University of South Bohemia in České Budějovice
Faculty of Science
Department of Zoology

RNDr. Thesis

A NEW METHOD FOR MARKING SLUGS BY UV-FLUORESCENT DYE

Mgr. Pavel Foltan

Supervisor: Mgr. Martin Konvička, Ph.D.
Consultant: doc. RNDr. Oleg Ditrich, CSc.

České Budějovice 2009

FOLTAN P, 2009. A new method for marking slugs by UV-fluorescent dye, – 9 pp. University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic.

Annotation:

A new method for cheap, fast and reliable slug marking, using biologically inert UV-fluorescent dye is presented. The dye remained visible for more than 3 months in all individuals marked. Use of the dye did not affect survival, spatial behaviour or daily activity of the slugs, nor the prey choice of the predatory beetle Pterostichus melanarius, under controlled semi-natural conditions. The new method will enable further investigations of absolute slug densities, diurnal activity, mobility and dispersal, using capture-recapture techniques. The resulting data would allow the construction of more exact forecasting models for the population dynamics of pest slugs, potentially leading to a reduction in the use of control agents, particularly chemical molluscicides.

Financial support:

The study was supported by Ministry of Education of the Czech Republic (MSMT 6007665801) and by the Grant Agency of the Czech Republic (GAČR) no. 526/09/1249.

Autor Declaration/prohlášení autora:

I hereby declare that I worked out this RNDr. thesis on my own, or in collaboration with the co-authors of the presented papers and manuscripts, usány only the cited literature.

I declare that in accordance with the § 47b of Act No. 111/1998 Coll. in its valid version, I agrese with the publication of my RNDr. thesis (in an edition made by removing marked parts archived by the Faculty of Science) in an electronic way in the public access to the STAG database run by the University of South Bohemia in České Budějovice on its web pages.

Prohlašuji, že jsem svoji rigorózní práci vypracoval samostatně nebo ve spolupráci se spoluautory přiložených prací, pouze s použitím citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své rigorózní práce (v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou) elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách.

V Českých Budějovicích dne 7.12.2009 .....

Pavel Foltan

Acknowledgements

I would like to thank Frantisek Sedlacek for providing UV-fluorescent dye, Simon Shayler for correcting the language, and Oldrich Cizek for a fruitful discussions. Thanks are also due to the Institute of Entomology for allowing use of their controlled-environment room, to the Czech Ministry of Education (MSM6007665801) for funding, Martin Konvička for guidance and to an anonymous referee for many helpful improvements to the manuscript.

A NEW METHOD FOR MARKING SLUGS BY ULTRAVIOLET-FLUORESCENT DYE

PAVEL FOLTAN[1,2] AND MARTIN KONVICKA[1,3]

[1]*Faculty of Biological Sciences, University of South Bohemia, Branisovska 31, CZ-37005 Ceske Budejovice, Czech Republic;*
[2]*I2L research Ltd, Capital Business Park, Wentloog, Cardiff CF3 2PX, UK;*
[3]*Institute of Entomology, Czech Academy of Sciences, Branisovska 31, CZ-37005 Ceske Budejovice, Czech Republic*

(Received 20 June 2007; accepted 24 November 2007)

ABSTRACT

We introduce a new, cheap, fast and reliable method for marking slugs, using biologically inert UV-fluorescent dye. The dye remained visible for more than 3 months in all individuals marked. Use of the dye did not affect survival, spatial behaviour or daily activity of the slugs, nor the prey choice of the predatory beetle *Pterostichus melanarius*, under controlled semi-natural conditions. The new method will enable further investigations of absolute slug densities, diurnal activity, mobility and dispersal, using capture-recapture techniques. The resulting data would allow the construction of more exact forecasting models for the population dynamics of pest slugs, potentially leading to a reduction in the use of control agents, particularly chemical molluscicides.

INTRODUCTION

Slugs are important pests that attack a wide range of agricultural and horticultural crops (Godan, 1983; Port & Port, 1986; Glen, 1989; Willis *et al*., 2006). Slug control is typically achieved by treating crops with biologically effective quantities of molluscicides (containing either metaldehyde, methiocarb or iron phosphate) or more recently by pathogenic nematodes (Ester & Wilson, 2005). Only the construction of reliable forecasting models for slug population dynamics can facilitate successful biological and integrated control of the slug pests and reduce common mass usage of molluscicides as a precautionary measure, rather than their prophylactic release (Bohan *et al*., 1997). In the studies of slug spatial and population dynamics (Barker, 1991; Symondson *et al*., 1996, 2002; Bohan *et al*., 1997, 2000; Shirley *et al*., 2001; Schley & Bees, 2002, 2003; Choi *et al*., 2004, 2006; Willis *et al*., 2006) the basic data originate mainly from either laboratory experiments, including gut-content analyses of slug predators, or from different sampling and trapping surveys, of which soil sampling and flooding techniques (Glen, Wiltshire & Milsom, 1992) have recently become the most commonly used. Slugs are usually strongly spatially aggregated (Bohan *et al*., 2000), and thus estimating slug density, dispersal or other population characteristics from randomized sampling in the field introduces important bias into the data.

Until now, studies of slug population dynamics, as well as studies of treatment effects on pest populations, have suffered a serious limitation resulting from the lack of reliable methods of individual marking for mark-recapture studies. Mark-recapture methods have undergone remarkable progress during the last 15 years, owing to the introduction of regression-based analytical techniques (Lebreton *et al*., 1992; Schwarz & Seber, 1999; Brooks, Catchpole & Morgan, 2000) and the development of appropriate software (e.g. White & Burnham, 1999). These developments now allow more flexible and reliable model selection and hence more detailed analyses of survival rate, capture probability, recruitment and movement probabilities, including testing the influences of external effects on animal demography parameters.

Correspondence: P. Foltan; e-mail: pavel_foltan@yahoo.co.uk

Crucial prerequisites of reliable marking are equal survival of marked and unmarked individuals, easy readability and no loss of marks, as well as quick and easy execution of marking and low cost (Petersen, 1896; Krebs, 1989; Schwarz & Seber, 1999; Williams, Nichols & Conroy, 2001). None of the methods used for slugs have so far met all these criteria. Richter (1976) marked slugs using liquid nitrogen. The mortality of treated animals was about 30% (Hogan & Steele, 1986) and the method was recommended for marking adult individuals only, as juveniles had to be remarked due to their rapid growth. Müller & Ohensorge (1985) applied methylene blue, but found this to be ineffective as the duration of the dye was only 6 weeks. Hogan & Steele (1986) used a Panjet dental inoculator for dye-marking of *Deroceras reticulatum* in the laboratory, but the marking of dark individuals proved very problematic. Moreover, in the field experiment, the slugs had to be intensively examined to reveal whether they were marked or not. Finally, Grimm (1996) proposed the use of magnetic transponders for individual marking of large arionid slugs. Advantages of her method included high number of different codes, long durability and reliable readability. However, the method is extremely expensive for mass usage and cannot be used for smaller species such as *D. reticulatum* (O. F. Müller, 1774), one of the most economically important slug pests in temperate climates (South, 1992).

In our study, we introduce a new, cheap, fast and reliable method for marking slugs, using biologically inert UV-fluorescent dye. We report on three experiments demonstrating that the marking technique does not influence survival, prey choice between marked slugs and unmarked controls by a generalized slug predator, the beetle *Pterostichus melanarius* (Illiger, 1798), or spatial behaviour or daily activity of marked slugs, under controlled semi-natural conditions.

METHODS

Sample localities, collection and experimental preparations

Adult slugs *Deroceras reticulatum*, *Arion vulgaris* Moquin-Tandon, 1855 (= *lusitanicus* auct. non Mabille, 1868) and *Limax cinereoniger* (Wolf, 1803), as well as *Pterostichus melanarius* beetles, were collected from arable fields around Ceske Budejovice, Czech Republic (49°02′N, 14°30′E). Both slugs and beetles

Journal of Molluscan Studies (2008) ***74****: 293–297. Advance Access Publication: 22 April 2008* *doi:10.1093/mollus/eym012*

were housed individually in clear plastic containers (8 × 10 × 5 cm deep) half filled with moist soil substrate (Aros Ltd) and maintained in a controlled environment (light:dark 12:12; 20 ± 1°C) prior to the experiment. The beetles were fed *ad libitum* on a diet of mixed pork and beef and then starved for 7 days, to ensure the same nutritional state in all individuals. The slugs were fed on fresh vegetables, also *ad libitum*.

UV Fluorescent dye

A 10% liquid solution of biologically inert powder Radglo® JST UV-fluorescent pigment (Radiant colour N.V.) was created by dilution in polyethylene glycol (av. mol. wt.: 400; Sigma–Aldrich). The solution was subsequently diluted 1:1 in distilled water.

Labelling

The dye was injected directly under the mantle using an insulin syringe at a very low angle to prevent injury to internal organs. To simplify manipulation, the slugs were cooled at 4°C for 20 min before dye application and held in a dry paper napkin during the application procedure. Injection into non-cooled individuals was also tested, but the manipulation was much complicated due to slugs rapidly escaping from the touch of the needle. When illuminated, using a mobile UV-lamp in the dark, the dye shone prominently through the mantle in the all marked individuals.

Deroceras reticulatum slugs were marked by an injection of 10 μl of the dye into the tail region. The larger species, *A. vulgaris* and *L. cinereoniger*, were injected twice into the mantle shield, twice into the tail region and twice into the middle of the foot (20 μl per single injection, six injections per individual). When illuminated by UV, all marking points were clearly visible.

Experiment 1: Does UV marking affect slug survival?

Twenty individuals of each slug species were marked. A further 20 individuals of each species were used as an untreated control. The slugs were continually housed individually in plastic containers half-filled with moist soil and fed on fresh vegetables, as described above. Numbers of surviving *L. cinereoniger* and *A. vulgaris* slugs were counted daily for 30 days, *D. reticulatum* for 3 weeks. Ten labelled *A. vulgaris* (having the lowest mortality in both the labelled and control groups) were then observed weekly for another 9 weeks to provide information on the stability of the dye in the slug body.

Experiment 2: Does UV marking influence defence abilities of the slug, or affect prey choice by a slug predator?

Experiments 2 and 3 were conducted under controlled conditions in mini-plots consisting of a glass container (40 × 60 × 40 cm deep) filled with a 10 cm layer of moist soil substrate and covered with mosquito netting on the top to prevent slugs from escaping. Barley seeds (approx. 500 per mini-plot) were inserted 1 cm deep into the soil and the barley was grown to a height of 10 cm before the experiment started.

Twenty *D. reticulatum* slugs were introduced into each of two containers for a 2-day period and allowed to become established and to locate possible refuges. Ten slugs were then removed from each container, labelled by the UV-dye and replaced 1 h before six *P. melanarius* beetles (3 males and 3 females) were introduced into each of the experimental containers. The experiment was then run for 7 days. At its termination, the beetles were removed and the surviving slugs collected from the surface and extracted from the soil by sieving.

Experiment 3: does UV marking influence daily activity or spatial behaviour of the slugs?

The experimental set-up is described under Experiment 2. Ten dye-labelled and ten control *D. reticulatum* were introduced into each of the two experimental containers. The number of labelled and control slugs apparent on the surface and vegetation or walls was counted after 1, 3, 6, 12, 24, 48, 72, 96, 120, 144 and 168 h using a mobile UV-lamp. The experiment was initiated 3 h after the start of the dark period (L:D 12:12; 20 ± 1°C). Thus, all observations, except the one after 12 h, were made during the dark period, when the maximum number of slugs can be expected to be active and feeding above the ground (Hommay, Lorvelec & Jacky, 1998; Grimm & Schaumberger, 2002).

RESULTS

Experiment 1

Numbers of surviving slugs were compared using Cox proportional hazard regressions for two groups (labelled *vs* not-labelled); the Cox–Mantel test was used to assess statistical significance.

Survival did not differ between the labelled and non-labelled individuals of all three slug species tested: *Deroceras reticulatum* ($P = 0.48$), *Limax cinereoniger* ($P = 0.69$) (Fig. 1). As 100% of *Arion vulgaris* survived in the both the labelled and control groups for the whole experimental period of 3 months, no statistical evaluation of their data was undertaken. The dye remained stable in 100% of surviving labelled individuals, with no signs of having been metabolized.

Experiment 2

Numbers of slugs surviving at the end of experiment, i.e. those that did not fall prey to the beetles, were compared using log-linear analysis. There was no difference in numbers of surviving/predated slugs between the experimental containers (partial/marginal $\chi^2 = 0.82/0.84$; partial/marginal $P = 0.36/0.36$) and between the UV-labelled/non-labelled individuals (partial/marginal $\chi^2 = 0.1/0.1$; partial/marginal $P = 0.76/0.76$).

Experiment 3

Repeated-measure ANOVA with repeated observations as 11 levels of dependent variable was used to compare the numbers of observed slugs with respect to slug position surface/vegetation and labelling.

As all slugs were placed on the surface at the beginning of the experiment and the slugs gradually became established and found refugia, numbers of observed slugs predictably declined in time (Fig. 2). Moreover, there was no difference in numbers of labelled and non-labelled individuals observed either on the surface or on the vegetation during the experimental period (Table 1, Fig. 2).

DISCUSSION

Previously developed marking techniques for slugs have several major disadvantages, such as low durability (Müller & Ohnesorge, 1985), poor readability of markings (Richter, 1976; Hogan & Steele, 1986), altered animal survival (Richter, 1976) or high cost (Grimm, 1996).

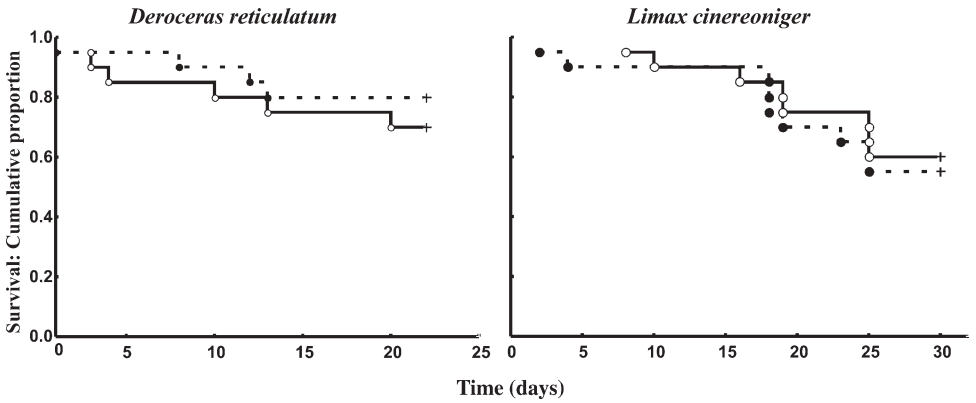

Figure 1. Survival of UV-fluorescent dye labelled (——) and non-labelled (------) *Deroceras reticulatum* and *Limax cinereoniger* slugs over a period of 25 or 30 days, respectively. The data for *Arion vulgaris* are not shown in the figure as 100% of the slugs survived for the whole experimental period.

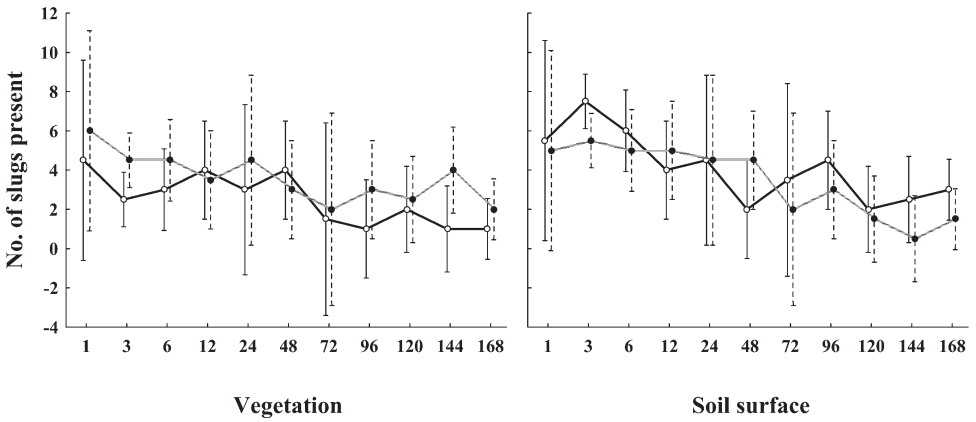

Figure 2. Number of UV-fluorescent-dye-labelled (——) and control slugs (------) apparent on the surface and vegetation/walls of two experimental containers, from the total number of 40 slugs added. The slugs were counted after 1, 3, 6, 12, 24, 48, 72, 96, 120, 144 and168 h by using mobile UV-lamp. Vertical bars denote 0.95 confidence intervals.

We have demonstrated that our cheap and fast marking technique using UV-fluorescent dye does not affect the survival of the three different slug species tested, including *Deroceras reticulatum* which cannot be marked using transponders because of its small body size. The pigment is non-toxic, safe and stable and has previously been used for tracking mammals (e.g. Lemen & Freeman, 1985) and frogs (Eggert, 2002) without causing any apparent harm. Nor does the minor injury caused by insertion of the needle seem to pose any threat to the slug's long-term survival, and the dye remained stable in all surviving individuals. Moreover, our marking technique did not increase susceptibility to predation by *Pterostichus melanarius*, which is probably because the UV-marking does not appear to affect the slugs' spatial behaviour or daily rhythms. Moreover, as there were six marking points present on larger *Limax cinereoniger* and *Arion vulgaris* slugs and there are eight recognizably different colours of dye produced, combining colour positions on the marking points would allow the individual marking of large numbers of individuals, at least in the larger species. However, it is important that new data on absolute densities obtained using our technique should be compared with the results of sampling using other techniques such as baiting (Duthoit, 1961; Webley, 1962; Bailey & Wedgewood, 1991) or trapping (Ökland, 1929; Purkhauser, 1991), in order to test its reliability.

As has previously been demonstrated by Grimm, Paill & Kaiser (2000), Grimm & Paill (2001) and Grimm (2001) for *A. vulgaris*, data on spatial position over time, and other aspects of behaviour of mark-recaptured slugs, can provide new insights into diurnal activity and resource use, hence opening a wide array of slug research possibilities so far largely

Table 1. Comparison of numbers of slugs observed with respect to slug position (surface/vegetation) and labelling by UV-fluorescent dye (repeated-measure ANOVA).

	SS	DF	MS	*F*	*P*
	1,022.73		1,022.73		<0.001
Intercept		1		79.65	
Marking: UV-labelled/not	1.14	1	1.137	0.09	0.78
Position: surface/ vegetation	11.64	1	11.64	0.91	0.39
Marking*Position	16.41	1	16.41	1.28	0.32
Error	51.36	4	12.84		
Time	131.02	10	13.10	8.94	<0.001
Time*Marking	3.114	10	0.31	0.21	0.99
Time*Position	26.61	10	2.66	1.82	0.09
Time*Position*Marking	29.34	10	2.93	2.0	0.06
Error	58.63	40	1.47		

Deroceras reticulatum slugs were introduced in two experimental containers. Number of UV-labelled and control slugs apparent on the surface and vegetation/walls was counted after 11 different time periods, from 1 to 168 h, using mobile UV-lamp. SS, sum of squares; MS, model sum of squares.

available only to those studying vertebrates (e.g. Ramsey, 2005) or insects (e.g. Nowicki *et al*., 2005).

The combination and comparison of existing and new methodologies could in turn permit the construction of more reliable forecasting models for slug populations, thus potentially leading to a reduction in the use any control agents, particularly chemical pesticides.

ACKNOWLEDGEMENTS

We would like to thank Frantisek Sedlacek for providing UV-fluorescent dye, Simon Shayler for correcting the language, and Oldrich Cizek for a fruitful discussions. Thanks are also due to the Institute of Entomology for allowing use of their controlled-environment room, to the Czech Ministry of Education (MSM6007665801) for funding, and to an anonymous referee for many helpful improvements to the manuscript.

REFERENCES

BAILEY, S.E.R. & WEDGWOOD, M.A. 1991. Complementary video and acoustic recordings of foraging by two pest species of slugs on non-toxic and molluscicidal baits. *Annals of Applied Biology*, **119**: 163–176.

BARKER, G.M. 1991. Biology of slugs (Agriolimacidae and Arionidae: Mollusca) in New Zealand hill country pastures. *Oecologia*, **85**: 581–595.

BOHAN, D.A., BRAIN, P., GLEN, D.M., WILTSHIRE, C.W., HUGHES, L., SHIRLEY, M. & PORT, G. 1997. Decision making in slug pest control: Slug population dynamics in space and time. *Aspects of Applied Biology*, **50**: 323–332.

BOHAN, D.A., BOHAN, A.C., GLEN, D.M., SYMONDSON, W.O.C., WILTSHIRE, C.W. & HUGHES, L. 2000. Spatial dynamics of predation by carabid beetles on slugs. *Journal of Animal Ecology*, **69**: 367–379.

BROOKS, S.P, CATCHPOLE, E.A. & MORGAN, B.J.T. 2000. Bayesian animal survival estimation. *Statistical Science*, **15**: 357–376.

CHOI, Y.H., BOHAN, D.A., POWERS, S.J., WILTSHIRE, C.W., GLEN, D.M. & SEMENOV, M.A. 2004. Modelling *Deroceras reticulatum* (Gastropoda) population dynamics based on daily temperature and rainfall. *Agriculture Ecosystems & Environment*, **103**: 519–525.

CHOI, Y.H., BOHAN, D.A., POTTING, R.P.J., SEMENOV, M.A. & GLEN, D.M. 2006. Individual based model of slug population and spatial dynamics. *Ecological Modelling*, **190**: 336–350.

DUTHOIT, C.M. 1961. Assessing the activity of the field slug in cereals. *Plant Pathology*, **10**: 165.

EGGERT, C. 2002. Use of fluorescent pigments and implantable transmitters to track a fossorial toad (*Pelobates fuscus*). *Herpetological Journal*, **12**: 69–74.

ESTER, A. & WILSON, M.J. 2005. Application of slug parasitic nematodes. In: *Nematodes as biological control agents* (Grewal, P.S., Ehlers, R.U. & Shapiro-Ilan, D. eds), 431–444. CABI Publishing, Wallingford.

GRIMM, B. 1996. A new method for individually marking slugs *Arion lusitanicus* (Mabille) by magnetic transponders. *Journal of Molluscan Studies*, **62**: 477–482.

GRIMM, B. 2001. Life-cycle and population density of the pest slug *Arion lusitanicus*, *Mabille* (Mollusca: Pulmonata) on grassland in Central Europe. *Malacologia*, **43**: 25–32.

GRIMM, B. & PAILL, W. 2001. Spatial distribution and home range of *Arion lusitancius*, Mabille in a grassland habitat. *Acta Oecologica*, **22**: 219–227.

GRIMM, B. & SCHAUMBERGER, K. 2002. Daily activity of the pest slug *Arion lusitanicus* under laboratory conditions. *Annals of Applied Biology*, **141**: 35–44.

GRIMM, B., PAILL, W. & KAISER, H. 2000. Daily activity of the pest slug *Arion lusitanicus* Mabille. *Journal of Molluscan Studies*, **66**: 125–130.

GLEN, D.M. 1989. Understanding and predicting slug problems in cereals. *Slugs and snails in world agriculture*. *BCPC Monograph*, **41**: 253–262.

GLEN, D.M., WILTSHIRE, C.W. & MILSOM, N.F. 1992. Some aspects of forecasting slug damage in arable crops. *Journal of Medical and Applied Malacology*, **4**: 147–152.

GODAN, D. 1983. *Pest slugs and snails*. Springer-Verlag, Berlin.

HOGAN, J.M. & STEELE, G.R. 1986. Dye-marking slugs. *Journal of Molluscan Studies*, **52**: 138–143.

HOMMAY, G., LORVELEC, O. & JACKY, F. 1998. Daily activity rhythm and use of shelter in the slugs *Deroceras reticulatum* and *Arion distinctus* under laboratory conditions. *Annals of Applied Biology*, **132**: 167–185.

KREBS, C.J. 1989. *Ecological methodology*. Harper Collins, New York.

LEBRETON, J.D., BURNAHAM, K.P., CLOBERT, J. & ANDERSON, D.R. 1992. Modeling survival and testing biological hypotheses using marked animals – a unified approach with case studies. *Ecological Monographs*, **62**: 67–118.

LEMEN, C.A. & FREEMAN, P.W. 1985. Tracking mammals with fluorescent pigments – a new technique. *Journal of Mammalogy*, **66**: 134–136.

MÜLLER, S.T. & OHNESORGE, B. 1985. Die Verwendung markierter Schnecken zur Populationsdichteabschstzung und zum Studium des Migrationsverhaltens von *Arion* sp. *Anzeiger fur Schädlingskunde und Pflanzenschutz*, **58**: 123–126.

NOWICKI, P., RICHTER, A., GLINKA, U., HOLZSCHUH, A., TOELKE, U., HENLE, K., WOYCIECHOWSKI, M. & SETTELE, J. 2005. Less input same output: simplified approach for population size assessment in Lepidoptera. *Population Ecology*, **47**: 203–212.

ÖKLAND, F. 1929. Quantitative Untersuchungen der Landschneckenfauna Norwegens. *Zeitschrift far Morphologie und Ökologie der Tiere*, **16**: 748–804.

PETERSEN, C.G.J. 1896. The yearly immigration of young plaice into the Limfjord from the German Sea. *Report of the Danish Biological Station*, **6**: 1–48.

PORT, C.M. & PORT, G.R. 1986. The biology and behaviour of slugs in relation to crop damage and control. *Agricultural Zoology Reviews*, **1**: 255–299.

PURKHAUSER, R. 1991. Testfolie zeigt rasch Schneckenbefall. *Der Pflanzenarzt*, **9–10**: 30.

RICHTER, K.O. 1976. A method for individually marking slugs. *Journal of Molluscan Studies*, **42**: 146–151.

RAMSEY, D. 2005. Population dynamics of brushtail possums subject to fertility control. *Journal of Applied Ecology*, **42**: 348–360.

SCHLEY, D. & BEES, M.A. 2002. A discrete slug population model determined by egg production. *Journal of Biological Systems*, **10**: 243–264.

SCHLEY, D. & BEES, M.A. 2003. Delay dynamics of the slug *Deroceras reticulatum*, an agricultural pest. *Ecological Modelling*, **162**: 177–198.

SCHWARZ, C.J. & SEBER, G.A.F. 1999. Estimating animal abundance: Review III. *Statistical Science*, **14**: 427–456.

SHIRLEY, M.D.F., RUSHTON, S.P., YOUNG, A.G. & PORT, G.R. 2001. Simulating the long-term dynamics of slug populations: a process-based modelling approach for pest control. *Journal of Applied Ecology*, **38**: 401–411.

SOUTH, A. 1992. *Terrestrial slugs: biology, ecology and control*. Chapman & Hall, London.

SYMONDSON, W.O.C., GLEN, D.M., WILTSHIRE, C.W., LANGDON, C.J. & LIDDELL, J.E. 1996. Effects of cultivation techniques and methods of straw disposal on predation by *Pterostichus melanarius* (Coleoptera: Carabidae) upon slugs (Gastropoda: Pulmonata) in an arable field. *Journal of Applied Ecology*, **33**: 741–753.

SYMONDSON, W.O.C., GLEN, D.M., IVES, A.R., LANGDON, C.J. & WILTSHIRE, C.W. 2002. Dynamics of the relationship between a generalist predator and slugs over five years. *Ecology*, **83**: 137–147.

WILLIS, J.C., BOHAN, D.A., CHOI, Y.H., CONRAD, K.F. & SEMENOV, M.A. 2006. Use of an individual-based model to

forecast the effect of climate change on the dynamics, abundance and geographical range of the pest slug *Deroceras reticulatum* in the UK. *Global Change Biology*, **12**: 1643–1657.

WILLIAMS, B.K., NICHOLS, J.D. & CONROY, M.J. 2001. *Analysis and management of animal populations*. Academic Press, San Diego.

WEBLEY, D. 1962. Experiments with slug baits in South Wales. *Annals of Applied Biology*, **50**: 129–136.

WHITE, G.C. & BURNHAM, K.P. 1999. Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46**: 120–139.