Seasonal Field Analyses of Fat Content, Live Weight, Dry Weight and Water Content of the Aphidophagous *Scymnodes lividigaster* (Mulsant) and Mycophagous *Leptothea galbula* (Mulsant) (Coleoptera : Coccinellidae)

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Abstract

Over 36 and 24 months respectively, both the aphidophagous *S. lividigaster* and the mycophagous *L. galbula* accumulated fat before periods of dormancy, but contained little fat after dormancy and during breeding. Over the whole period there were no significant differences between sexes or between years in either species, except that dormant females contained significantly more fat than males, and that during breeding male *S. lividigaster* contained significantly more than females. Subjective assessment of fat reserves gave the same results as did quantitative analysis. Live and dry weights of females were higher during breeding and dormancy than post dormancy; live weights of females were higher than those of males. Water content was lower during dormancy than during breeding. In *L. galbula*, seasonal changes were similar in a dry and in a well watered habitat; any differences appeared related to a higher survival rate in females compared with males, especially in the poorer habitat. In *S. lividigaster*, seasonal changes in fat content were negatively related to changes in abundance of an essential prey species.

Introduction

Fat accumulation before the onset of harsh environmental conditions ensures the survival of some ladybird species for periods as long as 9 months (Hodek and Cerkasov 1961; Hagen 1962). Increased fat accumulation is often associated with dormancy, including reproductive diapause (Hodek 1973).

Field studies during 1975–78 in the Sydney area have shown that the developmental cycle of the ladybirds *Scymnodes lividigaster* (Mulsant) and *Leptothea galbula* (Mulsant) are synchronized with the availability of essential aphid and fungal food supply. In the study area, *S. lividigaster* breeds in spring and in late summer–autumn when feeding on the aphid *Aphis eugeniae* van der Goot which infests the cheese tree, *Glochidion ferdinandi* (Muell. Arg.). By contrast, *L. galbula* breeds in summer–autumn on powdery mildew fungus, *Oidium* sp., infesting *Lonicera fragrantissima* Lindl. & Paxt. and cucurbits. The breeding periods are separated by dormancy, in summer and winter in *S. lividigaster*, and in winter only in *L. galbula*. Both species consume other food, such as pollen, during their developmental cycle (Anderson 1979).

Although fat accumulation is often associated with dormancy in northern-hemisphere species (Hodek 1973), no seasonal field studies of fat content in Australian ladybirds have been reported. Similarly, information on the seasonal availability in Australia of food essential to reproduction is lacking. Since changes

in live weight, dry weight, and water content are known to accompany changes in fat accumulation, fluctuations in these factors were also recorded concurrently with fat analysis. Fat content has previously been estimated by either subjective or quantitative methods (Hodek and Cerkasov 1961; El Hariri 1966*a*, 1966*b*). In the present study, both types of method were used simultaneously so that their effectiveness could be assessed.

Materials and Methods

Study Sites

S. *lividigaster* and L. *galbula* were both sampled four times per month at Chinaman's Beach reserve, an area of 6 ha at Middle Harbour, Sydney, from June 1975 to May 1978. L. *galbula* was also sampled, but with reduced frequency, from a garden at the University, which was cultivated with cucurbits inoculated with powdery mildew, from January 1977 to May 1978.

Subjective Estimation of Fat

Male and female ladybirds were dissected in Ringer's solution. Each fat body was examined and classified by appearance into four arbitrary classes:
   (1) Little fat; fat body not obscuring viscera and usually found as accumulations in the parietal layer.
   (2) Some fat often with accumulation in region of viscera, but not obscuring them.
   (3) Fat in quantity, with accumulations in viscera; fat body may obscure viscera but does not entirely fill abdomen, and often contains numerous small oil droplets. Seldom associated with gravid females.
   (4) Fat totally filling and distending abdomen, obscuring reproductive organs and alimentary canal; fat body often as massive oil droplets, which may exude on dissection. Never associated with gravid females.
   Results are expressed as percentage of the population per class per month; a rating of from 1 (class 1) to 4 (class 4) units is used to calculate total fat units per month for each sex and total fat units per individual per month.

Quantitative Estimation of Fat, Live Weight, Dry Weight and Water Content

Samples were collected every 4-8 weeks between June 1976 and July 1978. Procedures were adapted from those described by El Hariri (1966*b*). Individual ladybirds were weighed live on a Mettler H20T balance with a sensitivity of 10^{-5} g, sexed (in the case of S. *lividigaster*), killed by deep freezing and either processed immediately or deep frozen for later analysis.

   For the analysis, they were dried in small petri dishes for 48 h at 70°C, or until constant weight was achieved (24-37 h) to obtain dry weight and water content; then defatted in a Soxhlet apparatus with light petroleum (BP 40-60°C) for 24 h to remove phospholipids, neutral fats, fatty acids and other soluble substances; returned to the oven at 70°C for 24 h, and weighed to estimate defatted dry weight. Fat content was estimated by difference. L. *galbula* was sexed by dissection.

   Especially under humid conditions, dried insects were found to absorb moisture from the air. To avoid this, the covered glass petri dishes containing dried insects were cooled in a desiccator for 1 h after removal from oven and were then weighed.

   Teneral adults, identified by their soft, pale elytra and by a fat body similar to that of larvae, were excluded from analysis as they did not reflect the characteristics of the general population. Their fat content and dry weight are low and their water content high (El Hariri 1966*a*; Anderson, unpublished data).

Estimating Aphid Abundance

   A. *eugeniae* populations were estimated by subjective and by quantitative biomass methods (Anderson 1981*c*). Briefly, aphids were graded each week into six classes ranging from zero aphids (class 1) to a very heavy infestation (class 6). The biomass method involved weighing samples and estimating a total weekly biomass. Interpretation of results is based on monthly mean values.

The non parametric Sign test (*s* test), the Wilcoxon matched-pairs signed-rank test (ws test) and Spearman's rank correlation coefficient test (SR test) (Siegel 1956) were used in analysing the data, which showed no evidence of normal distribution, were inherently ranked, and had some small samples.

Results

Subjective Estimation of Fat Quantity

In the reserve, females of both species (Table 1) began to accumulate fat before winter dormancy (March, April). Fat increased rapidly until June and then decreased to a low point in September or October, during or before breeding. In *S. lividigaster* fat accumulated again in November or early December, before summer dormancy, and was depleted variably between January and March, before and during late summer–autumn breeding. Fat was accumulated by some *L. galbula* females in the summer breeding period (December–March), but not by the majority. In both species, males showed similar trends in fat accumulation to females, but the results for male *L. galbula* should be evaluated with caution because of the small samples. Over 36 months of study, the quantity of fat between the sexes in both species did not differ significantly ($P > 0.05$, ws test). However, when the data for reproductive and dormant periods were analysed separately, differences between sexes in fat accumulation were found. (In *S. lividigaster* the reproductive periods are October and February–March, dormancy December, April–July; in *L. galbula* the reproductive period is October–March, dormancy May–July.) *S. lividigaster* males had a significantly higher fat content than females during the reproductive periods ($P = 0.016$, *s* test). By contrast, females had significantly more fat than males during winter dormancy ($P = 0.01$, ws test). During summer and winter dormancy of *S. lividigaster* (treated together as the summer dormancy sample was too small for separate analysis), females also had significantly more fat than corresponding males ($P = 0.025$, ws test). In *L. galbula* the fat content of females was also significantly higher than that of males during winter dormancy ($P = 0.025$, ws test), but not during reproduction ($P > 0.05$, ws test).

Because the population dynamics of both species differed greatly between 1976 and 1977 (Anderson 1981a) overall fat accumulation in each year was compared by ranking data on fat units per month per individual. There was, however, no significant difference between the fat contents of individuals in the different years ($P > 0.05$, ws test).

Quantitative Estimations

(i) Fat content

In the reserve habitat, pooled data for both species showed that fat content was highest overall during winter predormancy and dormancy (April–July). In *S. lividigaster* only, fat content was also high in summer dormancy periods. Fat content was low in the months following dormancy and in breeding periods (Figs 1, 2). Individual male and female data showed similar trends to pooled data, but the small samples in some months make use of such data equivocal. Generally, the fat content of dormant females was greater than that of corresponding males but, by contrast, fat content in males exceeded that in females during reproduction.

Table 1. Seasonal variation in subjectively estimated fat deposits in *S. lividigaster* and *L. galbula*, 1975–78

Values are mean monthly scores on a range of from 1 to 4, with sample sizes in italics

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
S. lividigaster females												
1975	—	—	—	—	—	2·0 *(6)*	2·5 *(17)*	1·7 *(9)*	1·5 *(11)*	1·2 *(13)*	2·1 *(12)*	2·3 *(7)*
1976	1·6 *(10)*	1·0 *(14)*	1·2 *(10)*	3·1 *(23)*	3·3 *(9)*	3·6 *(11)*	2·4 *(26)*	1·5 *(16)*	1·1 *(14)*	1·4 *(12)*	1·7 *(13)*	1·9 *(18)*
1977	2·0 *(3)*	2·5 *(2)*	1·2 *(5)*	2·1 *(12)*	2·1 *(9)*	3·0 *(12)*	3·1 *(18)*	2·0 *(15)*	1·7 *(13)*	1·7 *(10)*	2·3 *(16)*	2·3 *(12)*
1978	2·5 *(10)*	1·6 *(16)*	1·8 *(11)*	3·0 *(14)*	3·4 *(14)*	—	—	—	—	—	—	—
S. lividigaster males												
1975	—	—	—	—	—	2·0 *(12)*	2·2 *(10)*	2·0 *(13)*	1·3 *(12)*	1·5 *(18)*	2·6 *(8)*	2·3 *(11)*
1976	1·8 *(4)*	1·1 *(30)*	1·3 *(16)*	2·9 *(39)*	3·2 *(6)*	3·3 *(10)*	2·3 *(12)*	1·6 *(11)*	1·3 *(12)*	1·5 *(12)*	1·6 *(8)*	2·1 *(10)*
1977	1·5 *(2)*	2·5 *(2)*	2·0 *(2)*	1·8 *(10)*	2·3 *(7)*	3·0 *(13)*	3·1 *(17)*	1·6 *(10)*	1·7 *(11)*	1·6 *(6)*	1·7 *(10)*	2·2 *(9)*
1978	2·6 *(9)*	1·6 *(9)*	1·4 *(7)*	1·8 *(11)*	2·3 *(12)*	—	—	—	—	—	—	—
L. galbula females												
1975	—	—	—	—	—	3·0 *(4)*	1·9 *(10)*	2·3 *(18)*	2·0 *(4)*	0 *(0)*	1·6 *(5)*	2·3 *(9)*
1976	1·8 *(9)*	2·0 *(3)*	1·5 *(4)*	3·0 *(23)*	3·5 *(10)*	3·6 *(17)*	2·6 *(17)*	1·5 *(17)*	1·8 *(12)*	1·3 *(6)*	1·5 *(12)*	1·6 *(17)*
1977	1·8 *(17)*	0 *(0)*	1·0 *(6)*	2·6 *(11)*	2·4 *(7)*	2·8 *(13)*	3·8 *(5)*	2·8 *(5)*	2·5 *(3)*	1·2 *(4)*	1·5 *(11)*	2·1 *(12)*
1978	2·3 *(4)*	2·3 *(4)*	2·5 *(4)*	4·0 *(1)*	4·0 *(4)*	—	—	—	—	—	—	—
L. galbula males												
1975	—	—	—	—	—	3·3 *(3)*	1·4 *(9)*	2·3 *(6)*	1·0 *(3)*	2·5 *(2)*	1·5 *(4)*	1·3 *(3)*
1976	2·0 *(3)*	1·0 *(1)*	2·0 *(3)*	2·5 *(12)*	0 *(0)*	3·1 *(8)*	1·7 *(6)*	1·2 *(5)*	1·3 *(3)*	2·0 *(4)*	2·5 *(8)*	1·3 *(13)*
1977	0 *(0)*	2·0 *(1)*	2·0 *(2)*	1·0 *(1)*	0 *(0)*	2·0 *(4)*	2·7 *(3)*	3·0 *(1)*	2·0 *(2)*	0 *(0)*	1·8 *(15)*	1·8 *(6)*
1978	2·0 *(5)*	2·8 *(4)*	1·4 *(5)*	3·0 *(2)*	3·4 *(8)*	—	—	—	—	—	—	—

With both ladybird species there was a high correlation between subjective and quantitative fat determinations by non-parametric ($S.$ $lividigaster$: n prs $=$ 21, R_s $=$ $0 \cdot 7720$, P $=$ $0 \cdot 01$; $L.$ $galbula$: R_s $=$ $0 \cdot 7079$, P $=$ $0 \cdot 01$) and parametric analyses ($S.$ $lividigaster$: r $=$ $0 \cdot 7854$, P $<$ $0 \cdot 001$; $L.$ $galbula$: r $=$ $0 \cdot 6990$, P $<$ $0 \cdot 001$). The coefficients were even higher when tests were repeated after exclusion of data for months having less than 10 specimens.
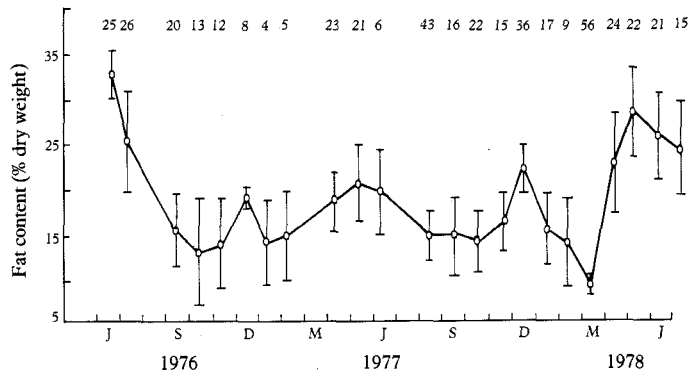
Fig. 1. *S. lividigaster*, monthly quantitative estimation of fat content as percentage of dry weight, pooled male and female data, from Chinaman's Beach reserve, 1976–78. Vertical lines represent ± 2 standard errors. Above the graph, numbers of individuals analysed each month.
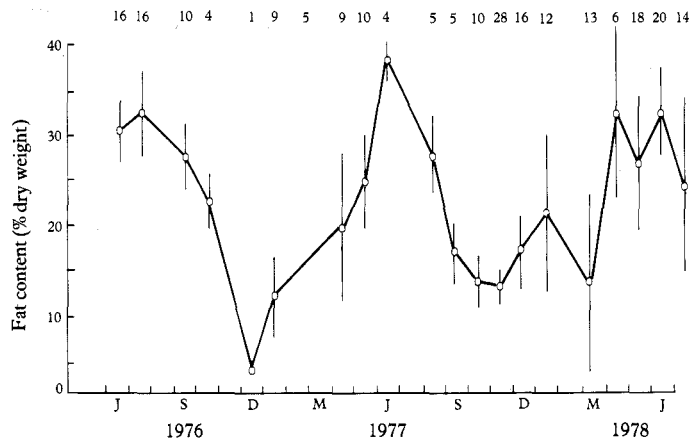

Fig. 2. *L. galbula*, monthly quantitative estimation of fat content as percentage of dry weight, pooled male and female data, from Chinaman's Beach reserve, 1976–78. Vertical lines represent ± 2 standard errors. Above the graph, numbers of individuals analysed each month.

(ii) *Live weight*

Mean female live weight (LW) displayed consistent seasonal trends in both species, rising in breeding periods and in most dormant periods. Generally, LW was lowest after dormancy. Female mean LW was significantly higher than that of males (*S. lividigaster* and *L. galbula*, P $=$ $0 \cdot 005$, ws test). In *S. lividigaster* (Fig. 3) female

LW exceeded that of males by an average of 16% and in *L. galbula* it did so by 26%. Trends in male LW were generally similar to those in females, but values tended to be very low in post-dormancy, and rose less in the breeding period than those of females.
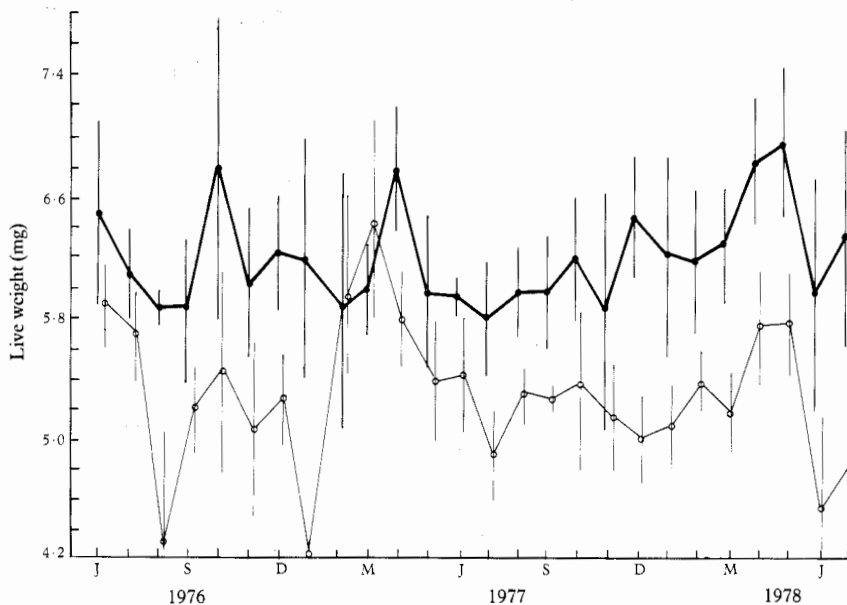

Fig. 3. *S. lividigaster*, monthly live weights of males (○) and females (●) from Chinaman's Beach reserve, 1976–78. Vertical lines represent ± 2 standard errors.
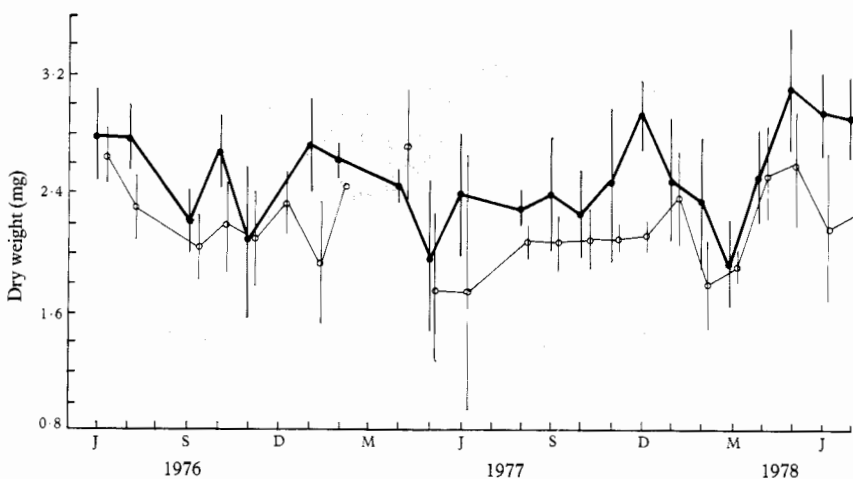

Fig. 4. *S. lividigaster*, monthly dry weights of males (○) and females (●) from Chinaman's Beach reserve 1976–78. Vertical lines represent ± 2 standard errors.

(iii) *Dry weight*

Mean female dry weight (DW) in both species varied less than LW, and generally tended to be high in the predormancy feeding and dormancy periods and low in post-breeding and post-dormancy periods, though interpretation of results be-

tween January and June 1977 is difficult because of the sample size. The seasonal changes in DW of male *S. lividigaster* were more marked than those in females (Fig. 4). Data on DW of male *L. galbula* were insufficient to allow interpretation.

(iv) *Water content*

No regular seasonal trends could be found when male and female data were considered separately. However, pooling the data showed that there were seasonal trends in water content (WC) in both species. In *S. lividigaster* (Fig. 5) mean values of less than 59% WC were nearly always associated with dormancy and high fat content; conversely, mean values of over 62% WC were associated with pre- or
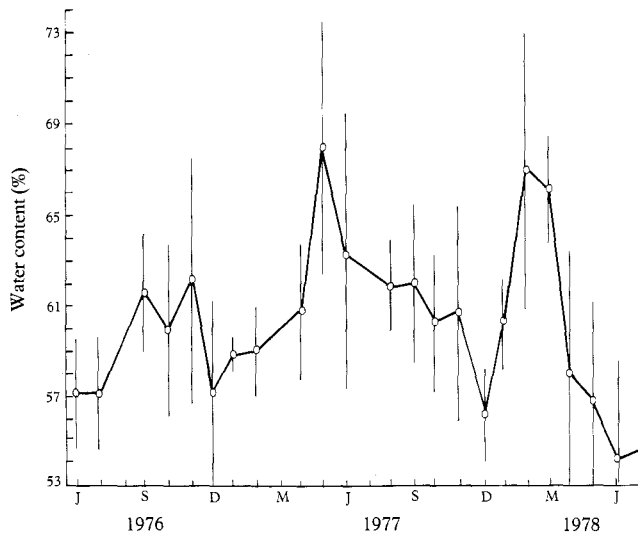

Fig. 5. *S. lividigaster*, monthly water content, pooled male and female data from Chinaman's Beach reserve 1976–78. Vertical lines represent ± 2 standard errors.

post-breeding periods, when ladybirds had large watery gut contents (Anderson, unpublished data). In breeding periods, WC values tended to lie between these extremes. In *L. galbula* WC values of over 62% were associated with predormancy feeding and breeding, and values under 60% with dormancy.

The data for individual male and female *S. lividigaster* (June 1976–April 1977) were examined by parametric methods to determine whether WC and fat were correlated. These factors were always negatively correlated, hence no further data was treated in this manner.

L. galbula *in the University Garden Habitat*

In the University garden nearly all data collected was related to breeding rather than dormancy periods, because the cucurbits and powdery mildew died in winter and the ladybirds dispersed. Comparison between *L. galbula* in the two study areas indicated that fat levels of ladybirds did not differ significantly between the habitats ($P > 0.05$, WS test). Within the garden habitat, female LW was consistently

and significantly higher (18%) than that of males ($P < 0.05$, ws test). Comparing the two habitats, the lw of garden females was higher than that of reserve females, but not significantly so ($P > 0.05$, ws test). However, male lw was significantly higher in the garden than in the reserve ($P = 0.01$, ws test). According to the ws test, dw of both males and females did not differ significantly between habitats ($P = 0.05$ for both sexes). By contrast, the s test showed a significant difference in male dw between habitats ($P = 0.035$), but not in female dw ($P > 0.05$).

Table 2. Fat content of *S. lividigaster* in relation to abundance of *Aphis eugeniae*

The subjective estimate of monthly abundance of *A. eugeniae* is based on a scale of from 1 to 6; the quantitative estimation is based on the total biomass in milligrams (x) of 68 sample branches, and expressed as log ($x + 1$)

Year	Month	Mean fat content in *S. lividigaster* (% DW)	*A. eugeniae* abundance	
			Subjective estimate	Quantitative estimation
1976	June	33·2	1·25	—
	July	25·7	1·25	—
	Aug.	—	1·50	—
	Sept.	16·3	2·25	—
	Oct.	12·9	3·50	4·96
	Nov.	13·5	2·00	2·26
	Dec.	19·7	1·25	0
1977	Jan.	14·0	1·50	0·87
	Feb.	15·1	1·00	0
	Mar.	—	2·50	2·75
	Apr.	18·1	4·25	5·65
	May	20·4	1·25	0·47
	June	19·5	1·00	0
	July	—	1·00	0
	Aug.	14·6	1·25	0·08
	Sept.	14·9	1·50	0·03
	Oct.	14·4	2·25	2·63
	Nov.	16·9	1·50	0·80
	Dec.	22·2	1·50	0
1978	Jan.	15·9	1·50	0·89
	Feb.	13·6	3·75	5·80
	Mar.	8·7	3·75	3·89
	Apr.	23·1	2·00	1·13
	May	28·1	2·75	5·18
	June	25·8	1·00	—
	July	24·7	1·00	—

Pooled wc data of males and females from the garden were difficult to interpret because of lack of continuity, but generally they indicated the same trends as that in the reserve. Comparison of wc data from both habitats showed no significant difference between them ($P > 0.05$, ws test).

Relationship between Fat Content of Ladybirds and Abundance of A. eugeniae *and* Oidium *sp.*

In *S. lividigaster*, when aphid food was most abundant (Anderson 1981c) the

fat content was reduced (Table 2). That is, fat content of *S. lividigaster* (quantitative data) and abundance of its aphid food (both subjective and quantitative data) were negatively and significantly correlated. With subjective data on aphids: $n = 23prs$, $R_s = -0.4573$, $P = 0.05$; with quantitative data: $n = 20prs$, $R_s = -0.4221$, $P = 0.05$. The fat content of *L. galbula* was also related to the type of food consumed. *Oidium* sp. was the major food consumed during breeding and was associated with lower fat content (although no quantitative data were collected). In both species the highest fat content was associated with periods of intense feeding, before dormancy, on alternative foods such as pollen (Anderson, unpublished data).

Discussion

Despite the obvious limitations of subjective methods, a significant correlation was found between the data from concurrent subjective and quantitative analyses for fat. In both *S. lividigaster* and *L. galbula*, consistent changes in the live weight, dry weight, fat and water contents of adult males and of adult females occurred seasonally.

Fat was accumulated before dormancy and was depleted greatly during dormancy. It was generally much lower during and after breeding periods. Fat accumulation before dormancy shows that dormancies of both species were reproductive diapauses, not quiescences, as suggested by Anderson and Richards (1977).

Analysis of a whole year's data indicated no difference between the fat contents of males and females; however, if reproductive and dormant periods were considered separately, differences in fat content between the sexes emerged. Females' reserves are drained for egg production during breeding, whereas male requirements are less. Females also require greater fat reserves than males on entering dormancy, so that they leave dormancy with some reserves for previtellogenesis and later vitellogenesis.

The fat content of individuals varied little between 1976 and 1977, even though the seasons and population levels differed markedly (Anderson 1981a). It appears that large changes in the size of ladybird populations were not accompanied by large changes in fat content of the individuals within the population. This suggests that availability of food regulates size of population but does not modify the food reserves of individuals.

Sexual dimorphism of body size was marked, particularly in *L. galbula*, and live weights showed consistent seasonal trends. Male and female LW often appeared to differ most after dormancy, possibly because of greater male activity at this time, associated with searching for a mate.

Female dry weights varied seasonally less than equivalent live weights. At the end of dormancy fat content in both sexes fell more quickly than DW. This was probably a reflection of a switch to protein metabolism, associated with breeding.

Use of the pooled data on water content for the two sexes indicated that there were seasonal trends in WC, closely related to the developmental cycle of the ladybirds. As the adult ladybird is relatively inexpansible, it was expected that WC would be negatively correlated with fat reserves, and the data are consistent with this hypothesis. No seasonal studies of WC have been previously reported, though Hagen (1962) and Hodek (1973) suggest that short-term changes in WC of aggre-

gating dormant ladybirds are related to environmental conditions such as drought.

The negative relationship between fat content in *S. lividigaster* and aphid abundance highlights the species' food specificity. Clearly, different roles are played by the essential and alternative foods in the developmental cycle (Anderson 1980). One might assume consumption of abundant essential food would cause fat deposition, but it does not. Instead, it stimulates reproduction. Fat deposition is associated with consumption of alternative food, such as pollen, in the predormancy feeding periods (Anderson, unpublished data). The difference in periodicity of body reserves of the two ladybird species in summer can be accounted for by the difference in periodicity of their essential food supplies. In summer, aphid food of *S. lividigaster* was absent, whereas there was always supply of *Oidium* sp. (albeit very variable) for *L. galbula*. Both essential foods were absent in winter. So far, there is no explanation for the mechanism by which the different metabolic pathways are stimulated by different types of food.

When data on *L. galbula* from the natural reserve habitat were compared with that for ladybirds from the well watered and tended garden habitat, many similarities in pattern of utilization of body reserves were apparent, particularly in the seasonal LW and DW of females. However, there were differences between the ability of the sexes to accumulate and retain reserves. Enhanced female survivorship is suggested by the lower LW and DW of males in the reserve compared with those in the garden habitat, and this may, in some way, account for the female-biased sex ratio of this species (Anderson 1981*b*).

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