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Short running title: Overwinter Survival in Stick Insects

Abstract:

Stick insects are found in a variety of habitats throughout New Zealand, including at least four species that occur at high altitudes. Here they face physiological challenges that differ from their typically warmer lowland habitats, but their strategies to deal with harsh winter conditions are not known. Autumn and winter field surveys, coupled with caging experiments, were conducted to determine which life stages are overwintering in montane and lowland habitats. Data loggers were placed for approximately one year at each site to measure the leaf litter and canopy microhabitat temperatures. From this, we have found that alpine and lowland stick insects persist in a variety of life stages throughout the year despite multiple exposures to freezing temperatures.

Keywords: Insect cold tolerance, microclimate, Phasmatodea, *Niveaphasma annulata*, *Micrarchus*

Introduction

Alpine habitats in New Zealand are relatively young (5 Ma), biologically diverse and contain representatives of all major insect lineages (Buckley & Simon 2007; Wharton 2011). To survive in this harsh environment, many insects have evolved a suite of physiological and biochemical adaptations. The best studied examples of this are the New Zealand alpine weta (*Hemideina maori* (Pictet & Saussure)) and the alpine cockroach (*Celatoblatta quinquemaculata* Johns) (Wharton 2011). Both of these species adopt the most common cold tolerance strategy in the southern hemisphere, freeze tolerance (Sinclair et al. 2003; Wharton 2011). However, the overwintering strategies of many other insects found at high altitudes in New Zealand have not been studied, and it is likely that other species are biochemically, evolutionary or ecologically constrained to avoid freezing through supercooling rather than tolerating internal ice formation (Sinclair et al. 2003).

At least four species of stick insect are found in montane habitats of the South Island of New Zealand (Dunning et al. 2013; Jewell & Brock 2002; O'Neill et al. 2009; Salmon 1991). Although nothing is known of their overwintering strategies, specimens in the New Zealand Arthropod Collection (NZAC) of mature adults and nymphs of *Niveaphasma annulata* (Hutton) and *Micrarchus* Carl have been collected above the tree line in spring and early summer, suggesting that they may have persisted there through winter. However, before investigating cold tolerance strategies in these stick insects, we must first determine the selective pressures that they face throughout the year, and the life stages that are present during the coldest months.

In other parts of the world, records of alpine stick insects are scant, with most Phasmid diversity occurring in lowland tropical forests of Southeast Asia and Central and South America (Otte & Brock 2005). High altitude stick insects are known from the Andes of South America, but nothing is known about their physiology or the microclimates they occupy. This includes the genera *Agathemera* Stål, *Peruphasma* Conle & Hennemann and *Monticomorpha* Conle & Hennemann. *Peruphasma marmoratum* Muranyi has been collected in the Venezuelan Andes at altitudes between 4,000 and 4,700 metres and may be an obligate alpine species. The genus *Agathemera* contains a number of species, some found in low altitude forests and some over 4,000 metres (Vera et al. 2012). In North America, the stick insect *Dicepheromera femorata* occurs at latitudes where winter temperatures are often below freezing for extended periods of the winter (B.J. Sinclair & M.L. McFarlane, unpublished observations). *D. femorata* overwinter as eggs (Giese & Knauer 1977), a common strategy among insects (Danks 2007; Rochefort et al. 2011). Overwintering insect eggs uniformly avoid freezing by supercooling (Sinclair et al. 2003).

In New Zealand, high altitude populations are reported from the genera *Micrarchus*, *Niveaphasma* and *Tectarchus* Salmon. The first montane species, *Micrarchus* nov. sp. 1 (NZAC voucher NZAC03000433), is restricted to the Kaikoura region of the South Island, where it has been collected from sea level to 1105 m (Mt. Fyffe). Its host plants include *Leptospermum scoparium*, *Kunzea ericoides*, *Muehlenbeckia* sp., and *Rubus* spp. As it has been recorded over a large altitudinal range, we consider it an ecological generalist.

The second observed montane species is referred to as *Micrarchus* nov. sp. 2 (NZAC03009458). Previously, it was incorrectly described by Salmon (1991) as *Mimarchus tarsatus* Carl, however, this name is a junior synonym of *Argosarchus horridus* (White) as established by Jewell and Brock (2002), and morphological and molecular studies now demonstrate it belongs in the genus *Micrarchus* Carl. It is commonly collected from *Leptospermum scoparium*, *Traversia baccharoides*, *Muehlenbeckia axillaris*, *Dracophyllum rosmarinioides* and *Gahnia* spp. Its range is restricted to Northwest Nelson, Nelson Lakes and Northern Westland (Salmon 1991; Dunning & Buckley unpublished observation). *Micrarchus* nov. sp. 2 has been collected as low as 650 metres on the Denniston Plateau, Westland and as high as 1400 metres on Mount Robert, Nelson Lakes National Park. Most records are between 900 and 1,300 metres in elevation and above the tree line; we therefore regard this species as a montane obligate.

Niveaphasma annulata (Hutton) is the third species to be found at high altitudes. Like *Micrarchus* nov. sp. 1 it is found in lowland habitats and we thus consider it a habitat generalist (Jewell & Brock 2002; O'Neill et al. 2009). *Niveaphasma annulata* has been collected from coastal areas just above sea level to the alpine zone up to 1,300 metres from Arthur's Pass National Park to coastal areas of the Southern South Island (Jewell & Brock 2002; O'Neill et al. 2009). This species appears to be absent from coastal and lowland Canterbury, Westland and the wetter areas of the Southern Alps. *Niveaphasma annulata* is commonly collected from *Leptospermum scoparium*, *Muehlenbeckia complexa*, *M. axillaris*, *Pimelea* sp. and *Rubus spp*. It is a geographic parthenogen with both sexual and asexual alpine populations (O'Neill et al. 2009). In contrast, both montane *Micrarchus* species always occur in sexual populations.

The fourth species to occur in montane areas is *Tectarchus salebrosus* (Hutton), which is found as high as 1,100 metres on the Seaward Kaikoura Range and close to sea level near Christchurch. This species has a broad altitudinal distribution, like *N. annulata* and

Micrarchus nov. sp. 1, but we did not conduct winter collections of this species and so we do not consider its ecology here.

To investigate whether *N. annulata, Micrarchus* nov. sp. 1 and *Micrarchus* nov. sp. 2 survive the winter as adults or as nymphs, we use both field cages and manual surveying in lowland and montane sites on the South Island of New Zealand. We also recorded microhabitat temperatures at each site to better understand abiotic selective pressures experienced by these species. These ecological data are key to determining the overwintering strategies of stick insects in New Zealand.

Methods

Data loggers

Temperature was measured at each site beginning in February and March 2011. Temperature recordings were made every 1.5 h using iButton thermochron data loggers (CD1992L, Maxim-Dallas Semiconductor) housed in rain covers made of ~40mm deep plastic cups open at one end (Figure 1). All loggers were placed out of direct sunlight and recorded for approximately one year, with the exception of Puhi Puhi, where temperature was only recorded from July 2011 to January 2012. At six sites (Sewell Peak, Mt. Arthur, Puhi Puhi, Seaward Moss, Nevis Rd., and Coach Rd) there was sufficient leaf litter at the base of the host plant to potentially shelter stick insects, and we buried data loggers here. These were paired with loggers placed in the centre of the plant canopy, between 0.5m and 1.0m high. In total three sets of paired loggers were deployed at each site. At the remaining sites (Ohau, Remarkables and Dunedin), there was insufficient leaf litter, therefore three solitary loggers were placed in a shielded location near the ground below three separate host plants. From these measurements, the mean temperature, mean daily average high and low, absolute high and low temperature, and number of recordings below 0˚C were calculated for each month using R (R Development Core Team 2012). At sites with paired loggers, we tested for differences between the annual minimum and annual maximum temperatures in the canopy and leaf litter using a one-sided Wilcoxon signed-rank test implemented in R.

Collections

References to undescribed species follow the recommendations in Leschen et al. (2009). We surveyed winter survivorship in *N. annulata*, *Micrarchus* nov. sp. 1, and. *Micrarchus* nov. sp.

2. Collection locales (Table 1, Figure 2) were chosen for their abundant summer (January – March) populations; in 2011 more than 50 individuals were observed at all sites in one or two nights (between 1 and 6 hours surveying). In summer 2012, we again found abundant populations at each site, indicating that the winter absences we report here were not permanent.

Winter field observations of *N. annulata*, *Micrarchus* nov. sp. 1 and *Micrarchus* nov. sp. 2 were conducted in April and July 2011 (Table 1). Insects were collected by eye (*N. annulata*) or by eye and using beat sheets (*Micrarchus*). All searches were conducted for 1.5 h to 4 h, and where live food plant could be located (i.e. this was not prevented by snow cover) this included a night collection, beginning at least one hour after dark.

Caging

In April 2011, *N. annulata* were placed into a cage at each of two field sites (Table 1). Cages were constructed of nylon insect mesh supported by plastic piping (Figure 1). These were anchored to the ground so that the entire host plant and surrounding ground vegetation was contained. At Seaward Moss, one cage enclosed an approximately 1 meter high *L. scoparium*. At Ohau, a slightly smaller cage enclosed a small *Rubus sp.* (bush lawyer). Cages were revisited and thoroughly inspected for insects in both the foliage and ground vegetation in July 2011.

Results and Discussion

Microhabitat temperatures

Across all sites, sub-zero temperatures were recorded at least once in between two and 12 months of the year (Table 2, 3). However, summer freezing incidences were rare. January, for example, had only two early morning freezing instances (lasting between 1.5 and 4.5 hours) at one alpine site (Nevis Rd., -0.1˚C) and two freezing instances at one lowland site (Seaward Moss, -1.4˚C). Mean temperatures in January ranged from +9.8˚C at Mt. Arthur to +15.6˚C at Kaikoura.

Winter temperatures were cooler, with mean July temperatures ranging from +0.5˚C (leaf litter , Mt Arthur and Nevis Rd.) to +4.5˚C (litter and at the ground, Seaward Moss and Dunedin). At Mt. Arthur, snow buffered the temperatures for an extended period, and temperature recorded by the data loggers was stable around 0˚C (+/- 2˚C) from 6 July to the

30 August in 2011. During this time, temperatures were warmer than the other montane sites, and some lowland sites (Figure 3, 4). To a lesser extent, snow cover at the Remarkables also reduced daily temperature fluctuations in winter.

Montane and lowland populations of *N. annulata* are genetically similar (Jewell & Brock 2002; O'Neill et al. 2009), thus we expect that all populations have the ability to survive similar conditions. In fact, the winter temperatures at some of the lowland sites can be nearly as cold as the montane sites. Seaward Moss (elevation 9m) had between two and 45 monthly freezing measures, and was the only site at which temperatures below freezing were recorded in all 12 months of the year (Table 3). Additionally, Seaward Moss had an average daily low canopy temperature of +1.0˚C in July, and an absolute minimum temperature of -6.7˚C in October (Figure 4). Although more freezing recordings were taken at montane sites, this was the coldest measurement anywhere in October, and demonstrates that overwinter survival in exposed lowland populations of *N. annulata* also requires adaptation to the cold.

In contrast to *N. annulata*, lowland populations of *Micrarchus* nov. sp. 1 (Table 2) collected at Puhi Puhi experienced relatively warm conditions, with mean July temperatures (+3.6˚C litter, +3.7˚C canopy) several degrees above those at Mt. Arthur (+0.5˚C litter, +0.3˚C canopy), although closer to those at Sewell Peak (+2.8˚C litter, +2.7˚C canopy). Incidences of freezing were also lower at Puhi Puhi in July (6-20, compared to 34 -106 at Mt. Arthur and Sewell, Table 2). Thus, in *Micrarchus*, lowland populations may experience less selective pressure to tolerate freezing than montane populations.

There was also evidence of thermal buffering in the leaf litter. In the canopy, minimum annual temperatures were significantly colder (W= 0, 11 df, p = 0.016) and maximum annual temperatures were significantly warmer (W = 21, 11 df, p = 0.016) than the leaf litter. Monthly extreme temperatures were generally buffered in the leaf litter by 1-3˚C, with this being most obvious at colder sites (Seaward Moss, Nevis, Sewell Peak and Mt. Arthur). There were also more sub-zero events in the canopy than the litter (Table 2, 3). Thus, except for extended cold periods, the litter at the base of the plants provides a habitat with less extreme temperatures than the canopy above. This microhabitat selection could be key to surviving both biotic and abiotic challenges in the environment (Sinclair 2001), but it is difficult to determine if this amount of thermal buffering is physiologically relevant. For example, in insects inhabiting tree bark microhabitats, thermal buffering may not be sufficient to provide a physiological benefit (Vermunt et al. 2012).

Caging

Despite temperatures that had already fallen to -2.4˚C, three nymphs out of the 25 stick insects that were caged in the autumn were located in winter at Ohau (Table 1). At Seaward Moss, both nymphs and adults were found in the cage, despite temperatures at this lowland site having reached -6.0˚C prior to collection. We also only recovered a portion of the caged individuals at Seaward Moss (14 placed, four recovered). The lower numbers of individuals recovered from both cages suggests that winter populations may be reduced relative to those found in the summer, but these collections show that both alpine and lowland *N. annulata* remain alive in the field despite being exposed to freezing conditions.

Autumn collections

In April, stick insects of all life stages were recorded at five of the six sites visited (Table 1). This included both alpine and lowland populations of *N. annulata,* and the sole *Micrarchus* site visited (*Micrarchus* nov. sp. 1, Puhi Puhi, lowland). The only site at which successful autumn collections were not made was the montane population at the Remarkables. This is possibly due to grass cover reducing the abundance of known food plants (*Pimelia sp., Muelenbeckia complexa* and *Rubus sp.*). It is unknown whether the insects had shifted to another host plant, or if they were only present as eggs.

Winter collections

In July, both adults (male and female) and late instar nymphs were actively moving around their host plants at five of the nine sites visited. *Niveaphasma annulata* was found at one of the three montane sites (Ohau, Table 1). We did not locate any individuals at the Remarkables or Nevis Rd, perhaps due to dense snow cover on the host plants (*Pimelea* sp. and *Rubus* sp., respectively). At both sites, we were able to remove snow and locate live plants, but the likelihood of doing so in a spot that happened to have a visible insect is low. Therefore, we could not determine if snow cover excluded adults and nymphs from this site during the winter or if it only made collection difficult. At Ohau there was less snow cover, and healthy host plants (*Rubus sp.*) were exposed during our collections. Here, we observed both adults and nymphs actively moving, and presumably feeding, at night, despite air temperatures of +3.5˚C during collection.

Active individuals of *N. annulata* were also located at two of three lowland sites (Seaward Moss and Dunedin, Table 1). At Seaward Moss, no active insects were observed on the food plants during the day or night, despite similar canopy temperatures (+3.4˚C). Rather, insects were found in thick tufts of grass at the base of the *L. scoparium*. This suggests that *N.*

annulata could utilize this microhabitat to avoid temperature extremes in the plant canopy, or perhaps to shelter from predators when the cold makes them less mobile. We cannot conclude that insects are not active on other nights in the winter, but, although diapause has not been observed in New Zealand insects (Dumbleton 1967), it is possible these individuals were quiescent. In Dunedin, not only did we observe several live nymphs and adults of both sexes, but also two copulating pairs at night, when temperatures were comparable to other sites (+3.3˚C). This suggests *N. annulata* is capable of continuous reproduction throughout the year, rather than mating seasonally, as is observed in many other insects (Kobayashi & Osakabe 2009; Tauber et al. 1986).

We found individuals of *Micrarchus* during the winter at two of the three sites visited, including one montane and one lowland population. *Micrarchus* nov. sp. 1 was readily found at lowland site of Puhi Puhi, where a total of 20 individuals were observed, including several active at night, when the canopy temperature measured +2.4˚C. Several individuals were also found at this site buried in the grass during the day, suggesting that they may use this habitat for protection, similar to *N. annulata*. However, as we also observed active individuals in the trees at night, this suggests they are sheltering at ground level during the day and moving into their host plants at night. We did not survey high altitude populations of *Micrarchus* nov. sp. 1, so we are unable to determine at what life stage this species over-winters at increased elevations.

Live animals were also found in one of the two montane populations of *Micrarchus* nov. sp. 2 surveyed in July. At Sewell Peak, 10 individuals were found during day and night searches. Although canopy temperature measured during collection were only slightly cooler than some sites (+2.5˚C), most individuals were located at the base of *L. scoparium* plants, either in the leaf litter and grass, or compressed a few centimetres into the mud at the base of the plant stem. A single nymph found in the leaves of a small bush during the day was the only individual observed in the canopy.

Winter collection of *Micrarchus* nov. sp. 2 at Mt. Arthur was prevented by large snow drifts. This site appears to have significant snow cover for a large portion of the winter, as was observed by Salmon (1991) in his discussion of what is now *Micrarchus* nov. sp. 2. Here, buffering by the snow kept this site relatively warm for much of the winter (Figure 3) such that the temperature extremes experienced by the Mt. Arthur population are less extreme than at Sewell Peak, and presumably other montane sites with less insulating snow. If insects do remain under the snow (which we could not determine), then buffered temperatures would mean that selection for cold tolerance would be weaker relative to Sewell Peak or the montane populations of *N. annulata*.

Conclusion

We have found that a variety of life stages overwinter in the three montane species surveyed: *N. annulata*, *Micrarchus* nov. sp. 1 and *Micrarchus* nov. sp. 2. There appears to be a reduction in the winter population abundance, as has been observed in other alpine insects in New Zealand (Sinclair et al. 2001). There was no clear relationship between altitude and mean temperature, but more freezing hours were recorded at high elevation. However, as both lowland and montane sites repeatedly experience sub-zero temperatures in winter, overwintering individuals in all populations must tolerate similar freezing conditions. Further investigation is now needed to determine how adults and nymphs survive freezing, and if that strategy is the same among stick insects species found in New Zealand.

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Table 1: Location, two-letter land area codes (Crosby et al. 1998), elevation, coordinates, summary of collections and caging. Sites with + are entirely parthenogenetic populations. Locales with heavy snow that prevented July collections are marked with a *. Grey shaded boxes indicate locales where cages were not placed. F = female, M= male and N= nymph.

Site	Elevation	Latitude, Longitude	April 2011, total	July 2011, total	Caged , April 2011	Cage, July 2011
Micrarchus nov. sp. 1						
Puhi Puhi Scenic Reserve, KA	290 m	-42.2397, 173.7528	Not visited	12 F, 5 M, 3 N		
Micrarchus nov. sp. 2						
Mt. Arthur, Kahurangi National Park, NN	1347 m	-41.1978, 172.7127	Not visited	0*		
Sewell Peak, the Paparoa Range, BR	736 m	-42.4052, 171.3424	8 F, 8 M, >5 N	2 F, 5 M, 3 N		
Niveaphasma annulata						
Lake **Ohau** ski field Rd., Mt. Sutton, MK	810 m	-44.2418, 169.8036	9 F, 11 M, 34 N	2 F, 1 M, 4 N	3 F, 7 M, 15 N	3 N
Rastus Burn Scenic Reserve, **Remarkables**, CO+	1031 m	-45.0267, 168.7852	0	0*		
Nevis Rd., Carrick Range, Bannockburn, CO+	604 m	-45.2594, 169.2112	2 F, >20 N	0*		
Malvern St., **Dunedin**, DN	49 m	-45.8501, 170.5044	Not visited	3 F, 15 M, 4 N		
Old **Coach Rd**. Track, Papatowai, SL	1 m	-46.5529, 169.4746	2 F, >30 N & M	0		
Seaward Moss Conservation Area, Invercargill, SL	9 m	-46.5419, 168.4339	16 F, 18 M, 22 N	4 F, 1 M, 2 N	3 F, 5 M, 6 N	3 F, 1 M

Table 2: Average frequency of observations of temperatures below 0°C at sites containing *Micrarchus*. Observations were made every 1.5 hours, and the average of three data loggers is presented. Localities are detailed in Table 1. Missing data are indicated by a dash (-).

Month	Sewell Peak	Sewell Peak	Mt. Arthur	Mt. Arthur	Puhi Puhi	Puhi Puhi
Habitat	Canopy	Litter	Canopy	Litter	Canopy	Litter
January	0	0	0	0	0	0
February	0	0	0	0	-	-
March	0	0	2.0	0	-	-
April	0	0	18.0	9.0	-	-
May	1.0	0	27.0	8.0	-	-
June	0.3	0	59.0	36.0	-	-
July	33.7	33.5	106.0	36.0	20.3	6.0
August	48.3	57.0	67.0	36.0	42.0	19.0
September	11.3	6.0	135.0	29.0	6.7	0.7
October	0	0	29.0	9.0	5.7	1.0
November	6.3	1.5	26.0	3.0	0	0
December	0	0	0	0	0	0

Table 3: Average frequency of observations of temperatures below 0°C at sites containing *Niveaphasma annulata*. Each measure represents a 1.5 hourly recording below 0˚C and is the average of three data loggers. Localities are detailed in Table 1.

Month	Coach Rd.	Coach Rd.	Seaward Moss	Seaward Moss	Dunedin	Nevis Rd.	Nevis Rd.	Remarkables	Ohau
Habitat	Canopy	Litter	Canopy	Litter	Ground	Canopy	Litter	Ground	Ground
January	0.4	0	2.5	2.0	0	0.7	0	0	0
February	0.8	0	3.0	1.7	0	0	0	0	0
March	1.2	0	7.0	3.3	0	0	0	0	0
April	5.0	0	29.5	25.7	0	20.0	12.3	4.3	1.3
May	2.0	0	2.0	1.3	0	10.7	5.3	2.0	0.0
June	12.6	0.3	58.0	45.0	0	41.0	32.0	26.7	3.0
July	4.2	0	37.5	33.7	5.5	223.0	185.7	39.3	127.3
August	8.4	0	35.0	24.3	6.0	129.0	128.3	79.7	78.0
September	4.4	0	14.0	11.3	0	57.0	52.3	17.7	11.0
October	4.0	0	28.0	24.3	0	11.7	9.0	0.7	0.3
November	4.4	0	10.0	5.0	0	11.3	7.7	2.0	5.7
December	4.8	0	4.0	1.7	0	0	0	0	0

Figure 1:

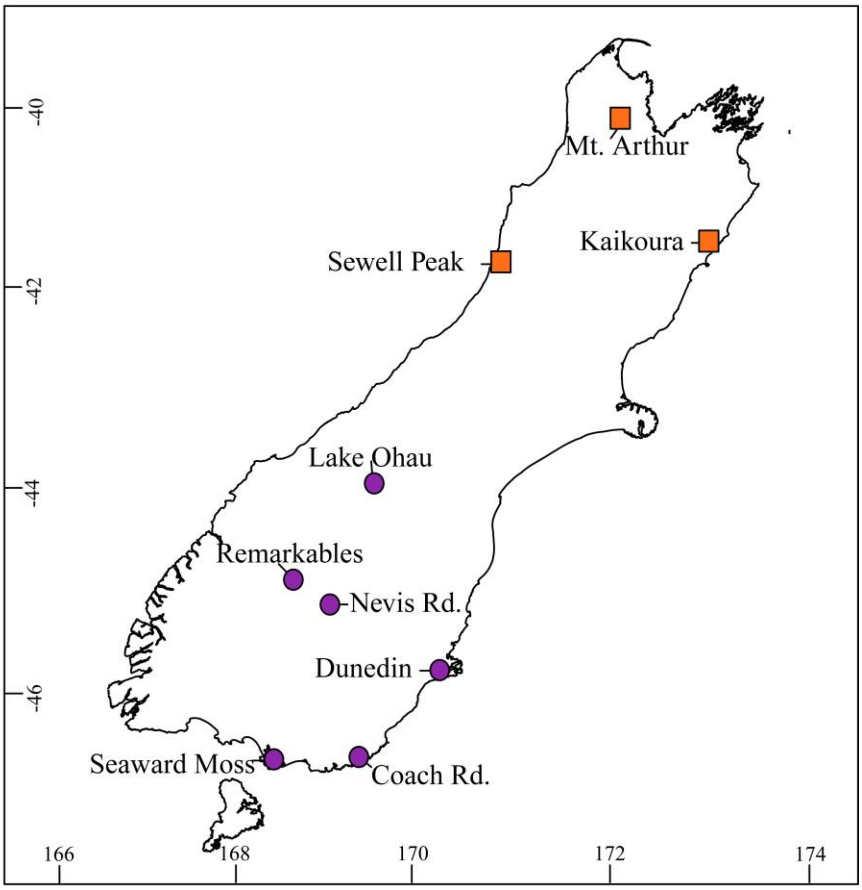
(a) An iButton thermochron data logger and plastic housing. The data logger is approximately 15 mm in diameter and was placed with the open end of the housing facing downwards.

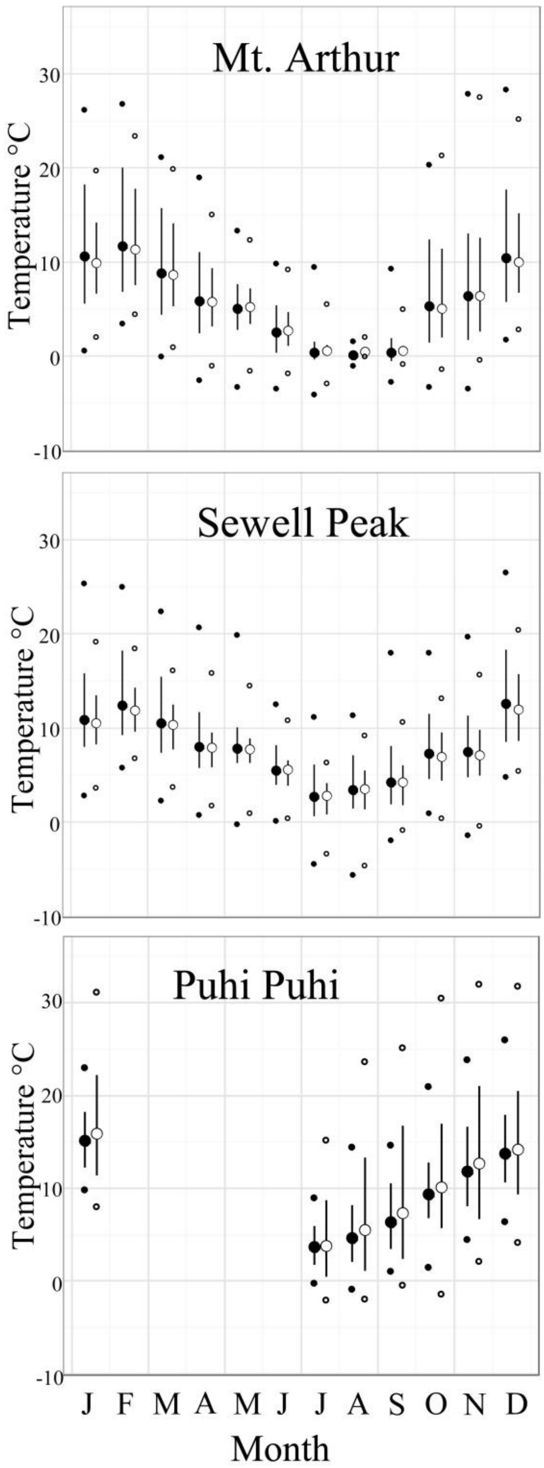
(b) A cage placed at Seaward Moss surrounded by *Leptospermum scoparium* host plants. The cage was constructed around a small *L. scoparium*, contained a known number of insects (Table 1), and was left in place from April to July 2011.

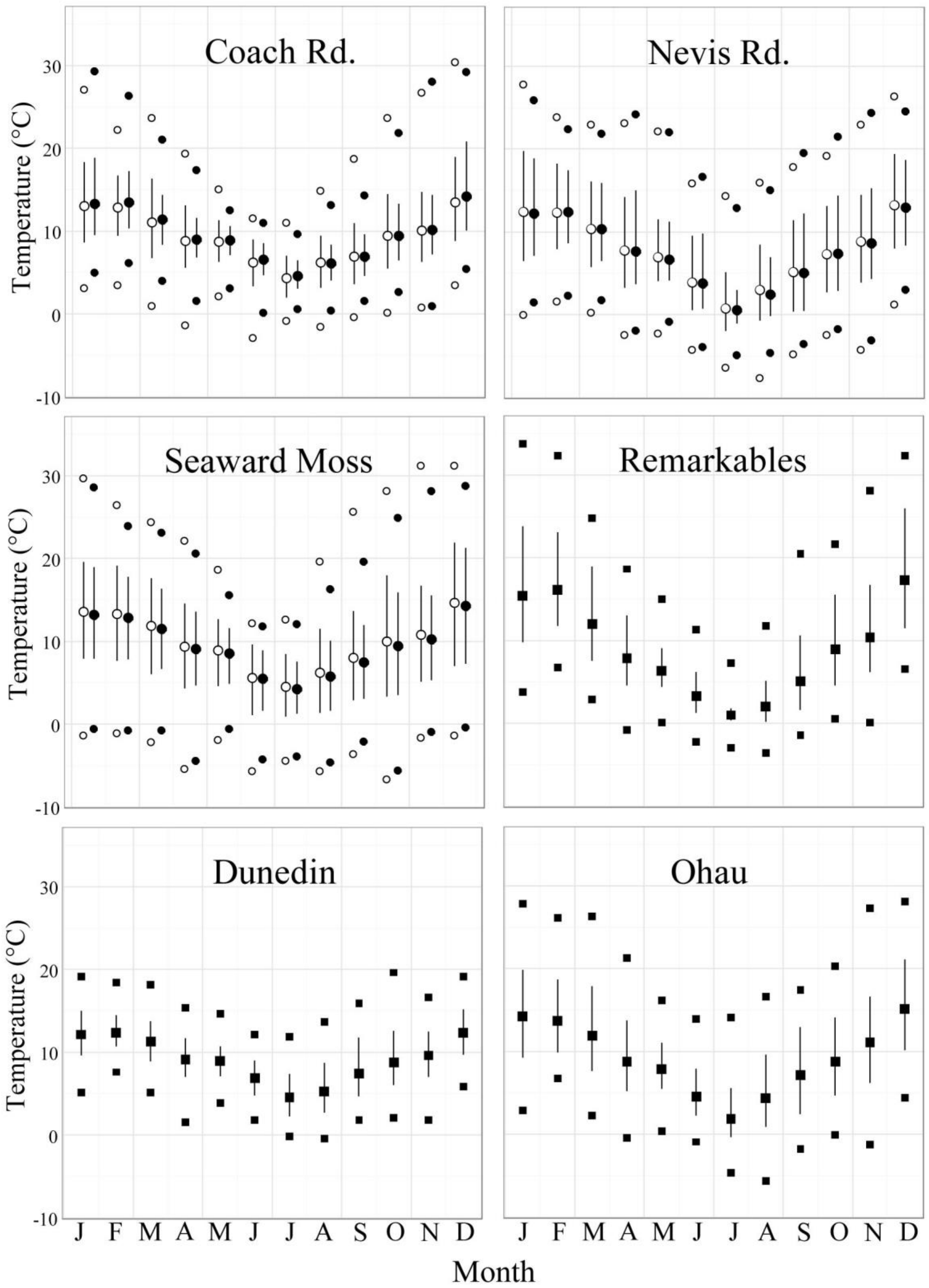
Figure 2: Map of collections sites containing *Niveaphasma annulata* (circles) and *Micrarchus sp*. (squares), coordinates are degrees S and E (WGS 1984 datum) and are detailed in Table 1.

Figure 3: Summary of mean monthly temperature recordings for sites containing *Micrarchus sp*. (large circles). Vertical lines span the mean daily maximum and mean daily minimum temperature for each month, and outer points indicate extreme monthly temperatures. Paired points for each month represent recordings from the plant canopy (open circles) and the leaf litter (dark circles).

Figure 4: Summary of mean monthly temperature recordings for sites containing *Niveaphasma annulata* (large circles). Vertical lines the span the mean daily maximum and mean daily minimum for each month, and outer points indicate extreme monthly temperatures. Paired points for each month represent recordings from the plant canopy (open circles) and the leaf litter (dark circles). Sites at which solitary data loggers were placed close to the ground, but not in litter, are represented by black squares.


Mt. Arthur
Kaikoura
Sewell Peak
Lake Ohau
Remarkables
Nevis Rd.
Dunedin
Seaward Moss
Coach Rd.
-40
-42
-44
-46
166
168
170
172
174


Mt. Arthur
Sewell Peak
Puhi Puhi
Temperature °C
30
20
10
0
-10
J F M A M J J A S O N D
Month


Coach Rd.
Nevis Rd.
Seaward Moss
Remarkables
Dunedin
Ohau
Temperature (°C)
30
20
10
0
-10
J F M A M J J A S O N D
Month

404