Life in the leaf-litter: a novel metal detector technique to investigate the over-wintering survival of rare, case-bearing beetle larvae

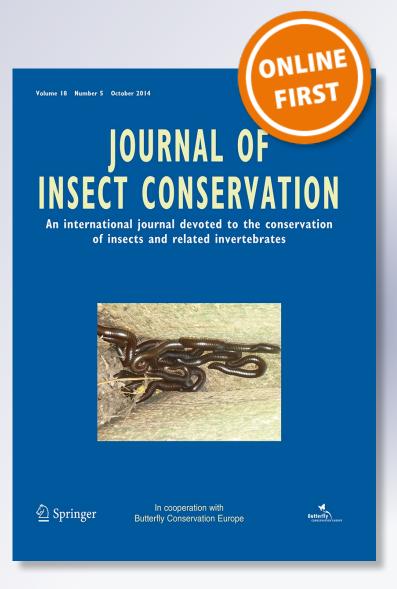
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ORIGINAL PAPER

Life in the leaf-litter: a novel metal detector technique to investigate the over-wintering survival of rare, case-bearing beetle larvae

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Abstract Investigating the ecology of leaf-litter dwelling insects is very difficult without destructive sampling of their habitat. Here, we describe the use of a metal-detector technique to study the overwintering survival of the casebearing, leaf-litter dwelling larvae of Cryptocephalus coryli (Coleoptera: Chrysomelidae). Three-hundred and thirty captive-reared larvae of this RDB-1 species were released at three sites and recovered on three occasions over a 6 month period. The number of recovered larvae at the three release sites was generally high and was always over 50 % of those remaining to be found. The technique also enabled predation to be investigated. After a period of 180 days, 50, 79 and 89.8 % of the released larvae that were known to have died had been preyed upon at the three study sites. Feeding tests (using baited larval cases), mammal trapping and pitfall trapping suggest the wood mouse (Apodemus sylvaticus) was the principal predator. This study also suggests that open areas with abundant bare ground may be optimal for the over-wintering survival of C. coryli larvae. The adult beetles make no known selection of the ground characteristics when ovipositing from their perches in host trees. Therefore, the remaining populations of this species may be dependent on the dynamic

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occurrence of suitable host trees on ground that is coincidentally suitable for larval development.

Keywords Chrysomelidae · *Cryptocephalus* · Rare insects · Over-wintering · Predation · Leaf-litter · Releases · Habitat requirements

Introduction

Studying the insect fauna of leaf litter presents several problems for the ecologist. Most techniques that are used in the study of leaf litter provide absolute population estimates (Mason 1970; Speight 1973; Southwood 1978). Other techniques such as Berlese funnels, etc. are behavioural based (Macfayden 1968). Trapping techniques such as pit falls rely on the animal taking action that leads to enumeration (Luff 1975; Southwood 1978). All these techniques involve a great deal of disturbance to the leaf litter habitat. Studying one particular species in the leaf litter is very difficult due to the problems associated with finding relatively small numbers of individuals in this type of situation. This is compounded by the fact that many leaf litter insects are also cryptically coloured. Typically, studies of leaf litter insects focus on biotic interactions and faunal diversity and there is a dearth of literature on the ecology of individual leaf litter species.

An important determinant of the viability of rare insect species in northern latitudes is the period of winter hibernation (Nicholls and Pullin 2000). Over-wintering survival has been the focus of many studies, principally of Lepidoptera larvae (Webb and Pullin 1996, 1998; Joy and Pullin 1999; Nicholls and Pullin 2000; Tanhuanpaa et al. 1999). In contrast, very little research has been undertaken on the over-wintering survival of beetles, other than those Author's personal copy

of economic importance. Some studies have focused on the micro-habitat preferences of Chrysomelidae larvae that over-winter at the base of their host-plants (e.g. Spring and Kok 1999), but literature dealing with the effects of predation on beetle larvae that spend all or part of their time in leaf litter appears to be non-existent. Determining the survival of larvae over this relatively long but inactive winter period is often particularly difficult due to many insects taking refuge in microhabitats which render their detection and recovery difficult.

Marking of individuals provides a means of monitoring over-wintering success in the leaf litter, but recovery of released individuals using visual searching can be destructive, and consequently does not allow repeat sampling. In an attempt to remedy this problem a novel technique was developed using the attachment of metal tags that can be found with a metal detector (Piper and Compton 2002). This allows efficient recovery of released insect larvae without destruction of their habitat, enabling in situ observation of individual insects that spend the winter period in the leaf litter.

Cryptocephalus (Coleoptera; Chrysomelidae) adults feed on leaves and petals and are generally oligophagous (Erber 1988). Their case-bearing larvae are found in leaf litter and eat mainly dried and decaying leaves, although fresh plant material may also be required before pupation (Masutti 1960). Adults are generally thermophilic and capable of flight, and can be found sitting in exposed positions on leaves. Females oviposit from such perches, covering each egg in faeces to form an egg case that is dropped onto the ground below. Once hatched, larvae carry the egg case and continue to add to it to form a case (Erber 1988). Over-wintering in northern latitudes takes place in the larval stage, with pupation and adult emergence in the spring/early summer (Steinhausen 1996). UK species are often univoltine, but some require more than 1 year to complete their development (Owen 1997, 1999, Pers. Obs.).

Overwintering larvae of *Cryptocephalus coryli*, an RDB1 species (Shirt 1987), were the focus of this study. Adults can be found mainly on *Betula pendula* growing in heathland or downland. This species has a biodiversity action plan, highlighting the need for the establishment of new populations and the enhancement of current populations. Both of these goals require an understanding of the ecological requirements of the larval stages if they are to be successful.

The aims of this study were as follows:

1. To monitor the survival of released *C. coryli* larvae during the over-wintering period and determine their sources of mortality.



Fig. 1 Cryptocephalus larval case showing metal tag on its dorsal surface

- 2. To assess the impact of predation on over-wintering larvae.
- 3. To determine if ground cover characteristics influence mortality.

Materials and methods

Rearing and tagging larvae for releases

Cryptocephalus coryli larvae were reared from eggs laid in captivity by females collected from a site in Lincolnshire (Kirkby Moor). Larvae were reared in captivity, but in containers that allowed them to experience natural daylight and temperature. *B. pendula* leaf litter and water were provided ad libitum until they reached their final instar. At this point they were tagged with small strips of metal (Fig. 1) as described in Piper and Compton (2002).

The tags used to label the larvae were 1 mm (\pm SD 0.2 mm) × 3 mm (\pm SD 0.2 mm) and 0.35 mg (\pm SD 0.07 mg). The tags were 1.18 % (\pm SD 0.36 %) of the final instar (including larval case) body weight (n = 10).

Release sites

Cryptocephalus coryli larvae were released at two sites in Lincolnshire (Laughton Forest 1, Laughton Forest 2 and Whisby) that are similar to other sites where wild populations of this species are still found today. Eighty, 70 and 180 larvae were released at Laughton Forest 1, Laughton Forest 2 and Whisby, respectively.

Laughton Forest is approximately 50 km from Kirkby Moor, the origin of the captive reared larvae. Forest Enterprise operations were reclaiming parts of the site as heathland. Two areas were used for the study (situated 90 m apart at their closest point). The first, Area 1, was a site with *B. pendula* scrub and appeared similar to the habitat currently occupied by *C. coryli* at Kirkby Moor. There was a diverse ground cover in this area with many *Molinia caerulea* tussocks, mosses and herbaceous species. Area 2 was a recently partially-cleared area with very little ground vegetation apart from isolated grass tussocks. This area would not be utilised by *C. coryli* adults because it is too early in the vegetation succession and lacks the small birch trees from which they oviposit.

Whisby Pits Nature Reserve is about 35 km from Kirkby Moor. The area chosen for the releases was a relatively uniform section of *B. pendula* scrub on a sand/ gravel surface with diverse ground vegetation consisting of grasses and many herbaceous plant species. The area, which is a reclaimed gravel extraction site, was chosen because of its superficial similarity to other localities that support this species, in particular the presence of a complex *B. pendula* scrub in south-facing, sheltered conditions.

These release sites could be categorised as two different types. The first of these is habitat that appears suitable for adult beetles, but regular surveying had shown that they were not being utilised (Laughton Forest 1 and Whisby). The second was habitat that is not utilised by adults, i.e. a recently cleared plantation (Laughton Forest 2). *C. coryli* larvae were released at Whisby and Laughton Forest on the 14th and 20th of November, respectively.

At each release site a grid of release points (8 at Laughton Forest 1; 7 at Laughton Forest 2 and 18 at Whisby), each separated by 2 m, was measured out and ten final instar larvae were released at each of these points.

Recovery of larvae

The larvae were recovered by using a sensitive hand-held metal detector (Pulse Technologies, Oxford, UK) that was swept over the release area. At Laughton Forest 1 and 2, recoveries were made on the 5th December 2000; 25th January 2001 and 23rd of May 2001. At Whisby recoveries were conducted on the 24th November 2000; 16th January 2001 and 8th of May 2001.

The microhabitats in which the larvae were found was recorded, together with their condition and, where appropriate, likely causes of death. Larvae were recorded as being still alive when the head capsule was visible at the aperture end of the case.

Ground cover

Ground cover characteristics were recorded for each point where larvae were released. A 50 cm² quadrat was placed at the release point and the percentage cover of grasses, mosses, herbs, bare ground and leaf litter was recorded. Leaf litter was recorded first and then removed if it was obscuring the ground flora. Maximum sward height was measured with a 30 cm ruler.

Predation

Released larvae were found to suffer frequent predation. In order to establish the species of predator, mammal trapping was carried out at the Laughton Forest site at the end of May 2001, the only site where this was permitted. 110 mammal traps (a mixture of Longworth traps and Sherman traps) were set out (electronic supplementary Figure S1) to sample the small mammal faunas in the two areas of Laughton Forest where the larvae had been released in November 2000, and either side of the track separating the two release sites (Transect A was established in Area 1 and Transect B in Area 2; see Figure S1). Twenty-five pitfall traps were also placed in each area to sample for surfaceactive, predatory arthropods (Figure S1).

In order to characterise the predation damage seen on recovered larval cases, single blow fly maggots were placed in empty C. coryli larval cases obtained from individuals that had died in captivity before release. The maggots were killed beforehand by placing them in boiling water. Twenty-five of these cases were then placed, singly, in some of the mammal traps, along with corn and blow fly pupae. Mammal traps were set during the evening and checked the following morning on five occasions. The captured mammals were marked by cutting fur from their hindquarters, enabling the number of individuals to be counted during the trapping period. If a trap with a mammal had included a C. corvli case then the case was recovered. Pitfall traps were also checked every morning over 5 days and the contents identified and then released in the field. Four Carabus problematicus (Carabidae) beetles were taken back to the laboratory and maintained in suitable conditions together with C. coryli cases, each filled with a maggot as before, for 2 weeks, in order to determine whether C. problematicus would prey upon the C. coryli.

Statistical analyses were conducted using Minitab (v16) and SPSS (v10.1).

Results

Recovery of released larvae

The number of recovered larvae at the three release sites was generally high (Table 1) and were always over 50 % of the larvae remaining to be found. There was a significant difference in the final numbers of individuals recovered across the three sites [Pearson $\chi^2(2) = 49.58$, P < 0.001]; only 57.14 % of released individuals were finally recovered at Laughton Forest Area 2, compared with 93.75 % at Laughton Forest Area 1 and 90.56 % at Whisby. Recovery

	Laughton Forest 1			Laughton Forest 2			Whisby		
	Recapture 1 (release + 15 days)	Recapture 2 (release + 70 days)	Recapture 3 (release + 180 days)	Recapture 1 (release + 15 days)	Recapture 2 (release + 70 days)	Recapture 3 (release + 180 days)	Recapture 1 (release + 10 days)	Recapture 2 (release + 65 days)	Recapture 3 (release + 175 days)
Unrecovered	20	11	5	20	36	30	72	76	17
Recovered	60	36	17	50	29	15	108	77	90
Alive	27	11	2	45	9	8	81	31	26
Dead	4	4	2	3	10	3	3	7	19
Predated	29	21	13	2	10	4	24	39	45

Table 1 Total numbers of Cryptocephalus coryli larvae that were recovered during each recapture episode at the Whisby and Laughton Forest sites

rates at Whisby were higher for recaptures 1 and 3 than for 2, potentially because recapture 2 took place during the coldest months of the year, when larvae would have been at their most inactive. Recovery of larvae at the Laughton Forest site showed the same pattern, with the lowest recovery rate again during recapture 2 (Table 1).

Predation rate of released larvae

Of the individuals known to have died across the three release sites, the rate of predation was highest in Laughton Forest Area 1, where after a period of 180 days 89.04 % of the released larvae had been preyed upon. In contrast, by this time, 50.00 % of released larvae had been preyed upon in Area 2 of the Laughton Forest site and 78.83 % at the Whisby site. There was, however, no significant difference across the three sites in the rate of predation [Pearson $\chi^2(2) = 2.75$, P = 0.25].

Damage to the larval cases ranged from a small hole in the posterior end of the case to almost complete fragmentation. On eight occasions only the metal tag fitted to the larval case was located. These loose tags were assumed to be artefacts of predation as a persistent predator would have been able to dislodge them.

Overall mortality

Following exclusion of individuals whose fate was unknown at the end of the recovery period, there was a significant difference between the final numbers of recovered larvae that had died or been predated across the three sites [Pearson $\chi^2(2) = 10.10$, P = 0.01]. Of the individuals of known fate, 97.33 % suffered mortality overall at Laughton Forest Area 1, compared to 80.00 % at Laughton Forest Area 2, and 84.05 % at Whisby.

Mammal and arthropod trapping

After 5 days of mammal trapping in Laughton Forest the only species recorded was the wood mouse (Apodemus

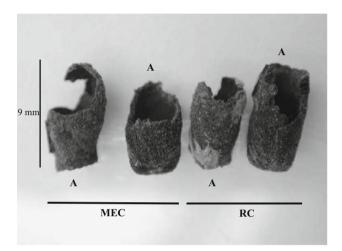


Fig. 2 Predated *Cryptocephalus coryli* larval cases. MEC = cases broken into by *Apodemus sylvaticus*; RC = predated cases recovered from the field using the metal detector. A = anterior end of case

sylvaticus). Area 1 yielded nine wood mice, but none were found in Area 2. Two and three mice were caught in Transects A and B respectively. The only large carabid that was found using the pitfall traps was a single specimen of *C. problematicus* in Area 1.

Characterising predation

Eight of the traps that were baited with *C. coryli* larval cases contained a wood mouse. The cases showed the same type of damage as larval cases predated in the field (Fig. 2). They had been chewed open and the maggot removed from the inside. Four *C. problematicus* offered baited *C. coryli* cases did not attempt to break into them.

Release site and ground cover characteristics

A summary of ground cover characteristics of the release points at the three sites is shown in Table 2. There was no significant difference between the sites in terms of maximum sward height [ANOVA, F(2, 30) = 2.81, P = 0.08], grass [F(2, 30) = 0.04, P = 0.96] and herbs [F(2, 30) = 0.70, P = 0.50]. Percentage moss cover between the sites was significantly different [F(2, 30) = 43.29, $P \le 0.001$], as was bare ground [F(2, 30) = 57.21, $P \le 0.001$] and leaf litter [F(2, 30) = 5.59, P = 0.01].

The majority (88.88 %) of release points at Whisby and all the release points at Laughton Forest Area 1 had 0-33 % bare ground. In contrast, 85.71 % of the release points at Laughton Forest Area 2 had 67-100 % bare ground. A binary logistic regression (SPSS version 10.1) was used to determine the relative effects of the measured ground cover characteristics at release points, on predation of released larvae. Data from the three sites were combined. Bare ground percentage cover was the most significant of the measured variables (Table 3). Predation was lowest at release sites that had the greatest proportion of bare ground. Leaf litter percentage cover was also significant but not selected for use in succeeding steps of the regression. Percentage bare ground cover was used to produce a classification that projected an overall correct group membership of 66 %. This suggests that the regression model based on ground cover was only a poor predictor of larval predation. Herb, moss and grass percentage cover showed no significant effect, and were not selected for use the in the forward conditional binary logistic regression.

Discussion

The proportion of released larvae recovered during the course of this study was high, but not all were recovered. Although many recovered larval cases exhibited evidence of predation, the non-recovered cases may have been moved to a safe place by a predator (Hanski 1992).

Some larvae may have been out of the range of the detection equipment. This would be particularly true for release sites where the ground was very uneven, with high rabbit activity producing narrow, deep divots, which would make larval detection very difficult. It is also possible that some larvae may have lost their metal tag; however, the recovery of a metal tag was interpreted as an artefact of predation.

Table 3 The relative significance of ground cover variables at larval release sites related to the final level of predated *Cryptocephalus coryli* larvae (forward conditional binary logistic regression output)

Variable	В	Standard error	Degrees of freedom	Significance
% Bare ground cover	.04	.01	1	<0.001
% Leaf litter cover	04	.02	1	0.02
Constant	36	.14	1	0.01

The number of recovered larvae was at its lowest during recapture period 2 (the coldest period of the study) suggesting the released larvae were perhaps seeking out warmer micro-climates.

The observations made concerning predation provided insights into the mortality factors affecting over-wintering *Cryptocephalus* larvae. The only small mammal captured during the trapping exercise at the Laughton Forest site was the wood mouse (*A. sylvaticus*). Wood mice are known to feed on insect larvae and pupae during the winter and early summer (Watts 1968; Green 1979; Hansson 1985). This rodent will break through a *C. coryli* larval case to eat the bait within, damaging the cases in the same way as predated larval cases recovered using the metal detector.

Many more wood mice were caught in Area 1 than Area 2 or transects A and B. Area 1 is essentially dense birch scrub with high proportion of low level cover, fallen timber and coppiced stools, that would provide refuges for foraging and nesting wood mice (Wilson 1992; Fitzgibbon 1997). In contrast, Area 2 is a recently cleared area with a large proportion of bare, homogeneous ground. Wood mice seemed to be absent from this area. This could be due to the area being more open, leaving the mice vulnerable to predation, and perhaps prompting them to avoid this area.

Transects A and B were placed in locations bordering the two study areas. Transect B was very overgrown and appeared to represent good mouse habitat. The low number of mice encountered here may be as a result of too much ground cover that may impede movement over the ground. Predation of released *C. coryli* larvae in Area 2 was apparent but very low. Refuges in close proximity to this

Table 2 Summary of ground cover data at Whisby, Laughton Forest 1 and Laughton Forest 2 release points

	Sward height (cm)	Moss (%)	Grass (%)	Bare ground (%)	Herbs (%)	Leaf litter (%)
Whisby	0.99 (±0.31) a	80.8 (±19.9) a	4.64 (±6.48) a	11 (±17.0) a	4.1 (±3.3) a	20.0 (±23.0) a
Laughton Forest Area 1	1.3 (±0.5) a	86.4 (±11.5) a	4.58 (±3.8) a	6.6 (±11.1) a	2.4 (±1.1) a	10.3 (±8.4) a
Laughton Forest Area 2	0.85 (±0.5) a	6.65 (±12.5) b	4.2 (±5.3) a	88 (±17.5) b	1.5 (±1.4) a	45.8 (±27.9) b

Letters after values indicate results of post hoc tests (LSD method following ANOVA). Values for each variable with similar letters were not significantly different; data are means followed by SD

area may enable mice to forage in this area as long as cover is nearby.

Vertebrate predators of insect pre-pupae or pupae are assumed to be generalists (Tanhuanpaa et al. 1999) as these immature stages are only available for a relatively short time compared to the length of the vertebrate life cycle.

Furthermore, vertebrate predators may be responsible for most mortality in post-diapause larvae (Duffey 1968; Webb and Pullin 1996) whilst arthropod natural enemies are to blame for most mortality in pre-diapause larvae (Webb and Pullin 1996). However, these studies relate to larvae with no protective cases. No direct evidence of arthropod predation was observed in the current study and it may be that a fully-grown or even a half grown C. coryli larva is protected inside its hard case from predatory arthropods, such as beetles. Indeed, large predatory beetles (e.g. Carabus sp.) primarily rely on soft-bodied invertebrates (e.g. earthworms and slugs) for their food (Jung 1940; Scherney 1959). Only mature larvae were released in the current study and it would be interesting to investigate the impact of invertebrate predators on young Cryptocephalus larvae.

In terms of other causes of mortality, some recovered cases had fungal hyphae protruding from the case aperture, but it was not established if these were saprophytic or entomophagous. All other dead, recovered larvae were simply found to be decayed or desiccated at the bottom of their case.

This study also yielded information on the microhabitat preferences of C. coryli larvae. During the course of the study mature larvae were found partly buried in bare ground or dense moss, leaving just the posterior end of the case sticking out. This behaviour was interpreted as the full-grown larvae preparing for pupation as the adults escape from the posterior part of their case by chewing a neat lid. If the three sites are combined, then one, three, and 19 larvae were found in this position during recaptures 1, 2 and 3 respectively. Only circumstantial evidence is available to support the idea that this behaviour may make this stage of the beetle more vulnerable to predation. It is interesting to note that many of the recovered cases of predated, fully developed larvae exhibited damage to the posterior end of the case, which is the part of the case above the ground surface and therefore possibly more vulnerable to predators. However, the larva may simply be more easily extracted by the predator from this end of the case. Further work would be needed to investigate this.

Very little movement of larvae was seen during the study, possibly due to the cold weather. Later instar larvae may also be more sedentary than early instars, especially first instar larvae. First instar larvae have been seen to be very mobile in captivity (Pers. obs.), but their dispersal ability in the wild remains to be seen. Monitoring young larvae would be technically difficult, but would surely yield interesting results.

Analysis of the ground cover variables in relation to larval predation showed that C. corvli larvae were more likely to be predated if there was less bare ground and leaf litter. Leaf litter coverage could be viewed as being very closely related to bare ground cover. A sward of vegetation may mean that fallen leaves never reach the soil and are more prone to being displaced by wind whilst balanced on the sward. Open ground will encourage a covering of leaf litter. Furthermore, habitat with a high percentage cover of bare ground does not represent optimal foraging habitat for small mammals (e.g. A. sylvaticus that were shown to break open baited C. coryli cases) for the reasons stated above. Predation of C. coryli larvae was lower in Area 2 of the Laughton Forest site (Fig. 2), which had a great deal of bare ground. This area is not the type of habitat where you would typically find C. coryli adults. Our data suggest that C. coryli adults oviposit in areas that are not optimal for larval development, but further study would be needed.

Adult *Cryptocephalus* females oviposit from host trees which fulfil their habitat requirements, i.e. typically small trees in warm, sheltered areas. The female makes no selection of the discrete oviposition site; the eggs are simply dropped onto the ground from the host plant. Habitats that are optimal for adult survival may be suboptimal for the larvae because of predator activity, as shown by the contrasting predation seen in the *C. coryli* release sites, Laughton Forest Area 1 and Area 2.

Remaining populations of this species may be dependent on the coincidental occurrence of suitable host trees on ground suitable for larval development. The problem that this coincidental arrangement produces for the long-term survival of this rare *Cryptocephalus* species is compounded by the very nature of the habitat on which they depend. This and many other *Cryptocephalus* species depend on fast growing trees encroaching into suitably warm areas of heathland/downland/wetland. This very dynamic process may facilitate the coincidental occurrence of good adult habitat with good larval habitat. Without management; however, the progressing, suitable front of the scrub transition will produce a mosaic of habitats that offer differing levels of adult and larval suitability where the juxtaposition of habitat suitable for adults and larvae is very rare.

Adults *Cryptocephalus* beetles have been shown to have very limited powers of dispersal and they move around areas of habitat using suitable host-plants as stepping stones (Piper and Compton 2003, 2010, 2013). The complexity of their habitat requirements and their limited powers of dispersal render them acutely vulnerable to extinction in an extremely fragmented landscape.

The goal of establishing new populations of these species may only be a success if recipient habitats can be found that optimally support both larvae and adults and can be managed in such a way to perpetuate the occurrence of good adult habitat and good larval habitat.

This study also suggests that to establish a population of *C. coryli* at a recipient site using larvae alone would require many more individuals than were used in this study. The combined effects of predation and other mortality factors result in a very small percentage of released larvae reaching adulthood. This could be partially overcome by rearing adults in captivity. However, it remains to be seen if an introduction of adults reared from a small founder population could propagate itself. To enhance the success of introductions or augment present populations of *C. coryli* sites would have to be suitable for both the adults and the larvae.

Finally, this technique and variations of thereof lends itself to investigating the ecology of other arthropods that live out all or part of their lives in the leaf litter or the upper levels of the soil. Obvious examples include the larval and pupal stages of Lepidoptera, Diptera and other Coleoptera, particularly rare species where the ecology of the immature stages is often very poorly known.

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