Response of the Agile Antechinus to Habitat Edge, Configuration and Condition in Fragmented Forest

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Abstract

Habitat fragmentation and degradation seriously threaten native animal communities. We studied the response of a small marsupial, the agile antechinus *Antechinus agilis*, to several environmental variables in anthropogenically fragmented *Eucalyptus* forest in south-east Australia. Agile antechinus were captured more in microhabitats dominated by woody debris than in other microhabitats. Relative abundances of both sexes were positively correlated with fragment core area. Male and female mass-size residuals were smaller in larger fragments. A health status indicator, haemoglobin-haematocrit residuals (HHR), did not vary as a function of any environmental variable in females, but male HHR indicated better health where sites’ microhabitats were dominated by shrubs, woody debris and trees other than *Eucalyptus*. Females were trapped less often in edge than interior fragment habitat and their physiological stress level, indicated by the neutrophil/lymphocyte ratio in peripheral blood, was higher where fragments had a greater proportion of edge habitat. The latter trend was potentially due to lymphophoenia resulting from stress hormone-mediated leukocyte trafficking. Using multiple indicators of population condition and health status facilitates a comprehensive examination of the effects of anthropogenic disturbances, such as habitat fragmentation and degradation, on native vertebrates. Male agile antechinus’ health responded negatively to habitat degradation, whilst females responded negatively to the proportion of edge habitat. The health and condition indicators used could be employed to identify conservation strategies that would make habitat fragments less stressful for this or similar native, small mammals.

Introduction

In studies examining habitat fragmentation and degradation effects on animals there has been a tendency to rely on distribution metrics (e.g. occurrence, abundance, density), without much reference to performance indices (e.g. litter size, survivorship, physiological stress). Fletcher et al. [1] noted that in 194 studies of fragment edge and area effects on vertebrates, distribution metrics were almost three times as common as performance indices, despite earlier authors suggesting that understanding how environmental factors limit a population or species’ range requires examination of population densities and at least one index of well-being (fecundity, parasite load, body condition, growth rate etc.) [2].

Decline and extinction of vertebrate populations in fragmented habitat is variously attributed to habitat change (loss, degradation, edge effects and isolation), altered species interactions (predation, parasitism etc.), changed behaviour (edge avoidance, disrupted dispersal, social relationships or resource-tracking), altered physiology (poor body condition and chronic physiological stress) and stochastic threats associated with small population size [1,3,4,5]. The area, spatial configuration, isolation and habitat degradation levels of fragments are considered the key environmental factors influencing these threatening processes [3,6,7,8,9]. However, the relative importance of the putative agents of population decline remain unclear and probably vary among taxa and landscapes [3]. Further research using diverse study areas and species is needed to properly evaluate this possibility.

We report elsewhere on performance and distribution differences between agile antechinus (*Antechinus agilis*, Family: Dasyuridae) populations living in fragmented and continuous *Eucalyptus* forest [10]. Here, we compare responses of this species to landscape configuration (e.g. fragment area, proportion of edge) and microhabitat variables [11] in an anthropogenically-fragmented landscape in order to identify possible causal relationships. The microhabitat variables were either living or dead vegetation. Abiotic features, such as rocks or human-made tracks, and features related to the presence of other animals, such as burrow entrances or dens, were never close enough to an antechinus trapping station to be recorded.

The agile antechinus is the most widespread and common native mammalian carnivore in much of our South Gippsland study area in south-east Australia [12]. It is locally common [12] and consequently not the focus of much conservation effort. However, there is a growing view in conservation biology that successful wildlife management should include a focus on common, native species, as it is preferable to prevent future decline rather than wait until such species become threatened before taking management action. The approach used here could easily be applied to other small mammals that are frequently the focus of fragmentation studies e.g. voles (subfamily *Arvicolinae*) and shrews (family *Soricidae*).
We examined one distribution metric and three independent performance variables in the agile antechinus: (1) relative abundance (based on trapping rates); (2) mass/size residuals (MSR), a well-established index of fat reserves in small mammals [13]; (3) erythrocyte indicators of health status, including a novel metric, haemoglobin-haematocrit residuals (HHR) and (4) leukocyte profile indicators of hypothalamus-pituitary-adrenal (HPA) axis-mediated stress (hereinafter physiological stress [14]). This last variable encompassed the neutrophil-to-lymphocyte ratio (N:L) and total neutrophil, lymphocyte and eosinophil concentrations in peripheral, circulating blood [15]. We used these estimates of population health status to address the following questions:

1) Are blood cell indicators of stress or health status correlated with estimated body condition (MSR)?
2) Do agile antechinus in Eucalyptus forest fragments use some microhabitats preferentially?
3) Are features of fragmented landscapes, such as edge habitat, fragmentation area, microhabitat heterogeneity etc., related to agile antechinus’ abundance, body condition and blood cell indicators of stress or health status?

Results

We captured 734 agile antechinus over 3,780 trap-nights at 30 study sites in 2007 and 2008; 165 males and 131 females were captured at fragment edges and 191 males and 247 females in the interior. Over the two study years, a subset of 263 individuals was measured for mass, morphometrics and haematological indicators of stress. Of these, 76 males and 45 females were captured in fragment edges and 71 males and 71 females in interiors. Relative abundance was calculated from capture rates for edge and interior populations (Table 1).

Relationship between blood cell variables and body condition

In females, the model including Ht best explained variation in body condition (indexed as MSR). As an AIC difference (ΔAIC) ≥ 2 is usually considered reasonable support for a model [16], the ΔAICs between models for female Ht and Hb can only be considered marginal (Ht-Hb ΔAIC = 2.0), whereas there is support for Ht being a better predictor of MSR (estimated fat reserves) than is HHR (Ht-HHR ΔAIC = 3.4). For males, the model including HHR best explained MSR, but the differences among models were not convincing (HHR-Hb ΔAIC = 0.9 and HHR-Ht ΔAIC = 2.1). None of the individual erythrocyte variables were significantly associated with MSR (all P > 0.05). For subsequent analyses we use HHR as a health status indicator, as it is the most readily interpretable of the three erythrocyte variables (Tables 2, 3 and 4).

There were no significant relationships between any of the female leukocyte variables and MSR. Male lymphocyte and eosinophil concentrations were significantly higher where MSR was greater (r = 0.20, P = 0.015 and r = 0.34, P = 0.036, respectively) (Tables 2, 3 and 4). However, these relationships were somewhat confounded, because all three variables were correlated with MONTH during the March–August trapping period (see below) and so were difficult to interpret.

Microhabitat preference

Agile antechinus were captured more often in traps whose local microhabitat was dominated by woody debris than in traps associated with any of the other microhabitat categories (P = 0.033) (Table 5). No other significant relationships between capture sites and microhabitat characteristics were evident.

Table 1. Summary of mean (± s.e.) values obtained for stress and condition indicators in this study.

| FRAGMENTS | | |
|---|---|---|
| **Sex** | **Response variable** | **Edge (<60 m)** | **Interior (>60 m)** |
| **FEMALES** | Relative abundance | 0.006±0.005 | 0.037±0.016 |
| | MSR (g): | −1.05±0.62 | −1.32±0.38 |
| | HHR (g L⁻¹): | −0.24±0.62 | +0.15±1.78 |
| | N:L ratio: | 0.822±0.091 | 1.003±0.158 |
| | Neutrophils (×10⁶ L⁻¹): | 1.39±0.21 | 1.30±0.24 |
| | Lymphocytes (×10⁶ L⁻¹): | 1.82±0.21 | 1.44±0.13 |
| | Eosinophils (×10⁶ L⁻¹): | 7.19±1.76 | 5.64±0.94 |
| **MALES** | Relative abundance | 0.004±0.002 | 0.013±0.010 |
| | MSR (g): | +1.58±0.63 | +2.13±0.70 |
| | HHR (g L⁻¹): | −1.17±1.51 | +1.28±2.03 |
| | N:L ratio: | 0.927±0.103 | 0.967±0.103 |
| | Neutrophils (×10⁶ L⁻¹): | 1.42±0.20 | 1.38±0.14 |
| | Lymphocytes (×10⁶ L⁻¹): | 1.61±0.15 | 1.59±0.13 |
| | Eosinophils (×10⁶ L⁻¹): | 8.02±1.21 | 9.19±1.43 |

*HHR: Haemoglobin-haematocrit residuals.

Table 2. Relationships between blood cell indicators of stress and health status and Mass-Size Residuals (g) for female agile antechinus.

| Blood cell indicator | Variables | df | t-value | P |
|---|---|---|---|---|
| Neutrophils | MONTH | 25 | −0.38 | 0.710 |
| Lymphocytes | MONTH | 25 | −0.42 | 0.681 |
| Eosinophils | MONTH | 25 | −0.49 | 0.625 |
| Haemoglobin (Hb) | MONTH | 25 | −0.43 | 0.669 |
| Haematocrit (Ht) | MONTH | 25 | −0.28 | 0.784 |
| HHR | MONTH | 25 | −0.43 | 0.672 |

*Linear mixed effect model results are shown. The df, t- and P-value are from restricted maximum likelihood models.

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(1) Relative abundance. Relative abundance was significantly greater for females in interior than EDGE habitat.

Table 3. Relationships between blood cell indicators of stress and health status and Mass-Size Residuals (g) for male agile antechinus.

| Blood cell indicator | Variables | df | t-value | P |
|----------------------|-----------|----|---------|---|
| Neutrophils          | MONTH     | 26 | 2.18    | 0.039 |
| Neutrophils (cells L\(^{-1}\)) | 112 | 1.14 | 0.257 |
| Lymphocytes          | MONTH     | 26 | 2.98    | 0.006 |
| Lymphocytes (cells L\(^{-1}\)) | 112 | 2.48 | 0.015 |
| Eosinophils          | MONTH     | 26 | 2.23    | 0.035 |
| Eosinophils (cells L\(^{-1}\)) | 112 | 2.14 | 0.035 |
| NL ratio             | MONTH     | 26 | 3.14    | 0.004 |
| (log)NL ratio        |           | 115 | −0.89 | 0.377 |
| Haemoglobin (Hb)     | MONTH     | 26 | 3.25    | 0.003 |
| Hb (g L\(^{-1}\))    |           | 105 | 1.15 | 0.251 |
| Haematocrit (HT)     | MONTH     | 26 | 3.17    | 0.004 |
| Ht                   |           | 105 | 0.40 | 0.689 |
| HHR                  | MONTH     | 26 | 3.35    | 0.003 |
| HHR (g L\(^{-1}\))   |           | 105 | 1.49 | 0.139 |

*aLinear mixed effect model results are shown. The df, t- and P-value are from restricted maximum likelihood models.

Table 4. Information-theoretic (AIC) parameters for blood cell measurements and time of year (MONTH) as explanatory models for Mass-Size Residuals (g).

| Sex   | Model                                      | AIC* |
|-------|--------------------------------------------|------|
| FEMALES | Neutrophils (cells L\(^{-1}\)) + MONTH | 491.1 |
|        | Lymphocytes (cells L\(^{-1}\)) + MONTH    | 491.0 |
|        | Eosinophils (cells L\(^{-1}\)) + MONTH    | 491.1 |
|        | (log)NL ratio + MONTH                      | 490.7 |
|        | Haemoglobin (Hb) (g L\(^{-1}\)) + MONTH    | 479.2 |
|        | Haematocrit (HT) + MONTH                   | 477.2 |
|        | HHR (g L\(^{-1}\)) + MONTH                 | 480.6 |
|        | Neutrophils (cells L\(^{-1}\)) + MONTH     | 789.8 |
|        | Lymphocytes (cells L\(^{-1}\)) + MONTH     | 784.9 |
|        | Eosinophils (cells L\(^{-1}\)) + MONTH     | 786.4 |
|        | (log)NL ratio + MONTH                      | 804.2 |
|        | Haemoglobin (Hb) (g L\(^{-1}\)) + MONTH    | 745.7 |
|        | Haematocrit (HT) + MONTH                   | 746.9 |
|        | HHR (g L\(^{-1}\)) + MONTH                 | 744.8 |

Interaction terms were examined and discarded from the models.

*bLinear mixed effect model results are shown. The AIC values are from maximum likelihood models (suitable for comparing models).

Table 5. Analysis of the difference between expected (number of traps set) and observed captures of agile antechinus as a function of microhabitat.

| Microhabitat          | Mean ± SE* | df | t-value | P  |
|-----------------------|------------|----|---------|----|
| DEAD EUCALYPT TREE    | 0.01 ± 0.07 | 180 | −0.17   | 0.866 |
| EUCALYPT (<2 m diam.) | −0.12 ± 0.24 | 180 | −0.43   | 0.665 |
| EUCALYPT (>2 m diam.) | 0.07 ± 0.16 | 180 | 0.32    | 0.750 |
| NON-EUCALYPT TREE     | −0.17 ± 0.10 | 180 | 0.20    | 0.842 |
| SHRUB                 | −0.14 ± 0.19 | 180 | −0.45   | 0.651 |
| TEATREE/PAPERBARK     | −0.36 ± 0.07 | 180 | −1.45   | 0.148 |
| WOODY DEBRIS          | 0.48 ± 0.27 | 180 | 2.14    | 0.033 |

*aMeans and SE are shown for residuals of number of captures (observed) and number of traps set (expected) in each microhabitat category (positive sign = greater than expected and negative sign = less than expected). doi:10.1371/journal.pone.0027158.t005

Male relative abundances were significantly greater where PC.2 was higher (r = 0.19, P = 0.002), although r was small. Core habitat area had a significant effect on male relative abundance (P < 0.001), but the relationship was complicated by a significant interaction with PC.3 (P = 0.002). A conditioning plot of CORE

Table 6. Relationships between relative abundance and environmental variables for agile antechinus.

| Sex | Explanatory variables | ρ  | IE | df | t-value | P  |
|-----|-----------------------|----|----|----|---------|----|
| Females | DI                   | 0.03 | 0.6 |    |         |    |
|        | DIST                  | 0.13 | 9.3 |    |         |    |
|        | EDGE                  | 0.26 | 29.8 | 144 | 5.04   | <0.001 |
|        | HETEROGEN.            | −0.02 | 1.6 |    |         |    |
|        | CORE                  | 0.27 | 34.3 | 27 | 1.91   | 0.066 |
|        | MONTH                 | 0.05 | 2.7 |    |         |    |
|        | PC.1                  | −0.14 | 9.6 |    |         |    |
|        | PC.2                  | −0.16 | 11.1 |    |         |    |
|        | PC.3                  | 0.01 | 1 |    |         |    |
| Males | DI                   | 0.1 | 3 |    |         |    |
|        | DIST                  | 0.08 | 7.7 |    |         |    |
|        | EDGE                  | 0.07 | 2.2 |    |         |    |
|        | HETEROGEN.            | 0.14 | 4.9 |    |         |    |
|        | CORE                  | 0.31 | 47.1 | 24 | 5.19   | <0.001 |
|        | MONTH                 | 0.21 | 15.1 |    |         |    |
|        | PC.1                  | −0.02 | 0.4 |    |         |    |
|        | PC.2                  | 0.19 | 12.2 | 24 | 2.44   | 0.022 |
|        | PC.3                  | 0.13 | 7.4 | 24 | −0.97  | 0.343 |
|        | CORE × PC.3           | na | na | 24 | 3.53   | 0.002 |

*Pearson’s correlation coefficients (ρ), the independent effect of variables from hierarchical partitioning (IE), and results of linear mixed effect model fitting are shown. Degrees of freedom, t-value and P-values are shown for variables that were selected for inclusion in the reduced LMEM using Akaike Information Criterion. doi:10.1371/journal.pone.0027158.t006
and PC.3 suggested that the effect of CORE on male relative abundance was generally positive, but that the slope of the effect was less pronounced where PC.3 was greater (Figure 1). (i.e. male abundance was higher in larger fragments except where PC.3 was high). The two most important variables for independently explaining male relative abundances were CORE (47.1%) and MONTH (15%) (Table 6).

(2) Fat stores. Estimated fat reserves (MSR) in females showed significant associations with habitat CORE ($r = -0.27$, $P = 0.001$), HETEROGEN ($r = 0.03$, $P = 0.003$), PC.1 ($r = 0.13$, $P = 0.008$) and PC.3 ($r = -0.05$, $P = 0.025$), although again most $r$ values were small. The variables with the most important independent effects on female MSR were CORE (36.8%) and HETEROGEN (16.8%).

In males, fat reserves were significantly associated with fragment DI ($r = -0.10$, $P = 0.034$), CORE ($r = -0.20$, $P = 0.037$) and MONTH ($r = 0.33$, $P = 0.002$). The interaction term DI×CORE required interpretation before the main effects were examined ($P = 0.059$). A conditioning plot of CORE and DI suggested that the effect of the former on male MSR was generally negative, but that the slope of the effect was less pronounced where DI was shallower (Figure 2) (i.e. fat reserves were smaller in agile antechinus in fragments with a greater core area, but only when the fragments also had a higher ratio of edge to interior habitat). The variables that best explained variation in male fat reserves were MONTH (42.0%) and CORE (16.7%) (Table 7, Figure 2).

(3) Haemoglobin/Haematocrit residuals. Female HHR was not significantly associated with any habitat variable.

**Figure 1.** Conditioning plot of CORE (ha) given PC.3 for male relative abundance. The top box shows regions of PC.3 for which relative abundance is plotted against CORE. The overlap in PC.3 is 25%. Conditioning plots show the range of a response variable (here, male relative abundance) for values of one explanatory variable (here, fragment core area, CORE) over given ranges of a second explanatory variable (here, vegetation condition index PC.3).

**Figure 2.** Conditioning plot of CORE (ha) given DI for male body condition index (MSR). The top box shows regions of DI for which MSR is plotted against CORE. The overlap in DI is 25%.
Variation in female HHR was best explained by HETEROGEN (27.1%) (Table 8).

In males, HETEROGEN (r = 0.07, P = 0.034), PC.2 (r = -0.15, \( P = 0.026 \)) and PC.3 (r = -0.11, \( P = 0.027 \)) were significantly associated with HHR, although the correlation coefficients were small. The variables that best independently explained variation in male HHR were PC.2 (19.4%) and HETEROGEN (16.2%) (Table 8).

(4) Neutrophil-to-lymphocyte ratio. Female N:L was strongly associated with DI (r = 0.53, P = 0.002). Variation in this stress index was best explained by DI (42.6%) and MONTH (25.5%). Male N:L was strongly associated with MONTH (r = 0.53, \( P < 0.001 \)). For males, the best independent, explanatory variables for N:L were MONTH (57.2%) and PC.2 (19.9%) (Table 9).

(5) Leukocyte concentrations. In both sexes, the only significant relationship between an environmental variable and the peripheral blood neutrophil concentration was for MONTH (March to August) (females r = 0.52, \( P < 0.001 \), independent effect = 63.0%; males r = 0.62, \( P < 0.001 \), independent effect = 63.6%) (Table 10).

In females, lymphocyte concentration was significantly associated with DI (r = -0.16, \( P = 0.008 \)), and although EDGE, MONTH, PC.1 and PC.2 were included in the best model, the interaction terms EDGE\( \times \)PC.1 and \( \text{MONTH}\times\text{PC.3} \) were also included. The relationship between PC.1 and lymphocyte concentration was positive in both interior and edge habitat, but more pronounced in populations living near forest edges (Figure 3). The relationship between PC.3 and lymphocyte concentration was difficult to interpret, as the correlation changed from positive to negative during the sampling period (Figure 3). The independent effects on female lymphocyte concentration were strongest for PC.3 (22.3%) and MONTH (19.6%). The best explanatory variables for male lymphocyte concentration were PC.1 (20.2%) and PC.3 (43.3%) (Table 11).

Neither male nor female eosinophil concentration showed a significant relationship with a potential explanatory factor, except for MONTH in males (r = 0.40, \( P < 0.001 \)). The variables that best independently explained variation in eosinophil concentration were PC.3 in females (27.1%) and MONTH (53.9%) in males (Table 12).

Discussion

Relationships between blood cell variables and estimated fat reserves of agile antechinus.

There was no convincing relationship between any immune cell variable and MSR in female agile antechinus. Male lymphocyte and eosinophil concentrations were higher when body condition indices were higher, but these associations were confounded by correlations between MSR, lymphocyte concentration and eosinophil concentration and time in the study period when trapping occurred (MONTH).

Haematocrit, Hb and HHR explained variation in MSR better than any of the leukocyte variables. In both sexes, HHR was positively correlated with MSR, implying that the amount of haemoglobin per unit of packed cell volume was greater in agile antechinus with larger lipid reserves. Theory and empirical
Effects of microhabitat on capture rates

Capture rates were higher than expected where trapping station microhabitat was dominated by woody debris (logs and fallen branches), so agile antechinus could have been foraging preferentially on or beside fallen timber. Such timber could provide arthropods, such as spiders and beetle larvae, which comprise most of the study species’ diet [22], as well as cover from predators [23]. Woody debris density contributed to PC.1 (loading = −0.39), but the latter did not significantly influence agile antechinus’ relative abundance in the various study sites. Thus although agile antechinus preferentially used microhabitats dominated by woody debris, fallen timber density per se did not affect their relative abundances at sites. In contrast, other studies [24,25,26] have found a positive association between Antechinus spp.’ abundance and/or site occupancy and fallen timber volume and/or density. Fallen timber can provide nest sites [27,28], but in our study area the equivalent of at least several home ranges and therefore survivorship or reproductive success e.g. by non-random movement due to a preference for complex microhabitats where predation risk was lower and food abundance higher [23]. This hypothesis could be addressed by (a) trapping agile antechinus and collecting microhabitat information over larger spatial scales (i.e. the equivalent of at least several home ranges and therefore >10 ha [29]), so that the confounding effect of movement into or across trapping grids is reduced [30], or (b) radio-tracking agile antechinus and documenting their movement patterns through the fragmented landscape [31].

Effects of fragment area on agile antechinus’ relative abundance

Agile antechinus’ relative abundance was positively associated with fragment area. Brown antechinus’ population densities also vary with fragment area, but Knight and Fox [28] suggested that the relationship may have been an indirect one, in which smaller fragments were more degraded and the resultant lower habitat complexity negatively affected population density. However, in the

**Table 9. Relationships between (log)Neutrophil:Lymphocyte ratio and environmental variables for agile antechinus.**

| Sex   | Explanatory variables | r   | IE  | df  | t-value | P    |
|-------|-----------------------|-----|-----|-----|---------|------|
| Females | DI                    | 0.53| 42.6| 23  | 3.54    | 0.002|
|        | DIST                  | −0.22| 4.5 |     |         |      |
|        | EDGE                  | −0.18| 7.8 | 85  | −1.23   | 0.222|
|        | HETEROGEN.            | 0.04| 0.7 |     |         |      |
|        | CORE                  | 0.33| 12  |     |         |      |
|        | MONTH                 | 0.39| 25.5| 23  | 1.84    | 0.079|
|        | PC.1                  | −0.1 | 1.1 |     |         |      |
|        | PC.2                  | 0.13 | 1.7 |     |         |      |
|        | PC.3                  | −0.08| 4.2 | 23  | −1.91   | 0.069|
| Males  | DI                    | 0.24| 7.9 |     |         |      |
|        | DIST                  | −0.24| 7.1 |     |         |      |
|        | EDGE                  | −0.06| 0.9 |     |         |      |
|        | HETEROGEN.            | 0.06| 0.7 |     |         |      |
|        | CORE                  | 0.02| 0.4 |     |         |      |
|        | MONTH                 | 0.53| 57.2| 24  | 4.82    | <0.001|
|        | PC.1                  | −0.14| 3.9 |     |         |      |
|        | PC.2                  | 0.35| 19.9| 24  | 1.23    | 0.229|
|        | PC.3                  | −0.04| 2.4 | 24  | −1.5    | 0.146|

*Pearson's correlation coefficients (r), the independent effect of variables from hierarchical partitioning (IE), and results of linear mixed effect model fitting are shown. Degrees of freedom, t-value and P-values are shown for variables that were selected for inclusion in the reduced LMEM using Akaike Information Criterion. doi:10.1371/journal.pone.0027158.t009

**Table 10. Relationships between neutrophils (cells·L⁻¹) and environmental variables for agile antechinus.**

| Sex   | Explanatory variables | r   | IE  | df  | t-value | P    |
|-------|-----------------------|-----|-----|-----|---------|------|
| Females | DI                    | 0.25| 6.8 |     |         |      |
|        | DIST                  | −0.01| 0.4 |     |         |      |
|        | EDGE                  | −0.02| 0.3 |     |         |      |
|        | HETEROGEN.            | 0.11| 1.7 |     |         |      |
|        | CORE                  | 0.22| 8.7 |     |         |      |
|        | MONTH                 | 0.52| 63  | 25  | 4.54    | <0.001|
|        | PC.1                  | 0.03| 1.4 |     |         |      |
|        | PC.2                  | 0.22| 10.3|     |         |      |
|        | PC.3                  | 0.21| 7.6 |     |         |      |
| Males  | DI                    | 0.15| 1.8 |     |         |      |
|        | DIST                  | −0.12| 2.3 |     |         |      |
|        | EDGE                  | 0.02| 0.4 |     |         |      |
|        | HETEROGEN.            | 0.12| 1.3 |     |         |      |
|        | CORE                  | 0.02| 1.2 |     |         |      |
|        | MONTH                 | 0.62| 63.6| 26  | 5.44    | <0.001|
|        | PC.1                  | −0.06| 0.8 |     |         |      |
|        | PC.2                  | 0.35| 17  |     |         |      |
|        | PC.3                  | 0.25| 11.7|     |         |      |

*Pearson's correlation coefficients (r), the independent effect of variables from hierarchical partitioning (IE), and results of linear mixed effect model fitting are shown. Degrees of freedom, t-value and P-values are shown for variables that were selected for inclusion in the reduced LMEM using Akaike Information Criterion. doi:10.1371/journal.pone.0027158.t010
present study the independent effect of core habitat area on relative abundance was strong (females = 34.3%; males = 47.1%) (Figure 4), suggesting that a direct effect was operating. Patch occupancy by agile antechinus in another fragmented forest was better explained by a combination of fragment area and vegetation composition than by either variable alone [32] and other investigations have reported equivocal effects of fragment size on agile antechinus’ abundance [33,34]. These varying findings could be attributable to differences in the environment (e.g. dry vs. wet sclerophyll forest, differences in rainfall or competitor species) or the time of year when sampling occurred.

The lower relative abundance of agile antechinus in small fragments could have been due to higher levels of predator intrusion from the agricultural matrix [35], altered emigration and/or immigration rates [36,37], greater competition with generalist species [38,39,40] or reduced and/or degraded resources [28]. Theoretical models predict, and there is evidence to support the occurrence of, proportionally greater emigration from, and reduced immigration into, smaller habitat patches [1]. The rationale here is twofold, namely that dispersers are more likely to encounter large than small patches (the ‘target effect’) [36,41], and patch-dwellers are probabilistically more likely to encounter boundaries in small than large patches, thus increasing the likelihood of emigration [37]. Given that male agile antechinus have an inherently strong propensity for dispersal [42], we might expect the effect to be stronger in males, as we observed (m = 47.1% cf. f = 34.3%).

Effects of habitat structure and heterogeneity on agile antechinus’ abundance and health

In both sexes of agile antechinus, PC.1, PC.2 and PC.3 had smaller independent effects on relative abundance than did CORE. This was surprising, given the prevailing opinion that Antechinus populations are strongly influenced by habitat complexity and structure [23,25,26,32,43]. The only clear support for this predominant view was that PC.2 was positively associated with male relative abundance, although its independent effect was only 12.2% (compared with 47.1% for CORE). The effect of PC.2 was that male relative abundance was higher where there were more Eucalyptus trees of >2 m in trunk diameter and fewer shrubs. Although large eucalypts could potentially contribute to nest-hollow, leaf litter and woody debris availability, the negative effect of shrub density on agile antechinus’ relative abundance was unexpected and its cause enigmatic.

Health status of agile antechinus (indexed by HHR) was associated with certain vegetation characteristics, although the relationship was not overly convincing for females. We expect HHR to be greater in individuals in good body condition and male HHR was higher where microhabitat heterogeneity was greater. Conceivably, heterogeneous habitat provided more foraging (and/or nesting) opportunities, so that the environment was generally less stressful. Male HHR was negatively associated with the vegetation descriptors PC.2 and PC.3 and Eucalyptus densities contributed to both of these indices. Thus males had a poorer health status in forest with denser stands of Eucalyptus. As capture rates of agile antechinus were higher in sites with more large eucalypts, it is plausible that there was an indirect effect of social stress or food competition on HHR when male densities were high.
Shrubs, woody debris and trees other than *Eucalyptus* contributed to *PC.2* and *PC.3*, so that a greater dominance of these microhabitat features was associated with better male health. Non-*Eucalyptus* tree species in the study area (e.g. *Cassinia* and *Olearia* spp.) frequently had fissured bark likely to harbour arthropod prey. Higher shrub density could contribute to better body condition in agile antechinus, as small mammals’ foraging bouts are typically longer [44] and arthropod abundance higher where shrub cover is greater. Higher shrub density could contribute to better body condition or possibly to habitat site dominance on agile antechinus’ abundance. Logs and fallen branches could also be promoting better health through increasing canopy cover [45].

### Edge effects on relative abundance, stress and body condition

The trapping rate of males was not influenced by edges, but female relative abundance was significantly and markedly lower (*IE* = 29.8%) at fragment edges than in interiors. Typically, two paradigms are invoked to explain animal population distribution and abundance patterns, species sorting and habitat selection. Species sorting is characterised by random dispersal followed by non-random survivorship. Habitat selection is characterised by individual dispersal and site occupancy based on perceived rather than realised habitat quality. Species' habitat perception is a product of prior selection processes [45].

Exercising the species sorting paradigm first, predation rates on birds’ nests are higher in edge than interior habitat in wet *Eucalyptus* forest [46]. The same might be true for agile antechinus’ tree-hollow nests, although most of Berry’s [46] birds were open-cup nesters whose nestlings were probably inherently more vulnerable than concealed antechinus young. However, if fewer dependent young survive at the edge than in the interior of fragments, over several generations this could lead to successively fewer females living in edge habitat because females normally remain in the natal home range throughout life [42,47]. Different predation rates could help explain the observed population differences, but could other factors be playing a role? Caughley et al.’s model [2] proposes that population density and condition of both male and female *Antechinus agilis* were influenced by sex and year. Examining the relationships between lymphocytes (cells·L⁻¹) and environmental variables for agile antechinus.

### Table 11. Relationships between lymphocytes (cells·L⁻¹) and environmental variables for agile antechinus.

| Sex    | Explanatory variables | *P* | IE df | t-value | *P* |
|--------|-----------------------|-----|-------|---------|-----|
| Females | DIST                  | 0.14| 2.3   | 0.006   |
|         | EDGE                  | 0.21| 1.3   | 0.08    |
|         | HETEROGEN.            | -0.01| 2.4  | 0.006   |
|         | CORE                  | -0.07| 2.4  | 0.002   |
|         | MONTH                 | 0.24| 1.96| 2.86  | 0.009 |
|         | PC.1                  | 0.24| 1.96| 0.005  |
|         | PC.2                  | 0.08| 5.7  | 0.6    |
|         | PC.3                  | 0.29| 2.23| 2.27  | 0.014 |
|         | DIST*×PC.1            | na  | na   | 0.27   | 0.004 |
|         | EDGE*×PC.3            | na  | na   | 0.27   | 0.004 |
| Males   | DIST                  | -0.14| 15.8| 0.008  |
|         | EDGE                  | 0.08| 7.1  | 0.005  |
|         | HETEROGEN.            | 0.07| 5.5  | 0.006  |
|         | CORE                  | -0.05| 2.3  | 0.006  |
|         | MONTH                 | 0.06| 2.2  | 0.08   |
|         | PC.1                  | 0.14| 20.2| 0.02   |
|         | PC.2                  | 0.24| 1.92| 0.004  |
|         | PC.3                  | 0.24| 1.92| 0.004  |

*Pearson’s correlation coefficients (r), the independent effect of variables from hierarchical partitioning (IE), and results of linear mixed effect model fitting are shown. Degrees of freedom, t-value and P-values are shown for variables that were selected for inclusion in the reduced LMEM using Akaike Information Criterion. doi:10.1371/journal.pone.0027158.t011

Examining the species sorting paradigm first, predation rates on birds’ nests are higher in edge than interior habitat in wet *Eucalyptus* forest [46]. The same might be true for agile antechinus’ tree-hollow nests, although most of Berry’s [46] birds were open-cup nesters whose nestlings were probably inherently more vulnerable than concealed antechinus young. However, if fewer dependent young survive at the edge than in the interior of fragments, over several generations this could lead to successively fewer females living in edge habitat because females normally remain in the natal home range throughout life [42,47]. Different predation rates could help explain the observed population differences, but could other factors be playing a role? Caughley et al.’s model [2] proposes that population density and condition of both male and female *Antechinus agilis* were influenced by sex and year. Examining the relationships between eosinophils (cells·L⁻¹) and environmental variables for agile antechinus.

### Table 12. Relationships between eosinophils (cells·L⁻¹) and environmental variables for agile antechinus.

| Sex    | Explanatory variables | *P* | IE df | t-value | *P* |
|--------|-----------------------|-----|-------|---------|-----|
| Females | DIST                  | 0.08| 2.7  | 0.08    |
|         | EDGE                  | 0.02| 0.3  | 0.08    |
|         | HETEROGEN.            | 0.08| 2.0  | 0.08    |
|         | CORE                  | 0.08| 0.7  | 0.08    |
|         | MONTH                 | 0.24| 13.8| 0.08    |
|         | PC.1                  | 0.14| 19.6| 0.08    |
|         | PC.2                  | 0.18| 19.6| 0.08    |
|         | PC.3                  | 0.26| 27.1| 0.08    |
|         | DIST*×PC.1            | na  | na   | 0.45   | 0.08 |
|         | PC.1×PC.2             | na  | na   | 0.6    |
| Males   | DIST                  | 0.09| 1.7  | 0.08    |
|         | EDGE                  | 0.07| 1.7  | 0.08    |
|         | HETEROGEN.            | -0.13| 1.7  | 0.08    |
|         | CORE                  | -0.13| 1.7  | 0.08    |
|         | MONTH                 | 0.48| 53.9| 0.08    |
|         | PC.1                  | 0.13| 2.9  | 0.08    |
|         | PC.2                  | 0.32| 19.2| 0.08    |
|         | PC.3                  | 0.24| 11   | 0.08    |

*Pearson’s correlation coefficients (r), the independent effect of variables from hierarchical partitioning (IE), and results of linear mixed effect model fitting are shown. Degrees of freedom, t-value and P-values are shown for variables that were selected for inclusion in the reduced LMEM using Akaike Information Criterion. doi:10.1371/journal.pone.0027158.t012
the sexes outside the breeding season is well-known in lek-breeding mammals [48,49]. The predation risk hypothesis [49,50] predicts that to maximize their fitness, females in lekking species should make more use of habitat with a lower predation-risk, whereas males should use habitat with more abundant foraging resources, even if predation-risk is also higher there. The rationale is that males in good condition can produce many more young than good condition females, so the potential fitness benefits of ‘riskier’ foraging are different for the sexes [49]. Forest-field ecotones are more resource-rich than forest interiors and invertebrate species richness generally declines with distance from edges in forests [51]; however, edge habitats also have higher nest predation rates, at least in birds, which may indicate greater predator activity in general [46,51,52]. At least one other study has also reported that female agile antechinus may generally occupy better quality habitat than males [32].

The hotspot theory of lek sitting [53] predicts that during the breeding season, males should aggregate where female traffic is greatest. Male brown and agile antechinus can move large distances during or before the breeding season [54,55]; it would be interesting to determine whether males living in edge habitat move into the fragment interior where female population density is higher immediately prior to, or at this time. Equally, females might forage nearer edges during lactation when metabolic demands are high, at least until young detach from the pouch (~5 weeks post-parturition) when the need to return to the nest to nurse them could restrict this behaviour [55].

Female N:L was significantly higher in fragments with a large proportion of edge habitat. Assuming that N:L was a positive index of stress, this finding implied that female agile antechinus found such fragments more stressful than those with more interior habitat. This might not be an effect of edges per se if females avoided edge habitat, limited availability of core habitat in more dissected fragments could have resulted in crowding, psychosocial stress and competition for nest sites and food in the interior.

**Effects of environmental features on stored lipid reserves**

Mass-size residuals, an estimate of stored fat reserves, allow us to make some inferences about whether per capita food resources varied among fragments. The most convincing significant relationship between MSR and a landscape variable was the negative association between MSR and core habitat area i.e. in larger fragments, the estimated stored lipid reserves were smaller. The association was strong in both sexes (females = 36.8%; males = 42.0%). Therefore nutritional stress was almost certainly not a factor causing the lower relative abundance of agile antechinus in smaller forest fragments. One possible explanation for this situation was that inter- and/or intra-specific competition for food was more pronounced in larger fragments. Experimental food provisioning suggests that inter-specific competition between agile antechinus and bush rats can be intense [39] and the latter were present in many of our study sites. Agile antechinus’ relative abundance was also greater in larger fragments, so intra-specific competition may also have contributed to the observed COR-MSR relationship.

**Environmental features affecting immune cell variables**

Female N:L was influenced by the proportion of edge habitat in a fragment, but male N:L was not convincingly correlated in a consistent manner with landscape configuration, proximity to forest edge or the vegetation composition indices (PC.1, PC.2, PC3).

Absolute leukocyte concentrations in peripheral blood can be more informative of population health status than N:L alone [56]. Neutrophil concentrations in both sexes were unaffected by any measured environmental variable, but they responded strongly to seasonality (females = 63.0%; males = 63.6%) i.e. concentrations increased during the March (post-dispersal) to August (pre-breeding season) sampling period. Numerical domination of neutrophils in peripheral blood may reflect greater innate
immunocompetence [57,58] and presumably the neutrophilia in agile antechinus later in the sampling period (July–August) resulted from neutrophil trafficking, production or release from bone marrow. This might constitute a form of ‘preparation’ for breeding and the synchronized breeding rut, during which physical contact among individuals, and hence the risk of disease transmission, probably increased.

Female lymphocyte concentrations responded to a broad set of environmental variables, including interactions between \textit{EDGE} and \textit{PC.1} and \textit{MONTH} and \textit{PC.3}. However, the only unambiguous relationship was that with the proportion of edge habitat in a fragment \((\text{JE} = 15.7\% )\). Trafficking of lymphocytes away from peripheral blood into the skin, lymph nodes and spleen, where they are more likely to be useful in the event of injury, is the most frequently cited mechanism underlying the increased N:L observed in chronically-stressed vertebrates [15,59,60,61]. Thus it appears likely that lymphopoenia produced the positive association between N:L and edge habitat in female agile antechinus.

Eosinophil concentrations were not convincingly related to any environmental variable. In males, they were higher nearer to the environment is more stressful. In fragments with a large proportion of edge habitat and microhabitat change in anthropogenically fragmented environments. Fragments with more edge and/or more degraded microhabitats affected population health indicators (MSR, N:L and HHR) negatively, whereas a smaller core area reduced population abundance. Potentially, populations inhabiting small fragments that exert greater edge effects and/or are more degraded experience the interactive effects of reduced population size and lowered body condition [63], making their conservation problematic.

In the present study, agile antechinus’ relative abundance decreased from core (larger, unsubdivided, forest fragments) to peripheral habitat (smaller fragments), whereas MSR was either constant (females) or increased (males) along such a gradient (Figure 4). From this dichotomy, Caughey et al.’s [2] model would suggest that the population limiting factor is probably a resource used consumptively or pre-emptively, which could be nesting sites for agile antechinus in small fragments. The model argues that if predation, disease or parasites are regulating a population, body condition should decline at the periphery of its range where the environment is more stressful. In fragments with a large proportion of edge, male MSR and female N:L decreased from interior to edge, so conceivably at forest edges such factors were limiting population density. Thus two regulating factors could be co-occurring: 1) limited nest-site availability in smaller fragments and 2) higher rates of predation or disease in edge habitat.

It is difficult to unravel the interacting effects of fragment area and proportion of edge. On balance, the simplest explanation is that predation rates were higher in fragments with more edge habitat and predation was holding population levels below that at which \textit{per capita} food availability would limit population size. European red foxes (\textit{Vulpes vulpes}) and feral cats (\textit{Felis catus}) were present and do prey upon \textit{Antechinus} spp [64,65]. If agile antechinus living near fragment edges were more exposed to such predators, it would help to explain why females apparently found forest edges more stressful than interiors.

**Conservation implications**

For the conservation management of agile antechinus in the study area, we suggest that preserving forest fragments with large core areas, a high level of microhabitat heterogeneity and a minimum of edge habitat would help to mitigate the negative effects of habitat fragmentation on this species. This conclusion is in accordance with theories of how anthropogenic habitat fragmentation affects native vertebrates [3], although we could only identify negative effects of habitat area reduction, increased patch dissection and lower microhabitat heterogeneity by examining relative abundance and multiple performance metrics.

**Materials and Methods**

**Study area and design**

Research involving live animals followed the guidelines approved by the American Society of Mammalogists [66] and was conducted in accordance with local animal ethics legislation. Trapping and data collection were conducted under Monash University Biological Sciences Animal Ethics Committee permits BSCI/2008/03 and BSCI/2006/05 and the Victorian Department of Sustainability and Environment permit 10003798. Effort was made to minimize suffering and stress experienced by animals during trapping and handling.

This study was conducted from April to August 2007 and March to August 2008 in South Gippsland, Victoria, Australia (Figure 5). We sampled thirty \textit{Eucalyptus} forest fragments dispersed in an anthropogenically-disturbed, agricultural landscape in an area bounded by the coordinates 38°35’25”S 145°41’41”E, 38°21’55”S 146°06’10”E, 38°37’19”S 146°28’20”E and 38°45’12”S 146°01’33”E. The fragments, 4.8 to 293.6 ha in area, were situated 2.1 to 38.6 km from any area of continuous forest (defined as >1000 ha of continuous, native treecover, Figure 5). Habitat similarity among study sites was achieved by restricting sites to forest stands composed of the three Ecological Vegetation Classes (EVC) [67] ‘Lowland Forest’, ‘Wet or Damp Forests (Wet)’ and ‘Wet or Damp Forests (Damp)’. Most sites contained a mixture of the first two, but some also contained small areas of the EVCs ‘Riparian Forests or Woodlands’ or ‘Rainforests’.

**Study Species**

The agile antechinus is a scansorial, nocturnal marsupial restricted to south-eastern Australia. Its diet comprises terrestrial invertebrates, supplemented by some small vertebrates and scavenging from carcasses [22]. Home range area can be up to 5 ha, but is more typically 1–3 ha [29,55]. Pre-1998 this species was considered part of the brown antechinus (\textit{A. stuartii}) species-complex [68]; the two species have very similar life-histories and morphology [12] and authors frequently cite studies of one when discussing theories about the other.

\textit{Antechinus} are unusual because they are semelparous [69]. A synchronized breeding rut in the Austral winter (in August in our study area) is followed by senescence and death of all males. During the 2–3 week breeding season, male foraging behaviour is reduced and lek behaviour occurs, apparently involving extended periods of male ‘vigilance’ in tree-hollow nests [70,71]. A negative nitrogen balance develops in males, which are eventually unable to obtain sufficient food for self-maintenance [72]. Sperm storage in females, relatively protracted oestrus (≥21 days in captivity) and promiscuous mating behaviour by both sexes generate a high level of intra-sexual competition among males, with larger individuals...
and those that mate closer to the time of a female’s ovulation typically sire more young [70,73,74]. After the weaning of young, a male-biased dispersal occurs in the Austral late summer [47] (January–February in the study area). Most females die after weaning their only litter [75], although a few breed in a second year (~15% in A. stuartii [76]).

Trapping protocols

Live-traps were baited with a mixture of rolled oats, peanut butter, water and vanillin. Sufficient bait was supplied for trapped agile antechinus and by-catch small mammals to eat ad libitum. To reduce the risk of animal death during trapping due to stress or inclement weather, traps were weather-proofed with a plastic bag and provided with bedding and a plastic refuge tube. They were set no earlier than 3 h before dusk and checked no later than 3 h after dawn.

Ten small (<30 ha), ten medium (30–60 ha) and ten large (60–300 ha) fragments were used and they were allocated randomly to a sequence for trapping. However, the fragment size categories were not used in data analysis because including the actual area of fragments as a covariate probably generated more accurate results. One trapping grid in each fragment was placed in edge (60 m from the forest-field ecotone) and one in interior habitat (always >80 m, often 200–400 m and sometimes up to 500 m from the ecotone). The two trapping grids had 21 traps each, arranged in three lines of seven (i.e. 21 traps in 4800 m²). Trapping was conducted for three successive nights in each fragment. We considered captures per trap-night to represent agile antechinus’ relative abundance and used this as an estimate of population density.

Lipid reserve estimation and haematological methods

All captured individuals were sexed by visual inspection. On each trapping day, the first two ‘new’ (i.e. not previously captured) agile antechinus captured at the edge and the first two in the interior of a fragment were measured to determine total and differential leukocyte counts, mass (±0.1 g) and linear distance from nose to vent (NV) (±0.1 mm). Only single morphometric measurements were taken, which is not ideal [77], but this was unavoidable; individuals were already subjected to prolonged handling during blood sampling such that the additional handling required for multiple measurements would likely have unreasonably stressed individuals. A <1 mm disc of pinna tissue was removed from a unique position to facilitate identification on recapture to ensure that recaptured individuals were not re-sampled.

Blood-sampling was conducted within 15 min of removing an antechinus from a trap. Blood was collected before measuring the animal’s mass and size to reduce the potentially confounding effects of handling stress and consequent leukocyte trafficking on leukocyte counts [60,61]. Blood volume collected never exceeded 100 μL (±0.1 g) and so was unlikely to have markedly affected subsequent measurement of mass. The possibility that trapping and/or handling stress could have influenced leukocyte measurements [76] is addressed in the Discussion.

Blood samples were collected by capillarity in heparinised microhematocrit tubes after puncturing one of the two lateral veins near the base of the tail with a 27 gauge needle. Whole blood haemoglobin concentration (Hb) (±0.1 grams per litre [gL⁻¹]) was determined immediately with a Hemocue 201+ haemoglobinometer (Hemocue®, Angelholm, Sweden). All other blood samples were stored on ice and processed within 10 h, and no
deterioration was observed. Haematocrit (Ht) (±0.1 mm) (%) was
determined by centrifugation for 3 min at 12,700 g. Hb and Ht
alone are potentially difficult to interpret, as high and low values
can be caused by several factors (e.g. anaemia, dehydration, 
disease) [21]. Therefore we derived an index of health status based
on Hb/Ht residuals, based on a similar principle to that used for
deriving MSR (see Discussion).

Blood smears for differential leukocyte counts were made by the
pull-wedge method [21] and stained with May-Grünwald-Geimsa
's head' to the 'tail' of each smear under 400 magnification. They
comprised >200 leukocytes and were all conducted by the same
person. Population mean neutrophil/lymphocyte ratios were
calculated from mean proportions of neutrophils and lymphocytes,
as averaging ratios can generate spurious results [79]. To make
total white blood cell counts (WBC), 5 μL of blood were diluted
with Natt and Herrick's solution at a ratio of 1:200 [80]. Counting
was conducted under 400 magnification using an improved
Neubauer haemocytometer (Blau Brand, Germany). Total neu-
 trophil, lymphocyte and eosinophil concentrations were derived
from total and differential leukocyte counts.

Mass-size residuals were derived by calculating the residuals of
body mass as a factor of NV. Ordinary least squares (OLS)
regressions were used to generate HHR and MSR [13].

Relationship of haematotogical variables to body
condition

We used linear mixed effect models (LMEM) to examine
whether erythrocyte variables, neutrophil, lymphocyte and
eosinophil concentrations or N:L ratio were significantly related
to the measured body condition index, MSR. In all LMEM, the
factor SITE (i.e. each fragment) was included as a random effect to
avoid pseudoreplication and the covariate MONTH [March = 3 to
August = 8] was included because there are biological reasons to
expect some variation in body condition to be explained by the
time of year [81]. Final models were validated graphically using
ordinary and standardized residuals [82].

Handling and trapping stress

Stress indices, such as N:L, can alter sufficiently rapidly to
potentially be confounded as baseline measures by the effect of
trapping and sometimes even of handling [78,83]. However, this is
not true of erythrocyte variables (e.g. HHR), in which it can take as
long as 48 h before a peak response to an acute stressor occurs
[84]. We eliminated the possibility that handling stress affected
N:L through a validation trial in which agile antechinus were
blood-sampled 0, 10, 20 and 30 min post-removal from a trap
[85]. Detecting trapping stress requires the immediate killing of
trapped animals to establish baseline values for each study site
[78], an impractical and ethically contentious procedure in our
investigation.

Arguably the most appropriate interpretation of N:L in the
present study is that it reflected an additive or multiplicative
response [86,87,88] to a combination of environmental and
trapping stress. We have no reason to think that time spent in traps
differed among sites. Moreover, trapping evoked a stress response
in meadow voles (Microtus pennsylvanicus) [78], but its magnitude
do not increase as a function of time spent in the trap (i.e. trapping
could be considered a uniform stressor). Therefore probably the
most accurate interpretation here is to view N:L as a positive index
of stress [15] and assume that significant differences in this ratio
among sites are more likely due to differences in background
environmental stress than in mean duration of trap occupancy.
However, stress responses have not been widely studied in free-
living, small mammals, so unexpected interactions of chronic and
acute stress could occur [89], and therefore the interpretation of
N:L presented here is necessarily tentative.

Response of agile antechinus to microhabitat, vegetation
features and landscape configuration

We documented the dominant local microhabitat in a 3 m
radius around each trapping station, using a system of 48
categories. The categories were devised during preliminary
fieldwork to record as much variation in microhabitats as possible,
but for analysis we used variable reduction methods (principal
component analysis, PCA, and model simplification [90]) to
reduce the number of categories to a manageable seven for
analysis and interpretation (Table 13). More details of these
categories can be obtained from the corresponding author on
request. We derived residuals of trap-nights conducted in a given
microhabitat (expected) and number of captures (observed) for
each microhabitat for each trapping grid (i.e. from a linear model
in which captures in microhabitats was treated as a function of
trap-nights in microhabitats).

Trapping stations represented pseudo-random samples of
microhabitat. Therefore we constructed vegetation feature indices
by applying a PCA to station microhabitat feature occurrences
(Table 13). We used PCA axes 1, 2 and 3 (PC1, 2 & 3), which
had Eigenvalues>1, as vegetation descriptors, and these were
included as explanatory variables in linear models examining
indices of agile antechinus' stress and condition. We were also
interested in whether habitat heterogeneity influenced agile
antechinus population health, and so used the 48 original
microhabitat categories to derive a Shannon’s heterogeneity index
for each site [91] which served as a habitat complexity index
(HETEROGEN) in analysis.

We use the term ‘configuration’ to encompass fragment area
and spatial configuration (shape and degree of isolation). Fragment
configuration data were obtained from online native vegetation
cover maps (1: 75,000) from the Victorian Department of
Sustainability and Environment (Forest-Explorer Online’ maps,
http://www.dse.vic.gov.au), estimated using ImageJ (http://
rsbweb.nih.gov/ij/) (measured in pixels and converted to appro-

| Table 13. Eigenvalues and component loadings from
principal component analysis of simplified microhabitat
variables. |
|--------------------------------------------------|
| Eigenvales | PC.1 | PC.2 | PC.3 |
| Eigenvalues | 1.55 | 1.47 | 1.20 |
| **Component loadings** | | | |
| DEAD EUCALYPT TREE | −0.17 | 0.12 | 0.76 |
| EUCALYPT (<2 m diam.) | 0.48 | 0.34 | −0.36 |
| EUCALYPT (>2 m diam.) | −0.39 | 0.41 | −0.23 |
| NON-EUCALYPT TREE | −0.49 | 0.31 | −0.29 |
| SHRUB | 0.13 | −0.63 | −0.18 |
| TEATREE/PAPERBARK | 0.38 | 0.37 | 0.36 |
| WOODY DEBRIS | −0.43 | −0.26 | 0.10 |

*Principal component axes 1–3 of 7 are shown. The dominant microhabitat was
recorded at each trapping station as one of 48 microhabitat categories. The
categories shown here are simplifications of the field categories derived by
a model simplification procedure. Trap station microhabitats were treated as
pseudo-random samples of the microhabitats in each study site. Bold values are
component loadings. >+/−0.40. 
doi:10.1371/journal.pone.0027158.t013
Habitat Fragmentation Effects on *Antechinus agilis*

We measured the following fragment variables: (a) largest inside circle (CORE, ha), (b) nearest neighbour', the distance (m) to the nearest *Eucalyptus* fragment of >5 ha (DIST) and (c) dissection index (DI; CORE is an estimate of unsubdivided fragment area, which we term 'core area'. Dissection Index was estimated by taking the ratio of the perimeter (P) of the fragment to the square root of its area (A) and scaling the results, so that for a circle DI = 1.0 and values >1.0 are increasingly dissected: DI = P / \(\sqrt{A}\) [92].

**Data analysis**

The responses of the sexes to habitat fragmentation were analysed separately, as the behaviour, morphology and physiology of male and female *Antechinus* differ markedly [81,83,94]. All data were analysed with R 2.11.1 [95] (packages ‘nlme’, ‘MuMIN’ and ‘hier.part’) and checked for normality and homoscedasticity. Relative abundance (RA; captures per trap-night) was square-root transformed and N:L was log 10-transformed to achieve normality where appropriate, but no other transformations were needed.

Linear mixed-effects models (using maximum likelihood) were applied to explanatory factors (EDGE response: edge or interior and MONT and covariates (DI, DIST, CORE, PC1, 2, 3 and HETEROGEN), and to response variables (RA, MSR, HHR and differential leukocyte parameters of stress) for all subsets model selection using the Akaike Information Criterion (AIC) ('dredge' in ‘MuMIN’). We checked for correlation structures in the data (sparsity, auto-regression et al.) and included these in the final model where warranted. Restricted maximum likelihood (REML) was used to generate the final models.

Where interactions among factors occur in linear models, they must be interpreted first [96], one consequence of which is that the main effects are not always interpretable. We used conditioning plots to examine interactions, but provide only provisional interpretations. We used hierarchical partitioning [97] to help infer the relative percentage of variation in each response variable that was explained by each predictor variable. In this procedure, if a variable has a total influence of 50% it indicates that it explained 50% of the variation explained by the cohort of explanatory variables used, not 50% of the total variation in the response variable. We report the independent effect (IE) of explanatory variables and consider variables with IE≥25% to have had a potentially important influence on the response variable in question.

**Acknowledgments**

Access was kindly granted by private landowners throughout the South Gippsland region and we also thank C. Rankin for access to South Gippsland Shire council reserves. Field accommodation was provided by Parks Victoria, J. and S. Bell, G. and J. Wallis, D. and M. Hook and D. Farrar. The support, co-operation and enthusiasm of many individuals and groups helped to facilitate this project, notably the South Gippsland Conservation Society, Venus Bay Landcare and Andrews Inlet Landcare. Special thanks are due to Katarina Achkar-Kerbai for fieldwork assistance.

**Author Contributions**

Performed the experiments: CPJ. Analyzed the data: CPJ. Contributed reagents/materials/analysis tools: AL, RDR. Wrote the paper: CPJ AL RDR.

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