Behavioural ecology and prey attraction of the New Zealand Glowworm *Arachnocampa luminosa* (Skuse)(Diptera: Mycetophilidae) in bush and cave habitats.

A thesis presented in partial
fulfilment of the requirements
for the degree
of Master of Science
in Zoology at
Massey University

Richard Adam Broadley
1998

To my father

Abstract

Larvae of the mycetophilid *Arachnocampa luminosa* (Skuse), known commonly as "glowworms", inhabit damp, sheltered and shaded places in bush, and caves. The glowworm is predaceous, and it lives within a mucus tube or gallery from which hang vertical "fishing lines" made from silk and sticky mucus. Invertebrates are captured on the fishing lines and hauled up by the larva and eaten.

Glowworms use bioluminescence to attract invertebrates. I tested the effectiveness of bioluminescence by comparing the numbers of invertebrates caught on transparent adhesive traps placed over glowworms with similar traps set over areas that glowworms had been removed from. Prey attraction was investigated in Reserve Cave, Waitomo, and in its bush-clad entrance over 200 days during "winter", "spring" and "summer." Traps placed over glowworms caught significantly more invertebrates overall per trap per day than control traps. Glowworms in bush attracted both greater numbers and types of invertebrates than glowworms in the cave. There were also significant seasonal differences in the numbers caught and types of invertebrates. Flying Diptera predominated in both bush (85% of the total catch) and cave (89%) habitats. Minor components consisted mainly of spiders (Araneae), Coleoptera, Hymenoptera, Orthoptera, Trichoptera, Gastropoda, Acariformes and Neuroptera. Confirmation that the attracted invertebrates were eaten by glowworms was demonstrated by collecting and examining glowworm faeces and identifying discarded material from their snares. This was done by placing blotting paper sheets under glowworms in cave and bush habitats during spring and summer. Most faecal material consisted of insect sensillae and spines, cuticle and compound eye cuticle, but discarded legs, antennae and wings were sometimes present either as parts or entire. Entire or fragmented millipedes were sometimes present, especially in the cave. Occasionally insect head capsules, thoraxes and abdomens were also discarded. Several small snail shells (Gastropoda) were found under bush glowworms and three entire insects were found under glowworms in the cave in summer. No adult *A. luminosa* were caught on adhesive traps, or identified in the material discarded from glowworm snares. Glowworms under adhesive traps appeared to be able to survive for long periods without food, especially those in the cave, which all survived with little or no food for 78 days.

At Waitomo, variation in light output by different glowworms affected the number of invertebrates that were attracted to the light and increased the variance in prey numbers between different glowworms. This was overcome at Waitomo by running the experiment for long periods of time in order to demonstrate attraction. Using live glowworms in such experiments was also labour intensive and time consuming. A light-emitting diode (LED) with a similar maximum wavelength to glowworms was used, to explore the possibility that it

could be used to sample the potential food of glowworms in areas where glowworms do not occur, such as some passages in caves. The suitability of these LEDs were tested by comparing catches in adhesive traps containing them with traps containing glowworms and traps without (controls). These were run in bush for 21 days and then a further 21 days in a cave passage at Piripiri Road Caves, Pohangina. Traps with LEDs caught a significantly greater total number of invertebrates overall than traps either with or without glowworms. However, there were no significant differences in the numbers of Mycetophilidae, other Diptera families and other invertebrates caught on the three trap types in bush or in the cave.

The prey recognition behaviour of *A. luminosa* larvae involves taste and/or smell. This was demonstrated by comparing the numbers of live and dead *Drosophila melanogaster*, and blotting paper that was both dry and soaked in crushed *D. melanogaster* juice, that were "hauled-up", "discarded", "left hanging" or "missing" the day after they were placed on the vertical fishing lines of larval snares. There were significant differences between responses of glowworms to dry paper, wet paper, and dry paper placed above *D. melanogaster* on fishing lines. Most (72%) of the pieces of paper with crushed *D. melanogaster* juice were hauled up into glowworm snares, and none were discarded, whereas dry pieces of paper were found hauled up 16% of the time but 40% of them had been discarded.

Remote recording of *A. luminosa* in both bush and cave habitats at Waitomo was done between 18/1/95 and 19/11/95. Observations were made using infra-red light and a TV camera which was sensitive to this light. A total of 934 individual "larva-hours" of activity were recorded, including 308 "larva-hours" of 4 glowworms in bush; 345 "larva-hours" from 4 glowworms in Demonstration Chamber of Glowworm Cave; 233 "larva-hours" from one glowworm in Reserve Cave; and 48 hours of several glowworms in Waitomo Waterfall Cave. Observations were also made of three adults emerging from their pupal exuviae. A male adult was observed alighting upon a female which had not glowed for about 53 minutes, and the pair copulated. This provides evidence to support the suggestion that adults may use olfactory organs in mate attraction. After copulating they were both eaten by a large predatory harvestmen (*Megalopsalis tumida* Forster). As a result of video-taping *A. luminosa* many observations were made and many types of behaviour were identified. The most obvious ones were production of bioluminescence, "fishing line construction", "defecation", "fighting" between pairs of larvae, "prey capture", and attempted capture of invertebrates by larvae. Behaviours recorded rarely were the eclosion of both male and female adult *A. luminosa* from pupae, mate attraction, and copulation. Glowworm larvae in bush glowed only at night. They usually became active late in the afternoon when they began to make fishing lines, repair snares, and void defecatory droplets. They started to glow up to an hour and a half after becoming active, and they

turned on their bioluminescence relatively quickly, from less than 15 seconds to about 1 minute for a bright light to be visible. At dawn, glowworms in bush took several minutes to fade out their lights, but on cold nights (~ < 6°C) they glowed only intermittently or not at all. Larvae in the Demonstration Chamber appeared to be disturbed by cave lights and wind currents apparently generated by the activities of humans in there. When cave lights were switched on for more than about 30% of the time (~ 20 minutes per hour), larvae did not glow brightly and spent little time making fishing lines (< 5% of time per hour). However, there is no way to be certain if this disturbance was detrimental to their overall well-being. The larva in Reserve Cave glowed on average only between 13:00 and 02:00 on only four out of eleven days, and did not appear to glow brightly compared with glowworms either in bush or in Glowworm Cave. However, it was not possible to determine whether this behaviour was typical of other glowworms living in the cave. Only one observation of prey capture was made, and this occurred in bush. It appeared to be a small winged dipteran. Three other partial observations were made of insects being hauled-up. Observations were made in bush at night of spiders which appeared to move accidentally through glowworm snares, breaking the delicate fishing lines. However they were not caught and eaten by glowworms. On one occasion, a spider was attacked by a glowworm when it touched its snare, but it was strong enough to break free. Fighting between larvae usually occurred when a larva moved part-way out of its gallery to search the substrate for new points of attachment for its snare and fishing lines, and then accidentally touched the snare of its neighbour. This indicates that larvae fight to increase the size of their territory or to protect their own territory. Fighting larvae glowed brilliantly and would snap at each others heads with their jaws, and occasionally tried to pull each other out of their snares. These fighting episodes usually concluded when one of the larvae retreated, but often the fights would resume some time later. Larval cannibalism was not observed, although on one occasion a larva was bitten on its body by an intruder which had moved completely out of its snare. Defecation was observed on ten occasions. In bush, larvae either voided excretory droplets out of the snare or produced them so they hung on fishing lines. In the latter case the larvae then lengthened the fishing lines until the droplets made contact with the substrate. In caves the glowworms cut and dropped entire fishing lines with droplets on them, or they left them hanging within the snare.

Starch-gel electrophoresis of allozymes showed that populations of glowworms tend to be genetically discrete but with no particular geographic or ecological structuring between them. This was demonstrated by collecting *A. luminosa* larvae from many cave and bush sites in the North Island. Average Standard Genetic Distances between populations were determined (Nei, 1975) and the results were subjected to

cluster analysis using average pair group clustering with the package M.V.S.P. This showed that geographically adjacent glowworm populations did not tend to be more similar genetically than distant populations and that glowworm populations did not cluster into bush and cave populations. However, the high degree of polymorphism (range ~ 38% to ~ 86%) and heterozygosity (range ~ 3% to ~ 18%) suggests gene flow occurs regularly between glowworm populations, and does not support the notion that cave and bush forms should be regarded as distinct species or subspecies.

Acknowledgements

Financial support for this research was provided by the (formerly) T.H.C. Waitomo Caves Hotel, Waitomo Caves Management Committee, Ruapuha Uekaha Hapu Trust, Department of Ecology at Massey University, and a graduate research award from the Massey University Graduate Research Fund.

Without the kind help of all of the following people this work would not have been possible - to all of you, thanks a lot: Bryson Anderson for showing me the glowworms up at Waituna West; Ian Andrew and Jay McCartney at Massey for identifying invertebrates. John Ash and Pete Chandler and guides of Blackwater Rafting for loaning me helmets, lamps, and an inflatable raft when needed, and also for granting me a free trip; Penny Aspin for taking cave photographs. Nick Broomfield at Massey for expertise in the editing of video tapes; Alan Carpenter and Case van Epenhausen for providing me with a bucket full of insect adhesive at short notice; Kate Banbury for information regarding lighting in Glowworm Cave; Stuart Burgess at the National Institute of Water and Atmospheric Research Ltd (NIWA) in Wellington for Waitomo rainfall data; Nicci Coffey for helping to lug equipment into Reserve Cave; Peter Dimond for his hospitality and for providing me with space for all my gear in the Museum of Caves and use of the workshop there; Allan Goss for allowing me to conduct research on his land at Piripiri Road Caves; Sacha Hall at the Carter Observatory in Wellington for various atmospheric data; Shane Hatcher for providing limitless supplies of *Drosophila* at a moments notice; Ian Henderson for much-needed help with statistical analyses; Dave Hurst for help collecting samples in the field and caving adventures; Jens Jorgensen for constructing various ingenious pieces of equipment from scrap material; Bruce Rapley, Dexter Muir, and Wyatt Page for their kind help with constructing the camera casing, infra-red light sources, light traps and various other pieces of equipment, and for teaching me some useful skills i.e. how to use a lathe and a soldering iron; Oliver Paskins for helping collect inverts off adhesive traps and insect collecting expeditions; Steve Pilkington for providing transport to get most of the gear up to Waitomo; Chris Pugsley for some brain-picking and use of his cave map; Ray Scrimgeour of DoC Te Kuiti for giving the all clear to conduct research at Reserve Cave; Ashley Shaw of the New Zealand Speleological Society for permission to use cave maps; Allan Singleton at NIWA for providing me with atmospheric data for Glowworm Cave; Dave Smith for a guided tour around several caves at Waitomo; Cam Speedy of Department of Conservation Taupo/Tongariro Conservancy for granting me permission to collect glowworm samples from Okupata; Robert Tahi and guides of Glowworm Cave for advice and assistance; Tamsin Ward-Smith for her help and assistance; Dave Williams, former manager of the Waitomo Caves Hotel for allowing me to stay at the cottage for free throughout the field work and for much

needed local knowledge; and to the many travellers I met at the hostel and everybody else who took an interest in what I was doing, and lent a hand.

Thanks especially to my family for their support, and to my supervisors Dr Ian Stringer and Dr Alastair Robertson, who introduced me to a brilliant thesis topic.

Contents **Page**

List of Figures

List of Tables

Overleaf *A boat-load of visitors gaze up at hundreds of pinpoints of bioluminescence produced by* Arachnocampa luminosa *larvae in the famous grotto of Glowworm Cave, Waitomo (Printed by permission Waitomo Museum of Caves).*

I wish I was a glowworm
For they are never glum
How can you be unhappy
When the sun shines out your bum?

- Ricardo Palmer, Entomologist, Museum of New Zealand

Chapter 1 GENERAL INTRODUCTION

In 1887, Fred Mace, an English surveyor, and Tane Tinorau, a Maori Chief, constructed a raft which they used to make the first exploration of what is now known as Glowworm Cave at Waitomo. The spectacular display of blue-green bioluminescence produced by larvae of *Arachnocampa luminosa* (Skuse), that inhabit the ceiling of the "Grotto" is now a drawcard for visitors from all around the world. This is now an important component of the local economy, providing both revenue and employment (Arrell, 1984).

A. luminosa is an unusual member of the dipteran family Mycetophilidae, or "fungus gnats." Indeed, of the approximately 2000 described species worldwide only 10 or 11 are known to be luminescent (Lloyd, 1978). Coupled with this, *A. luminosa* is considered to be the "ultimate" evolutionary form in relation to its fungus-eating ancestors (Jackson, 1974), in that the larva is carnivorous, and apparently uses bioluminescence to attract prey which it traps in its sticky mucus snare. As a consequence much has already been published on its unusual habits (see Meyer-Rochow, 1990 & Pugsley, 1983).

However, many aspects of glowworm ecology remained unanswered.

The aims of my research were as follows;

1. Investigate what glowworms catch, what they eat and what is discarded both in caves and bush, and estimate prey availability in Glowworm Cave and in the nearby bush.
2. Determine how effective glowworm bioluminescence is at attracting prey both in caves and in bush.
3. Investigate how glowworms detect when prey is caught in their webs.
4. Examine differences between cave and bush glowworms.
5. Compare the behaviour of undisturbed glowworms with those disturbed by human activity.

Unlike workers in the past, I made most of the behavioural observations remotely by using infra-red time-lapse video recording equipment to reduce observer interference.

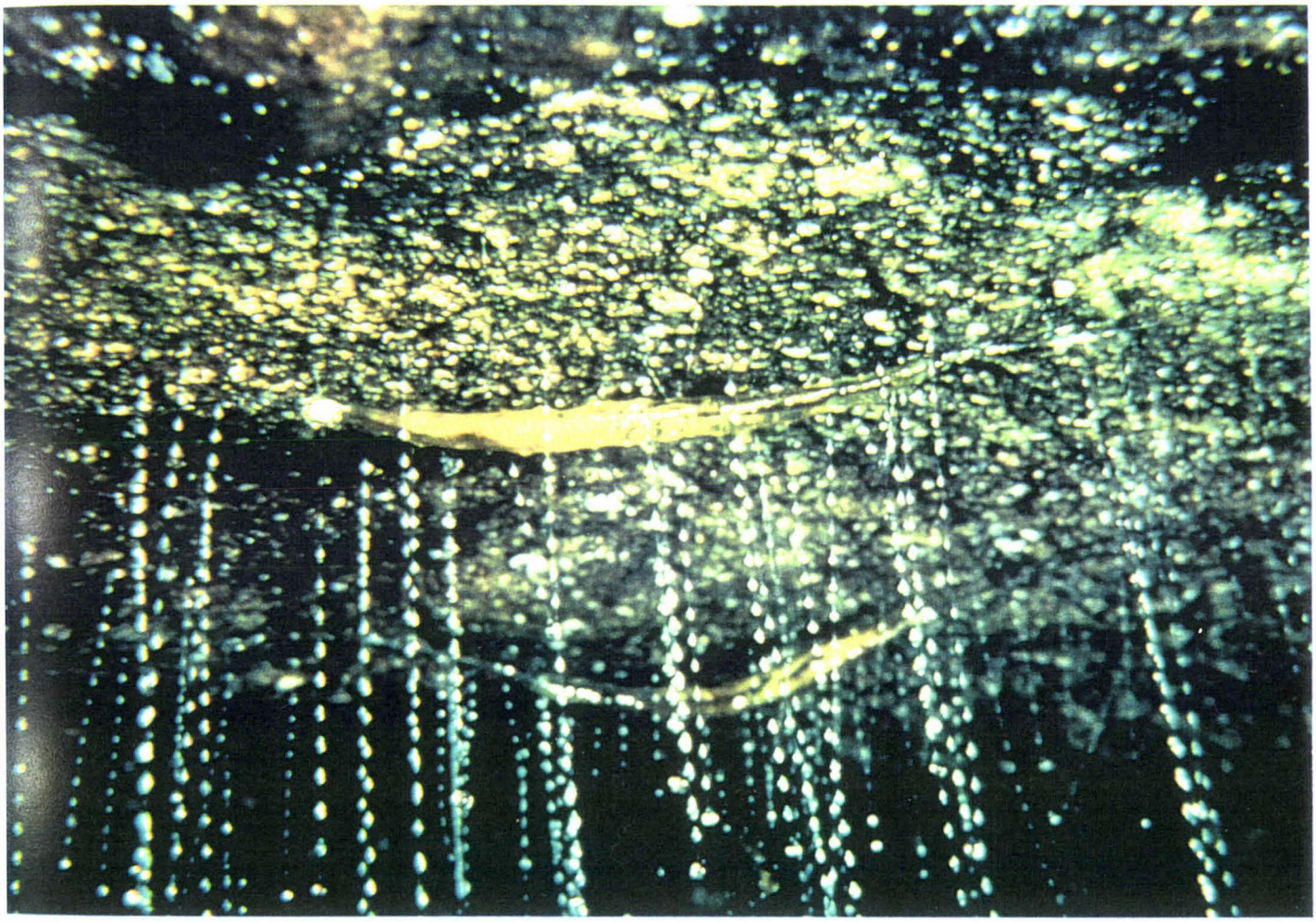


Figure 1.1 *Larvae of* Arachnocampa luminosa *(pictured about 3 times normal size), known commonly as glowworms. Each larva lives within a hollow mucus gallery suspended from the substrate by a web of fine branching threads. Numerous vertical silk threads or fishing lines beaded with sticky droplets hang from the web to form the snare. The snare is used for catching insects attracted to bioluminescence produced by these larvae. Once an insect becomes entangled in the sticky fishing lines it is hauled up by the larva and eaten (printed by permission Waitomo Museum of Caves).*

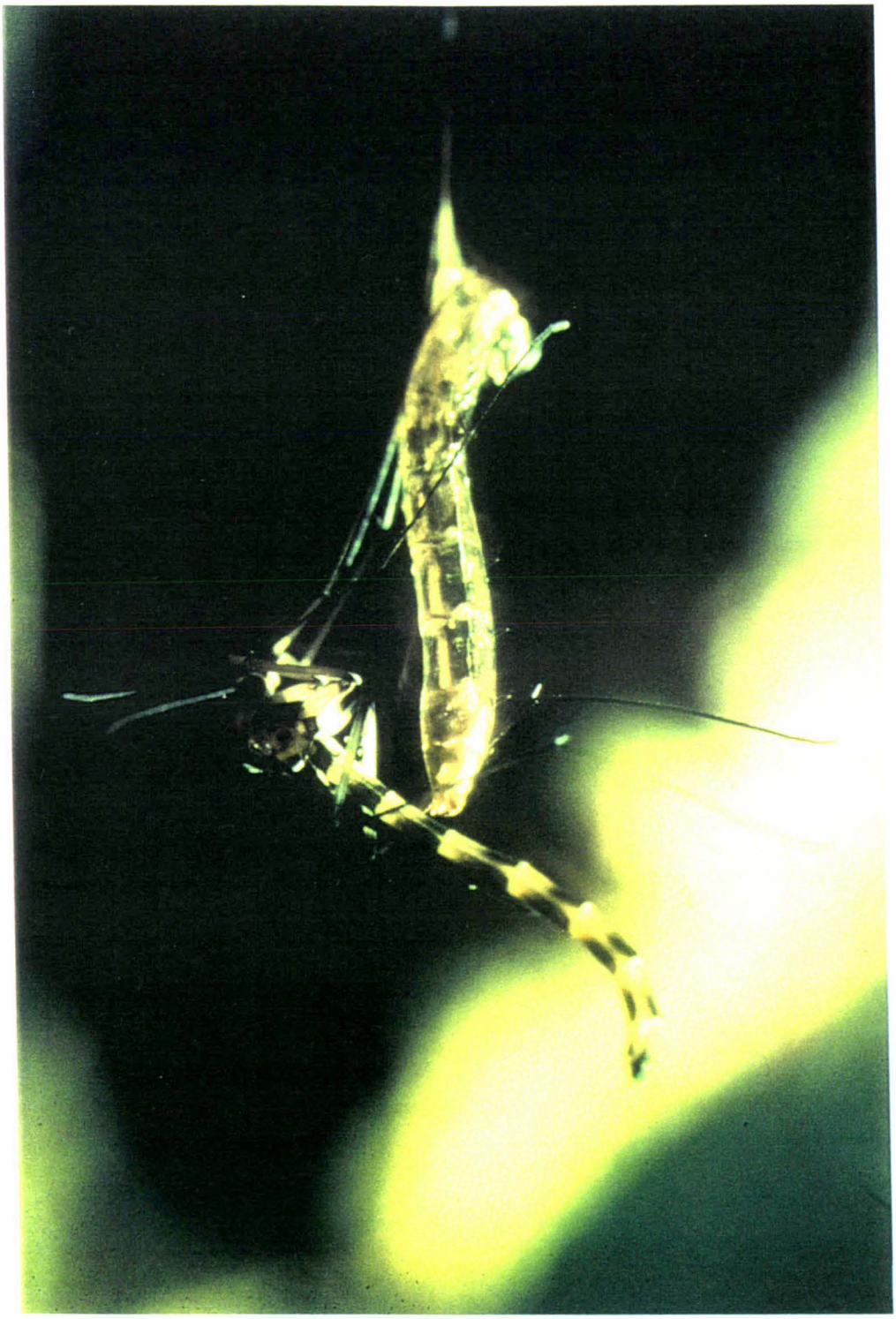


Figure 1.2 *A male* Arachnocampa luminosa *adult clings to a female pupa (pictured about 6 times normal size). Once the female adult has emerged from the pupal case they will mate (printed by permission Waitomo Museum of Caves).*

Chapter 2 PREY ATTRACTION BY GLOWWORMS IN A CAVE AND IN THE BUSH-CLAD CAVE ENTRANCE

Introduction

Larvae of the New Zealand Glowworm *Arachnocampa luminosa* (Skuse) "occur in caves, unused mining tunnels, along stream banks, in damp bush-clad ravines, in damp, shady crevices and under tree-fern fronds in rain forest, often forming quite impressive displays with their myriad twinkling bluish-green lights" (Richards, 1960). Meyrick (1886) was first to suggest that the larva might be ". . . carnivorous, feeding on minute insects, which it entangles in the slimy network; and I conjecture that it uses its lamp (as I do mine) to attract them, or perhaps, to see to eat them." Larvae were reported to feed upon a variety of invertebrates (Norris, 1894; Edwards, 1933; Gatenby, 1959; Richards, 1960). Their prey are hauled up after becoming entangled in the sticky vertical fishing lines (Gatenby & Cotton, 1960; Richards, 1960; Stringer, 1967). In addition, further evidence that glowworms eat their prey comes from examinations of the gut contents of larvae by Wheeler & Williams (1915) and Gatenby (1959), which revealed pieces of chopped up insects.

The bioluminescence of *A. luminosa* has been assumed to be used for attracting prey ever since Meyrick (1886) first suggested this. However, we know little about how effective this bioluminescence is as an attractant, or of the types of invertebrates that are attracted to it. Previous accounts of what are caught are derived from invertebrates found in fishing lines (Norris, 1894; Richards, 1960; Stringer, 1967; Pugsley, 1984) and from insects trapped in areas where glowworms occur (Pugsley, 1984; Oxenham, 1985).

Bioluminescence of *A. luminosa* has a maximum wavelength of 487 nm (Shimomura et al, 1966). Such light, of generally shorter wavelengths signifies "open space" (Mazokhin-Porshnyakow, 1969) and, at least in caves, such shorter wavelengths provide a strong phototactic stimulus to the larvae of aquatic insects that accidentally enter caves, but whose adults "wish to get out" (Meyer-Rochow & Eguchi, 1984). May (1963) reported that trogloxenes[1] formed approximately half of the total cave fauna collected in the Port Waikato - Piopio limestone area of New Zealand. Most of these were Coleoptera, Diptera, Trichoptera and Ephemeroptera. The only information about attraction of insects to lights of different wavelengths in caves is by Stringer & Meyer-Rochow (1994). They worked in a Jamaican Cave and reported that flying Diptera were most strongly attracted to light traps emitting wavelengths between 412-531 nm (blue-green).

[1] According to May (1963) *trogloxenes* "are creatures of the outside which enter caves for various reasons but do not, as a rule, complete their life cycle there."

Besides species of *Arachnocampa*, which occur in both New Zealand and Australia (Harrison, 1966), only two other predaceous luminescent web-spinning Mycetophilidae are known (Lloyd, 1978; Pugsley, 1983; Meyer-Rochow, 1990). However, only one of these, *Orfelia fultoni* (Fisher), uses its light as a lure to attract potential prey (Sivinski, 1982). The larvae of *O. fultoni* inhabit small cavities in soil, mosses, dead wood, or crevices between stones in North America's Appalachian mountains (Fulton, 1941). Little is known about the other bioluminescent mycetophilid *Keroplatus sesioides* which was reported from Sweden by Wahlberg (1848). According to Meyer-Rochow (1990) these larvae inhabit webs on the underside of fungi and emit a weak bluish-white light from the entire larval body.

This chapter sets out to firstly, demonstrate whether the glowworm bioluminescence attracts invertebrates, and secondly, to determine what types of invertebrates are attracted to glowworm bioluminescence. Attraction was tested by comparing the numbers of invertebrates caught on transparent adhesive traps placed over glowworms with similar traps set over areas after glowworms had been removed from them. This was done in both bush and cave locations. Behavioural observations of glowworms hauling up insects caught in their fishing lines and eating them are presented separately in Chapter 5. Confirmation that the attracted invertebrates are eaten by glowworms was attempted by collecting and examining glowworm faeces and identifying discarded material from their snares. The collection was made by placing several sheets of blotting paper under glowworm snares in bush and cave habitats.

Materials & Methods

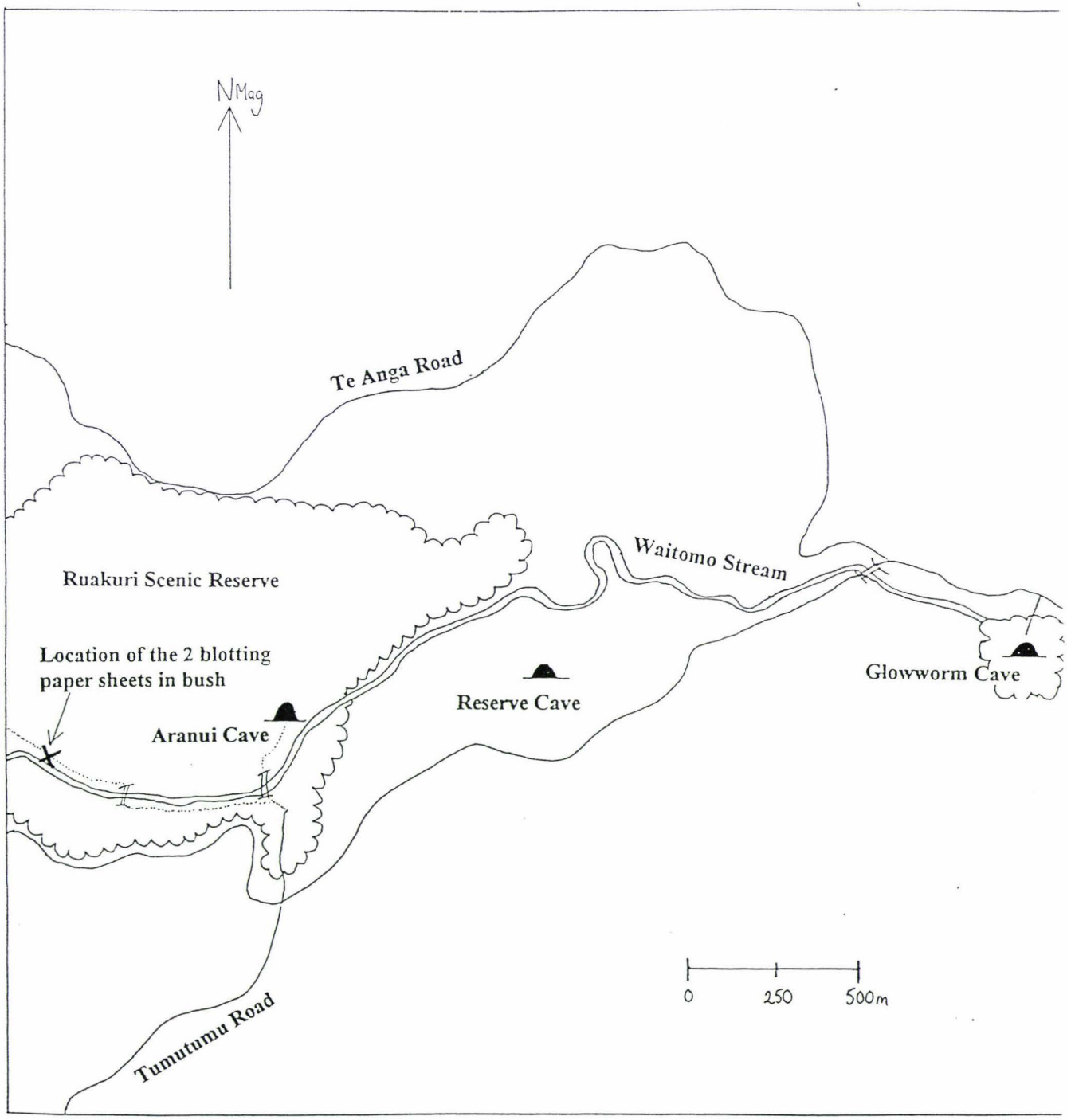
Trapping Experiments

All trapping was done in Reserve Cave, Waitomo (NZMS 260 S16, 927 246)(Fig. 2.1 & 2.2) because this cave is seldom visited despite easy access. It has a large population of *A. luminosa* larvae both at the bush-clad entrance (used here as the 'bush' location) and at the far end of the cave (~ 500 m in from the entrance) (Fig. 2.3). Traps were run simultaneously in both locations.

Invertebrates were caught in transparent adhesive traps. These were placed so each completely surrounded a glowworm larva together with its entire snare. Each adhesive trap was made from the top two-thirds of a 3-litre clear plastic fruit juice bottle (~ 120 mm long, ~ 90 mm wide, ~ 180 mm deep). The screw caps were removed to leave a ~ 32 mm diameter opening at the end of a neck - Fig. 2.4). These adhesive traps were also used as controls when set over areas after removal of the glowworms. Foam rubber was glued to the cut surface of the base of each trap to form a close seal with the substratum. A pair of aluminium hooks riveted on either side of the trap allowed the trap to be held in place by tie-wires. These tie-wires were attached to the substrate with non-toxic 'Emerkit'® (S. Austin Carr & Co Ltd., Auckland, New Zealand) epoxy resin putty. A thin coating of 'Tanglefoot'® (The Tanglefoot Co.,Grand Rapids, MI 49504, USA) insect adhesive was applied to the outer surface of each adhesive trap. Traps were left for between 60 and 78 days. All invertebrates caught on these traps were cleaned and stored in vials of kerosene before they were counted and identified. Many invertebrates were damaged during removal from the traps, so in most cases they were identified only to family.

Twelve sites with glowworms and sixteen control sites where the glowworms were removed prior to placement of the traps were randomly selected from those positions occupied by glowworms within reach at the bush location. Fourteen sites with glowworms and fifteen control sites were similarly randomly selected along the length of the stream at the far end inside Reserve Cave (Fig. 2.3). The glowworms selected for setting adhesive traps over were all of about the same size, but were relatively small (~ 15 - 25 mm) compared to full grown larvae (~ 30 - 40 mm). This was done to ensure larvae would not pupate during the trapping period. Traps were set on 4/7/95 and left for 60 days during winter. They were then set again on 9/9/95 and left for 62 days during spring, and finally set on 10/11/95 for 78 days during summer. Traps were set at the same sites each time. One glowworm site and four of the control sites in the bush were prone to water runoff in winter and this destroyed the invertebrates trapped in the adhesive. The data from these sites was not used and the sites were not used again in spring and summer. Four of the glowworms disappeared

Figure 2.1 **Location of Reserve Cave and the bush sites in Ruakuri Scenic Reserve, Waitomo.** **Adapted from Waitomo Visitor Information Map.**



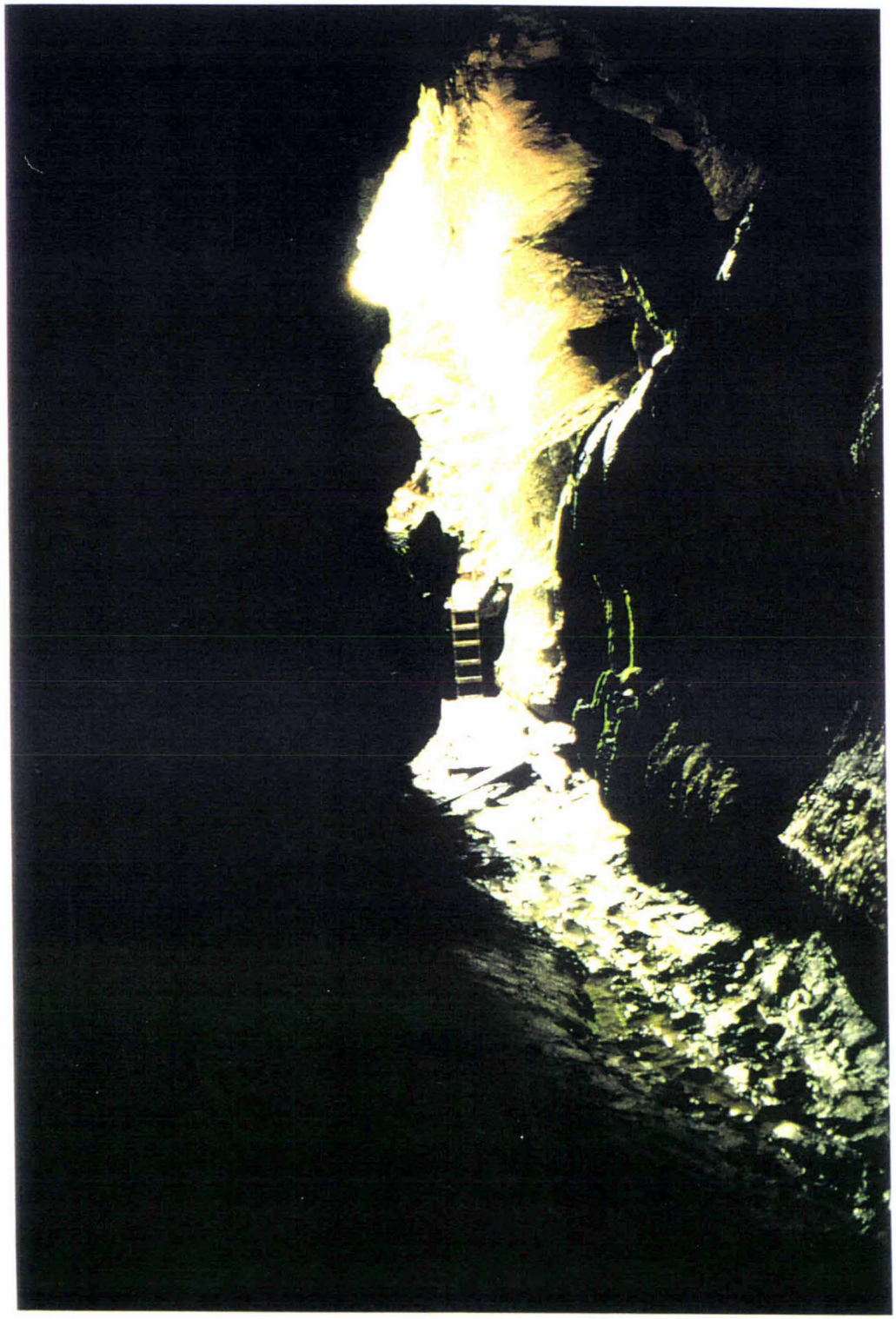


Figure 2.2 *A view of the bush-clad entrance to Reserve Cave, Waitomo, taken from about 20 metres inside the cave. Access to the cave involved descending a steep muddy track and an ~ 8 foot ladder at the bottom (pictured). Before entering the cave the track passes close by bush where adhesive traps were set (out of view). Trapping of cave invertebrates took place approximately 500 metres deeper within the cave. Glowworms were plentiful at both locations.*

Figure 2.3 **Location of both bush and cave sites at Reserve Cave, Waitomo, where invertebrates were collected from adhesive traps containing glowworms and from areas where glowworms had been removed. The location of blotting paper sheets used to collect glowworm faeces and discarded material are also shown.** Map redrawn with permission from New Zealand Speleological Society (NZSS) map by L. Fow and P. Dimond (1960).

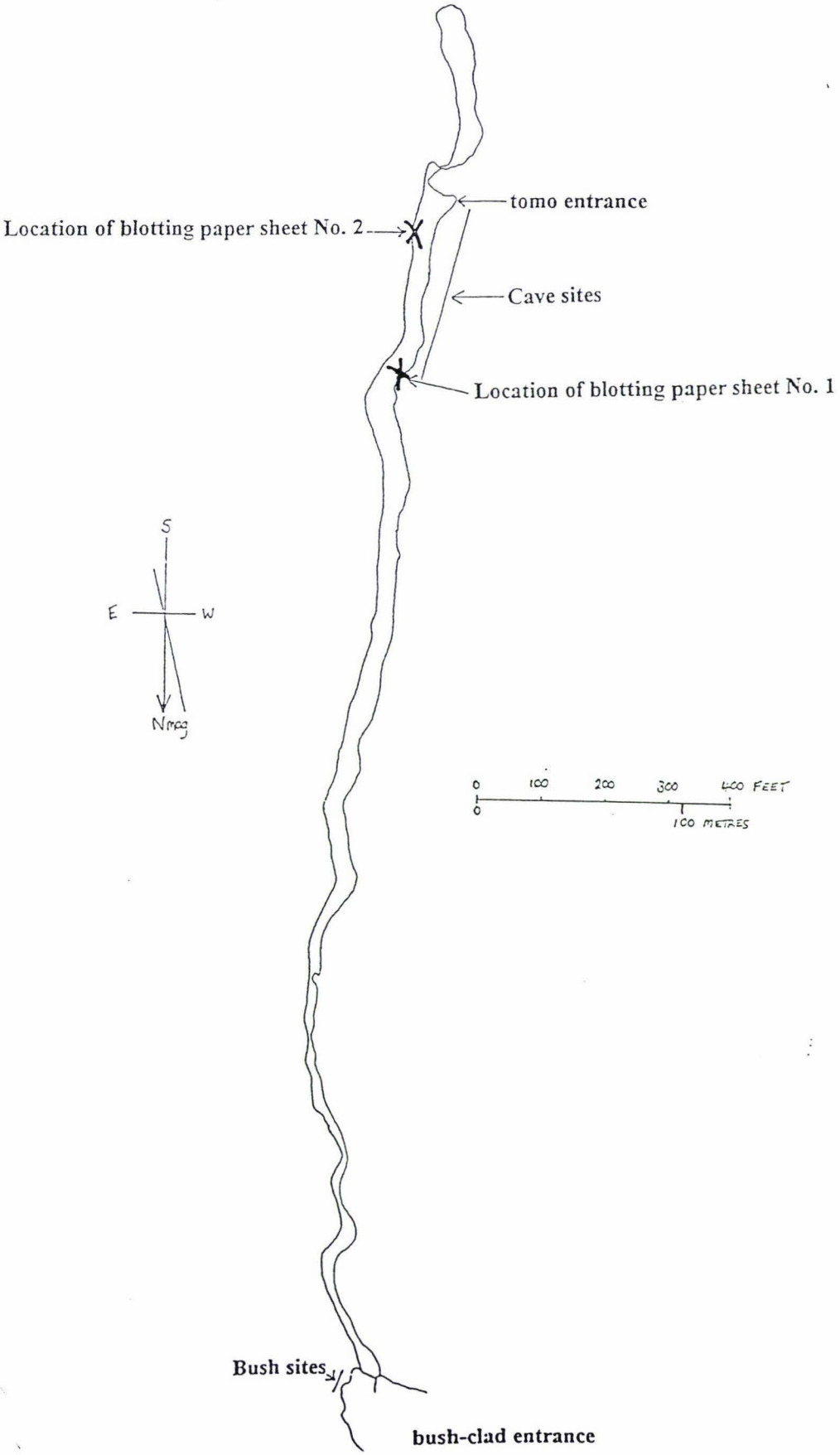
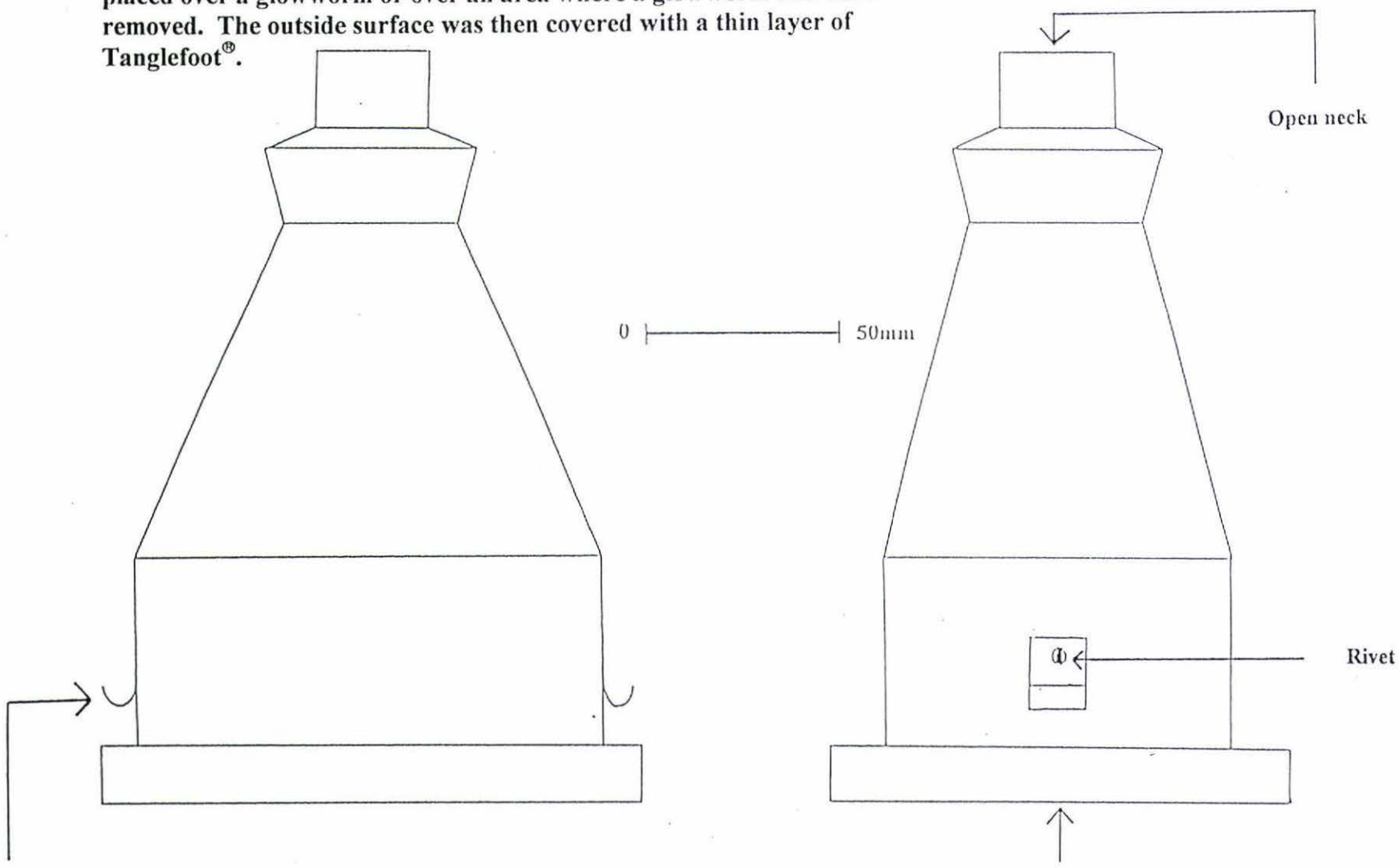


Figure 2.4 **Diagram of a transparent adhesive trap. Each trap was made from the upper two-thirds of a plastic soft drink bottle. The trap was either placed over a glowworm or over an area where a glowworm had been removed. The outside surface was then covered with a thin layer of Tanglefoot®.**



from within their traps in the bush during summer so the data from these traps were not included in the statistical analysis.

Statistical analysis was done using SYSTAT®. The ANOVA was performed on log-transformed (log n + 0.01) numbers of invertebrates caught on the traps per day.

Collection of faeces and other material discarded from glowworm snares

Faeces and other material discarded from the snares of glowworms in both cave and bush habitats were collected on sheets of blotting paper, 570 mm long by 220 mm wide. A sheet was placed under each of two groups of 3 and 10 glowworms at the end of Reserve Cave close to where the adhesive traps were set (Fig. 2.3). The sheet at one of the cave sites was protected from mud and surface water by placing it on an aluminium foil tray resting on the cave floor beneath a group of 10 glowworms. Wire and Emerkit® were used to suspend an aluminium tray to support the second sheet of blotting paper in Reserve Cave. This was positioned ~ 300 mm beneath a group of 3 glowworms that were located further towards the far end of the cave. Here it was halfway between the first sheet and where the stream entered at a tomo[1] (Fig. 2.3).

It was not possible to collect faeces and discarded material from the snares of glowworms in the bush-clad entrance to Reserve Cave because larval snares were positioned mostly in small crevices in the rock face, where material falling from them did not reach the ground. Also, a considerable amount of water runoff fell in these areas and this would have destroyed sheets of blotting paper. Two sheets of blotting paper were therefore positioned in bush nearby in Ruakuri Scenic Reserve under a suitable sheltered ledge (Fig. 2.2). Both sheets were placed on aluminium foil beneath groups of six glowworms.

All sheets of blotting paper were left in place over spring, then collected and replaced at the start of summer. Discrete clusters of droplets on the blotting paper were presumed to have originated from a single glowworm above them. These droplets were counted and, where possible, the contents of the droplets and any discarded material were identified.

[1] *Tomo* is a Maori word meaning sink hole or shaft.

Results

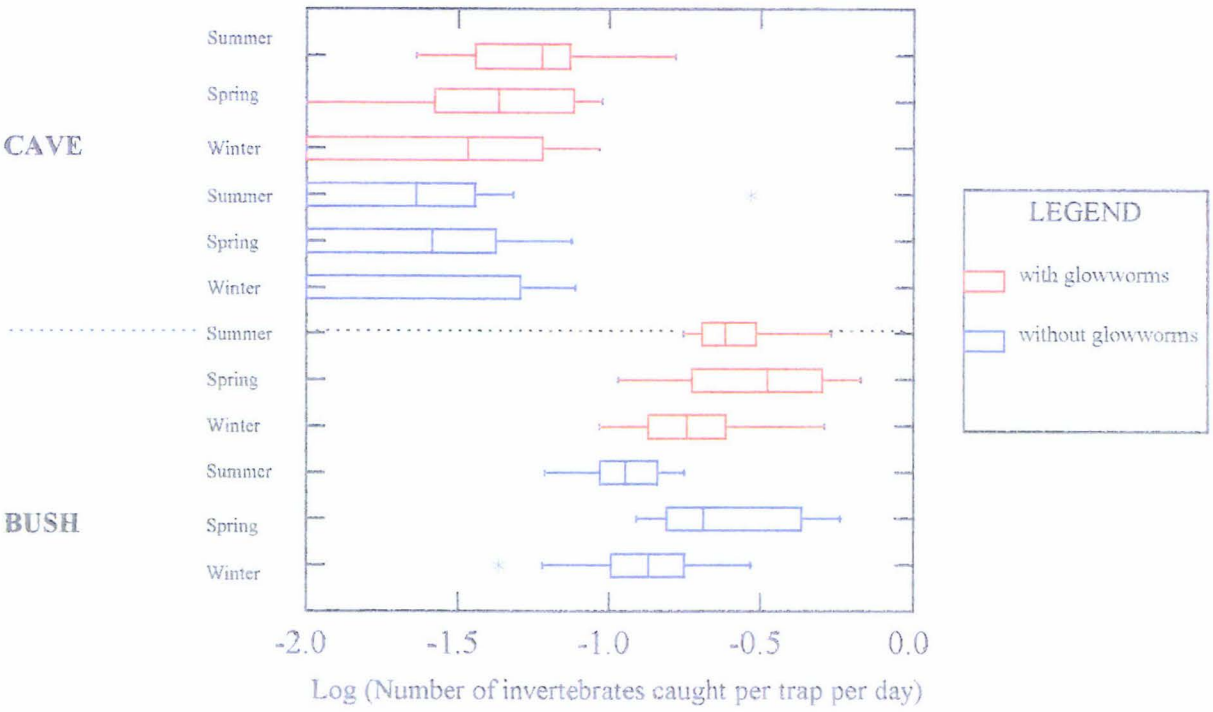


Figure 2.5 *Total numbers of invertebrates collected per day from adhesive traps in Reserve Cave and in the bush-clad entrance to Reserve Cave, Waitomo. Results are shown for winter, spring and summer. Medians, quartiles and ranges are shown and asterisks indicate outliers. The upper outlier (cave; summer) was due to the presence of numerous Empididae (Diptera) that were caught on several of the control traps in Reserve Cave during summer.*

Comparison between the numbers of invertebrates caught on traps in bush and in the cave

Traps placed over glowworms at both bush and cave sites caught significantly more invertebrates per trap per day overall than control traps (ANOVA: $P < 0.01$, Table 2.1). This demonstrates that glowworm bioluminescence does attract invertebrates. Significantly fewer invertebrates (182) were collected from adhesive traps in Reserve Cave compared with the number caught on traps in the bush (890)(Table 2.1). Cave traps caught only 20% of the total numbers of invertebrates caught in the bush.

Source	DF	Mean-Square	F-Ratio	P	% Variance Explained
HABITAT (bush & cave)	1	23.780	266.937	0.000	86.868
TREATMENT (glowworm-occupied & control)	1	2.003	22.486	0.000	7.317
SEASON (winter, spring & summer)	2	0.340	3.821	0.024	2.484
HABITAT × TREATMENT	1	0.012	0.130	0.719	0.044
HABITAT × SEASON	2	0.243	2.727	0.069	1.775
TREATMENT × SEASON	2	0.165	1.851	0.161	1.205
HABITAT × TREATMENT × SEASON	2	0.042	0.472	0.625	0.307
ERROR	141	0.089			

Table 2.1 *Analysis of Variance of the numbers of invertebrates caught per trap in bush and cave habitats, using traps occupied by glowworms or no glowworms (controls) during winter, spring & summer.*

Adhesive traps in the bush-clad entrance to Reserve Cave caught significantly more invertebrates per trap per day than adhesive traps within Reserve Cave ($P < 0.01$, Table 2.1: "Habitat"), showing that there is a significantly greater number of invertebrates as potential prey for glowworms in bush than there are for glowworms in the cave. The numbers of invertebrates caught per trap per day also differed significantly between the three seasons ($P < 0.05$, Table 2.1: "Season").

Glowworms within Reserve Cave attracted more invertebrates on average per trap per day in summer than they did in either spring or winter (Fig. 2.5). In contrast, bush glowworms attracted more invertebrates in spring, than they did in either summer or winter. However, the "Habitat × Season" interaction was not quite significant, nor were any other of the interaction terms (Table 2.1).

Prey and prey availability in bush

In the bush, Diptera clearly predominated in the catch of all adhesive traps (86% of 890 invertebrates, Table 2.2). Glowworm-occupied traps caught 59% of all the Diptera collected from all traps here. Overall, glowworm-occupied traps caught a larger total number of dipterans, more dipteran families and more invertebrates in total than the control traps. Overall, 17 Diptera families were collected from traps occupied by glowworms over all three seasons, whereas 14 Diptera families were collected from control traps. In these traps the greatest diversity of dipteran families occurred in winter (14), followed by spring (11) then summer (10). Diptera most frequently attracted to glowworm bioluminescence were Sciaridae, followed in

Table 2.2 Invertebrates captured on traps placed over *A. luminosa* larvae and over areas where larvae have been removed from

ORDER	FAMILY	Bush, over glowworm				Bush, no glowworm				Cave, over glowworm				Cave, no glowworm			
		Winter 4/7 - 26/8/95 12 traps	Spring 9/9 - 10/11/95 11 traps	Summer 23/11 - 9/2/96 7 (11) traps	Total	Winter 4/7 - 26/8/95 16 traps	Spring 9/9 - 10/11/95 10 traps	Summer 23/11 - 9/2/96 10 traps	Total	Winter 4/7 - 26/8/95 14 traps	Spring 9/9 - 10/11/95 14 traps	Summer 23/11 - 9/2/96 14 traps	Total	Winter 4/7 - 26/8/95 15 traps	Spring 9/9 - 10/11/95 15 traps	Summer 23/11 - 9/2/96 15 traps	Total
Diptera	Calliphoridae										1		1				
	Cecidomyiidae	1			1	4			4	4			4		4		4
	Chironomidae	1			1												
	Culicidae	1			1							4	4			1	1
	Dolichopodidae	18	51	42(47)	111(116)	20	48	20	88								
	Empididae	2			2		1		1		2	12	14			22	22
	Heleomyzidae	10	15	3(4)	28(29)	18	14	4	36								
	Muscidae						1		1								
	Mycetophilidae	18	7	8(10)	33(35)	21	16	1	38		4	3	7		7		7
	Phoridae	1			1			2	2								
	Psychodidae	5	21	14(16)	40(42)	2	8		10		2		2			3	3
	Rhagionidae		1		1												
	Sciaridae	17	71	27(38)	115(126)	12	41	8	61	11	20	20	51	13	9	4	26
	Simuliidae	1			1							1	1				
	Stratiomyiidae		1	1	2			1	1								
	Tanyderidae	6	1	2	9	2		4	6								
	Teratomyzidae		13	8	21			3	3								
	Tipulidae	4	13	14(17)	31(34)	3	11	9	23	3		3	6	1			1
	Trichoceridae	37	10	2	49	24	5	2	31			2	2				
	Unidentified	1	4	0(1)	5(6)	5	3		8	1	1	4	6				
	Total	123	208	121(146)	452(477)	111	148	54	313	20	29	53	98	18	16	30	64
Araneae		6	18	3(5)	27(29)	2	5	7	14					1	1		2
Coleoptera		2	6	5(8)	13(16)		1	10	11	1	1	2	4			2	2
Hymenoptera		7	3	2(4)	12(14)	2	4		6			3	3				
Orthoptera			2	2(4)	4(6)	3	1		4			1	1				
Trichoptera				1(4)	1(4)			1	1		1	2	3			3	3
Cl. Gastropoda		3		2(4)	5(7)		1		1								
Homoptera				1(4)	1(4)			3	3								
Acariformes		1	1	0(1)	2(3)	1	2	1	4								
Neuroptera				2(3)	2(3)		2	1	3								
Opiliones				1	1	1		1	2								
Collembola		1			1	1			1								
Cl. Diplopoda		1			1			1	1								
Hemiptera				1	1			1	1								
Isopoda		1			1	1			1								
Plecoptera										2			2				
Total		22	30	20(39)	72(91)	11	16	26	53	3	2	8	13	1	1	5	7
All Invertebrates		145	238	141(185)	524(568)	122	164	80	366	23	31	61	111	19	17	35	71

Bracketed figures denote numbers of invertebrates collected from traps including the 4 where larvae disappeared.

order by Dolichopodidae, Trichoceridae, Psychodidae and Mycetophilidae (Fig. 2.6). However, the frequency of capture of these families varied seasonally with Trichoceridae being caught most often in winter, Sciaridae being caught most often in spring and Dolichopodidae being caught most often in summer.

In control traps, 14 dipteran families were caught over the entire trapping period. Dolichopodidae were most commonly trapped, followed by Sciaridae, Mycetophilidae, Heleomyzidae and Trichoceridae. There were, however, no significant seasonal differences, although Trichoceridae were caught most frequently in winter and Dolichopodidae in spring and summer (Table 2.2).

Traps occupied by glowworms in the bush also caught 58% of all the non-dipteran invertebrates. Spiders (Araneae) were the most frequently trapped, particularly in spring. Most of these were also Symphytognathidae. Traps occupied by glowworms caught about 190% more spiders and about 200% more Hymenoptera than control traps, although the total numbers of spiders and Hymenoptera collected from adhesive traps in the bush were low (41 and 18 respectively, Table 2.2).

Ground-dwelling invertebrates such as small snails (Gastropoda), mites (acariformes), Collembola, millipedes, the predatory harvestmen *Megalopsalis tumida* (Forster) (Opiliones), cave weta (Orthoptera: Rhaphidophoridae) and isopods contributed less than 4% of the total number of invertebrates caught in traps occupied by glowworms. Small numbers of other non-dipteran insects were also caught on adhesive traps in the bush during summer. These were 2 Trichoptera, 2 Hemiptera, 4 Homoptera and 5 Neuroptera. These did not appear to show any preference for glowworm-occupied traps or control traps (Table 2.2), although statistical power to detect such a difference was low because of the small sample size. The dipteran families Heleomyzidae and Mycetophilidae were the only invertebrates that were caught with any frequency which appear not to display attraction for traps occupied by glowworms (Fig. 2.6). No adult *A. luminosa* were caught in any adhesive traps.

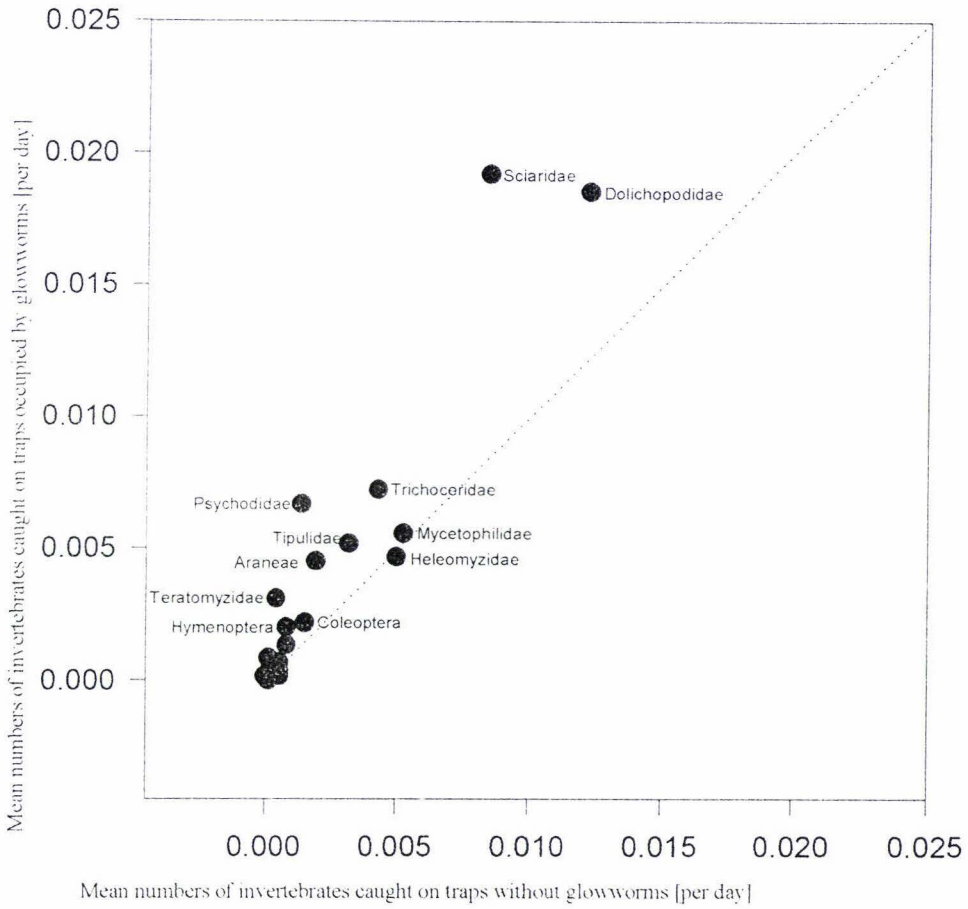


Figure 2.6 *Relative numbers of invertebrates caught per day on traps containing glowworms and control traps in the bush-clad entrance to Reserve Cave, Waitomo, during winter, spring and summer. The dotted line indicates equal numbers of invertebrates captured on adhesive traps occupied by glowworms and control traps (no glowworms).*

Prey and prey availability in Reserve Cave

In Reserve Cave, 89% of the invertebrates caught on adhesive traps were Diptera and glowworm-occupied traps in Reserve Cave contained 62% of the total dipteran catch.

Ten dipteran families were captured on traps occupied by glowworms over the three seasons (Table 2.2). Sciaridae (52% of the total glowworm-occupied trap catch) were most frequently caught followed by Empididae (14%), Mycetophilidae (7%), Tipulidae (6%)(Fig. 2.7) and Cecidomyiidae and Culicidae (both 4%). Seven dipteran families were collected from glowworm-occupied traps in summer, and this was almost double the number of families collected in both winter and spring (both 4). Overall, greater numbers of

invertebrates were collected from adhesive traps in the cave in summer (53% of the total cave catch), than in spring (26%) or winter (23%) respectively (Table 2.2). In contrast, only seven dipteran families were caught on control traps within the cave. Sciaridae (41% of the total control trap catch), Empididae (34%) and Mycetophilidae (11%) were caught most frequently, as was the case in the bush, then Cecidomyiidae (6%) and Psychodidae (5%). Sciaridae and Tipulidae display a positive association with adhesive traps occupied by glowworms (Fig. 2.7) whereas Empididae appear to avoid traps occupied by glowworms in Reserve Cave. Mycetophilidae show no preference for either traps occupied by glowworms or control traps (Fig. 2.7) and no *A. luminosa* adults were caught on adhesive traps.

A total of only 20 non-dipterans were trapped within the cave over the entire trapping period. These included 6 beetles, 3 Hymenoptera, 6 Trichoptera, 2 spiders, 2 Plecoptera and a single cave weta (Orthoptera: Rhaphidophoridae) (Table 2.2). 65% of these were caught in summer, 20% in winter and 15% in spring.

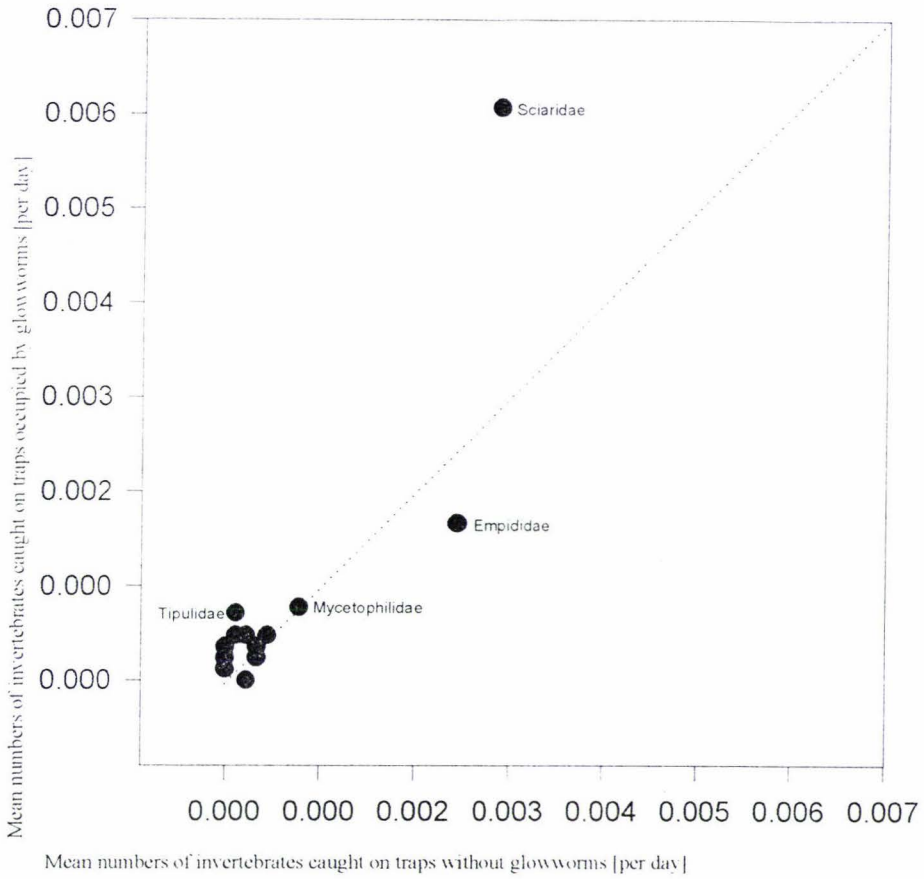


Figure 2.7 *Relative numbers of invertebrates caught on traps containing glowworms and control traps per day in Reserve Cave, Waitomo during winter, spring and summer. The dotted line indicates equal numbers of invertebrates captured on traps occupied by glowworms and on control traps (no glowworms).*

Differences in the types of invertebrates caught in bush and cave habitats

No Chironomidae, Dolichopodidae, Heleomyzidae, Muscidae, Phoridae, Rhagioniidae, Stratiomyiidae, Tanyderidae or Teratomyzidae were caught on adhesive traps in the cave, whereas all were caught on traps in the bush (Table 2.2). Also, few Psychodidae (10% of the total bush & cave trap catch), Tipulidae (13%) and Trichoceridae (2.5%) were collected from traps in the cave. In contrast, few Empididae were collected from traps in bush compared with the number collected from traps in the cave (3 and 36 respectively) predominantly in summer (Table 2.2). This indicated that the diversity of Diptera in the cave was reduced compared with the bush. A similar reduced diversity amongst other invertebrates occurred in the cave. Thus

Gastropoda, Homoptera, Acariformes, Neuroptera, Opiliones, Collembola, Diplopoda and Hemiptera were caught only in traps in the bush. Only two Plecoptera were caught on cave traps whereas none were found in bush traps. Also, six Trichoptera were caught in the cave whereas two were caught in the bush. Few Araneae (5% of the total bush and cave trap catch), Coleoptera (25%), Hymenoptera (17%) and Orthoptera (12.5%) were caught in the cave.

Comparison of Invertebrate Catch Rates and Glowworm Defecation Rates

There was a 26% decrease in the number of invertebrates caught per trap per day in the bush between spring and summer, whereas there was an increase of 131% in Reserve Cave in the number of invertebrates caught per trap per day in Reserve Cave (Table 2.3). Similarly, there was a 14% decrease in the number of excretory droplets discarded by glowworms in Ruakuri Scenic Reserve between spring and summer, whereas in Reserve Cave glowworms discarded ~153% more droplets in summer than in spring (Table 2.4). There appears to be some correlation between the numbers of invertebrates caught daily in traps occupied by glowworms and the numbers of excretory droplets discarded by them but this is not significant.

Table 2.3 *Means, ranges and standard errors (SE) of numbers of invertebrates caught per day in glowworm-occupied traps.*

	Mean	Range	SE
Bush; in spring	0.349	0.097 - 0.661	0.061
Bush; in summer	0.258	0.154 - 0.526	0.049
Cave; in spring	0.037	0 - 0.081	0.007
Cave; in summer	0.056	0.013 - 0.154	0.012

	Mean	Range	SE
Bush; in spring	0.175	0.03 - 0.4	0.036
Bush; in summer	0.15	0.09 - 0.26	0.017
Cave; in spring	0.135	0.05 - 0.21	0.014
Cave; in summer	0.288	0.22 - 0.41	0.022

Table 2.4 *Means, ranges and standard errors (SE) of numbers of droplets collected per day per glowworm from beneath glowworms.*

Material discarded from glowworm snares

Material that was stuck together into a cluster beneath a glowworm was presumed to be from a discrete excretory droplet. Most of this material consisted of well-chewed parts of insects (Table 2.5). Finely cut insect sensillae and spines were always present and cuticle and compound eye cuticle were common to most clusters. Legs, antennae and wings that appeared to have been discarded separately from the faecal droplets were sometimes present either as parts or as entire animals. In addition, entire wings of Mycetophilidae (not *A. luminosa*), Psychodidae and Sciaridae were found under glowworms in Ruakuri Scenic Reserve whereas Psychodidae and Sciaridae wings were found under glowworms in the cave (Table 2.5). Entire or fragmented millipedes were sometimes present, especially in the cave. Occasionally insect head capsules, thoraxes and abdomens were also discarded. Several small snail shells (Gastropoda) were found under bush glowworms and three entire insects were found in the cave in summer. All of the three latter insects, including one psychodid and one empidid were dry and had no contents.

Table 2.5 *Material identified from blotting paper sheets placed under bush and cave glowworms. Numbers indicate the numbers of such fragments found.*

Discarded material	Bush - spring	Bush - summer	Cave - spring	Cave - summer
Antennae	+		+	
Cuticle	+	+	+	+
Cuticle - eye	+	+	+	+
Sensillae + spines	+	+	+	+
Leg parts	+	+	+	+
Mandibles				+
Wing fragments			+	
Wing membrane			+	
Whole wings;				1
Mycetophilidae	1			
Psychodidae	2		1	
Sciaridae		2	1	
Arthropod abdomen	1			1
Arthropod head	1	3	2	1
Arthropod thorax	1	1		
Whole exuviae;			1	1
Empididae				1
Psychodidae				1
Coleoptera elytra	1			
Gastropoda shells	1	3		
Millipedes		1	+	3

+ indicates material that was present but not able to be counted.

Discussion

I have demonstrated that glowworm bioluminescence certainly attracts prey as first suggested by Meyrick (1886). This conclusion is based upon the significantly larger number of invertebrates caught in adhesive traps occupied by glowworms compared with controls. There is also clear evidence from an examination of discarded faecal material that at least some of these invertebrates are hauled up by glowworms and eaten. This was true both in the bush and cave locations. Most of the invertebrate families were caught in larger numbers in adhesive traps occupied by glowworms, and Sciaridae appear to be most commonly attracted to glowworm bioluminescence (Figs. 2.6 & 2.7) whereas Dolichopodidae and Teratomyzidae, although not found on adhesive traps in the cave, also displayed a positive attraction to glowworm bioluminescence in the bush. Confirmation that some of those families of Diptera encountered most frequently on adhesive traps occupied by glowworms in the bush (Sciaridae, Dolichopodidae, Trichoceridae, Psychodidae and Mycetophilidae)(Table 2.2) are indeed eaten by glowworms was demonstrated when several discarded Mycetophilidae, Psychodidae and Sciaridae wings were found among glowworm excretory droplets on blotting paper beneath larvae in Ruakuri Scenic Reserve (Table 2.5). These dipteran adults are common in damp, shady places so they are likely to occur where glowworms are found. In Australia they are often attracted to light traps and have crepuscular or nocturnal flight activity patterns (Colless & McAlpine, 1991). Some of these families also occur in caves elsewhere in the world together with predatory web-building mycetophilid larvae. Sciaridae, Psychodidae and Mycetophilidae occupy North American caves along with non-bioluminescent larvae of *Macrocera nobilis* Johnson (Peck & Russell, 1976) whereas Sciaridae were a minor component of the flying insects attracted to blue-green light in Dromilly Cave, Jamaica where there were large concentrations of non-bioluminescent *Neoditomyia farri* Coher larvae (Stringer & Meyer-Rochow, 1994). Sciaridae were the family I found most frequently on adhesive traps in Reserve Cave and May (1963) reported *Sciara* sp. as permanent occupants of caves in the Waitomo district. However, Pugsley (1984) found no sciarids in Glowworm Cave.

It appears that Empididae actively avoid glowworm bioluminescence (Fig. 2.7), because almost twice as many empids were caught during summer on control traps than on those occupied by glowworms (Table 2.2). However, there is evidence that Empididae may be eaten by glowworms because one was found on blotting paper under a glowworm in the cave during summer (Table 2.5). It is also possible that this may have died there naturally. Most Empididae were collected from two adhesive traps located at the far end of the cave near the tomo entrance (Fig. 2.3). These had probably entered the cave as adults because no aquatic

Empididae larvae have been identified in New Zealand. Empid larvae are common amongst vegetation or plant debris in many open or forested streams (Winterbourne & Gregson, 1989). According to Smith (1989) "the adults frequently swarm on the surface", so it seems likely that these flies entered the cave by accident via the tomo, which is why so many were caught on the two traps located nearby.

Significantly more invertebrates were trapped in the bush-clad entrance to Reserve Cave than within the cave itself. This agrees with the general situation that species numbers are often greater in cave entrance zones than either cave or surface habitats (Howarth, 1981). In general, cave ecosystems are comparatively simple in relation to surface ones because they are often limited by allochthonous energy inputs from soil and surface ecosystems (see review by Howarth, 1983). In the bush itself, there were relatively large differences in summer between daily catch rates for glowworm-occupied and control traps (Fig. 2.5). This suggests that glowworms were more effective at attracting invertebrates during this season than during spring and winter, although glowworm-occupied traps in the bush caught a greater mean number of invertebrates per day in spring than in the summer (Table 2.3).

A smaller number of dipteran families were caught on adhesive traps in Reserve Cave than on traps in the bush, although Diptera formed a slightly greater proportion (89% as opposed to 85% in bush) of the total number of invertebrates attracted to glowworms in the cave. This resulted because Gastropoda, Homoptera, mites, Neuroptera, Opiliones, Collembola, Millipedes, Hemiptera and Isopoda were not caught on cave adhesive traps. This does not necessarily mean these insects were absent from the cave altogether. Millipedes and their body parts were reported in glowworm snares in Kauri Park, Auckland, by Stringer (1967). These were a common component of material discarded from glowworms at site 1 in Reserve cave (Fig. 2.3). On a few occasions the predatory harvestmen *M. tumida* were seen around a group of glowworms although none were caught on adhesive traps at this site. The small numbers of crawling invertebrates caught on adhesive traps in Reserve Cave indicates that cave glowworms are probably more dependent upon the winged adults of aquatic insects that emerge inside the cave (Pugsley, 1984; Oxenham, 1985). However, such non-flying invertebrates may fall into the glowworm snare from above (Stringer, 1967), so the adhesive traps would not have sampled such behaviour.

There was a longheld assumption that the principal food of bush-dwelling glowworms are flightless soil arthropods. This appears to be based upon the work of Norris (1894), who mentioned that glowworms will attack crustaceans, and records of prey caught in fishing lines in Kauri Park, Auckland by Stringer (1967). In fact, these small crawling arthropods, thought by Gatenby (1959) to be negatively phototrophic, formed a

minority of animals caught on traps in the bush (Table 2.2). This was further substantiated by observations derived from remote-recording glowworms in bush (Chapter 5).

Adults of *A. luminosa* were not found on adhesive traps or in the material discarded from the webs of glowworm larvae. The eyes of *A. luminosa* adults can detect glowworm bioluminescence (Meyer-Rochow & Waldvogel, 1979), but it is not known whether the adults are attracted to this light, or whether they simply blunder into larval fishing lines. Adults have been found stuck in larval webs (Chapter 5; personal observation; Gatenby, 1959; Pugsley, 1984), but according to Richards (1960) "most of the flies caught usually manage to break free." Stringer & Meyer-Rochow (1994) caught no web-spinning mycetophilids in either light traps or adhesive traps in Dromilly Cave, Jamaica, but this may have been because these flies were a relatively rare component of the flying fauna. It is interesting to note that Mycetophilidae as a family indicated no preference nor aversion to adhesive traps occupied by glowworms in either bush or cave habitats (Figs. 2.6 & 2.7).

Although only eight gastropods were caught on adhesive traps in the bush, seven of these were caught on traps occupied by glowworms. On the basis of this information, and the several empty gastropod shells presumably discarded from glowworms in Ruakuri Scenic Reserve it appears that they are attracted to glowworm bioluminescence, and are probably eaten. Interestingly, von Berg (1978) in an Electro-retinogramme study of the eye of the *Helix pomatia* L., (Gastropoda) found that the maximum sensitivity was about 475 nm, and this is very close to the 487 nm maximum wavelength emitted by *A. luminosa*.

Considerably more spiders were caught on traps occupied by glowworms than on control traps in the bush, especially during spring. Most were Symphytognathidae, which construct small webs among mosses or liverworts in areas of high relative humidity (Forster, 1967). Although they appear to be attracted to bioluminescence, it seems unlikely that these small spiders prey upon glowworm larvae. In bush, spiders appeared to accidentally move into and out of larval snares at night, breaking the delicate fishing lines in the process (Chapter 5). However, there was no evidence that spiders prey upon glowworms. In fact, small spiders may occasionally form part of the glowworm diet because one larva was observed attacking a spider in bush (Chapter 5). Pugsley (1984) found no evidence that spiders prey upon glowworms in the Glowworm Cave. On many occasions I noted that spiders spun their webs over areas where glowworm larvae occur. This indicates a possible exploitation by spiders of prey attracted to glowworm bioluminescence and confirms several other reports of this behaviour (Meyrick, 1886; Gatenby, 1959; Stringer, 1967; Morley, 1993).

Twice as many Hymenoptera were caught on traps occupied by glowworms in the bush compared with those caught on control traps (Table 2.2). Also, three Hymenoptera were collected from traps occupied by glowworms in the cave. As far as I am aware, no Hymenoptera have been reported to prey upon *A. luminosa* except for a species of Belytidae described by Marshall (1892) that Hudson (1892a; 1892b) found parasitised glowworm pupae in the Botanical Gardens, Wellington.

Very few weta (Orthoptera) were captured on adhesive traps in both bush and cave habitats. This may be because weta become easily entangled within the sticky fishing lines produced by larvae of *A. luminosa* (Chapter 5), and probably avoid them so they are often not found in areas where glowworms occur (Richards, 1956; Pugsley, 1980).

Pugsley (1984) reported that the distribution of glowworms in caves in New Zealand depends upon the availability of food. The adhesive traps must have prevented glowworms from capturing and assimilating prey items by trapping invertebrates attracted to glowworm bioluminescence within them. It seems unlikely that many flying insects would pass through the open end of an adhesive trap to become entangled in the sticky fishing lines. Also, I did not find any invertebrates entangled in the fishing lines, or identify any discarded material on the inside surfaces of these traps. It therefore seems that glowworms can survive for long periods without food. Certainly, all of the glowworms that were enclosed within adhesive traps in the cave during summer survived, despite apparently having received very little food for 78 days. Four of the eleven glowworms in the bush were not present when I removed the adhesive traps at the end of summer, so the data from these sites were not used (Table 2.2). I could not find any pupal exuviae or remains of them, so I can only assume that they had died or moved out. Climatic conditions are generally more stable in caves than on the surface (Pugsley, 1980), so bush glowworms under adhesive traps not only went without food for 78 days, but also had to tolerate a wider range of environmental conditions, including overall higher mean temperatures during summer. It also seems likely that this may have had an effect on their light production (Chapter 3).

To conclude, the significant differences between numbers and types of invertebrates collected from glowworm-occupied and control adhesive traps in both bush and cave locations demonstrate that glowworms attract invertebrates with their light. Glowworms in the bush attracted both greater numbers and types of invertebrates than glowworms inside adhesive traps within the cave. Flying Diptera comprised the bulk of invertebrates attracted to glowworm bioluminescence, and formed a greater proportion of invertebrates attracted to glowworms in the cave because many non-dipterans that were collected from bush adhesive traps

Chapter 3 ATTRACTION OF INVERTEBRATES TO GLOWWORMS AND ARTIFICIAL LIGHT SOURCES AT PIRIPIRI ROAD CAVES, POHANGINA

Introduction

Glowworm bioluminescence attracts some invertebrates as demonstrated by placing transparent adhesive traps in front of glowworms and comparing the catches with other transparent adhesive traps that lacked glowworms (Chapter 2). However, live glowworms vary their light production over time. Evidence for this is based upon personal observation (Chapter 5) and anecdotal reports by Gatenby (1959), Richards (1960), Stringer (1967), Meyer-Rochow & Waldvogel (1979) and Pugsley (1984). Such variation in light output could be expected to reduce the numbers of invertebrates that are attracted to the light leading to an increase in the variation between numbers of invertebrates attracted to different glowworms. In Chapter 2 this was overcome by running the experiment for long periods of time in order to demonstrate attraction. Using live glowworms in such experiments is also labour intensive and time consuming.

If an artificial light source with very similar characteristics to glowworm bioluminescence could be used, then this has the potential to be used to sample the potential food of glowworms in areas where glowworms do not occur, such as some passages in caves. Such a method could be used to determine if the lack of glowworms was due to a lack of suitable food. A light-emitting diode (LED) is available which fits this criterion. It has a similar maximum wavelength to glowworms (LEDs: max. 470 nm; Glowworms: max. 487 nm, Shimomura et al, 1966).

Here, I attempt to compare the use of adhesive traps containing LEDs along with traps containing glowworms and traps without (controls).

This approach allows 1) the standardization of the light source used to attract invertebrates in both cave and bush locations; and 2) comparison of the frequency and types of invertebrates attracted both to glowworm bioluminescence and LEDs.

A number of problems needed to be solved before attempts could be made to collect invertebrates using LEDs. Bush glowworms, unlike cave glowworms, are largely nocturnal and produce bioluminescence only at night (Chapter 5). They begin glowing when the reflected light intensity is less than 10 lux (Richards, 1960; Stringer, 1967). Also, according to Gatenby (1959) glowworms can fade out their light slowly over about a minute. Dusk light levels were measured using a luxmeter to confirm these observations. A light-

sensitive photoswitch was therefore built (Fig. 3.1 & 3.2), that switched on the LEDs when the reflected light intensity fell below 10 lux, and switched them off again when this intensity rose above 15 lux. The photoswitch circuit incorporated a 55 second ramp time delay so that the intensity of the LEDs changed gradually when they were switched on or off.

Figure 3.1 **The light-sensitive photoswitch equipment used to switch LEDs on at dusk and off at dawn at Piripiri Road Caves, Pohangina. This was designed and constructed by Wyatt Page, Bruce Rapley and Dexter Muir at the Department of Production Technology, Massey University.**

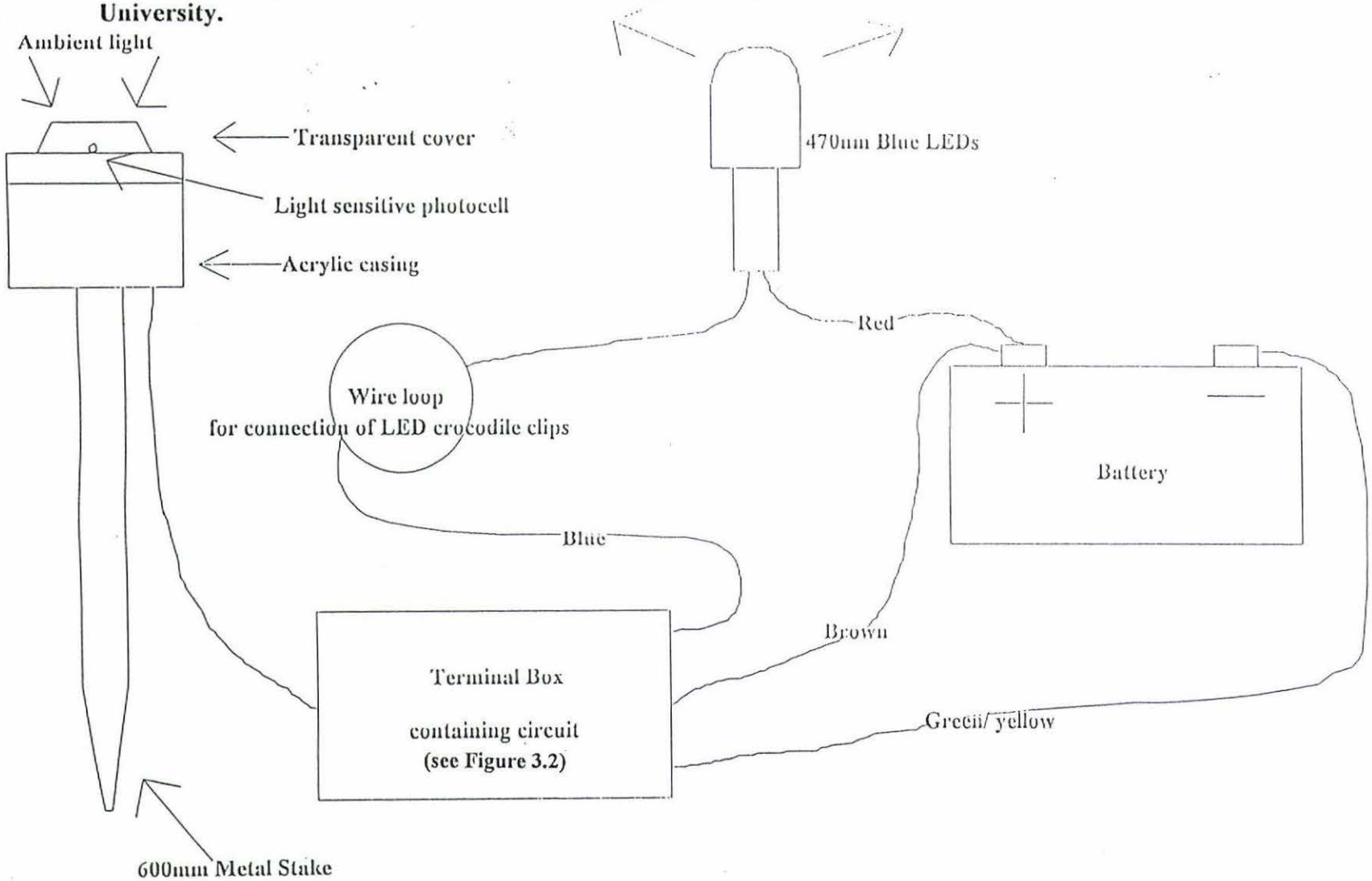
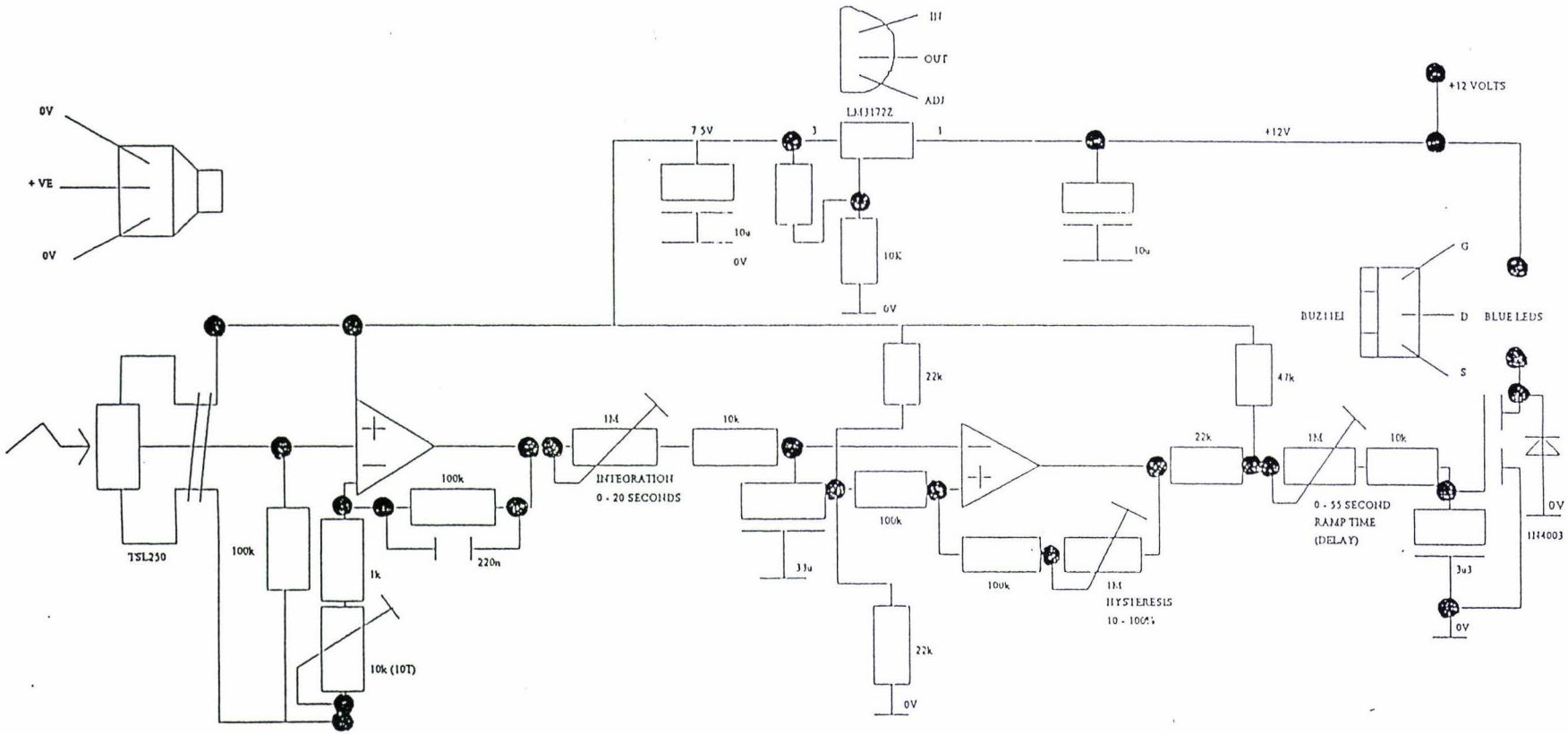


Figure 3.2 **Circuit diagram of the light-sensitive photoswitch used in Figure 3.1. The unit was designed and constructed by Wyatt Page, Bruce Rapley and Dexter Muir at the Department of Production Technology, Massey University.**



Materials & Methods

This research was conducted within a tunnel passage off Bridge Cave at Piripiri Road Caves, Pohangina (NZMS 260 T23, 1:50, 000; E641 N254) and in the nearby bush (Fig. 3.3). Descriptions of these caves, together with maps, are given in Pearce (1988).

Adhesive traps identical to ones used in Reserve Cave, Waitomo, were used. Details of their construction, and the recovery of invertebrates caught on them at Waitomo are given in Chapter 2.

The location chosen for setting out the adhesive traps in the bush was a wet vertical rock face surrounded by overhanging vegetation. This was about 60 metres north of the natural bridge at Bridge Cave and directly above the submergence of the Te Ano Whiro Stream (Fig. 3.3). Adhesive traps were set in the bush from 24/5/96 to 14/6/96. The second location chosen was a cave passage about 30 metres in length, which runs approximately south-south-west to the Te Ano Whiro Stream (Fig. 3.3). This passage was chosen because the glowworms within it were accessible and it was close to the bush location (40 metres). Although the cave passage was relatively short in length, very little light reached the area where the adhesive traps were set and most *A. luminosa* larvae there were observed to glow during the day. Adhesive traps were set here from 17/6/96 to 8/7/96.

The artificial light sources used to attract invertebrates were blue diffused 5 mm LEDs (Kingbright®). These have an emission angle of 50 degrees and a maximum wavelength of 470 nm. Each LED was installed within a watertight PVC tube (80 mm long, 52 mm diameter) with a transparent perspex face through which the light shone (Fig. 3.4). They were each suspended within a transparent adhesive trap coated with Tanglefoot® (see full description in Chapter 2), by the cables that connected them to a 12 volt battery.

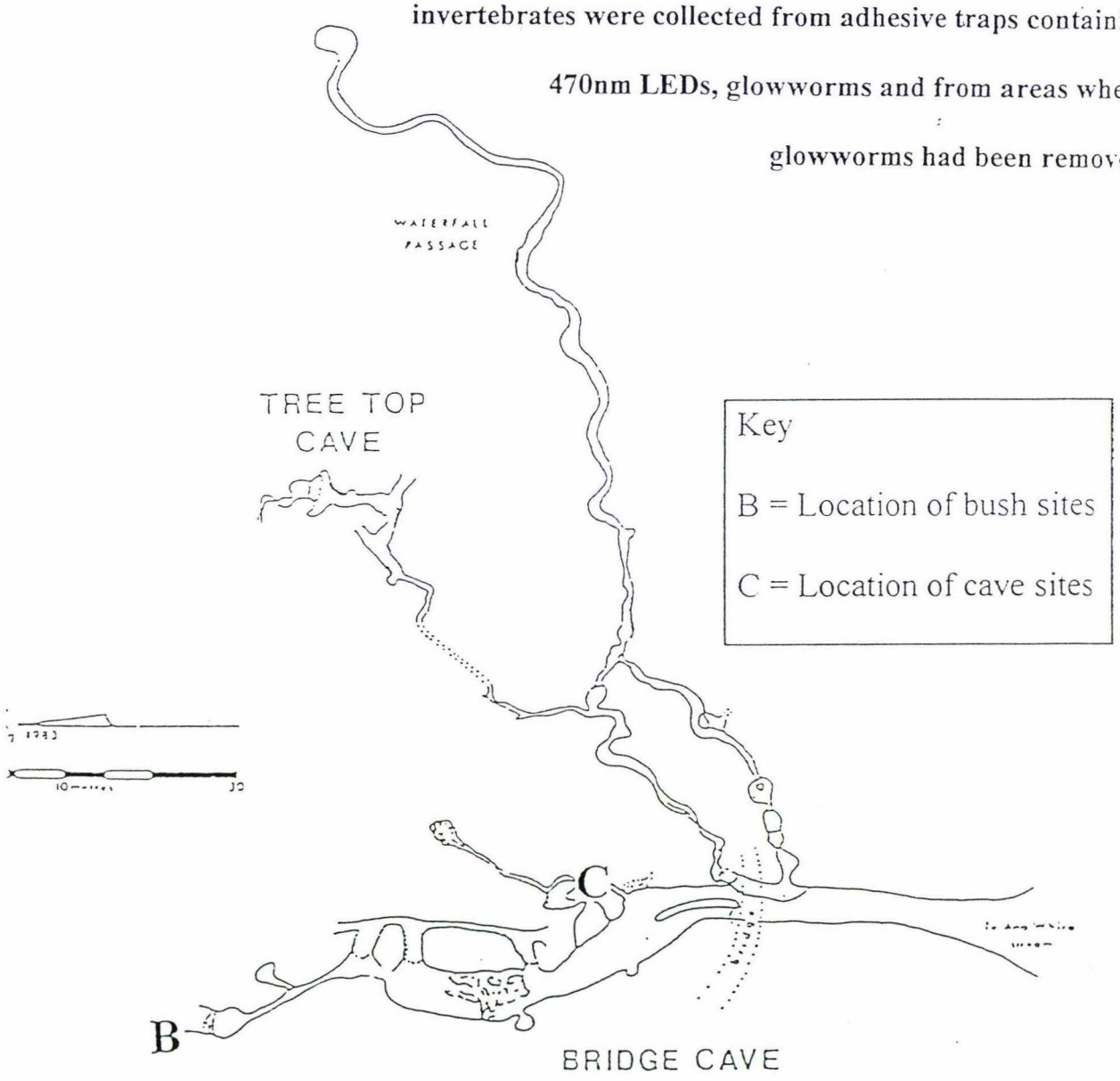
At each location eighteen glowworms were randomly selected from those within reach then pieces of tie-wire were attached to the substrate with 'Emerkit'® on either side of them. Twelve of these glowworms were then removed. Six of these sites were destined to be controls and six were destined to have an LED positioned over them. After the Emerkit® had hardened (24 hours), adhesive traps coated with fresh Tanglefoot® were then attached. The remaining six traps were wired into position over glowworms (Fig. 3.5 & 3.6).

Relative humidity and temperature were recorded using a thermohydrograph (Sato® Ratona; 7 day). This was situated 5 metres from the rock face on the ground at the bush location. It was then moved to a rock ledge in the cave passage approximately 1 metre from the adhesive traps on 17/6/96.

Most invertebrates caught in adhesive traps were identified to genus and species.

It was not possible to undertake a comprehensive analysis of all invertebrates caught on adhesive traps because of the relatively small numbers collected. Therefore, Chi-square and G-tests (Sokal & Rohlf, 1995) were used to test differences in the total numbers of invertebrates, and total numbers of mycetophilids and other Diptera caught in these traps.

Figure 3.3 Location of both bush and cave sites at Piripiri Road Caves, Pohangina, where invertebrates were collected from adhesive traps containing 470nm LEDs, glowworms and from areas where glowworms had been removed



Redrawn from Pearce (1988).

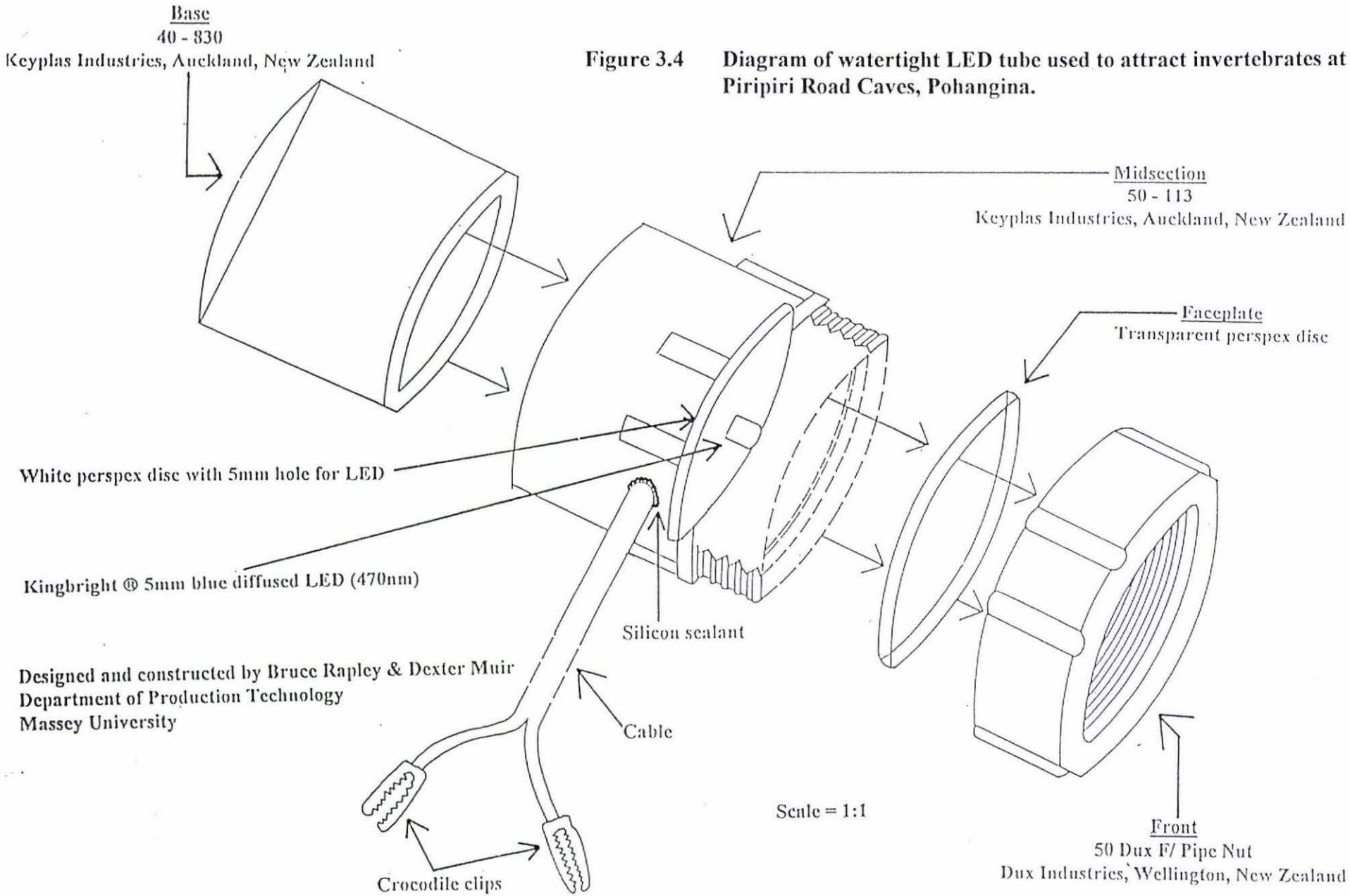


Figure 3.4 **Diagram of watertight LED tube used to attract invertebrates at Piripiri Road Caves, Pohangina.**

Figure 3.5 *Bush location at Piripiri Road Caves, Pohangina, showing all 18 transparent adhesive traps wired onto the rock face. The cables are connected to blue LEDs in six of the traps. Six traps surround glowworms and six are unoccupied (controls).*

Figure 3.6 *Time exposure of the bush location at night, showing the location of both LEDs and some of the glowworms within adhesive traps. Pinpoints of light surrounding the adhesive traps indicate the location of other* Arachnocampa luminosa *larvae.*

Results

Table 3.1 Invertebrates captured on adhesive traps placed over 470 nm LEDs, glowworms and over areas where glowworms have been removed.

CONTENTS OF TRAPS				TYPES OF TRAP							
				BUSH (24/5/96 - 14/6/96)				CAVE (17/6/96 - 8/7/96)			
Order	Family	Genus	Species	LEDs	Glowworms	Controls	Total	LEDs	Glowworms	Controls	Total
Araneae	Cycloctenidae	Cycloctenus		1			1				
	Linyphiidae	Mynoglenes	diloris	1			1				
	Salticidae				1		1				
	Theridiidae				1		1				
	Unidentified			1			1	1	1		2
		Total Araneae		3	2		5	1	1		2
Coleoptera	Chrysomelidae	Longitarsus			1		1				
	Curculionidae			1			1				
	Leiodidae			1			1				
	Staphylinidae			1			1				
		Total Coleoptera		3	1		4				
Collembola				1			1				
Diplopoda				4		1	5				
Diptera	Chironomidae			2			2				
	Dolichopodidae	Achalcus		3	1	1	5				
		Sympycnus		2		1	3				
		Total Dolichopodidae		5	1	2	8				
	Ephydridae	Hydrellia	enderbii						1		1
	Heleomyzidae	Allophylopsis	bivittata			1	1				
	Lauxaniidae	Sapromyza	dichromata		2		2				
	Mycetophilidae								1	1	2
		Aneura							1		1

Table 3.1 cont'd Invertebrates captured on adhesive traps placed over 470 nm LEDs, glowworms and over areas where glowworms have been removed.

CONTENTS OF TRAPS				TYPES OF TRAP							
				BUSH (24/5/96 - 14/6/96)				CAVE (17/6/96 - 8/7/96)			
Order	Family	Genus	Species	LEDs	Glowworms	Controls	Total	LEDs	Glowworms	Controls	Total
Diptera	Mycetophilidae	Cycloneura		1			1				
		Exechia				1	1				
		Mycetophila		3	1	2	6	5	2	4	11
		Mycetophila	colorata	1		1	2				
		Mycetophila	fagi	1	1		2		1		1
		Mycetophila	filicornis	1		1	2	1			1
		Mycetophila	fumosa	1	1		2				
		Mycetophila	grandis		1		1			1	1
		Mycetophila	marginepunctata	1			1	1		1	2
		Mycetophila	phyllura		1		1	2	2	1	5
		Mycetophila	subspinigera	1			1	1			1
		Mycetophila	vulgaris	1			1				
		Plaurocrypta	immaculata					1			1
		Zygomyia	albinotata	2	2	2	6	1	1		2
		Zygomyia	fusca					1			1
		Zygomyia	trifasciata	1			1				
		Total Mycetophilidae		14	7	7	28	13	8	8	29
	Phoridae			1			1				
	Psychodidae			1			1				
		Psychoda	harrisi	1	1		2				
		Total Psychodidae		2	1	3					
	Sciaridae	Sciara		1	2	2	5				
		Sciara	marcilla	1			1				
		Sciara	rufulenta	1	1	1	3				
		Total Sciaridae		3	3	3	9				
	Sciomyzidae	Huttonina			1		1				
	Teratomyzidae	Teratomyza	neozelandica	1			1				
	Thaumaleidae	Austrothaumalea	crosbyi		1		1				

Table 3.1 cont'd Invertebrates captured on adhesive traps placed over 470 nm LEDs, glowworms and over areas where glowworms have been removed.

CONTENTS OF TRAPS				TYPES OF TRAP							
				BUSH (24/5/96 - 14/6/96)				CAVE (17/6/96 - 8/7/96)			
Order	Family	Genus	Species	LEDs	Glowworms	Controls	Total	LEDs	Glowworms	Controls	Total
Diptera	Tipulidae				1		1				
		Amphineurus		1			1				
		Leptotarsus	halteratus	1			1				
		Molophilus			1		1				
		Total Tipulidae		2	2		4				
		Total Diptera		30	18	13	61	13	9	8	30
Ephemeroptera				1			1		1		1
	Leptophylebiidae	Deleatidium	myzobranchia		1		1				
		Total Ephemeroptera		1	1		2		1		1
Hemiptera	Lygaeidae				1		1				
Hymenoptera	Diapriidae	Stylaclista			1		1				
Lepidoptera larvae					1		1				
Neuroptera	Hemerobiidae	Micromus	tasmaniae					1			1
Plecoptera	Notonemouridae	Spaniocerca	zelandica	1		1	2				
Trichoptera	Philopotamidae	Hydrobiosella		1		1	2		1	1	2
	Rhyacophilidae	Tiphobiosis								1	1
		Total Trichoptera		1		1	2		1	2	3
TOTAL NUMBER OF INVERTEBRATES				44	24	17	85	15	12	10	37

Results

Traps with LED's caught a significantly greater total number of invertebrates than traps either with or without glowworms ($\chi^2 = 8.66 > 5.99$; 2 D.f.).

Differences between the types of invertebrates collected from adhesive traps in bush and in the cave

Significantly more invertebrates were captured on bush adhesive traps than on traps in the cave ($G = 19.33$; $p = 0.05$; 1 D.f.). Dipterans collected from cave traps formed a higher proportion of the total numbers of invertebrates (81%) compared with 72% in bush. However, bush adhesive traps caught 12 families of Diptera whereas those in the cave caught only 2. More Mycetophilidae were caught in the cave than in bush (Table 3.1), although there were no significant differences between the numbers of mycetophilids caught in cave and bush habitats ($G = 0.017$; $p = 0.05$; 1 D.f.).

Prey and prey availability in bush

A total of 85 invertebrates were collected from all three types of trap at the bush location (Table 3.1), and most of these (71.8%) were flying Diptera. Spiders (Araneae) and millipedes (Diplopoda) were the next most abundant, but only amounted to 5.9% each. Other invertebrates caught were beetles (Coleoptera; 4.7%) followed by Ephemeroptera, Neuroptera, Plecoptera and Trichoptera (2.4% each). A single specimen each of Collembola, Hemiptera, Hymenoptera, and a Lepidoptera larva were also caught.

Twelve families of Diptera were trapped in the bush (Table 3.1). Mycetophilidae were caught most frequently (45.9% of Diptera) and other families well represented were Sciaridae (14.8% of Diptera), Dolichopodidae (13.1%), Tipulidae (6.6%), Psychodidae (4.9%), Chironomidae (3.3%) and Lauxaniidae (3.3%). Single specimens of Heleomyzidae, Phoridae, Sciomyzidae, Teratomyzidae and Thaumaleidae were also caught. Traps over glowworms caught the greatest number of dipteran families (9), whereas eight families were caught in traps with LED's and only four families in the control traps (Table 3.1).

Small numbers of Chironomidae (2), Phoridae (1) and Teratomyzidae (1) were collected only from LED traps (Table 3.1). One or two Lauxaniidae, Sciomyzidae and one Thaumaleidae were caught only on glowworm-occupied traps. A single Heleomyzidae fly was caught on one control adhesive trap (Table 3.1).

Low numbers of spiders and beetles were caught and all of these were on traps either containing LEDs or glowworms (Table 3.1). Four of the five millipedes were caught on LED traps, and the remaining one on a control trap. There were no significant differences between the numbers of Mycetophilidae, other Diptera families and other invertebrates caught on the three trap types in the bush ($G = 1.24$; $p = 0.05$; 4 D.f.).

Prey and prey availability in the cave

A total of 37 invertebrates were collected from adhesive traps in the cave passage. Ephydridae and Mycetophilidae comprised ~ 81% of the total cave catch, and relatively few other invertebrate types were caught. These included small numbers of Trichoptera (~ 8.1%), Araneae (~ 5.4%), and single specimens of Ephemeroptera and Neuroptera (Table 3.1). There were no significant differences between the numbers of Mycetophilidae, other Diptera and other invertebrates caught between the three trap types in the cave ($G = 2.22$; $p = 0.05$, 4 D.f.). None of the Mycetophilidae caught were *A. luminosa*.

Relative humidity and temperature in bush and in the cave

Relative humidity varied little within and between both bush (82 - 83.5%) and cave (78 - 80%) locations. The maximum of 83.5% occurred at the bush site between 7 - 9/6/96 and coincided with a period of rainfall. Temperature varied little between locations. Cave temperatures ranged between 0.5 - 7 ° C and bush temperatures ranged between -0.5 to 8 ° C. However the mean daily change in temperature was greater in the bush (1.9 ° C) than in the cave (0.7 ° C).

Discussion

Insect neuromuscular performance, and flight in particular, is influenced by temperature (May, 1985). Low temperatures and short trapping periods (21 days as opposed to 200 days at Waitomo, Chapter 2) are probably why so few invertebrates were collected on the adhesive traps at Pohangina. Such small numbers are probably why no significant differences were found between the numbers of invertebrates collected from glowworm-occupied and control traps at both bush and cave locations. However, in both locations traps containing glowworms did collect a greater total number of invertebrates than control traps (Table 3.1). It seems likely that if the traps had been left for longer these differences may have become significant as was the case at Waitomo (Chapter 2).

Significantly fewer invertebrates were caught on traps containing glowworms in the bush than on traps containing LEDs. Once switched on, the LEDs emitted light constantly at an apparently greater intensity than the bioluminescence of glowworms (see Fig. 3.6). Bush glowworms, on the other hand, "do not always turn on their lights" (Gatenby, 1959), and they "often shield their lights by retreating into a crack or tunnel in the substrate." Certainly at Waitomo bush glowworms did not glow brightly all night, at least in May (Chapter 5). Such behaviour would reduce their potential to attract prey. Another factor that could influence this is the report by Hudson (1950) that larvae cease to shine on very cold nights. Certainly low temperatures were experienced at Pohangina at both bush and cave locations over the trapping period. There is also anecdotal evidence, often conflicting, that light production is controlled by many other factors. Richards (1960) reported that larval *A. luminosa* can control the intensity of the glow and Gatenby (1959) and Pugsley (1984) also report that small larvae and hatchlings can produce light apparently as bright as larger larvae. In contrast, Richards (1960), Stringer (1967) and Meyer-Rochow & Waldvogel (1979) noted that larger glowworms generally produce a more intense light than smaller ones. In addition, Richards (1960) noted that a lack of food makes a glowworm glow more brilliantly, whereas Meyer-Rochow & Waldvogel (1979) stated that older and well-fed larvae shine the brightest. Finally, Meyer-Rochow & Waldvogel (1979) also considered that the time of day also influences the brightness of the light.

However, glowworms under adhesive traps at the bush location still caught twice the number of invertebrates collected from traps occupied by glowworms in the cave passage.

Based on this evidence, blue LEDs appear to be suitable to use for sampling the types of invertebrates attracted to glowworms in the bush.

All of the invertebrate orders caught on bush traps at Pohangina were also caught at Waitomo, with the exception of a small number of Ephemeroptera which were only caught at Pohangina (Table 3.1; Chapter 2). Diptera were caught most frequently during both studies, although they formed a smaller proportion of invertebrates caught on bush traps at Pohangina (72% of the total bush catch, Table 3.1) than during the Waitomo study (85% of the total bush catch, Chapter 2). Mycetophilidae, Sciaridae, Dolichopodidae, Tipulidae and Psychodidae were caught most often in bush at Pohangina (Table 3.1) and a similar pattern occurred in bush at Waitomo, where Sciaridae, Dolichopodidae, Trichoceridae, Psychodidae and Mycetophilidae were caught most often. However, at Waitomo the frequency of capture of these families differed significantly between seasons (Chapter 2).

Mycetophilidae were collected in equal numbers from glowworm-occupied and control traps, which suggests that they were not influenced by larval bioluminescence. This was confirmed during the considerably longer Waitomo study (Chapter 2).

According to Pugsley (1980) the main reproductive period of the annual life cycle of *A. luminosa* in Glowworm Cave, Waitomo, occurred between June and October in 1979. It is therefore surprising that no adults were caught in cave or bush adhesive traps during this study, as trapping took place during June and one week of July 1996. No adults were caught during the Waitomo study either (Chapter 2). Certainly some glowworm adults have been reported to fly into the sticky fishing lines of the larva (Gatenby, 1959; Richards, 1960; Pugsley, 1984). I observed one *A. luminosa* adult flying into fishing lines in a cave at Waitomo, but it is not known if it was eaten by a larva or managed to escape (Chapter 5).

The only other similar study to mine was reported by Sivinski (1982). He used adhesive traps to capture arthropods attracted to luminescent web-spinning mycetophilid larvae *Orfelia fultoni* (Fisher), which inhabit small cavities in soil, mosses, dead wood, or crevices between stones in North America's Appalachian mountains (Fulton, 1941). He placed transparent and blackened adhesive traps over larvae and over areas where larvae had been removed from, and reported that several *O. fultoni* adults were caught on transparent traps occupied by larvae.

In summary, glowworms in bush habitats attract significantly more invertebrates, especially Diptera, compared with glowworms in caves. After comparison with the longer Waitomo study it appears that Mycetophilidae, Sciaridae, Dolichopodidae, Tipulidae, Psychodidae and Trichoceridae are caught most frequently on traps occupied by glowworms in bush. However, Mycetophilidae are not influenced by the presence of glowworm bioluminescence. Blue LEDs appear to be suitable for sampling the types of invertebrates attracted to glowworm bioluminescence, at least in bush. It would nevertheless be useful to

carry out a longer, more comprehensive study in both bush and cave habitats to obtain more evidence for this.

Chapter 4 PREY RECOGNITION BY GLOWWORMS

Introduction

The larva of the New Zealand Glowworm *Arachnocampa luminosa* (Skuse) "lives in a nest made from its own clear secretions. Each nest consists of a long tubular gallery suspended from the substrate by an irregular web of fine branching threads. Vertical threads with evenly spaced sticky droplets hang from the web to form the snare" (Stringer, 1967). Edwards (1924) erected the genus *Arachnocampa* for the species *luminosa* because of the "spider-like habits of the larva, forming webs and using them for the capture of insect prey." Invertebrates attracted to larval bioluminescence consist mainly of Diptera (see Chapters 2 & 3).

It is generally thought that glowworm larvae detect prey entangled in their snares by registering vibrations produced by the movements of these insects. This is based upon behavioural observations by Marshall (1892), Norris (1894), Gatenby (1959), Richards (1960) and Meyer-Rochow (1990). The neuro-sensory basis for such vibration detection are unknown although Gatenby (1959) reported "hair-like projections" or setae in the anal papillae of larvae, which he proposed were used to register vibrations made by ensnared prey. However, he also found other chordotonal sense organs without setae in the anal papillae of larvae. He hypothesized that these also function for the detection of vibration, although he "could not provide any evidence for this" (Gatenby, 1959). Ganguly (1960) carried out a more detailed investigation into the scolopale (chordotonal) sense organs in the anal papillae *of A. luminosa* larvae, but she "could not suggest any definite function."

More recently, Meyer-Rochow (1990) reported that glowworms can be 'tricked' into biting inanimate objects such as pieces of leaves that had been rubbed in crushed house flies, if these were stuck to fishing lines. He therefore showed that smell or taste is also involved, at least at close range. However, this has never been demonstrated experimentally. This chapter gives the results of experiments designed to demonstrate how glowworms detect prey, and how they discriminate between both prey and non-prey items.

Materials & Methods

Thirty glowworms of about the same size were collected from the ceiling of Glowworm Cave, Waitomo about 10 metres from the stream entrance (Fig. 4.1) on 23/3/96. Each glowworm was removed with a sterile toothpick and placed in an individual 'Prespak'® P35 plastic container (Biolab Scientific, Palmerston North, New Zealand) together with a damp wad of cotton wool to provide a high relative humidity. Glowworms become torpid and die in low humidity (Pugsley, 1984). The glowworms were transported back to a Constant Temperature (CT) Room (~ 15 ° C, no light) at Massey University the next day. It was hoped that this procedure would reduce the risk of infection by fungal pathogens such as *Tolypocladium* sp. (Samson, 1984).

All observations in the CT Room were made with a 12 volt spot-light covered with a 679 nm interference filter (Ealing, U.K.). Meyer-Rochow & Eguchi (1984) reported that light of a wavelength longer than 660 nm does not elicit measurable responses in the eyes of male adult *A. luminosa*, and Stringer (1967) found that dim red light "usually allowed up to five minutes observation, during which the larvae seemed to be little affected."

On 25/3/96 glowworms were transferred with sterile toothpicks from Prespak® containers to individual observation chambers (Fig. 4.2). Each consisted of an inverted lower half of a transparent 1.5 litre plastic soft-drink bottle. A ceiling of plaster of paris lined the top as a substrate for the glowworm and this was prevented from falling out by a wire loop that was embedded in the plaster and tied off through small holes in the base of the bottle. Bottles were kept on aluminium foil trays filled with wet sand, and strips of blotting paper were glued to the inside surface of each bottle with methyl cellulose paste to facilitate water movement between the sand and plaster ceiling. This kept the relative humidity in the bottles high. A ~ 50 mm diameter hole was cut into the side of each plastic bottle to allow access. The hole was blocked with a wad of cotton wool when access was not required to minimize moisture loss.

Each glowworm was first starved for 10 days before each trial, and then fed with 1 adult *Drosophila melanogaster* (Biosuppliers, Auckland, New Zealand) per week. This ensured that the nutritional status of all glowworms were the same prior to the commencement of prey recognition experiments, which took place between 3/6/96 and 12/7/96.

A variety of objects were placed on the sticky vertical fishing lines of larval snares. *D. melanogaster* that had been killed by freezing and live *D. melanogaster* were used to investigate the possible effect of live prey on glowworm behaviour. Pieces of blotting paper soaked in freshly crushed *D. melanogaster*, and dry pieces of

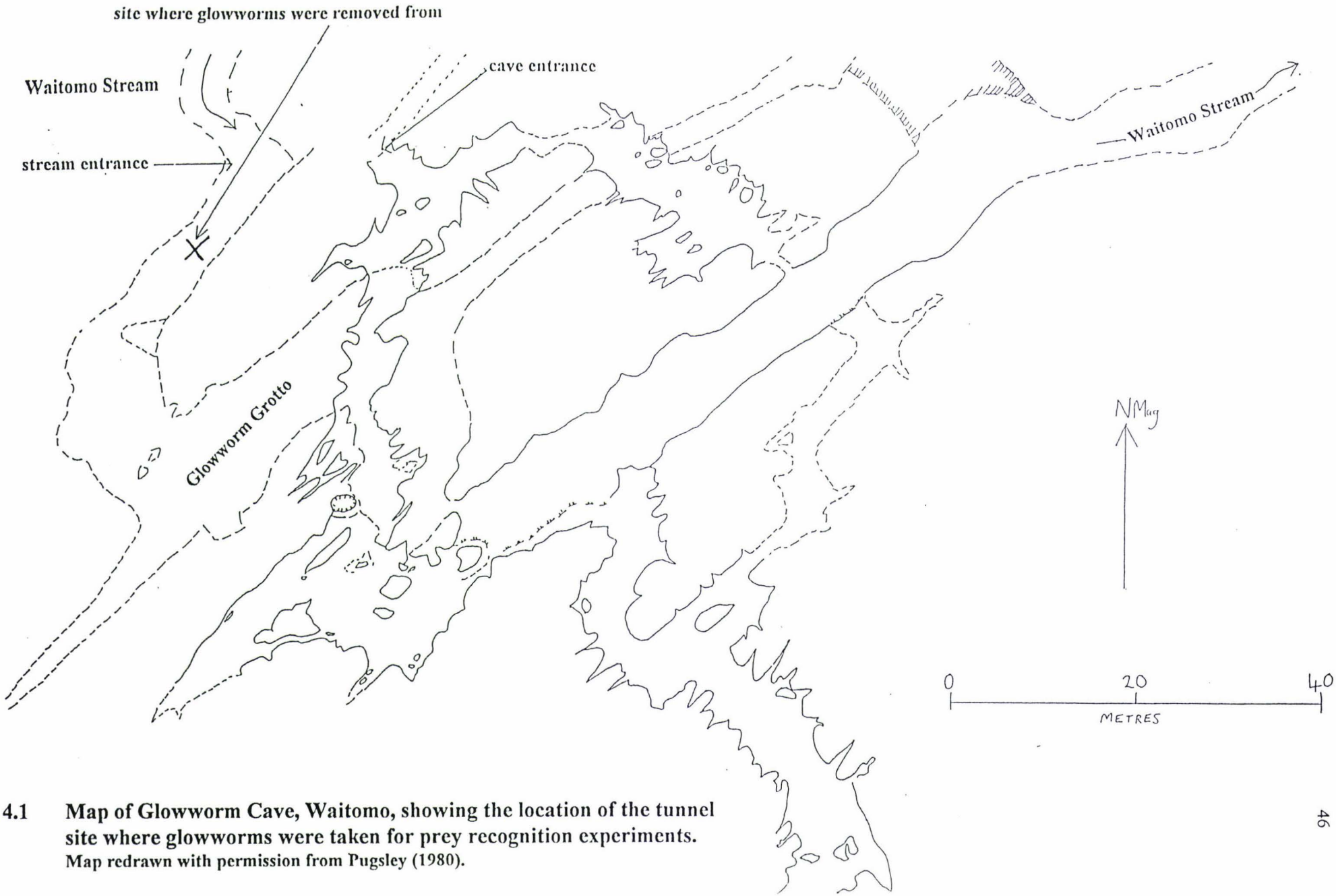
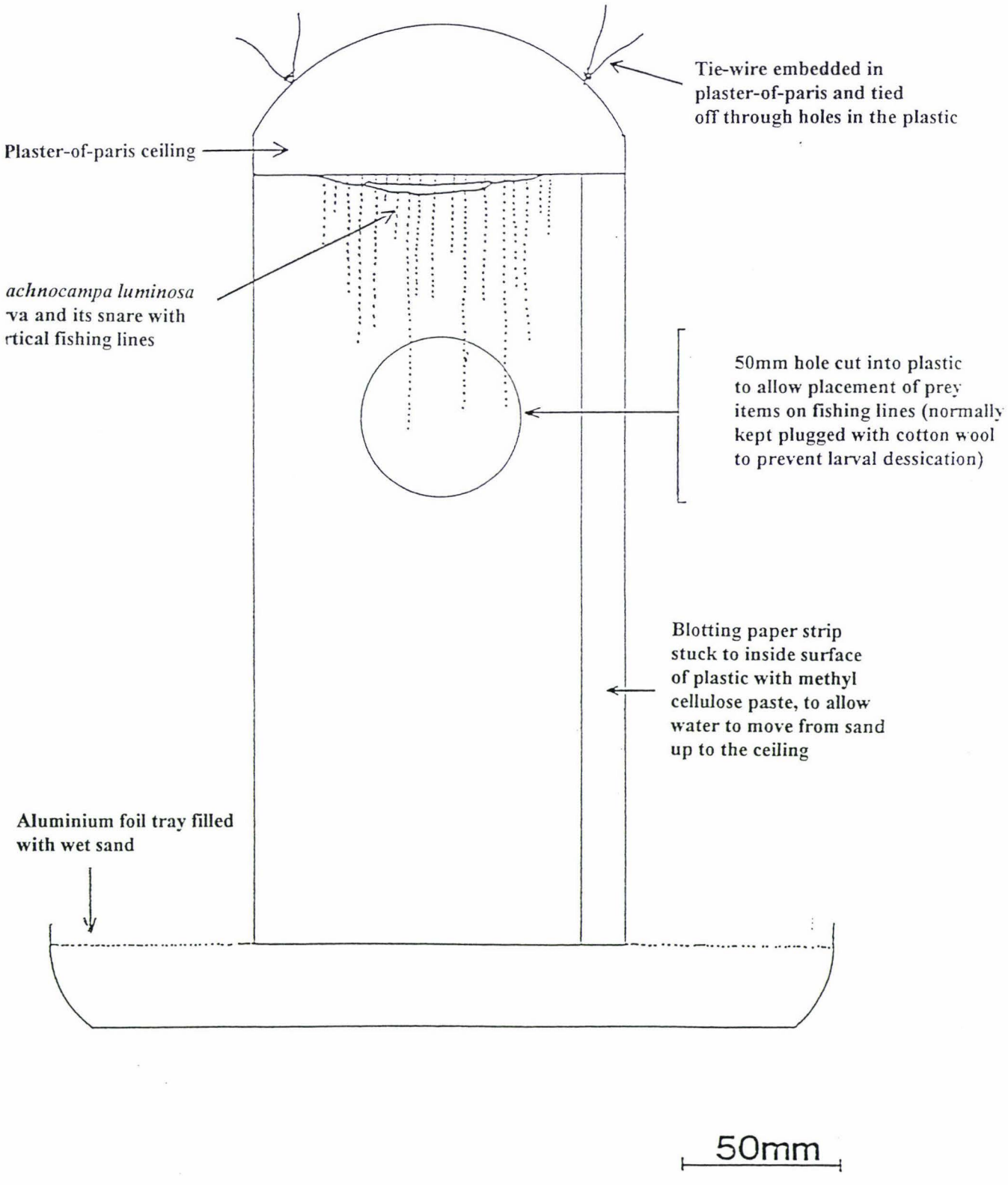


Figure 4.1 **Map of Glowworm Cave, Waitomo, showing the location of the tunnel site where glowworms were taken for prey recognition experiments.**
Map redrawn with permission from Pugsley (1980).

Figure 4.2 Diagram of an observation chamber, constructed from an inverted 1.5 litre transparent plastic soft-drink bottle with its top cut off, used to house a glowworm larva while conducting prey recognition experiments.



blotting paper of the same mass[1] as *D. melanogaster* were used to investigate the hypothesis that smell or taste may play a role in glowworm prey recognition behaviour. Responses to moving prey were tested further when I placed dry pieces of paper between glowworms and live *D. melanogaster*.

Before I could place the live *D. melanogaster* on the fishing lines of glowworm snares within each observation chamber, I had to first capture them. Capturing live *D. melanogaster* with forceps proved impossible because I always crushed them. I therefore anaesthetized them first with Ethyl acetate, so that I could easily manipulate them with forceps before they recovered. Anaesthetized *D. melanogaster* usually revived and commenced struggling a few minutes after they were placed on fishing lines.

During the first 7 days a nightscope (Varo® Noctron V) with an infra-red light source was used to observe glowworm behaviour. Observations were conducted for 1 hour following the treatments. The nightscope was mounted upon a trolley which could be wheeled along a track parallel to the five chambers so that glowworms could be observed at leisure. I also checked on the status of prey-items the day after they had been placed on fishing lines (Table 4.1).

Behavioural responses to prey-items were as follows. "Hauled up" prey-items were found adhering to either the gallery itself, or just near it, some distance higher than the point where they had been placed the day before. Prey-items were "discarded" from the snare on to the sand below. Prey-items "left hanging" were not moved from the point on the snare where they were placed. "Missing" prey-items could not be found anywhere within an observation chamber.

Treatments were performed once a day. Both the order and type of treatment each glowworm received was determined randomly, but glowworms did not receive the same treatment twice. Once five treatments had been performed, the corresponding glowworms were not disturbed until the following day. The next day changes to the status of prey-items were noted (missing, discarded, hauled up, left hanging), and treatments were performed on the next five glowworms.

Pieces of blotting paper were put under each observation chamber on the wet sand after day 5 to catch faeces and discarded material.

Differences between responses to treatments were analyzed using Chi-square.

[1] 10 *D. melanogaster* were anaesthetized and weighed. The mean mass of a single *D. melanogaster* was 0.001 g.

Table 4.1 The status of prey items the day after they had been placed upon glowworm fishing lines

Day	Live fly[1]	Dead fly[1]	Dry paper	Wet paper	Dry paper above a live fly[1]
1	Missing	Left hanging	Discarded	Left hanging	Paper left hanging, fly left hanging
2	Left hanging	Missing	Left hanging	Left hanging	Paper discarded, fly missing
3	Hauled up	Left hanging	Discarded	Hauled up	Paper hauled up, fly missing
4	Missing	Missing	Discarded	Hauled up	Paper hauled up, fly left hanging
5	Missing	Hauled up	Left hanging	Left hanging	Paper discarded, fly hauled up
6	Hauled up	Left hanging	Left hanging	Left hanging	Paper hauled up, fly hauled up
7	Missing	Left hanging	Left hanging	Hauled up	Paper left hanging, fly left hanging
8	Missing	Left hanging	Discarded	Left hanging	Paper left hanging, fly left hanging
9	Missing	Left hanging	Left hanging	Hauled up	Paper left hanging, fly left hanging
10	Left hanging	Left hanging	Left hanging	Hauled up	⊗
11	Hauled up	Missing	Left hanging	Left hanging	Paper left hanging, fly missing
12	Left hanging	Hauled up	⊗	⊗	⊗
13	⊗	Missing	Discarded	⊗	Paper hauled up, fly missing
14	Missing	Hauled up	Discarded	Hauled up	Paper hauled up, fly missing
15	Missing	Missing	Hauled up	Hauled up	Paper discarded, fly missing
16	⊗	Missing	Left hanging	⊗	Paper hauled up, fly missing
17	Missing	Left hanging	⊗	Hauled up	Paper hauled up, fly missing
18	Left hanging	Left hanging	Hauled up	Hauled up	⊗
19	Left hanging	⊗	Discarded	Hauled up	Paper hauled up, fly missing
20	Missing	Left hanging	Discarded	⊗	Paper hauled up, fly missing
21	⊗	⊗	Left hanging	Hauled up	⊗
22	⊗	⊗	⊗	Hauled up	⊗
23	Missing	Missing	Hauled up	Hauled up	Paper hauled up, fly missing
24	Left hanging	Missing	⊗	Hauled up	Paper hauled up, fly hauled up
25	Missing	Missing	Discarded	Hauled up	Paper hauled up, fly missing
26	Missing	⊗	Left hanging	Hauled up	⊗
27	Missing	⊗	Discarded	Hauled up	⊗
28	Left hanging	Missing	Left hanging	Hauled up	⊗
29	⊗	Missing	⊗	⊗	Paper hauled up, fly missing
30	⊗	⊗	Hauled up	Left hanging	⊗

⊗ This symbol represents glowworms that were either pupating, had emerged as adults, or had died, or where there were not enough fishing lines to place prey-items.

1. *Drosophila melanogaster*

	Live *D. melanogaster*	Dead *D. melanogaster*	Live *D. melanogaster* below paper	Total
Missing	14	11	13	38
Hauled up	3	3	3	9
Left hanging	7	10	6	23
Total	24	24	22	70

Table 4.2 *The status of live* D. melanogaster, *dead* D. melanogaster *and live* D. melanogaster *hanging below pieces of paper the day after they had been placed on glowworm fishing lines.*

There were no significant differences between responses of glowworms to live or dead *D. melanogaster* (χ^2 = 1.806; p = 0.05; 4 Df)(Table 4.2). They were never discarded but over half of them were missing from observation chambers the day after they had been placed on glowworm snares (Table 4.2). Faecal droplets containing insect sensillae and cuticular material often appeared on blotting paper sheets beneath larval snares.

There were significant differences between responses (χ^2 = 21.67; p = 0.05; 4 Df) of glowworms to dry paper, wet paper, and dry paper placed above *D. melanogaster* on fishing lines (Table 4.3). Most (72%) of the pieces of paper with crushed *D. melanogaster* juice were hauled up into glowworm snares, and none were discarded. Dry pieces of paper were hauled up 16% of the time but 40% of them were discarded. However, when a live *D. melanogaster* was placed below a piece of paper then most (61%) of the pieces of paper were hauled up, and only 14% of these were discarded (Table 4.3).

	Dry paper	Paper crushed in *D. melanogaster* juice	Dry paper hanging above live *D. melanogaster*	Total
Hauled up	4	18	13	35
Left hanging	11	7	5	23
Discarded	10	0	3	13
Total	25	25	21	71

Table 4.3 *The status of pieces of dry paper, paper crushed in* D. melanogaster *juice and dry paper hanging above live* D. melanogaster *the day after they had been placed on glowworm fishing lines.*

About one-third of the total number of *D. melanogaster* and pieces of paper were found left hanging in glowworm snares (Table 4.2 & 4.3) the day after they had been placed there. However, dead *D. melanogaster* and dry pieces of paper were left hanging in snares more frequently (Table 4.1).

During the course of these experiments three of the glowworms became torpid and died and a further ten glowworms pupated. The latter did so mostly towards the end of the experimental period (Table 4.1). The duration of the pupal stadium ranged from 8 to 16 days, and had a mean duration of 13 days. All 10 adult *A. luminosa* that emerged were male.

Discussion

Previous reports (Marshall, 1892; Gatenby, 1959; Richards, 1960; Meyer-Rochow, 1990) suggested that glowworms quickly respond to prey-items on the adhesive mucus droplets of fishing lines, and to the struggling movements of entangled live flies. This was based upon anecdotal reports such as "when touched the threads are drawn up suddenly into the web" (Marshall, 1892) and "the larva is not alarmed until its snare is touched" (Gatenby, 1959). Richards (1960) reported that following a tug on a fishing line by a midge, a glowworm larva moved along its nest to the appropriate fishing line and hauled in the midge "in a little over one minute", and according to Meyer-Rochow (1990) "the glowworm larva reacts to the initial impact vibrations of prey within a few seconds to a few minutes." Certainly at Waitomo a larva in bush appeared to bite an insect about ten seconds after it had flown into the snare, but on another occasion in Waitomo Waterfall Cave a large mayfly (Ephemeroptera) was left hanging for at least two hours before it was hauled up (Chapter 5). On one occasion I did observe a *D. melanogaster* that I had just stuck to a fishing line move its wings at high speed, whereupon the glowworm moved immediately to the correct line and hauled it in and devoured it. However, most of the time my one-hour observation period elapsed without any apparent movement by glowworms towards their prey-items.

It is possible that this general lack of movement was attributable in part to the vibrations I produced when I opened and closed the CT Room door before commencing treatments each day, and that these vibrations were sufficient to alarm the larvae. When experimenting with captive glowworms Gatenby (1959) reported that "lighted larvae on a table in a darkroom soon doused when their table was accidentally shaken." Certainly on occasion many of the glowworms in their observation chambers faded their lights rapidly when I entered the room. The lack of response by glowworms to prey-items was the reason why I instead decided to check on the status of prey-items the day after they had been placed on larval snares.

It appears from my experiments that live *D. melanogaster* have no significant effect on glowworm prey recognition behaviour (Table 4.2). Several key factors may explain this. Firstly, it was suggested by Jackson (1974) that prey mass can play a role in determining responses of larvae of a non-bioluminescent predatory mycetophilid *Orfelia aeropiscator* Jackson larvae to insects caught in their fishing lines. Like *A. luminosa*, *O. aeropiscator* also has free-hanging vertical fishing lines. Jackson (1974) found that "when a large prey-item struck a fishing line and began struggling, the larva would rapidly approach the line and haul it in. However, small gnats did not elicit immediate attention and sometimes remained uneaten for hours." Certainly the *D. melanogaster* I was using can be considered small given their mean mass of 0.001 g.

Another possibility is that the live *D. melanogaster*, which are diurnal, did not move in darkness as much as they would in light. Thus, their lack of movement coupled with their small mass may have significantly reduced their chances of being detected.

However, according to Gatenby & Cotton (1960) and Peck & Russell (*Macrocera nobilis* Johnson; 1976), leaving captive prey on fishing lines for extended periods of time is thought to be a precautionary measure to ensure that prey will become more entangled, and possibly die before any attempt is made to consume it. When a stunned house fly, *Musca domestica*, was placed on a glowworm snare, Gatenby and Cotton (1960) found the glowworm never came down suddenly, "but this may have been due to the fact that the captive larvae were over fed, or were merely cautious." However, Gatenby & Cotton (1960) did consider the fly to be "somewhat too large." Indeed, 58% of live *Drosophila* (as opposed to 45% of dead *D. melanogaster*) were often missing the day after I had placed them on fishing lines (Table 4.2). This suggests that they were being eaten because blotting paper placed under the observation chambers had faecal droplets directly beneath larval snares. Upon microscopical inspection these droplets often contained insect sensillae and cuticular material. On several occasions at Waitomo both bush and cave glowworms were observed voiding excretory droplets onto the substrate below (Chapter 5).

Many of the live *D. melanogaster* that I stuck to fishing lines usually ceased struggling a few minutes after I had placed them there, although they may not have been dead. As mentioned previously, Drosophila are diurnal insects, and so may not have moved as much in darkness as they would have if the room was lit. However, Stringer (1967) also carried out observations of captive glowworms which he fed *Drosophila* and psychodids. He reported that the prey "often recommences struggling when it is bitten and dies fairly quickly." According to Meyer-Rochow (1990) this behaviour to remain motionless benefits the captured insect in two ways. Firstly, it "appears to be a widespread, instinctive reaction of insects when trapped (in the web of a spider also) as to alarm the owner of the web as little as possible." Secondly, "the captured insect reduces the chances of coming into contact with neighbouring lines or silk strands and thus becoming even more helplessly entangled."

One suggestion (Gatenby, 1959) is that snared prey soon cease struggling because the mucus droplets of fishing lines somehow immobolizes them, although he found that freshwater protozoa and aquatic larval nematodes immersed in the liquid mucus were not killed. Buston (1933) reported that fluid from the snares of predaceous *Platyura* and *Ceroplatus* larvae was acidic with a pH of 1.8 because of the presence of oxalic acid. *A. luminosa's* fishing lines were analysed and found to have a pH of 3.6, "but the presence of oxalic

acid was not confirmed" (Pugsley, 1983). More recently, Meyer-Rochow (1990) reported that he had identified low molecular weight proteins in the mucus droplets of the fishing lines of *A. luminosa* "that could possibly have an anaesthetic effect."

The *D. melanogaster* that were eaten and the pieces of paper that were discarded first appeared to have been hauled up. Evidence that insects are first hauled up by glowworms before being eaten is supported by one observation of prey capture in bush, and of three insects stuck on fishing lines that were observed being hauled up by glowworms in caves at Waitomo (Chapter 5). Also, one-third of the total number of *D. melanogaster* and of the total number of pieces of paper were found the day after left hanging where they had been placed on fishing lines (Table 4.2 & 4.3). From these observations it therefore appears that glowworms do not discriminate between the prey-items that they haul up. However, smell and/or taste appears to play a role because more pieces of paper with crushed *D. melanogaster* juice were hauled up (18) than dry pieces of paper (4), and these were never discarded (Table 4.3). This confirms observations by Meyer-Rochow (1990) that glowworms can be "tricked" to bite inanimate objects such as pieces of leaves or tiny twigs previously rubbed in crushed houseflies and were placed on fishing lines.

The conclusion from these experiments is that it is unclear whether or not glowworms use tactile senses to discriminate between live and dead prey caught in their webs. However, I have demonstrated that glowworm prey recognition behaviour does involve the senses of taste and/or smell.

Chapter 5 OBSERVATIONS DERIVED FROM REMOTE RECORDING OF *ARACHNOCAMPA LUMINOSA* IN BUSH AND CAVES AT WAITOMO

Introduction

The New Zealand Glowworm *Arachnocampa luminosa* (Skuse) (Diptera: Mycetophilidae) inhabits dark and damp areas such as caves and unused railway and mining tunnels, as well as places that are damp and sheltered in bush such as stream banks, bush-clad ravines, and along road cuttings. The larva lives within a tubular mucus gallery which is suspended from the substrate, and many vertical silken fishing lines beaded with sticky droplets hang from it. A light organ at the end of its body is formed from the distended ends of Malpighian tubules (Green, 1979). The blue-green bioluminescence has a maximum wavelength of 487 nm (Shimomura et al. 1966). This is used to attract prey, most of which are Diptera (Chapters 2 & 3). These fly into the sticky snare and are hauled up by the larva and eaten (Gatenby & Cotton, 1960; Richards, 1960; Stringer, 1967).

Glowworms are nocturnal insects which often inhabit places which can be difficult for humans to get to. *A. luminosa* larvae, together with pupae and adults are also relatively small (Richards, 1960), and consequently workers must get close to them to make accurate observations. Indeed, many workers have reported difficulties when attempting to investigate the behavioural ecology of *A. luminosa*. According to Hudson (1950), "the investigation of the habits and life-history of the New Zealand glow-worm has proved by far the most difficult problem I have had to face during the many years I have been privileged to work at the Entomology of New Zealand." Richards (1960) reported that glowworms are "very difficult to study unless they are caged", and Gatenby (1959) stated that it is difficult to make accurate observations of glowworms because in caves "the roof is often dripping with water, and the banks slippery."

Previous observations of *A. luminosa's* behaviour were carried out both with captive specimens (Hudson, 1950; Gatenby & Cotton, 1960; Stringer, 1967) and at sites where they are found (Meyrick, 1886; Norris, 1894; Hudson, 1950; Gatenby, 1959; Gatenby & Cotton, 1960; Richards, 1960; Stringer, 1967), using artificial light sources visible to humans. However, there are many anecdotal reports that these light sources affect *A. luminosa's* behaviour (Hudson, 1950; Gatenby, 1959; Gatenby & Cotton, 1960; Richards, 1960; Stringer, 1967), and this therefore calls into question the reliability of any behavioural observations using visible light.

Experiments conducted by Meyer-Rochow & Eguchi (1984) demonstrated that the eyes of adult male *A. luminosa* are maximally sensitive to light of 540 nm (green); with further response peaks at 460 nm (bluegreen) and in the near ultraviolet. Furthermore, Meyer-Rochow & Waldvogel (1979) reported that both larval and adult cave *A. luminosa* exhibit behavioural reactions to these light wavelengths. However, "light of a wavelength longer than 660 nm does not elicit measurable responses" (Meyer-Rochow & Eguchi, 1984).

Technological advances in the field of night vision have allowed ecologists to overcome the difficulties of observing nocturnal animals such as *A. luminosa* (see Burton & Taylor, 1983). This chapter presents findings obtained from remote-recording the behaviour of *A. luminosa* larvae, pupae and adults in both bush and cave habitats at Waitomo. Observations were made using infra-red light-sources apparently not visible to *A. luminosa*, and a TV camera which is sensitive to this light.

Materials & Methods

Remote recording of *A. luminosa* was done between 18/1/95 and 19/11/95 using the equipment shown in Figure 5.1. Larvae observed were on the ceiling of the Demonstration Chamber in Glowworm Cave, Waitomo (NZS 260 S16, 943 248)(Fig. 5.2), in the bush-clad entrance to Reserve Cave, Waitomo, and at two sites within Reserve cave (NZS 260 S16, 927 246)(Fig. 5.3), in Waitomo Waterfall Cave (NZS 260 S16, 909 249)(Fig. 5.4), and at two sites in bush at Ruakuri Scenic Reserve, Waitomo (Fig. 5.5). Larvae were recorded at all of these locations, but pupae and adults were recorded only within Reserve Cave and in its bush-clad entrance.

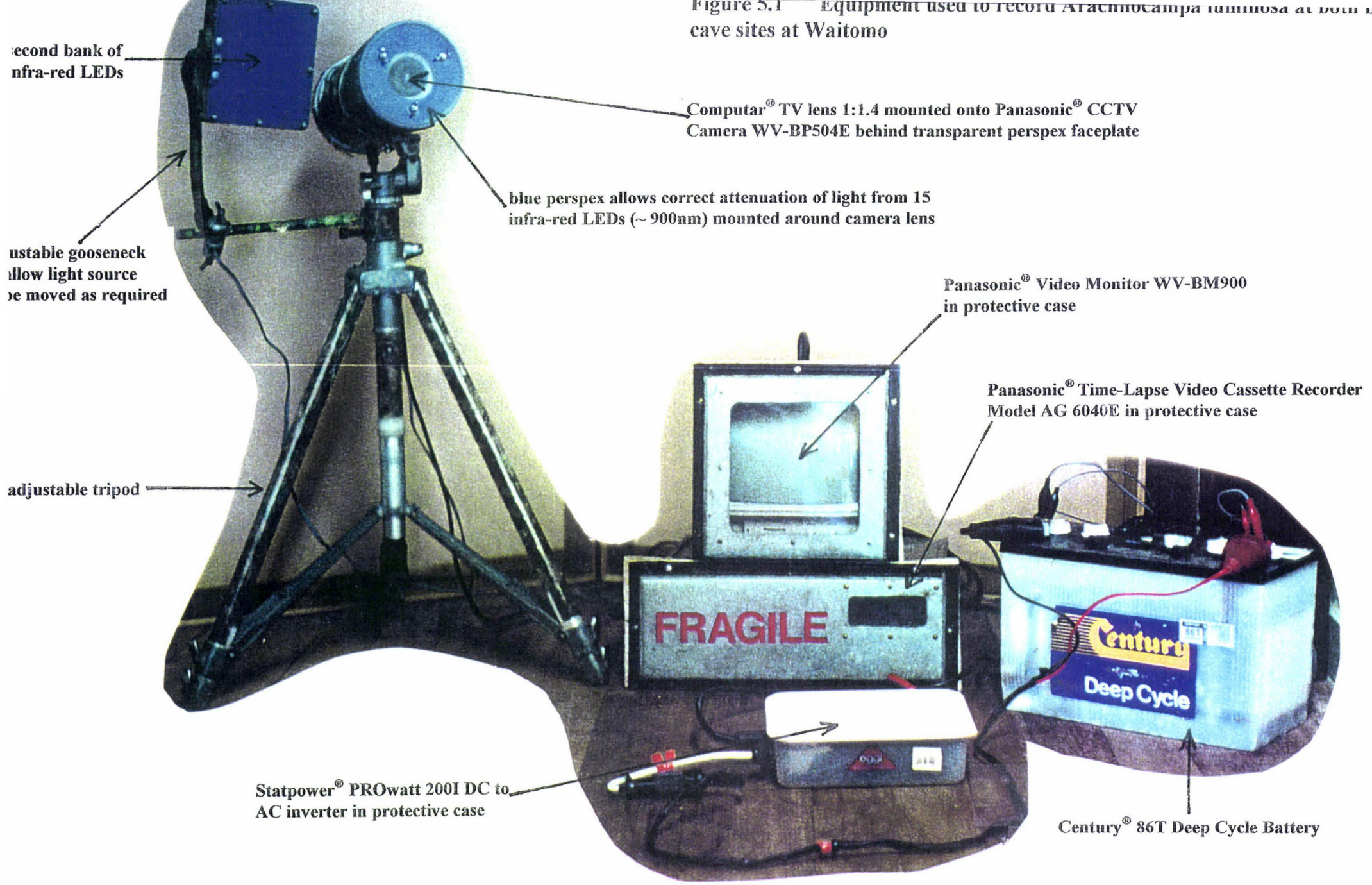
The camera lens was positioned as close to *A. luminosa* as possible. This was because a) *A. luminosa* and their prey are of a small size, and b) a wide-angle camera lens was used. Often the perspex faceplate was as close as 50 mm from *A. luminosa*, but it was never close enough to make contact with the animal or its snare. When recording larvae, a site was usually chosen where 2 or sometimes 3 larvae were close enough together to enable them to be simultaneously recorded.

The video-recorder was set to record 48 hours on a 3 hour tape. This made efficient use of a 3 hour video cassette, whilst also recording the behaviour of *A. luminosa* in detail. Once set up, the equipment was visited only to exchange batteries and to replace cassettes every two days. Sometimes, unpredictably, the battery was flat before its replacement was due, and consequently recording had stopped. In some instances, the battery had run flat almost 2 hours before replacement and occasionally up to 5 hours of glowworm behaviour had not been recorded.

Video cassettes were analysed using slow motion and frame by frame playback. Data recorded with observations were the date, time and duration of each behaviour to the nearest second. When and for how long *A. luminosa* produced bioluminescence was measured. Many types of behaviour were identified. The most obvious ones were; "fishing line construction", when a larva leaned out of its gallery, produced a fishing line and then withdrew back into its snare; "defecation" when a larva released an excretory droplet from its snare; "fighting" between pairs of larvae; "prey capture", and attempted capture of spiders by larvae. Behaviours recorded rarely were the eclosion of both male and female adult *A. luminosa* from pupae; mate attraction, and copulation.

Bush temperatures were recorded at the entrance to Glowworm Cave (Fig. 5.2) which is approximately 2.5 kilometres away from Ruakuri Scenic Reserve (Fig. 5.5).

Figure 5.1 Equipment used to record Arachnocampa luminosa at both bush and cave sites at Waitomo



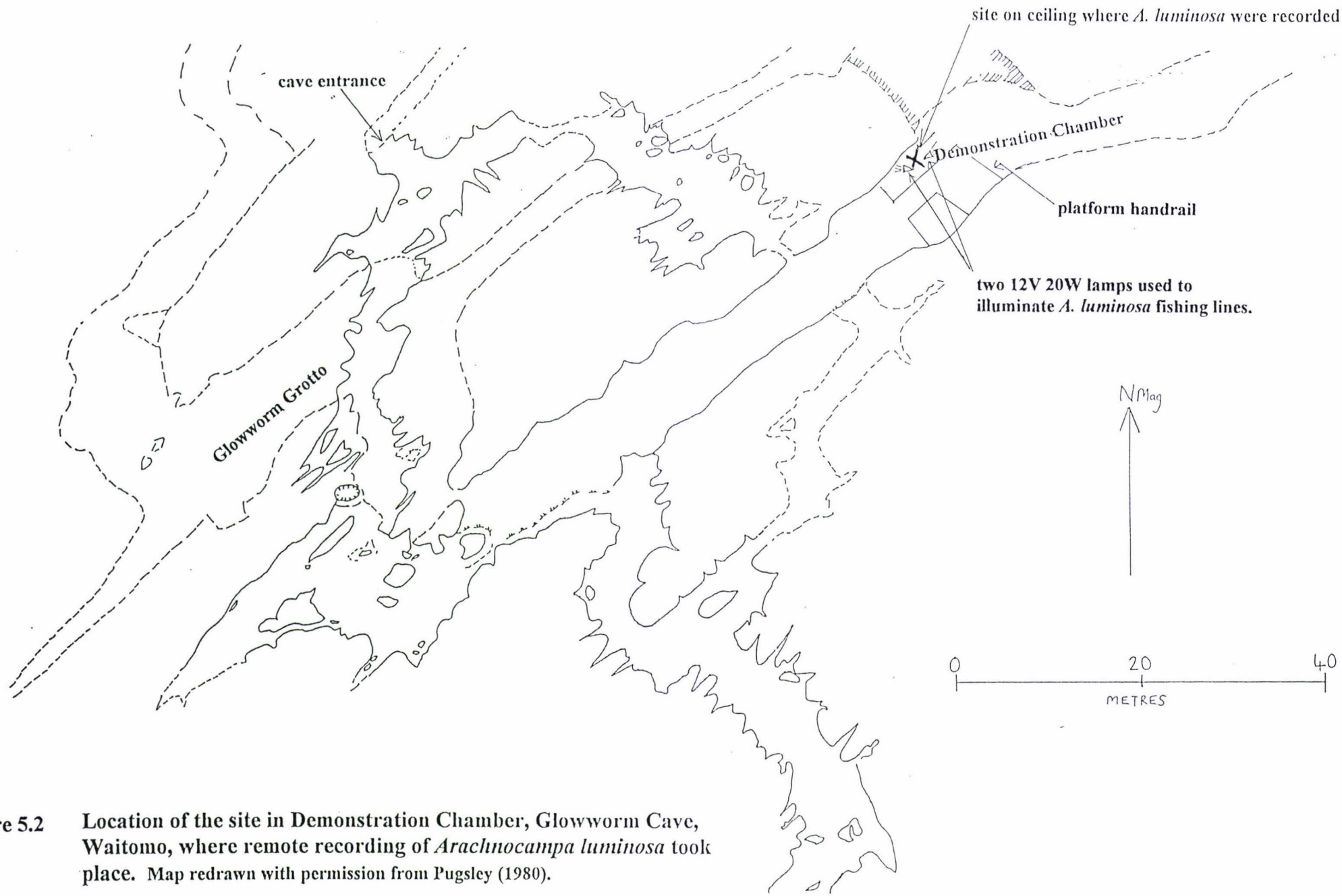


Figure 5.2 **Location of the site in Demonstration Chamber, Glowworm Cave, Waitomo, where remote recording of *Arachnocampa luminosa* took place.** Map redrawn with permission from Pugsley (1980).

Figure 5.3 **Location of both bush and cave sites at Reserve Cave, Waitomo, where remote recording of *Arachnocampa luminosa* pupae, adults and a single larva took place.** Redrawn with permission from New Zealand Speleological Society (NZSS) map by L. Fow and P. Dimond (1960).

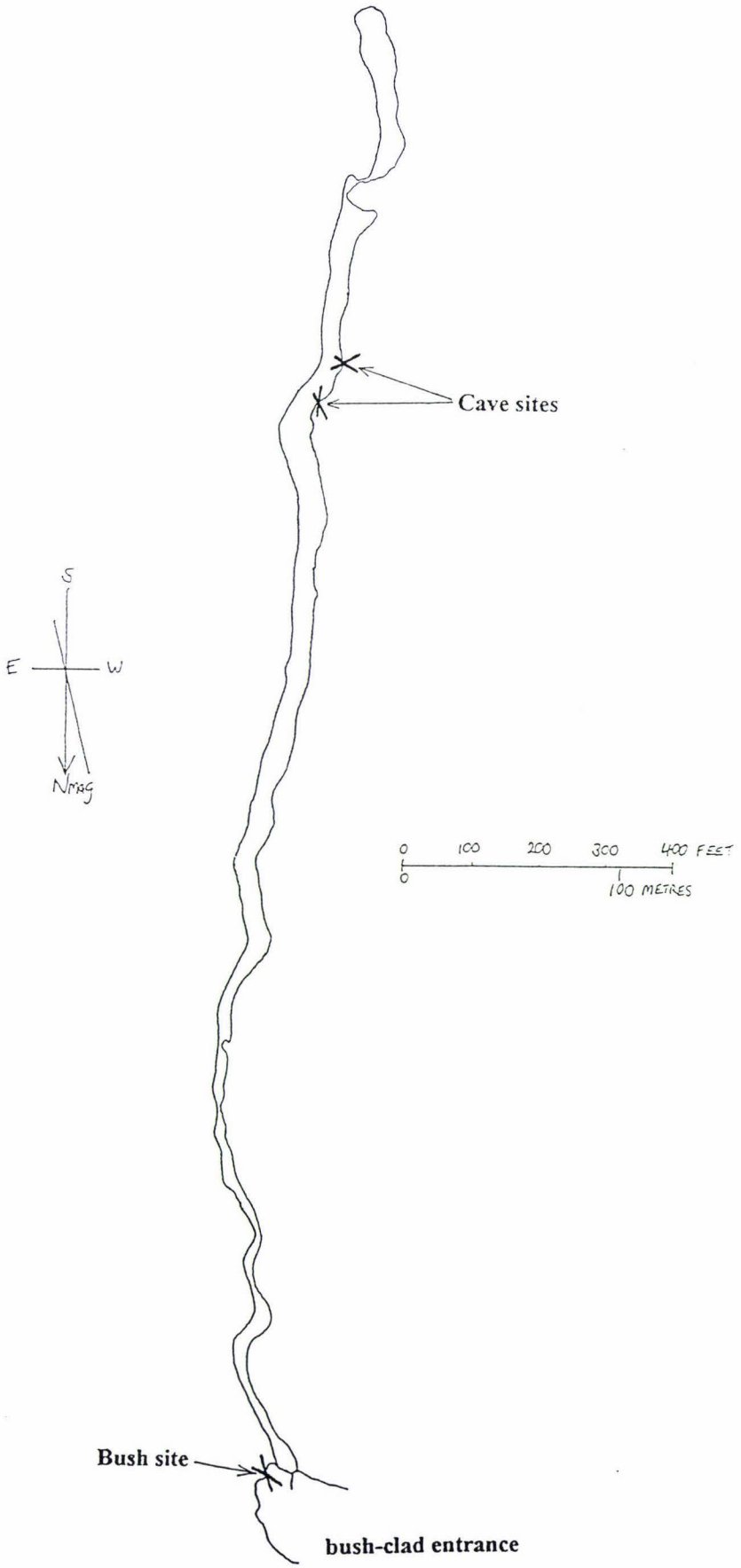


Figure 5.4 **Location of the site in Waitomo Waterfall Cave, where remote recording of *Arachnocampa luminosa* took place.** Map redrawn with permission from New Zealand Speleological Society (NZSS).

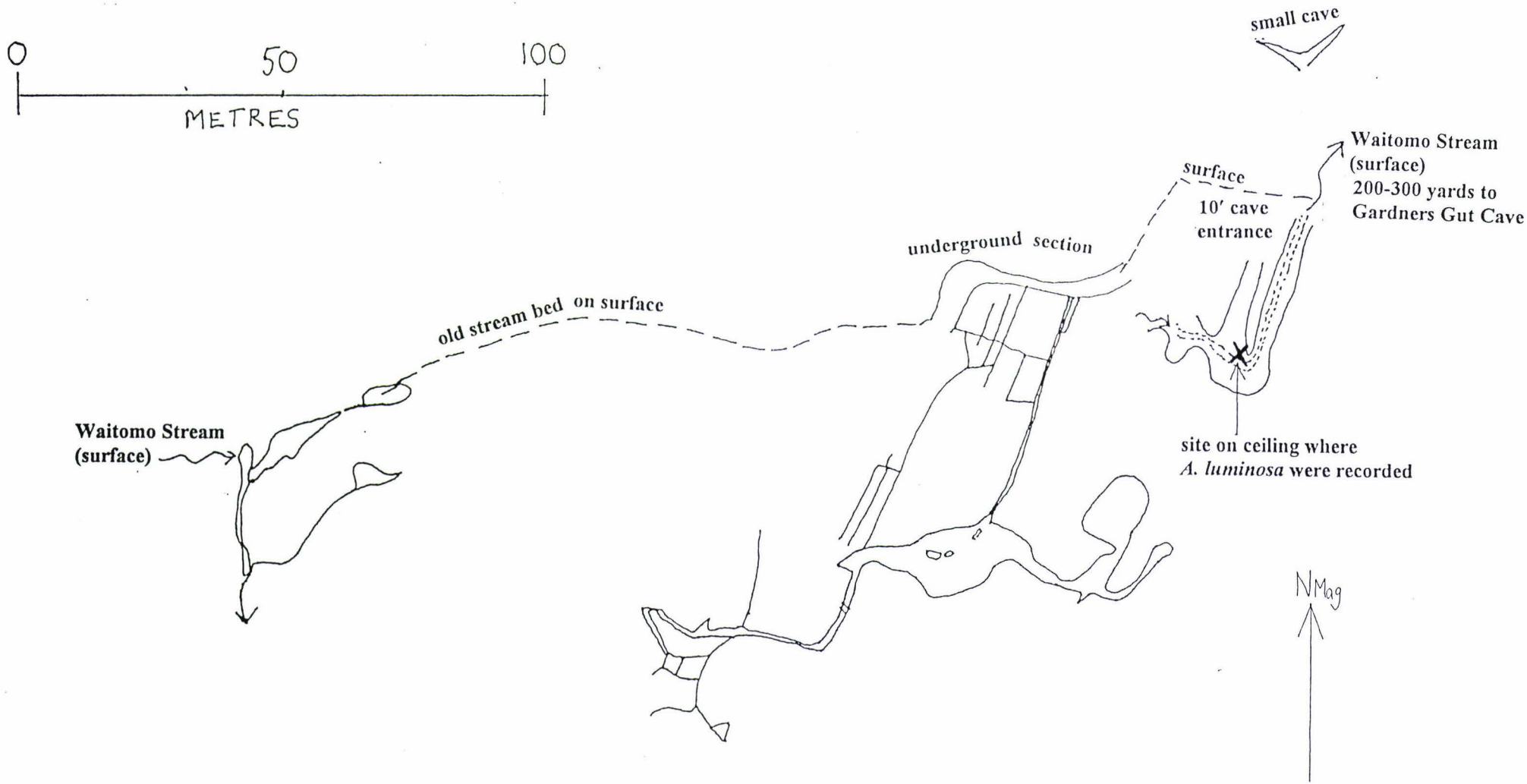
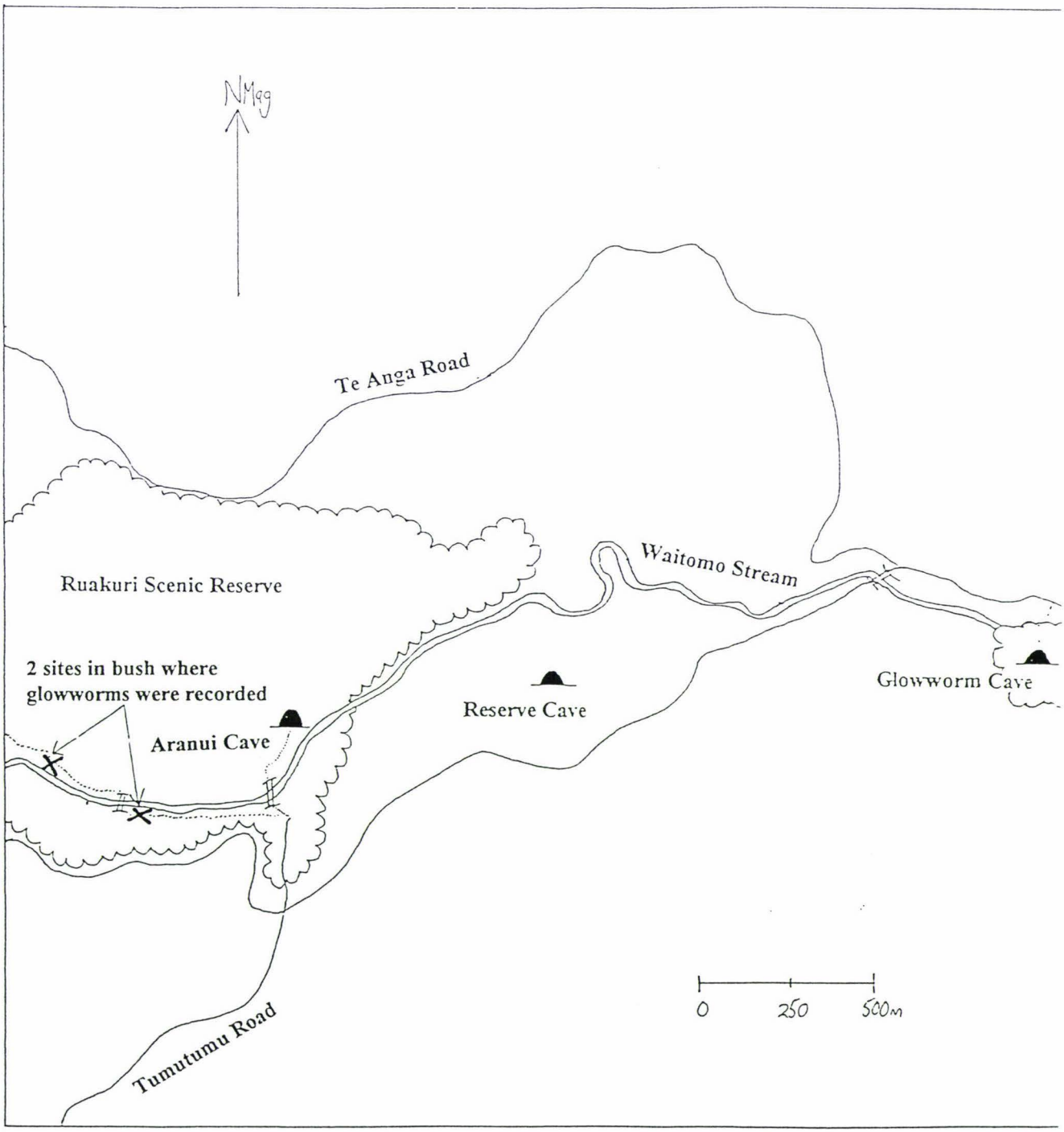


Figure 5.5 **Location of Glowworm Cave, Reserve Cave, and the bush sites in Ruakuri Scenic Reserve, Waitomo, where remote recording of glowworms took place.** Adapted from Waitomo Visitor Information Map.



The effects of disturbance by humans upon larvae in Glowworm Cave were also investigated. This was carried out by analysing video tape to determine when two small 20 Watt lights in the ceiling of Demonstration Chamber (Fig. 5.2) were switched on, and comparing this data with the behavioural patterns of larvae. These lights are used many times during the day to allow groups of visitors to view numerous fishing lines that hang from the ceiling.

Results

Video-taping of larvae

A total of 638 hours of larval behaviour were recorded over the 10 month period. However, two or more glowworms were sometimes filmed simultaneously, so 934 individual "larva-hours" of activity were recorded. On-site study resulted in 308 "larva-hours" being recorded from 4 glowworms in bush; 345 "larva-hours" from 4 glowworms in Glowworm Cave and 233 "larva-hours" from one glowworm in Reserve Cave. Several glowworms in Waitomo Waterfall Cave were also recorded over a 48 hour period.

Behaviour of pupae and adults

The first *A. luminosa* pupa observed in Reserve Cave was female. It was suspended from the cave wall about 25 metres from the site where recording of the larva took place (Fig. 5.3). This female pupa was video-taped for 163 hours then the adult emerged and copulated with a male fly. The female took 13 hours and 23 minutes to eclose during which she glowed intermittently on 12 occasions. The male alighted upon the female 13 hours and 8 minutes after eclosion began and copulation commenced 13 hours later. Immediately after copulating both adults were caught and eaten by a large predatory harvestmen (*Megalopsalis tumida* Forster). The second pupa observed was also female, but this one was attached to a rockface in the bush-clad entrance to the cave (Fig. 5.3). After 168 hours of video taping most of its eclosion was recorded. A male pupa in Reserve Cave was video taped for ~ 144 hours until the adult emerged. It glowed three times during eclosion and the adult flew off 43 hours after eclosion started.

Only one *A. luminosa* adult caught in fishing lines was observed, in Waitomo Waterfall Cave. It is not known whether it was eaten by a larva or managed to escape capture because it disappeared from view.

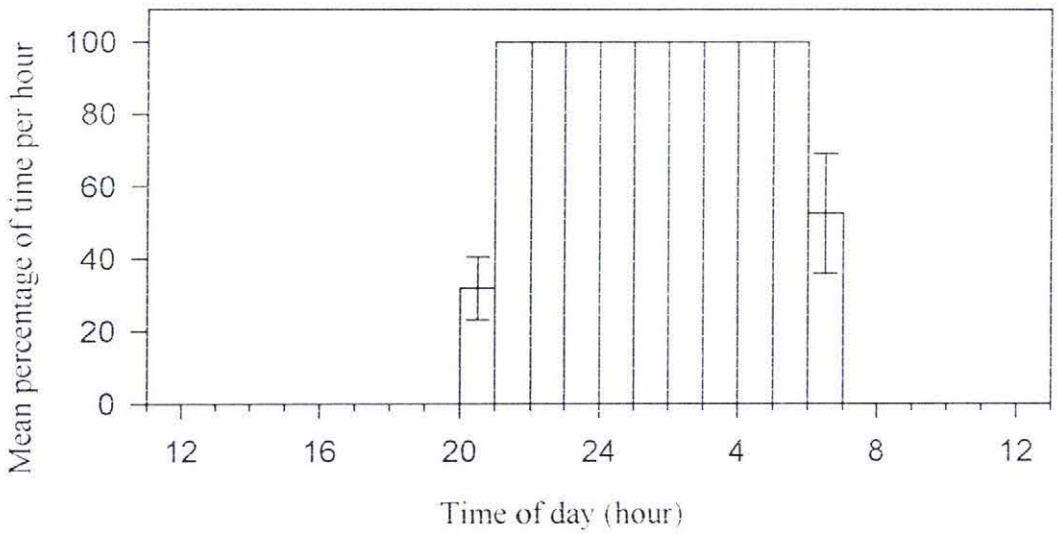
GLOWWORM BIOLUMINESCENCE

In bush

Glowworms in bush turned on their lights at or after sunset (range 0 to 42 minutes after sunset; Appendix) and turned them off again before sunrise (range 10 hours 37 minutes to 4 minutes; Appendix). On four nights individual larvae started to glow 15 to 20 minutes before other larvae nearby, and on four other nights all larvae appeared to start glowing within about 1 minute of each other (Appendix). They produced a "bright" light from a non-glowing state relatively quickly, over a period of less than 15 seconds to about 1 minute. Glowworms in bush took several minutes to turn out their lights, during which their glow slowly faded.

The two glowworms observed in bush in February produced bioluminescence between 20:31 and 06:42 the next day (Fig. 5.6). Both larvae glowed continuously once they started glowing and their bioluminescence remained at about the same brightness throughout the night.

Figure 5.6 *Mean hourly percentage of time two larvae spent glowing in bush at Ruakuri Scenic Reserve, Waitomo, between 23 and 25.2.95. Standard error bars are also shown.*



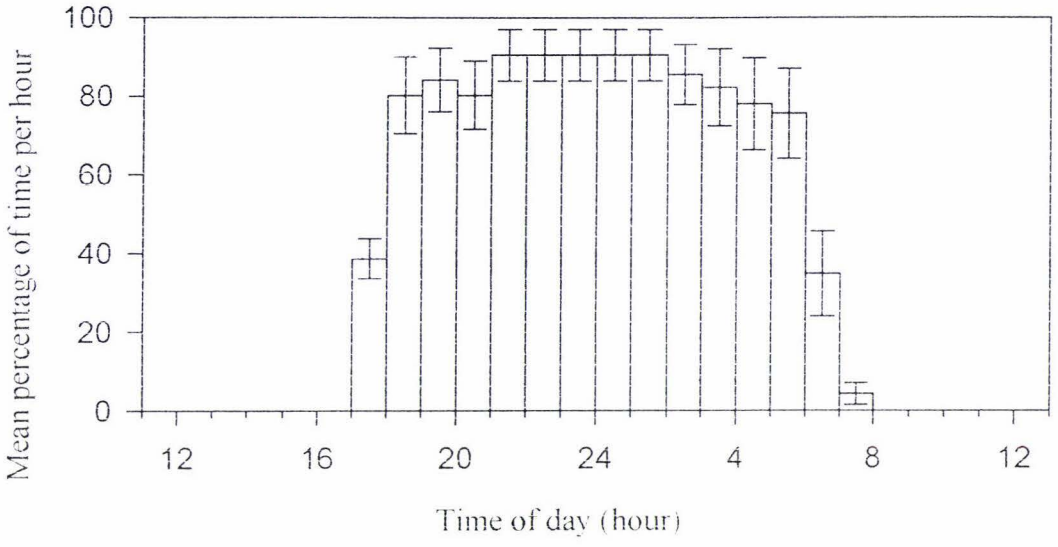


Figure 5.7 Mean hourly percentage of time two larvae spent glowing in bush at Ruakuri Scenic Reserve, Waitomo, between 9 and 15 5 95. Standard error bars are also shown.

In May, the two glowworms observed in bush produced bioluminescence between about 17.22 and 07 05 the next day (Appendix). However, they did not glow all of the time once they started (Fig. 5.7). On the nights of 10/5/95 and 11/5/95 both larvae either stopped glowing for some periods or glowed only faintly. Larvae glowed constantly when the temperature range was ~ 18° C - 12° C at sunset and diminished to a minimum of ~ 6° C at sunrise. On the night of 11 - 12/5/95 one of the two larvae glowed intermittently for periods of time (1 hour 29 minutes; 19 minutes; 10 minutes; 9 hours 41 minutes) when the temperature range was ~ 10° C at sunset to ~ 6° C at sunrise (Appendix). On 10/5/95 both larvae ceased glowing after doing so for 4 and a half hours when the temperature dropped below about 6° C. The temperature range that night was ~ 9° C to ~ 2° C (Appendix). The possible effects of temperature on bioluminescence is shown in Fig. 5.8.

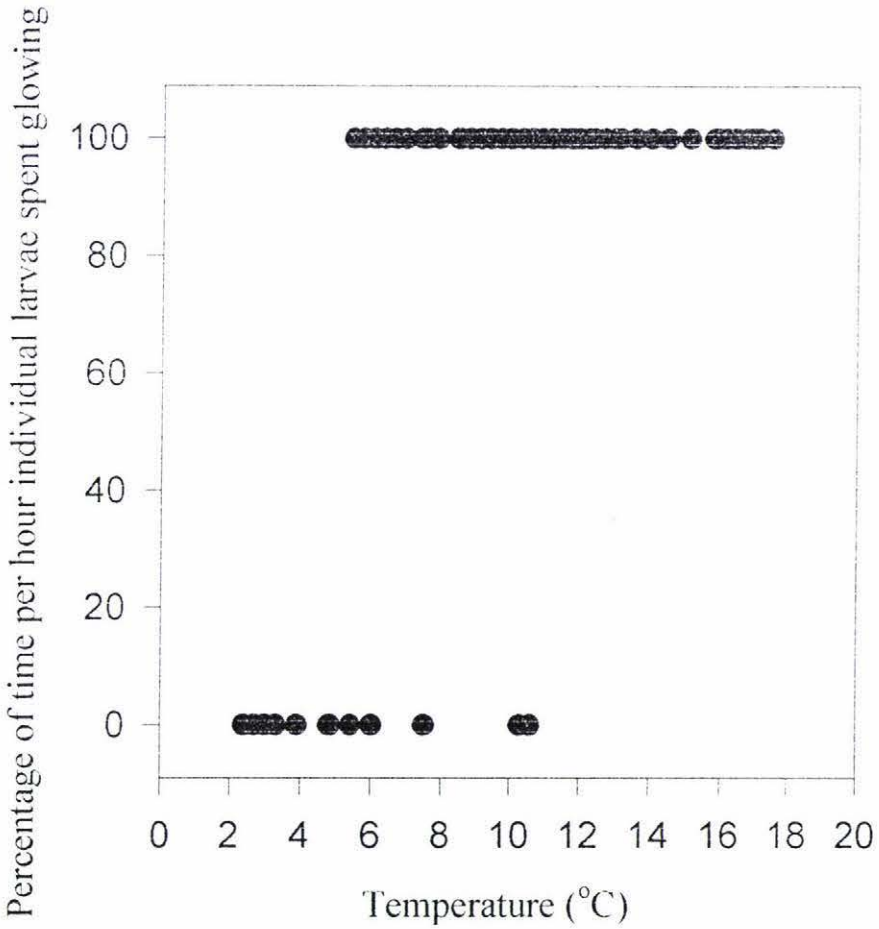
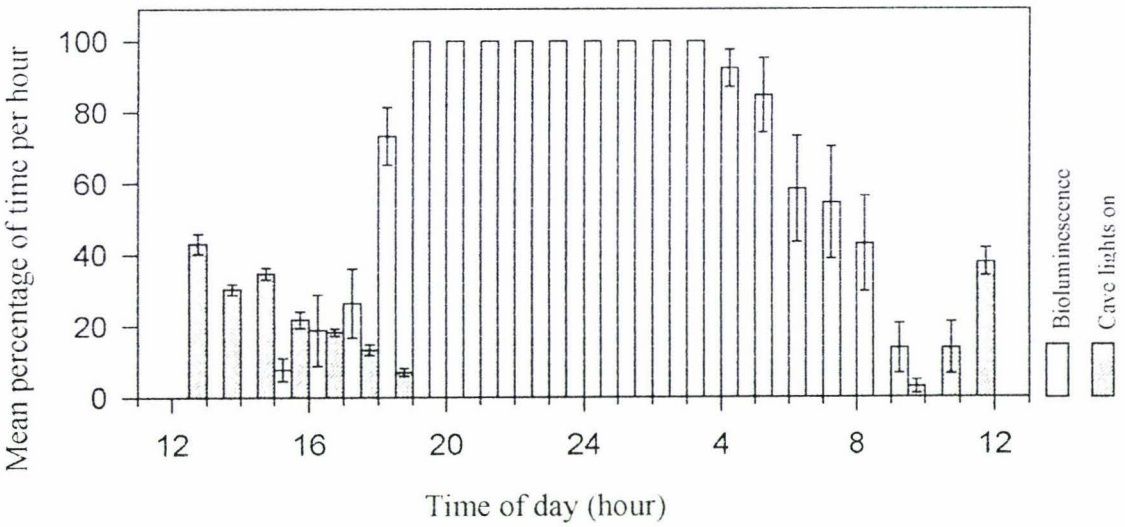


Fig. 5.8 *The possible effect of temperature on the percentage of time per hour* A. luminosa *larvae were observed to spend glowing in bush at Ruakuri Scenic Reserve, Waitomo. Temperature measurements were taken each hour in bush outside the entrance to Glowworm Cave approximately 2.5 kilometres away.*

In Glowworm Cave

Larvae glowed brightly most of the time between 15:00 and 10:00 (Fig. 5.9), and all did so between 19:00 and 04:00 (Fig. 5.9). Their bioluminescence was often particularly intense between 19:00 and 22:00. Between 10:00 and 15:00 the larvae never stopped glowing altogether, but their lights often appeared very dim. This was the time when cave lights were switched on most frequently although they were switched on intermittently between 09:00 and 19:00 (Fig. 5.9).

Figure 5.9 *Mean percentage of time per hour that four A. luminosa larvae spent glowing brightly in Demonstration Chamber of Glowworm Cave, Waitomo. Observations were made between 2 - 5 4 95 and 19 - 23 5 95. Standard error bars are also shown.*



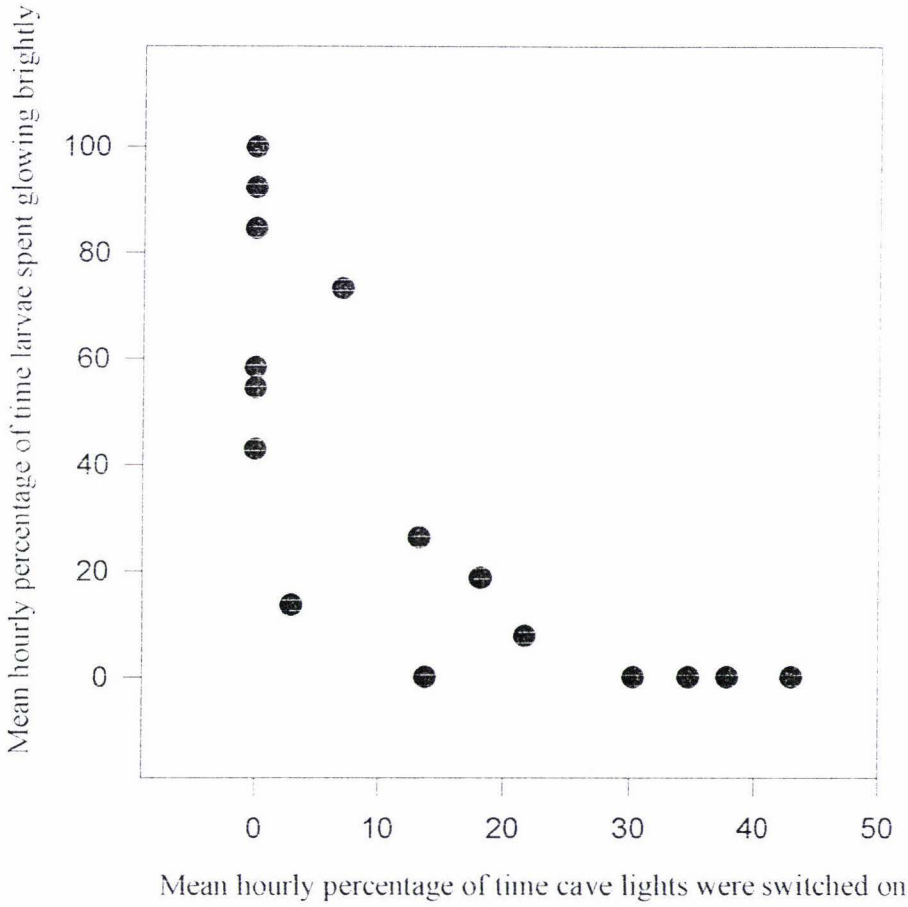


Figure 5.10 *Relationship between the mean percentage of time per hour spent by four* A. luminosa *larvae glowing brightly in Demonstration Chamber of Glowworm Cave, Waitomo, and the mean hourly percentage of time that the cave lights were switched on. Observations were made between 2 - 5 4 95 and 19 - 23 5 95.*

When cave lights were switched on for more than about 30% of the time (~ 20 minutes per hour), larvae in the cave glowed only faintly. However, the effect of the cave lights on larval bioluminescence was complicated by the possible effect of wind currents produced by tourists on glowworms. The relatively static fishing lines always began to sway rapidly from side to side a few seconds before the cave lights were switched on, and this sometimes tangled them. When the timer switched the lights off again five minutes later these swaying movements often continued. The lines swayed constantly and the larvae only glowed dimly between 10:00 and 15:00 each day.

In Reserve Cave

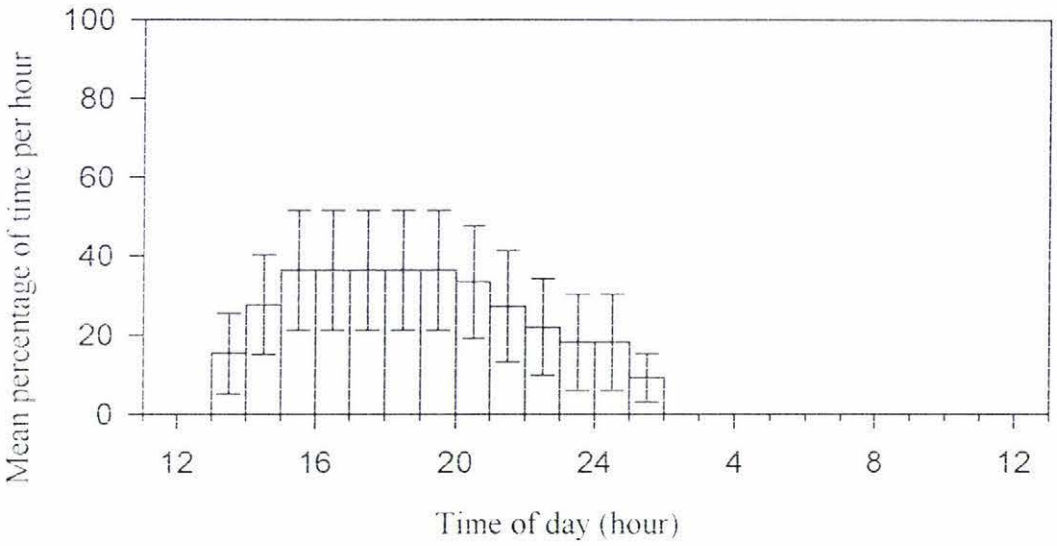


Figure 5.11 *Mean percentage of time one* A. luminosa *larva spent glowing per hour in Reserve Cave, Waitomo. Observations were made between 7 - 19 11 95. Standard error bars are also shown.*

Only one larva was observed in Reserve Cave. This larva glowed during only four out of the eleven days of observation. All of its bioluminescence occurred between 13:00 and 02:00 (Fig. 5.11). Data from the days when the larva was not glowing is incorporated into Figure 5.11 to keep the results comparable with those obtained for both bush and Glowworm Cave. This reduced the mean percentage of time the larva glowed each day (Fig. 5.11). For instance, when this larva did glow between 15:00 and 20:00 then it glowed constantly and not ~ 21 - 50% of the time as shown in Figure 5.11. The larva never appeared to glow very brightly compared with glowworms at the other two locations, except on five occasions when it fought with another larva that was out of view. When this occurred its light was intense.

MAKING FISHING LINES

How glowworms make fishing lines

Hundreds of observations of individual larvae making fishing lines were made. The general behavioural pattern based upon these observations is described here. When a larva made a fishing line it first moved out of its gallery until the anterior half of its body hung vertically (Fig. 5.15). Peristaltic waves of contraction passed rhythmically along its body until a large globule of mucus appeared in its jaws. The larva lowered this a short distance on a silk strand after rocking its head backwards and forwards about 20 times. It took the glowworm about 60 to 90 seconds to produce this globule which was about twice the size of the regular sticky droplets which followed. Regular sticky droplets took about 8 to 16 seconds each to produce (3 - 4 peristaltic cycles). When the fishing line was near completion the glowworm slowly reversed backwards into its gallery whilst adding to the length of the fishing line. It then attached it to the gallery suspensory threads. Fishing lines took variable lengths of time to complete (Table 5.1). Usually a larva first appeared to attach a suspensory thread to a point on the substrate above or beside the snare and then fastened this to the gallery. A fishing line was then made and attached to this point of intersection. This sometimes lead to conflict when a larva that was searching the substrate around the snare for a place to attach a suspensory thread touched the snare of its neighbour (see "Observations of Larvae Fighting").

In Glowworm Cave, on eleven occasions, larvae hauled in tangled fishing lines before replacing them with new ones. On eight occasions glowworms there were also observed moving fishing lines a few millimetres from their original attachment points to new points in the snare.

The longest fishing line observed was over 1 metre in length. This was hanging from the ceiling of Waitomo Waterfall Cave on 26/6/95 and it had a single sticky droplet at the bottom with no regular sticky droplets along its length. The next day this fishing line had disappeared. It had probably broken under its own weight.

Comparisons between the lengths of time spent making fishing lines by glowworms in bush, Glowworm Cave and Reserve Cave.

On average, bush glowworms spent the shortest period of time overall making fishing lines (mean = ~ 7 minutes) whereas larvae in Glowworm Cave took a mean of ~ 11 minutes and the glowworm in Reserve Cave spent an average ~ 15 minutes (Table 5.1) making fishing lines. Larvae in Glowworm Cave spent the shortest period of time making a fishing line (~ 21 seconds; Table 5.1) followed by bush glowworms (~ 36 seconds; Table 5.1) and the larva in Reserve Cave (~ 2 minutes; Table 5.1).

Table 5.1 *Time (mm:ss) spent making individual fishing lines by glowworms in bush, Glowworm Cave and a glowworm in Reserve Cave.*

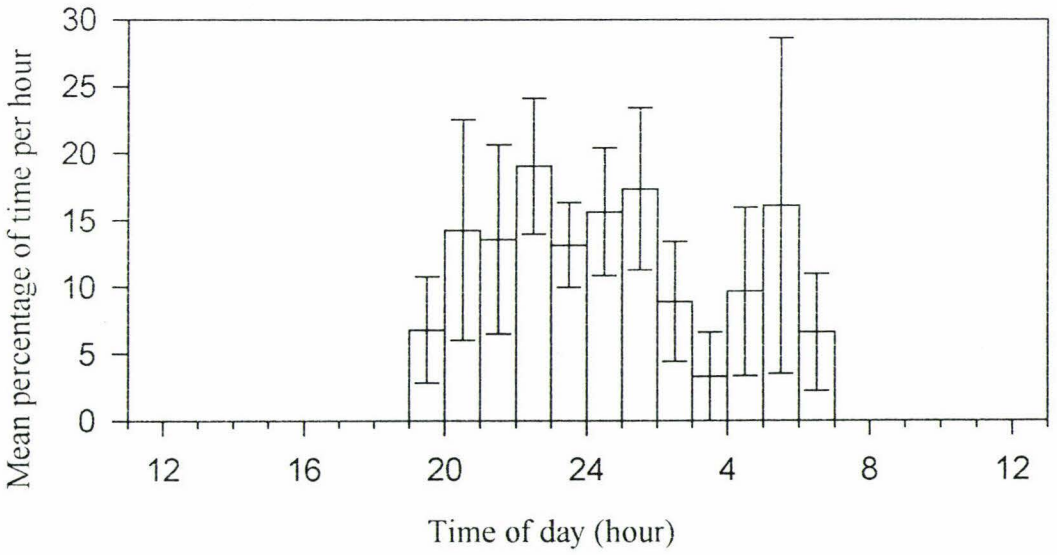
Location	*N*	Mean	Range	SE
Bush	223	06:41	00:36 - 16:30	00:13
Glowworm Cave	202	10:31	00:21 - 32:06	00:33
Reserve Cave	39	14:42	02:19 - 25:54	00:56

When glowworms in bush made fishing lines

On seven occasions individual larvae in bush began making fishing lines up to forty-five minutes before sunset (Appendix). On seven other occasions glowworms in bush began to make fishing lines up to 54 minutes after sunset. Individual larvae were observed making fishing lines on only five occasions after sunrise. However, they did not make them more than 50 minutes after sunrise (Appendix). A mean of 15 fishing lines were made by each glowworm during the night (range: 5 ~ 28).

In February, glowworms constructed fishing lines between about 19:00 in the evening and 07:00 the next day (Fig. 5.12). The amount of time they spent making fishing lines increased to a maximum between 22:00 and 23:00 when about 14 - 24% of their time was spent making fishing lines (Fig. 5.12). Thereafter the proportion of time spent diminished slightly until dawn, but there was a small increase in activity between 05:00 and 06:00 (Fig. 5.12).

Figure 5.12 *Mean hourly percentage of time spent by two* A. luminosa *larvae constructing fishing lines in bush at Ruakuri Scenic Reserve, Waitomo, between 23 - 25.2.95. Standard error bars are shown.*



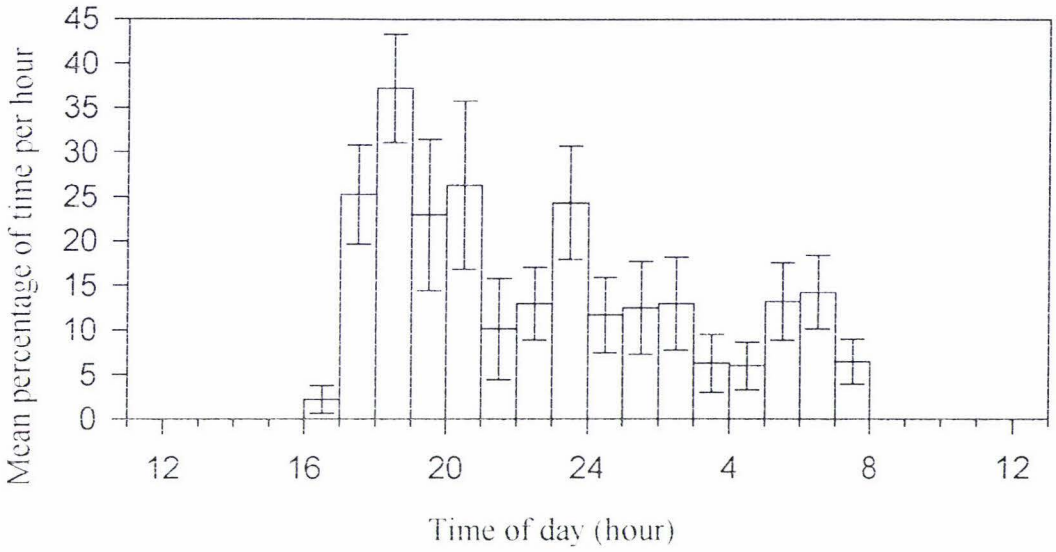


Figure 5.13 *Mean hourly percentage of time spent by two* A. luminosa *larvae constructing fishing lines in bush at Ruakuri Scenic Reserve, Waitomo, between 9 and 15 5 95. Standard error bars are shown.*

In May, individual larvae made fishing lines between about 16:00 and 08:00 the next day (Fig. 5.13). The amount of time they spent making fishing lines increased rapidly to a maximum between 18:00 and 19:00 when about 32 - 44% of their time was spent making them (Fig. 5.13). Between 21:00 and dawn the proportion of time spent by larvae making fishing lines was between about 3 and 17%, with the exception of a small increase in activity between 23:00 and midnight (17 - 30%)(Fig. 5.13).

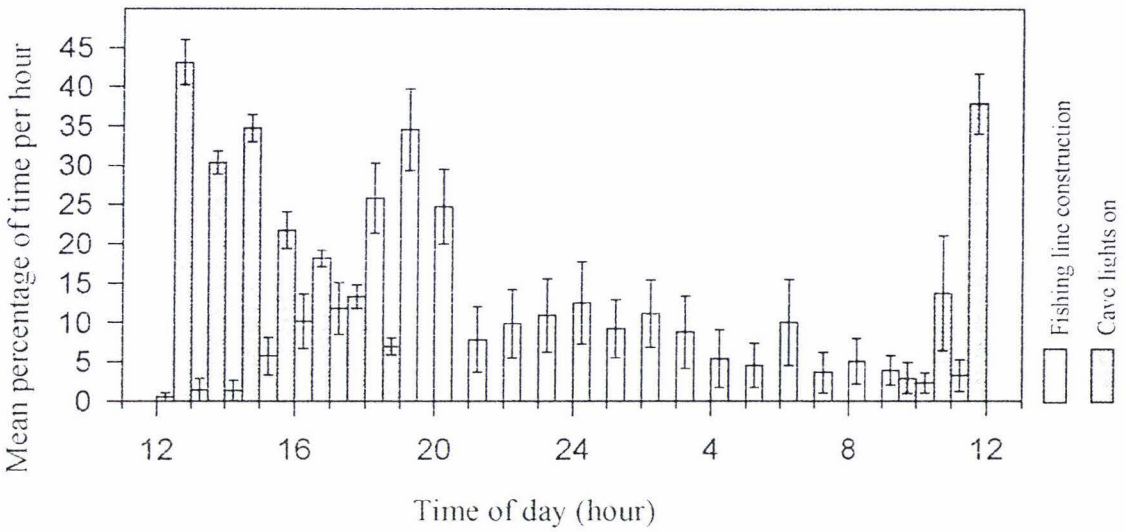


Figure 5.14 *Mean percentage of time per hour spent by four* A. luminosa *larvae constructing fishing lines in the Demonstration Chamber of Glowworm Cave, Waitomo, between 2 - 5 4 95 and 19 - 23 5 95. The percentage of time when the cave lights were switched on is also shown. Standard error bars are included.*

Larvae in Glowworm Cave constructed fishing lines throughout the entire day, although most of this activity occurred between 18:00 and 21:00 when ~ 22 to 37% of their time was spent doing this (Fig. 5.14). Following this, fishing line construction diminished with ~ 1 to 15% of their time spent in this activity (Fig. 5.14).

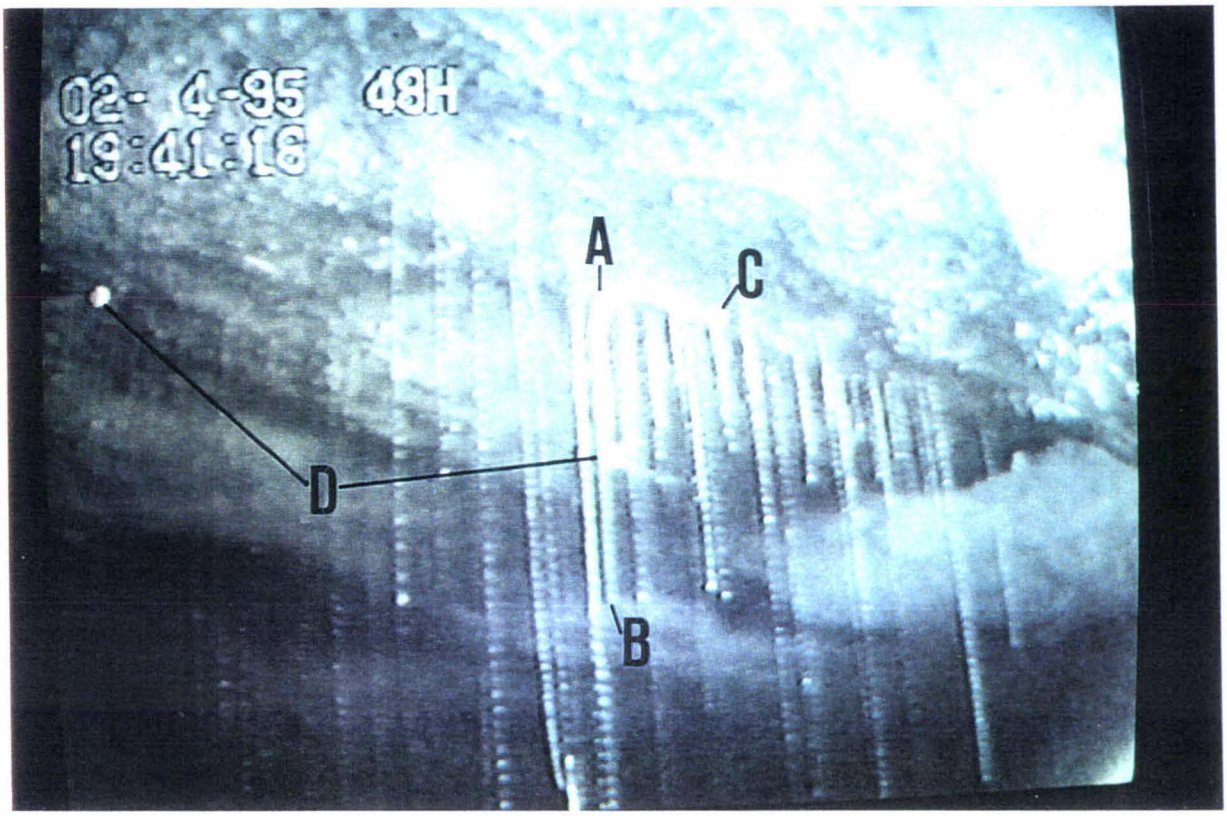


Figure 5.15 A. luminosa *larva (~ 20 mm long; A) constructing a fishing line (B) in Demonstration Chamber of Glowworm Cave. The light organ (C) is brightly glowing while the larva hangs from its gallery. This posture is typical of larvae engaged in this behaviour. Two brightly glowing light organs nearby (D) indicate the location of other* A. luminosa *larvae.*

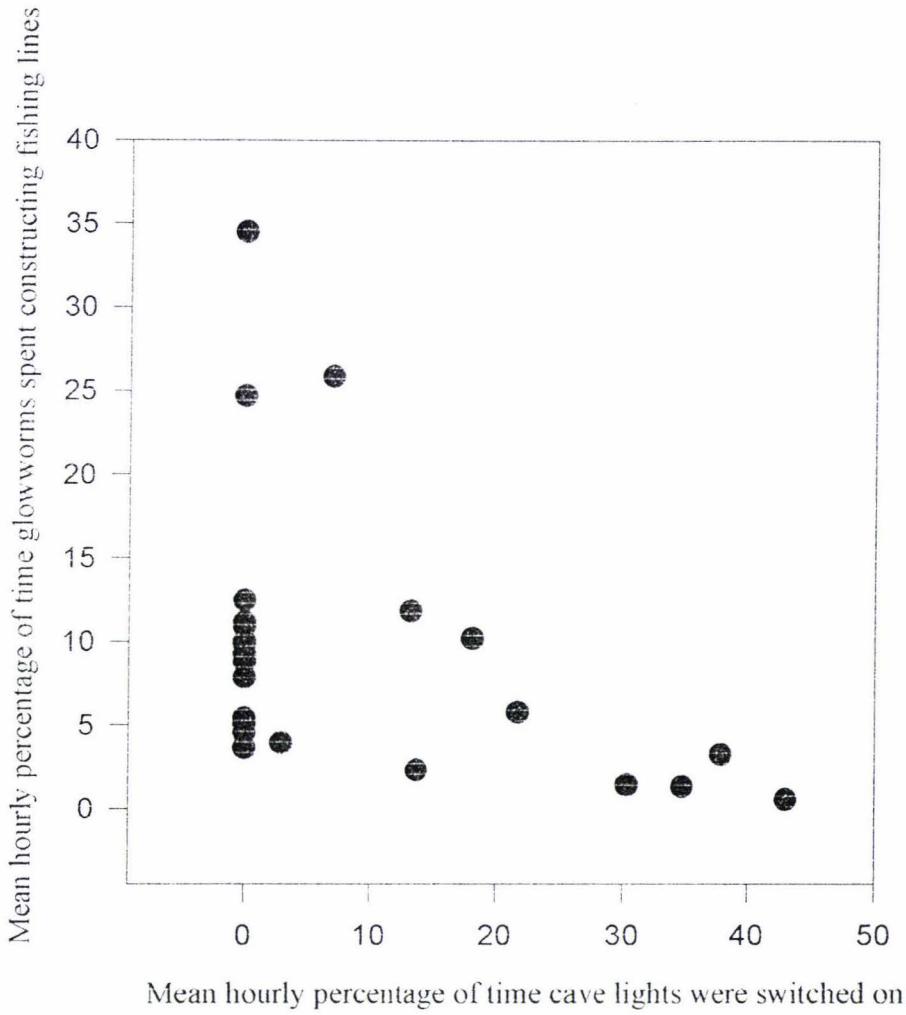


Figure 5.16 *Relationship between the mean percentage of time per hour spent by four* A. luminosa *larvae constructing fishing lines and the mean hourly percentage of time cave lights in Demonstration Chamber of Glowworm Cave, Waitomo, were switched on between 2 - 5/4/95 and 19 -23/5/95.*

When the cave lights were switched on for less than about 30% of the time the glowworms did not appear to significantly alter the amount of time they spent making fishing lines. However, when the lights were switched on for more than about 30% of the time glowworms spent only 0 - 5% of the time making fishing lines.

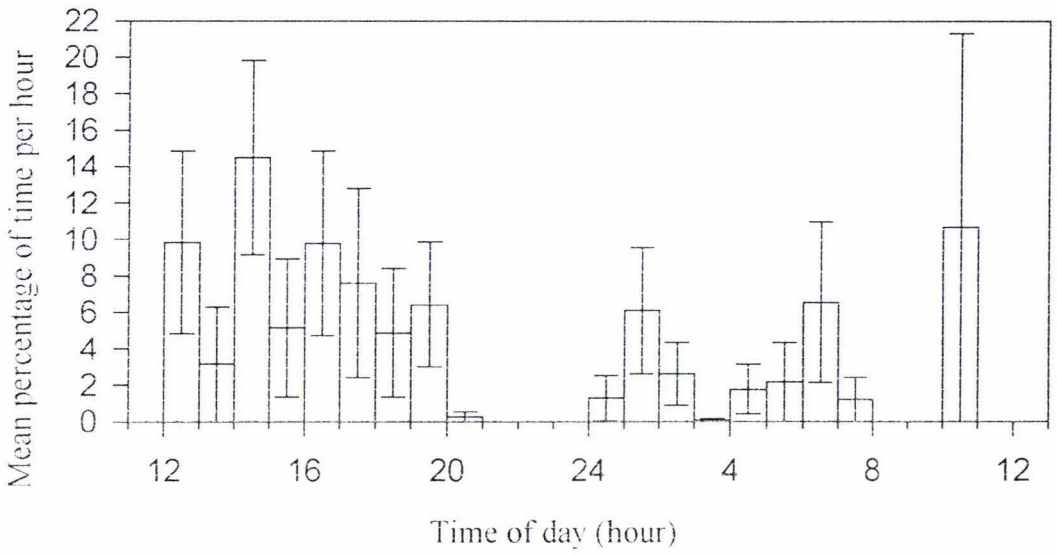


Figure 5.17 *Mean hourly percentage of time spent by one* A. luminosa *larva constructing fishing lines in Reserve Cave, Waitomo, between 7 11 95 and 19 11 95. Bars indicate standard error.*

Only one glowworm was observed in Reserve Cave. This larva appeared to construct fishing lines randomly throughout the day, but mostly between 12:00 and 20:00, when it spent about 2 to 20% of the time making fishing lines. It did not make any fishing lines between 21:00 and midnight, 08:00 and 10:00, and 11:00 and 12:00 over 12 days (Fig. 5.17).

PREY CAPTURE

Prey capture was observed in its entirety on only one occasion. The insect appeared to be a small dipteran which flew into a larval snare in bush. It was hauled up by the larva and eaten. Insects were, however, commonly observed flying past the camera at night in bush. Another three insects were observed being hauled up on fishing lines by individual glowworms but they were out of view when they were first snared. These were two mayflies (Ephemeroptera) in Waitomo Waterfall Cave, and a small winged insect in Glowworm Cave. What appeared to be two small flatworms ~ 20 mm long were observed in bush at night moving over the substrate around larval snares. They were attacked by glowworms but they were not caught and eaten by them. In Waitomo Waterfall Cave a dead weta (Orthoptera) was observed caught in a snare, and the glowworm was missing from its gallery. One *A. luminosa* adult was observed flying into fishing lines in Waitomo Waterfall Cave, but is not known if it was captured and eaten by a larva. On seven occasions spiders were observed moving through glowworm snares in bush. On one of these occasions the glowworm attacked the spider after it touched the snare, but the spider escaped, apparently unharmed. All of these observations are described in more detail below.

The one complete prey capture observed in bush at night occurred when what appeared to be a dipteran 2 - 3 mm long flew into several fishing lines about 30 mm below the gallery (Fig. 5.18). The glowing larva reacted within three seconds by turning around within its gallery. It then moved about half-way out of its gallery until it was hanging vertically. The glowworm reached the struggling insect and appeared to bite it ten seconds after it flew into the snare (Fig. 5.18). The glowworm then pulled its prey about 10 mm further up into the snare. Over the next hour and a half the glowworm fed on the captured insect, while still hanging part way out of its gallery. While the larva was feeding it continued to glow, but not as brightly as before the insect was captured. It paused during feeding only once (~ 13 minutes), when a spider moving behind the snare touched some of the fishing lines. When this happened the larva glowed brilliantly. When the larva finished feeding, it withdrew into its gallery and one minute later began to repair its snare and construct new fishing lines.

In Waitomo Waterfall Cave there is a dense population of glowworms on the ceiling, and at least in summer insects were commonly observed in their snares. On 21/2/95 the camera was focussed upon a large mayfly (Ephemeroptera) in a fishing line about 15 cm below the gallery. The mayfly often appeared to move its wings and legs. Two hours later the glowworm began to haul in the mayfly, and it took the larva 5 minutes to haul it up to the gallery. Unfortunately the cave ceiling obscured a view of the larva feeding upon the captured insect. Two days later another glowworm in the cave hauled up a mayfly similar to the one already

mentioned, but it was not possible to know how long it had been caught before this because it was out of view. The glowworm took four minutes to haul up the mayfly and the captured insect did not appear to struggle.

In Glowworm Cave one larva was observed hauling up a tiny winged insect (~ 2 - 3 mm long) caught on a fishing line. The glowworm took two minutes to do this. Unfortunately it was not possible to know how long it had been caught for because the captured insect was out of camera range. The glowworm spent 15 minutes eating it and there did not appear to be any remains of its meal left in the snare afterwards.

On 26/6/95 in Waitomo Waterfall Cave a dead cave weta (Orthoptera) was observed hanging from the tattered remains of a snare by its hind legs, but the glowworm was missing from its gallery. The next day the weta had disappeared from the snare but the larva had not returned.

An *A. luminosa* adult which was glowing on the wing was observed caught in larval fishing lines in Waitomo Waterfall Cave. It was not possible to identify the sex of the adult. It flew upwards towards the cave ceiling, while still attached to the end of the fishing line. On two occasions it managed to break free from the fishing lines and got caught again before it disappeared from view.

In the Grotto of Glowworm Cave on 4/7/95 one glowing adult was observed on the wing flying sluggishly upwards towards the ceiling and the many glowing larvae inhabiting it. Its light went out before it reached the larvae so it is not known what happened to it.

In bush at night spiders were commonly observed moving over the substrate around glowworm snares. On seven occasions they moved over or through the fishing lines. The spiders appeared to do this accidentally and were too large and powerful to become caught in the fishing lines. No spiders were observed in Glowworm Cave, or in Reserve Cave, although they were caught in adhesive traps there (Chapter 2 & 3), but cave weta (Orthoptera) and the harvestmen *M. tumida* were occasionally observed moving through fishing lines in Reserve Cave.

In bush at night a spider with a body length of about 10 mm moved into a narrow crevice behind some fishing lines. Several seconds later the spider retraced its path out of the crevice and was attacked by the glowworm (Fig. 5.19). The glowworm appeared to be holding on to one of the spiders legs, and would not let go, but the spider was strong enough to pull free. The glowworm was elongated to about one and a half times its normal length while it was holding on to the spider for 68 seconds. However, when the spider pulled free the glowworm rebounded back into its snare, and seemingly unaffected, began repairing damage to it.

On one occasion, when a spider moved into some fishing lines the glowworm immediately moved part-way out of its gallery and began to haul up one of the fishing lines that the spider had touched, but the spider moved away. When a spider did this on another occasion the larva glowed brightly, but did not move, and the spider proceeded to clean itself to remove a sticky fishing line, then left. The next day a large mygalomorph spider moved over a glowworm snare, then moved off out of view. It did not destroy the snare. The larva glowed brightly for about six minutes afterwards. Twenty minutes after this first encounter what appeared to be the same spider again touched the side of the snare. This time the larva, brightly glowing, turned around within its gallery to face the spider, then moved half-way out of its gallery. Unfortunately the anterior half of the larva and the spider were out of view. The next evening a spider moved over a snare and proceeded to clean itself to remove fishing lines. After about half an hour it moved away, clumsily blundering through the same snare again. The glowworm rapidly turned around within its gallery to face the disturbance each time the spider touched its snare. When a small spider moved into a snare the following night, the glowworm lit up brightly, turned around within its gallery and immediately went to the fishing line that the spider had touched. The larva then moved part-way out of its gallery, apparently to check for captured prey, but the spider had moved off. The glowworm then began repairing parts of the snare that the spider had damaged.

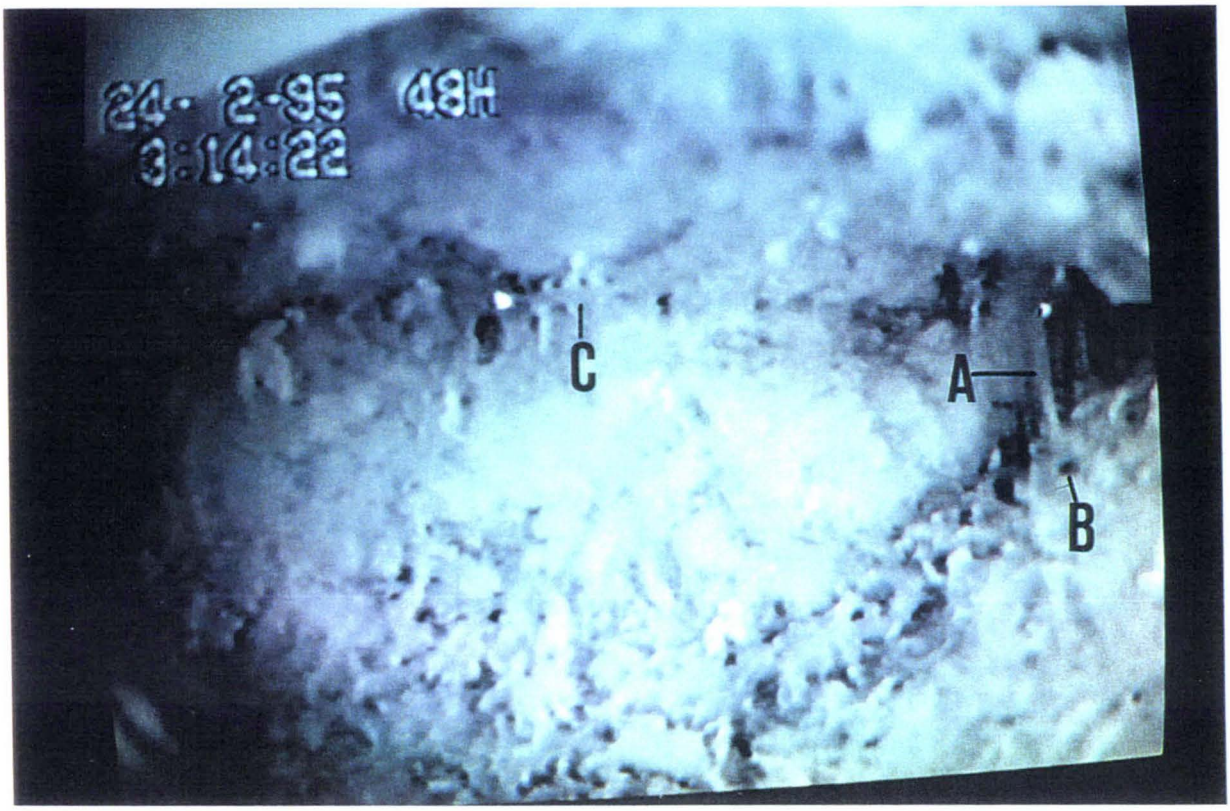


Figure 5.18 *A glowworm (A) in bush at night reaches down to bite a small insect (B) 10 seconds after it had flown into the sticky fishing lines. The larva then pulled its prey up into the snare before spending an hour and a half consuming it. Another brightly glowing* A. luminosa *larva (C) rests in its snare nearby.*

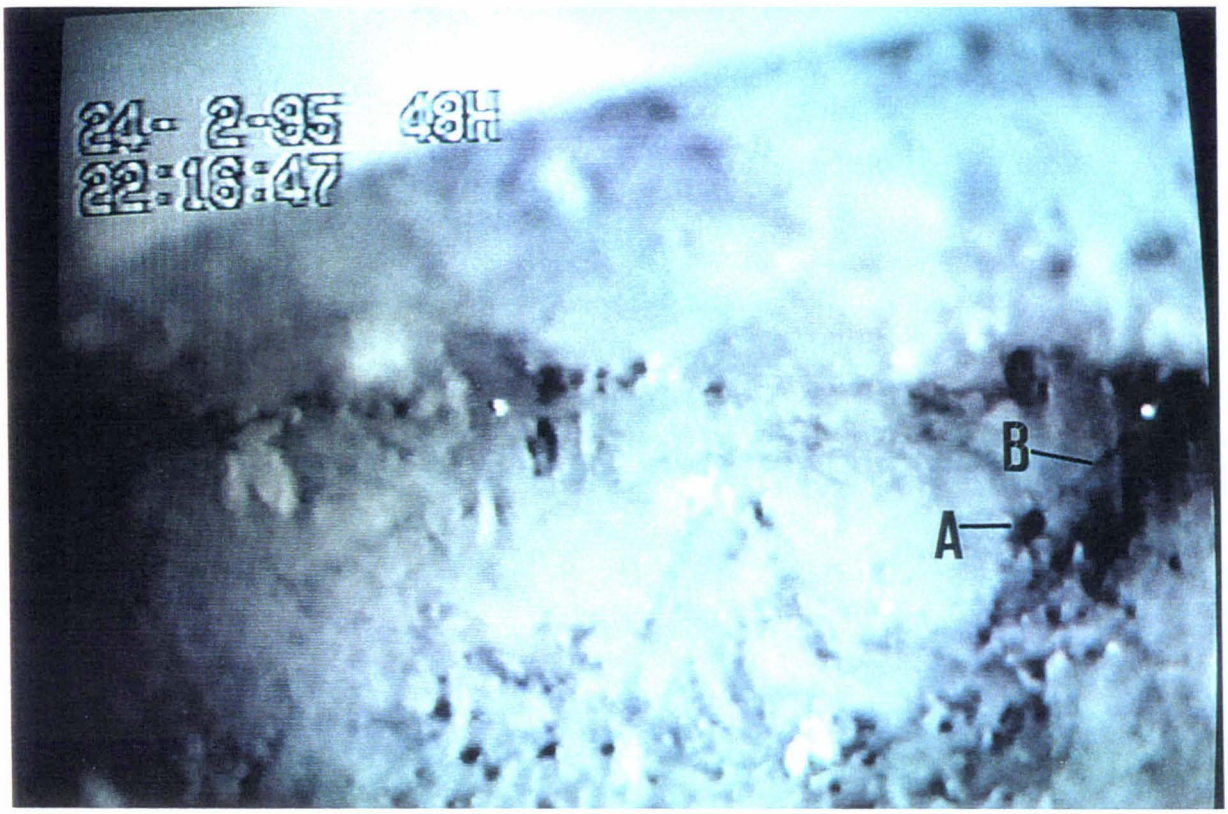


Figure 5.19 *A spider (A) in bush at night that had wandered into a crevice behind a curtain of fishing lines was attacked by the glowworm (B) as it moved back out. However, the spider was strong enough to break free, and subsequently moved quickly away.*

OBSERVATIONS OF LARVAE FIGHTING

Glowworms recorded during this study were chosen because they were usually close enough together to be recorded simultaneously. Fighting between larvae, however, was uncommon, despite the many hours of glowworm behaviour that was recorded at each location (Table 5.2). Individual fights between glowworms varied from 1 second to 1 hour and 25 minutes (Table 5.2).

Prior to fighting a larva usually moved part-way out of its gallery to search the substrate for a site to attach a suspensory thread (see "The Snare"), and appeared to accidentally touch the snare of a neighbouring glowworm. The neighbour would glow brilliantly and move part-way out of its gallery to snap at the intruder with its jaws and fighting between the two would ensue. On one occasion two larvae were observed pulling a silk strand strung between their jaws as if competing in a tug-o-war, each trying to pull the other from its snare. Larvae would fight for variable lengths of time (Table 5.2). Cannibalism was not observed, but on one occasion a larva was injured by its attacker. This was recorded in Glowworm Cave when a larva that had left its snare out of view moved into a snare being observed on camera (Fig. 5.20). However, instead of snapping at each others jaws, which usually occurs, the intruder in this case bit the larva on its body behind its head. The larva quickly recoiled upon being bitten, but appeared to survive the attack. The intruder moved out of view of the camera in the direction it had come from.

Table 5.2 *Means, ranges and standard errors (SE) of the lengths of time (h:mm:ss) that pairs of glowworms recorded at each location were observed fighting for.*

Location	*N*	Mean	Range	SE
Bush	7	0:38:00	0:00:01 - 1:25:10	0:12:00
Glowworm Cave	12	0:17:00	0:03:35 - 0:44:21	0:04:00
Reserve Cave	5	0:06:06	0:00:01 - 0:20:03	0:03:38

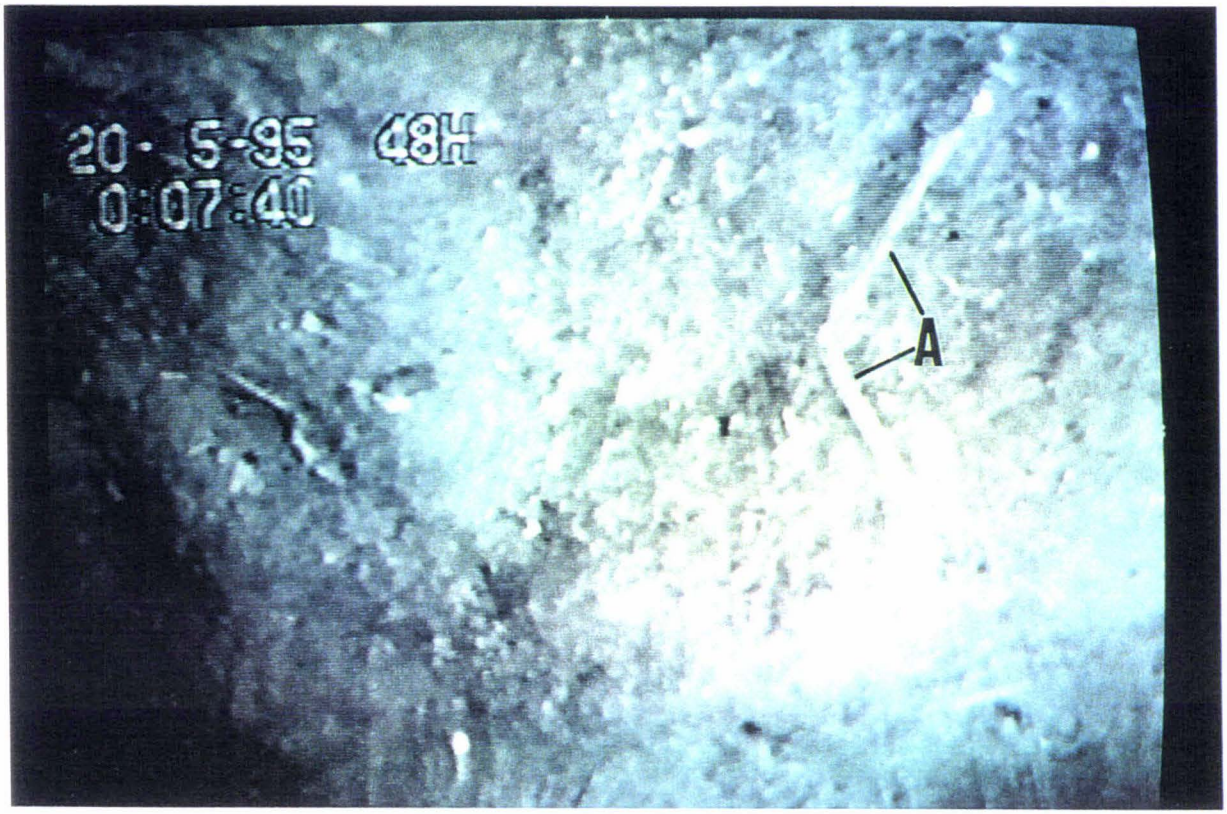


Figure 5.20 *A pair of* A. luminosa *larvae (A) fighting in Demonstration Chamber of Glowworm Cave. Note the intensity of their lights. The larva at top had moved out of its own snare, and into the neighbour's. The two glowworms began to fight, but the glowworm at top bit the body of the other glowworm with its jaws. The lower larva immediately recoiled from this attack. The larva at top then moved back in the direction it had come from. The lower larva appeared to survive the attack, although it did not move and glowed only faintly for many hours afterwards.*

SANITATION OF THE SNARE

Observations of *A. luminosa* larvae defecating were made on five occasions in bush, four occasions in Glowworm Cave and once in Reserve Cave. When defecating, each glowworm moves head-first part-way out of its gallery, then turns around in its gallery to hang tail-first half-way out of it. It then begins muscular contractions and voids an excretory droplet before moving back up into its gallery. In bush, larvae either void excretory droplets out of the snare or hang them on fishing lines. In the latter case the larvae then lengthen the fishing lines until the droplets make contact with the substrate. However, in caves the glowworms cut and drop entire fishing lines with droplets on them, or else they leave the droplet hanging within the snare. This behaviour took variable lengths of time to complete (Table 5.3). Observations made are described below.

The first observation in bush was made at 21:00 on 23/2/95. A glowworm moved half-way out of its gallery as if it was going to make a fishing line, but instead it moved back up into its gallery, so only about a third of its body remained hanging. Then it turned around and lowered the distal half of its body out of its gallery. The larva began to make muscular contractions and a dark mass of faecal material was clearly visible in the posterior quarter of its body through the almost transparent cuticle. However, the larva did not void this material, but instead moved back up into the gallery again, turned around, and moved head first down to the same point. The larva appeared to be checking the fishing lines. Two minutes later the glowworm moved further along its gallery, and repeated the same behavioural pattern, but on this occasion the faecal material moved through its body and was voided as a large excretory droplet. The glowworm spent about 50 seconds voiding this droplet. It fell onto the substrate below the nest, where half a dozen other droplets were also clearly visible (Fig. 5.21). Before the larva moved back into its gallery it again turned around, and appeared to be checking the fishing lines. The second observed defecation occurred precisely 24 hours later when the same glowworm voided an excretory droplet after 90 seconds of muscular contractions, onto a fishing line ~ 25 mm below the gallery and the substrate below it. The larva then turned around and lowered the fishing line until the droplet made contact with the substrate. A third observation in bush was made after 112 seconds of muscular contractions, when a larva appeared to void an excretory droplet 20 mm below the gallery onto a fishing line. This time the glowworm left the excretory droplet hanging in the snare. Forty-eight hours later, to the minute, the same glowworm repeated this behaviour, onto another fishing line. With muscular contractions it took the glowworm 140 seconds to void the excretory droplet. This time the fishing line with the excretory droplet broke and fell to the substrate below. The final observation in bush was made

on 14/5/95 when a larva voided an excretory droplet which ran part-way down a fishing line. The glowworm then lengthened the fishing line until the droplet made contact with the substrate below the snare.

The first observed defecation in Glowworm Cave was made on 2/4/95 at 15:39. A larva voided an excretory droplet into one of its fishing lines, but left the droplet suspended in its snare. When a second larva there was observed to void an excretory droplet into a fishing line it turned around, then moved part-way out of the gallery and appeared to check the fishing line. This larva was furthest from the camera, and its fishing lines were not clearly visible so it is not known if the droplet was discarded from the snare, or remained on a fishing line. On 5/4/95 at 12:20 a third observation was made when a larva defecated onto a fishing line. Likewise, this droplet was left suspended where it was. The last observation in Glowworm Cave was made on 22/5/95 at 08:00, when a larva hauled up one of its fishing lines. It appeared to use the silk and mucus to construct a very short fishing line before it turned around and defecated. Unfortunately, this was precisely the moment the batteries were changed for the equipment, so it is not known what happened to the droplet. When recording began about 3 minutes later the droplet was not visible in the snare.

One observation of defecation in Reserve Cave was made, on 10/11/95 at about 21:25, when the larva defecated into a fishing line, which it cut and dropped from the snare.

Table 5.3 *Means, ranges and standard errors (SE) of the lengths of time (mm:ss) glowworms recorded at each location took to dispose of excretory droplets.*

Location	*N*	Mean	Range	SE
Bush	5	06:00	00:58 - 13:47	02:12
Glowworm Cave	4	03:00	01:13 - 05:56	01:00
Reserve Cave	1	-	03:29	-

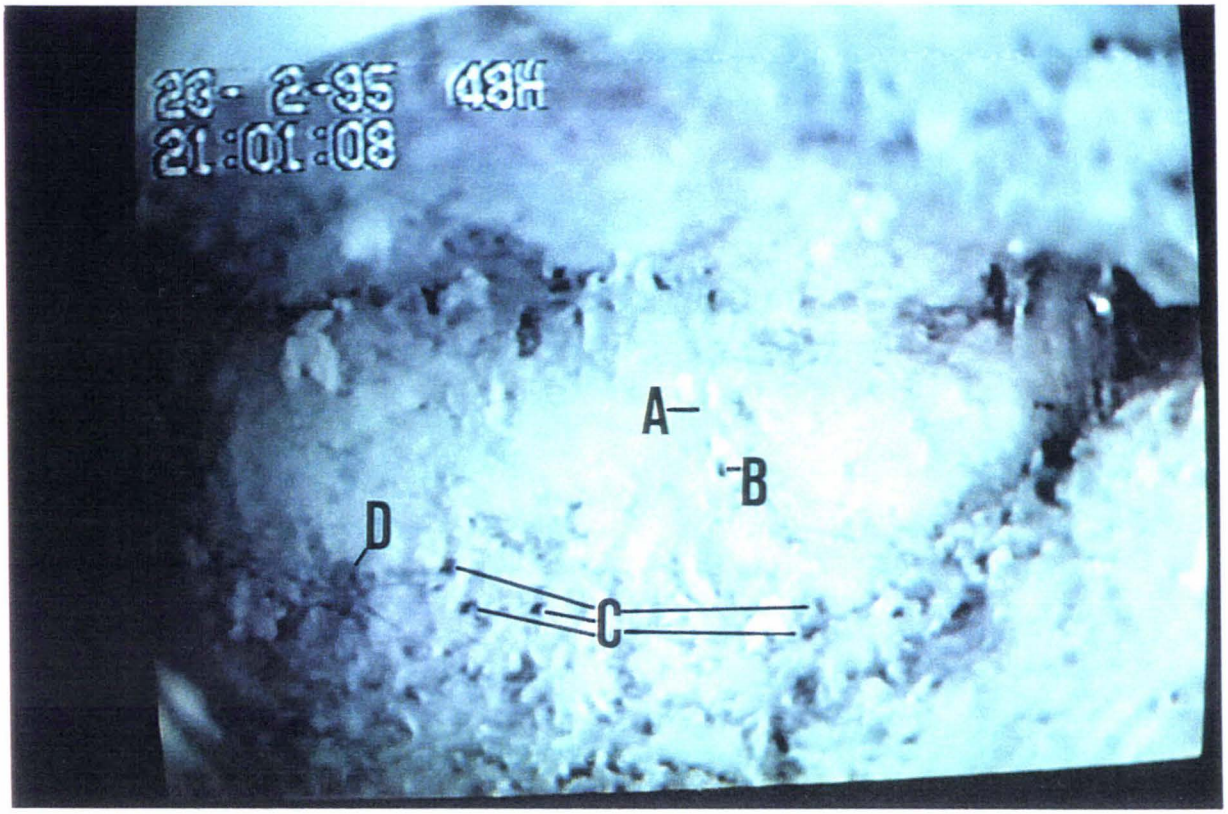


Figure 5.21 *A brightly glowing larva (A) whilst hanging backwards from its gallery at night in bush releases an excretory droplet (B) onto the substrate below. Other excretory droplets from previous occasions also litter the substrate and are clearly visible (C). Note the small spider (D) nearby.*

A. LUMINOSA PUPAE

Observations of three pupae were made during this study. The first was female. It was recorded in Reserve Cave for 163 hours before the adult emerged and copulated with a male fly (see "Mate Attraction and Copulation"). The female took 13 hours and 23 minutes to eclose during which she glowed intermittently on 12 occasions. The male alighted upon the female 13 hours and 8 minutes after eclosion began and copulation commenced 13 hours later. Immediately after copulating both adults were caught and eaten by a large predatory harvestmen *M. tumida* (see "Predation"). The second pupa was also a female. It was in the bush-clad entrance to the cave. Most of its eclosion was observed after 168 hours of recording. The third pupa observed was male and eclosed after 144 hours of recording. The adult emerged and flew off. However, observations made of the latter two pupae were incomplete. Observations made of the first female pupa are described overleaf. The latter two pupae are described here.

Video-taping of a ~ 14 mm long female pupa began in the bush-clad entrance to Reserve Cave at 16:40 on 29/6/95. The distal tip of the pupa began to twitch at about 18:20 on 5/7/95 and the pupa glowed faintly for about 30 seconds starting at 01:31 on 6/7/95. It glowed faintly again at 01:55 for one minute, and again from 03:23 until 03:25. At 12:24 the pupa began to turn clockwise on its suspensory thread so that by 13:21 it was horizontal. The wings emerged at 13:34 and the legs emerged one minute later. At this stage the emerging adult was vertical again, but its head faced downwards. From 15:10 the distal tip of the exuviae still with the abdomen in began to bend towards the head of the adult, until 15:38 when it was adjacent to the head. The emerging adult glowed once from 15:13 to 15:18, but it was so faint as to be almost unnoticeable. Unfortunately, there was power loss to the equipment from 15:39 onwards, so the remainder of its eclosion was not video-taped.

On 21/6/95 video-taping of an ~ 11 mm long male pupa began in Reserve Cave. It appeared inactive until 09:35 on 26/6/95, when the distal tip of the pupa began to slowly twitch and glow faintly. This twitching was so slow that it is probably not detectable by the naked eye. At 11:30 the pupa began to turn clockwise on its suspensory thread from the vertical. The wings emerged eleven minutes later when the adult was horizontal and at 11:50 it was upside-down. Legs began to emerge from the pupal exuviae at 12:40, and at 13:00 the abdomen appeared to glow faintly along its entire length. By 14:00 both the head and the distal tip of the abdomen were almost touching, so that the adult formed an inverted U shape. The light organ was also glowing at this time. Unfortunately, there was power loss to the equipment between 16:30 and 14:40 on the 27/6/95. When video-taping recommenced again the adult was clinging to the empty pupal exuviae, and remained there until 06:19 on 28/6/95 when it flew away.

MATE ATTRACTION AND COPULATION

Observations of an emerging female that subsequently copulated with a male fly are described here. Video-taping of a female pupa in Reserve Cave commenced on 23/3/95. The distal tip of the pupa began to twitch and slowly move from at least 07:00 on 29/3/95, but it was not glowing. By 13:00 its abdomen began to twitch more noticeably. The pupa started its turn from the vertical position at 14:27 and was horizontal by 14:41. Ten minutes later the head and thorax of the pupa were vertical, but its abdomen was bent over at about a 45° angle. The adult wings emerged from the pupal exuviae at 14:55, and it glowed briefly for the first time for three minutes at 15:13. By 15:50 the legs were clearly visible, and 15 minutes later the distal part of the abdomen began to move down towards the head so that the emerging adult formed an inverted U shape. The adult remained in this position until 03:50 on 30/3/95, when it emerged fully from its pupal exuviae. Between 17:14 and 02:42 on 30/3/95 the emerging adult glowed intermittently on eleven occasions from a few seconds to four minutes. On one of these occasions it glowed because a harvestmen (*M. tumida*) brushed against it with one of its legs.

Fifty-three minutes after the emerging female last glowed (03:35) a male *A. luminosa* adult flew up to and alighted upon the female. Five minutes later one or both of the pair began to glow, but because they were close together it was not possible to identify which. At 03:50 the female emerged fully from her pupal exuviae and copulation subsequently occurred as described below. From then on both glowed intermittently, although the female usually glowed for longer and brighter than the male.

Copulation between the pair appeared to start at 16:30 on 30/3/95, when there were periods of intense glowing, which occurred with greater frequency as time passed. The couple ceased copulation at 05:15 on 31/3/95, but remained together. The male then stopped glowing, but the female glowed intermittently on 14 occasions for 10 seconds duration each time. At 05:48 the pair rapidly separated. The female alighted upon the cave wall and glowed for 10 seconds, while the male dropped down a short distance and remained clinging to the empty pupal exuviae.

PREDATION

133 seconds after the pair separated from each other a fast-moving predatory harvestmen *M. tumida* snatched the female and then ten seconds later the pupal exuviae with the male still clinging to it in its chelicerae and moved along the cave wall. However, when the harvestmen moved off with its prey the pupal exuviae on its suspensory cord would not pull free and it dropped back down where it had been hanging. At the same time one of the predator's legs appeared to brush against the suspensory cord of the pupal case. The pupal exuviae rocked backwards and forwards in a pendulum type motion, which caused the harvestmen to snatch the exuviae again in its chelicerae, but it discarded this in favour of the two adults in its chelicerae, which it proceeded to eat. Forty minutes later the harvestmen finished eating the adults because its chelicerae were no longer moving. Using its chelicerae it then appeared to pull at the empty pupal exuviae from its attachment point on the cave wall. However, the pupal exuviae was camouflaged with the cave wall, so it is not known if it was successful in pulling it free.

Discussion

I have confirmed that *A. luminosa* larvae were largely active at night in bush and that they only glow at night as was first reported by Hudson (1886). During the day they were generally inactive, except when they occasionally turned around in their galleries. According to Stringer (1967) they do this when alarmed, but no apparent causes could be determined for this from my remote recording results.

Glowworms in bush usually became active late in the afternoon when they began activities such as making fishing lines, repairing snares and voiding defecatory droplets. This was similarly reported by Stringer (1967). They started to glow up to an hour and a half after becoming active. The bioluminescence was turned on relatively quickly so that it took less than 15 seconds to about 1 minute for a bright light to be visible. This conflicts with a report by Gatenby (1959) who stated that "in the evening the light comes on slowly." At dawn, however, glowworms in bush took several minutes to fade out their lights, and this agrees with Gatenby's (1959) observations. In February, glowworms in bush usually glowed about the same brightness all night. But on three nights in May they either glowed faintly for periods or stopped glowing altogether. Other workers have also observed larvae that have stopped glowing for a period (Gatenby, 1959; Stringer, 1967) and according to Edwards (1924) larvae "cease to shine on cold nights." Certainly two larvae that began glowing at dusk ceased glowing after four and a half hours when the temperature dropped below about 6° C. The temperature readings were, however, taken in bush outside the entrance to Glowworm Cave about 2.5 kilometres away from where the bush glowworms were recorded. It is, however, unlikely that the temperatures at the two sites differed much.

Larvae in the Demonstration Chamber of Glowworm Cave appear to be disturbed by human activity. Certainly larvae do not glow brightly when artificial lights are switched on for more than 30% of the time. They also spend little time (< 5%; Fig. 5.16) making fishing lines. Perhaps more disturbing to the larvae were the wind currents associated with the periods when the chamber lights were switched on. These appear to be generated by the movements or breathing of humans in the chamber area. The wind was sufficient to tangle the fishing lines of the snares, and this disturbance was probably why larvae spent the shortest periods of time making fishing lines in comparison to larvae at the other two locations (Table 5.1). Larvae in Glowworm Cave were also observed occasionally moving whole fishing lines short distances (~ 5 mm) around their snares, perhaps to prevent them tangling further. However, there is no way to be certain if this disturbance is detrimental to their overall well-being. The lighting system currently being used in Demonstration Chamber was installed in 1992 (K. Banbury, personal communication) and many people pass

through the cave each year. Yet despite this disturbance the glowworm population there appears to be a healthy one.

Cave-dwelling glowworms were reported to glow continuously (Gatenby, 1959; Richards, 1960) but the single larva observed in Reserve Cave glowed on average only between 13:00 and 02:00, and only on four out of the eleven days it was observed. It also did not appear to glow brightly compared to the glowworms at the other two locations. This indicates perhaps that glowworm bioluminescence in Reserve Cave is not as essential for the capture of prey as it is for larvae in Glowworm Cave and in the bush. However, it is not possible to say whether this behaviour is typical of other glowworms living in the cave because this was the only glowworm observed. A more comprehensive study of larvae in this cave is required before their behavioural patterns can be properly determined.

Only one observation of prey capture was made in the 934 "larva-hours" that were video-taped during this study. This occurred in bush and the prey appeared to be a small winged dipteran. Certainly Diptera were found to form the majority of invertebrates attracted to glowworm bioluminescence in both caves and bush during adhesive trapping experiments (Chapters 2 & 3). Three other partial observations were made of insects being hauled-up by larvae but it was not possible to know how long they had been stuck to the fishing lines. That only four observations of prey being hauled-up were made provides further evidence that glowworms may have to survive for long periods without food. They appear very capable of doing this since during adhesive trapping experiments glowworms under adhesive traps in Reserve Cave survived with little or no food for 78 days (Chapter 2).

Spiders appeared to blunder into glowworm snares at night in bush, but they were never captured and eaten. On the one occasion when a spider was observed to be attacked by a glowworm, the spider escaped, apparently unharmed. Both spiders and glowworms seem to co-exist together, at least in bush. Indeed, there are no other reports which suggest that spiders are part of the glowworms' diet, or that spiders prey upon *A. luminosa*. In the bush-clad entrance to Reserve Cave, most of the spiders caught on transparent adhesive traps occupied by glowworms were Symphytognathidae, which were probably too small to prey upon glowworm larvae (Chapter 2). Pugsley (1984) also found no evidence that spiders prey upon glowworms in Glowworm Cave. Spiders, however, often spin their webs over areas where glowworms occur (personal observation; Meyrick, 1886; Gatenby, 1959; Stringer, 1967; Morley, 1993). Since glowworms and spiders appear to compete for the same types of food this may indicate a possible exploitation by spiders of prey attracted to glowworm bioluminescence, although they could also be using the same physical areas. It is

interesting to note that Mansbridge (1933) found small spiders on two occasions in the snares of larvae of *Macrocera stigma* Curt. These small predaceous non-bioluminescent mycetophilids live under logs and boulders in the South and South-East of England. He reported that "they lived together in the same web in the laboratory, and the spider was easily able to move about the *Macrocera* web without getting entangled." However, he did not notice any special relationship between them.

In Reserve Cave weta (Orthoptera) and harvestmen (*M. tumida*) were sometimes observed moving past the larval snare, and on occasion they accidentally moved through the fishing lines, breaking and tangling them in the process. This must be a nuisance to the glowworms, which repair this damage to their snares, and to the weta and harvestmen, which are in danger of becoming helplessly entangled in the sticky fishing lines. Evidence that this may occur is based upon my observation in Waitomo Waterfall Cave of a large dead weta in a glowworm snare, although there was no sign of the larva. It is likely that the struggling weta pulled the larva from its snare, or that the larva quickly moved away to a new site to build a new snare as sometimes happens according to Richards (1956).

Glowworms recorded during my study were chosen because they were close enough together to be recorded simultaneously. Fighting between them, however, was relatively uncommon despite the many hours of glowworm behaviour that were recorded at each location (Table 5.2). Fighting mostly occurred when a larva moved part-way out of its gallery to search the substrate for new points of attachment for its snare and fishing lines, and then accidentally touched the snare of its neighbour. This indicates that larvae may fight to increase the size of their territory or to protect their own territory. Fighting larvae glow brilliantly and snap at each others heads with their jaws, and occasionally try to pull each other out of their snares. These fighting episodes usually concluded when one of the larvae retreated, but often the fights would resume again some time later. On one occasion in Glowworm Cave, I observed a larva that was bitten on its body by an intruding larva which had moved completely out of its snare. This was the nearest thing to larval cannibalism that I observed. On the basis of these observations I must agree with Pugsley's (1984) suggestion that "larvae maintain uniform spacing by aggressive territorial defence, extending occasionally to cannibalism."

According to Stringer (1967) glowworm "larvae are extremely sensitive and are easily disturbed during defecation and detailed observation of it has so far been impossible." The remote recording equipment used during this study has enabled ten observations of defecation to be made. Glowworms appeared to employ four different methods of disposing of faecal material. In bush, where snares were close to the substrate beneath them, larvae either voided excretory droplets out of the snare or hung them on fishing lines. In the

latter case the larvae then lengthened the fishing lines until the droplets made contact with the substrate. However, in caves the glowworms cut and dropped entire fishing lines with droplets on them, or else they simply left the droplet hanging within the snare as reported by Stringer (1967).

In the total darkness of Reserve Cave I recorded a male *A. luminosa* adult alight upon an emerging female which had not glowed for about 53 minutes. This observation conflicts with suggestions that the light of the female is used as a mate attraction device (Hudson, 1950; Gatenby, 1959; Richards, 1960; Meyer-Rochow & Eguchi, 1984; Meyer-Rochow & Waldvogel, 1979). In any case if the emerging female did glow then this would be largely blocked by the recording equipment so any adult males on the wing would probably not see her. An alternative hypothesis to the use of light as a mate attraction device was first proposed by Richards (1960) who suggested that olfactory organs may play an important role in mate attraction. Evidence for this was demonstrated by an examination of the antennae of male flies, which possess more and structurally different hairs from those of females (I. Stringer, personal communication). Use by female flies of an olfactory mate attraction mechanism would be more logical because if light was used the male flies would surely be in danger of becoming entangled in the fishing lines of the predaceous larvae. Indeed, a glowing *A. luminosa* adult was observed caught on a larval fishing line in Waitomo Waterfall Cave. It was not possible to identify the sex of the adult, but it was strong enough to fly up to the cave ceiling whilst stuck to the fishing line. It is not known if it was eaten by a larva or managed to evade capture because it disappeared from view. Adults are probably not attracted to larval bioluminescence because during a total of 221 days of trapping in both bush and cave habitats no *A. luminosa* adults were caught on transparent adhesive traps containing glowworms (Chapter 2 & 3).

It was interesting to observe the predation of both *A. luminosa* adults in Reserve Cave by the large harvestmen *M. tumida*. Richards (1960) first reported *M. tumida* as a predator of adult *A. luminosa* in Glowworm Cave, but it appears that my study is the first time that actual predation was recorded. The attack appears to have been triggered by the brief glowing movement of the female fly when she separated from her mate and alighted upon the cave wall. Indeed, *M. tumida* are reported to exhibit a positively phototactic response to a dim artificial "glowworm" light (Meyer-Rochow & Liddle, 1988). However, this may not have been the only cue used by the harvestmen to orientate towards its prey on the cave wall as the legs, and particularly the long second pair, possess a variety of tactile and chemo- receptors (Cloudsley-Thompson, 1968).

Appendix

Comparison between the time of day individual bush glowworms were observed to start glowing, the total time they spent glowing, and the time of day they stopped glowing; with sunset, sunrise, temperature range, rainfall and moon phase data. Each line represents observations of a single glowworm. All times are hh:mm. Sunset and sunrise data were recorded at Otorohanga which is approximately 12.5 km away. Sunset and sunrise, together with moon phase data were provided courtesy of the Carter Observatory in Wellington. Rainfall was recorded at THC Waitomo Caves Hotel at 9:00 each day and represents rainfall during the previous 24 hours. This data was provided courtesy of the National Institute of Water and Atmospheric Research Ltd.

Date (evening - next morning)	Sunset	Time larva started to glow	Total time spent glowing	Time larva stopped glowing	Sunrise	Temp. range (°C) from sunset to sunrise	Rainfall (mm)	Moon phase
23 - 24/2/95	20:11	20:50	9:52	6:42	6:55	18 - 16	5.4	Full moon
23 - 24/2/95	20:11	20:50	9:52	6:42	6:55	18 - 16	5.4	Full moon
24 - 25/2/95	20:09	20:31	10:10	6:41	6:56	16 - 11	nil	Last quarter
24 - 25/2/95	20:09	20:32	9:30	6:02	6:56	16 - 11	nil	Last quarter
09 - 10/5/95	17:26	17:34	12:28	6:02	7:06	12 - 6	0.2	First quarter
09 - 10/5/95	17:26	17:35	12:17	5:52	7:06	12 - 6	0.2	First quarter
10 - 11/5/95	17:25	17:46	4:14	22:00	7:07	9 - 2	nil	First quarter
10 - 11/5/95	17:25	18:06	2:23	20:30	7:07	9 - 2	nil	First quarter
11 - 12/5/95	17:24	17:34	10:16	5:50	7:08	10 - 6	nil	First quarter
11 - 12/5/95	17:24	17:50	11:39	6:18	7:08	10 - 6	nil	First quarter
12 - 13/5/95	17:23	17:30	13:35	7:05	7:09	11 - 9	1.3	First quarter
12 - 13/5/95	17:23	17:30	11:16	6:20	7:09	11 - 9	1.3	First quarter
13 - 14/5/95	17:22	17:22	13:43	7:05	7:10	15 - 7	1.1	First quarter
13 - 14/5/95	17:22	17:35	12:50	6:25	7:10	15 - 7	1.1	First quarter
14 - 15/5/95	17:21	17:26	13:14	6:40	7:11	14 - 9	nil	First quarter
14 - 15/5/95	17:21	17:39	13:01	6:40	7:11	14 - 9	nil	First quarter

Chapter 6 ARE *ARACHNOCAMPA LUMINOSA* IN BUSH GENETICALLY ISOLATED FROM THOSE FOUND IN CAVES? AN INVESTIGATION USING STARCH-GEL ALLOZYME ELECTROPHORESIS

Introduction

Larvae of the New Zealand Glowworm *Arachnocampa luminosa* (Skuse)(Diptera: Mycetophilidae) are found in caves, unused mining tunnels, and damp and sheltered places in bush, such as along stream banks and road cuttings. Richards (1960) reported that there are marked differences in size between the pupae and adults of *A. luminosa* from caves and bush. Significant differences in size between cave and bush dwelling larvae were also reported by Morley (1993). Such a size difference probably lead to the suggestion that more than one species of *Arachnocampa* exists in New Zealand (Gatenby, 1959; 1960a; 1960b), but Harrison (1961) appears to have discounted this. However, the ecotypic differentiation between cave and bush glowworms indicates that there is a possibility of separate cave and bush ecotypes.

Glowworms in caves generally have to contend with a lower prey density (Chapter 2 & 3), and higher mortality from fungal pathogens such as *Tolypocladium* sp (Samson, 1984), desiccation, and flooding (Pugsley, 1984). It would therefore be interesting to know whether caves are recolonised naturally by *A. luminosa* adults from populations located outside them. This question is of particular interest to managers of Glowworm Cave, Waitomo, where a major flood could reduce population numbers considerably (Meyer-Rochow, 1990). Indeed, Pugsley (1984) observed the Tunnel, Jetty and Demonstration Chambers occasionally filled to the roof with water. I too observed this several times during my study.

May (1971) suggested that bush glowworms are important in maintaining the population in Te Anau Cave, Fiordland, and that lights left on continuously outside the cave entrance prevented *A. luminosa* adults from migrating into the cave during the decline of glowworm numbers which occurred there between 1966 and 1970. However, there appears to be no evidence that such a migration of adults into the cave actually occurs.

A. luminosa adults of both sexes are very sluggish flyers, although male flies are the more active (Richards, 1960; personal observation). Male flies also live longer than females (4 days & 76 hours respectively;

Richards, 1960). The more active, longer-lived males are therefore more likely to act as agents of gene flow than females, but this is only possible if females are already present in a locality.

If adults from bush do migrate into caves where there are *A. luminosa* and vice-versa, there is no evidence that mating occurs. To this end Richards (1960) conducted an experiment where she put a female fly that had emerged from a pupa collected in bush at Waitomo in a container with two male flies from Waitomo Cave. Mating did not take place and the female died four hours later.

This chapter sets out a preliminary investigation into gene flow between glowworms in bush and in caves. The aim was to determine how genetically isolated cave *A. luminosa* are and whether there was evidence of regional geographic separation. This was accomplished by collecting *A. luminosa* larvae from many bush and cave sites in North Island, and carrying out starch-gel allozyme electrophoresis, which to our knowledge has not been done before.

Materials & Methods

Samples of 30 glowworm larvae were collected from the wall of the Grotto in Glowworm Cave, Waitomo; and another 30 also from the ceiling of the cave about 10 metres from the stream entrance (NZS 260 S16, 943 248)(Fig. 6.1). The same number of larvae were also collected from the bush alongside the walkway to Aranui Cave in Ruakuri Scenic Reserve (NZS 260 S16 922 244)(Fig. 6.2); the ceiling of Moa's Hook in Frazers Bluff Cave, Okupata (NZS 260 S19 282 373)(Fig. 6.3); and in bush from a road cutting of Access Road No. 2 leading to Frazers Bluff Cave about 5 km from State Highway 47; a cave passage about thirty metres in length which runs approximately south-south-west to the Te Ano Whiro Stream at Piripiri Road Caves, Pohangina (NZS 260 T23, 641 254)(Fig. 6.4); and in bush alongside a stream near Totara Reserve, Pohangina (NZS 260 T23, 533 175)(Fig. 6.5).

Each glowworm was collected with a sterile toothpick and carefully placed in an individual Prespak® P35 plastic container (Biolab Scientific, Palmerston North, New Zealand) together with a damp wad of cotton wool to provide a high relative humidity. Glowworms become torpid and die in low humidity (Pugsley, 1984). The glowworms were transported back to Massey University either immediately or the following day.

In the Lab each glowworm was placed into a plastic auto-analyser cup with 20 µl of distilled water and ground into a thick soup with a glass stirring rod. Samples were then transported on ice to a - 80° C freezer where they were stored until analysis.

Throughout our experiments we used starch-gel electrophoresis (see Appendix for a detailed account of methods and recipes). Levels of polymorphism and heterozygosity in the populations were determined as outlined in Hartl (1980). Average Standard Genetic Distance between each glowworm population was also determined as described by Nei (1975). Cluster analysis was done using average pair group clustering with the package M.V.S.P.

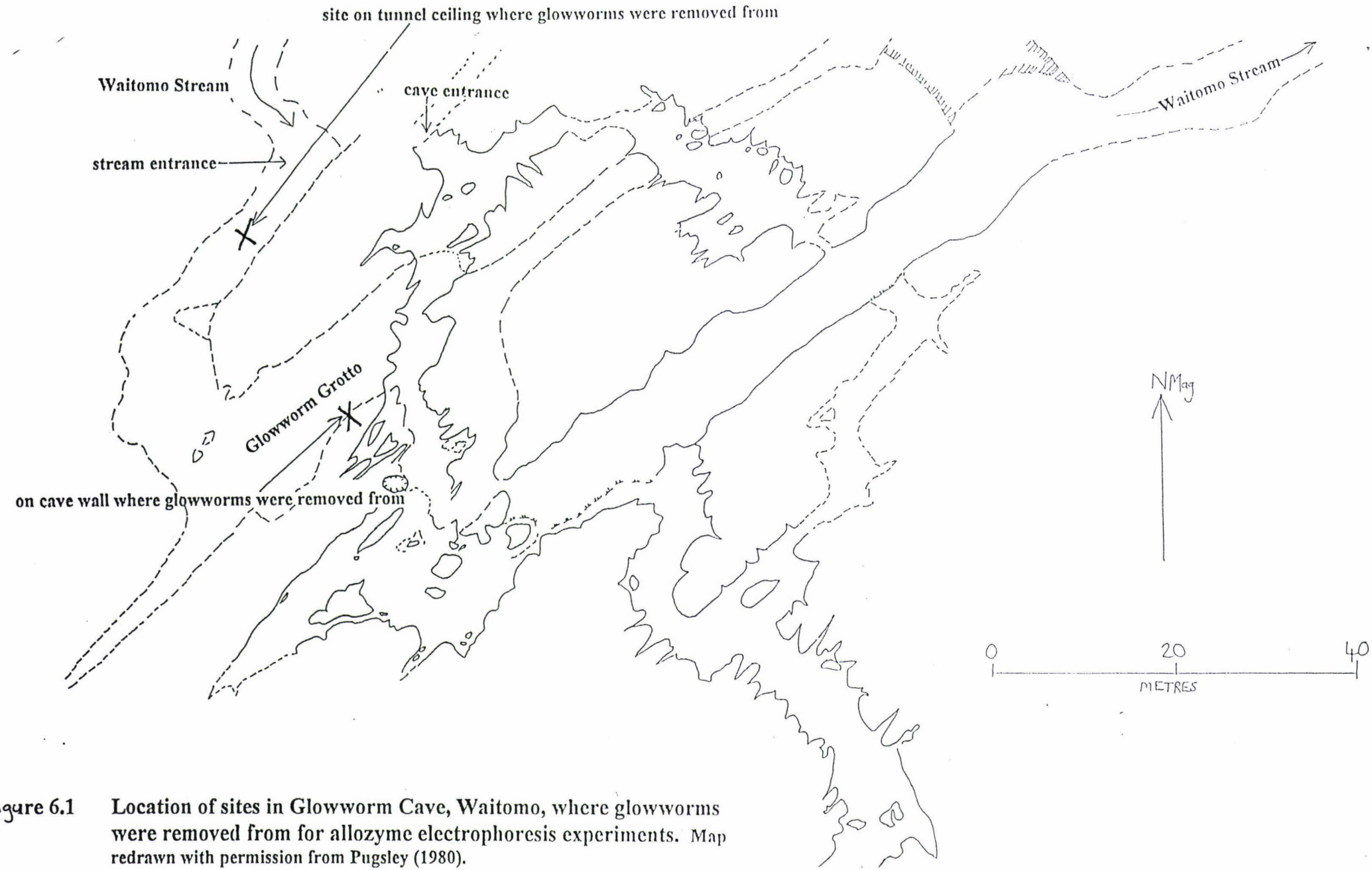


Figure 6.1 **Location of sites in Glowworm Cave, Waitomo, where glowworms were removed from for allozyme electrophoresis experiments.** Map redrawn with permission from Pugsley (1980).

Figure 6.2 Location of the site in Ruakuri Scenic Reserve, Waitomo, where glowworms were removed from for allozyme electrophoresis experiments. Adapted from Waitomo Visitor Information Map.

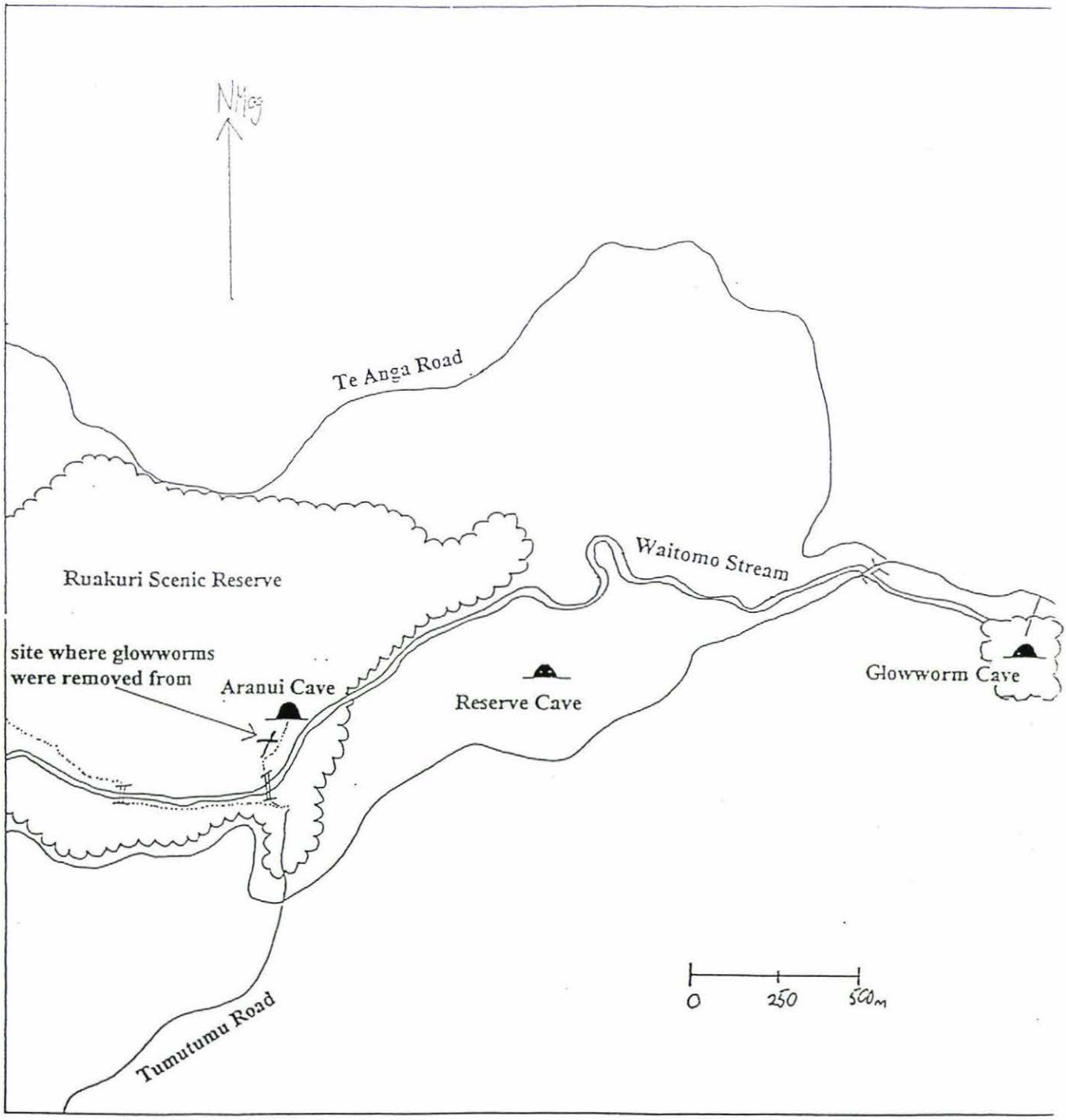


Figure 6.3 **Location of the site in Frazers Bluff Cave, Okupata, Tongariro, where glowworms were removed from for allozyme electrophoresis experiments.** Map redrawn with permission from New Zealand Speleological Society (NZSS).

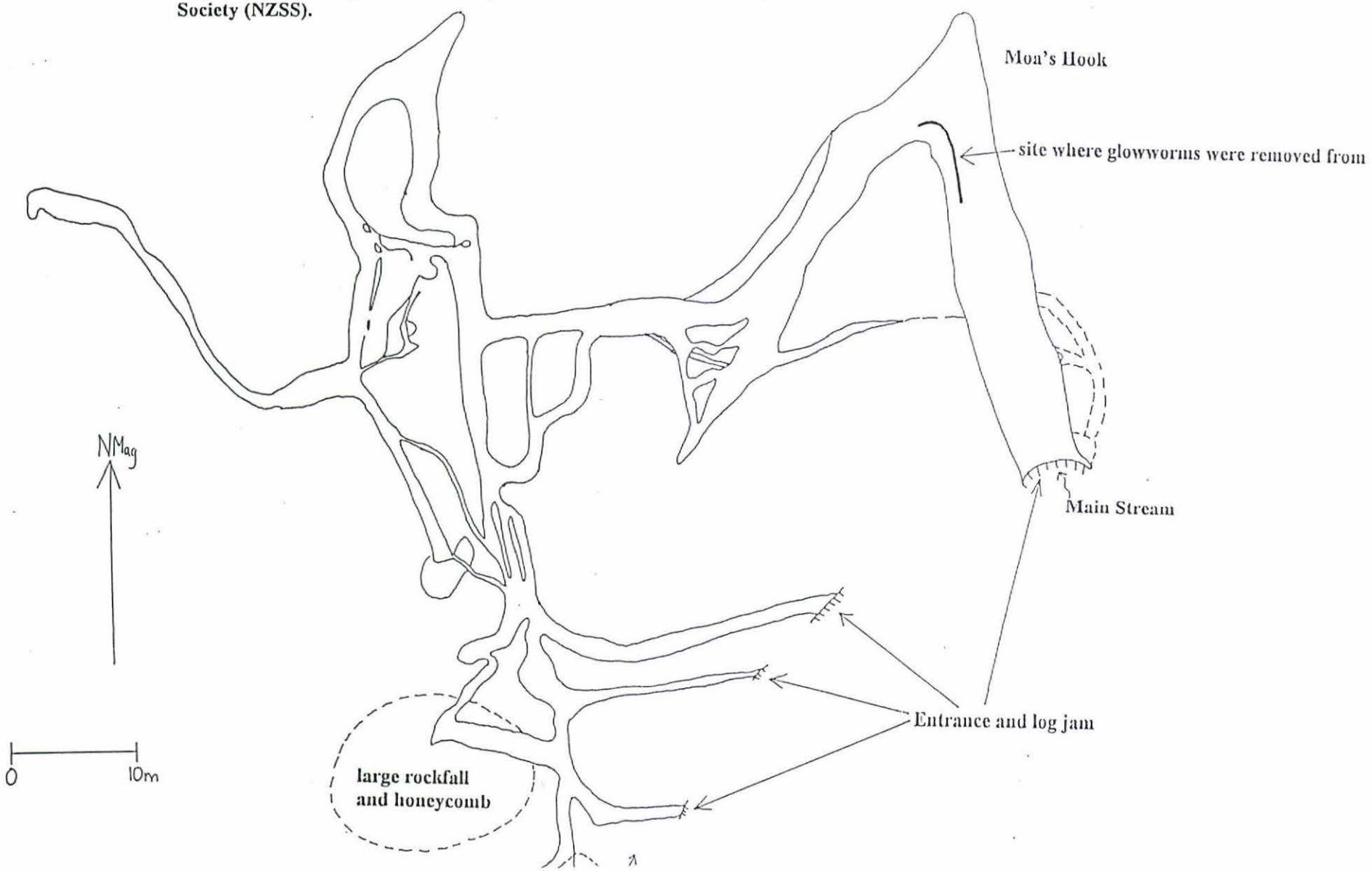
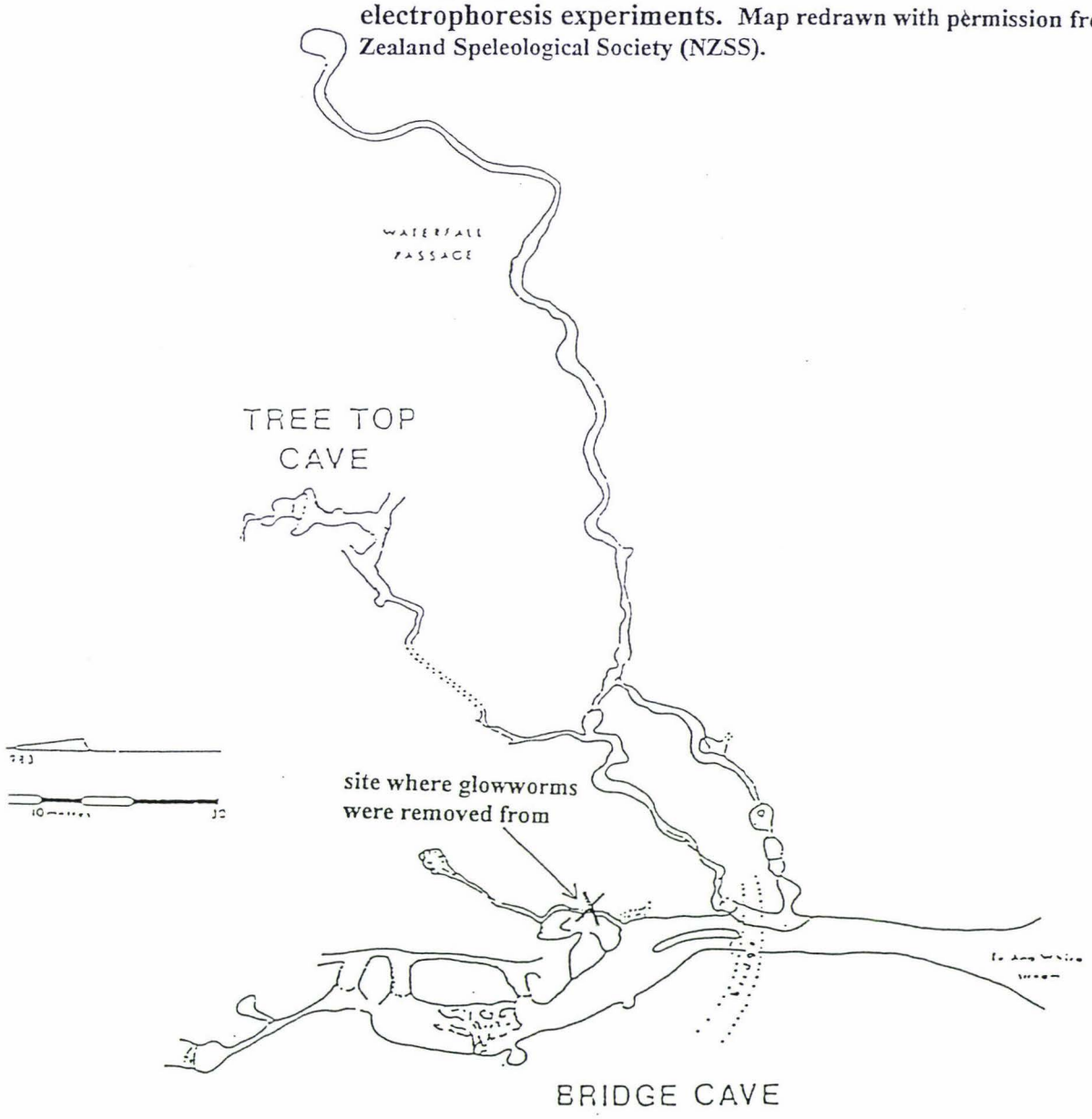
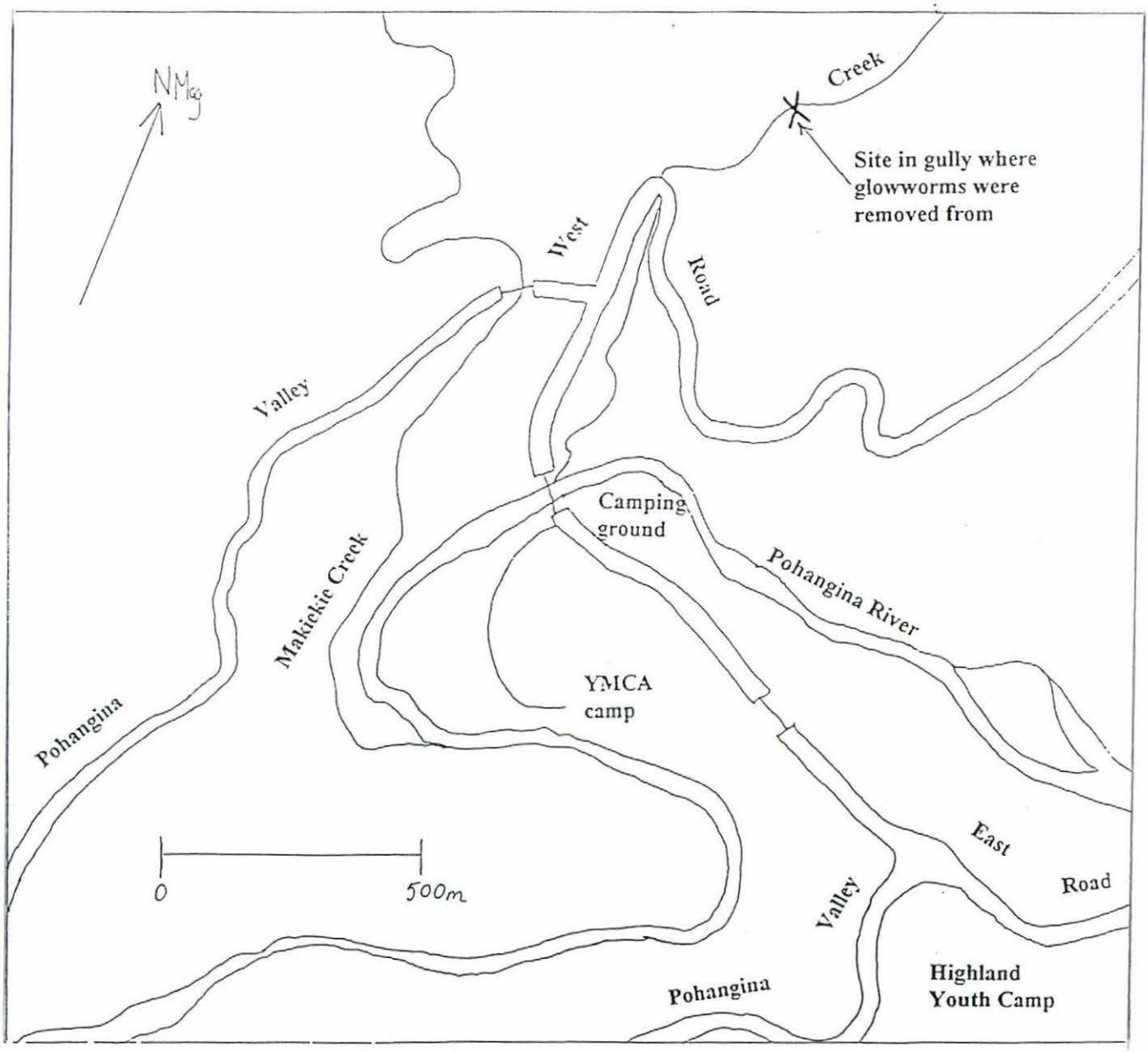


Figure 6.4 **Location of the site in a tunnel passage at Piripiri Road Caves, Pohangina, where glowworms were removed from for allozyme electrophoresis experiments.** Map redrawn with permission from New Zealand Speleological Society (NZSS).



Redrawn from Pearce (1988).

Figure 6.5 Location of the bush site near Totara Reserve, Pohangina, where glowworms were removed from for allozyme electrophoresis experiments.



Enzyme	Abbreviation
Glucose phosphate isomerase	PGI
Glucose-3-phosphate dehydrogenase	G_3PDH
Malate dehydrogenase/Malic enzyme	MDH/ME
Diaphorase	DIA
Glutamate-oxaloacetate transaminase	GOT
β-*N*-Acetylglucosaminidase	HEX
Lactate dehydrogenase	LDH
Phosphogluconate dehydrogenase	6PGD
Phosphoglucomutase	PGM
Glucose-6-phosphate dehydrogenase	G_6PDH

Table 6.1 *The 10 enzymes and their standard abbreviations, which glowworm samples were run successfully in.*

Samples were always run successfully in GOT enzyme, followed by PGI (88% of the total number run with PGI), 6PGD (85%), HEX (73%) and MDH/ME (64%), then to a lesser degree DIA (44%), G_3PDH (42%), LDH (38%), PGM (37%) and G_6PDH (32%). However, most bands were difficult to score because they were often not well resolved within the gel.

Results

The glowworm populations that were sampled yielded a large number of polymorphic loci. GOT had 4 alleles (Table 6.2); PGI had 7 alleles; 6PGD had 1 allele; HEX had 4 alleles; MDH/ME had 5 alleles; DIA had 6 alleles; G_3PDH had 2 alleles; LDH had 4 alleles; PGM had 4 alleles; and G_6PDH had 3 alleles (Table 6.2).

Most of the glowworm populations showed a high percentage of polymorphism with a mean of 66.3% overall (Table 6.3). Bush glowworms from Pohangina were most polymorphic (P = 85.7%; Table 6.3); followed by bush glowworms from Okupata (P = 80%); cave glowworms from Pohangina (P = 75%); cave entrance glowworms from Waitomo (P = 70%); cave glowworms from Waitomo (P = 60%); cave glowworms from Okupata (P = 56%); and bush glowworms from Waitomo (P = 37.5%)(Table 6.3).

There was a relatively wide range of heterozygosity between glowworm populations (~ 3% to ~ 18%), with a mean heterozygosity of 8.3% over all 7 populations (Table 6.3).

The average standard genetic distance (D) values within glowworm populations showed the greatest differences occurred between glowworms collected from bush at Okupata and Waitomo (D = 0.08694; Table 6.4), whilst those collected from the cave at Okupata and in the cave entrance at Waitomo had the least differences (D = 0.00273; Table 6.4).

Overall dissimilarities are illustrated by the cluster analysis shown in Figure 6.6. Glowworms from adjacent bush and cave habitats were not very similar whereas some populations of glowworms that were considerable distances apart are more similar (i.e. Waitomo, Okupata and Pohangina; Figure 6.6). However, bush glowworms at Okupata are most dissimilar to other glowworm populations, especially bush glowworms at Pohangina, cave glowworms at Pohangina, and bush glowworms at Waitomo.

Table 6.2 *Frequency of alleles derived from running glowworm extract from several bush and cave populations in starch-gel electrophoresis, for 10 enzymes. Data is shown only for the buffer systems that they were most successful in (in brackets).*

Location of glowworm population	Cave; Waitomo	Cave entrance; Waitomo	Bush; Waitomo	Cave; Okupata	Bush; Okupata	Cave; Pohangina	Bush; Pohangina
Enzyme;							
PGI (AC); Alleles and frequency	1b; 16c; 1ce	24c	1b; 15c	1bc; 12c	1ac; 16c	13c; 3d; 1cd	1ac; 20c; 3d
G_3PDH (TC); Alleles and frequency	3b	1ab; 9b	2b	4b	5b	3b	-
MDH/ME (AC); Alleles and frequency	1b; 7c; 4d	13c; 2cd; 6d	8c; 3cd	12c; 6d	17c; 1d	8c; 1cd	1ac; 14c; 1cd; 4d
DIA (RW); Alleles and frequency	14c; 1cd	1ab; 3b; 2bc; 10c	10c	1bc; 7c	1ab; 6c	1abc; 1b; 2bc; 6c	1b; 13c
GOT (TC); Alleles and frequency	2b; 1bc; 2c	3b; 2bc	-	1ab; 4b	1ab; 4b	-	-
HEX (TC); Alleles and frequency	8b	1ab; 7b	2b	5b	1a; 4b	2b; 6bc	1ab; 3b; 4bc
LDH (AC); Alleles and frequency	1a; 1b; 2c	8b; 3c	7b	4b	1a; 7b; 1c	1bc; 7b	4b; 2c
6PGD (AC); Alleles and frequency	4a	5a	-	5a	3a	-	-
PGM (RW); Alleles and frequency	4b	14b	5b; 1bc	5b; 1bc	1ab; 3b	8b	1a; 4b
G_6PDH (RW); Alleles and frequency	1a; 6b	4a; 9b	1b	-	3a; 1b	3a; 9b; 1ab	3b

Table 6.3 *Percentage of polymorphic loci (P) and average heterozygosity (H) for each glowworm population (based upon the data in Table 6.2).*

Location of glowworm population	Cave; Waitomo	Cave entrance; Waitomo	Bush; Waitomo	Cave; Okupata	Bush; Okupata	Cave; Pohangina	Bush; Pohangina	Mean
Percentage of loci found to be polymorphic (P)	60	70	37.5	56	80	75	85.7	66.3
Percentage of loci that were heterozygous in an average individual (H)	2.67	8.075	5.5	6.3	6.52	17.775	10.96	8.3

Table 6.4 *The average Standard Genetic Distance (D) between each glowworm population, based on allele frequencies from the 10 loci (enzymes) shown in Table 6.2. A high D value means two populations are more different than two populations with a lower D value. See Figure 6.6 for cluster analysis.*

	Cave; Waitomo	Cave entrance; Waitomo	Bush; Waitomo	Cave; Okupata	Bush; Okupata	Cave; Pohangina	Bush; Pohangina
Cave; Waitomo	0						
Cave entrance; Waitomo	0.01596	0					
Bush; Waitomo	0.03358	0.02769	0				
Cave; Okupata	0.00967	0.00273	0.02007	0			
Bush; Okupata	0.01987	0.02148	0.08694	0.02617	0		
Cave; Pohangina	0.01655	0.02163	0.00343	0.01255	0.08525	0	
Bush; Pohangina	0.02285	0.00343	0.03360	0.04537	0.08437	0.01867	0

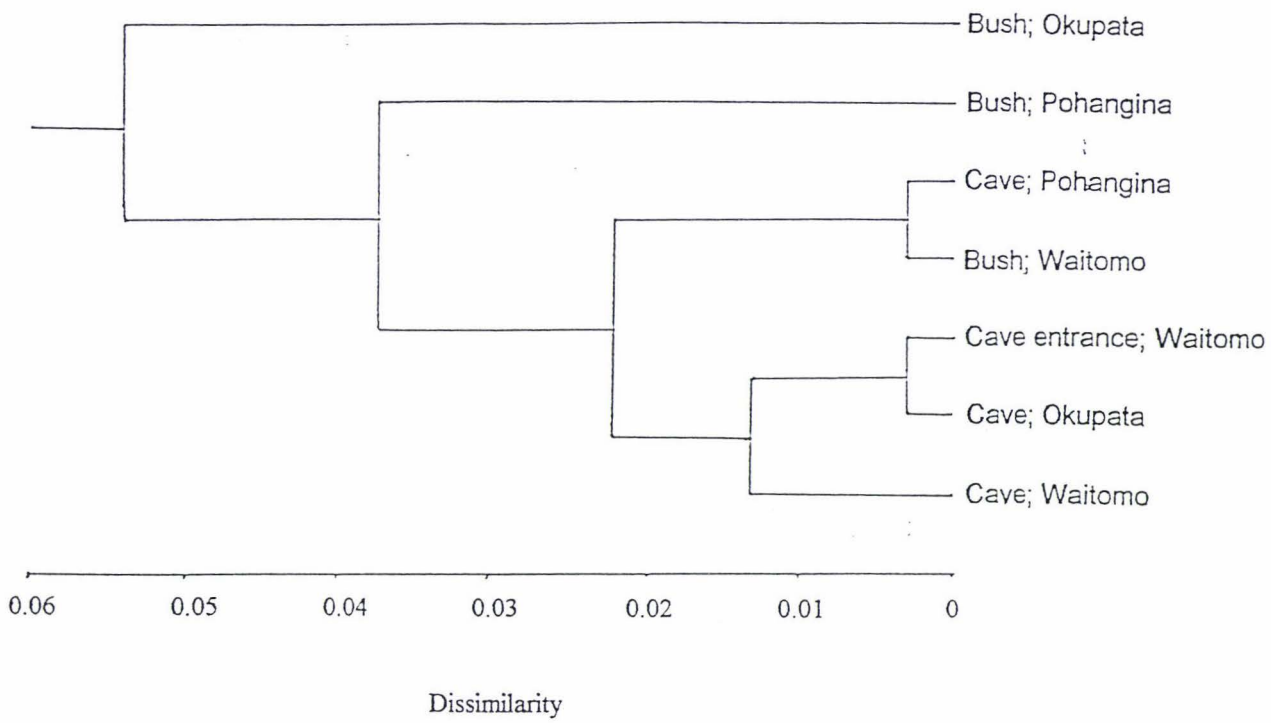


Figure 6.6 *Cluster analysis of Average Standard Genetic Distances (D) between seven bush and cave glowworm populations. This is based upon the data presented in Table 6.4.*

Discussion

It appears that there is a pattern of discrete glowworm populations but that there is no particular geographic or ecological structuring between them. There is no tendency for populations most adjacent geographically to be most similar genetically (Figure 6.6), nor for populations from similar environments in cave or bush to share genetic affinity. Indeed, some bush and cave populations, such as cave glowworms at Pohangina and bush glowworms at Waitomo, which are located hundreds of kilometres apart from one another, are more similar (Figure 6.6). Glowworm populations in bush also appear to be more dissimilar to each other than to those in caves (Table 6.4; Figure 6.6). However, the standard genetic distances between all of the populations are relatively small (range ~ 0.00273 D to 0.08694 D; Table 6.4). According to Nei (1976) the genetic distance obtained is generally 0.00 ~ 0.05 D between races, 0.02 ~ 0.20 D between subspecies, 0.1 ~ 2.0 D between species, and more than 1 D between genera. Three of the D values obtained during this study were greater than 0.05 and less than 0.20 (Table 6.4), and since bush glowworms at Okupata feature all three times here it appears that they could be a subspecies of *A. luminosa* (see Figure 6.6). Besides these differences, nine other D values obtained were within the region of overlap between race and subspecies (D = 0.02 ~ 0.05), and another nine differed at race level only (D = 0 ~ 0.02; Table 6.4). Unfortunately, the distinction between local races and subspecies is not altogether clear. Nei (1976) defines a race as a group of populations of a species that are geographically separated from other such groups and that differ in allele frequencies; if they differ enough in easily observable characteristics, they are often given Latin names and formerly recognised as subspecies. The degree of polymorphism and heterozygosity observed during this investigation suggests that gene flow occurs regularly between populations. Gene flow acts to increase polymorphism within populations and reduce the differences between them (Hartl, 1980; Slatkin, 1985).

Two of the three pairs of glowworm populations that differ at subspecies level are from bush habitats (Table 6.4), but no significant difference in larval size between geographically separated bush populations has yet been determined. Another confounding factor when trying to determine how cave and bush glowworms differ in easily observable characteristics is whether a glowworm found at a cave entrance is a 'bush' glowworm or a 'cave' glowworm. Certainly at Piripiri Road Caves, Pohangina, glowworms in cave passages, cave entrances and also in bush do not appear to form discrete populations and can not therefore be considered to be geographically separated from each other.

To conclude, the data does not support the notion that cave and bush forms should be regarded as distinct species or subspecies. Genetic differences between populations of glowworms found in caves and in bush are

generally restricted to race level only. Some geographically separated populations may differ genetically at subspecies level, but they can not be formerly recognised as subspecies because bush and cave glowworms do not differ enough in easily observable characteristics.

Appendix

In the Lab each glowworm was placed into an auto-analyser cup containing 20 µl of distilled water. Larvae were then ground up into a thick soup with the end of a glass stirring rod. Only live glowworms were used, and the cups were kept on ice. Samples were stored until analysis at - 80° C.

Three buffer systems were used. These were lithium-borate (Ridgeway RW), a discontinuous system; and morpholine-citrate (AC) and tris citrate (TC), which are continuous systems (see Appendix for recipes). 12% (w/v) starch-gels were prepared (StarchArt® Corporation, Smithville TX 78957, U.S.A.) using a microwave.

The power supply (Bio-Rad® Model 1000/500, Richmond, CA 94804, U.S.A.) for the AC buffer was set to 40 mA while the power supply for the other systems was set to 120 mA.

BUFFER SYSTEMS

Lithium borate gel buffer (pH 8.5*) (RW GB 8.5)

Citric Acid Monohydrate	2.10	g
Tris	7.27	g
Electrode Buffer	20.00	mls
Distilled Water	2,000.00	mls

* pH with Sodium hydroxide

Lithium borate electrode buffer (pH 8.1*) (RW EB 8.1)

Lithium hydroxide	5.04	g
orthoBoric Acid	37.10	g
Distilled Water	2,000.00	mls

* pH with Hydochloric acid

Morpholine citrate gel buffer (pH 6.0) (AC GB 6.0)

3-aminopropyl morpholine*	0.50	mls
Citric Acid Monohydrate	0.84	g
Distilled Water	2,000.00	mls

* used to pH gel buffer

Morpholine citrate electrode buffer (pH 6.1) (AC EB 6.1)

3-aminopropyl morpholine	22.00	mls
Citric Acid Monohydrate	16.80	g
Distilled Water	2,000.00	mls

Tris citrate gel buffer (pH 8.0) (TC2 GB 8.0)

Tris	5.54	g
Citric Acid Monohydrate	2.20	g
Distilled Water	2,000.00	mls

Tris citrate electrode buffer (pH 8.0*) (TC2 EB 8.0)

Tris	166.40	g
Citric Acid Monohydrate	60.00	g
Distilled Water	2,000.00	mls

* pH with Hydrochloric Acid (conc.)

ENZYMES

PGI (GPI) - Glucose phosphate isomerase

Fructose-6-phosphate-Na2	20.00	mg
G_6PDH	20.00	units
MTT	10.00	mg
NAD	10.00	mg
PMS	2.00	mg
Magnesium Chloride	1.00	ml
SB2 8.0	25.00	mls

G_3PDH - Glucose-3-phosphate dehydrogenase

Aldolase	50.00	units
Arsenic Acid	0.08	g
Fructose-1,6-diphosphate	0.10	g
MTT	10.00	mg
NAD	10.00	mg
PMS	2.00	mg
SB2 8.0	25.00	mls

MDH/ME - Malate dehydrogenase/Malic enzyme

MTT	10.00	mg
NAD	10.00	mg
PMS	2.00	mg
Magnesium Chloride	0.10	g
NADP	5.00	mg
SB2 8.0	50.00	mls
dl-malic acid	0.15	g

DIA - Diaphorase

2-6-dichlorophenol-indo-phenol	~ 2.00	mg
MTT	10.00	mg
NADH	10.00	mg
SB2 8.0	25.00	mg

GOT - Glutamate-oxaloacetate transaminase

Distilled Water	20.00	mls
Fast garnet GBC salt*	0.06	g
SB1 8.5	20.00	mls
a-ketoglutaric acid	0.05	g
l-Aspartic acid	0.10	g

* Add all but Fast garnet salt to stain tray. pH to 8.0 with weak Sodium hydroxide. Add salt 15-20 minutes after incubation begins.

HEX - ß-*N*-Acetylglucosaminidase

ATP	0.13	g
G_6PDH	40.00	units
Glucose	0.20	g
MTT	10.00	mg
Magnesium Chloride	0.10	g
NAD	10.00	mg
PMS	2.00	mg
SB2 8.0	25.00	mls

LDH - Lactate dehydrogenase

Lactic acid	0.15	g
MTT	10.00	mg
NAD	10.00	mg
PMS	2.00	mg
SB2 8.0	50.00	mls

6PGD - Phosphogluconate dehydrogenase (decarboxylating)

6-phosphogluconic acid	20.00	mg
MTT	10.00	mg
Magnesium Chloride	0.10	g
NADP	5.00	mg
PMS	2.00	mg
SB2 8.0	50.00	mls

PGM - Phosphoglucomutase

G_6PDH	20.00	units
Glucose-1-Phosphate G 1259	0.15	g
MTT	10.00	mg
Magnesium Chloride	0.10	g
NAD	10.00	mg
PMS	2.00	mg
SB2 8.0	25.00	mls

G_6PDH - Glucose-6-phosphate dehydrogenase

Glucose-6-phosphate	50.00	mg
MTT	10.00	mg
Magnesium Chloride	0.10	g
NADP	5.00	mg
PMS	2.00	mg
SB2 8.0	50.00	mls

STAIN BUFFERS

SB2 8.0

Distilled Water	1,000.00	mls
Hydrochloric Acid (conc.)	~ 2.60	mls
Tris	6.07	g

SB1 8.5

Distilled Water	1,000.00	mls
Hydrochloric Acid (conc.)	~ 1.10	mls
Tris	6.07	g

AMBER BOTTLE SOLUTIONS

Magnesium Chloride	100	mg/ml
MTT	10	mg/ml
NAD	10	mg/ml
NADP	5	mg/ml
PMS	5	mg/ml

LYCOPHILISED ENZYMES

Aldolase	50	units/ml
Malic Dehydrogenase	200	units/ml
Isocitric Dehydrogenase	60	units/ml
G_6PDH	40	units/ml

References

ARRELL, R. (1984): *Waitomo Caves - A Century of Tourism*. Waitomo Caves Society Inc. Waitomo Caves. New Zealand.

BURTON, J. & TAYLOR, K. (1983): The Electronic Eye. P. 156-163 *In: Nightwatch - The natural world from dusk to dawn*. Roxby Nightwatch Limited. London. 190pp.

BUSTON, H. W. (1933): Note on the chemical nature of the fluid from the webs of larvae of *Platyura* and *Ceroplatus*. *Transactions of the Royal Society of London 81*: 90-92.

CLOUDSLEY-THOMPSON, J. L. (1968): *Spiders, scorpions, centipedes and mites*. Oxford. Pergamon Press.

COLLESS, D. H. & MCALPINE, D. K. (1991): Diptera. P. 717-786 *In: The Insects of Australia: a textbook for students and research workers Vol. 2*. 2nd edition. Melbourne University Press.

EDWARDS, F. W. (1924): A note on the New Zealand glow-worm (Diptera: Mycetophilidae). *Annals and Magazine of Natural History 9* (14): 175-179.

EDWARDS, F. W. (1933): The New Zealand glow-worm. *Proceedings of the Linnean Society of London 146*: 3-10.

FORSTER, R. R. (1967): *The Spiders of New Zealand Part I*. Otago Museum Bulletin No. 1. Dunedin. 124pp.

FULTON, B. B. (1941): A luminous fly larva with spider traits (Diptera: Mycetophilidae). *Annals Entomological Society of America 34*: 289-302.

GANGULY, G. (1960): Notes on the histology and anatomy of the larva of *Bolitophila luminosa* of New Zealand. *Journal of the royal microscopical society 79*: 137-154.

GATENBY, J. B. (1959): Notes on the New Zealand glow-worm, *Bolitophila* (*Arachnocampa*) *luminosa*. *Transactions of the Royal Society of New Zealand 87*: 291-314.

GATENBY, J. B. (1960a): The Australasian Mycetophilid Glowworms. *Transactions of the Royal Society of New Zealand 88*: 577-593.

GATENBY, J. B. (1960b): The New Zealand Glow-worm. *Tuatara 8*: 86-92.

GATENBY, J. B. & COTTON, S. (1960): Snare building and pupation in *Bolitophila luminosa*. *Transactions of the Royal Society of New Zealand 88*: 149-156.

GREEN, L. F. B. (1979): The fine structure of the light organ of the New Zealand Glow-worm *Arachnocampa luminosa* (Diptera:Mycetophiladae). *Tissue & Cell 11*(3): 457-465.

HARRISON, R. A. (1961): Notes on the taxonomy of the New Zealand glow-worm *Arachnocampa luminosa* (Skuse)(Diptera: Mycetophilidae). *Transactions of the Royal Society of New Zealand, Zoology 1*: 197-201.

HARRISON, R. A. (1966): Australian glow-worms of the genus *Arachnocampa* Edwards (Diptera: Mycetophilidae). *Pacific insects 8*: 877-883.

HARTL, D. L. (1980): *Principles of Population Genetics*. Sinauer Associates Inc. Massachusetts. 488pp.

HOWARTH, F. G. (1981): Lava-tube ecosystem as a study site. P. 222-230 *In*: Mueller-Dombois, D.B; Carson, K.W. Stroudsburg, H.L. *(Eds): Island Ecosystems: Biological Organization in Selected Hawaiian Communities 15*. Hutchinson Ross.

HOWARTH, F. G. (1983): Ecology of Cave Arthropods. *Annual Review of Entomology 28*: 365-389.

HUDSON, G. V. (1892a): Note on *Tanyzonus bolitophilae*, Marshall, parasitic in the New Zealand glow-worm. *Entomologist's monthly magazine 28*: 277 - 278.

HUDSON, G. V. (1892b): Note on an ant-like insect (*Betyla fulva*, Cameron) parasitic in the New Zealand glowworm. *Transactions of the New Zealand Institute 25*: 164.

HUDSON, G. V. (1950): The natural history of the New Zealand glow-worm. P. 15-37 *In*: *Fragments of New Zealand Entomology*. Ferguson & Osborn. Wellington.

JACKSON, J. F. (1974): Goldschmidt's Dilemma resolved: Notes on the larval behaviour of a new neotropical web-spinning Mycetophilid (Diptera). *American midland naturalist 92*: 240-245.

LLOYD, J. E. (1978): Insect Bioluminescence. P 241-272 *In*: Herring, P. (Ed) *Bioluminescence in Action*. Academic Press. London. New York.

MANSBRIDGE, G. H. (1933): On the biology of some Ceroplatinae and Macrocerinae (Diptera, Mycetophilidae). *Transactions of the Royal Entomological Society of London 81*: 75-90.

MARSHALL, T. A. (1892): A new genus and species of *Belytidae* from New Zealand. *Entomologist's monthly magazine 28*: 275-277.

MAY, B. M. (1963): New Zealand Cave Fauna II - The limestone caves between Port Waikato and Piopio districts. *Transactions of the Royal Society of New Zealand (Zoology) 3*: 181-204.

MAY, B. M. (1971): Cave Fauna. *New Zealand Speleological Bulletin 4 (79):* 571-576.

MAY, M. M. (1985): Chapter 12 - Thermoregulation. P. 507-552 *In*: KERKUT G.A.; GILBERT L.I. (Eds): *Comprehensive Insect Physiology, Biochemistry and Pharmocology 4*. Pergamon Press. Oxford.

MAZOKHIN-PORSHNYAKOV, G. A. (1969): *Insect Vision*. New York, Plenum.

MEYER-ROCHOW, V. B. (1990): *The New Zealand Glowworm*. Waitomo Caves Museum Society Inc. New Zealand. 60pp.

MEYER-ROCHOW, V. B. & EGUCHI, E. (1984): Thoughts on the possible function and origin of bioluminescence in the New Zealand glowworm *Arachnocampa luminosa* (Diptera: Keroplatidae), based on electrophysiological recordings of spectral responses from the eyes of male adults. *New Zealand Entomologist 8*: 111-119.

MEYER-ROCHOW, V. B. & LIDDLE, A. R. (1988): Structure and function of the eyes of two species of opilionid from New Zealand glow-worm caves (*Megalopsalis tumida*: Palpatores, and *Hendea myersi cavernicola*: Laniatores). *Proc. R. Soc. Lond* B (233): 293-319.

MEYER-ROCHOW, V. B. & WALDVOGEL, H. (1979): Visual behaviour and the structure of dark and light-adapted larval and adult eyes of the New Zealand Glowworm *Arachnocampa luminosa* (Mycetophilidae: Diptera). *Journal of Insect Physiology 25*: 601-613.

MEYRICK, E. (1886): A luminous insect larva in New Zealand. *Entomologist's Monthly Magazine 22*: 266-267.

MORLEY, C. (1993): Variances in nest size of two different populations of the New Zealand glowworm; (*Arachnocampa luminosa*). Unpublished Ecology Project. Massey University. New Zealand.

NEI, M. (1975): *Molecular Population Genetics and Evolution*. American Elsevier. New York.

NEI, M. (1976): Mathematical models of speciation and genetic distance. P. 723-765 *In*: KARLIN, S. & NEVO, E. (Eds.) *Population Genetics and Ecology*. Academic Press. New York.

NORRIS, A. (1894): Observations on the New Zealand glow-worm *Bolitophila luminosa*. *Entomologist's Monthly Magazine 30*: 202-203.

OXENHAM, H. E. (1985): The Biology of the Chironomidae of the Waitomo Stream. Unpublished M.Sc Thesis. University of Waikato. New Zealand.

PEARCE, K. (1988): Piripiri Road Caves, Pohangina Valley, Palmerston North. *New Zealand Speleological Society Bulletin 8*(148): 229-234.

PECK, S. B. & RUSSELL, D. R. (1976): Life history of the fungus gnat *Macrocera nobilis* in American caves (Diptera: Mycetophilidae). *The Canadian Entomologist 108*: 1235-1241.

PUGSLEY, C. W. (1980): Ecology of the New Zealand Glowworm *Arachnocampa luminosa* (Skuse) (Diptera: Mycetophilidae) in tourist caves at Waitomo. Ph.D. Thesis. University of Auckland. New Zealand.

PUGSLEY, C. W. (1983): Literature review of the New Zealand glowworm *Arachnocampa luminosa* (Diptera: Keroplatidae) and related cave-dwelling Diptera. *New Zealand Entomologist 7*(4): 419-424.

PUGSLEY, C. W. (1984): Ecology of the New Zealand Glowworm, *Arachnocampa luminosa* (Diptera: Keroplatidae), in the Glowworm Cave, Waitomo. *Journal of the Royal Society of New Zealand 14*(4): 387-407.

RICHARDS, A. M. (1956): The life history and ecology of two species of Rhaphidophoridae in Waitomo Caves. Unpublished Ph.D. Thesis. Victoria University. Wellington. New Zealand.

RICHARDS, A. M. (1960): Observations on the New Zealand Glow-worm *Arachnocampa luminosa* (Skuse) 1890. *Transactions of the Royal Society of New Zealand 88*: 559-574.

SAMSON, R. A. (1984): Entomopathogenic Species of the Hyphomycete Genus *Tolypocladium*. *Journal of Invertebrate Pathology 43*: 133-139.

SHIMOMURA, O.; JOHNSON, F. H.; HANEDA, Y. (1966): Observations on the Biochemistry of luminescence in the New Zealand Glowworm *Arachnocampa luminosa*. P. 487-494 *In*: JOHNSON, F. H. & HANEDA, Y. (Eds). *Bioluminescence in Progress*. Princeton University Press. New Jersey.

SIVINSKI, J. (1982): Prey attraction by luminous larvae of the fungus gnat *Orfelia fultoni*. *Ecological Entomology 7*: 443-446.

SLATKIN, M. (1985): Gene Flow in Natural Populations. *Ann. Rev. Ecol. Syst. 16*: 393-430.

SMITH, K. G. V. (1989): Family Empididae. P. 382 *In*: EVENHUIS, N.L. (Ed): *Catalog of the Diptera of the Australasian and Oceanian Regions*. Bishop Museum Press and E.J. Brill. Honolulu.

SOKAL, R. R. & ROHLF, F. J. (1995): Chapter 17 - Analysis of Frequencies. *In*: *Biometry - The Principles and Practice of Statistics in Biological Research*. 3rd Edition. 887 pp.

STRINGER, I. A. N. (1967): The larval behaviour of the New Zealand glow-worm *Arachnocampa luminosa*. *Tane 13*: 107-117.

STRINGER, I. A. N. & MEYER-ROCHOW, V. B. (1994): Attraction of flying insects to light of different wavelengths in a Jamaican cave. *Memoires de Biospeologie 21*.

VON BERG, V. E. (1978): The Action Spectrum of the Eye of *Helix pomatia* L. *Zool. Jb. Physiol. Bd 82*: 483-492.

WAHLBERG, P. F. (1848): Ytterligare bidrag till kännedonnen om Svamp-myggan *Ceroplatus sesioides*. *Kongl. Vetenskap. Akad. Handl*: 317-327.

WHEELER, W. M., WILLIAMS, F. X. (1915): The luminous organ of the New Zealand glow-worm.

Psyche 22: 36-43.

WINTERBOURNE, M. J. & GREGSON, K. L. D. (1989): Guide to the Aquatic Insects of New Zealand.

Bulletin of the Entomological Society of New Zealand 9. 95pp.