IS Protocol SPITTLE BUGS

Version 1.1 (updated Dec 2001)

Aim To estimate an index of nymph density of two species of spittle bug (Neophilaenus lineatus and Philaenus spumarius) and to estimate the proportions of colour morphs of the adults of P. spumarius

Rationale Two species of spittle bug, *Philaenus spumarius* and *Neophilaenus lineatus*, are both widespread and common throughout the United Kingdom. Their nymphs, which are xylem feeders, are surrounded by a mass of froth or spittle which the nymphs produce by forcing air into a fluid exuded from the anus. As well as providing some protection from predators, the presence of the spittle makes the nymphs visible and therefore relatively easy to sample. There is a solid background of published ecological work on these species both at lowland and at upland sites (eg Whittaker 1965, 1969, 1970, 1971). In addition to estimating an index of nymph density, the Protocol involves the sampling of colour morphs of *P. spumarius* adults. The colour polymorphism is mainly determined by a series of closely linked genes; it is likely that the proportions of morphs are environmentally determined (Whittaker 1968) and will therefore be good indicators of environmental change.

Method Populations of *P. spumarius*, which occurs on dicotyledons, and *N. lineatus*, which occurs on monocotyledons, should be monitored annually. Counts of the spittle produced by nymphs of these species are used as an index of nymph density. In addition, the proportions of the different colour morphs of adult *P. spumarius* should be estimated at each sampling location once each year. Monitoring of spittles should preferably be on the TSS but otherwise on an area at the site where they are known to occur.

Equipment

For supplier of sweep nets, please see Appendix II. Quadrats are easily made from wood or metal.

Nymphs

Location

In mid-June 20 quadrats, each 0.25 m^2 are placed randomly in permanent grassland or in natural vegetation in the vicinity of the TSS. They are marked, their positions recorded, and they are re-visited annually. If a permanent grid is available this can be used in conjunction with random numbers to place the quadrats. Otherwise quadrats can be thrown 'randomly', making sure that no visual reference is made to the vegetation. If monocotyledons and dicotyledons have distinctly different spatial distributions at the site, 20 samples should be taken in each vegetation type, recording *P. spumarius* from the quadrats containing dicotyledons and *N. lineatus* from those containing moncotyledons.

Sampling

The number of spittles occurring in each quadrat is counted separately for dicotyledons (*P. spumarius*) and monocotyledons (*N. lineatus*).

In addition, a random sample of 50 spittles of each species is taken to correct quadrat samples for the mean number of nymphs per spittle. This is done by throwing the 0.25 m quadrat without visual reference to the vegetation or spittle presence and then removing all spittles within the quadrat, keeping separate those spittles collected from monocotyledons (*N. lineatus*) and dicotyledons (*P. spumarius*). This is repeated until enough spittles are found. If spittle density is low and most of the quadrats have no spittles, then the first 50 spittles of each

species encountered, ie without reference to quadrats, can be used, making
sure that there is no bias towards large, conspicuous spittles. The number of
spittles and the time taken to find and collect them should be recorded. A
maximum of 2 hours should be spent searching. These samples are preserved
for counting and determination in the laboratory. Nymphs are preserved using a
mixture of 70% alcohol, 5% glycerol and 25% water. An absolute minimum
sample of 30 spittles of each species is required.

Adults

Location

In late August, sweep net samples of adults are taken in the vicinity of, but not directly on, the TSS. Sampling must be in the same area each year and must be done when the vegetation is dry.

Sampling

A minimum of 50 individual adults of *P. spumarius* is required. <u>The number of</u> <u>adults and the time taken to find and collect them should be recorded. A</u> <u>maximum of 2 hours should be spent searching.</u> Samples should be stored dry, for later determination, in small pill boxes which are then stacked inside a larger container to which naphthalene is added. Each adult is allocated to one of the colour morphs using the methods explained in Appendix II; the numbers of males and females allocated to each morph type are recorded separately and the identified specimens should be stored for future reference and confirmation.

Time Spittle counts		2 h/site/year
	Sweep netting	1 h/site/year

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References Whittaker, J.B. 1965. The distribution and population dynamics of *Neophilaenus lineatus* (L.) and *N. exclamationis* (Thun.) (Homoptera: Cercopidae) on Pennine moorland. *Journal of Animal Ecology*, **34**, 277-297.

Whittaker, J.B. 1968. Polymorphism of *Philaenus spumarius* (L.) (Homoptera: Cercopidae) in England. *Journal of Animal Ecology*, **37**, 99-111.

Whittaker, J.B. 1969. Quantitative and habitat studies of froghoppers and leafhoppers (Homoptera: Auchenorrhncha) of Wytham Woods, Berkshire. *Entomologist's Monthly Magazine*, **105**, 27-37.

Whittaker, J.B. 1970. Cercopid spittle as a microhabitat. Oikos, 21, 59-64.

Whittaker, J.B. 1971. Population changes in *Neophilaenus lineatus* (L.) (Homoptera: Cercopidae) in different parts of its range. *Journal of Animal Ecology*, **40**, 425-443.

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Figure 13. Colour morphs of Philaenus spumarius

Appendix I. Identification of colour morphs of *Philaenus spumarius*

Specimens of the common morphs of *Philaenus* are provided for each ECN site as a guide to identification of the collections.

The specimens are labelled as follows.

1	POP	populi	7 PRA	praeusta
2	TRI	trilineata	8 LAT	lateralis
3	MAR	marginella	9 QUA	quadrimaculata
4	FLA	flavicollis	10 ALB	albomaculata
5	GIB	gibba	11 LOP	leucopthalma
6	LCE	leucocephala	12 TYP	typica

Some points regarding morph identification need to be stressed.

- You will not be able to assign every specimen to one of the illustrated morphs.
- Freshly emerged specimens are referred to as teneral, and may be soft and of a greenish straw colour; the integument hardens quite quickly but it takes some time for the darker pigments to develop fully.
- Figure 13 illustrates the dark parts as black but this will not always be so. You may find many specimens where the colour has not reached its full development of intensity and others which are brown or dark brown rather than black. It is not the intensity/density of the colour which is important but the distribution pattern of that colour.
- Surprisingly one of the most difficult morphs to distinguish accurately is *typica*. In most parts of England *typica* is the most abundant morph (between 50% and 90% of the population) so you should soon become familiar with it. Note that in Figure 13 the dark areas are indicated by stippling. This is deliberate. Look for a zig-zag pattern consisting of stippling, concentrated into narrow, variegated bands, giving the appearance of cross-gartering. Pale specimens of the two four-spotted morphs *flavicollis* and *quadrimaculata* are very easy to confuse with *typica*, look for the contrast between the areas of solid dark and pale colour in these two morphs and the pale diamond half way along the centre line of the elytra in *typica*.
- Specimens of *praeusta* are difficult to come by because this is the scarcest of all the morphs, but it is so distinctive that you should have no difficulty in recognising it if you find it.
- All the illustrations show the eyes as white, indicating that they are pale. Many of the reference specimens collected to provide you with reference material have dark eyes and you may find dark-eyed specimens yourself. We have disregarded eye colour in selecting reference specimens and even some of the *leucopthalma* have dark eyes. If you come across dark-eyed specimens, assign them to the morph which they would otherwise fit.

Some practical tips

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- You will need good illumination such as an anglepoise or similar desk lamp.
- Specimens can be quite easily handled with a small (size 1) paint brush with which they can either be pushed around or picked up by entanglement of the legs.

Females are much easier to identify than males. Start by sorting your collection into separate groups of males and females. To assist with this the reference specimen of *typica* is a female and the *trilineata* is a male (the other morphs are a mixture of sexes).

By eye

Divide your group of females into four sections

Pale specimens	Dark specimens with pale fronts
trilineata	Dark specimens with dark fronts

Having sorted into the four groups start with the pale-headed dark group and split it into *marginella, leucocephala* and spotted forms.

Then split the entirely dark group into lateralis, leucopthalma and spotted forms.

There will be some specimens in both groups that you will not be able to 'rough sort' by eye.

Using a lens (a x-10 hand lens is quite sufficient)

Check ALL the specimens, group by group, starting with the dark ones which you have just sorted into morphs. You will probably find that one or two have been sorted into the wrong morphs and you can now correct this and also sort the ones which were not separable by eye.

Using the lens, return to the group of pale specimens which you sorted first. Some of these will now turn out to be immature specimens of the darker morphs but under the lens you should be able to see the beginnings of the pattern.

Having dealt with the females, now sort the males in the same way. This will not be quite so easy but having 'got your eye in' with the females will help considerably. Keep the results for females and males separate for entry on the recording form.

In recording your results the most important distinctions to make are into the groups:

Pale (*typica*, *populi*) Striped (*trilineata*, *marginella*, *lateralis* together with *praeusta*) Melanics (the rest) but be as accurate as you can.



	Appendix II.	Equipment details
Supplier	Sweep nets (Catalogu	ue no. E679) are obtainable for approximately £50 from:
	Watkins & Doncaster The Naturalists Four Throws Hawkhurst Kent TN18 5ED, UK	

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Specification of results and recording conventions

The measurement variables listed below are those required for each IS sampling location at an ECN Site. Sites submitting data to the ECNCCU should refer to the accompanying Data Transfer documentation for the specification of ECN dataset formats, available on the restricted access Site Managers' extranet. Contact <u>ecnccu@ceh.ac.uk</u> if you need access to this documentation.

The first 4 key parameters uniquely identify a sample or recording occasion in space and time, and must be included within all datasets:

- <u>Site Identification Code</u> (e.g. T05)
- Core Measurement Code (e.g. PC)
- Location Code (e.g. 01)
- Sampling Date (/time)

Unique code for each ECN Site Unique code for each ECN 'core measurement' Each ECN Site allocates its own code to replicate sampling locations for each core measurement (e.g. for different surface water collection points) Date on which sample was collected or data recorded. This will include a time element where sampling is more frequent than daily

ECNCCU 2001

Core measurement: invertebrates – spittle bugs (IS Protocol)

The following variables are recorded annually - nymphs in mid-June, adults in late August:

Variable	Units	Precision of recording
For nymph recording		
Site Identification Code		
Core Measurement Code		
Location Code		
Sampling date		
Vegetation mixed or segregated	character code (M or S)	
Quadrat ID	character code (Qn) ¹	
Species code	BRC code ²	
Species name	genus species	
Number of spittles	count	1
From random-throw exercise		
Number of spittles found (by species)	count	1
Time taken to record spittles	hours:mins	
Total number of nymphs found (by species)	count	1
For P. spumarius adult morph recording		
Site Identification Code		
Core Measurement Code		
Location Code		
Sampling date		
Morph	3-character code ³	
Number of males	count	1
Number of females	count	1
Time taken to collect adults	hours:mins	

Recording forms

Three types of form are available from the CCU:

- 1. Nymphs: quadrat sampling where the vegetation is mixed
- 2. Nymphs: quadrat sampling where the vegetation is segregated
- 3. Adults (sweep-netting).

Notes

- 1. Quadrats should be numbered from Q1 to Q20, or from Q1 to Q40, depending on whether the vegetation is mixed or separate. Numbering is unique within each ECN site.
- 2. The coding system should follow the standard currently used by the Biological Records Centre, ITE Monks Wood, Abbots Ripton, Huntingdon, Cambs PE17 2LS, UK.
- 3. Adult colour morphs of *P. spumarius* should be coded as follows:
 - POP populi PRA praeusta lateralis LAT
 - TRI trilineata MAR marginella
 - FLA flavicollis
 - GIB gibba
- ALB albomaculata LOP leucopthalma
- LCE leucocephala UUU Unallocated morph
- TYP typica

QUA quadrimaculatus