

**CIRCADIAN RHYTHMS AND EFFECTS OF DIFFERENT
DIETS ON THE DEVELOPMENT AND REPRODUCTION OF
NABIS KINBERGII (HEMIPTERA: NABIDAE)**

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SUMMARY

Nabis kinbergii is a native polyphagous predator in Australia. It has been found in all states and territories of Australia. *N. kinbergii* has been regarded as an efficient predator of many insect pests in lucerne, cotton and particularly brassica crops. The circadian rhythms, the effects of different prey on development and reproduction, and prey preferences of *N. kinbergii* have not been studied in South Australia. These are the subjects of this thesis.

N. kinbergii is more active at night than in the day. They seemed to be still more frequently at dawn and more active at dusk under natural environmental conditions. Yet, their behaviour was possibly different when they were held in a controlled environment with artificial light. Under both natural and artificial lighting conditions, they would spend more time moving on plants at night. They spent less time moving than other activities. Furthermore, they were more active during the second day of the observations, probably because of the hunger.

A mixed diet including *Plutella xylostella*, *Myzus persicae* and *Brevicoryne brassicae* brought the most significant positive influences to the development, survival and longevity of *N. kinbergii*. It had a shorter preoviposition period and greater egg production when fed on *P. xylostella* than when fed on *M. persicae*. Among the three prey, *B. brassica* was the poorest food because the survival rate between egg hatch and adult eclosion was only 7.5 %, compared to 85 %, 92.5 % and 97.5 % when *N. kinbergii* fed on *M. persicae*, *P. xylostella* and a mixed diet, respectively.

Evidence of prey preference was exhibited by *N. kinbergii*. *B. brassica* seemed to be the least preferred food. *N. kinbergii* possibly attacked less mobile prey and delayed eating prey with poor nutrition or that were toxic. In wind tunnel experiments, *N. kinbergii* may have been attracted by plant volatiles rather than prey odour.

These findings may help to refine the timing of experiments and improve the understanding of the role of this predator in integrated pest management.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available in all forms of media, now or hereafter known.

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CHAPTER 1

GENERAL INTRODUCTION

The Pacific damsel bug, *Nabis kinbergii* Reuter (Hemiptera: Nabidae), is a common and widespread species in Australia, New Zealand and some islands in the western Pacific (Strommer, 1988). It has been collected from all Australian states and mainland territories (Woodward & Strommer, 1982). *Nabis kinbergii* is the only macropterous species in the genus *Nabis* in Australia (Strommer 1988). This species has been previously been identified under the names *Nabis capsiformis* Germar, *Tropiconabis capsiformis* (Germar), *Sastrapada nigrolineata* Distant, *Nabis nigrolineata* (Distant), *Nabis nigrolineatus* (Distant), *Tropiconabis nigrolineatus* (Distant), and *Nabis tasmanicus* Remane (Strommer, 1988; Woodward, 1982; Woodward & Strommer, 1982). Woodward (1982) indicated that *Nabis capsiformis* does not occur in Australia or New Zealand. He demonstrated that *N. kinbergii* is similar in appearance to *N. capsiformis*, but significant differences in male and female genitalia exist between the species.

Nabis kinbergii is considered to be a common predator on a wide range of insect pests on brassica, lucerne and cotton in Australia (Leathwick & Winterbourn, 1984; Ma *et al.*, 2005; Wade *et al.*, 2005). There is a diverse range of pest insects it attacks, including occasional and minor pests that cause yield losses and are difficult to control in agricultural and horticultural crops (Flint, 1987; Leathwick & Winterbourn, 1984). These pests include diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), cabbage white butterfly, *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae), cabbage aphid, *Brevicoryne brassica* (Linnaeus) (Hemiptera: Aphididae), and green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) on brassica crops (Ma *et al.*, 2005); blue-green aphid, *Acyrtosiphum kondoi* Shinji (Hemiptera: Aphididae), pea aphid, *Acyrtosiphum pisum* (Harris) (Hemiptera: Aphididae), and Australian crop mirid, *Sidnia kinbergi* (Stål) (Hemiptera: Miridae) on lucerne (Siddique & Chapman, 1987; Ma *et al.*, 2005); and cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Wade *et al.*, 2005). Among the pests, the diamondback moth is perhaps the most difficult to control (Talekar & Shelton, 1993). In Australia, this insect pest was first reported in 1893 (Waterhouse, 1990) and has subsequently spread to all parts of Australia (Waterhouse & Norris, 1987). Although many methods have been developed to control *P. xylostella*, serious crop damage from this pest still occurs in many countries due to its biology and frequent evolution of insecticide resistance (Endersby *et al.*, 2004). To develop effective pest control methods and minimize insecticide applications,

biological control approaches using predators could be one good strategy for suppression of the diamondback moth and other damaging pests. However, utilization of predators for pest control might be not effective unless the behaviours of both the predators and their prey are understood.

Various behavioural activities of nabids have been described in the literature for more than 60 years. They included foraging, feeding, oviposition, flight, and locomotion (e.g., Dicker, 1946; Fewkes, 1961; Hormchan *et al.*, 1976; Siddique, 1985). These activities are influenced by both biotic and abiotic factors. The biotic factors include prey availability, prey types and prey habitat (Siddique, 1985). The abiotic factors involve environmental conditions such as temperature and light. Among environmental conditions, the effects of temperature (Braman *et al.*, 1984; Guppy, 1986; Nadgauda & Pitre, 1986) on nabid activity have been more extensively studied than those of photoperiod. Many activities of insects are governed by light/dark cycles or so-called circadian rhythms (Howlader & Sharma, 2006; Walker, 1977; Yamano *et al.*, 2001; Zhang *et al.*, 2006). For example, some nabids are more commonly collected on plants at night than during the day (Dicker, 1946; Leathwick & Winterbourn, 1984). These observations may indicate that the timing of behavioural experiments on these predators may influence the outcome. Thus, experiments were conducted to elucidate the circadian rhythms of *N. kinbergii* (Chapter 2).

Nabids are known to be polyphagous predators (Lattin, 1989), i.e. predators that can eat a wide range of insect species. Unlike specific predators, generalist predators have more opportunities to survive when their common food sources in the field are scarce (Eubanks & Denno, 2000). Moreover, the population densities of a generalist predator might increase by consuming alternative prey before the major target pest arrives in a crop (Settle *et al.*, 1996). However, not all prey species are equally nutritious. For instance, pea aphids, *A. pisum* (= *Microsyphum pisum*) and lygus bugs, *Lygus hesperus* (Knight) and *Lygus elisus* Van Duzee (Hemiptera: Miridae) could support the development of *Nabis alternatus* Parshley (Hemiptera: Nabidae), but alfalfa weevil larvae, *Hypera postica* (Gyllenhal) (= *Phytonomous posticus*) (Coleoptera: Curculionidae) could not (Taylor, 1949). In addition, the other parameters of life history such as survival, longevity and fecundity are affected differently by different food sources (Hormchan *et al.*, 1976; Nadgauda & Pitre, 1986; Siddique & Chapman, 1987). Thus, understanding the effects of different prey species may provide a means to improve the management of native and introduced populations of natural enemies in the field as well as in greenhouses. Furthermore, understanding of the consequences of consumption of different prey species on survival, growth, and reproduction is likely to

provide helpful information for developing mass rearing techniques. Therefore, the effects of selected prey species on the development, survival and fecundity of *N. kinbergii* were investigated (Chapter 3).

Like many other predators, nabids exhibit preferences for different prey species when given a choice. For instance, the attack rate of *Nabis americanoferus* Carayon on *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) was different from that on *L. hesperus* (Propp, 1982). In addition, Ma *et al.* (2005) reported that *N. kinbergii* consumed significantly more *P. xylostella* than *P. rapae*. However, the authors did not investigate the underlying reasons for the preference. Insect predators may select their prey by distinguishing their characteristics such as mobility, nutritional quality, appearance, odour, and taste (Eubanks & Denno, 2000). Knowledge of the prey preference may help to improve the understanding of the foraging behaviour of insect predators, which is considered to be central to insect ecological studies (Fellowes *et al.*, 2005). Therefore, experiments were conducted to investigate prey preferences of *N. kinbergii* (Chapter 4).

Investigations of the circadian rhythms, the effects of prey types and prey preferences are the primary topics of this thesis. The aims of this project were focused on answering three main questions:

1. Are the behavioural activities of *N. kinbergii* expressed in circadian rhythms?
2. Which types of prey are suitable for the development and reproduction of *N. kinbergii*?
3. Which factors affect the prey choice of *N. kinbergii*?

The results have important implications for sampling, rearing, predator-prey dynamics, and ultimately the efficacy of biological control. Overall, the research reported here may allow us to develop better pest management strategies against insect pests.

CHAPTER 2

CIRCADIAN RHYTHMS

1. INTRODUCTION

Behavioural, physiological and biochemical processes that are repeated in daily 24 hour cycles are called circadian rhythms. Behavioural rhythms are found in many species, both unicellular and multicellular (Sarov-Blat *et al.*, 2000). In insects, circadian rhythms affect many activities such as foraging, feeding, oviposition, flight and locomotion (Howlader & Sharma, 2006; Walker, 1977; Yamano *et al.*, 2001; Zhang *et al.*, 2006). These activities have been documented in many different insect species. The rhythms in foraging activity have been well studied in bees. For instance, foragers of the eusocial bee, *Scaptotrigona Scaptotrigona depilis* (Hymenoptera: Apidae) are always found in the hive at sunset after seeking food. This cycle is synchronised with the light/dark cycle and temperature does not seem to influence it (Bellusci & Marques, 2001). In addition to bees, other insect species have to forage for their food, thus they may exhibit circadian rhythms in their foraging activities. The feeding activity of many insects is also influenced by circadian rhythms. For instance, grasshoppers, *Poeciloceris hieroglyphicus* (Acrididae: Pyrgomorphinae), showed rhythmic feeding activity that started in the early morning and peaked after sunrise (Abushama, 1968). The New Zealand kiwifruit leafroller, *Cnephasia jactatana* (Lepidoptera: Tortricidae), reached peak foraging activity at the end of night time although feeding also occurred during the day (Jimenez-Perez *et al.*, 2002). The reproduction of a number of insect species also exhibits circadian rhythms. In particular, oviposition rhythms have been extensively studied in flies. Paranjpe *et al.* (2004) found evidence suggesting that the vinegar fly, *Drosophila melanogaster* (Diptera: Drosophilidae), lays more eggs in the dark than in the light. They reported that oviposition of *D. melanogaster* is rhythmic under either constant light or constant darkness. They suggested that the oviposition rhythms in *D. melanogaster* are regulated by multi oscillators. Among the behaviours of insects, flight and locomotion are the best known for their rhythmicity. This is possible due to the ease of recording. Many species of flies, such as *D. melanogaster* and house fly, *Musca domestica* (Diptera: Muscidae), exhibit circadian rhythms in locomotory activity (Cymborowski *et al.*, 1994; Parker, 1962). Among these, the rhythm of locomotory activity of *M. domestica* is diurnal (Parker, 1962), whereas *D. melanogaster* has a bimodal pattern (Cymborowski *et al.*, 1994). Some insects exhibit unimodal patterns of activity. Unimodality can occur when the locomotory activity reaches a peak around the light to dark transition (Helfrich-Forster, 2000; Lazzari, 1992). For

example, males of the apple leafminer, *Phyllonorycter ringoniella* (Lepidoptera: Gracillariidae), exhibit strong rhythmicity in flight activity with a peak in the early morning (Sekita, 2000). Thus, the effects of circadian rhythms on behaviours of insects vary among species. These effects need to be studied on a species by species basis.

Circadian rhythms in insects have been reported to be influenced by environmental conditions, particularly light/dark cycles and temperature. For example, the circadian rhythms of *D. melanogaster* are regulated by both light and temperature (Tomioaka *et al.*, 1998). The photoperiod may affect the developmental time, mortality, longevity, oviposition, flight/walking, feeding and other activities (Brodsgaard, 1994; Jimenez-Perez *et al.*, 2002; Pearsall, 2002). The threshold photophase at which insects are affected may be long or short. The activities of some insects are promoted more by longer periods of light, but others benefit more from longer nights. For instance, the milkweed bug, *Oncopeltus fasciatus* (Hemiptera: Lygaeidae), increased its feeding rate when reared under a 16L:8D photoperiod compared with that when reared under a 12L:12D photoperiod (Caldwell & Dingle, 1967). In contrast, a 12L:12D photoperiod brought positive effects to the locust, *Locusta migratoria* (Orthoptera: Acrididae), rather than a 16L:8D photoperiod (Rodgers *et al.*, 2006). Some insect predators seem to be more active in cycles with a long dark period (Ortega-Escobar, 2002; Yamashita & Nakamura, 1999). Apart from the length of light hours, the light intensity can also contribute to variation of insect rhythmic behaviour. For example, the flight activity the dwarf honeybee, *Apis florea* (Hymenoptera: Apidae), had a positive correlation with light intensity (Abrol, 2006). Light intensity also affects the foraging activity of nocturnal and crepuscular bees (Kelber *et al.*, 2006). Furthermore, the quality of light is important in the activities of insects. For example, it has been suggested that the frequency of flicker in artificial lighting can affect the behaviour of insects (Shields, 1980). As Shields (1980) suggested, natural light should be used preferentially in experimentation because artificial light can have a strong influence on the behaviour of insects. Therefore, the qualities of light sources should be considered before doing experiments.

The other important factor that affects the rhythmic behaviour of insects is the temperature. The temperature may influence mating, feeding, oviposition and other activities, including locomotion and flight. For instance, the time from adult eclosion to the first mating of *O. fasciatus* was three to four days longer at low temperature (23 °C) than at high temperature (27 °C) under the same photoperiod (Caldwell & Dingle, 1967). Furthermore, the interaction between light and temperature may also influence activity patterns of insects. For example, the first mating by milkweed bugs was seven days earlier when reared under a 16L:8D

photoperiod at 27 °C than when reared under a 12L:12D photoperiod at 23 °C. In addition, in four different conditions involving two temperature regimes and two photoperiods, the feeding activity in the milkweed bug rose sharply to a maximum in the dark and decreased when the lights came on (Caldwell & Dingle, 1967). Current results indicate that temperature and photoperiod should be included in any detailed behavioural rhythmic experiments.

Many studies of circadian rhythms have been done on flies and other insect pests, but few involve insect predators. Some studies on common predators such as spiders are available. Among these, studies of rhythms in locomotion and feeding behaviour are noteworthy, because they reflect on the predatory behaviour of the spiders. Ortega *et al.* (1992) and Ortega-Escobar (2002) studied the ground-living spider, *Lycosa tarantula* (Araneae: Lycosidae), to determine whether or not its locomotory activity shows rhythmicity, and what environment conditions may affect its circadian rhythms. They showed that the locomotory activity of *L. tarantula* was primarily nocturnal and exhibited a circadian rhythm. Rhythms in predatory behaviour may be harder to document because they depend on parameters involving prey type, density and behaviour. Ortega-Escobar (2002) noted that some spiders are active in the day and suggested that the absence of prey at night might cause this exceptional behaviour. In spiders, there are some diurnal species such as the jumping spider, *Menemerus confusus* (Araneae: Salticidae), while others, such as *Araneus ventricosus* (Araneae: Araneidae) and *Cupiennius salei* (Araneae: Ctenidae), are nocturnal (Walla *et al.*, 1996; Yamashita & Nakamura, 1999). In addition, some spiders are active both at night and in the day such as the web-building species *Argiope* spp (Araneae: Araneidae)(Yamashita, 1987). Daily rhythms affect locomotory activities in other spider species including *Frontinella pyramitela* (Araneae: Linyphiidae) and *Argyrodes trigonum* (Araneae: Theridiidae) (Suter, 1993). Apart from the above studies, there is little detailed information about the behavioural rhythms of other insect predators, although some authors report that predaceous insects are active during certain times of the day. For some predaceous insects, an effect of circadian rhythms on reproductive behaviour has been demonstrated. For example, in the ladybird beetle *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae) overall oviposition was significantly greater in the dark phase when placed under a 14L :10D photoperiod (Omkar & Singh, 2007). Nocturnal predaceous insects are more active at night, and their feeding and locomotory activities occur mostly under low light intensity. This implies that ecological and behavioural studies of nocturnal species done during the day may give unreliable results. Therefore, it is necessary to assess whether insects either are nocturnal or diurnal before such studies are done.

Regarding the activity of damsel bugs (Hemiptera: Nabidae), there are some studies on foraging behaviour but only one on circadian rhythms (Wade *et al.* 2005). Almost all studies on foraging behaviour of predatory bugs have been conducted during the day. Therefore, this does not give an overview of all the activities of these predators. However, evidence of circadian patterns was obtained during some of these studies. Dicker (1946) reported that *Nabis lativentris* was active at night, however he noticed that its nocturnal activity occurred specifically on cold nights. In addition, Fewkes (1961) suggested that nabids may primarily be nocturnal, which was deduced from the fact that more nabids were collected during night-time sweeping. Both observations do not give direct evidence for the rhythms of nabids. The nocturnal activity of these bugs should be determined in order to understand their predatory behaviour, as this could be helpful for the development of pest management strategies. Furthermore, a study of nabids as predators of lucerne aphids suggested that their predation rate at night was higher than during the day (Leathwick & Winterbourn, 1984). This study also showed that the numbers of nabids that were sampled at night were four times more than those collected during the day, corroborating Fewkes's (1961) conclusions. However, the activity patterns of nabids may be dependent on the species. Among six species of nabids collected in German lucerne (alfalfa) during three years, only *Nabis major* was hard to sample by sweep net during the daytime (Roth, 2003). The nocturnal activity of nabids was also observed on soybean and corn crops in United States (Pfannenstiel & Yeorgan, 2002). Over 80% of the feeding activity was between 21:00 h and 06:00 h. Furthermore, the predation of nabids on eggs of prey reached a peak at midnight. The largest difference between day and night time feeding activity was found for the nymphs of nabids (Pfannenstiel & Yeorgan, 2002). Another study from United States used direct observation, to demonstrate that nabids were seen throughout the day, but were in greater abundance in the evening (Costamagna & Landisa, 2007). In contrast, Wade *et al.*, (2005) reported that the activity of *N. kinbergii* did not differ between day and night. The authors studied three main activities by direct observation: stationary, feeding and walking. Among these activities, *N. kinbergii* spent most of the time standing still (Siddique, 1985; Wade *et al.*, 2005). According to Siddique (1985), *N. kinbergii* has two peaks of walking at about 12:00 noon and at 20:00 hr. He also reported that *N. kinbergii* walked more if no food was provided, particularly in the morning. With respect to feeding activity, Siddique (1985) noted that timing of feeding was influenced by the time of releasing predator into the feeding arena. However, the wings of the *N. kinbergii* that were used in his study were clipped and this may have caused unusual behaviour. In addition, the two hour intervals between observations may not have reflected precisely the amount of time this nabid spent on each activity. Therefore, the continuous observations should be done to determine the circadian rhythms of *N. kinbergii*.

It is clear that studies so far have included too many uncontrolled variables to allow proper assessment of circadian rhythms in *N. kinbergii*. This lack of information is addressed in this study, and the results can be used to develop further studies to assess the effectiveness of this species as a predator of insect pests. The activity patterns of *N. kinbergii* were observed in both uncontrolled and controlled environments. In addition, the influence of different light/dark cycles on the activity patterns was examined.

2. MATERIALS AND METHODS

2.1 General rearing methods

Adult *N. kinbergii* were collected from broccoli crops at Virginia, and lucerne crops at Murray Bridge and Netherby, South Australia. These were maintained on three prey species that infested canola: larval diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae); cabbage aphid, *Brevicoryne brassicae*; and green peach aphid, *Myzus persicae* (Hemiptera: Aphididae). Rearing cages with canola and lucerne were kept at 25 °C, under a 14L:10D photoperiod. Adult *N. kinbergii* that were the offspring of field-collected nymphs and adults were used for all experiments.

2.2 Observational arena

In order to determine the circadian rhythms of *N. kinbergii*, individuals were kept in a clear plastic jar (7 cm diameter, 14 cm height). The jar was inverted and the top was removed and covered with mesh (32.5 strands/cm) for ventilation. The experiment was done with lucerne plants, *Medicago sativa* (Fabaceae), because all parts of lucerne such as stems and leaves are thin and small, which makes it possible to continuously observe all nabid behaviour. Blue-green aphid, *Acyrtosiphon kondoi* (Hemiptera: Aphididae), was used as the prey in this work. This experiment assumed that the activity of *N. kinbergii* on lucerne plants is similar to that which is observed on other plants. A shoot of lucerne with 30 blue-green aphids was kept fresh by wrapping the stem in absorbent cotton and placing it in a vial of water. This vial was inserted into a hole in the centre of a circular piece of foam rubber. Then the vial and foam were inserted into the bottom of the inverted plastic jar. The night before a video recording, an adult nabid was placed in an empty cup. This individual was introduced into the jar at the beginning of the observation (Figure 2.3).

2.3 Video recording

There were three experiments under natural and artificial light, each with a different photoperiod. Nabid behaviour was recorded using a video camera with a charge-coupled device (CCD) sensor that is sensitive to infrared light (528F – Yoko, Panasonic, Taiwan). The camera was connected to a time lapse video recorder (AG -TL500BE, Panasonic, Osaka, Japan) in order to record the behaviour of nabids (Varley *et al.*, 1994). Each observation was conducted over 48 hours. The insects were not disturbed by the presence of a human observer throughout the recording period. With the aid of infrared light provided by 8 IR light emitting diodes, the behaviour of each nabid was filmed in the dark without it being disturbed. In order to make sure the camera could view the entire arena, a mirror was positioned next to the jar to enable the far side of the plant to be viewed. The behaviour of nabids was divided into two main categories (Table 2.1). Observations from videotapes were reviewed and data were recorded using a Psion Workabout (Psion PLC, UK) which was programmed with The Observer[®] software (Version 3.0, Noldus Information Technology B.V., Wageningen, The Netherlands) (Hagler *et al.*, 2004; Tillman & Mullinix, 2003) (Figure 2.1).

Experiment 1. Natural light conditions

The foraging behaviour and circadian rhythms of *N. kinbergii* were first studied under natural lighting conditions. This work was conducted near a window inside a room at the Waite Campus of the University of Adelaide where natural light was available. Sunrise and sunset times were 06:47 – 07:06 h and 17:17 – 17:40 h, respectively (~ 10.5L:13.5D photoperiod). The temperature during the experiment was uncontrolled and varied between 22 and 27 °C. Observations started at 9 AM and terminated two days later at 9 AM. There were 8 replicates, 6 females and 2 males. Each observation involved different *N. kinbergii*, aphids and lucerne shoots on separate days.

Experiment 2. Artificial light conditions, 14L:10D photoperiod

Similar observations as described above were conducted in a controlled environment under artificial lighting. Preliminary experiments indicated that *N. kinbergii* is disturbed by the 50 Hz flicker of standard fluorescent lighting, so a high frequency lighting system that powered 4 fluorescent lamps was used to illuminate the experimental arena (Appendix 1). The lighting system automatically came on at 6:00 AM and went off at 8:00 PM (14 hour photophase). Observations started at 5 PM and terminated two days later at 5 PM. A 0.1 A minilamp simulated moonlight and was on throughout the recording. This lamp provided sufficient dim light to allow filming of the observational arena. Therefore, the infrared light was not used in these observations. The temperature was constant at 25 °C. There were 20 replicates, 10 females and 10 males.

Experiment 3. Artificial light condition, 12L:12D photoperiod

To confirm the influence of light on the circadian rhythms, *N. kinbergii* was placed under a 12 hour photophase. *N. kinbergii* used for the 12 hour photophase experiment were reared on *A. kondoi* in a rearing cage. This cage was placed into the recording room four or more days before an experiment. This difference in rearing method was necessitated by the availability of only one room that had a 12L:12D photoperiod. The nabids could be reared on blue-green aphids without being attended for four or more days at a time, while the rhythms of other individuals were being recorded. The lights came on at 8:00 AM and went off at 8:00 PM. Observations started at 5 PM and terminated two days later at 5 PM. The number of replicates was 10, 4 females and 6 males. All insects were recorded one at a time.

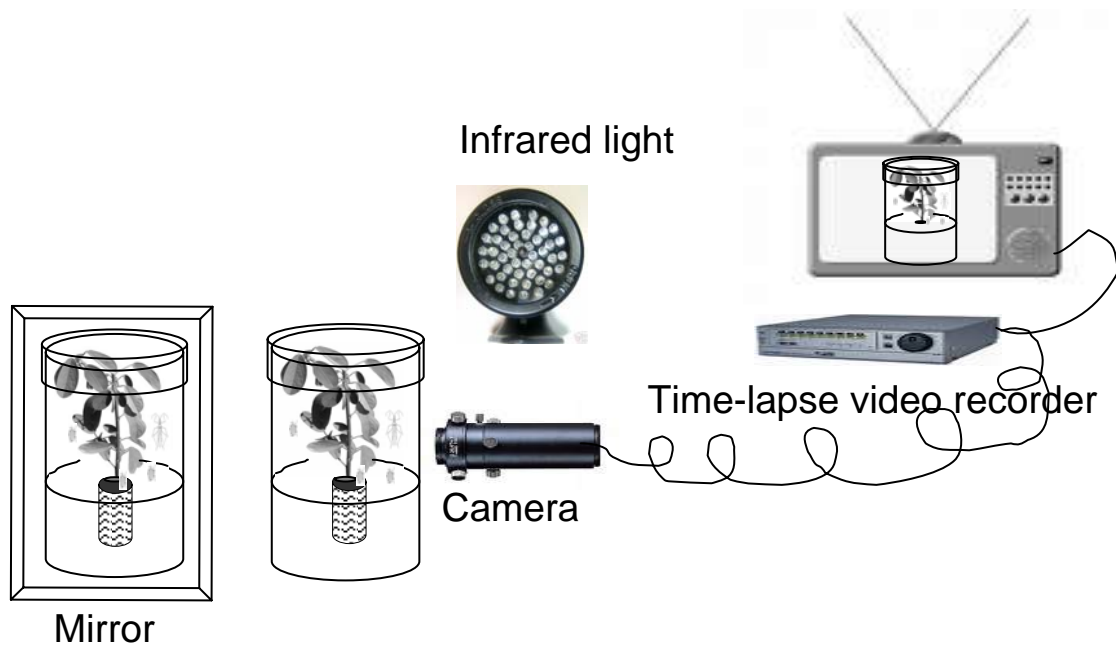


Figure 2.1 Diagram of video recording the behaviour of *N. kinbergii* under natural light conditions.

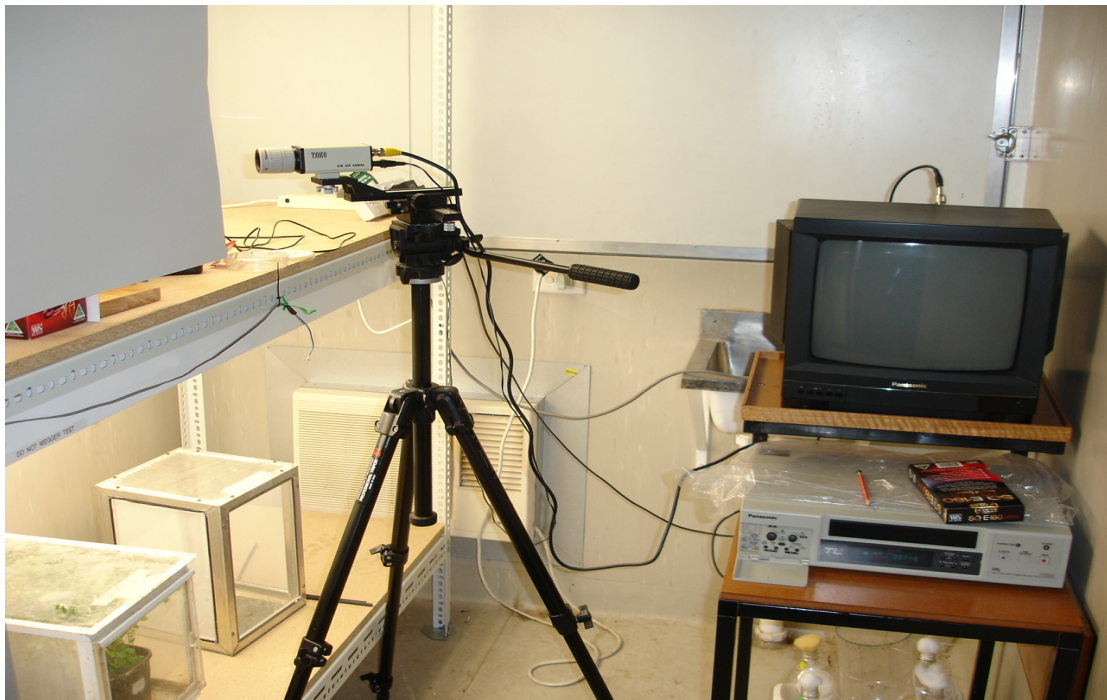


Figure 2.2 The observational room for recording behaviour of *N. kinbergii* under artificial light. Note the screen in front of the observational arena allowed the room to be checked without the observer being seen by the insects.



Figure 2.3 The feeding arena for *N. kinbergii* in video recording experiments, with the mirror image on the left.

Table 2.1 Description of the behaviours of *N. kinbergii* foraging for blue green aphids on lucerne shoots inside a 0.5 L plastic jar.

Category of behaviour	Component of behaviour	Description of behaviour
Location	On plant	<i>N. kinbergii</i> located on plant
	Off plant	<i>N. kinbergii</i> located on jar wall, lid or sponge.
Activity	Moving	<i>N. kinbergii</i> moves its position within the arena
	Still	<i>N. kinbergii</i> does not move

2.4 Data analysis

Daily patterns of activity were analysed by non-linear regression using the sigmoidally transformed cosine curve as the model (Marler *et al.* 2006). The activity data from each experiment were pooled and expressed as fraction of insects moving and the location data were expressed as fraction of insects on plants at any given time. The midpoint of the period over which each fraction occurred was taken as the time in this analysis. For example, if three insects out of 20 were active starting at 2.20 hours and then four insects were active starting at 2.22 hours, then the data point for this interval would be (2.21, 0.15). Three different model transformations were fitted to each data set, i.e., the Hill Function, the anti-logistic function and the arctangent transformation (Marler *et al.* 2006). As the fitted curves were virtually identical for all models, only the results for the Hill function are reported here. The fitted model for the Hill function is given by:

$$r(t) = \text{min} + \text{amp} * h(c(t)) \quad [1]$$

$$h(c(t)) = [c(t)+1]^\gamma / (m^\gamma + [c(t)+1]^\gamma) \quad [2]$$

$$c(t) = \cos([t-\phi]2\pi/24) \quad [3]$$

where

equation [1] is the full regression model,

equation [2] is the Hill-transformed cosine model,

equation [3] is the basic cosine model,

min is the minimum of the fitted curve,

amp is the amplitude of the fitted curve,

m is a constant,

γ is a parameter that determines the steepness of the curve,

ϕ is the time at which the curve reaches its maximum, and

t is the elapsed time in hours.

Data were analysed using non-linear regression with JMP (version: 4.0.2; SAS Institute, Cary, North Carolina, U.S.A.). In order to visualise the results, the fitted curves were plotted with both the raw data and 9-point moving means.

3. RESULTS

Experiment 1. Natural light conditions

The sigmoidally transformed cosine curve fitted the fraction of *N. kinbergii* moving during the two-day observation period (Figure 2.4, Table 2.2), indicating that there was a circadian rhythm of behaviour of *N. kinbergii*. Individuals were more active at night than during the day. However, *N. kinbergii* was moving during both day and night, not only at night. There were some discrepancies between the fitted curve and the activities of the nabids when compared to the 9-point moving means. In addition, there was a sudden increase in activity at the light/dark transition right after dusk. *N. kinbergii* was present on plants more often at night (Figure 2.5, Table 2.3).

Experiment 2. Artificial light conditions, 14L:10D photoperiod

As in experiment 1, *N. kinbergii* exhibited a circadian rhythm in movement behaviour, being more active in the dark phase than in the light phase as indicated by a sigmoidally transformed cosine curve that was fitted to the data (Figure 2.6, Table 2.2). The movements of *N. kinbergii* occurred during both day and night. There was also a pronounced change in the frequency of movement at the light/dark transition in the second day when *N. kinbergii* increased or decreased its activity..

Male *N. kinbergii* displayed similar circadian rhythms to females (Figure 2.7 & 2.8, Table 2.2).

Unlike the experiment conducted under natural lighting conditions, no circadian pattern of presence on plants was detected in this experiment (Figure 2.9, Table 2.3).

Experiment 3. Artificial light conditions, 12L:12D photoperiod

The sigmoidally transformed cosine curve fitted the data on fraction of *N. kinbergii* moving over the 48 hr observation period. This indicated *N. kinbergii* also displayed the circadian rhythm of behaviour under the 12L:12D photoperiod. In the other words, there was more activity of *N. kinbergii* at night than in the day (Figure 2.10, Table 2.2). During a day, *N. kinbergii* was less active in the morning than in the afternoon. During two days of observation, *N. kinbergii* was much less active during the first day than the second day.

Like experiment 2, *N. kinbergii* did not exhibit a circadian pattern of presence on plants (Figure 2.10, Table 2.3), but movement between the plant and the other parts of the arena were more frequent during the second day.

Overall, there was a consistent pattern of behaviour of *N. kinbergii* in all experiments. *N. kinbergii* was active during both day and night, but it was more active at night than during the day. Within the same day, *N. kinbergii* was less active in the morning than in the afternoon. Between two days of observation, *N. kinbergii* was less active during the first day than the second day. In addition, *N. kinbergii* spent less time moving than on other activities (Figure 2.11). Among three experiments, the biggest difference in percentage of moving between the first day and the second day occurred in a 12L:12D photoperiod (Figure 2.11).

Table 2.2 Parameter estimates for the Hill Function transformation in the sigmoidally transformed cosine curve that described the circadian rhythms of movement by *Nabis kinbergii* under experimental conditions.

Parameter	10.5L:13.5D natural light		14L:10D artificial light: Combined sexes		14L:10D artificial light: Females		14L:10D artificial light: Males	
	Estimate	Approx. Std. Error	Estimate	Approx. Std. Error	Estimate	Approx. Std. Error	Estimate	Approx. Std. Error
Phi (ϕ)	14.0471	0.0193	8.4253	0.0083	7.5994	0.0212	7.2978	0.0681
Gamma (γ)	99.112	45.255	228.85	89.38	269.52	246.27	30.767	12.583
<i>m</i>	0.9950	0.0051	1.178505	0.0022	1.2450	0.0054	1.1879	0.0182
<i>min</i>	0.2392	0.0040	0.313303	0.0017	0.3827	0.0033	0.3029	0.0035
<i>amp</i>	0.2349	0.0056	0.163193	0.0026	0.14686	0.0053	0.1149	0.0052

Table 2.3 Parameter estimates for the Hill Function transformation in the sigmoidally transformed cosine curve that described the circadian rhythms of plant occupation by *Nabis kinbergii* under experimental conditions and 10.5L:13.5D natural light.

Parameter	Estimate	Approx. Std. Error
Phi (ϕ)	13.1050	0.0878
Gamma (γ)	0.6655	0.1595
m	1.0030	0.1854
min	0.1158	0.0158
amp	0.44288	0.0634

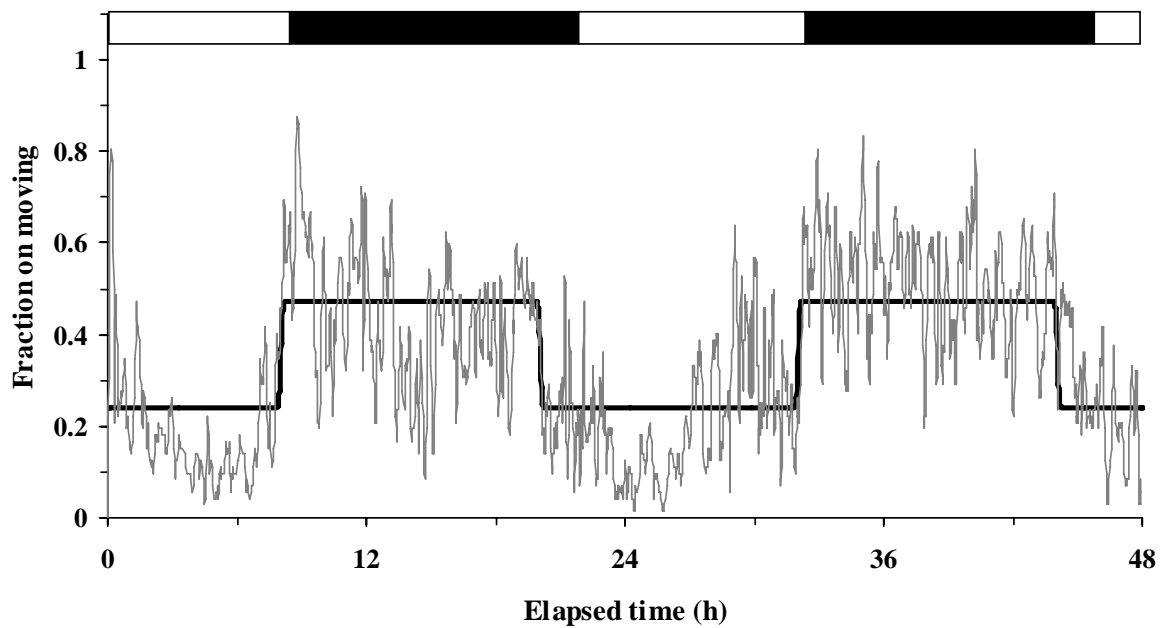
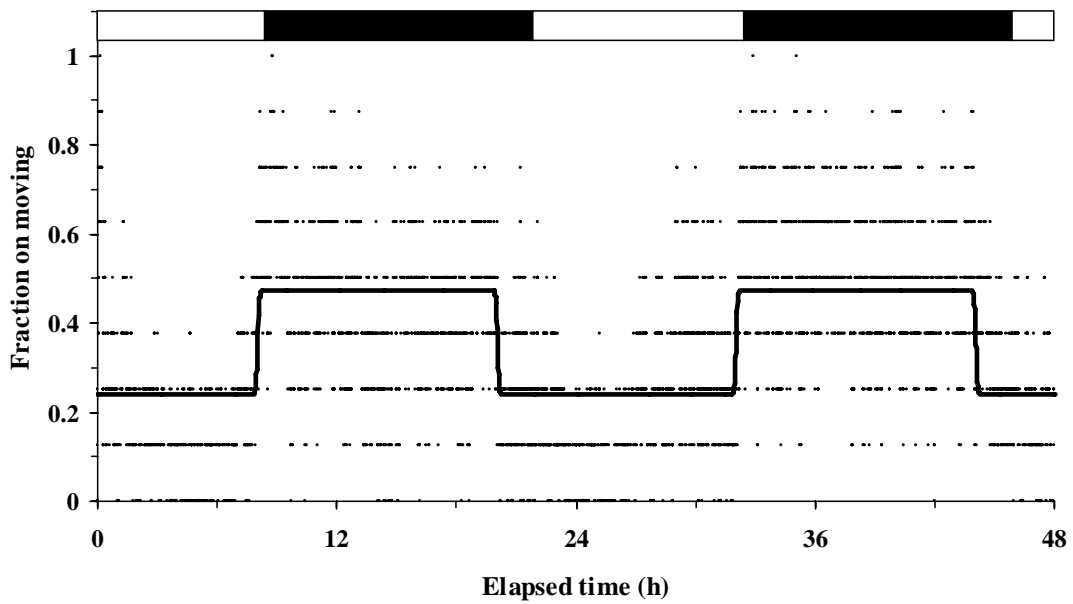


Figure 2.4 The fraction on moving of *N. kinbergii* ($n = 8$) under natural light (10.5L:13.5D). Observations were made for 48 hours and are expressed as raw data (upper figure) and 9 point mean (lower figure). The black and white bars indicate night time and day time, respectively. Experiments started at 9:00 AM.

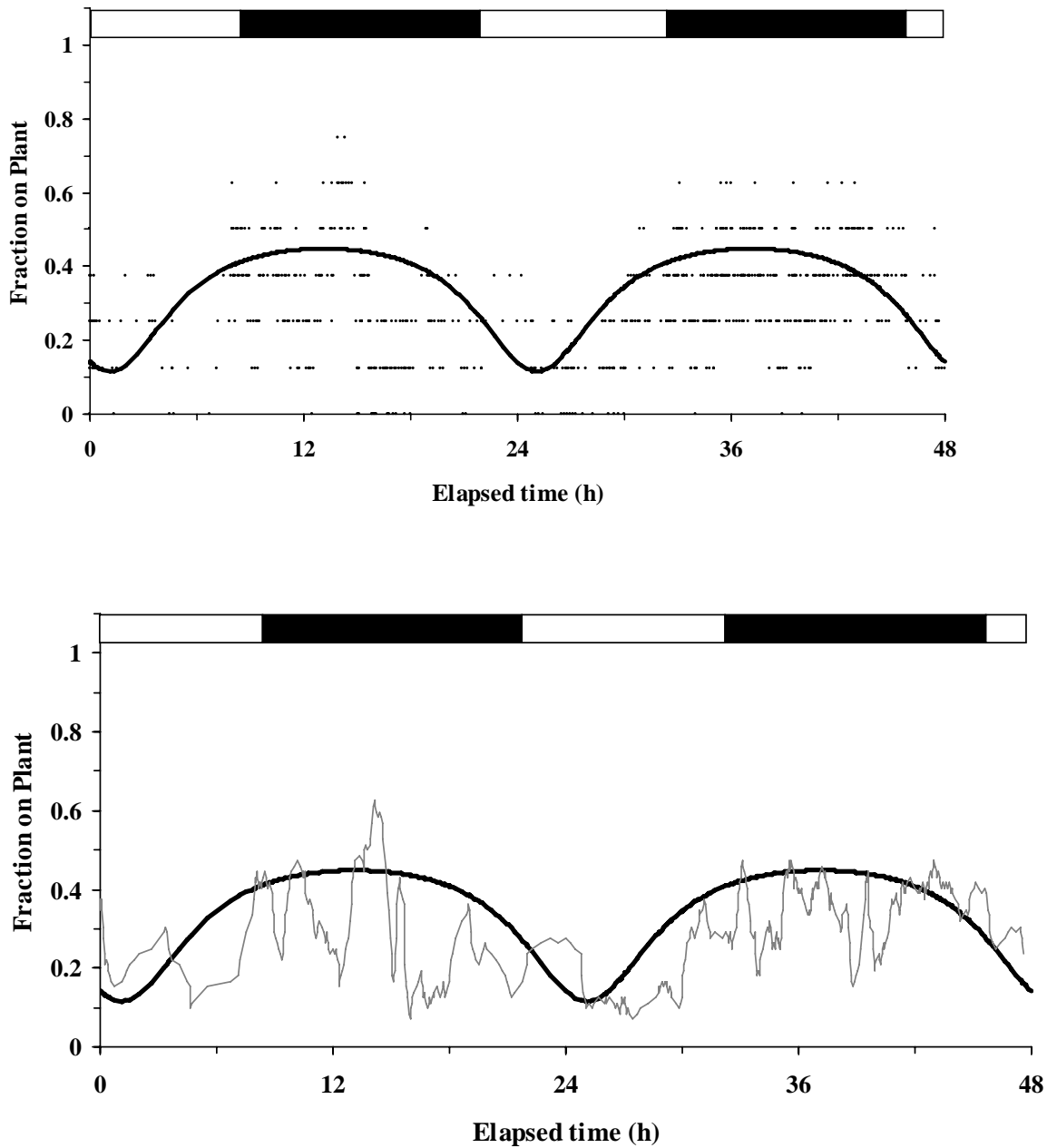


Figure 2.5 The fraction on plant of *N. kinbergii* (n = 8) under natural light (10.5L:13.5D). Observations were made for 48 hours and are expressed as raw data (upper figure) and 9 point mean (lower figure). The black and white bars indicate night time and day time, respectively. Experiments started at 9:00 AM.

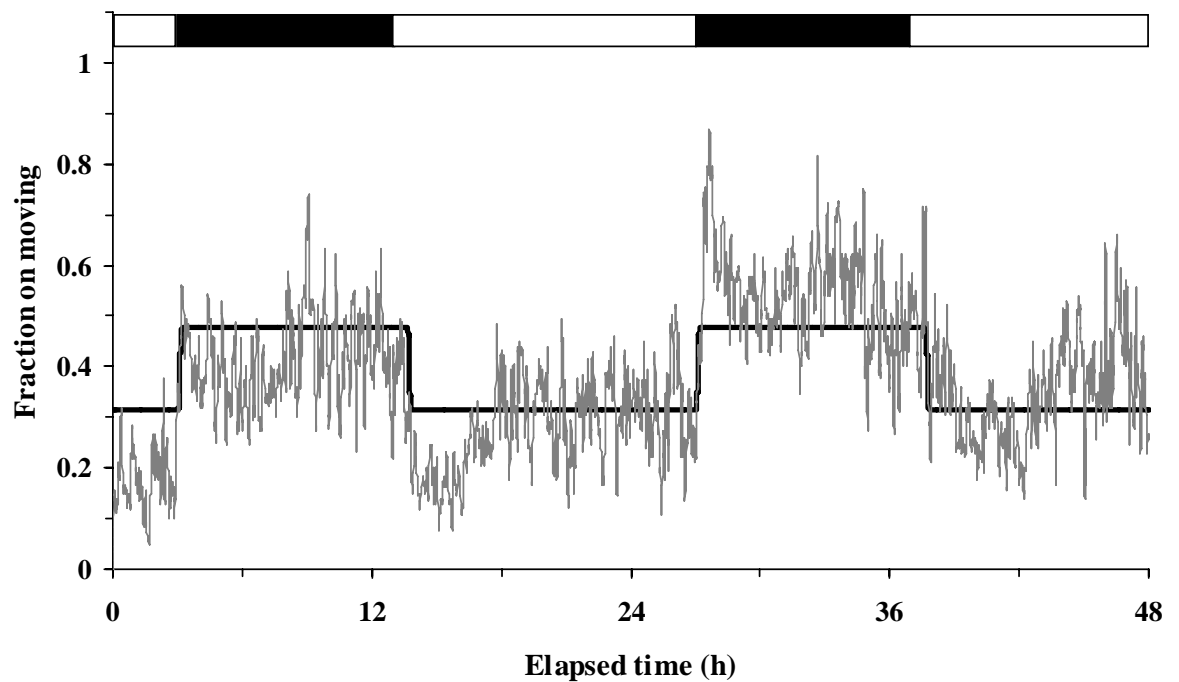
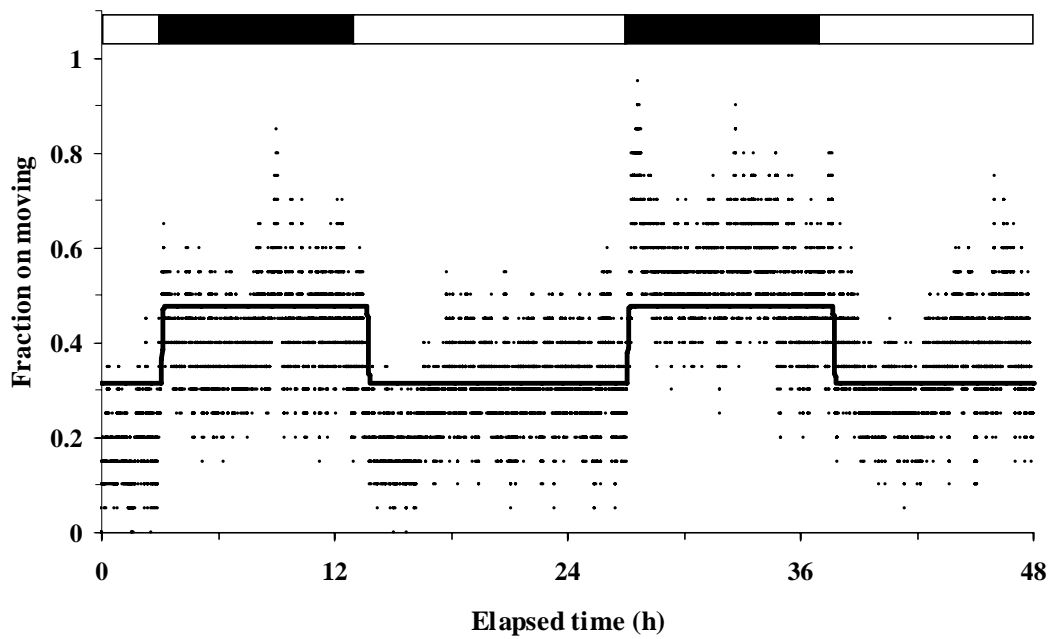


Figure 2.6 The fraction on moving of *N. kinbergii* ($n = 20$) under artificial light (14L:10D). Observations were made for 48 hours and are expressed as raw data (upper figure) and 9 point mean (lower figure). The black and white bars indicate night time and day time, respectively. Experiments started at 5:00 PM.

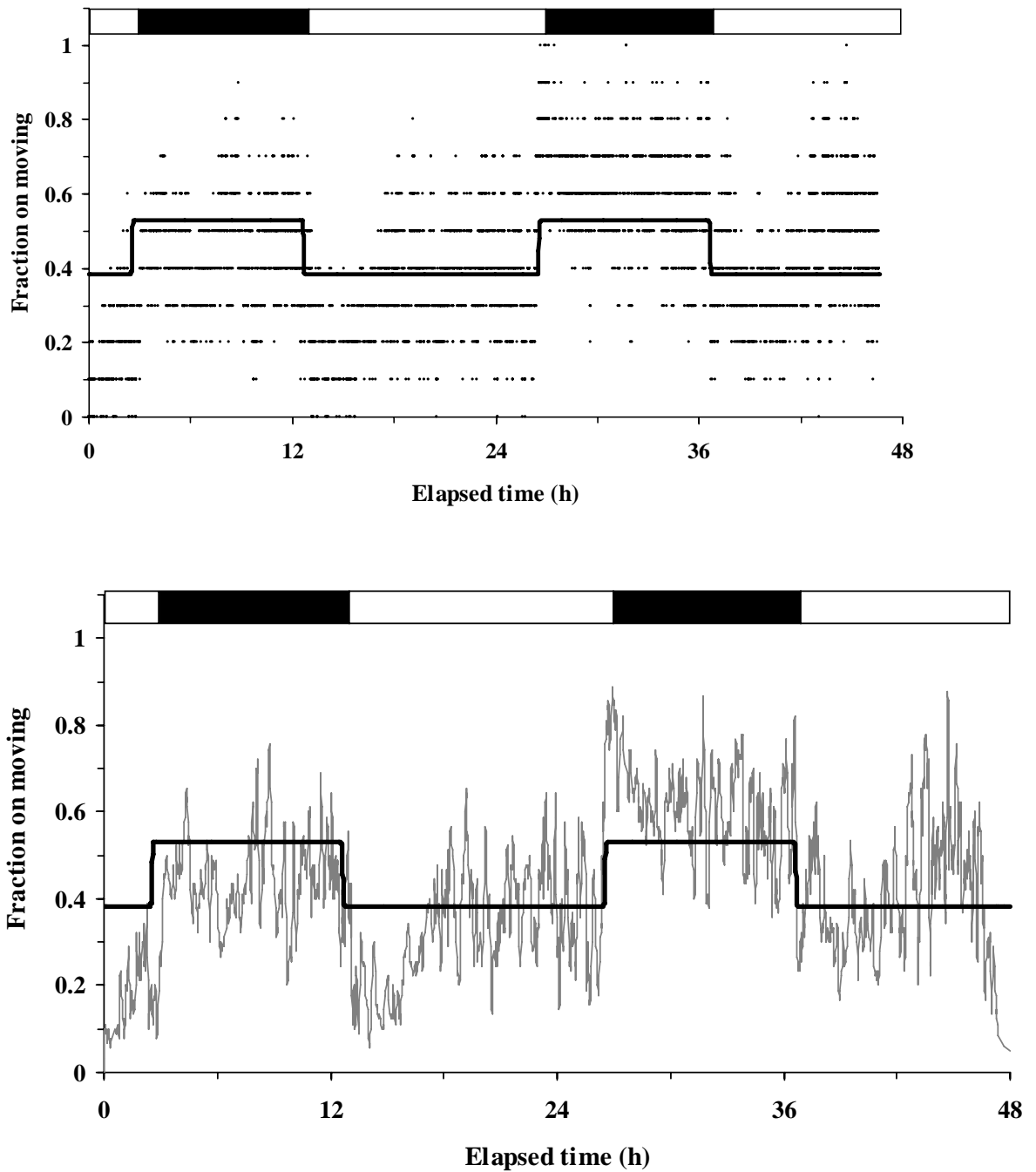


Figure 2.7 The fraction on moving of female *N. kinbergii* ($n = 10$) under artificial light (14L:10D). Observations were made for 48 hours and are expressed as raw data (upper figure) and 9 point mean (lower figure). The black and white bars indicate night time and day time, respectively. Experiments started at 5:00 PM.

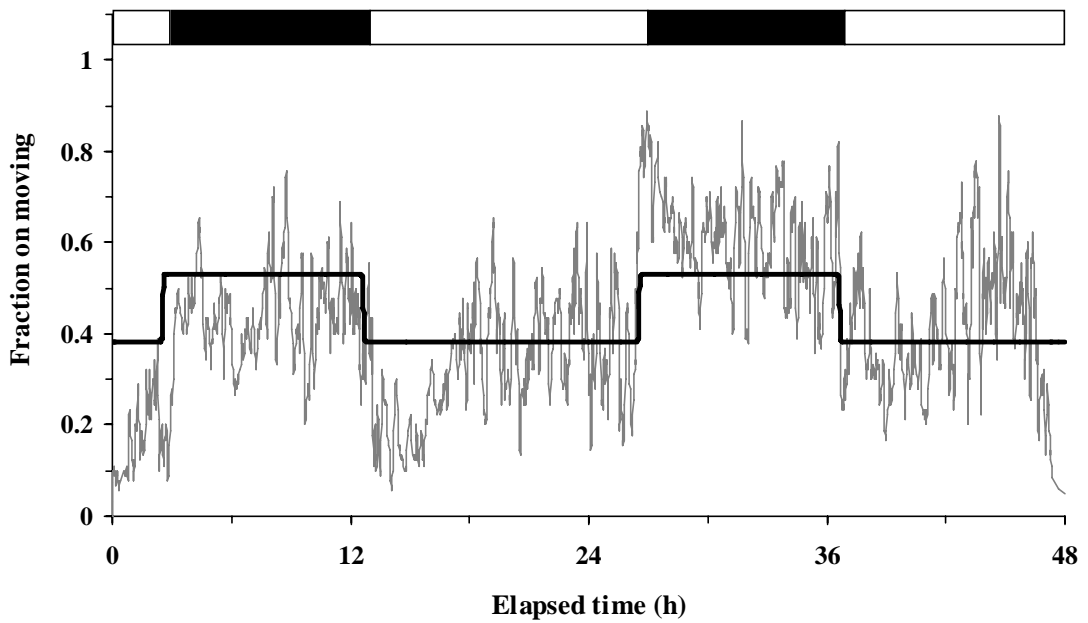
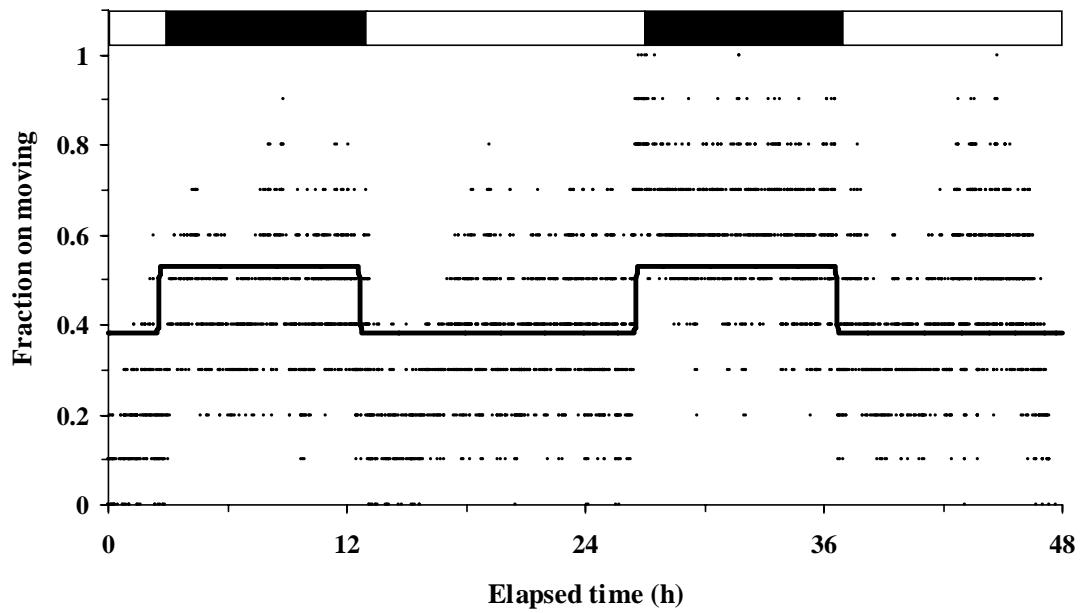


Figure 2.8 The fraction on moving of male *N. kinbergii* ($n = 10$) under artificial light (14L:10D). Observations were made for 48 hours and are expressed as raw data (upper figure) and 9 point mean (lower figure). The black and white bars indicate night time and day time, respectively. Experiments started at 5:00 PM.

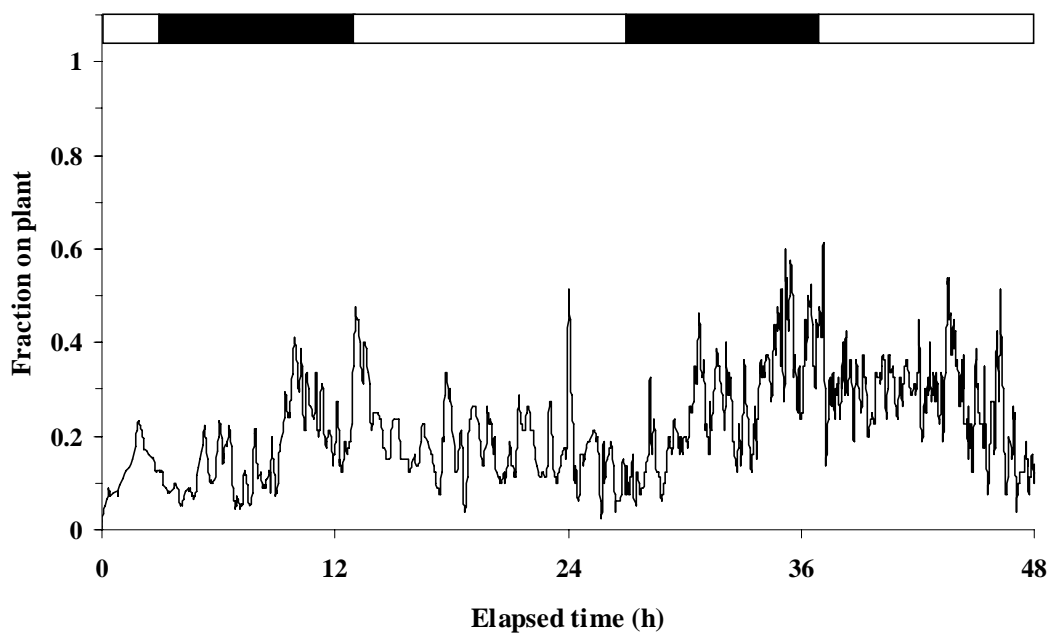
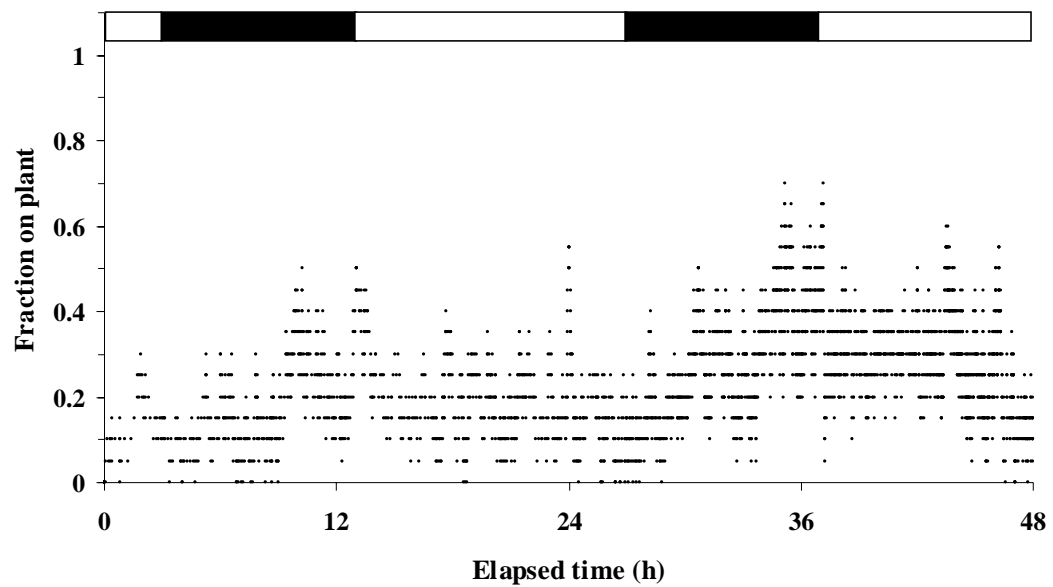


Figure 2.9 The fraction on plant of *N. kinbergii* (n = 20) under artificial light (14L:10D). Observations were made for 48 hours and are expressed as raw data (upper figure) and 9 point mean (lower figure). The black and white bars indicate night time and day time, respectively. Experiments started at 5:00 PM.

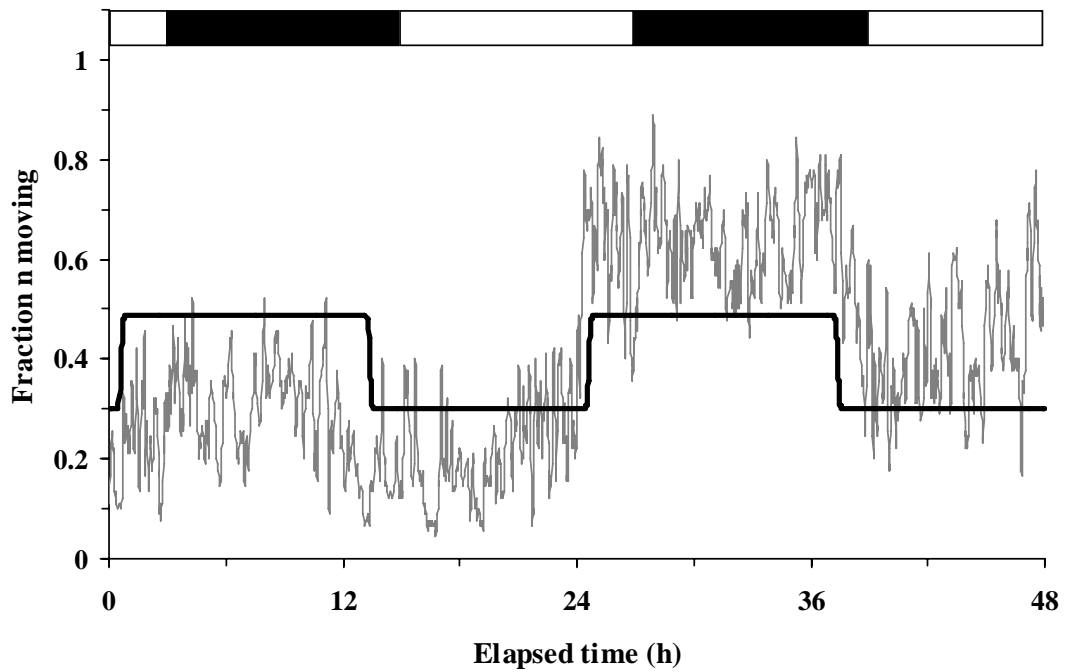
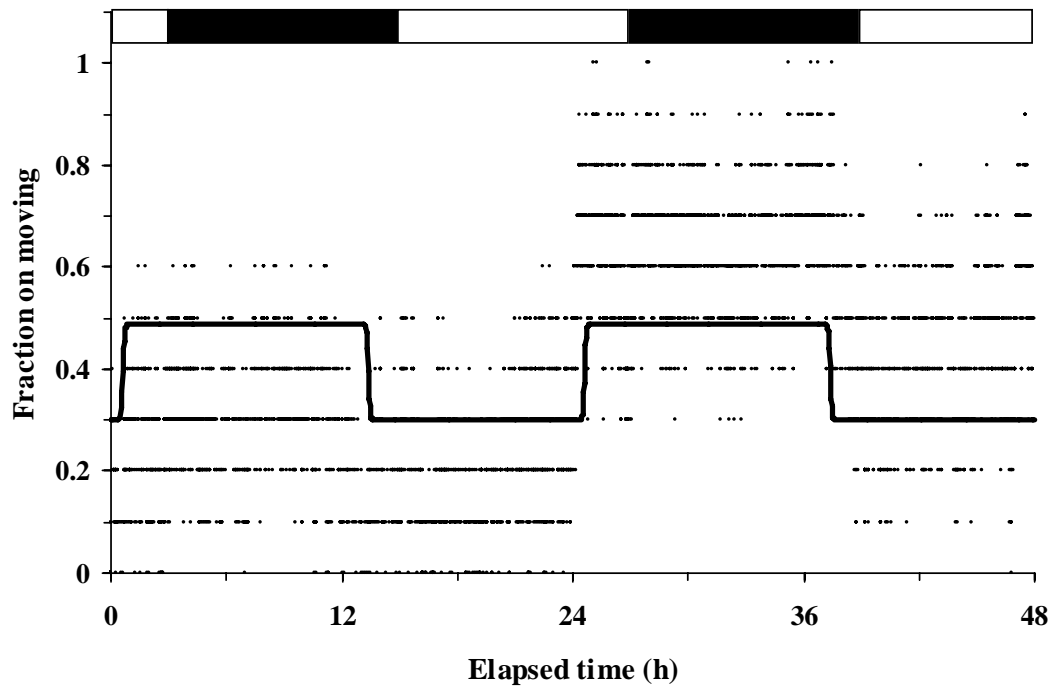


Figure 2.10 The fraction on moving of *N. kinbergii* ($n = 10$) under artificial light (12L:12D). Observations were made for 48 hours and are expressed as raw data (upper figure) and 9 point mean (lower figure). The black and white bars indicate night time and day time, respectively. Experiments started at 5:00 PM.

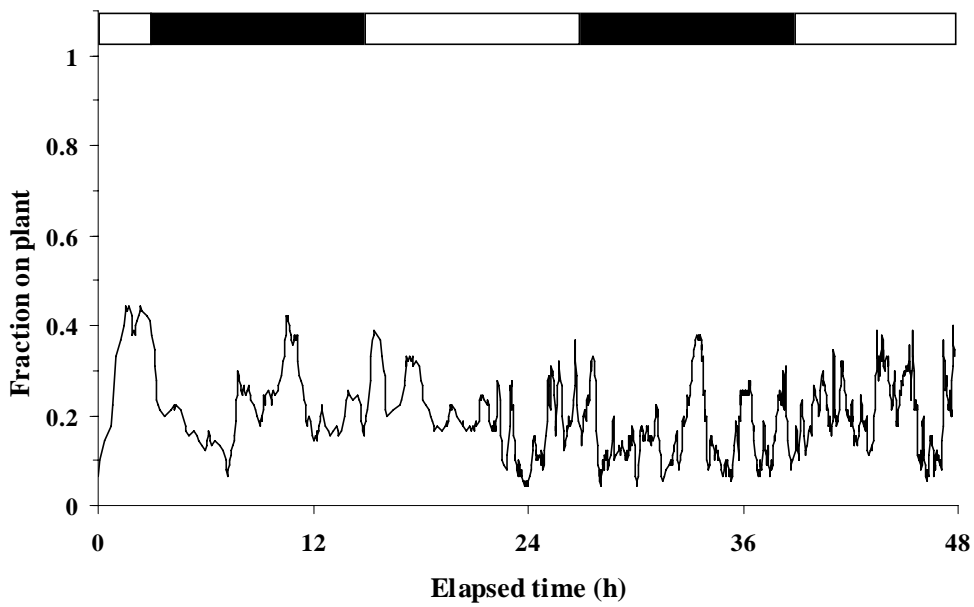
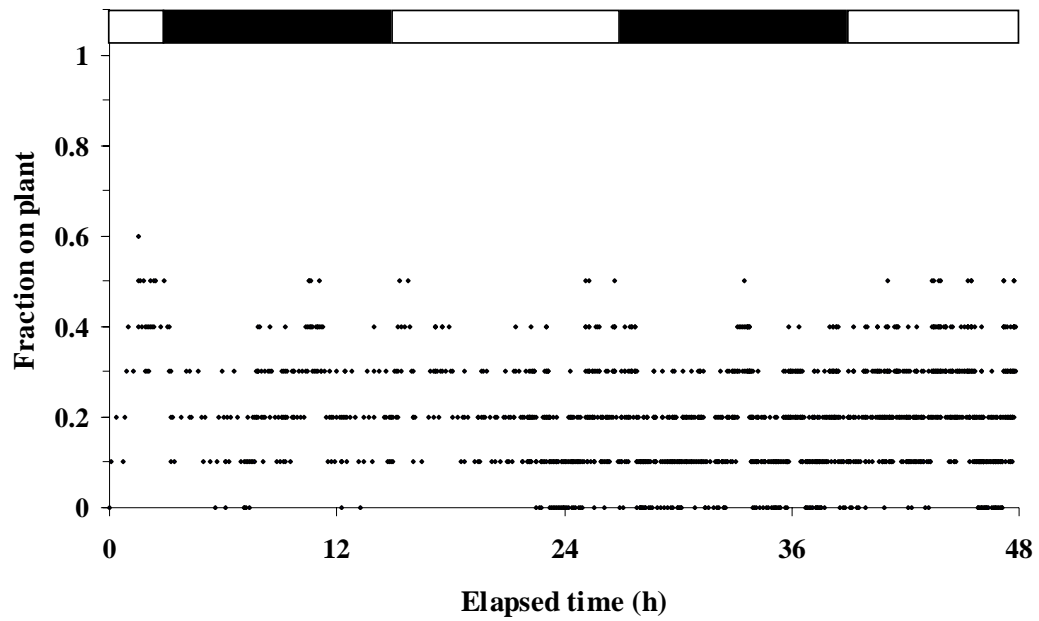


Figure 2.11 The fraction on plant of *N. kinbergii* ($n = 10$) under artificial light (12L:12D). Observations were made for 48 hours and are expressed as raw data (upper figure) and 9 point mean (lower figure). The black and white bars indicate night time and day time, respectively. Experiments started at 5:00 PM

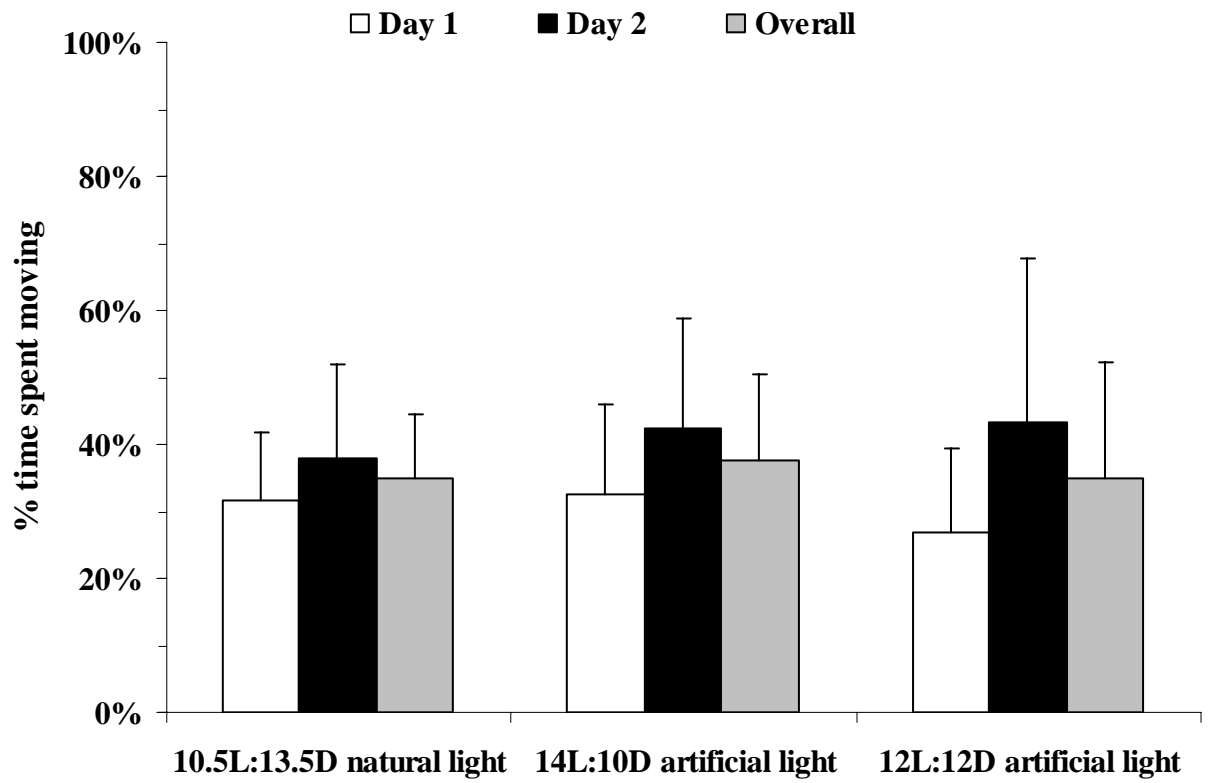


Figure 2.12 Overall mean (\pm SD) percentage of time spent moving by *N. kinbergii* observed under different light conditions: 10.5L:13.5D, natural light (n = 8); 14L:10D, artificial light (n = 20), 12L:12D, artificial light (n = 10).

4. DISCUSSION

The sigmoidally transformed cosine curves (Figures 2.4, 2.5, 2.6 & 2.10) fitted the frequency of movement data for *N. kinbergii*, which indicates that they behaved differently in the light than in the dark. They seemed to spend more time moving in the dark under both natural and artificial lighting. This indicates that activity of nabids occurs mainly in the night time. This is in accordance with results from Leathwick & Winterbourn (1984), who demonstrated that *N. kinbergii* had a greater feeding rate on lucerne aphids at night than that during the day. The results reported here also agree with observations conducted by Dicker (1946) and Fewkes (1961). According to Dicker (1946), *N. lativentris* exhibited its greatest nocturnal activity some time after dusk. Although Fewkes (1961) did not come to any conclusions about the nocturnal activity of *Nabis* spp, his data showed higher collection rates at night, which may reflect higher mobility in the upper parts of the plants. Furthermore, 80% of the feeding activity of nabids on soybean and corn was recorded at night between 9:00 PM and 6:00 AM (Pfannenstiel & Yeargan, 2002). By contrast, Wade *et al.*, (2005) concluded that the time of day did not significantly affect activities of *N. kinbergii*. They found no difference in the activities of *N. kinbergii* on cotton between night and day (Wade *et al.*, 2005). However, similar to Siddique's (1985) work, *N. kinbergii* behaviour in Wade *et al.*'s (2005) study was not recorded continuously. Also, periodic observations could have disturbed the nabids and affected their activity patterns.

With respect to the low levels of activity in the morning, Siddique (1985) observed that the time *N. kinbergii* spent moving in the morning was less than at other times of the day. This result is in line with present study. However, he suggested that the cold temperature in the morning was the main factor affecting the crawling pattern of nabids. Because the temperature in the present study was constant, it is unlikely that the low morning activity is a consequence of temperature fluctuations alone. In addition, compared to our results, the timing of the period spent motionless was quite different in Siddique's study. Motionless periods in Siddique's study were longer at midnight than in the afternoon and evening, whereas in our study *N. kinbergii* was very active at midnight. Furthermore, *N. kinbergii* was less active in the morning than in the afternoon in all experiments reported here (with the exception of the first day under the natural photoperiod), which indicates that there is further complexity in the circadian rhythms of behaviour. By contrast, Wade *et al.* (2006) observed no effect of time of day between 06:00 and 18:00 (daylight hours) on capture of immature *N. kinbergii* with a beat sheet in cotton at Jimbour, Queensland, Australia. This suggests that circadian rhythms of behaviour do not affect sampling efficiency in cotton.

Wade *et al.*, (2005) reported that *N. kinbergii* spent most of the time (ca. 80%) stationary during experimental observations. However, the time *N. kinbergii* spent moving presented here was more than 30 % (Figure 2.11). A possible explanation is that both of the earlier studies the activities of *N. kinbergii* (Siddique, 1985; Wade *et al.*, 2005) were observed by the naked eye, hence it is hard to distinguish the difference between completely motionless and standing still with small movements.

The possible difference between the two days of observation indicated that *N. kinbergii* was likely more active during the second day than the first day. A possible explanation is that there was less food available during the second day. There was also a difference in handling of nabids before videoing between the 12L:12D photoperiod and the other two experiments. Nabids used in experiments under 14L:10D and 10.5L:13.5D photoperiods were reared in small containers with larval diamondback moth and green peach aphids provided as food, while the nabids under the 12L:12D photoperiod were fed on blue-green aphids in a big cage. Diamondback moth has been known to be a more nutritional food for *N. kinbergii* than aphids (Siddique & Chapman, 1987; see Chapter 3). Thus, the nabids reared under the 12L:12D photoperiod were possibly more hungry and more used to actively searching for their aphid food than the nabids used in the other experiments. Many insects can learn the attributes of resources and can exploit them better as a result (Bell, 1990), which may have been the case in this instance. Predators with previous experience of searching for the same prey would find prey faster and more effectively than those without experience as a consequence of their experience with the prey's habitat, appearance and behaviour (Nomikou *et al.*, 2005). In each experiment, 30 blue-green aphids were provided at the start of the experiment. It is possible that nabids that were fed on *P. xylostella* and *M. persicae* (10.5L:13.5D and 14L: 10D) did not eat as many aphids during the first day because they were not as hungry. In contrast, the nabids that were fed *A. kondoi* (12L:12D) may have consumed more aphids during the first day, either as a result of greater hunger or prior learning associated with feeding on this species. In this case, they would increase their movement in search of food during the second day because of hunger. Similarly, the predator *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) had a higher feeding rate on aphids in the day time since they had difficulties with prey capture in the dark phase (Perdikis *et al.*, 2004). The circadian rhythm of searching behaviour in the mantid *Hierodula crassa* (Mantodea: Mantidae) is affected by hunger, so both time of day and gut contents determine prey capture rates (Holling, 1966). Siddique (1985) also observed that nabids without prey crawled significantly longer in the morning than nabids with prey. Thus, *N. kinbergii* in a 12L:12D photoperiod may have found prey

easier or faster than others and produced a shortage of food during the second day. In brief, the physiological state of insects also may affect the searching behaviour of this predator.

It was possible to see the effect of light on the behaviour of *N. kinbergii*, particularly when darkness was approaching. They increased the speed and frequency of moving, in other words they were more active at the light-dark transition. Nabids have compound eyes that are known to be sensitive to photoperiodic signals (Morita & Numata, 1999; Nakamura & Hodkova, 1998). Thus, the behavioural activities of nabids might be affected by the duration of daylight hours. The similar overall of percentage of time spent moving among experiments shows that the number of light hour hours seems not to be an important factor that affects the overall activity of *N. kinbergii*.

The influences of the different types of light on behaviour of insects have been demonstrated, particularly artificial light (Shields, 1989). Artificial light has a different spectral composition from natural light. This may lead insects to behave differently, particularly those insects that use visual cues to locate their food (Shields, 1980). The light system used in this study was established with the aim to minimise the disruption of the insects' behaviour as Shields (1989) suggested (Appendix 1). However, the amplitude of the fitted regression line was greater under natural lighting than under artificial lighting. This suggests that circadian rhythms were more distinct under natural light (Table 2.2). Despite that, there was not much difference in overall activities of predators between natural light and artificial light. The difference that occurred at 12 hour photophase may have come from the physiological state of the insects, as discussed above, and uncontrolled fluctuations in temperature under natural lighting. The temperature under the natural photoperiod (22 – 27 °C) was not constant as that in the controlled photoperiods (constant 25 °C). The results suggest that there is a circadian rhythm if the bugs obtain enough food at night but even then they may get hungry during the afternoon. If they do not get enough food at night, their circadian rhythm may become less distinct, and they are likely to hunt more readily during the daytime.

There were no differences in activity between males and females. According to Ma *et al.*, (2005), consumption rates of females are higher than those of males. This may suggest that females are more efficient in hunting for prey. Ideally, this should be confirmed by experiments using larger numbers of nabids and by keeping track of the movements and numbers of prey items consumed by each individual. It was impossible to clearly record the predatory behaviour of *N. kinbergii* with the equipment that was used in this study due to limitations in the resolution of video images.

The foraging behaviour of predators can also be influenced by other factors such as plant structure, prey density and distribution. The more complex the plant structure, the more time is likely to be spent searching for prey. In addition, plant structure has an effect on prey capture success. For example, the rate of parasitism of *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) decreased on complex plants compared with simple plants (Gingras & Boivin, 2002). The plants used in the present study were simpler than those used in either Wade *et al.*'s (2005) or Siddique's (1985) studies. Only one lucerne shoot was used in each observation compared with a cotton plant in a pot (Wade *et al.*, 2005) and two lucerne shoots (Siddique, 1985) used in the earlier studies. As Perdikis *et al.* (2004) suggested, prey distribution and density are factors that influence the searching behaviour of predators. Other studies showed that the predatory behaviour of the predatory mites *Phytoseiulus persimilis*, *Typhlodromus occidentalis* and *Amblyseius andersoni* depend on prey density and distribution (Zhang *et al.*, 1992).

The nabids seemed to spend more time on the plants in the night than in the day in one experiment (10.5L:13.5D). The present result shows that there is no distinct increase in time spent on plant when lights go on or off. Only the tendency to be on plants increased in the dark phase. This indicates that feeding activities and/or movements of nabids occurred more often in low light intensity. Similarly, Wade (2005) found more *N. kinbergii* on the plant at night than during the day. However, he did not provide any explanation for this finding. According to Fewkes (1961), the vertical movements of nabids are possibly a reaction to changing environmental conditions. This is in agreement with the results presented here: In the controlled environment (constant 25 °C, 14L:14D and 12L:12D), the photoperiod did not influence the presence of *N. kinbergii* on and off the plant, whereas in the natural lighting conditions there was a greater presence on the plant during the dark when temperatures were somewhat lower. In addition to vertical movement, nabids may move onto the plant to search for prey. Almost all blue-green aphids remained on the plant, unless nabids disturbed them. After being disturbed, they recovered very quickly and came back onto the plant. Thus, the movements of nabids onto plants may be influenced by changing environmental conditions and hunger. However, Wade *et al.* (2005) reported that *N. kinbergii* killed more larvae of bollworm in the day time than at night. In addition, he found more bollworm larvae on fruit during the day than at night. He also observed that significantly more nabids on plants during the night than during the day. Therefore, his results may have been influenced by the behaviour of the prey and the structures of the cotton plants. Similarly, Leathwick &

Winterbourn (1984) collected more lucerne aphids (1.8 times) and fewer nabids (4 times) in the day time than at night.

The underlying behavioural pattern of these predators shows a circadian rhythm that seems to be influenced by photoperiod. Therefore, the time of the day is a crucial element to be considered when conducting behavioural experiments on these natural enemies. In particular, experiments in the early morning are likely to yield little results due to the nabid's relative inactivity at this time of the day.

CHAPTER 3

DEVELOPMENT, SURVIVAL AND REPRODUCTION OF *NABIS KINBERGII* WHEN FEEDING ON DIFFERENT PREY

1. INTRODUCTION

Most insect predators are polyphagous (Symondson et al., 2002). They can consume a variety of species that differ in nutritional value. Some prey species may be toxic or offer poor nutrition to a predator. Such food may cause slow growth, prevent complete development or restrict reproduction in predators. For instance, some aphids are known to be toxic to ladybirds, spiders, harvestman and carabid beetles (Hodek & Honc'k, 1996; Toft, 2005). In contrast, other prey are non-toxic and highly nutritious to predators. These types of prey can promote higher developmental rates, longevity, survival and fecundity of predators. For instance, the big-eyed bug, *Geocoris punctipes* (Hemiptera: Lygaeidae) can complete development, reach maturity and have greater longevity when it feeds on corn earworm eggs, *Helicoverpa zea* (Lepidoptera: Noctuidae) instead of pea aphid, *Acyrtosiphon pisum* (Hemiptera: Aphididae) (Waddill and Shepard, 1974). Likewise, minute pirate bug, *Orius insidiosus* (Hemiptera: Anthocoridae) was reported to have higher fecundity when it fed on soybean thrips, *Neohydatothrips variabilis* (Thysanoptera: Thripidae), than when it fed on soybean aphids, *Aphis glycines* (Hemiptera: Aphididae) (Reitz et al., 2006). On the other hand, the nutritional requirements of insect predators vary among species. With one prey species, a particular predator may complete development, but with another it may not. For example, pea aphid has been considered to be a toxic prey species for *G. punctipes*, but it is suitable for female ladybird, *Harmonia axyridis* (Coleoptera: Coccinellidae), to lay eggs (Evans & Gunther, 2005). Similarly, mixing prey species can give positive effects to one predator, but negative or neutral effects to another. For instance, *Lycosa* sp. had higher survival and growth rate when fed on a combination of crickets, fly grubs, mealworms and beetles than when fed on crickets only (Uetz et al., 1992). Toft & Wise (1999) reported that the aphid *Aphis nerii* (Homoptera: Aphididae) is toxic to wolf spiders *Schizocosa* sp. (Araneae: Lycosidae) whether it was consumed alone or mixed with higher quality prey. Apart from prey species, other factors that could affect survival and reproduction of predators include temperature, prey quantity and oviposition substrate (Gotoh et al., 2004; Mori et al., 2005). The evidence suggests that development and reproduction of predators are likely to be affected by different types of prey, including different species, in single or mixed diets. Therefore, the responses of a particular predator with a particular prey type need to be

quantified in order to understand how the predator will respond to the nutritional resources that are offered by the prey.

Nabis kinbergii is a polyphagous predator that can consume a wide range of prey species. The results of gut content analysis conducted by Hosseini (2007) showed that *N. kinbergii* collected in brassica crops in South Australia consumed four pest species: cabbage aphid, *Brevicoryne brassicae* (Homoptera: Aphididae); green peach aphid, *Myzus persicae* (Homoptera: Aphididae); diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae); and cabbage white butterfly, *Pieris rapae* (Lepidoptera: Pieridae). In addition, *N. kinbergii* is known to feed on insect pests of lucerne (Leathwick & Winterbourn, 1984) and cotton (Leathwick & Winterbourn, 1984; Wade *et al.*, 2005). Like other predators, the development of *N. kinbergii* has been found to be correlated with prey species. *Nabis kinbergii* spent more time in the 5th instar when reared on pea aphid compared with Australian crop mirid, *Sidnia kinbergi* (Hemiptera: Miridae) and *P. xylostella* (Siddique, 1985). The effects of prey species on development have also been observed in other nabids, including *Nabis alternatus*, *Nabis americanoferus* and *Nabis roseipennis* (Guppy, 1986; Nadgauda & Pitre, 1986). There may be both positive and negative effects of different prey species on the developmental time of nabids in general and *N. kinbergii* in particular. *N. kinbergii* may develop faster when feeding on one prey species, but respond poorly with another prey. This should be investigated in more detail with *N. kinbergii*.

In addition to influencing the developmental time, the prey species can affect the survival of the immature stages of nabids. Some species are a favourable food source that promote the survival of a predator during development, but others can contribute to the death of young individuals. Siddique (1985) reported that the number of nymphs of *N. kinbergii* that died before reaching adulthood was the highest when fed on *A. pisum* (83%) compared with individuals fed on *S. kinbergi* (28%) and *P. xylostella* (and 27%). Similarly, some *N. alternatus* reached maturity when reared on *A. pisum* (= *Microsiphum pisi*) and lygus bugs, *Lygus hesperus* and *Lygus elisus*, but they all died when reared on alfalfa weevil larvae, *Hypera postica* (= *Phytonomus posticus*) (Coleoptera: Curculionidae) (Taylor, 1949). Information on the effects of prey species on immature survival is needed for rearing nabids and other predators.

Prey quality can have positive or negative effects on the longevity of a predator. Female *N. kinbergii* fed on *P. xylostella* lived two times longer (58.6 days) than those fed on *A. pisum* (24.7 days) (Siddique & Chapman, 1987). At 20 °C, *N. roseipennis* lived up to 71 days with

nymphs of *L. lineolaris* as food, whereas they lived up to just 58 days if larval *H. virescens* were their diet. However, there was no difference in quality of the two diets when experiments were conducted at 25 °C and 30 °C (Nadgauda & Pitre, 1986). Surprisingly, beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), that had eaten *Bt*-cotton did not influence the longevity of *Nabis* sp. compared with the control, indicating that the plant variety did not affect the nutritional quality of the prey in this instance (Ponsard *et al.*, 2002).

Longevity sometimes varies between the two sexes of a predator.. With *L. lineolaris* and *H. zea* (Lepidoptera: Noctuidae) as prey, adult female *Tropiconabis capsiformis* lived an average of 6 days longer than males (Hormchan *et al.*, 1976).

Along with longevity, the fecundity of nabids can be influenced by different diets. For example, in a study conducted by Siddique (1985), *N. kinbergii* was fed on three prey species at 27-28 °C. The average number of eggs laid per day was 11.7, 14.5 and 20.0 when reared on *A. pisum*, *S. kinbergii* and *P. xylostella*, respectively. In addition, the total number of eggs laid per female *N. kinbergii* was greater when it fed on *P. xylostella* than when it fed on *A. pisum* (averaging 794 eggs compared with 198 eggs). In contrast, under laboratory conditions (28 °C, 15L:9D photoperiod) *T. capsiformis* laid just 105 eggs when 2nd instar *L. lineolaris* and larval *H. zea* served as food (Hormchan *et al.*, 1976). Taylor (1949) observed that *N. alternatus* laid an average of 127 eggs during their lifetime when reared on a range of alfalfa pests at 19.5-31 °C. When this species fed on *Estigmene acrea* (Lepidoptera: Arctiidae) at 28 °C, the number of eggs was about 223 eggs per female (Perkins & Watson, 1972). These results suggest that prey species was the main factor which affected the reproduction rate of *N. alternatus* rather than temperature. In general, prey species is likely to be a key factor that influences the fecundity of predators.

Some researchers have found that a mixed diet is more beneficial than a single diet on the life history characteristics of a predator (Butler & O'Neil, 2007; Evans *et al.*, 1999). However, Siddique (1985) found that there was no difference between a mixed diet and a single diet.

In addition to prey species, the reproductive rates of nabids also change in response to changes in temperature, quantity of prey, and ovipositional substrate. For instance, the rate of reproduction of *N. kinbergii* (= *N. capsiformis*) was quite sensitive to temperature (Samson & Blood, 1979). They laid more eggs when caged at 23 °C and 28 °C than those caged at 18 °C. Similarly, egg production of three *Nabis* species (*N. americanoferus*, *N. roseipennis* and *N. rufusculus*) was higher at 27 °C than at 21 °C (Braman & Yeagan, 1988). Food availability

was one factor contributing to the reproductive rate of female nabids. Siddique & Chapman (1987) reported that there was a positive correlation between rate of reproduction and rate of prey consumption of *N. kinbergii*. Moreover, the size and quality of the ovipositional substrate can affect the reproductive rate of a predator. For example, the greatest number of eggs of *N. americoferus* were laid on the stems of alfalfa when their diameters were 0.8 – 1.9 mm (Guppy, 1986). Braman & Yeargan (1988) stated that type of oviposition substrate contributed to the overall fecundity of *N. americoferus*, but not *N. roseipennis* and *N. rufusculus*. *Nabis americoferus* laid more eggs on soybean than on green bean. Thus prey species and a range of other factors are likely to be involved in determining the reproductive rate of *N. kinbergii*.

The effects of different diets on the fitness of *N. kinbergii* were examined by conducting two experiments. The first was carried out to determine how diet affects the developmental time, survival and longevity of *N. kinbergii*. Four diets were used in this experiment: *P. xylostella*, *B. brassicae*, *M. persicae* and a mixed diet that combined these three prey species. The second experiment was conducted to determine the effects of diet on the fecundity of *N. kinbergii*. One of two different prey species, *P. xylostella* and *M. persicae*, were fed to the nabids in this experiment.

2. MATERIALS AND METHODS

Experiments were carried out in a plant growth cabinet (Phoenix Biosystems, Envirotec PTY LTD, Australia). The environmental conditions were as follows:

Day temperature:	25 °C
Night temperature:	22 °C
Lights on:	7:00 AM
Lights full:	7:30 AM
Lights twilight:	8:30 PM
Lights off:	9:00 PM

The first experiment was conducted to determine the effects of prey species on development and survival. A newly hatched individual *N. kinbergii* was placed into a 100 ml clear plastic cup with a filter paper in the bottom. All newly hatched predators were collected from a colony that was reared on a mixture of cabbage aphids, green peach aphids and diamondback moths. The lid of the cup had pin holes for ventilation. A 20 mm fresh stem of canola was introduced into the cup as food for prey. The stem of canola and the filter paper were replaced daily. Each nabid was provided with one of four diets: *P. xylostella* only, *M. persicae* only, *B. brassicae* only and a mixed diet (a combination of *P. xylostella*, *M. persicae* and *B. brassicae*). All prey came from colonies that were maintained on canola in rearing cages. An excess number of prey was provided daily (approximately 20 and 6 for aphids and diamondback moths, respectively). The size of prey was matched to the stage of *N. kinbergii*. For example young larval *P. xylostella* were provided to young nabids and 3 – 4th instar *P. xylostella* was provided to mature nabids. The number of prey eaten was recorded at first, however it was hard to identify whether prey died from being attacked by nabids or from another cause. Furthermore, the reproduction of green peach aphids made it impossible to determine exact numbers that were attacked by nabids. Therefore, the attempt to determine numbers of prey consumed by nabids was abandoned. The status of *N. kinbergii* was recorded daily including its moulting and survival. The developmental time, longevity and survival of each individual was recorded. The experiment ended when *N. kinbergii* died. There were 40 replicates for each treatment.

The duration in days of each instar and the overall developmental time of *N. kinbergii* supplied with different diets was analysed by one-way ANOVA. The adult longevity of females and males on each diet was compared by using *t*-tests ($P < 0.05$).

The second experiment was conducted to determine the influence of different diets on the reproduction of *N. kinbergii*. First instar *N. kinbergii* were divided into two groups. One of them was fed on *P. xylostella*, and the other was fed on *M. persicae*. *B. brassicae* was not included in this experiment because *N. kinbergii* did not usually reach maturity when it was reared on this prey species. Each nabid was provided with an excess number of prey. The size of prey was matched with the size of predator as above. Males and females from these two groups were put into 750 ml plastic jars in pairs with a stem of canola about 5 cm long as an oviposition substrate. Any dead male *N. kinbergii* was replaced by another male. A wet cotton wick was also placed into the jar to provide moisture. Food and fresh canola stems were replaced daily. The number of eggs was recorded daily. The canola stems with eggs were placed into 100 ml plastic cups and monitored daily for 14 days. There were 23 and 17 replicates for *P. xylostella* and *M. persicae*, respectively.

The possibility of a difference in the frequency of oviposition between treatments was tested with a Chi-squared test. Differences in the overall numbers of eggs laid and % egg hatch were analysed with *t*-tests.

3. RESULTS

There were significant differences in the developmental times of *N. kinbergii* when reared on four different diets (Table 3.1). Some statistical differences were detected in the developmental times of each instar. Some of these differences were within the margin of error because the insects were checked only once each day. Clear significant differences were found in overall developmental times. The overall developmental times of *N. kinbergii* fed on *P. xylostella* and mixed prey (about 15 days) were significantly shorter than those fed on *B. brassicae* and *M. persicae* (approximately 20 days).

The longevity of adult *N. kinbergii* was also influenced by the types of food provided. The number of days female and male *N. kinbergii* lived was similar on any given diet, but differences among diets were observed (Table 3.2). It was not possible to compare effects of all diets on the difference in longevity between the sexes because the number of *N. kinbergii* reared on *B. brassicae* was not large enough (one female, two males). Adult *N. kinbergii* had the greatest longevity with the mixed diet, whereas the poorest diet was with *B. brassicae*.

When the total time from first instar to death was analysed, survival of *N. kinbergii* on the mixed diet was found to be longest, followed by *P. xylostella*, *M. persicae* and *B. brassicae* (Figure 3.1). The longest living individual of *N. kinbergii* reared on *B. brassicae* reached only 33 days of age. In contrast, the longest a *N. kinbergii* fed on the mixed diet lived was 175 days. Nabids that had been fed the mixed diet lived significantly longer than those that had been fed *P. xylostella* (Wilcoxon $\chi^2 = 203.98$, 3 *df*, $P < 0.0001$).

Table 3.1 Duration in days of each instar of *N. kinbergii*, supplied with different prey species under laboratory conditions (22-25 °C, 14L:10D). Means within a row, followed by the same letter are not significantly different ($P < 0.05$).

Instar	<i>B. brassicae</i>	<i>P. xylostella</i>	<i>M. persicae</i>	Mixed diet
	Mean \pm SE (n)	Mean \pm SE (n)	Mean \pm SE (n)	Mean \pm SE (n)
1 st	4.18 \pm 1.85a (28)	2.93 \pm 0.08b (40)	2.72 \pm 0.07b (39)	2.18 \pm 0.06c (40)
2 nd	4.17 \pm 0.33a (18)	2.45 \pm 0.08c (40)	3.38 \pm 0.09b (37)	2.35 \pm 0.08c (40)
3 rd	3.33 \pm 0.26a (12)	2.21 \pm 0.07c (38)	2.66 \pm 0.13b (35)	3.00 \pm 0.08ab (39)
4 th	3.25 \pm 0.26ab (4)	2.92 \pm 0.08b (37)	3.29 \pm 0.08a (34)	3.13 \pm 0.08ab (39)
5 th	4.67 \pm 0.63b (3)	4.76 \pm 0.10b (37)	8.09 \pm 0.28a (34)	5.10 \pm 0.08b (39)
1 st -5 th	20.0 \pm 0.58a (3)	15.24 \pm 0.12b (37)	19.71 \pm 0.54a (34)	15.77 \pm 0.20b (39)

Table 3.2 Comparison of adult longevity of females and males that had been reared on different diets under laboratory conditions (22-25°C, 14L:10D).

	<i>B. brassicae</i>	<i>P. xylostella</i>	<i>M. persicae</i>	Mixed diet
	Mean \pm SE (n)	Mean \pm SE (n)	Mean \pm SE (n)	Mean \pm SE (n)
Female	14 (1)	72.36 \pm 3.60 (25)	17.90 \pm 1.29 (20)	128.5 \pm 5.45 (24)
Male	10 (2)	69.50 \pm 6.46 (12)	17.00 \pm 1.70 (14)	123.67 \pm 4.66 (15)
<i>t</i>		0.386742	0.421556	0.674369
<i>P</i>		> 0.05	> 0.05	> 0.05

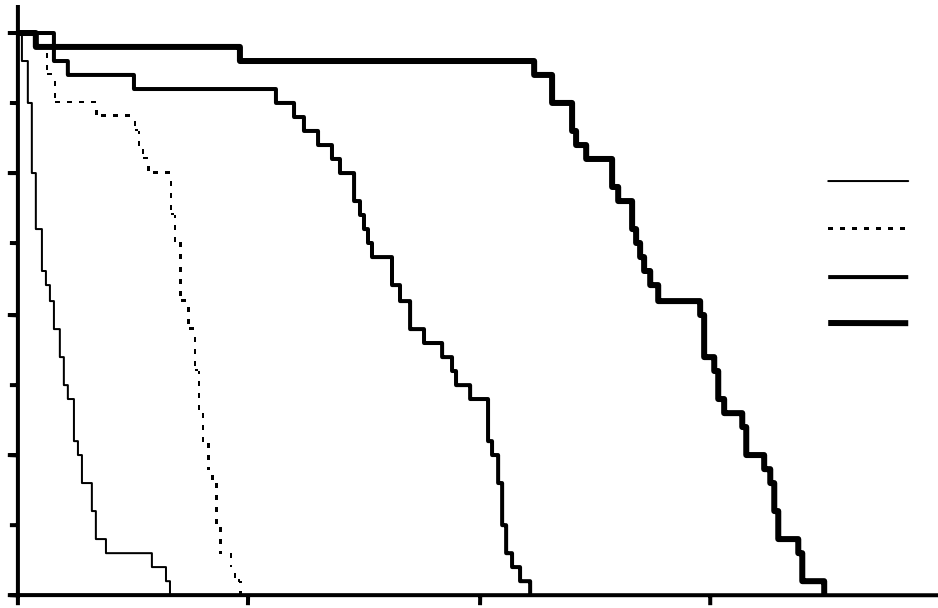


Figure 3.1 Survival from egg hatch of *N. kinbergii* under laboratory conditions (22-25 °C, 14L:10D).

Reproduction was affected by the diet of the *N. kinbergii*. The first female laid eggs on the second day after pairing, but the consistently greater oviposition started from day four for *N. kinbergii* fed on *P. xylostella* and day six for those fed on *M. persicae* (Figure 3.2). The fraction of females that laid eggs in both treatments was not significantly different (Table 3.3). However, there was a significant difference in the number of eggs laid per day per female between the two treatments. More eggs were laid in a day by females fed on *P. xylostella* than on *M. persicae*. Both groups of females laid eggs every day from the beginning of oviposition. Overall egg numbers during the 14 days were significantly lower when females were reared on *M. persicae*. The percentage of eggs hatched was significantly lower when females were reared on *M. persicae* than when they were reared on *P. xylostella*.

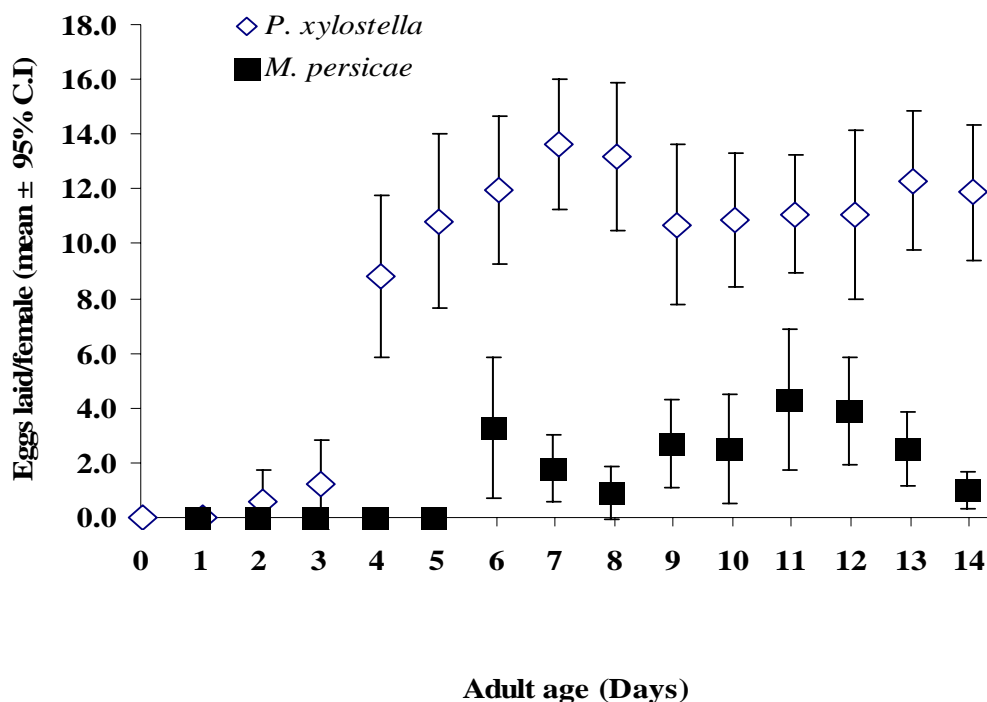


Figure 3.2 Number of eggs laid per day per female when reared on different prey species under laboratory conditions (22-25 °C, 14L:10D). Data are plotted as mean number of eggs laid per female. Only females that laid eggs were included in the analysis.

Table 3.3 Egg production of female *N. kinbergii* fed on two different prey species (*P. xylostella* and *M. persicae*) within 14 days of eclosion under laboratory conditions (22-25 °C, 14L:10D). Only females that laid eggs were included in the analysis.

	<i>P. xylostella</i> (n = 23)	<i>M. persicae</i> (n = 17)	Test statistic	P-value
No. females that laid eggs	23	10	$\chi^2 = 2.88$	$P = 0.09$
Mean overall egg numbers	128.13	22.90	$t = 10.71$	$P < 0.001$
% egg hatch	80.95	53.75	$t = 2.57$	$P < 0.05$

4. DISCUSSION

The larval development of predators depends on prey suitability (Cabral *et al.*, 2006). The present results show that there are significant differences in developmental times of *N. kinbergii* among four diets. With an excess number of prey and the same rearing environmental conditions, the difference is certainly due to the different prey types. *P. xylostella* appeared to be a highly suitable food for *N. kinbergii*, producing the shortest larval developmental time. Cabral *et al.*, (2006) concluded that less suitable food leads to longer developmental times. Thus, the two aphids *B. brassicae* and *M. persicae* are the less suitable food for the development of *N. kinbergii*. In line with this result, Siddique & Chapman (1987) observed that *A. pisum* was a less favourable food for development of *N. kinbergii* than *P. xylostella*. They reported that the time from egg to adult was longer when *N. kinbergii* fed on *A. pisum*. According to Siddique & Chapman (1987), prey quality is a factor that affects the larval development of *N. kinbergii*. A similar conclusion was made by many authors who worked on other predators such as ladybird beetle larvae (Cabral *et al.*, 2006, Hodek & Honc'k, 1996). Thus, it is reasonable to conclude that the nutritional value of *P. xylostella* is higher than *B. brassicae* and *M. persicae*. *N. kinbergii* had the same developmental time with single prey *P. xylostella* and the mixed diet. This indicates that mixing of prey species in this study did not have a positive effect on the larval development of *N. kinbergii*. Similarly, Toft & Wise (1999) reported that the development of spiderlings of the wolf spider, *Schizocosa* sp., was not different between a mixed diet of the collembolan *Tomocerus bidentatus* (Collembola: Entomobryidae) and fruit flies, and a single diet of *T. bidentatus*. Thus, the development of larval *N. kinbergii* is likely influenced by the different prey species, but a mixed diet does not necessarily increase the developmental rate over a single highly nutritious prey species.

The survival rate of nymphs can indicate how the quality of prey influences a predator. Only 7.5 % of *N. kinbergii* reared on *B. brassicae* reached adulthood. The survival rate on the other three diets was much higher (Figure 3.1). It is likely that this difference is indicative differences in quality of the food, and that *B. brassicae* is either nutritionally deficient or even toxic. Similar results have been found from other predator – prey systems. Similar to *B. brassicae*, *A. pisum* was the food that led to the highest mortality rate to immature *N. kinbergii* (Siddique, 1985). In addition, the rate of nymphal survivorship of *T. capsiformis* was 19 % at the 1st instar and decreased to 0 % at 5th instar when *Lygus lineolaris* and *H. zea* served as food (Hormchan *et al.*, 1976). The survival rate may have been affected by the rearing environment of the prey, particularly by the type of host plant. Siddique (1985) stated

that the number of nabids that reached maturity increased significantly when reared on aphids on whole lucerne plants. The survival percentage was 80 % instead of 17 %. This suggests that the nutritional value of prey may have been affected by host plants (Giles *et al.*, 2002). For example, growth of larvae of the predatory gall midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) was faster when feeding on *M. persicae* or *B. brassicae* on cv. Derby Day compared with cv. Ruby Ball (Verkerk *et al.*, 1998). *B. brassicae* and *M. persicae* used for this experiment were cultured on canola plants, therefore it is unlikely the host plant was responsible for the poor development of *N. kinbergii* when it was reared on *B. brassicae*.

The quality of food also influenced the longevity of adult *N. kinbergii*. Adults that fed on *B. brassicae* lived up to 11.3 days, much shorter than when fed on *P. xylostella* or mixed diet. Some researchers have indicated that the number of prey consumed affects the longevity of a predator (Butler & O'Neil, 2007; Gotoh *et al.*, 2006). However, in this study, *N. kinbergii* was supplied with an excess number of prey items every day, so there was no shortage of food. Siddique & Chapman (1987) reported that the longevity of *N. kinbergii* when reared on *P. xylostella* was approximately 13 days shorter than reported here. Bernays *et al.* (1994) considered that a single diet may only bring a limitation of nutrients to generalist predators compare with mixed diets. However, the longevity of adult *N. kinbergii* when feeding on the mixed diet in this study was much longer than any other. Similarly, a mixed diet has also shown to be beneficial for the survival of predators, such as wolf spiders, *Lycosa* spp. (Uetz *et al.*, 1992). However, the effect of prey combination may depend on the predator and on the range of prey species involved. A negative effect possibly occurs if there is a toxic prey in the mixture. Siddique (1985) used the mixture of *A. kondoi* and *A. pisum* to feed *N. kinbergii*, but it was not as beneficial a food source for *N. kinbergii* compared with *P. xylostella*. In addition, Toft and Wise (1999) suggested that predators did not benefit from either a combination of high quality and toxic prey or from the toxic prey alone. In the present study, *B. brassicae*, although not a good food for nabids, did not seem to be highly toxic, because the mixed diet of prey used in this study did not lead to a reduction in longevity or higher mortality of *N. kinbergii*. However, the number of *B. brassicae* consumed by nabids was not recorded. *N. kinbergii* may have consumed *P. xylostella* and *M. persicae* only; therefore the benefit might be due to the combination between *P. xylostella* and *M. persicae*. Current evidence suggests that a mixture of prey types is one factor that results in significantly different longevity of adult *N. kinbergii*.

There was no sex difference in longevity of adult *N. kinbergii*. On all four diets, the longevity of adult females and males was similar. There are few life history studies on other nabids

which have observed differences in longevity of the sexes. For instance, Hormchan *et al.* (1976) reported that female *T. capsiformis* lived approximately 6 days longer than males. Perkins & Watson (1972) observed that the longevity of females *N. alternatus* was two days longer than that of males. In line with present study, Wiedenmann & O'Neil (1990) recorded that females and males *N. roseipennis* had a similar longevity when the boll weevil, *Anthonomus grandis* (Coleoptera: Curculionidae), served as food. Thus, any difference in longevity between males and females is probably dependent on species.

Differences among food sources could be also considered as one of the main factors that has an effect on the fecundity of nabids. In addition, the longevity of adults fed on *M. persicae* was much shorter than those fed on *P. xylostella*. Therefore the testing of the difference in fecundity was ceased 14 days after pairing. The results indicate that the preoviposition period of *N. kinbergii* feeding on *M. persicae* was two days longer than when fed on *P. xylostella*. This result agrees with the observation of Siddique (1985). He stated that the preoviposition period of *N. kinbergii* was approximately four days when male and female were reared on *P. xylostella*. The significant difference in overall egg numbers laid within 14 days between two types of prey indicates that *P. xylostella* is a better food for egg production of *N. kinbergii*. Siddique & Chapman, (1987) found a total of approximately 800 eggs was laid when *P. xylostella* served as prey, much superior to when *A. pisum* was supplied as food. Similarly, there was a significant difference in the number of eggs laid per female per day, even though only those females that laid eggs were included in the analysis. Siddique & Chapman (1987) also found that *N. kinbergii* laid more eggs per day when fed on *P. xylostella*. However, compared to this investigation, more eggs were laid in a day by *N. kinbergii* feeding on *P. xylostella* in Siddique's work (14.6 eggs/day compared with 11.5 eggs/day). This was also greater when *N. kinbergii* was provided *A. pisum* (9.6 eggs/day) than *M. persicae* (6.3 eggs/day). These differences might arise from the lower temperature that was used in this study, because *N. kinbergii* lays more eggs at moderately higher temperatures (Siddique, 1985). In addition, the greatest difference between the two studies was the source of *N. kinbergii*. *N. kinbergii* in Siddique's work was fed on *P. xylostella* before being divided into two groups. One group of adults was subsequently fed on *A. pisum* whilst the other was fed on *P. xylostella*. The two groups of nabids in the present study were fed on different single diet during their entire life. All females reared on *P. xylostella* laid eggs, while only 59% of females that had been reared on *M. persicae* laid eggs. However, these percentages were not significantly different, possibly due to insufficiently high statistical power. Siddique (1985) did not report data on the effects of diet on occurrence of egg-laying. It is possible that all females in his study laid eggs because they were fed on *P. xylostella* before feeding on other

prey (*A. pisum*). Apart from that, the percentage of egg hatch was also recorded in this study. It was suggested that the number of eggs hatched also may differ due to prey quality. The percentage hatch was greater when nabids were provided *P. xylostella* (81 %) as prey which is similar to Siddique's result (79 %). In general, varying prey quality influences the different parameters of reproduction of *N. kinbergii*.

Thus, this study shows that the diet is crucial to both juvenile development and adult fecundity and survival. Mass breeding of *N. kinbergii* and possibly other predators should involve a mixed diet for optimal results.

CHAPTER 4

PREY PREFERENCE AND FACTORS THAT AFFECT PREY PREFERENCE OF *NABIS KINBERGII*

1. INTRODUCTION

Prey preference has been recognised as an important characteristic of insect predators and has been extensively studied for many different insect families (Reitz *et al.*, 2006; Rieger, 1990; Heong *et al.*, 1991; Waldbauer & Friedman, 1991). The reason for this is that understanding prey preference of a predator may enable new pest management strategies to incorporate predators more effectively, particularly in biological control (Holt & Lawton, 1994; Waldbauer & Friedman, 1991; Symondson *et al.*, 2002). For instance, it is important to know whether predators can survive when their food source is scarce (Eubanks & Denno, 2000). Moreover, the population density of a predator might increase by consuming alternative prey before a major target prey species arrives (Settle *et al.*, 1996).

The preference of predators may be affected by a number of factors. Among these are characteristics of the prey such as mobility, nutritional quality, colour, odour and taste, which probably play an important role (Eubanks & Denno, 2000). Prey preference may also arise through the metabolic route (Waldbauer & Friedman, 1991). This means that predators may prefer to hunt prey that are nutritious and non-toxic. Some insects appear to search for food from which they derive nutritional benefit (Waldbauer & Friedman, 1991). In contrast, insects may avoid food that is toxic (Stamp & Meyerhoefer, 2004). In addition, prey preference is considered to be a consequence of active choice, passive selection, or both (Provost *et al.*, 2006a; Onkonburi & Formanowicz, 1997). Active choice is when predators can determine which prey they need, such as highly nutritious species, whereas passive selection happens when predators consume less self-defensive prey (Toft, 1997).

There have been many studies about prey preference and the mechanisms of prey selection have been investigated for many species of predatory arthropods. For instance, prey preference studies have been conducted on wolf spiders (Punzo & Preshkar, 2002; Heong *et al.*, 1991), lady beetles (Provost *et al.*, 2006b), carabid beetles (Mundy *et al.*, 2000), and bugs (Baez *et al.*, 2004), but there is little information about prey choice, or the mechanisms of prey selection, by the Pacific damsel bug, *N. kinbergii*. Regarding prey choice, Ma *et al.* (2005) stated that *N. kinbergii* exhibits a preference for diamondback moth, *P. xylostella* over

cabbage white butterfly, *P. rapae*. In addition, Siddique & Chapman (1987) indicated that *N. kinbergii* prefers pea aphid, *A. pisum* to Australian crop mirid, *S. kinbergi*. Beside these reports, a number of studies have been conducted on other nabids such as *N. roseipennis*, *N. americanoferus* and *N. alternatus*. Waddill & Shepard, (1974) found that *N. roseipennis* consumes the first three larval stages of Mexican bean beetle, *Epilachna varivestis* (Coleoptera: Coccinellidae), but Wiedenmann & O'Neil (1990) stated that *N. roseipennis* only fed on *A. grandis* when given both prey species. *N. roseipennis* in one study did not exhibited a preference for different prey species (Medal *et al.*, 1997). There was no difference between predation rates of *N. roseipennis* on two alternative prey, *Pseudoplusia includens* (Lepidoptera: Noctuidae) and *Helicoverpa zea* (Lepidoptera: Noctuidae), and the focal prey *Spissistilus festinus* (Hemiptera: Membracidae). Another species, *N. americanoferus* has been shown to prefer certain prey species. In this species, attack rates on *Spodoptera exigua* (Lepidoptera: Noctuidae) differed to those on *Lygus hesperus* (Hemiptera: Miridae) (Propp, 1982). When *N. alternatus* was given a choice between eggs of big eyed-bug *Geocoris punctipes* (Hemiptera: Geocoridae) and *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), it preferred the eggs of *P. gossypiella*. Thus, it can be said that nabids often display a prey preference.

However, the factors that affect prey choice of nabids have not been widely examined. Siddique & Chapman (1987) considered that the behaviour of prey may cause the different attack rates of *N. kinbergii* when presented with two types of prey. They suggested that *N. kinbergii* has higher attack rate on pea aphid compared to Australian crop mirid, because pea aphid is less active. Similarly, Southwood & Leston (1959) stated that the movement of prey may be a crucial cue used by nabids to track prey. In addition, prey vigour and defensiveness can also affect prey preferences. *N. roseipennis* has been reported to prefer larvae infected with nuclear polyhedrosis virus (NPV) over healthy larvae of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) (Young & Kring, 1991). The authors suggested that the decrease in self-defence of infected larvae may be the main reason for the preference. In agreement with that suggestion, Nadgauda & Pitre (1986) indicated that *N. roseipennis* consumed more first instar *Heliothis virescens* (Lepidoptera: Noctuidae) than those of *Lygus lineolaris* (Hemiptera: Miridae) because young larvae of *H. virescens* are less active. Likewise, *N. roseipennis* met difficulties when faced with the stronger defence of the large larvae of *H. virescens* (Nadgauda & Pitre, 1978; Southwood & Leston, 1959). Therefore, as the movement, defensiveness and quality of prey may affect the prey selection of *N. kinbergii*, they should be examined to determine how they affect behaviour.

According to Waldbauer & Friedman (1991), sensory stimuli contribute to the preference of insects as well as metabolic demands. For instance, some predators use vibrational, olfactory or visual cues to detect their prey (Barth, 1986; Hostettler & Nentwig, 2006; Le Ru & Makosso, 2001; Laubertie *et al.*, 2006). Among sensory stimuli, vibration serves as one of the cues some predators use to find prey within a habitat. Vibration is the sound that is produced by prey when they move over the plants or when they chew or suck plants. Hemipteran predators such as *Podisus maculiventris* (Hemiptera: Pentatomidae) have been reported to use vibrational stimuli for prey location (Pfannenstiel *et al.*, 1995). Freund & Olmstead (2000) indicated that vibrational signals were probably critical for *N. americanoferus* in locating the chewing larvae of *Colias eurytheme* (Lepidoptera: Pieridae). With large and well-developed eyes, visual cues are considered to be an important cue that hemipterans use to locate their prey (Parker, 1969). In addition, vision is crucial in avoiding enemies such as *Nabidula subcoleoprata* (Hemiptera: Nabidae) and *Sinea diadema* (Hemiptera: Reduviidae), in contrast to olfactory cues detected by the antennae that seem to be important in habitat location (Freund & Olmstead, 2000). Siddique (1985) considered that tactile stimuli appear to be a main factor that affects searching behaviour of *N. kinbergii*. He also concluded that *N. kinbergii* can not detect chemical stimuli from the surface of prey if not touching it. However, all *N. kinbergii* used in his study were impaired by removal of their antennae. This may have affected their normal behaviour. It can be said that the factors that affect prey preference of *N. kinbergii* when given a choice among various prey need to be studied further.

In order to elucidate the above aspects of the behaviour of *N. kinbergii*, the present study addressed three main questions: (1) Which prey do *N. kinbergii* prefer among *P. xylostella* (highly nutritious, faster moving), *M. persicae* (lower nutrition, slow moving) and *B. brassicae* (very low nutrition or toxic, slow moving)? (2) Does prey mobility affect prey selection? (3) Is prey odour alone or combined with chemical cues from the host-plant a factor that influences prey preference?

2. MATERIALS AND METHODS

2.1 Prey preference of *N. kinbergii*

Different feeding arenas may affect the prey selection of predators. Therefore, laboratory experiments to investigate the prey preferences of *N. kinbergii* were carried out in two types of arenas. One was an empty small plastic Petri dish (50 mm diameter, 12 mm height); the second was a similar Petri dish with a canola stem (20 mm long). The experiments were conducted at 25 °C under fluorescent lights (Appendix 1). In order to test the prey preference of *N. kinbergii*, one specimen of each prey species (late instars of *P. xylostella*, *M. persicae* and *B. Brassicae* taken from the same colony as described in Chapter 2) was introduced to an arena. The biomass of prey was not considered to this experiment. One adult *N. kinbergii* was then released into it 30 minutes after introducing the prey. All *N. kinbergii* were collected from the colony and starved for 24 hours before the onset of the experiment. The observation of an individual treatment was stopped when the first prey was caught by *N. kinbergii*. There were 34 and 30 replicates for the first and second arena, respectively.

Differences in the numbers of prey that were attacked first by *N. kinbergii* were analysed using *Chi*-squared tests to determine which species is preferred.

2.2 Prey mobility

To investigate whether mobility of prey affected the preference of *N. kinbergii*, two mobile and two immobilised late instar larval *P. xylostella* were provided to an adult *N. kinbergii*. In order to immobilise *P. xylostella*, healthy larvae were placed in a freezer at around 0 °C for half an hour. This did not kill any larva. The experiment was conducted in the laboratory at 25 °C under fluorescent light. The arena was a small plastic Petri dish (50 mm diameter, 12 mm height). *N. kinbergii* was starved 24 hours before being used in the experiment. There were 40 replicates. The experiment was stopped when the first larva was caught or if one of the immobilised *P. xylostella* became active.

The numbers of immobilised and active larvae of *P. xylostella* that were attacked by *N. kinbergii* were compared using a Binomial test ($P = 0.05$).

2.3 Prey odour

To determine whether prey odour affected the prey preference of *N. kinbergii*, an experiment was conducted in a wind tunnel (for details, see Keller 1990). The temperature was 25 °C with a 12 hour photoperiod. Two square pots of canola (12 cm x 12 cm x 14 cm tall) were placed into the wind tunnel near the upwind end of the test section.

One pot contained a canola plant with approximately 30 larval *P. xylostella*, and the other contained a plant that was not infested. The plants were replaced each time when a new group of *N. kinbergii* were introduced. A piece of paper (29.7 cm x 42 cm) was suspended between the pots and a wooden stand downwind that was at the same height as the rims of the pots. The paper served as a bridge that connected the stand with the canola pots. Five adult *N. kinbergii* that had been starved for 24 h were released onto the downwind end of the paper. The location of *N. kinbergii* was checked after 30, 60, 120 min and at the termination of the experiment after 24 hours. The location of *N. kinbergii* was classified as follows:

- On the stand: *N. kinbergii* was present on the stand
- On the paper: *N. kinbergii* was present on the paper between the stand and plant pots
- On the wall: *N. kinbergii* was present on the wall of wind tunnel
- On the ground: *N. kinbergii* was present on the bottom of wind tunnel
- On the clean plant: *N. kinbergii* was present on the clean plant or the pot
- On the plant with *P. xylostella*: *N. kinbergii* was present on the pot or the plant with *P. xylostella*

There were five replicates of this experiment.

A second experiment was carried out to determine if *N. kinbergii* preferred to move onto a canola plant infested with approximately 30 larval *P. xylostella* or a lucerne plant infested with an undetermined number of *A. kondoi*. The set-up for this experiment was the same as above. There were eight replicates of this experiment.

For both experiments, the number of *N. kinbergii* that moved onto each plant and that were found on or off plants were analysed using a Binomial test ($P = 0.05$).



Figure 4.1 Wind tunnel experiment set up to test for attraction to prey odour in *N. kinbergii* between canola infested with *P. xylostella* and lucerne infested with *A. kondoi*.

3. RESULTS

There were a significant differences in the frequency at which *N. kinbergii* first attacked the different prey species in the empty arena (Table 4.1). Comparison between the different prey items showed that the number of *N. kinbergii* that first attacked *B. brassicae* was the lowest and significantly lower than those first attacked *M. persicae* (binomial test with Bonferroni correction, $P < 0.01$).

Table 4.1. Prey preference of *N. kinbergii* in the two different arenas

Arena	n	No. of prey that was first attacked by <i>N. kinbergii</i>			df	χ^2	P
		<i>P. xylostella</i>	<i>M. persicae</i>	<i>B. brassicae</i>			
Empty	34	9	21	4	2	13.47	<0.01
With canola stem	30	15	12	3	2	7.80	<0.05

A preference for prey was found for the arena with the canola stem (Table 4.1). However, in this case, a significant difference in attack frequency was found both between *P. xylostella* and *B. brassicae* and between *M. persicae* and *B. brassicae*.

There was no statistical significant difference between the numbers of immobilised (25) and active (15) larvae of *P. xylostella* that were attacked by *N. kinbergii* (Binomial test, $P > 0.05$).

The number of *N. kinbergii* found on canola with *P. xylostella* (10 out of 25 individuals) was higher than those found on canola without *P. xylostella* (3 out of 25 individuals) after 24 h. However, this difference was not significant (Binomial test, $P > 0.05$).

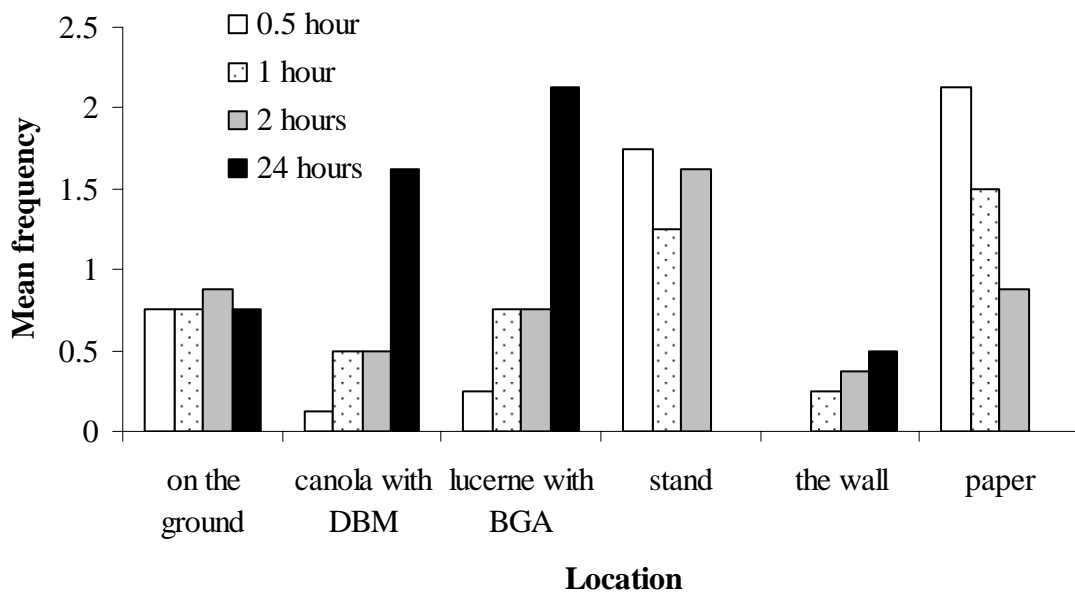


Figure 4.2 Movement of *N. kinbergii* in the wind tunnel at 25 °C, with a 12L:12D photoperiod. (DBM: diamondback moth, BGA: blue-green aphid)

There was movement of *N. kinbergii* during the time of observation among different locations. Almost all *N. kinbergii* were found on the paper 0.5 h after release. In addition, some of the nabids had moved down to the wooden stand and the ground (Fig. 4.2). The number of *N. kinbergii* that had moved to the plants within the first 30 min was low (mean frequencies of 0.125 and 0.25 for canola and lucerne, respectively). After one and two hours, slightly more nabids had reached the plants (0.5 for canola and 0.75 for lucerne). In addition, nabids were found in all locations after two hours. After 24 hours, the number of nabids located on the plants was the highest. In total, about 75 % of nabids occupied plants after 24 h (12 h of dim light). There was no difference between the number of *N. kinbergii* which moved to canola plants (13) with *P. xylostella* and those which moved to lucerne (17) with *A. kondoi* (Binomial test, $P>0.05$). After 24 hours, *N. kinbergii* was found significantly more often on the plants (30) than off plants (10) (Binomial test, $P<0.01$).

4. DISCUSSION

Nabis kinbergii fed on all three brassica insect pests (*P. xylostella*, *M. persicae* and *B. brassicae*). However, among these, *M. persicae* and *P. xylostella* were more often attacked first. Thus, *N. kinbergii* appeared to prefer to some prey species over others. This is similar to the findings of Siddique & Chapman (1987), who reported that *N. kinbergii* exhibited strong preference for *A. pisum* over *S. kinbergi*. Furthermore, *P. xylostella* was also considered to be a more preferred food of *N. kinbergii* (Ma *et al.*, 2005). An earlier study by Jessep (1964) also showed that *B. brassicae* was not the first choice of nymphs of *N. capsiformis* and, although they consumed either nymphs or adults of *B. brassicae*, they did not attack them readily. These results suggest that there is an aversion to some species of prey in nabids. However, the reason behind this aversion is not known. Reasons for prey preference and for prey aversion are discussed below.

The mechanisms of prey preference may be affected by the mobility, size, odour, colour, nutritional quality and taste of prey (Bonacci *et al.*, 2006; Eubanks & Denno, 2000; Bilde & Toft, 1994; Nadgauda & Pitre, 1978). Among the three prey used in this study, the movements of *B. brassicae* and *M. persicae* seemed to be similar and slower than *P. xylostella*. If nabids preferred to attack more mobile prey, more *P. xylostella* larvae would have been consistently killed first. But the number of *P. xylostella* first attacked was equal to or smaller than that for *M. persicae*. Thus, this suggests that nabids may prefer to attack less mobile prey. Larvae of *P. xylostella* moved about the feeding arena frequently, and some larvae moved to the lid of container and were harder to catch. More importantly, the prey mobility study showed that immobile larvae of *P. xylostella* were attacked more frequently than mobile larvae, although there was no significant difference, possibly because of a lack of statistical power. Siddique & Chapman (1987) also reported that *N. kinbergii* had a lower attack rate on *S. kinbergi* compared with *A. pisum* because *S. kinbergi* was more active. Similarly, Young & Kring (1991) concluded that the NPV infected larvae with slow movement were attacked by *N. roseipennis* more often than healthy larvae. In addition to mobility, the efficiency of escape may help prey survive. The results of the experiment conducted in the arena with a canola stem showed more *P. xylostella* and less *M. persicae* were caught, which differed from the results in the simpler arena. It might be the case that *M. persicae*, with their small body size and the similar colour to canola, could hide better in the arena with canola. In contrast, *P. xylostella* with a larger body size, may have been found more easily. Thus, it is possible that the evasive or defensive ability of the active prey in reaction to predator attack can be sufficient to influence predation.

Along with prey mobility, the size of prey may be one of the cues that influences prey choice by nabids. The capture rate of *N. kinbergii* on different sizes of prey was not the subject of experiments in this study. In spite of this, the results showed that *N. kinbergii* seemed to prefer catching smaller prey in the empty arena. More *M. persicae* (21 out of 34 replicates) were consumed than *P. xylostella* (9 out of 34 replicates). This point was supported by similar results of Ojeda Pena (1971) who observed that small larvae of *Bucculatrix thurberiella* (Lepidoptera: Lyonetiidae) were the preferred food for *N. capsiformis* rather than large larvae. Samson & Blood (1980) suggested that *N. kinbergii* is more likely to consume more young than old larvae of *H. punctigera*.

The prey selection of a predator may be affected by prey quality (Montserrat *et al.*, 2006). Waldbauer & Friedman (1991) suggested that consumers would forage for food from which they derived positive benefits. For instance, the lycosid *Pardosa ramulosa* appeared to search for prey for according to their nutritional benefits (Greenstone, 1979). Similar foraging behaviour occurred in social wasps, *Polistes fuscatus* (Hymenoptera: Vespidae), when they were presented with non-toxic and toxic prey. The wasps preferred to catch the non-toxic prey to toxic prey (Stamp & Meyerhoefer, 2004). Thus, *P. xylostella* would be predicted to have been the preferred prey in the present study because it has the highest nutritional value for nabids among the three prey (see Chapter 3). However, *P. xylostella* was not preferred over *M. persicae* (Table 4.2). Toft (1999) also concluded that the spiders could not distinguish the most nutritional prey from a selection of high and low quality prey. Similarly, the nutritional quality of prey was not considered to be a criterion in prey selection of big-eyed bugs *G. punctipes* (Eubanks & Denno, 2000). It is possible that *N. kinbergii* was not able to select the best quality food (*P. xylostella*), but they were able to respond to poor quality food (*B. brassica*). The recognition of poor or toxic prey was discussed by Toft (1999), who suggested that prey aversion can be developed by a predator because of the relationship between the characteristics of prey such as morphology/behaviour and taste/odour. In support of that, Waldbauer & Friedman (1991) indicated that arthropods can learn to distinguish prey species using sensory stimuli. In this study, *M. persicae* and *B. brassicae* seemed to be similar in behaviour and morphology, but the nabids declined to eat *B. brassicae*. Therefore, it is likely that the nabids could perceive the difference between the two prey items, possibly using taste and/or odour. *B. brassicae* has been known as a toxic or unpalatable food to some predators (Tsaganou *et al.*, 2004; Hodek & Honc'k, 1996). According to Hodek & Honc'k (1996), waxy aphids, like *B. brassicae*, may cause difficulties in handling for predators and make an unpalatable food. In support of this, one study found that predators that use visual cues to

locate prey attack more wax-less than waxy aphids (Moss *et al.*, 2006). Another possible explanation is that *B. brassicae* emits allomones that are not attractive to predators, whereas *M. persicae* produced (*E*)- β -farnesene that is attractive to natural enemies (Francis *et al.*, 2004). In addition, taste may make a difference in prey consumption. Thus, *B. brassicae* could not only be a toxic prey species, it also seems to be an unpalatable food for some predators, including *N. kinbergii*.

In order to test whether the prey odour influenced the behaviour of nabids, two wind tunnel experiments were conducted in the present study. The results showed that most nabids had moved onto plants after 24 h. The results might indicate that nabids were attracted by plant odour rather than prey odour. However, the movement did not occur immediately after the insects were released, but occurred in the dim light. It is possible that the initial lack of movement was influenced by the light. This is indicated by the observation that the nabids, upon release in the wind tunnel, immediately moved down to the stand or the lower side of the paper, presumably to escape the light of fluorescent lights. In such a lower position, they could not smell the volatiles released by plants or the insects feeding on them. At night, they moved up again where they could again smell the odours. They then moved forward to the plants when it became dark. This experiment was inconclusive, and further research is necessary to determine whether nabids use odour as a cue for locating prey.

Although more nabids were found on canola with *P. xylostella* larvae than on the uninfested canola), this difference was not significant. This lack of significance maybe caused by low statistical power, therefore further research should be done. It is possible that they could not find any prey on the clean canola and then moved to another plant. In line with this suggestion, the number of nabids that were found on lucerne and canola with prey was similar (17 and 13 individuals, respectively). In addition, a possible explanation for the density of nabids after 12 hours of dim light is the diel vertical movement of nabids. A study showed that more *Nabis fereus*, *Nabis flavomarginatus* and *Nabis rugosus* moved to upper grass layers at night (Fewkes, 1961).

Freund & Olmstead (2000) stated that *N. capsiformis* used vibrational and chemical cues to locate their prey. The authors observed that blinded nabids could find chewing insects. Freund and Olmstead (2000) also showed that vision is not important in locating prey patches in Heteroptera, but antennal olfaction is. In particular, *N. subcoleoprata* and *S. diadema* exhibited significant differences in ability to detect prey using antennal olfaction compared with using vision. However, the nabids in the previous study may not have behaved normally

because they had neither antennal olfaction nor vision. Although the importance of visual cues was not tested in this study, *N. kinbergii* appeared to prefer small and immobile prey to large and mobile prey. Moreover, the number of *M. persicae* was first attacked decreased when a stem of canola served as a shelter. These observations suggest that they use vision as one cue to select prey.

As regards active choice and passive selection, nabids rejected apparently toxic food (*B. brassicae*) and preferred less self-defensive prey (*M. persicae* and *P. xylostella*). Therefore it is likely that prey preference of nabids is the result of both of active choice and passive selection.

CHAPTER 5

GENERAL DISCUSSION AND FUTURE RESEARCH

1. GENERAL DISCUSSION

The objectives of this thesis were to investigate the circadian rhythms, the effects of prey types on growth, development, survival and reproduction, and prey preferences of *Nabis kinbergii*, with a view to providing insight into the predatory behaviour of this insect. The results of this research could ultimately allow us to develop better pest management strategies against pests like the diamondback moth.

Pacific damsel bug was found to be not strictly nocturnal. Under all conditions, it tends to be more active at night. However, under a Spring light/dark cycle (12L:12D), *N. kinbergii* continued to be active during the day when food was scarce (Chapter 2). Although *N. kinbergii* seems to respond to the change of light, there were not clearly two peaks of activity. The response only occurred as the light was off. Therefore, *N. kinbergii* is not a bimodal predator. The hunger state may play an important role in the timing of searching of this predator, but the rhythm of *N. kinbergii* is not an expression of a food demand cycle. The results show that *N. kinbergii* displays circadian rhythms, especially with reasonable food availability. This suggests that the rhythms of *N. kinbergii* are driven by both exogenous (direct response to environmental change: photoperiod and prey availability) and endogenous (internal oscillations) mechanisms. Although the rhythm is probably controlled by an endogenous oscillation (Saunders, 1982), it is affected by the changes of temperature and light intensity. In particular, the decrease of temperature at night and a sudden change of light intensity at dawn and dusk appear to influence circadian rhythms. The effects of different temperature regimes on the rhythms of *N. kinbergii* were not tested in this research. However, under the constant temperature in a controlled environment, *N. kinbergii* did not exhibit as strong a circadian as that in natural lighting conditions with the lower temperatures at night. This agrees with the results of Dicker (1946), since he reported that *N. lativentris* was most often found after dusk, particularly on a cold night. Similarly, Fewkes (1961) reported that the movements of *N. flavomarginatus*, *N. rugosus* and *N. ferus* were strongly affected by the decrease of evening temperatures. Nevertheless, in a controlled environment with a constant temperature (25 °C), *N. kinbergii* tended to be more active at night than during the day. In addition, in the experiment with a 12L:12D photoperiod *N. kinbergii* had the same trend as with a 14L:10D photoperiod during the first day, but food availability or past experience with

prey may have caused an increase in activity during the second day. It seems that environmental factors affect behavioural rhythms of this species. The results indicate that *N. kinbergii* may not be seen or sampled accurately during the day. Therefore, it may not be recognised as an important predator if sampling only occurs during daylight.

Pacific damsel bug was considered to be a not very active predator by Siddique (1985). He concluded that this predator does not spend much time searching for prey. Wade *et al.* (2005) also reported that *N. kinbergii* was almost stationary over the time of observation. They also reported that feeding and walking time was less than 10 % of total time. Meanwhile, resting time was approximately 80 %. These authors suggested that *N. kinbergii* is not an active predator. However, both previous results were observed with observations at intervals, not with continuous observations like this study. Therefore, the previous results may not reflect the actual time spent in each activity. The results reported here partly agree two previous studies because *N. kinbergii* in this work had a high frequency of moving in the feeding arena at night. It is known that most of the feeding by some nabids takes place during the active period (Harker, 1956).

It is clear that prey quality affects the fitness of *N. kinbergii* (Chapter 3). The fitness components include longevity, developmental time and fecundity, which are the main parameters contributing to the health of a population. The present results show that diamondback moth is a good quality prey species for *N. kinbergii*. *Nabis kinbergii* lived longer and had a greater reproduction rate when fed on diamondback moth than when fed on green peach aphid or cabbage aphid. In line with this conclusion, Siddique (1985) indicated that diamondback moth is a good prey for rearing *N. kinbergii*. He established a colony of this lepidopteran to build up the culture of nabids. In contrast, cabbage aphid is determined to be an inferior food for this predator. Similar to cabbage aphid, Siddique (1985) also found that pea aphid does not support development of *N. kinbergii*. Having either of these pest species as prey, the survival of nymphal *N. kinbergii* was low. On the other hand, mixed diets that included high quality (diamondback moth), medium quality (green peach aphid) and low quality prey (cabbage aphid) delivered positive effects to *N. kinbergii*, rather than any single prey species. This may indicate that different prey species provide different nutritional values to *N. kinbergii*. The results have important implications for biological control by this predator in the field or in greenhouses. Populations of *N. kinbergii* are likely to thrive when several species of prey are common. On the other hand, *N. kinbergii* is unlikely to specialise on any one prey species, so it is unlikely to deliver effective pest control alone. However, *N. kinbergii* may combine with specialist natural enemies to control pests.

The study shows that preference is one characteristic of *N. kinbergii*. For instance, this predator prefers larvae of the diamondback moth and green peach aphids to cabbage aphids. In line with this, Ma *et al.*, (2005) also found that diamondback moth is preferred by *N. kinbergii*, compared with cabbage white butterfly. However, the research reported here and earlier does not provide any certain explanation of the mechanisms that drive the prey preference of *N. kinbergii*. The wind-tunnel experiment showed that prey odour does not seem to be a main factor that this predator relies on to find prey. Siddique (1985) concluded that *N. kinbergii* uses mainly tactile stimuli to search for prey. Therefore, he concluded that mobile prey is more easily killed than sedentary prey. In contrast, the research reported in this thesis demonstrated that fast moving prey (diamondback moth) is not necessarily caught more easily than slow moving prey (green peach aphid). In addition, the present study shows that more immobile prey (frozen diamondback moth) might be attacked more often than mobile prey (fresh diamondback moth). The circadian rhythm experiments showed that *N. kinbergii* is more active at night, which means that it searches for prey more frequently at night than during the day. However, *N. kinbergii* can not see in complete darkness. It may detect food not only via visual cues. Thus, more research is needed to clarify the searching behaviour of this insect. In addition, prey characteristics may affect the prey selection of *N. kinbergii*. There is no data to show that *N. kinbergii* can choose the optimal diet in a mixture of different prey. However, this research demonstrates that *N. kinbergii* benefits from a mixed diet. Prey preference in this species is suggested by the apparent avoidance of *B. brassicae*. Such prey preferences may lead *N. kinbergii* to control some pests like diamondback moth more so than others like aphids.

In general, the environmental conditions, physiological state and resource supply may affect the behavioural activities of *N. kinbergii*.

2. FUTURE RESEARCH

Future research should consider other factors that affect circadian rhythms, the range of prey attacked by it, the nutritional ecology of *N. kinbergii*, and the behavioural mechanisms that drive prey choices.

This research reveals the need for some research to investigate the elements that contribute to the circadian rhythms of this predator. The research challenge for the future is to detect what oscillators control the circadian rhythms of this predator. The effects of food source should be eliminated in further work to confirm the circadian rhythms of this predator. In addition, study of the free-running behaviour of *N. kinbergii* is needed to determine how a physiological clock might affect circadian rhythms by putting animal into constant dark (DD) or constant light (LL) (Saunders, 1982). This will clarify the extent to which *N. kinbergii* is a night-active insect. Apart from the movement activity reported in the present study, daily rhythms in feeding and reproduction should also be investigated to obtain more detailed information about the behavioural rhythms of this predator. This may help to understand the population interactions of this species with others, which relates to predator-prey dynamics. The results of such research would give scientists a better understanding how *N. kinbergii* modulates its biology and ecology at the individual as well as the population level. This could be applied to biological control via mass release of this predator into fields and greenhouses.

It is important to note that in Australia *N. kinbergii* has been reported to be a predator in brassica, lucerne and cotton. However, *N. kinbergii* feeds on other insects in other crops like lucerne, as well as in natural ecosystems. Further studies of the wide prey range of *N. kinbergii* are needed to provide an indication of its effectiveness as a predator in other agricultural and horticultural crops.

Current evidence indicates that prey quality is crucial in contributing to the development of a predator, but the reason is still not known yet. Therefore, the nutrition which is essential for *N. kinbergii* to reach optimal fitness should be determined. This kind of information could be used to produce artificial diets for culturing this and other beneficial predators if there is a lack of prey.

More conclusive experiments should be conducted to determine the key factors that affect the preference and searching behaviour of *N. kinbergii*. Although, Siddique (1985) reported that tactile stimuli are important cues for locating prey by *N. kinbergii*, his study was done with

aphid liquid, not real insects. In addition, previous research was not carried out comparing different prey species with different characteristics.

The research in this thesis has only been done under laboratory conditions. The results may not reflect exactly what is happening in the field. Therefore, behavioural experiments need to be conducted in natural settings to confirm that observations from laboratory studies apply in the field.

APPENDIX

Lighting was provided by a control system that simulated dusk and dawn conditions. Daytime lighting was provided by four fluorescent lamps (GE Tri-Tech F36T8/840). These were powered by solid state ballasts (PCA ECO 18-58W 220-240 V 50/60/0 Hz dimmable ballast; Tridonic.Atco GmbH & Co KG, Dornbirn, Austria) that flicker at 40-100 Hz. This is greater than the flicker fusion frequency of insect eyes (Shields 1989). An electronic ballast controller (DDBC1200; Dynalite, Mascot, NSW, Australia) operated by an astronomical time clock (DTC602; Dynalite, Mascot, NSW, Australia) provided dimming functions. Full lighting was provided when the controller delivered the maximum 255 units of power. Dusk conditions were simulated by decreasing the lamp power by 1 unit every seven seconds such that the lamps went from full power to off over a 30 minute period. Relative light levels were not linear; the 50% light level occurred 5 minutes after the dusk cycle commenced.

At the end of the dusk period, there was an abrupt change from low level lighting to darkness. Dawn conditions were the reverse of dusk. The photoperiod was considered to last from when lights went on at the start of the dawn period until they were completely off at the end of simulated dusk. This system could be programmed to deliver any desired photoperiod.

Text written by Michael Keller

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