

Methods for rearing and observations on the life history of *Ctenognathus novaezelandiae* (Fairmaire) (Coleoptera: Carabidae)

Bruce A. Philip¹ and Elisabeth P. J. Burgess²

¹MAF Biosecurity New Zealand, P. O. Box 2526, Wellington, New Zealand

²The Horticulture and Food Research Institute of New Zealand Ltd, Private Bag 92 169, Auckland, New Zealand. E-mail: eburgess@hortresearch.co.nz

Abstract

Methods are given for maintaining a laboratory colony of the carabid *Ctenognathus novaezelandiae* and incidental data on its life history are presented. Adults of *C. novaezelandiae* were collected from Woodhill pine forest, north of Auckland, and kept in the laboratory at 18°C with a 16:8 h light:dark cycle. The adults were gregarious and so were kept on moist compacted peat in groups of up to 15 males and 15 females and fed artificial diet-reared *Spodoptera litura* (Lepidoptera: Noctuidae) larvae, or forest floor invertebrates (mostly amphipods) with or without fresh tobacco leaf-reared *S. litura*. Eggs hatched after approximately 9 days. Larvae were cannibalistic and thus reared individually on either *S. litura* larvae and *Teleogryllus commodus* (Orthoptera: Gryllidae) nymphs grown on artificial diet, or *S. litura* grown on tobacco leaf (although few carabid larvae pupated on this diet). There were three larval instars. Mean duration of the larval stage for individuals reared on artificial diet-fed *S. litura* caterpillars and *T. commodus* crickets was 82.2 ± 2.8 days, and pupal duration was 11.8 ± 0.3 days. For larvae reared on tobacco-fed caterpillars, first and second instar durations were 17.2 ± 0.66 and 24.8 ± 1.39 days respectively. Third instar duration could not be reliably determined because of the low numbers that pupated on this diet. Adults lived for up to a year and females fed with forest floor invertebrates laid a mean of 32.3 eggs each, 65% of which hatched.

Keywords: carabid beetle, New Zealand, endemic, laboratory rearing method

Introduction

Carabid beetles are considered useful ecological indicator species and a key taxonomic group for assessing biodiversity and environmental impacts (Lopez *et al.* 2005; Boscaini *et al.* 2000). We used

larvae and adults of *Ctenognathus novaezelandiae* (Fairmaire 1843), a carabid endemic to New Zealand and found in coastal lowland forests throughout the North Island (Larochelle & Larivière 2001), in laboratory experiments to investigate the tri-trophic impacts of transgenic insect-resistant plants (Glare *et al.* 2004; Burgess *et al.* 2008). In order to carry out this research, we first had to develop a rearing method for this species. Many species of carabids have been reared in the laboratory, at least for part of their life cycle. Both larvae and adults are typically kept on a substrate of soil or peat, and can be fed on a wide range of food, such as live or dead invertebrates, processed meat and dog food (Weseloh 1998; Weseloh 1996; Malausa 1977; Goulet 1976; Tomlin 1975). There are, however, no published reports of rearing methods for New Zealand species. In this paper, we report on a laboratory rearing method for *C. novaezelandiae* based on published techniques but adapted for this species and our bioassay requirements, and we make some basic life history observations.

During our tri-trophic experiments, larvae and most adults were reared on larvae of the common cutworm *Spodoptera litura* (Lepidoptera: Noctuidae) fed on tobacco leaves (either non-transgenic or expressing one of two insecticidal proteins), which proved to be a suboptimal diet for the carabids. Adult beetles in several treatments were reared on a more suitable diet of field-collected forest floor invertebrates, or a combination of these and tobacco-fed caterpillars. Adults not used in experiments were fed largely on *S. litura* larvae and black field cricket *Teleogryllus commodus* (Orthoptera: Gryllidae) nymphs reared exclusively on optimised artificial diets. *C. novaezelandiae* larvae not used in the experiment were reared on larvae of the tomato fruitworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) and cricket nymphs, both of which had been fed artificial diets.

The tri-trophic experiments were not designed to produce life history data, and the information presented here is the result of incidental observation and is necessarily limited. Because carabid growth and performance were compromised by the presence of prey that had received tobacco in their diet, only data from adults whose diet included forest floor invertebrates are presented. Larvae did not receive forest floor invertebrates, and were reared on invertebrates grown either on tobacco or on artificial diet. Data from both groups are presented here as some information was not recorded for larvae fed only with invertebrates grown on artificial diet. Thus some results may be influenced by the suboptimal diet that included tobacco-fed prey, but should nevertheless provide some useful new information on this species.

Recently-emerged field-collected adults and their larval offspring were used in the experiments; thus it was not essential to develop a continuous colony in the laboratory. However, we believe the techniques presented here for collecting and maintaining adults, collecting eggs, and rearing larvae provide a strong basis for a continuous laboratory colony method.

Materials and Methods

Field Collection/Sex Determination

Recently-emerged *C. novaezelandiae* adults were collected from pitfall traps, from underneath fallen branches, and from the litter around tree trunks on the sandy floor of the coastal Woodhill *Pinus radiata* plantation north of Auckland (Lat. 36° 44'S, Long. 174° 23'E). Collections were made over three consecutive years (2001 to 2003) at different times during spring and early summer (the earliest at the end of October, the latest at the beginning of January). The sex of each beetle was determined by examination of the protarsi and ambulatory setae on the last abdominal segment. Segments 1-4 of male protarsi are widened and possess 2 rows of scale-like setae on the underside, while female protarsi are narrower (unmodified) and without the scale-like setae. Males typically have 2-4, though occasionally up to 6, ambulatory setae on the posterior margin of the last abdominal sternite, while females have 8 or more. (A. Larochelle, pers. comm.)

Rearing

Rearing of all life stages was carried out at 18±1°C in a 16:8 h light:dark photoperiod.

Adults

Beetles collected from the field were kept in unventilated plastic boxes (300 x 210 x 90 mm) containing approximately 1 cm of moistened, compressed peat covered with a double layer of moistened paper towel. The peat was compacted as tightly as possible (using pressure applied with the back of a spoon) to ensure eggs were laid on the surface, and not into the peat, where they were very difficult to find. Adults were fed twice a week, and each time the beetles were fed, the paper towel was replaced. Approximately 15 females and 15 males were kept per box for up to 90 days. In order to gather data on oviposition, beetles were then divided into smaller groups of five females and five males and transferred to smaller ventilated boxes (150 x 100 x 50 mm), although this would not be necessary for general rearing purposes.

Beetles were fed on three different diets: a combination of early third instar *S. litura* caterpillars (usually frozen and thawed, but occasionally live) reared on artificial diet, and frozen and thawed second instar 8-day-old black field cricket nymphs, or frozen and thawed forest floor invertebrates, or a combination of forest floor invertebrates and *S. litura* reared on fresh tobacco leaf. *S. litura* larvae, if not fed tobacco, were reared on an agar-based diet containing lima bean, wheatgerm, brewers yeast, linseed and wheatgerm oils, vitamins and preservatives (McManus & Burgess 1995). Crickets were reared on a diet of lucerne meal, whole wheat grains, oatmeal and dog biscuits (Pedigree Meaty Bites®) (adapted from Singh & Charles 1975).

The forest floor invertebrates were collected from the same site at Woodhill forest as the beetles and were likely to form the major part of the natural diet of *C. novaezelandiae*. Live invertebrates were collected once a week from dry rectangular pitfall traps (310 x 245 x 130 mm). To prevent the trapped invertebrates from desiccating, several centimetres of loose pine litter was used to line the floor of each trap, and humidity was raised by placing them over water. This was done by cutting five 65 mm diameter holes into the bottom of each trap and sealing these with fine steel mesh. Each was then fitted inside a second trap without holes and the gap (approximately 20 mm deep) between the two trap bottoms was filled with water. To prevent rainwater from filling the outer trap above the level of the bottom of the inner collecting trap and drowning the invertebrates, drain holes were made in the sides

of the outer trap just below the level of the bottom of the inner collecting trap. At collection, most of the litter was removed, leaving a thin layer of litter containing the invertebrates in the bottom of the trap, which was emptied into 5-litre plastic cylinders with clip-on lids for transfer to the laboratory. Invertebrates were also collected by sieving forest floor litter. Initial sieving was undertaken in the forest by shovelling litter into a large canvas funnel incorporating a wide-mesh sieve (7 mm) to remove larger litter items. The material that passed through the funnel was then sieved again through a finer mesh (2 mm), allowing the sand to drop through, leaving a mix of fine litter and invertebrates in the sieve. This was transferred into a large plastic bin for transport to the laboratory.

The invertebrates from sieved litter and pitfall traps were separated from litter and debris overnight in a Berlese/Tullgren funnel (Southwood 1978). Prey items found to be unpalatable to *C. novaezealandiae* or unmanageable, such as ants, weevils, wireworms, earthworms and harvestmen, were removed, and the remaining invertebrates frozen as prey. The bulk of prey invertebrates offered were sand hoppers (Crustacea: Malacostraca: Amphipoda), along with some isopods (Malacostraca: Isopoda), and a small number of bristletails (Insecta: Archaeognatha).

Eggs

Eggs, laid singly in cells constructed of peat, were collected from the adult beetle boxes twice a week. They were found by searching the underside of the paper towel by eye for the peat cells, and by gently disturbing the surface of the peat by lightly running the flat end of metal spatula back and forth over it, which opened the cells and exposed the eggs. They were sterilised in 0.1% (v:v) aqueous sodium hypochlorite (5 min), then rinsed in tap water (2 x 10 min). As neonate larvae will cannibalise eggs and other larvae, eggs were kept individually on a piece of moist paper towel in a sealed 1.5 ml clear plastic sample cup (Sarstedt).

Larvae/pupae

Upon hatching, neonate larvae were individually placed in 35 ml clear plastic coulter cups (LP Italiana SPA) containing several layers of moistened paper towel and closed with a clip-on plastic lid. Individual rearing was maintained throughout the larval stage to avoid cannibalism. They were fed *S. litura* larvae

reared on fresh tobacco leaf, or a combination of *H. armigera* larvae and *T. commodus* nymphs raised on artificial diets (*H. armigera* were fed the same lima bean diet as *S. litura* – see *Adults* section above). Frozen and thawed prey was offered on a piece of filter paper every two to three days. The paper towelling was replaced as necessary, generally every feeding day except when the larvae were still very small. Pupae were maintained individually in their larval rearing containers on clean, moist paper towel.

Results and Discussion

Adults

There was no difference between the longevity of males and females in the laboratory. Mean longevity of adults in the laboratory was 314 days. These beetles may have been adults for up to a month or so before collection, since adults begin to emerge from pupae in spring (A. Laroche, pers. comm.), suggesting that adult *C. novaezealandiae* could live for approximately a year.

Rearing containers for oviposition, each starting with five females and five males, yielded a mean of 161.6 (s.e. = 18.2) eggs per container, i.e., a mean of 32.3 eggs per female. As females were kept in groups, it was not possible to measure individual variability in fecundity. This is likely to be an underestimate of potential fecundity, as some of the females would have died while still reproductively active. Additionally, it is likely that some eggs were cannibalised in rearing boxes before collection.

Egg-laying began 80-140 days after collection, depending on whether the adults had been collected from the field in spring or early summer. Thus, egg-laying commenced at the same time each year, between mid March and early April, i.e., six to seven months after the adults would have emerged in the field. This is likely to be the time of year egg-laying commenced in the field at Woodhill Forest, as inferred by the appearance of neonate larvae in sieved litter (Philip and Burgess 2008b).

Eggs

Eggs were laid individually between the peat and paper towel (either on the surface of the peat or attached to the underside of the paper towel) in a cell constructed of fine fragments of peat stuck together. They were white when laid, oblong and approximately 3 mm long. As they matured, they

darkened to a light golden brown. Approximately a day before hatching, the pigmented mandibles of the larvae became visible. Sixty-five percent of collected eggs from adults receiving forest floor invertebrates in their diet hatched, after a mean of approximately 9 days. Because eggs were collected only twice a week, and hatching scored only three times a week, it was not possible to calculate a precise figure for egg duration.

Larvae/pupae

Carabid larvae are typically reared individually on a peat or soil substrate (Weseloh 1996, Goulet 1976), but for experimental purposes, we devised a method to keep them in layers of paper towel. For colony maintenance purposes, rearing on peat/soil would be preferable, as paper towel needs to be changed frequently. For bioassay purposes, however, it is easier to find the larvae and uneaten food for weighing using the method described above.

Like most carabids (Forsythe 1987), *C. novaezelandiae* has three larval instars. Moulting was recorded when observed in one group of larvae fed caterpillars reared on tobacco leaf. Moulting from both first to second and second to third instar was recorded in some larvae (N = 40). Mean durations of the first and second instars were 17.2 days (s.e. = 0.66), and 24.8 days (s.e. = 1.39) respectively. Unfortunately, only four larvae reared on tobacco-fed *S. litura* pupated, even though some lived as long as 177 days, and only one of these was observed to moult into the third instar, providing no reliable record of third instar duration. Mean larval duration (i.e., hatching to pupation), of the four that did pupate, was 91.8 days (s.e. = 5.4). Three of these emerged as adults, two after 10 days, and one after 12 days.

In contrast, 25 of 30 larvae fed with caterpillars and crickets grown on artificial diets pupated after a mean of 82.2 days (s.e. = 2.8), and adults emerged from all after a mean of 11.8 days (s.e. = 0.3). Moulting was not recorded in these larvae, so the duration of their larval instars is unknown.

Conclusions

We have developed a laboratory-based rearing method for *C. novaezelandiae*. For our bioassays, most individuals were fed, at least in part, with prey reared on fresh tobacco leaf; adults were kept in small groups to allow greater replication; and

larvae kept in layers of paper towel. For simple colony purposes, tobacco-fed prey should be avoided and field-collected invertebrate prey or prey fed with optimised artificial diet used instead. Larvae should be kept on moist peat or soil, and adults kept in larger groups of more than five males and five females. We found approximately 15 of each sex in a container 300 x 210 x 90 mm to be convenient. Furthermore, because adults collected in spring/summer do not lay eggs until autumn, it would be more efficient, when starting a colony, to collect adults from the field in autumn when they are reproductively active.

Acknowledgements

We thank Andre Larochele for advice about field collection of *C. novaezelandiae*, for identifications and training us in sex determination, and for general carabid information. This project was funded by the Foundation for Research Science and Technology, New Zealand under contract C06X0222.

References

- Boscaini A, Franceschini A, Maiolini B. 2000.** River ecotones: carabid beetles as a tool for quality assessment. *Hydrobiologia* 422: 173-181.
- Burgess EPJ, Philip BA, Christeller JT, Page NEM, Marshall RK, Wohlers MW. In press.** Tri-trophic effects of transgenic insect-resistant tobacco expressing a protease inhibitor or a biotin-binding protein on adults of the predatory carabid beetle *Ctenognathus novaezelandiae*. *Journal of Insect Physiology*.
- Forsythe TG. 1987.** Common Ground Beetles. *Naturalists' Handbook* 8. Richmond Publishing Co. Ltd (Surrey). Corbet SA, Disney RHL.
- Glare TR, O'Callaghan M, Malone LA, Burgess EPJ, Philip BA. 2004.** Measuring environmental impacts of genetically modified crops in New Zealand. In *GM crops – ecological dimensions (volume 74)* (eds HF van Emden & AJ Gray) pp. 91-99. The Association of Applied Biologists, Warwick, United Kingdom.
- Goulet H. 1976.** A method for rearing ground beetles (Coleoptera: Carabidae). *The Coleopterists Bulletin* 30(1): 33-36.
- Larochele A, Larivière M-C. 2001.** Carabidae (Insecta: Coleoptera): catalogue. *Fauna of*

New Zealand No. 43. Manaaki Whenua Press. 285 pp.

- Lopez MD, Prasifka JR, Bruck DJ, Lewis LC. 2005.** Utility of ground beetle species in field tests of potential nontarget effects of Bt crops. *Environmental Entomology* 34(5): 1317-1324.
- Malausa JC. 1977.** The rearing of carabid beetles: in the context of mass-rearing. *Annales de Zoologie, Ecologie Animale* 9(3): 497-505.
- McManus MT, Burgess EPJ. 1995.** Effects of the soybean (Kunitz) trypsin inhibitor on growth and digestive proteases of larvae of *Spodoptera litura*. *Journal of Insect Physiology* 41: 731-738.
- Philip BA, Burgess EPJ. 2008.** Observations on the ecology and behaviour of *Ctenognathus novaezelandiae* (Fairmaire) (Coleoptera: Carabidae). *New Zealand Entomologist* 31: 41-46.
- Singh P, Charles JG. 1975.** A list of laboratory cultures and rearing methods of terrestrial arthropods in New Zealand. *Bulletin 3, Entomological Society of New Zealand*. 30pp.
- Southwood TRE. 1978.** Ecological methods with particular reference to the study of insect populations. Chapman and Hall (London). 524 pp.
- Tomlin AD. 1975.** Notes on the biology and rearing of two species of ground beetle, *Pterostichus melanarius* and *Harpalus pensylvanicus* (Coleoptera: Carabidae). *Canadian Entomologist* 107: 67-74.
- Weseloh RM. 1996.** Rearing the cannibalistic larvae of *Calosoma sycophanta* (Coleoptera: Carabidae) in groups. *Journal of Entomological Science* 31(1): 33-38.
- Weseloh RM. 1998.** An artificial diet for the larvae of *Calosoma sycophanta* (Coleoptera: Carabidae), a gypsy moth (Lepidoptera: Lymantriidae) predator. *Journal of Entomological Science* 33(3): 233-240.