OCCURRENCE AND EFFECT OF TEMPERATURE REGIMES ON FOUR SPECIES OF FLY (DIPTERA) FOUND WITH *CULICOIDES BREVITARSIS* KIEFFER (CERATOPOGONIDAE) IN BOVINE DUNG

A.L. Bishop, H.J. McKenzie, I.M. Barchia and A.M. Harris

NSW Agriculture, PO Box 581, Gosford, NSW, 2250

Summary

The seasonal occurrence and the effects of temperature regimes on development, survival and emergence are given for *Psychoda* sp. (Psychodidae), *Australosepsis niveipennis* (Becker) (Sepsidae), *Leptocera mirabilis* (Collin) (Sphaeroceridae) and *Pyrellia tasmaniae* Macquart (Muscidae) breeding in bovine dung in the Hunter Valley, NSW. The data were then used to consider the possible interaction between these species and arbovirus vector, *Culicoides brevitarsis*, at the southern limits of its distribution. Like *C. brevitarsis*, *P. tasmaniae* was largely absent from dung in winter. The other species were present continuously. *A. niveipennis* and *P. tasmaniae* increased in number from spring to autumn. *Psychoda* sp. and *L. mirabilis* were limited to peaks in spring and autumn. The occurrences of *A. niveipennis, Psychoda* sp. and *L. mirabilis* were each related to average monthly temperature. No occurrence of the four species was related to rainfall. Each species was present when *C. brevitarsis* re-establishes in the study area each season. All except *Psychoda* sp. developed in a temperature range (17°C to 36°C) similar to that of *C. brevitarsis*. *Psychoda* sp. was active at 15°C but was inactive above 32°C. Development times for each species decreased as temperatures increased. These were different between species and these differences were maintained over the temperature range tested. Some development of juveniles occurred below temperatures inhibiting adult emergence which could be induced by raising the temperature above 17°C. Despite close similarities in time, space and responses to temperature, the fly fauna endemic in fresh dung was not considered as important to the re-establishment, growth and survival of *C. brevitarsis*.

INTRODUCTION

Several species of fly (Diptera) use dung at some stage during their life cycle (Colless and McAlpine 1991). Some are pests that affect humans and animals (e.g. Hughes *et al.* 1972). Others appear to simply use dung as habitat or food.

Culicoides brevitarsis Kieffer (Diptera: Ceratopogonidae) breeds in discrete pats of (bovine) dung (Cannon and Reye 1966) and is a vector of several viruses affecting livestock in Australia (Muller *et al.* 1982). It occurs as far south as the central coastal region of NSW (Murray and Nix 1987) where it commonly fails to survive winter and then re-establishes each season by migrating from endemic areas further north (Bishop *et al.* 1996a). In the Hunter Valley, there is a fly fauna coincident with *C. brevitarsis* in dung soon after it is deposited (Bishop *et al.* 1996b). The larvae mix spatially with *C. brevitarsis* (Bishop *et al.* 1996b) but apart from their taxonomy, general biology and the bio-geography of the Sepsidae (Colless 1980), there is little information on each species that could be used to explain and compare their ecologies. Any species that compete with, predate on or alter the environment of *C. brevitarsis* could be important, especially at times when the vector re-establishes or low temperatures start limiting the chances of its surviving at the southern end of its distribution.

Responses to temperature and seasonal activity by *C. brevitarsis* in dung have been studied over several seasons in the central coastal region of NSW. (Bishop *et al.* 1996b; Bishop *et al.* 1996c). These studies enabled the concurrent acquisition of data on other species of fly. Information on the seasonal occurrence and the effect of temperature on the development, survival and emergence of these flies was collected and was related to information on *C. brevitarsis*.

MATERIALS AND METHODS

The dung was from beef cattle grazing on native pasture grasses in the Hunter Valley. Laboratory rearing and temperature studies were undertaken in constant temperature (CT) rooms and cabinets at constant light.

Seasonal occurrence

Dung was sampled each fortnight from June 1992 to April 1994. Five paddocks (23 ha to 40 ha) on one farm were selected as permanent sampling areas to ensure that cattle were always present in at least one paddock. The dung was about three days old and of uniform size (approximately equal to the bottom of a 10 L opaque plastic bucket) when collected. Five dung pats were collected with a shovel in each paddock where dung was present and placed separately in buckets. The buckets were returned to the laboratory and set up as emergence chambers with collection containers (Dyce and Marshall 1988). Chambers were placed in a room maintained between 20°C and 25°C at constant light. Collection containers were removed for counting twice weekly for six to eight weeks until no flies had emerged for several days. Flies in containers were immobilised with CO_2 and transferred to plastic petri dishes containing 70% alcohol for counting.

Weather data were obtained from the C.B. Alexander Agricultural College at Tocal (near Maitland).

Analysis of data: Average monthly air temperature, numbers of flies and total monthly rainfall were graphed for comparison. The relationships between the counts, temperature and rainfall were then examined using generalised additive modelling (Hastie and Tibshirani 1990) where spline functions were used initially. Because the errors were highly overdispersed, count data were transformed by logarithm and errors were assumed to be normally distributed. The final model fitted was:

log (y) = Polynomial (Temperature) + Spline (Rainfall).

Responses to temperature

Dung pats were marked in the field when fresh and then sampled two days later to allow time for the flies to oviposit. Two cores were taken from each marked pat (Bishop *et al.* 1996b) with a 15 cm long piece of PVC pipe (internal diameter 10.5 cm; area 86.6 cm^2). Cores from different pats, were paired and placed in 10 L opaque plastic buckets to form composite samples. Buckets were brought to the laboratory, set up as emergence chambers with collection containers and allocated at random to treatments. A series of temperature experiments was undertaken as there were not enough cabinets to hold all the proposed treatments. Humidity and moisture were assumed high and constant in the chambers (Bishop *et al.* 1996c). Flies caught in containers were collected daily, immobilised with CO_2 and counted.

Experiment 1. Batches of five emergence chambers were kept for 28 days at 5°C, 12°C, 17°C, 20°C or 25°C. All chambers were placed at 25°C at 28 days where they were held for a further 28 days to stimulate any inhibited larvae to emerge.

Experiment 2. Batches of five chambers were kept for 28 days at 15°C, 17°C, 20°C, 25°C or 28°C. All chambers, except those at 28°C, were then transferred to 25°C for a further 28 days.

Experiment 3. Batches of five chambers were monitered for 56 days at 25°C, 28°C, 32°C or 36°C. Five chambers at 40°C were monitored for 28 days and then transferred to 25°C for a further 28 days.

Experiment 4. Experiments 1–3 showed that 17°C was close to the lower limit for the emergence of all species of fly and that 28°C was the best temperature at which inhibited larvae could be stimulated to emerge. Accordingly, experiment 4 was designed to consider the effect of exposure time on emergence and survival at 17°C. Twenty-four chambers were held at 17°C for varying periods and four chambers were held at 28°C. At 28 days, four of the 24 chambers at 17°C were transferred to 28°C. This was repeated at 42, 56, 70 and 84 days with four chambers remaining at 17°C. The experiment ended at 112 days.

Analysis of data

Thresholds for emergence: Average times to the first emergence of *C. brevitarsis* (Bishop *et al.* 1996c) and four other species of fly were calculated at each temperature. Data were $\log_e$ transformed and the linear effect of temperature on all five species modelled at the same time to give one coefficient of determination. Times to first emergence and the order of emergence of each species were compared.

Effects of temperature: Each treatment of the four experiments had cumulative emergence data modelled according to the logistic function:

$$y = C / (1+\exp [-B (x-D])$$

where C = total emergence, B = rate of emergence, x = days and D = inflexion point for the explanatory variable (or time to reach 50% emergence) (Draper and Smith 1981). The generalised linear modelling technique was used to fit the above function to cumulative data with six or more points using an assumption that errors followed a Poisson distribution (McCullagh and Nelder 1989). Treatments were compared at 28 and 56 days in experiments 1–3 and 28 days and 42 days in experiment 4 by calculating confidence regions round the total number emerging and the time to 50% emergence from day 1 for each species. The time to 50% emergence was used as the measure of development time due to temperature treatment. The standard errors of the parameter estimates were adjusted for over- or under-dispersion where necessary.

RESULTS

Seasonal occurrence

Seven species of fly were relatively abundant in three-day-old bovine dung. *Sepsis nitens* Wiedemann (Sepsidae) and an unidentified Sphaerocerid were only recorded occasionally. Distribution and temperature data on *C. brevitarsis* were described separately (Bishop *et al.* 1995; Bishop *et al.* 1996c). The seasonal occurrence of the four other species is given on a $\log_{10}$ scale and compared with weather data from the study area in figure 1. *Psychoda* sp. (Psychodidae) was present each month and was most abundant in spring and autumn. *Australosepsis niveipennis* (Becker) (Sepsidae) was present each month and gradually increased in abundance from spring to autumn. *Leptocera mirabilis* (Collin) (Sphaeroceridae) was present throughout the year (although it was apparently absent in January 1995) with peaks in abundance in spring/summer and autumn. *Pyrellia tasmaniae* Macquart (Muscidae) was largely absent from dung in winter. It reappeared in September and then gradually increased in number to peaks in autumn.

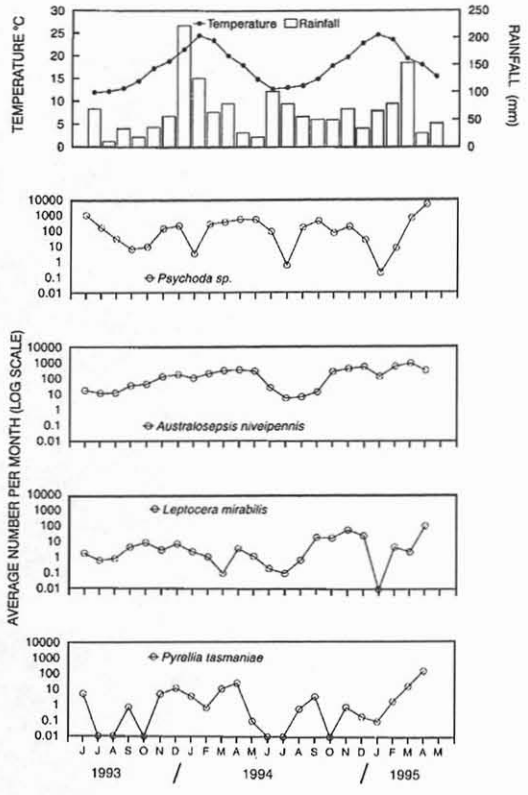

Figure 1. Seasonal distributions of four species of fly breeding in bovine dung in relation to average monthly temperature and total monthly rainfall in the Hunter Valley.

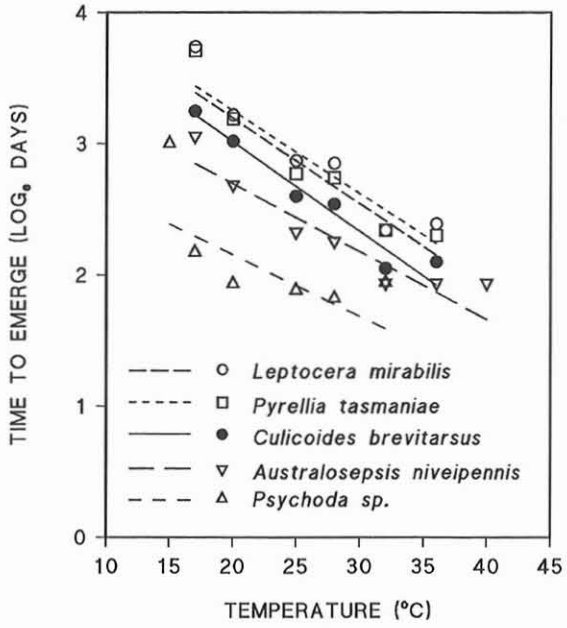

Figure 2. The linear effect of temperature on the time to first emergence of five species of fly breeding in bovine dung.

Average monthly temperatures at Tocal were close to the historical means (Dutton *et al.* 1993). They ranged between 10°C and 15°C (winter), 15°C and 20°C (spring and autumn) and 20°C to 25°C (summer). Rainfall was variable and no visual associations with occurrence were obvious. Spring rainfall was less than average on both occasions but was above or below average on one or other of the summer, autumn and winter seasons. Counts of *A. niveipennis, L. mirabilis* and *Psychoda* sp. were positively related ($P<0.05$) to monthly temperature (Deviance ratios were 25.84, 4.63 and 3.67 respectively, df 2, 15). No relationships were established with rainfall.

Responses to temperature

Thresholds for emergence: The effect of temperature on the time ($\log_e$ days) to the first emergence of five fly species (including *C. brevitarsis)* is shown in figure 2. Times to emergence were different between species and the emergence sequence was consistent over the temperature range. The model gave an $r^2 = 0.77$. Time to emergence decreased with increasing temperature until various upper limits were reached. Lower temperature limits were reached by about 17°C for all species except *Psychoda* sp.

Effects of temperature: Four species are arranged in the order that they first emerged from the dung and the results for each given separately.

Highest numbers of *Psychoda* sp. were recorded at 25°C with lower and upper limits to emergence at 15°C and 32°C respectively (table 1). Development times ranged from 5.9 to 11.3 days between 17°C and 32°C and was two to three times longer at 15°C. The species was abundant at 17°C and all adults emerged within 20 days at the temperatures tested. There were therefore no effects of time treatments in experiment 4 (table 5).

Numbers of *A. niveipennis* were uniformly high between 25°C and 36°C (table 2). Fewer adults emerged, or emergence was delayed at 17°C, 20°C and 40°C. Some juveniles survived for 28 days at 15°C and emerged as adults nine days after the temperature was raised to 25°C. Development times decreased from lowest to highest temperature but were variable at the same temperature in different experiments. Adults emerged naturally at 17°C between 19 and 34 days after dung deposition (tables

2 and 5). Numbers at 17°C increased significantly two days after the temperature was raised to 28°C at 28 days but most had emerged before the 42-day treatment. All individuals emerged within 46 days.

L. mirabilis adults mainly emerged between 20°C and 36°C (table 3). Some juveniles survived at least 28 days at 17°C and 15°C. They emerged four to twelve days after the respective temperatures were raised to 25°C. Development times decreased as the temperature increased in each experiment and were similar at the same temperature in different experiments. The data from experiment 4 could not be analysed because of low numbers and zero counts. However, some *L. mirabilis* started to emerge naturally at 17°C at 43 days (table 5) and several days after the chambers were moved to 28°C at 28 and 42 days. All individuals had emerged within 49 days.

Table 1. Total numbers of *Psychoda* sp. emerging and development times (D = days to 50% emergence after the deposition of bovine dung) at different temperature regimes at 28 days. Means in columns with different letters have treatment differences determined by 95% confidence regions.

	Experiment Number					
	1[t]		2[t]		3[t]	
Temperature	28d	D	28d	D	28d	D
5°C[#]	0*					
12°C[#]	0*					
15°C[#]			69.1a	21.3a		
17°C[#]	2323.7a	6.7a	178.2b	11.3b		
20°C[#]	536.4b	6.9a	241.8b	9.4c		
25°C	2868.2c	6.8a	393.4c	7.5d	398.2a	6.9a
28°C			212.9b	5.9e	39.8b	6.9a
32°C					14.1*	6.5*
36°C					0*	
40°C[#]					0*	

[#] Treatment moved to 25°C after 28 days

[t] No change after 28 days

* Insufficient or no data for inclusion in the analysis

Table 2. Total numbers of *Australosepsis niveipennis* emerging and development times (D = days to 50% emergence after the deposition of bovine dung) at different temperature regimes at 28 and 56 days. Means in columns with different letters have treatment differences determined by 95% confidence regions.

	Experiment Number						
	1[t]		2			3[t]	
Temperature	28d	D	28d	56d	D	28d	D
5°C[#]	0*						
12°C[#]	0*						
15°C[#]			0*	31.8a	37.1a		
17°C[#]	156.1a	19.0a	40.3a	209.3b	30.7b		
20°C[#]	351.4b	14.0b	644.5b	947.3c	26.0c		
25°C	427.7b	9.2c	1100.2c	1100.2cd	20.0d	568.4a	12.5a
28°C			1105.8c	1105.8d	17.9e	560.6a	11.0a
32°C						585.2a	8.1b
36°C						528.0a	7.0b
40°C[#]						43.0*	7.0*

[#] Treatment moved to 25°C after 28 days

[t] No change after 28 days

* Insufficient or no data for inclusion in the analysis

Table 3. Total numbers of *Leptocera mirabilis* emerging and development times (D = days to 50% emergence after the deposition of bovine dung) at different temperature regimes at 28 and 56 days. Means in columns with different letters have treatment differences determined by 95% confidence regions.

	Experiment Number							
	1			2			3[t]	
Temperature	28d	56d	D	28d	56d	D	28d	D
5°C[#]	0*	0*						
12°C[#]	0*	0*						
15°C[#]				0*	5.9a	40.8a		
17°C[#]	0*	2.6a	32.2a	0*	5.6a	35.1b		
20°C[#]	4.7a	4.8b	25.8b	13.9a	15.0b	25.4c		
25°C	4.0a	4.0b	15.4c	5.1b	5.1a	18.9d	33.9a	16.8a
28°C				8.0b	8.0a	17.3d	49.2b	15.9a
32°C							30.8a	11.1b
36°C							15.8c	12.2c
40°C[#]							0*	

[#] Treatment moved to 25°C after 28 days

[t] No change after 28 days

* No data for inclusion in the analysis

Table 4. Total numbers of *Pyrellia tasmaniae* emerging and development times (D = days to 50% emergence after the deposition of bovine dung) at different temperature regimes at 28 and 56 days. Means in columns with different letters have treatment differences determined by 95% confidence regions.

	Experiment Number							
	1			2			3[t]	
Temperature	28d	56d	D	28d	56d	D	28d	D
5°C[#]	0*	0*						
12°C[#]	0*	0*						
15°C[#]				0*	0*			
17°C[#]	0*	8.4a	29.6a	0*	30.1a	30.9a		
20°C[#]	19.4a	19.4b	20.7b	109.9a	109.9b	23.9b		
25°C	20.4a	20.4b	13.9c	145.9b	145.9c	16.2c	28.2a	13.0a
28°C				63.5c	63.5d	16.0c	36.4b	12.0b
32°C							42.4c	9.9c
36°C							25.8a	9.9c
40°C[#]							0*	

[#] Treatment moved to 25°C after 28 days

[t] No change in number after 28 days

* No data for inclusion in the analysis

P. tasmaniae was abundant and active at temperatures between 20°C and 36°C (table 4). Some juveniles survived at 17°C for 28 days in experiments 1 and 2. They emerged 24 to 48 hours after the temperature was raised to 25°C. Development times decreased as the temperature increased and were similar at the same temperature in the different experiments. Low numbers emerged naturally at 17°C with a development time of 40.3 days (table 5). Adults started to emerge in 24 to 48 hours when the temperature was raised after 28 days at 17°C. A few individuals also emerged after 42 days although most had emerged earlier from this treatment as the development time was 38.6 days. All individuals emerged within 45 days.

Table 5. Total numbers of four species of fly emerging and development times (D = days to 50% emergence after the deposition of bovine dung) at 28°C, 17°C and after being moved to 28°C after being kept at 17°C for different times. Means in columns with different letters have treatment differences determined by 95% confidence regions.

	Psychoda sp.		*A. niveipennis*		*L. mirabilis**		*P. tasmaniae*	
Treatment	**Number**	**D**	**Number**	**D**	**Number**	**D**	**Number**	**D**
28°C	115.8a	7.2a	564.1a	18.7a	7.9	19	103.2a	17.0a
17°C → 28°C at								
28 days	0*		684.3b	30.9b	2.4	36	34.3b	32.2b
42 days	0*		25.3c	36.0c	0.9	44	1.6c	38.6c
56 days	0*		0*		0		0*	
17°C	163.0b	11.9b	219.1d	34.3c	1.4	43	6.3c	40.3c

* Insufficient or no data for inclusion in the analysis

DISCUSSION

Of the flies found in fresh dung, *Psychoda* sp., *A. niveipennis* and *L. mirabilis* were each present throughout the year. *P. tasmaniae* was absent from dung in winter but probably survived nearby as it re-appeared in September. Overwintering of *P. tasmaniae* as pupae was possible as the larvae leave dung and pupate in the soil. In contrast, *C. brevitarsis* commonly fails to survive winter in the study area which it recolonises during spring and summer (Bishop *et al.* 1995). After it re-establishes, *C. brevitarsis* increases progressively in number to peaks in autumn until slowed or stopped by low temperatures near winter. *A. niveipennis* and *P. tasmaniae* developed in a similar manner but, while *L. mirabilis* also appeared capable of progressive growth it was not as active in summer as the temperature data would suggest.

The lower limit to activity for all species except *Psychoda* sp. was about 17°C. This was not a developmental threshold as some larvae developed at lower temperatures which apparently inhibited emergence. *C. brevitarsis* has a similar response to temperature partly because it has different developmental thresholds at different stages of its life cycle (Allingham 1991). *Psychoda* sp can be active in winter (Colless and McAlpine 1991) and was the least tolerant of high temperatures. This was reflected in reduced activity in summer and possibly explains why its females appear to oviposit beneath the dung pat (Bishop *et al.* 1996b). Each of the other species responded to temperatures up to 36°C with *A. niveipennis* the only species to emerge at 40°C.

Psychoda sp. had fully emerged before 28 days at 17°C and was therefore unaffected by changes in temperature imposed as treatments in experiment 4. *A. niveipennis* and *P. tasmaniae* were similar to *C. brevitarsis* in that they had apparently completed larval development at 17°C but were inhibited at the pupal stage (i.e. they emerged 24 to 48 hours after temperatures were raised after 28 days). In contrast, *L. mirabilis* took several days to emerge after the temperature was raised. It was therefore either inhibited in the larval stage or exhibited some form of diapause. Development of each species slowed further below 17°C and none survived at a constant 12°C.

A. niveipennis appeared to develop at different rates at the same temperature in different experiments. Dung used in these experiments was collected over a long period and was progressively exposed to temperatures declining towards winter. Field exposure was necessary to allow time for flies to oviposit and declining temperature during the oviposition period has been shown to have a significant effect on the development of *C. brevitarsis* (Bishop *et al.* 1996c). A similar effect may have been possible with *A. niveipennis*. Development would therefore have been affected early in the life cycle. Conversely, the development of *P. tasmaniae*, *Psychoda* sp. and *L. mirabilis* was consistent across the experiments.

The fly fauna as given is only part of the total zoological fauna possibly affecting the development of *C. brevitarsis*. It probably does not represent fully the total number of fly species capable of breeding in dung as oviposition was limited to two-three days. However, it was the dominant fly fauna present when *C. brevitarsis* is ovipositing and undergoing the major part of its development. The fauna was taxonomically diverse and, while species consistently varied in the time and sequence of emergence as adults, they mix spatially with *C. brevitarsis* as larvae. *C. brevitarsis* enters the system each season when the other flies are already active and develops within a range of temperatures similar to most of the other species. Despite being in a situation where the other flies could negatively affect *C. brevitarsis*, it successfully re-establishes and undergoes active population growth each season. It is absent in dung

only when most of the other species have decreased in number and their activity towards winter.

ACKNOWLEDGMENTS

We thank the staff of the C.B. Alexander Agricultural College for their valuable cooperation. The comments of Ms C. Wilson and Dr A.D. Clift were greatly appreciated during the preparation of this manuscript. Funding was received from the Meat Research Corporation.

REFERENCES

Allingham, P.G. (1991). Effect of temperature on late immature stages of *Culicoides brevitarsis* (Diptera: Ceratopogonidae). *J. Med. Ent.* **28**:876–881.

Bishop, A.L., Barchia, I.M. and Harris, A.M. (1995). Last occurrence and survival during winter of the arbovirus vector *Culicoides brevitarsis* at the southern limit of its distribution. *Aust. Vet.* J. **72**: 53–55.

Bishop, A.L., Kirkland, P.D., McKenzie, H.J. and Barchia, I.M. (1996a). The dispersal of *Culicoides brevitarsis* in eastern New South Wales and associations with the occurrences of arbovirus infections in cattle. *Aust. Vet. J.* **73:**174–178.

Bishop, A.L., McKenzie, H.J., Barchia, I.M., Murison, R. and Spohr, L.J. (1996b). Positions of juvenile stages of *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) and of four other flies in bovine dung. *Aust. J. Entomol.* **35**:209–212.

Bishop, A.L., McKenzie, H.J., Barchia, I.M. and Harris, A.M. (1996c). Effect of temperature regimes on the development, survival and emergence of *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) in bovine dung. *Aust. J. Entomol.* **35**:361–368.

Cannon, L.R.G. and Reye, E.J. (1966). A larval habitat of the biting midge *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae). *J. Ent. Soc. Qld.* **5**: 7–9.

Colless, D.H. (1980). Biogeography of Australian Sepsidae (Diptera). *Aust. J. Zool.* **28**: 65–78.

Colless, D.H. and McAlpine, D.K. (1991). Diptera. In *The Insects of Australia*, Vol. II. CSIRO, Division of Entomology, Melbourne University Press, Carlton, Vic.

Draper, N. and Smith, H. (1981). *Applied Regression Analysis*, Second Edition. Wiley and Sons, New York.

Dutton, T., Archer, C., Muddle, R. and Bruwer, D. (1993). *The Climate at Tocal*. NSW Agriculture Agdex 020, ISSN 0813–7162.

Dyce, A.L. and Marshall, B.D. (1988). An early record of *Culicoides* species (Diptera:Ceratopogonidae) developing in dung of game animals in southern Africa. *Onderstepoort J. Vet.* Res. **56**: 85–86.

Hughes, R.D., Greenham, P.M., Tyndale–Biscoe, M. and Walker, J.M. (1972). Synopsis of observations on the biology of the Australian bushfly (*Musca vetustissima* Walker). *J. Aust. Ent. Soc.* **11**: 311–331.

McCullagh, P. and Nelder, J.A. (1989). *Generalised Linear Models*, 2nd Edn. Chapman and Hall, London.

Muller, M.J., Standfast, H.A., St. George, T.D. and Cybinski, D.H. (1982). *Culicoides brevitarsis* (Diptera: Ceratopogonidae) as vectors of arboviruses in Australia. Proc. 3rd Symp. on Arbovirus Research in Australia, Brisbane, pp 43–49. (Eds T.D. St George and B.H. Kay).

Murray, M.D. and Nix, H.A. (1987). Southern limit of distribution and abundance of the biting midge *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae), in south-eastern Australia; an application of the GROWEST model. *Aust. J. Zool.* **35**: 575–585.

This page left blank intentionally