer traps were 1.7 m from the edge of the plot.

To manipulate flatworm densities, flatworms found under traps in the 'low' designated plots were collected, pooled and then distributed evenly among the 'high' designated plots. This procedure began on 27 January 1999 and ceased 30 October 2002 (3 years and 9 months representing 196 weekly visits). Flatworm numbers continued to be monitored after this, at weekly intervals until 5 March 2003, with some additional samples taken at fortnightly and then monthly intervals until 3 July 2003.

Temperature at the soil surface was measured by means of a TinyTag datalogger (Gemini Data Loggers (UK) Ltd.; www.geminidataloggers.com) placed under a shelter trap at the edge of the experiment.

Earthworm extraction

Earthworms were sampled using the mustard expulsion technique (Gunn 1992; Chan and Munro 2001). Five square steel quadrats (dimensions 0.5 × 0.5 m = 0.25 m^2) were arranged in an X pattern across the plot, with 3 m between quadrats, and pressed 2–3 cm into the soil. Inside the quadrats, foliage was cut to ground level using handheld grass clippers and the ground soaked with 5 l of a mustard solution (6 g l^{-1}) (Colman's Double Superfine Mustard Powder, Norwich, UK). A secondary dose of the mustard solution was applied 15 min later and any further worms collected. Earthworms expulsed by the irritant were stored in 4 % formalin. Later, they were patted dry using paper towels, counted and weighed. Adults were identified to species using the key of Sims and Gerard (1999) and juveniles to genus.

Earthworm extractions took place in the spring and autumn, when earthworms were most active, over 3 years. The specific dates were: 6 April 2000; 26 October 2000; 5 April 2001; 25 October 2001; 12 April 2002; 30 October 2002. The positions of the quadrats were altered for each sampling date to ensure that a different area of ground was sampled each time.

Analyses

Arthurdendyus triangulatus and earthworm densities and biomass were subjected to one-way ANOVA in randomised blocks, according to the experimental design. Count data are typically best analysed with generalized linear mixed models as this allows the handling of non-normal data, such as Poisson distributed counts (Bolker et al. 2009). In this case though, samples were collected from five quadrats (mustard expulsion) and averaged/adjusted to give densities per m^2. In addition, when looking at ecotypes or individual species, to allow for an overall analysis, data were averaged over the 3 years. Where data are averaged, the central limit theorem gives that they will tend to normality. As earthworm populations undergo a seasonal cycle, spring and autumn extractions were then analysed separately. Although samples were taken in three consecutive years, year was not included in the analyses as a factor because earthworm recovery by mustard expulsion is much influenced by preceding weather conditions and, although comparative amongst treatments at that point in time, may not reflect yearly populations.

The juvenile groups, *Allolobophora*, *Aporrectodea* and *Lumbricus*, were also subjected to ANOVA according to the experimental design. As sexual morphology is often used to identify earthworms, identification of juveniles is difficult and imprecise. However, for *Aporrectodea* and *Lumbricus*, as these groups comprised several species and different ecotypes, they were divided into two weight classes. Large-designated *Lumbricus* juveniles were those above 0.758 g at this particular location. This was done to separate off the anecic *L. terrestris* juveniles and was calculated by taking the mean weight of the next largest *Lumbricus* species, *Lumbricus festivus*, and adding 2 × SD = 0.464 + (2 × 0.147) = 0.758 g (Table 1; 95 % of values lie within 2 SD of the mean). Therefore any *Lumbricus* juveniles above this weight were likely *L. terrestris*. Similarly for *Aporrectodea* juveniles, any over 0.646 g were considered *Ap. longa* juveniles.

Table 1 Mean individual wet weights of the eight commonest adult earthworm species (incl. gut contents) and *A. triangulatus* after storage in formalin

Earthworm species	Mean weight (g)	n	SD
Adults			
Al. chlorotica	0.164	4,195	0.034
Ap. caliginosa	0.336	1,344	0.155
Ap. limicola	0.246	328	0.079
Ap. longa	1.445	175	0.385
L. castaneus	0.099	1,818	0.030
L. festivus	0.464	367	0.147
L. rubellus	0.363	1,358	0.122
L. terrestris	2.576	243	0.831
Flatworm			
A. triangulatus	0.685	69	0.380

Earthworm ecotype biomass in plots was related to flatworm densities, derived from quadrat expulsion and from shelter trapping, by linear regression. The assumptions of regression (normality and homoscedasticity) were checked by plotting the residuals against fitted values and in a normal probability plot. To examine effects on earthworm community structure, species composition in plots was analysed by principal components analysis (PCA) using the sums of squares and products matrix. The resultant PC scores were scaled to lie between ±1 and the original variables (i.e. species counts) were correlated back against the PC scores.

As this study looks at the effects of *A. triangulatus* predation on several earthworm species, many statistical results are generated. As the number of tests increase, then the likelihood of a type 1 error increases. One mechanism to accommodate for this is to adjust the *P* value using Bonferroni corrections. It was decided against this approach as Bonferroni adjustments are overly conservative, especially for ecological field data and where tests are interrelated (Moran 2003). The reader is therefore asked to consider the pattern of the results, rather than focussing on individual tests.

Analyses were conducted in GenStat (VSN International Ltd, UK; www.vsni.co.uk) and data graphed using Prism (Graphpad Software, USA; www.graphpad.com).

Results

Manipulation of flatworm densities

Flatworm numbers under shelter traps were greater during the first 6 months of the experiment, peaking at 40.2 ± SEM 3.43 in the 'high' plot in April 1999, but thereafter remained reasonably constant from year to year. However, there was a marked seasonal pattern to the numbers of flatworms recovered from under traps. Their numbers generally increased in the spring, although there was considerable variation from week-to-week, to crash sharply in mid-summer (July–August), when soil temperatures were at maximum. The numbers of flatworms under traps then rose again as the weather became cooler during September and October (Fig. 1).

Despite the fluctuations in flatworm numbers throughout the year, the intended manipulation of flatworm densities into 'high', control and 'low' treatments was consistent and statistically significant (Fig. 1). Across the whole period of the manipulation, the overall mean number of flatworms in the 'high' treatment was 13.5, in the control 8.2 and in the 'low' 3.8 (F = 43.05, *df* = 2, 16, $P < 0.001$; pooled SEM = 0.746). Comparison of means using Fisher's LSD method, showed that each treatment was separate from the others.

After the manipulation ceased on 30 October 2002, the separation in flatworm numbers between the 'high', control and 'low' treatments broke down (Fig. 1). On the final sample, taken on 3 July 2003, there were more flatworms in the 'low' plots than in

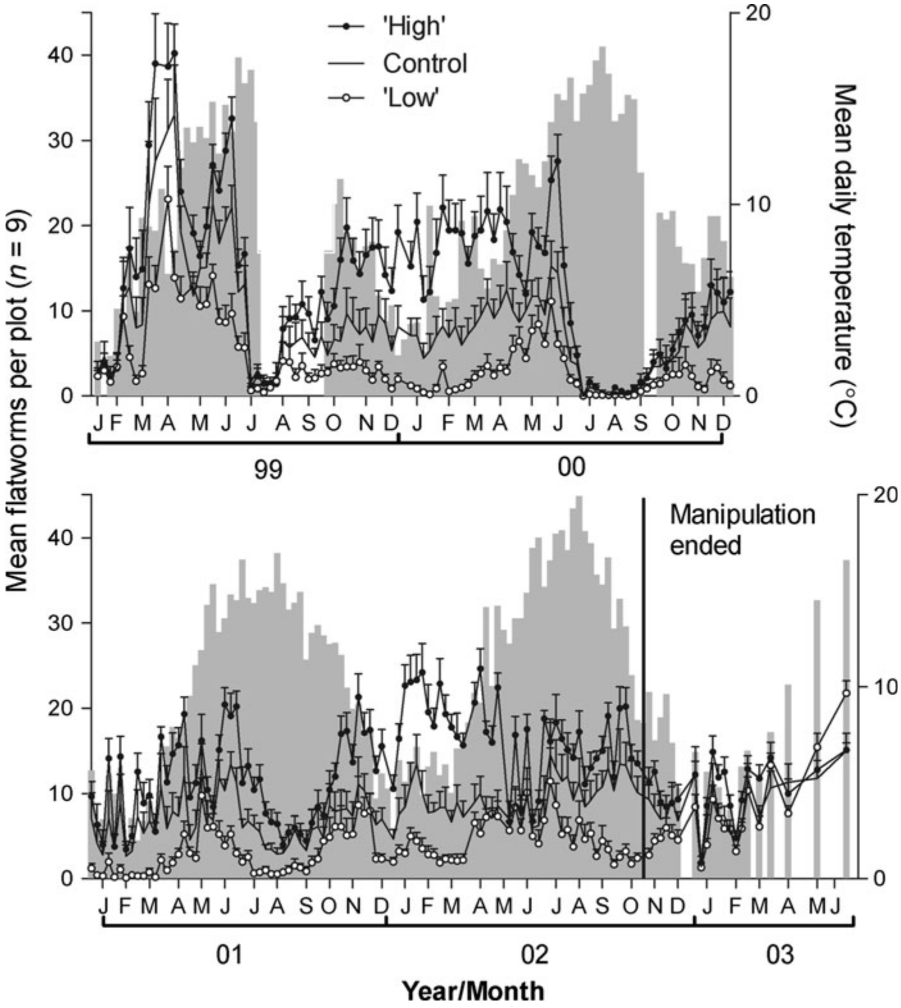

Fig. 1 *A. triangulatus* phenology derived from shelter trap catches and soil surface temperature (*grey bar*, *right hand axis*) on the manipulation experiment. Treatments are: 'high' (*filled circle with line*), control (*line*) and 'low' (*open circle with line*) flatworm densities achieved through physically moving flatworms each week. *Error bars* are SEM. The *solid vertical line* denotes the date when the manipulation process ended (30 October 2002)

the other treatments (F = 8.11, *df* = 2, 16, *P* = 0.004).

Flatworms were also recovered using the mustard expulsion technique. Compared to the shelter traps, densities were comparatively low with, for example, the control plots averaging 0.3 *A. triangulatus* per m^2 and even in the high plots there were less than 0.5 per m^2. In the spring samples, there was significantly less flatworm biomass in the 'low' treatment (F = 3.66, *df* = 2.16, *P* = 0.049). For the autumn samples, there was no significant difference between treatments.

Effects of differing flatworm densities on earthworms

The density of earthworms recovered was generally low at around 120 per m^2. However, this did vary between sampling dates, with the samples taken on 25 October 2001 having a grand mean of 246 earthworms per m^2 (Fig. 2).

In all sampling dates, there were no significant effects of treatments on overall earthworm densities (Fig. 2). However, for earthworm biomass, there was an effect of treatments, with the 'high' flatworm density plots having less earthworm biomass than the other treatments. This effect was statistically significant at the 5 % level for samples collected on 5 April 2001 (*P* = 0.021) and 12 April 2002 (*P* = 0.009; Fig. 3). If the significance level is extended to 10 % (*P* < 0.1), then four out of the six dates showed a treatment effect. On all sampling dates, there were significant block effects, demonstrating the spatial heterogeneity of earthworm counts.

Impact on earthworm ecotypes and species

Twelve earthworm species were recovered during mustard expulsion. Samples were mostly juvenile *Aporrectodea* (32 %) and juvenile *Lumbricus* (21 %), with *Al. chlorotica*, *Lumbricus castaneus*, *Ap. caliginosa* and *L. rubellus*, prevalent amongst the adults (Table 2). The pattern with earthworm biomass was slightly different. Juvenile *Aporrectodea* (26 %) and *Lumbricus* (22 %) again made up the bulk of samples. However for adults, although *Al. chlorotica* comprised the greatest portion of biomass at 11 %, *L. terrestris* made up 10 % of the biomass despite only being 1 % of the species present (Table 2). This is because earthworm species vary greatly in size and weight. Adult *L. terrestris* were on average 16 times heavier than *Al. chlorotica* (Table 1). In total, adult and juvenile anecic species (*L. terrestris*, *Ap. longa*)

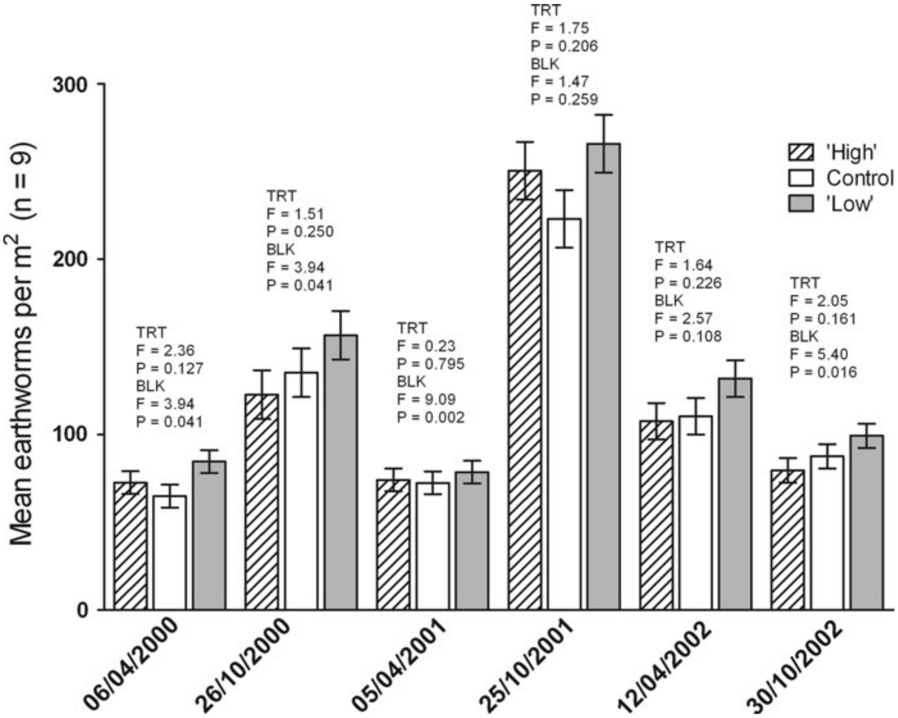

Fig. 2 Mean earthworm densities (m^{-2}) expulsed by mustard solution in the manipulation experiment. Treatments are: 'high' (*striped bar*), control (*open bar*) and 'low' (*filled bar*) flatworm densities achieved through physically moving flatworms each week. Statistics given above *bars* are treatment (TRT) and block (BLK) effects derived from ANOVA, with 2, 16 and 8.16 df respectively. *Error bars* are pooled SEM

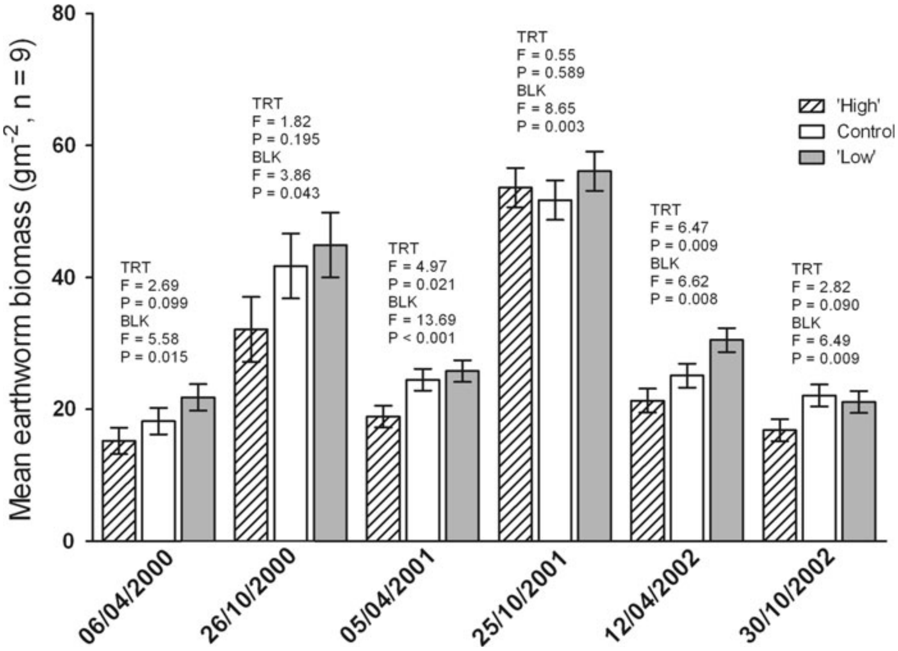

Fig. 3 Mean earthworm biomass (gm^{-2}) expulsed by mustard solution in the manipulation experiment. Treatments are: ‘high’ (*striped bar*), control (*open bar*) and ‘low’ (*filled bar*) flatworm densities achieved through physically moving flatworms each week. Statistics given above *bars* are treatment (TRT) and block (BLK) effects derived from ANOVA, with 2, 16 and 8.16 df respectively. *Error bars* are pooled SEM

comprised 4 % of earthworm numbers recovered but 26 % of biomass (Table 2).

The adult earthworm species found were assigned to three ecotypes (epigeic, endogeic and anecic) following the designation of Bouché (1971, 1977) and as listed by Fraser and Boag (1998) (Table 3). In terms of density, endogeic earthworms were most abundant, followed by epigeic, with anecic species numbers comparatively low (Fig. 4). However, the biomass of the three ecotype groupings was more similar (Fig. 5).

There were different treatment effects depending on the earthworm ecotype. For epigeic and endogeic species, there was no significant effect of altered flatworm density on earthworm densities (Fig. 4) or biomass (Fig. 5). For anecic species however, there were fewer earthworms ($P = 0.049$) and less biomass ($P = 0.016$) in the ‘high’ flatworm density plots in the spring samples (Figs. 4, 5), compared to the other treatments.

The direct relationship in the plots between flatworm density/shelter trap counts and ecotype biomass reflected the treatment effects revealed by ANOVA. For epigeic species, there was a weakly positive relationship between flatworm density and epigeic biomass (Fig. 6a; F = 4.24, *df* = 1, 25, $P = 0.050$). There was no relationship between flatworm density and endogeic species. However, there was a significant negative relationship between flatworm counts and anecic biomass (Fig. 6a; F = 17.60, *df* = 1, 24, $P < 0.001$), with the x-intercept, when y = 0, at 1.08 for anecic biomass. A similar pattern emerged in comparing the numbers of flatworms under shelter traps with earthworms recovered from the plots. There was no relationship with epigeic or endogeic species but there was a significant negative relationship between the presence of flatworms under shelter traps and anecic biomass (Fig. 6b; F = 16.50, *df* = 1, 24, $P < 0.001$). The x-intercept for the regression line was at 2.22.

Impact on individual earthworm species biomass

For most earthworm species there were no effects of altered *A. triangulatus* density on biomass (Table 4). The exception to this was the significant reduction in *L. terrestris* adult and juvenile biomass in the high flatworm density treatment, compared to the other treatments, in the spring samples. There was a similar reduction in large *Aporrectodea* juvenile biomass, although this was not quite significant at the 5 % level ($P = 0.057$).

For *L. castaneus*, there was less biomass ($P = 0.041$; Table 4) in the control plots compared to the ‘high’ or ‘low’ flatworm density plots in the autumn samples. It is possible this could be a type 1 error,

Table 2 Earthworm species composition in percentages of earthworm samples collected by mustard expulsion (n = 24,952)

Earthworm species	Spring (%)		Autumn (%)		Total (%)	
	Number	Biomass	Number	Biomass	Number	Biomass
Adults						
Al. chlorotica	12.4	7.5	19.3	13.6	16.8	11.3
L. castaneus	5.0	2.0	8.6	3.6	7.3	3.0
Ap. caliginosa	4.5	5.5	5.9	8.6	5.4	7.4
L. rubellus	6.0	8.1	5.1	8.1	5.4	8.1
L. festivus	0.9	1.7	1.8	3.5	1.5	2.8
Ap. limicola	1.2	1.2	1.4	1.4	1.3	1.3
L. terrestris	1.1	10.4	0.9	10.2	1.0	10.3
Ap. longa	0.5	2.4	0.8	5.2	0.7	4.2
Ap. rosea	0.3	0.1	0.2	0.1	0.2	0.1
O. cyaneum	0.2	1.2	0.2	1.0	0.2	1.1
O. tyrtaeum	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Satchellius mammalis	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Juveniles						
Aporrectodea juv. total[a]	36.1	26.1	30.1	25.6	32.3	25.8
(*Aporrectodea* juv. >0.646 g)	(1.0)	(3.8)	(1.0)	(4.2)	(1.0)	(4.0)
(*Aporrectodea* juv. ≤0.646 g)	(35.1)	(22.3)	(29.1)	(21.4)	(31.3)	(21.8)
Lumbricus juv. total[a]	27.9	31.9	17.4	15.5	21.2	21.5
(*Lumbricus* juv. >0.758 g)	(2.3)	(12.1)	(0.9)	(4.9)	(1.4)	(7.6)
(*Lumbricus* juveniles ≤0.758 g)	(25.6)	(19.8)	(16.5)	(10.6)	(19.8)	(13.9)
Allolobophora juv.	3.6	1.4	8.1	3.3	6.5	2.6
Octolasion juv.	0.2	0.5	0.2	0.4	0.2	0.5

[a] Juvenile *Aporrectodea* weighing greater than 0.646 g are considered *Ap. longa* and juvenile *Lumbricus* weighing over 0.758 g are *L. terrestris*. See text for details

Table 3 Allocation of earthworm species to ecotype, following the designation of Bouché (1971, 1977) as listed by Fraser and Boag (1998)

Ecotype	Species
Epigeic	*L. castaneus, L. rubellus*, *L. festivus*, *S. mammalis*
Endogeic	*Al. chlorotica*, *Ap. caliginosa*, *Ap. limicola*, *Ap. rosea*, *O. cyaneum*, *O. tyrtaeum*
Anecic	*L. terrestris*, *Ap. longa*

although a similar but non-significant result is found for the spring biomass of *Al. chlorotica* ($P = 0.088$).

Earthworm community structure

PCA gave three components accounting for 62.83 (PC1), 23.84 (PC2) and 6.59 % of the variation. The first two components therefore accounted for 86.67 % variation. Treatments were not strongly distinguished in the ordination plot (Fig. 7), although seven of the nine 'high' treatment plots were negatively associated with PC1 and most of the 'low' plots (8 from 9) were positively associated with PC2. For PC1, there were negative vector loadings for *A. triangulatus*, *L. festivus*, *L. rubellus*, *Lumbricus* juveniles (≤0.758 g) and *L. castaneus*. All other earthworm groups contributed positively to PC1. PC2 is most strongly influenced by epigeic species (*Lumbricus* juveniles (≤0.758 g), *L. castaneus*) and endogeic species (*Aporrectodea* juveniles (≤0.646 g), *Al. chlorotica*) with little or negative contributions from anecic species or *A. triangulatus*.

PCA clearly grouped earthworm species according to the three ecotypes. The anecic species (*L. terrestris*, *Ap. longa*, adults and juveniles) were directly opposed to *A. triangulatus* along the PC1 axis (Fig. 7). Conversely, the epigeic species, *L. festivus* and *L. rubellus*, grouped with *A. triangulatus*.

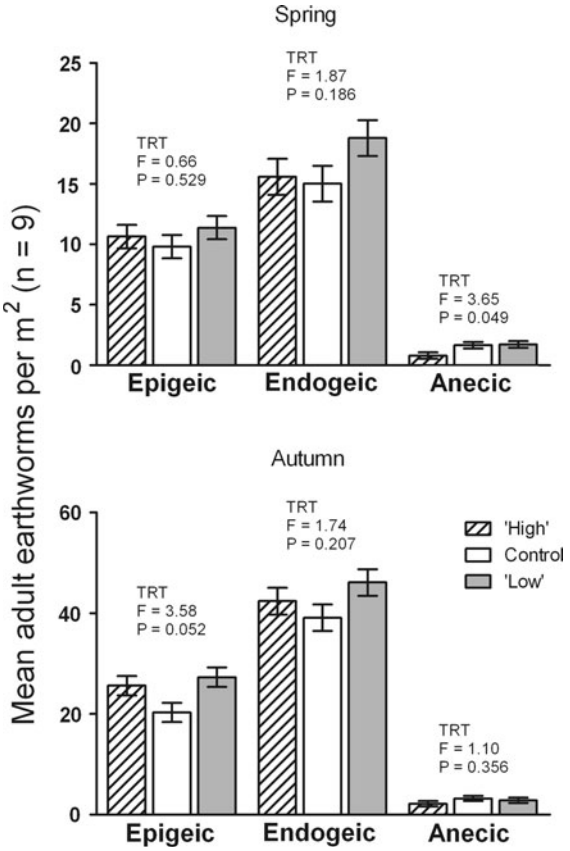

Fig. 4 Mean adult earthworm densities (m^{-2}) expulsed by mustard solution, divided into ecotype and summarised for spring and autumn samples. Treatments are: 'high' (*striped bar*), control (*open bar*) and 'low' (*filled bar*) flatworm densities. Statistics given above *bars* are treatment (TRT) effects derived from ANOVA, with 2, 16 df. *Error bars* are pooled SEM

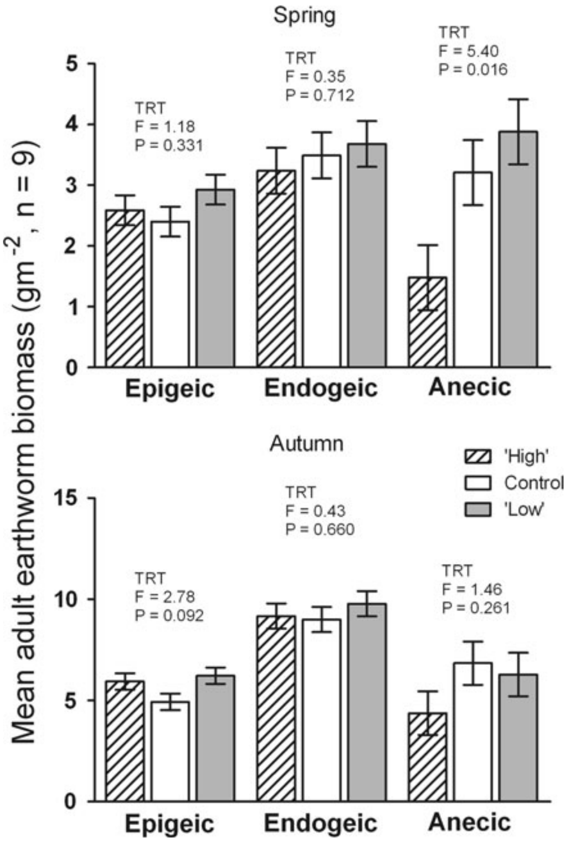

Fig. 5 Mean adult earthworm biomass ($g\ m^{-2}$) expulsed by mustard solution, divided into ecotype and summarised for spring and autumn samples. Treatments are: 'high' (*striped bar*), control (*open bar*) and 'low' (*filled bar*) flatworm densities. Statistics given above *bars* are treatment (TRT) effects derived from ANOVA, with 2, 16 df. *Error bars* are pooled SEM

Discussion

This study provides a quantitative assessment from a 4-year manipulative experiment on the impact of an invasive species, *A. triangulatus*, on its earthworm prey in the field. The weekly transfer of flatworms from 'low' to 'high' plots created differential flatworm densities. Despite some initial concerns that *A. triangulatus* would move between plots, shelter trap samples consistently indicated that flatworm densities were about three times greater in the 'high' allocated plots compared to the 'low'. This was also shown in the numbers of flatworms expelled by mustard solution in quadrats. The separation into different flatworm densities was maintained, despite the clear seasonal cycle demonstrated by *A. triangulatus* (Fig. 1). As *A. triangulatus* are susceptible to raised temperatures and associated soil moisture deficits (Boag et al. 1998), the marked decline in shelter trap counts during mid-summer probably reflects *A. triangulatus* moving deeper in the soil when it is dry and not outright mortality. This is supported by the resumption of greater flatworm numbers under the traps in early autumn, when conditions became cooler and soil more moist. After the manipulation process ended in October, the flatworms recolonised the 'low' density plots in the spring to the point where the 'low' plots had significantly more flatworms than the others. This was only significant in the last week of the study but may suggest that the flatworms were redistributing according to the greater levels of earthworm prey in the 'low' density plots; albeit, since this sample was

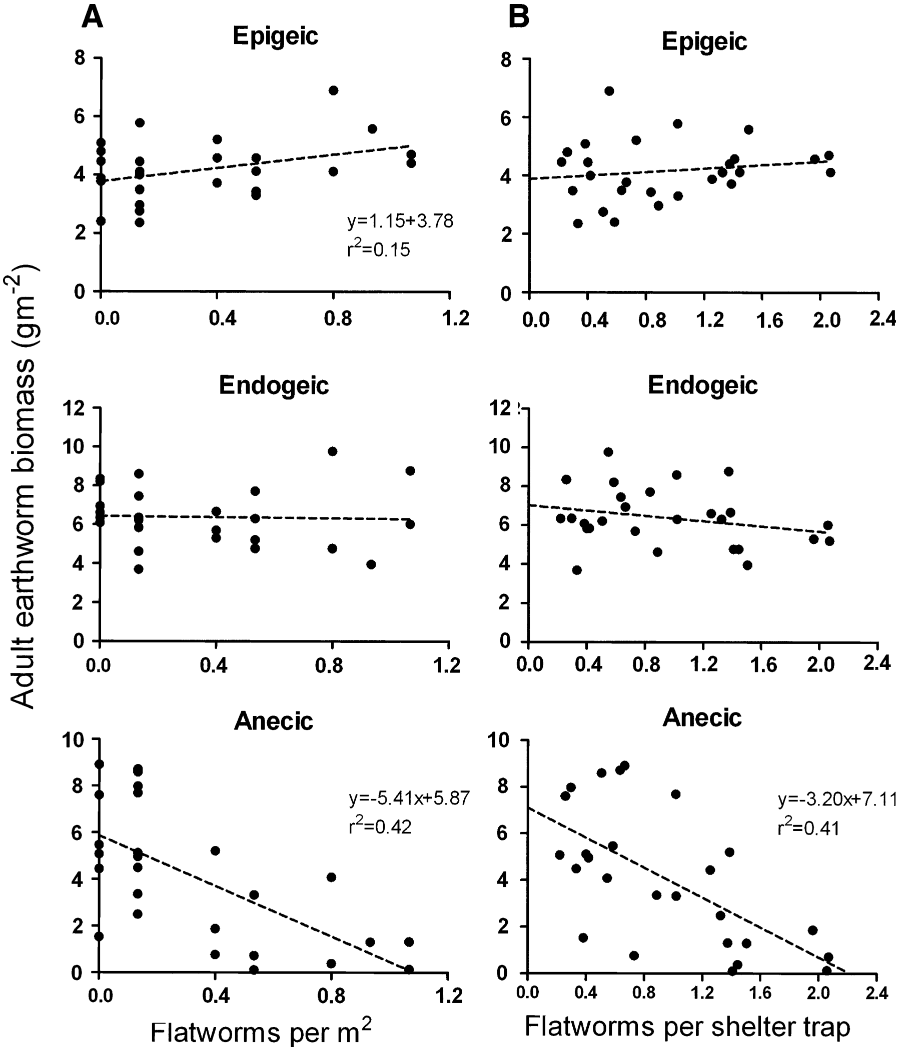

Fig. 6 Relationships between *A. triangulatus* and adult earthworm ecotype biomass in plots (n = 27). The independent variable in graphs (**a**) are overall average flatworm densities derived from mustard expulsion. In graphs (**b**) it is overall average flatworm numbers derived from shelter traps counts and expressed as flatworms per shelter trap. Two outliers are excluded from the anecic graphs: **a** coordinates 0.53, 11.53; **b** coordinates 0.83, 11.53

taken 8 months after the manipulation ended, at a relatively slow rate.

The most notable findings from this experiment were that increased *A. triangulatus* density did not lead to a reduction in overall earthworm numbers but did lead to a significant reduction in numbers of the anecic earthworm species, *L. terrestris* and *Ap. longa*. This in turn reduces earthworm biomass because anecic species are considerably larger than epigeic or endogeic species. *L. terrestris* is the largest indigenous earthworm species in the British Isles (Sims and Gerard 1999). For example, in this study the average weight of an individual adult *L. terrestris* was 2.576 g; making it approximately 16 times heavier than *Al. chlorotica* at an average of 0.164 g or 8 times heavier than *Ap. caliginosa* (0.336 g), which were the commonest earthworms. The size though of *L. terrestris* at this site was comparatively small. In laboratory conditions, *L. terrestris* easily reach 5–7 g (Butt 1993; Butt 2011). As a whole, anecic species, including juveniles, represented 4 % of the earthworm count but 26 % of the biomass.

The impact of *A. triangulatus* predation on *L. terrestris* was most obvious in the spring. This could be for two reasons: greater flatworm activity during the cooler winter months or greater earthworm mortality from other causes during the winter, i.e. the earthworm populations would be at a low point in their cycle and thus more vulnerable to predation. Both of these contentions would be supported from the data. Flatworm numbers derived from shelter traps, show the main period of surface activity of flatworms was

Table 4 Mean earthworm biomass (g m^{-2}) expulsed by mustard solution in three treatments: 'high', control and 'low' flatworm density

Earthworm species	Season	Treatment			Pooled SEM	*P*
		'High'	Control	'Low'		
Al. chlorotica	Spr	1.63	1.45	1.96	0.153	0.088
	Aut	5.02	4.76	5.62	0.354	0.242
Ap. caliginosa	Spr	0.95	1.45	1.27	0.225	0.317
	Aut	3.24	3.11	3.34	0.457	0.934
Ap. limicola	Spr	0.33	0.26	0.25	0.049	0.494
	Aut	0.51	0.53	0.52	0.074	0.977
Ap. longa	Spr	0.34	0.62	0.62	0.174	0.441
	Aut	1.80	2.26	1.85	0.553	0.817
L. castaneus	Spr	0.46	0.40	0.47	0.057	0.691
	Aut	1.41	1.06	1.56	0.130	**0.041**
L. festivus	Spr	0.45	0.35	0.32	0.085	0.546
	Aut	1.37	1.19	1.35	0.113	0.449
L. rubellus	Spr	1.68	1.64	2.14	0.225	0.247
	Aut	3.15	2.69	3.31	0.254	0.232
L. terrestris	Spr	1.14	2.59	3.27	0.524	**0.032**
	Aut	2.56	4.59	4.42	0.834	0.195
Allolobophora juveniles	Spr	0.26	0.35	0.35	0.040	0.218
	Aut	1.22	1.12	1.46	0.141	0.246
Aporrectodea juveniles >0.646 g	Spr	0.56	0.92	1.08	0.152	0.057
	Aut	1.06	1.90	1.77	0.313	0.158
Aporrectodea juveniles ≤0.646 g	Spr	4.66	4.74	5.55	0.373	0.209
	Aut	7.05	8.44	8.79	0.675	0.188
Lumbricus juveniles >0.758 g	Spr	1.28	3.27	3.60	0.649	**0.046**
	Aut	1.58	2.21	1.78	0.431	0.581
Lumbricus juveniles ≤0.758 g	Spr	4.28	4.14	4.82	0.426	0.510
	Aut	3.63	3.87	4.45	0.415	0.381

Probabilities in bold are <0.05

from October to June. So the earthworms sampled in spring would have been exposed to predation during the autumn and winter. Conversely, earthworm counts were generally greater in the autumn samples.

As mentioned in the introduction, other workers have postulated that the anecic species, *L. terrestris* and *Ap. longa*, may be more susceptible to flatworm predation because of their feeding activity on the soil surface and burrow construction (Fraser and Boag 1998). Anecic species construct vertical, semi-permanent burrows that may allow easy access by flatworms. In contrast, epigeic species burrow little, whilst endogeic species produce extensive horizontal burrows. In addition to availability for predation, the second reason why anecic species may be more vulnerable to *A. triangulatus* is because they are relatively slow to mature and reproduce. For example, under outdoor conditions *L. terrestris* and *Ap. longa* produce 8–13 cocoons per worm per year whereas *Ap. caliginosa* and *Al. chlorotica* (endogeics) both produce c. 27 and *L. rubellus* (epigeic) produces 79–106 (Evans and Guild 1948; Satchell 1967). It may be that epigeic earthworms have the reproductive potential to absorb *A. triangulatus* predation pressure compared to the slower-reproducing anecic species. Predation pressure on epigeics can be severe. *L. rubellus* and *Ap. caliginosa* and were predated proportionately more than *L. terrestris* by golden plover (*Pluvialis apricaria*; Bengtson et al. 1978), with the impact of bird feeding halving the earthworm population

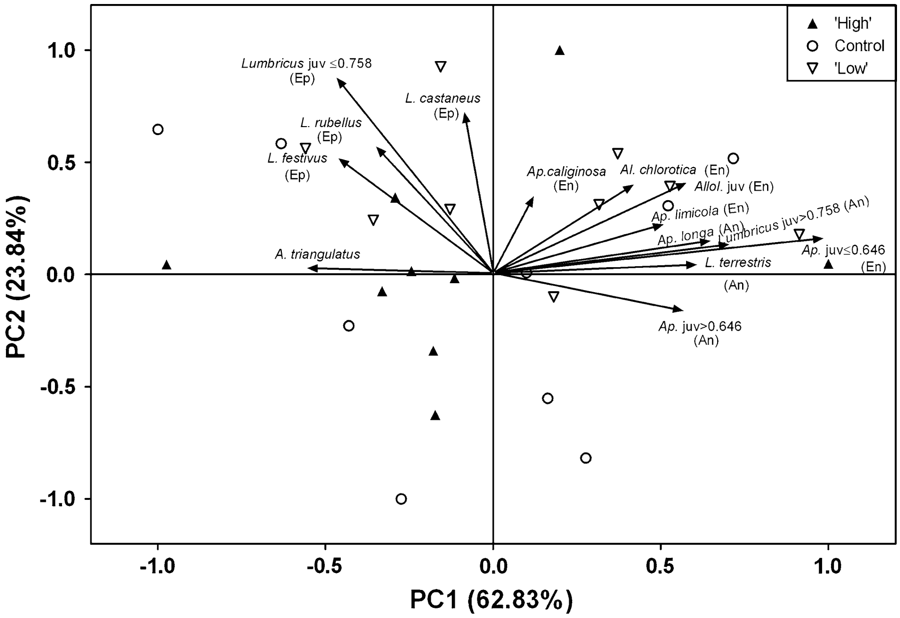

Fig. 7 Principal components analysis biplot with *A. triangulatus*/earthworm species vectors and treatment plots (denoted: 'high' *filled triangle*, control *open circle*, 'low' *inverted open triangle*). *Letters in parentheses* after species names denote ecotype: *An* anecic, *Ep* epigeic, *En* endogeic

(Bengtson et al. 1976). It would seem that vertical burrowers such as *L. terrestris* can rapidly withdraw below the probing depth of farmland birds. Such an escape response, however, would be ineffective when confronted with *A. triangulatus*.

Aside from the anecic species, most other earthworm species showed little impact of altered flatworm density in the ANOVAs. However, there was a significant positive relationship between adult epigeic biomass and *A. triangulatus* counts derived from mustard expulsion (Fig. 6). In the PCA, *L. festivus* and *L. rubellus* grouped with *A. triangulatus* and were disparate from the anecic species. This would suggest that *L. festivus* and *L. rubellus* may benefit from the absence of *L. terrestris* and *Ap. longa*, because of reduced competition. In a review of inter and intra-specific competition in earthworms, Uvarov (2009) considered *L. rubellus* to be a vigorous competitor of *L. terrestris*, as both feed on litter material. In laboratory culture experiments, the presence of *L. rubellus* retarded growth, maturation and cocoon production by *L. terrestris* (Lowe and Butt 2002a, b). Whereas, Shuster et al. (2001) found that additions of *L. terrestris* to field plots reduced the abundance and biomass of *L. rubellus*. There has been little work on the competitive aspects of *L. festivus*. A correlation matrix using earthworm species data from 200 Scottish fields, found a negative association between *L. festivus* and *O. cyaneum*, but not with other species (Jones et al. 2001). This does not mean that *A. triangulatus* does not predate *L. rubellus* or *L. festivus*. Rather, the trade-off between increased predation and reduced competition was, at this locality, advantageous for these earthworms.

Several caveats ought to be mentioned when interpreting the results from this experiment. Firstly, earthworm populations in this field would already have been exposed to *A. triangulatus* predation. *A. triangulatus* has been monitored in this field since 1984 (Blackshaw 1995) and it would seem reasonable to assume that flatworms have been there for at least 15 years. Therefore, this experiment was monitoring the effects of *A. triangulatus* on an already depleted earthworm population. It would be expected that the effects of enhanced *A. triangulatus* predation on an unexposed earthworm population would be more severe. Another factor that may account for the low earthworm counts was the use of the mustard solution technique for sampling. Expulsion sampling is considered less effective than hand-sorting (Lee 1985; Bartlett et al. 2010) and dependent on soil conditions (e.g. in saturated soils it is difficult for the mustard solution to penetrate). However, it has the advantages of being non-destructive and comparatively quick, which were prerequisites for this study. It is unfortunate though that mustard extraction is less effective at sampling *Octolasion tyrtaeum* compared with hand sorting (Lawrence and Bowers 2002). A similar species, *O. cyaneum*, is considered less susceptible to *A. triangulatus* predation because it is deep-dwelling and parthenogenetic (Blackshaw and Stewart 1992; Blackshaw 1995). Too few numbers of *O. cyaneum* were recovered in this study to draw any meaningful conclusions.

Another aspect that should be considered is the effects of the manipulation process on *A. triangulatus* behaviour. Flatworms were collected each week, pooled and redistributed according to the experimental design. Any spatial population structuring would therefore be broken down. In common with most soil invertebrates, *A. triangulatus* demonstrates an aggregated spatial distribution, possibly related to soil moisture, refugia or the presence of earthworms (Boag et al. 1999, 2005, 2010a; Green and Murchie 2006). In addition, there is tentative evidence of interactions between flatworms. Green and Murchie (2006) found a negative relationship between *A. triangulatus* counts and mean weight, meaning that under shelter traps there were either few large flatworms or many small ones. However, any effects from the manipulation process on flatworms were perhaps more likely to decrease predation than enhance it, so the overall results from this experiment are unaffected. Earthworm spatial distribution too has a potential impact on the results, although this is accommodated to some measure through blocking. Earthworm dispersal should also be considered; as movement between treatments could confound the results. Eijsackers (2011) reviews earthworm colonisation and gives the annual dispersal rates of earthworms introduced into worm-free soils. In grassland these are 1.5–11 m per year depending on species. In established soils, these dispersal rates are likely to be much less and probably within the bounds of the plots (8 m by 8 m).

So, despite some compromises in the experiment design, this study provides clear evidence that *L. terrestris* and *Ap. longa* populations are at disproportionate risk from *A. triangulatus* predation. The danger with this scenario is that it could provide a mechanism for local extinction of these species. If *A. triangulatus* preyed equally on all earthworm species, then population dynamics theory would predict that an equilibrium between predator and prey densities would be attained, otherwise any predator population that overexploited its prey would itself decline. However, if some earthworm species are more susceptible than others, then there is the risk that *A. triangulatus* may severely deplete that species but sustain their numbers by feeding on less-susceptible prey. Furthermore, *A. triangulatus* is persistent in the environment because they can reabsorb tissues to sustain themselves during starvation; remaining reproductively capable after 4–6 months starvation (Baird et al. 2005a, b) and surviving for over 1 year in the laboratory (Blackshaw 1992).

The flatworm densities recorded in the high plots were on average less than 0.5 flatworms per m^2, although in individual plots they went up to 1.1 per m^2 (Fig. 6). Such densities are comparable with those found in agricultural systems. It should though be recognised that the traps within the experimental plots could alter flatworm densities in the surrounding soil, either positively by providing additional refugia or negatively by concentrating the flatworm population under traps. In a survey of grassland fields in Northern Ireland, Murchie et al. (2003) recovered flatworms at densities up to 0.8 flatworms per m^2, by formalin sampling field centres. In plots near to the location of the experiment, Blackshaw (1995) found naturally-occurring populations of *A. triangulatus* up to 6 per m^2, and in untreated orchard plots, flatworm densities derived from mustard expulsion were 9.3 per m^2 (Murchie and Mac An tSaoir 2006). The highest *A. triangulatus* densities, of 110 per m^2, were recorded in the Faroe Islands, where flatworms accumulated under overturned turf in the 'Reimavelta' method of potato cultivation (Christensen and Mather 1998). Extrapolating from laboratory flatworm feeding rates, Blackshaw (1991) suggested that a flatworm population of 6.5 per m^2 could devour an average UK grassland earthworm population within 1 year. The results from the linear regressions of flatworm density versus earthworm counts/biomass in this study, suggest that adult anecic earthworm populations will be almost wholly depleted at flatworm densities above 1 per m^2, as sampled by mustard expulsion, or consistent counts of above 2 flatworms per shelter trap (360 × 240 mm). A simple comparison between the 'high' and 'low' plots in this experiment suggests that enhancing flatworm density led to a 20 % reduction in earthworm biomass (total earthworm biomass recovered from high plots = 1,799 g, and the low = 2,261 g). Since the majority of this loss in earthworm biomass will be anecic species, it is worthwhile to consider again the regression model. Here, a value of 0.8 flatworms per m^2 (as found in natural field infestations) gives a predicted adult anecic biomass

(±SE) of 1.54 ± 0.746 g; whereas at zero flatworms the prediction is 5.87 ± 0.616 g. This gives a mean reduction of 74 % with 95 % confidence limits equivalent to a 33 and 100 % reduction. Given that 26 % of earthworm biomass in this study was anecic species, this calculation gives an overall reduction in earthworm biomass of 19 % (74 % × 26 %), which is very close to the 20 % calculated directly from the plot biomass weights.

The wider implications of flatworm predation can be considered in the light of these results, albeit with the proviso that the earthworm population response to flatworm predation is unlikely to be truly linear. Nevertheless, an overall earthworm biomass reduction of 20 % and a reduction in anecic biomass of 74 % provide useful starting points. Where *A. triangulatus* is common, agriculture is often pasture-based: for example, in Northern Ireland 78 % of the farmed area is under grass (DARD 2011). The positive influence of earthworms on plant growth has been widely reported in the scientific literature (Lee 1985; Edwards and Bohlen 1996; Scheu 2003), yet the contribution of earthworms to grass yield in managed systems is difficult to estimate. Much information comes from microcosm based studies (e.g. Boyle et al. 1997) and the introductions of lumbricid earthworms to Australasian pastures or Dutch polder soils (e.g. Stockdill 1982; Hoogerkamp et al. 1983; Baker et al. 1999, 2006). On the other hand, no reduction in grass yield was recorded after 20 years absence of earthworms due to phorate treatment, although there was degradation of soil structure including a considerable build-up of thatch on the soil surface (Clements et al. 1990, 1991). A similar accumulation of thatch was seen in fields and orchard plots infested with *A. triangulatus* (Blackshaw 1995; Murchie and Mac An tSaoir 2006).

Reports of damage to plants are as yet largely anecdotal. There are suggestions that fields infested with *A. triangulatus* have poorer soil drainage and are more susceptible to waterlogging and colonisation by *Juncus* rushes (Alford 1998; Jones et al. 2001). It is considered that surface waterlogging could both reduce oxygen diffusion rates inhibiting root growth and increase run-off, potentially exacerbating flooding and surface water pollution (Haria 1995). However, in comparisons with flatworm-infested and non-infested areas of the same field, Haria et al. (1998) found that *A. triangulatus* presence increased saturated hydraulic conductivity. They put this down to *A. triangulatus* predation removing earthworms from burrows and thus allowing water to move more freely. The authors commented though that in the long-term hydraulic conductivity would decline as earthworm created macropores collapsed and were not replaced.

Earthworms are important prey items for many farmland birds, mammals and invertebrates, and it is probable that *A. triangulatus* predation has knock-on effects on these other predators (Alford et al. 1995; Alford 1998). Data on such impacts are lacking, with one exception. In two Scottish glens, there was a significant negative relationship between the presence of *A. triangulatus* and moles (*Talpa europaea*; Boag 2000). Earthworms, in particular *L. terrestris*, are the major food source for moles (Mellanby 1971) and their distribution and abundance are dependent on earthworm availability (Funmilayo 1977). Similarly, in a review of vertebrate predation on earthworms, *L. terrestris* is cited as an important food source for red foxes (*Vulpes vulpes*) and especially European badgers (*Meles meles*), because of their comparatively large size and surface activity (MacDonald 1983). Much work has shown that *L. terrestris* is a major component of badgers' diets (Kruuk 1978; Kruuk et al. 1979; Kruuk and Parish 1981; Neal 1988), although the extent to which badgers rely on earthworms varies between regions (Goszczyński et al. 2000), and in Ireland earthworms contributed little to dietary bulk (Cleary et al. 2009). What is clear though is that *A. triangulatus* predation will reduce *L. terrestris* availability to badgers. If *L. terrestris* is a major food source in that area, there is the possibility that this will alter badger foraging behaviour, with implications for management of bovine tuberculosis (Tolhurst et al. 2009).

In conclusion, this study supports the contention of Fraser and Boag (1998) that *L. terrestris* in particular is most at risk from *A. triangulatus* predation. The impact of this on grassland productivity and wildlife is likely to be chronic and insidious. Management options for *A. triangulatus* are limited (Blackshaw 1996), but it may be that earthworm prey adapt to *A. triangulatus* predation, either behaviourally or physiologically. The *L. terrestris* found at the experimental site were markedly smaller than those reared in laboratory cultures. There are many factors that could influence this (e.g. food availability) but it is possible that this could be a response to *A. triangulatus* predation. Darwin (1859) remarked on the inability of New Zealand species to establish in Great Britain, in comparison with the success of European introductions into New Zealand. *A. triangulatus* is a counter to this. Boag et al. (2010b) pointed out that Europe has a depauperate fauna of terrestrial flatworms and that all North American species are exotic introductions; which compares to extensive fauna in the southern hemisphere. New Zealand, for example, has over 100 species of terrestrial flatworm (Johns 1998). The implication is that there is a readily available niche for southern terrestrial flatworms to exploit in Europe and North America (Boag et al. 2010b). The spread of *Bipalium* spp. across America would seem to support this (Winsor 1983; Ducey et al. 2005, 2007), perhaps facilitated by the spread of *L. terrestris* as suitable prey, which ironically is a problematic invasive species in North America itself (Heimpel et al. 2010). At present *A. triangulatus* has a restricted range to the British Isles and Faroe Islands. Why it has not established in continental Europe is puzzling and is either to do with trade or climatic conditions (Murchie 2010). The effects of climate change, particularly milder wetter winters, may well alter this.

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References

Alford DV (1998) Potential problems posed by non-indigenous terrestrial flatworms in the United Kingdom. Pedobiologia 42(5–6):574–578

Alford DV, Hancocks PJ, Parker WE (1995) The potential impact of New Zealand flatworm (*Artioposthia triangulata*) on agriculture and the environment in England and Wales. Project Number OCS 9323. Ministry of Agriculture, Fisheries and Food, Chief Scientists Group, Cambridge, UK

Anonymous (1964) Annual progress report on research and technical work. Agricultural Entomology Division, Ministry of Agriculture, Belfast

Baird J, Fairweather I, Murchie AK (2005a) Long-term effects of prey-availability, partnering and temperature on overall egg capsule output of 'New Zealand flatworms', *Arthurdendyus triangulatus*. Ann App Biol 146(3):289–301. doi:10.1111/j.1744-7348.2005.040095.x

Baird J, McDowell SDR, Fairweather I, Murchie AK (2005b) Reproductive structures of *Arthurdendyus triangulatus* (Dendy): seasonality and the effect of starvation. Pedobiologia 49(5):435–442. doi:10.1016/j.pedobi.2005.05.003

Baker GH, Carter PJ, Barrett VJ (1999) Influence of earthworms, *Aporrectodea* spp. (Lumbricidae), on pasture production in south-eastern Australia. Aust J Agric Res 50(7):1247–1257. doi:10.1071/AR98182

Baker GH, Brown G, Butt K, Curry JP, Scullion J (2006) Introduced earthworms in agricultural and reclaimed land: their ecology and influences on soil properties, plant production and other soil biota. Biol Invasions 8(6):1301–1316. doi:10.1007/s10530-006-9024-6

Bartlett MD, Briones MJI, Neilson R, Schmidt O, Spurgeon D, Creamer RE (2010) A critical review of current methods in earthworm ecology: from individuals to populations. Eur J Soil Biol 46(2):67–73. doi:10.1016/j.ejsobi.2009.11.006

Bengtson SA, Nilsson A, Nordström S, Rundgren S (1976) Effect of bird predation on lumbricid populations. Oikos 27(1):9–12. doi:10.2307/3543424

Bengtson SA, Rundgren S, Nilsson A, Nordström S (1978) Selective predation on lumbricids by golden plover *Pluvialis apricaria*. Oikos 31(2):164–168. doi:10.2307/3543559

Blackshaw RP (1989) The effects of a calcareous seaweed product on earthworm populations in grassland soil. Biol Agric Hortic 6(1):27–33

Blackshaw RP (1990) Studies on *Artioposthia triangulata* (Dendy) (Tricladida, Terricola), a predator of earthworms. Ann App Biol 116(1):169–176. doi:10.1111/j.1744-7348.1990.tb06596.x

Blackshaw RP (1991) Mortality of the earthworm *Eisenia fetida* (Savigny) presented to the terrestrial planarian *Artioposthia triangulata* (Dendy) (Tricladida, Terricola). Ann App Biol 118(3):689–694. doi:10.1111/j.1744-7348.1991.tb05358.x

Blackshaw RP (1992) The effect of starvation on size and survival of the terrestrial planarian *Artioposthia triangulata* (Dendy) (Tricladida, Terricola). Ann App Biol 120(3):573–578. doi:10.1111/j.1744-7348.1992.tb04917.x

Blackshaw RP (1995) Changes in populations of the predatory flatworm *Artioposthia triangulata* and its earthworm prey in grassland. Acta Zool Fenn 196:107–110

Blackshaw RP (1996) Control options for the New Zealand flatworm. In: Brighton crop protection conference, pests & diseases, Brighton, UK, 18–21 November 1996. BCPC, pp 1089–1094

Blackshaw RP, Stewart VI (1992) *Artioposthia triangulata* (Dendy, 1894), a predatory terrestrial planarian and its potential impact on lumbricid earthworms. Agric Zool Rev 5:201–219

Blackshaw RP, Moore JP, Alston R (1996) Removal trapping to control *Artioposthia triangulata*. Ann App Biol 129(2): 355–360. doi:10.1111/j.1744-7348.1996.tb05758.x

Bloch D (1992) A note on the occurrence of land planarians in the Faroe Islands. Fródskaparrit 38:63–68

Boag B (2000) The impact of the New Zealand flatworm on earthworms and moles in agricultural land in western Scotland. Asp Appl Biol 62:79–84

Boag B, Yeates GW (2001) The potential impact of the New Zealand flatworm, a predator of earthworms, in Western Europe. Ecol Appl 11(5):1276–1286. doi:10.2307/3060919

Boag B, Palmer LF, Neilson R, Chambers SJ (1994) Distribution and prevalence of the predatory planarian *Artioposthia triangulata* (Dendy) (Tricladida: Terricola) in Scotland. Ann App Biol 124(1):165–171. doi:10.1111/j.1744-7348.1994.tb04124.x

Boag B, Evans KA, Neilson R, Yeates GW, Johns PM, Mather JG, Christensen OM, Jones HD (1995) The potential spread of terrestrial planarians *Artioposthia triangulata* and *Australoplana sanguinea* var. *alba* to continental Europe. Ann App Biol 127(2):385–390. doi:10.1111/j.1744-7348.1995.tb06682.x

Boag B, Palmer LF, Neilson R, Legg R, Chambers SJ (1997) Distribution, prevalence and intensity of earthworm populations in arable land and grassland in Scotland. Ann App Biol 130(1):153–165. doi:10.1111/j.1744-7348.1997.tb05791.x

Boag B, Yeates GW, Johns PM (1998) Limitations to the distribution and spread of terrestrial flatworms with special reference to the New Zealand flatworm (*Artioposthia triangulata*). Pedobiologia 42(5–6):495–503

Boag B, Jones HD, Neilson R, Santoro G (1999) Spatial distribution and relationship between the New Zealand flatworm *Arthurdendyus triangulata* and earthworms in a grass field in Scotland. Pedobiologia 43(4):340–344

Boag B, Deeks L, Orr A, Neilson R (2005) A spatio-temporal analysis of a New Zealand flatworm (*Arthurdendyus triangulatus*) population in western Scotland. Ann App Biol 147(1):81–88. doi:10.1111/j.1744-7348.2005.00017.x

Boag B, Mackenzie K, McNicol JW, Neilson R (2010a) Sampling for the New Zealand flatworm. Proc Crop Prot North Brit 2010:45–50

Boag B, Neilson R, Jones HD (2010b) Quantifying the risk to biodiversity by alien terrestrial planarians. Asp Appl Biol 104:55–61

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24(3):127–135. doi:10.1016/j.tree.2008.10.008

Bouché MB (1971) Relations entre les structures spatiales et fonctionelles des ecosystems, illustrées par le role pédobiologique des vers de terre. In: Pesson P (ed) La Vie dans les Sols, Aspects Nouveaux, Études Experimentales Gauthier-Villars, Paris, pp 187–209

Bouché MB (1977) Strategies lombriciennes. In: Lohm U, Persson T (eds) Soil organisms as components of the ecosystem, vol 25. Ecological bulletins (Stockholm). Swedish Natural Science Research Council, Stockholm, pp 122–132

Boyle KE, Curry JP, Farrell EP (1997) Influence of earthworms on soil properties and grass production in reclaimed cutover peat. Biol Fertil Soils 25(1):20–26. doi:10.1007/s003740050274

Butt KR (1993) Reproduction and growth of three deep-burrowing earthworms (Lumbricidae) in laboratory culture in order to assess production for soil restoration. Biol Fertil Soils 16(2):135–138. doi:10.1007/bf00369415

Butt KR (2011) Food quality affects production of *Lumbricus terrestris* (L.) under controlled environmental conditions. Soil Biol Biochem 43(10):2169–2175. doi:10.1016/j.soilbio.2011.06.021

Cannon RJC, Baker RHA, Taylor MC, Moore JP (1999) A review of the status of the New Zealand flatworm in the UK. Ann App Biol 135(3):597–614. doi:10.1111/j.1744-7348.1999.tb00892.x

Chan K-Y, Munro K (2001) Evaluating mustard extracts for earthworm sampling. Pedobiologia 45(3):272–278

Christensen OM, Mather JG (1998) The 'New Zealand flatworm', *Artioposthia triangulata*, in Europe: the Faroese situation. Pedobiologia 42(5–6):532–540

Cleary GP, Corner LAL, O'Keeffe J, Marples NM (2009) The diet of the badger *Meles meles* in the Republic of Ireland. Mamm Biol 74(6):438–447. doi:10.1016/j.mambio.2009.07.003

Clements RO, Murray PJ, Bentley BR, Henderson IF (1990) Herbage yield and botanical composition over 20 years of a predominantly ryegrass sward treated frequently with phorate pesticide and 3 rates of nitrogen-fertilizer. J Agric Sci 115:23–27. doi:10.1017/S0021859600073871

Clements RO, Murray PJ, Sturdy RG (1991) The impact of 20 years' absence of earthworms and three levels of N fertiliser on a grassland soil environment. Agric Ecosyst Environ 36(1–2):75–85. doi:10.1016/0167-8809(91)90037-X

DARD (2011) Statistical review of Northern Ireland agriculture 2010. Department of Agriculture and Rural Development, Policy and Economics Division, Belfast

Darwin CR (1859) On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London

Ducey PK, West L-J, Shaw G, De Lisle J (2005) Reproductive ecology and evolution in the invasive terrestrial planarian *Bipalium adventitium* across North America. Pedobiologia 49(4):367–377. doi:10.1016/j.pedobi.2005.04.002

Ducey PK, McCormick M, Davidson E (2007) Natural history observations on *Bipalium* cf. *vagum* Jones and Sterrer (Platyhelminthes: Tricladida), a terrestrial broadhead planarian new to North America. Southeast Nat 6(3):449–460. doi:10.1656/1528-7092(2007)6[449:nhoobc]2.0.co;2

Dynes C, Fleming CC, Murchie AK (2001) Genetic variation in native and introduced populations of the 'New Zealand flatworm', *Arthurdendyus triangulatus*. Ann App Biol 139(2):165–174. doi:10.1111/j.1744-7348.2001.tb00393.x

Edwards CA, Bohlen PJ (1996) Biology and ecology of earthworms, 3rd edn. Chapman & Hall, London

Eijsackers H (2011) Earthworms as colonizers of natural and cultivated soil environments. Appl Soil Ecol 50:1–13. doi:10.1016/j.apsoil.2011.07.008

Evans AC, Guild WJ (1948) Studies on the relationships between earthworms and soil fertility. IV. On the life cycles of some British Lumbricidae. Ann App Biol 35(4): 471–484

Fraser PM, Boag B (1998) The distribution of lumbricid earthworm communities in relation to flatworms: a comparison between New Zealand and Europe. Pedobiologia 42(5–6):542–553

Funmilayo O (1977) Distribution and abundance of moles (*Talpa europaea* L.) in relation to physical habitat and food supply. Oecologia 30(3):277–283. doi:10.1007/bf01833635

Gibson PH, Cosens D, Buchanan K (1997) A chance field observation and pilot laboratory studies of predation of the New Zealand flatworm by the larvae and adults of carabid and staphylinid beetles. Ann App Biol 130(3):581–585. doi:10.1111/j.1744-7348.1997.tb07684.x

Goszczyński J, Jedrzejewska B, Jedrzejewski W (2000) Diet composition of badgers (*Meles meles*) in a pristine forest and rural habitats of Poland compared to other European populations. J Zool 250(4):495–505. doi:10.1111/j.1469-7998.2000.tb00792.x

Green NC, Murchie AK (2006) In-field distributions of the New Zealand flatworm, *Arthurdendyus triangulatus* (Dendy). Proc Crop Prot North Brit 2006:57–62

Gunn A (1992) The use of mustard to estimate earthworm populations. Pedobiologia 36(2):65–67

Haria AH (1995) Hydrological and environmental impact of earthworm depletion by the New Zealand flatworm (*Artioposthia triangulata*). J Hydrol 171(1–2):1–3. doi:10.1016/0022-1694(95)02734-7

Haria AH, McGrath SP, Moore JP, Bell JP, Blackshaw RP (1998) Impact of the New Zealand flatworm (*Artioposthia triangulata*) on soil structure and hydrology in the UK. Sci Total Environ 215(3):259–265. doi:10.1016/s0048-9697(98)00126-0

Heimpel G, Frelich L, Landis D, Hopper K, Hoelmer K, Sezen Z, Asplen M, Wu K (2010) European buckthorn and Asian soybean aphid as components of an extensive invasional meltdown in North America. Biol Invasions 12(9):2913–2931. doi:10.1007/s10530-010-9736-5

Hoogerkamp M, Rogaar H, Eijsackers HJP (1983) Effects of earthworms on grassland on recently reclaimed polder soils in the Netherlands. In: Satchell JE (ed) Earthworm ecology: from Darwin to vermiculture. Chapman and Hall, London, pp 85–105

Johns PM (1998) The New Zealand terrestrial flatworms: a 1997–1998 perspective. Pedobiologia 42(5–6):464–468

Johns PM, Boag B (2003) The spread and distribution of terrestrial planarians (Turbellaria: Tricladida: Geoplanidae) within New Zealand. N Z J Ecol 27(2):201–206

Johns PM, Boag B, Yeates GW (1998) Observations on the geographic distribution of flatworms (Turbellaria: Rhynchodemidae, Bipaliidae, Geoplanidae) in New Zealand. Pedobiologia 42(5–6):469–476

Jones HD, Boag B (1996) The distribution of New Zealand and Australian terrestrial flatworms (Platyhelminthes: Turbellaria: Tricladida: Terricola) in the British Isles—the Scottish survey and MEGALAB WORMS. J Nat Hist 30(7):955–975. doi:10.1080/00222939600770511

Jones HD, Santoro G, Boag B, Neilson R (2001) The diversity of earthworms in 200 Scottish fields and the possible effect of New Zealand land flatworms (*Arthurdendyus triangulatus*) on earthworm populations. Ann App Biol 139(1):75–92. doi:10.1111/j.1744-7348.2001.tb00132.x

Kruuk H (1978) Foraging and spatial organisation of the European badger, *Meles meles* L. Behav Ecol Sociobiol 4(1):75–89. doi:10.1007/bf00302562

Kruuk H, Parish T (1981) Feeding specialization of the European Badger *Meles meles* in Scotland. J Anim Ecol 50(3):773–788. doi:10.2307/4136

Kruuk H, Parish T, Brown CAJ, Carrera J (1979) The use of pasture by the European badger (*Meles meles*). J Appl Ecol 16(2):453–459. doi:10.2307/2402521

Lawrence AP, Bowers MA (2002) A test of the 'hot' mustard extraction method of sampling earthworms. Soil Biol Biochem 34(4):549–552. doi:10.1016/s0038-0717(01)00211-5

Lee KE (1985) Earthworms, their ecology and relationships with soils and land use. Academic Press, London

Lillico S, Cosens D, Gibson P (1996) Studies of the behaviour of *Artioposthia triangulata* (Platyhelminthes; Tricladida), a predator of earthworms. J Zool 238(3):513–520

Lowe CN, Butt KR (2002a) Growth of hatchling earthworms in the presence of adults: interactions in laboratory culture. Biol Fertil Soils 35(3):204–209. doi:10.1007/s00374-002-0471-7

Lowe CN, Butt KR (2002b) Influence of organic matter on earthworm production and behaviour: a laboratory-based approach with applications for soil restoration. Eur J Soil Biol 38(2):173–176. doi:10.1016/s1164-5563(02)01141-x

MacDonald DW (1983) Predation on earthworms by terrestrial vertebrates. In: Satchell JE (ed) Earthworm ecology, from Darwin to vermiculture. Chapman and Hall, London, pp 393–414

Mellanby K (1971) The mole. The new naturalist series. Collins, London

Moore JP, Dynes C, Murchie AK (1998) Status and public perception of the 'New Zealand flatworm', *Artioposthia triangulata* (Dendy), in Northern Ireland. Pedobiologia 42(5–6):563–571

Moran MD (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100(2):403–405. doi:10.1034/j.1600-0706.2003.12010.x

Murchie AK (2010) Between two stools: dealing with the problem of the New Zealand flatworm. Asp Appl Biol 104:73–78

Murchie AK, Mac An tSaoir S (2006) High densities of 'New Zealand flatworms', *Arthurdenyus triangulatus* (Dendy), in experimental orchard plots in Northern Ireland and implications for thatch formation. Tearmann 5:23–28

Murchie AK, Moore JP, Walters KFA, Blackshaw RP (2003) Invasion of agricultural land by the earthworm predator, *Arthurdendyus triangulatus* (Dendy). Pedobiologia 47(5–6):920–923

Neal E (1988) The stomach contents of badgers, *Meles meles*. J Zool 215(2):367–369. doi:10.1111/j.1469-7998.1988.tb04904.x

Satchell JE (1967) Lumbricidae. In: Burges A, Raw F (eds) Soil biology. Academic Press, London, pp 259–322

Scheu S (2003) Effects of earthworms on plant growth: patterns and perspectives. Pedobiologia 47(5–6):846–856. doi:10.1078/0031-4056-00270

Sherlock E, Carpenter D (2009) An updated earthworm list for the British Isles and two new 'exotic' species to Britain from Kew Gardens. Eur J Soil Biol 45(5–6):431–435. doi:10.1016/j.ejsobi.2009.07.002

Shuster WD, Subler S, McCoy EL (2001) Deep-burrowing earthworm additions changed the distribution of soil organic carbon in a chisel-tilled soil. Soil Biol Biochem 33(7–8):983–996. doi:10.1016/S0038-0717(01)00002-5

Sims RW, Gerard BM (1999) Earthworms. Notes for the Identification of British Species, vol 31 (Revised). Synopses of the British Fauna (New Series). Field Studies Council, Shrewsbury

Stewart VI (1993) The biology of the terrestrial planarian *Artioposthia triangulata* (Dendy, 1894) and its Genetic Variation in Colonized Habitats. PhD Dissertation, The Queen's University of Belfast, Belfast

Stockdill SMJ (1982) Effects of introduced earthworms on the productivity of New Zealand pastures. Pedobiologia 24(1):29–35

Tolhurst BA, Delahay RJ, Walker NJ, Ward AI, Roper TJ (2009) Behaviour of badgers (*Meles meles*) in farm buildings: opportunities for the transmission of *Mycobacterium bovis* to cattle? Appl Anim Behav Sci 117(1–2):103–113. doi:10.1016/j.applanim.2008.10.009

Uvarov AV (2009) Inter- and intraspecific interactions in lumbricid earthworms: their role for earthworm performance and ecosystem functioning. Pedobiologia 53(1):1–27. doi:10.1016/j.pedobi.2009.05.001

Willis RJ, Edwards AR (1977) The occurrence of the land planarian *Artioposthia triangulata* (Dendy) in Northern Ireland. Irish Nat J 19(4):112–116

Winsor L (1983) A revision of the cosmopolitan land planarian *Bipalium kewense* Moseley, 1878 (Turbellaria, Tricladida—Terricola). Zool J Linn Soc-Lond 79(1):61–100. doi:10.1111/j.1096-3642.1983.tb01161.x