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**General biology and reproductive fitness of  
Tasmanian lacewing, *Micromus tasmaniae* Walker**

**Anand Yadav**

**2009**

*To my parents*

**General biology and reproductive fitness of  
Tasmanian lacewing, *Micromus tasmaniae* Walker**

A thesis presented in partial fulfillment of  
the requirements for the degree of

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in

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**Anand Yadav**

**2009**

## ABSTRACT

Tasmanian lacewing, *Micromus tasmaniae* Walker, is an important predator of a number of economically important pests such as aphids. This study was conducted to investigate some aspects of general biology and factors affecting the reproductive fitness of this species. Emergence of *M. tasmaniae* peaked 3 h before light off and there was no significant difference in emergence patterns between males and females. Males became sexually mature earlier than females. Mating success significantly increased from the first to the eleventh hour after lights on. Predation, development and oviposition of *M. tasmaniae* were affected when reared under different photoperiods [i.e. 24:0, 16:8, 12:12, 0:24 h (light:dark)]. Results indicate that no individuals entered diapause at either an immature or adult stage. *M. tasmaniae* larvae could feed in both the photophase and scotophase and late instar larvae consumed significantly more aphids than early instar larvae. *M. tasmaniae* reared at 16:8 h developed faster and had lower mortality, heavier adult body weight and higher reproductive output in terms of fecundity and fertility rate. Therefore, mass-rearing programmes are recommended to be carried out at 16:8 h to obtain the higher quality of individuals and faster increase of populations. The larger-the better theory predicts that the reproductive fitness is positively linearly associated with body size or weight. However, the body weight of female *M. tasmaniae* had no effect on the reproductive fitness in terms of fecundity, fertility, fertility rate, oviposition period and longevity. The male body weight may contribute to the population growth of *M. tasmaniae* as the average females that mated with average or heavy males had significantly higher fecundity, fertility and fertility rate and longer reproductive period. These results suggest that development of any control method that should selectively mass-produce heavy and average individuals in the laboratory would help increasing *M. tasmaniae* quality and populations. *M. tasmaniae* is a polygamous species. Results indicate that female remating either with the same or different males was crucial for maximizing their reproductive success. Males could inseminate up to eight females and father about one thousand offspring during their life span.

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## CHAPTER 1

### GENERAL INTRODUCTION

#### 1.1 Introduction

The massive overuse and frequent misuse of chemical pesticides in pest control in 1940s and 1950s have caused problems in agro-ecosystems, such as pesticide resistance, secondary pest outbreaks, human health hazards and environmental pollution (Luck et al. 1977; DeBach and Rosen 1991). Entomologists have been seeking alternative techniques to control insect pests; and integrated pest management has been advocated by scientists utilizing all suitable and compatible techniques to combat the increasing pest problems (Dent 1991). Many parasitoids and predators have been successfully employed in pest management programmes in different crops (Dent 1991).

In the family Hemerobiidae, many brown lacewings are considered to be beneficial as predators of a wide range of small insect pests. This family is widespread and contains around 560 described species in 27 genera and 10 subfamilies (Oswald 1993a, 1993b, 1994; New 2001a; Grimaldi and Engel 2005). The larvae of many species, especially of the genus *Micromus* and *Hemerobius*, commonly prey upon economically important pest insects in the agriculture, horticulture, and forest environments (Monserrat 2001). Amongst different natural enemies of aphid species, Tasmanian lacewing, *Micromus tasmaniae* Walker (Neuroptera: Hemerobiidae), and green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), are two of the major predators and have received greater attention in augmentative and conservation biological control of aphids in different crops.

The Tasmanian lacewing, *M. tasmaniae* is widely distributed in New Zealand and all mainland states and Tasmania in Australia (Wise 1963, 1973, 2000). It is recognized as a significant predator on aphids in New Zealand (Rohitha and Penman 1986) and Australia (Milne and Bishop 1987). In New Zealand, *M. tasmaniae* is common and abundant on low vegetation and shrubs, often in association with aphids on roses, brassicas, cereals, and lucerne (*Medicago sativa* L), as well as other crops (Butcher et al. 1988; Minks and Harrewijn 1987); it may also occur on apple (Collyer



and van Geldermalsen 1975) and some native trees (New 1984). Its ability to control blue green lucerne aphid, *Acyrothosiphon kondoi* Shinji, and pea aphid, *A. pisum* Harris, on lucerne has been evaluated (Cameron et al. 1983; Leathwick 1989; Rohitha and Penman 1986). Its importance in integrated pest management has been suggested (Rumpf et al. 1997). In New Zealand, the biology and ecology of *M. tasmaniae* are studied to some extent in the field (Hilson 1964; Leathwick and Winterbourn 1984) and laboratory (Islam and Chapman 2001).

## **1.2 Importance of *M. tasmaniae* in Biological Control of Aphids**

There are about 4000 species of aphids in the world, and in only one superfamily, for example the Aphidoidea, over 250 species are economically important pests of agricultural and horticultural crops (Blackman and Eastop 2000). There is an increasing number of attempts on biological control of aphids because of the consequence of the increased incidence of invasion of new areas/countries by aphid species (Carver 1989). For example, in New Zealand, blue green lucerne aphid was first reported in late 1975 (Kain et al. 1977) and by late autumn of 1976 it had spread through most of the lucerne-growing areas of New Zealand. Another example is the pea aphid which was first detected in Auckland, New Zealand in October 1976 (Archibald et al. 1979) and dispersed quickly and presented in most parts of South and North Islands, such as the Waikato, Bay of Plenty, Nelson and Christchurch during 1977 (Archibald et al. 1979). It arrived in New South Wales, Australia in January 1980 (Milne 1982).

In New Zealand, both blue green lucerne aphid and pea aphid are the major pests on lucerne that reduce the yields and quality (Kain et al. 1977; Kain and Biggs 1980). Kain et al. (1979) indicate that infestation with blue green lucerne aphids and pea aphids may result in large losses in herbage production (30~60%) and reduction in plant density (18%). In the United States, pea aphid was responsible for lucerne production losses of \$60 million in 1963 (Harper et al. 1978). Moreover, the field and glasshouse studies indicate that infestation of blue-green lucerne aphid and pea aphid caused increased coumestrol levels in severely aphid-damaged lucerne which were usually high enough to impair ewe fecundity (Kain and Biggs 1980).

In Eastern Australia, *M. tasmaniae* along with other natural enemies are capable of making a significant contribution to lucerne aphid control, mainly blue green lucerne aphid, pea aphid and spotted lucerne aphid *Therioaphis trifolii* (Monell) (Milne and Bishop 1987). In New Zealand, the ability of *M. tasmaniae* to control blue green lucerne aphid, *Acyrothosiphon kondoi* Shinji, and pea aphid, *A. pisum* Harris, on lucerne has been demonstrated (Cameron et al. 1983; Leathwick 1989; Rohitha and Penman 1986); and *M. tasmaniae* along with hover fly, *Melanostoma fasciatum* (Macquart) (Diptera: syrphidae), are found to contribute more towards the control of *T. trifolii* than do other natural enemies (Rohitha et al. 1985). *M. tasmaniae* has successfully suppressed the populations of lettuce aphids, *Nasonovia ribisnigri* (Mosley), in the spring crops over four consecutive years in Auckland (Workman et al. 2004; Cameron et al. 2007) and in the spring and summer crops in Canterbury and Horowhenua without the use of insecticides (Walker et al. 2005). Larvae and adults of *M. tasmaniae* are particularly effective predators in lettuce due to their ability, particularly the larvae, to move down into the heart of the lettuce plant where the lettuce aphids feed and reproduce (Workman et al. 2004; Cameron et al. 2007). By inhabiting the heart of lettuce the predators remain protected from topically applied insecticides.

*Micromus tasmaniae* has been considered as a significant predator of aphids on lucerne in New Zealand (Rohitha and Penman 1986). It is a generalist feeder and disperses readily on to low vegetation as a normal component of their habitats (New 2002). Both features are important in biological control, in which a broad spectrum of management activities to enhance the impacts of *M. tasmaniae* may be available (New 2002). Moreover, Hemerobiids are usually preponderant over some other natural enemies because they may develop and reproduce under relatively low temperatures (Neuenschwander et al. 1975; 1976), which allow them to effectively work as biological control agents much earlier in the season before other natural enemies are active. The abundance of *M. tasmaniae* in New Zealand and cooler regions of Australia implies that it could parallel some North American hemerobiidae in being a useful control agent over the cooler parts of the year (New 2002).

Due to the importance of *M. tasmaniae* in biological control of insect pests, large numbers are required for augmentative biological control programmes (New

2002). Recently, many studies have focused on the mass rearing of *M. tasmaniae* (Horne et al. 2001) and it is likely to receive more attention in the next few years (New 2002). Studies on effect of artificial food sprays (Mensah 1997) and floral resources (Robinson et al. 2008; Robinson 2009) on the reproductive fitness have provided useful information to improve the mass rearing of *M. tasmaniae* in the laboratory. Hussein (1984) has developed the technique for the mass release of eggs of *M. tasmaniae* in greenhouses which can be applied in the pest management programmes for control of pests on potatoes in Australia.

### **1.3 Importance of the Study of Reproductive Fitness of a Predator**

In predators, the success in progeny production relies on the consumption of preys which will directly reduce the prey populations. Thus, study of reproductive fitness is an important key to the understanding of how predators can influence the population dynamics of their preys and the structure of the insect communities in which they live (Stewart et al. 1991; van Alphen and Jervis 1996), and to the determination of what behaviours predators adopt in order to maximize their reproductive success. Moreover, studying how predators optimize their reproductive output should provide means to improve pest control efficacy in biological control programmes.

Until the 1990s, behavioural ecologists largely focused on developing and testing theoretical predictions for the fitness consequences of behaviour but devoted less effort to the studying of behavioural mechanisms employed by the animals in order to optimize their reproductive output (Anon 2005). In the last decade, behavioural ecologists have tried to integrate the study of behavioural mechanisms with the functional approach to study the fitness consequences of behaviour (Anon 2007). In *M. tasmaniae*, the effect of prey type on the reproductive output is studied and the lower development thresholds for different life stages were determined in the laboratory (Samson and Blood 1980; Syrett and Penman 1981; New 1984). The sex ratio of *M. tasmaniae* has also been recorded in the field; however, little information is available on its biology (Horne et al. 2001) that may be applied in improving mass rearing in the biological control programmes.

## 1.4 Relevance of This Study

Success in biological control is often dependent on a thorough understanding of biology and ecology of the beneficial species and pests. Few studies have investigated the reproductive biology and fitness of *M. tasmaniae*, limiting the design, improvement and implementation of biological control strategies.

The study of *M. tasmaniae* reproductive fitness is relevant because:

(1) Lucerne, with its high nitrogen fixation capacity, can improve soil quality, which consequently enhances agricultural profitability (Hanson et al. 1988). In New Zealand, it may give high yields of nutritious fodder that often out-yields pasture by 50~100% (McSweeney and Dunbier 1978; Rive 2003). However, some pests, such as blue-green lucerne aphid and pea aphid have continually caused serious damage resulting in the reduction of fresh or hay production and quality. Although the resistant lucerne cultivars have been introduced (Dunbier and Easton 1982) and better management practices have been developed (Kain and Trought 1982), around a third of lucerne grown in New Zealand is still the cultivar 'Wairau', which is susceptible to all major pests (Rive 2003). For example, in Palmerston North, New Zealand, the number of blue green lucerne aphid can be as high as 120 individuals per stem of 'Wairau', in spring and summer 2003 (He 2008), which is four times higher than the critical control threshold (Kain and Trought 1982). Therefore, augmentation of field *M. tasmaniae* populations may be an appropriate option which is relevant to mass-rearing.

(2) *Micromus tasmaniae* is already regarded as valuable contributors to pest management in numerous crops in New Zealand and the feasible mass production technology for lacewing could be useful in biological control programmes (New 2002). Lucerne is drought resistant (Seigler 2005), it is expected that more lucerne will be grown in drier regions if the global temperature continues to increase due to the 'global greenhouse effect' (He 2008). Moreover, in New Zealand, apple is one of the major pipfruits and New Zealand produces around 5% of the global trade in apples (The Encyclopaedia of New Zealand 2008). However, recently, pipfruit growers have raised concerns about woolly apple aphid outbreaks in 2008, due to the presence of

live aphids on the exported apples from Hawke's Bay, which has resulted in the suspension of apples access into the Chinese market (Daily New Zealand News 2008). The Chinese market is worth about NZ\$18 million in apple exports in 2008 (Daily New Zealand News 2008). *M. tasmaniae* is one of the potential predators of woolly apple aphids in the orchards (Collyer and van Geldermalsen 1975). It is thus valuable to find strategies to improve the success of aphid control using *M. tasmaniae*, especially in cropping systems where lucerne and apples are grown for many years.

(3) Development of IPM techniques, including the application of biological control agents, can reduce the amount of chemical used and increase the value of the harvested produce. The study of *M. tasmaniae* reproductive fitness provides information for the understanding of its biology and the development of environmentally friendly IPM practices. The general biology of *M. tasmaniae* has been studied to some extent (Hilson 1964; Samson and Blood 1979; Syrett and Penman 1981; Leathwick and Winterbourn 1984; Rousset 1984; New 1984; Elkarmi et al. 1987; Leathwick 1989; Islam and Chapman 2001) which may be applied to optimize the mass rearing environmental conditions. However, little information is available on what factors and how they may affect its reproductive fitness, such as body size and mating status. Unraveling the factors which govern the reproductive behaviour and its impact upon the reproductive fitness of *M. tasmaniae* will provide valuable information for IPM developers in improving mass rearing quality and biological control strategies.

## **1.5 Aim and Objectives of This Study**

The aim of this research is to provide useful information on the biology and reproductive fitness of *M. tasmaniae* with four objectives

- a) To understand the effect of photoperiod on the development and reproduction of *M. tasmaniae*;
- b) To investigate how and to what extent the body weight affects the reproductive fitness of *M. tasmaniae*;

- c) To determine the effect of female multiple mating on the reproductive fitness of *M. tasmaniae*;
  
- d) To examine the insemination capacity of male *M. tasmaniae* and its effect on female reproductive output.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Introduction

This chapter reviews the current knowledge on the family Hemerobiidae that is relevant to my studies. Special references are given to known biology of *M. tasmaniae*. However, when appropriate or necessary, I included examples of the family chrysopidae.

#### 2.2 Taxonomy of *M. tasmaniae*

The genus *Micromus* has about 100 species, but species recorded from South America may belong to *Nusalala* (Monserrat et al. 2001). Four species of Hemerobiidae are recorded in New Zealand (Wise 1973; New 1988): *M. tasmaniae*, *M. bifasciatus* Tillyard, *Drepanacra binocula* Newman and *Wesmaelius subnebulosus* (Stephens) (= *Boriomyia maorica* Tillyard). *M. tasmaniae* is more common in New Zealand (Wise 1963, 1973).

*Micromus tasmaniae* (Walker, 1860) has been known by many synonyms (New 1988) including:

*Hemerobius tasmaniae* Walker, 1858

*Micromus froggatti* Banks, 1909

*Micromus tasmaniae* (Walker): Tillyard, 1916

*Micromus perkinsi* Banks, 1939

*Nesomicromus tasmaniae* (Walker): Kimmins, 1958

*Austromicromus tasmaniae* (Walker): Nakahara, 1960

The classification for this species is:

Order: Neuroptera

Superfamily: Hemerobioidea

Family: Hemerobiidae

Subfamily: Microminae

Genus: *Micromus*

Species: *tasmaniae*

### 2.3 Identification of *M. tasmaniae*

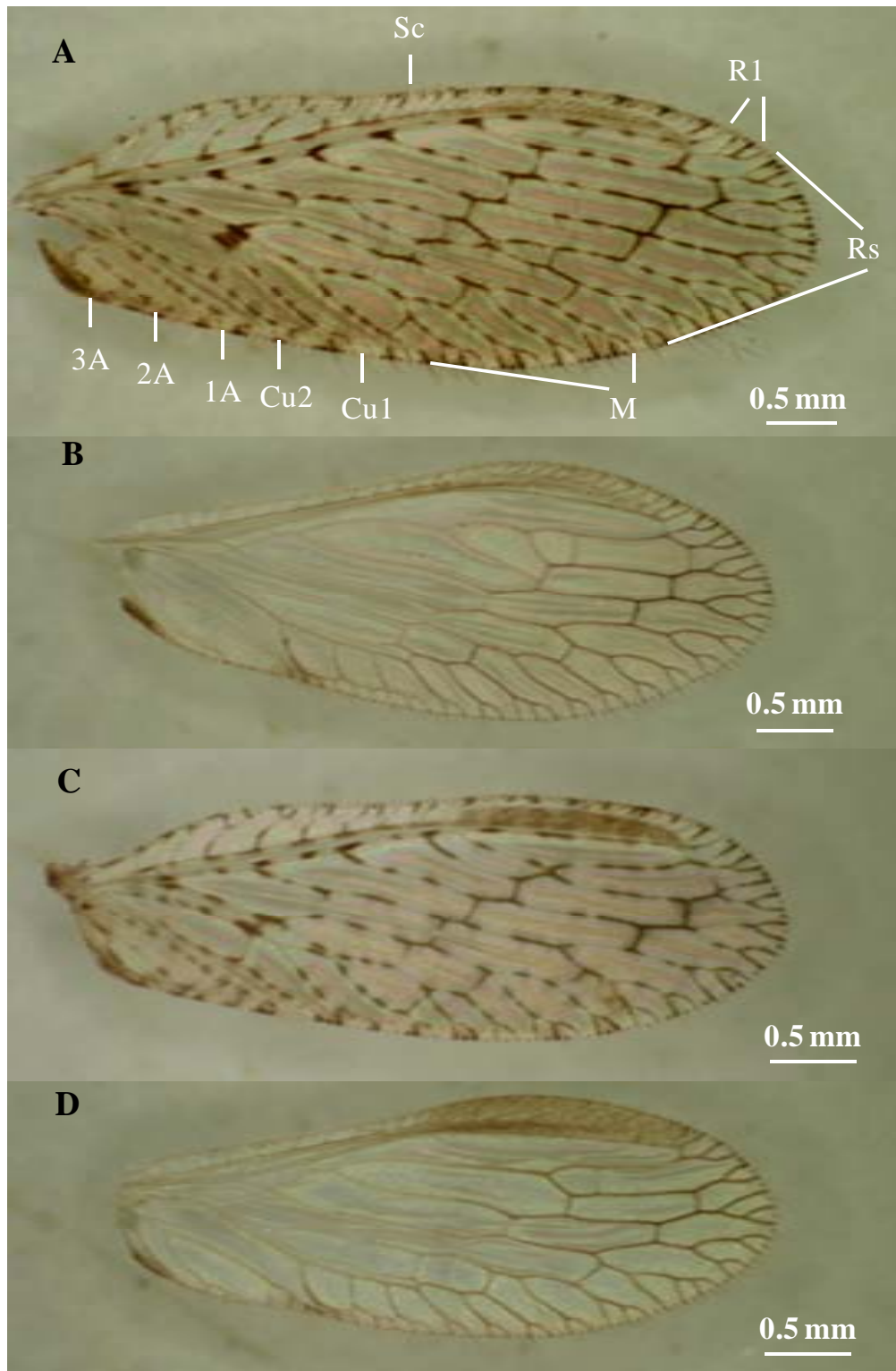
Hemerobiids are seldom green or other colours. As their common name implies, they are most commonly brownish or greyish and generally small in size, with the forewing length 3-18 mm (Oswald 1993b). There are various hemerobiids and they can be differentiated by their wing venation (Oswald 1993b).

There are only two *Micromus* species in New Zealand, i.e. *M. tasmaniae* and *M. bifasciatus* Tillyard (Wise 1973; New 1988); the former is Australian origin (Wise 1995, 2000) and the latter is New Zealand endemic (Tillyard 1923). The difference between these two species is described by Tillyard (1923) and Wise (2000).

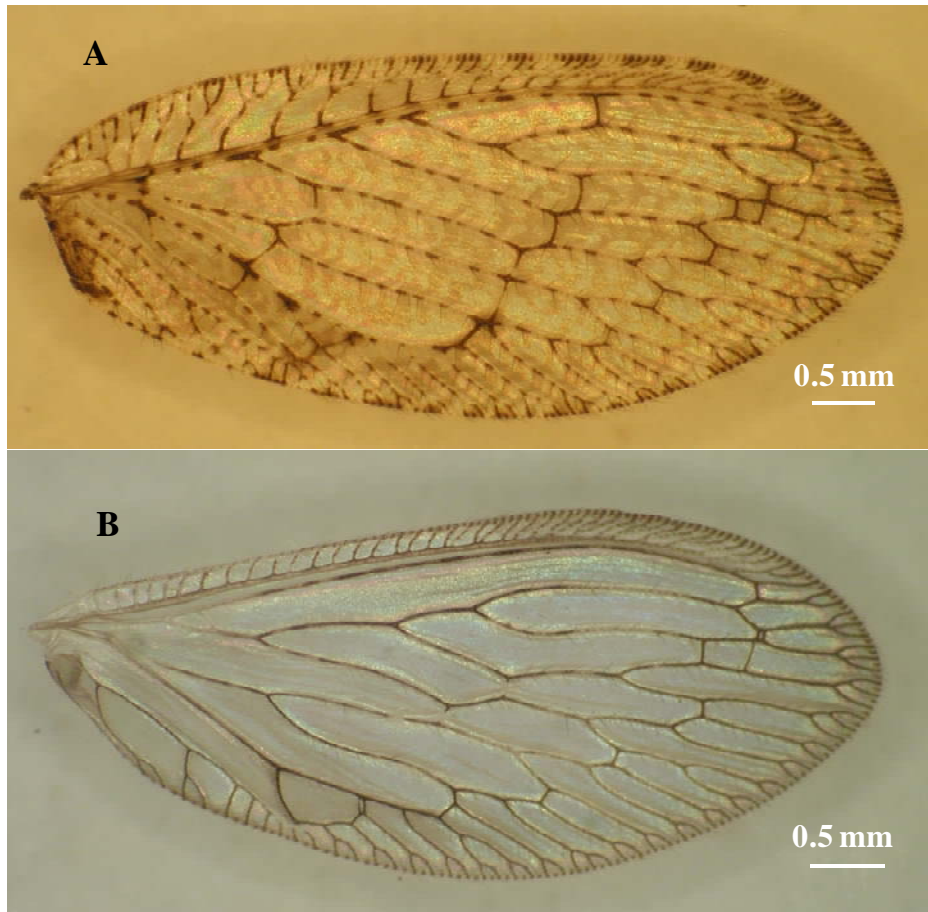
*Micromus tasmaniae* is a very uniform species in appearance and the adults are buff to pale brown; females vary in the anterior wing by the lighter or darker fine, transverse, diagonal lines depending on the colour on the two gradate series of cross-veins (Figure 2.1A) (New 1988; Wise 2000). Longitudinal veins of forewing possess conspicuous intermittent dark lines and bases of Rs branches are very dark (Figure 2.1A) (New 1988). Hindwing venation is pale greyish brown and gradates usually darkened (Figure 2.1B) (New 1988). The wings of *M. tasmaniae* male are presented in Figures 2.1C-D.

The forewing and hindwing of *M. bifasciatus* is shown in Figure 2.2. *M. bifasciatus* is a larger species, varying in appearance with anterior wings with or without the dark stripes (Wise 2000). The dorsal head pattern is a little variable but the pronotum consistently has a dark brown median longitudinal patch (Wise 2000). The varied nigroscriptus pattern in the anterior wings is apparently formed by suppression of the bifasciate pattern and an extension of the long dark basal mark to the posterior margin; when wings are closed this appears as a saddle-like mark (Wise 2000). Hindwing is hyaline, pterostigma testaceous; frenulum is well developed (Figure 2.2B) (Tillyard 1923). Male wings are similar to female, but less darkly banded (Tillyard 1923). Appendages are large, somewhat spatulate, turned vertically downwards (Tillyard 1923).





**Figure 2.1** Wings of *M. tasmaniae*: (A) female forewing, (B) female hindwing, (C) male forewing and (D) male hindwing. A = anal vein, Cu = cubitus, M = media, R1 = Radius1, Rs = radial sector and Sc = subcosta. All pictures were taken in the present study.

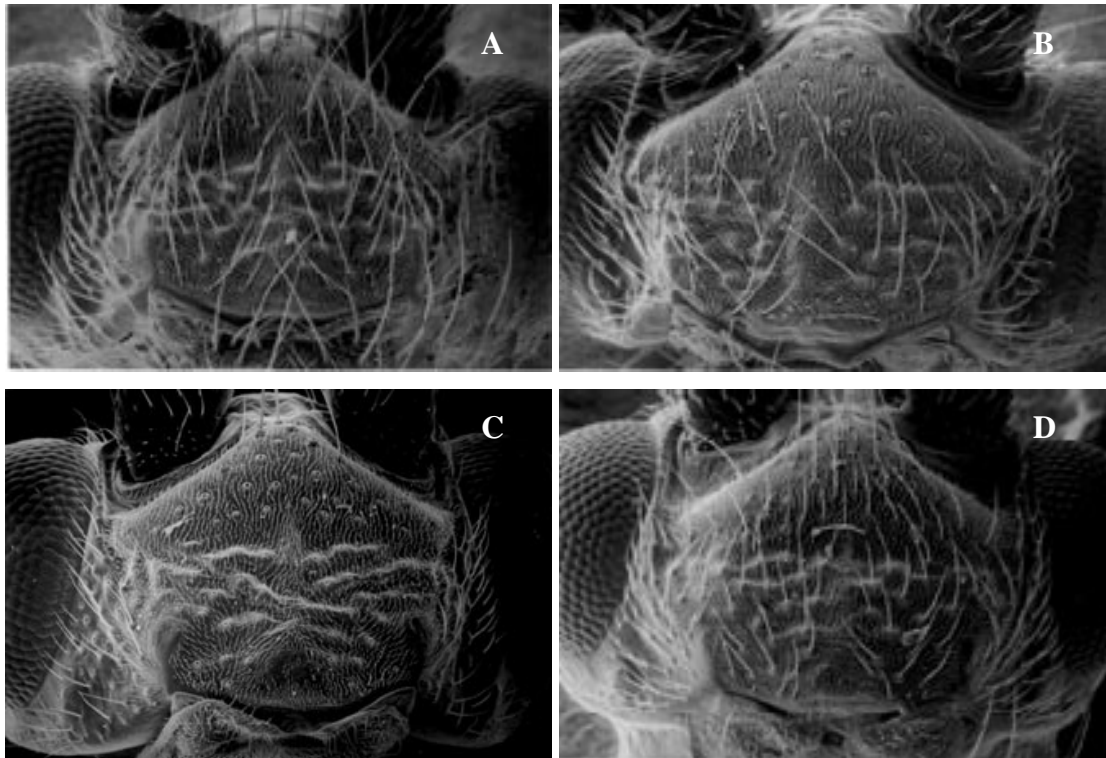


**Figure 2.2** Wings of *M. bifasciatus* female: (A) forewing and (B) hindwing. All pictures were taken in the present study. (*M. bifasciatus* female was collected in a lucerne field on 19 December 2007, Bunnythorpe, Manawatu, New Zealand).

The eyes of adult *M. tasmaniae* are black, usually with greenish iridescence, face is pale, genae is sometime slightly darkened and vertex usually darkened (New 1988). Antennae are greyish brown and the scape is sometimes darkened. Pronotum has a broad dark strip on either side of the middle line and legs are generally pale (New 1988). In *M. bifasciatus*, the head, antennae, thorax and abdomen are of bright medium brown; epicranium has two dark brown patches; eyes are dark brown; and legs are testaceous (Tillyard 1923).

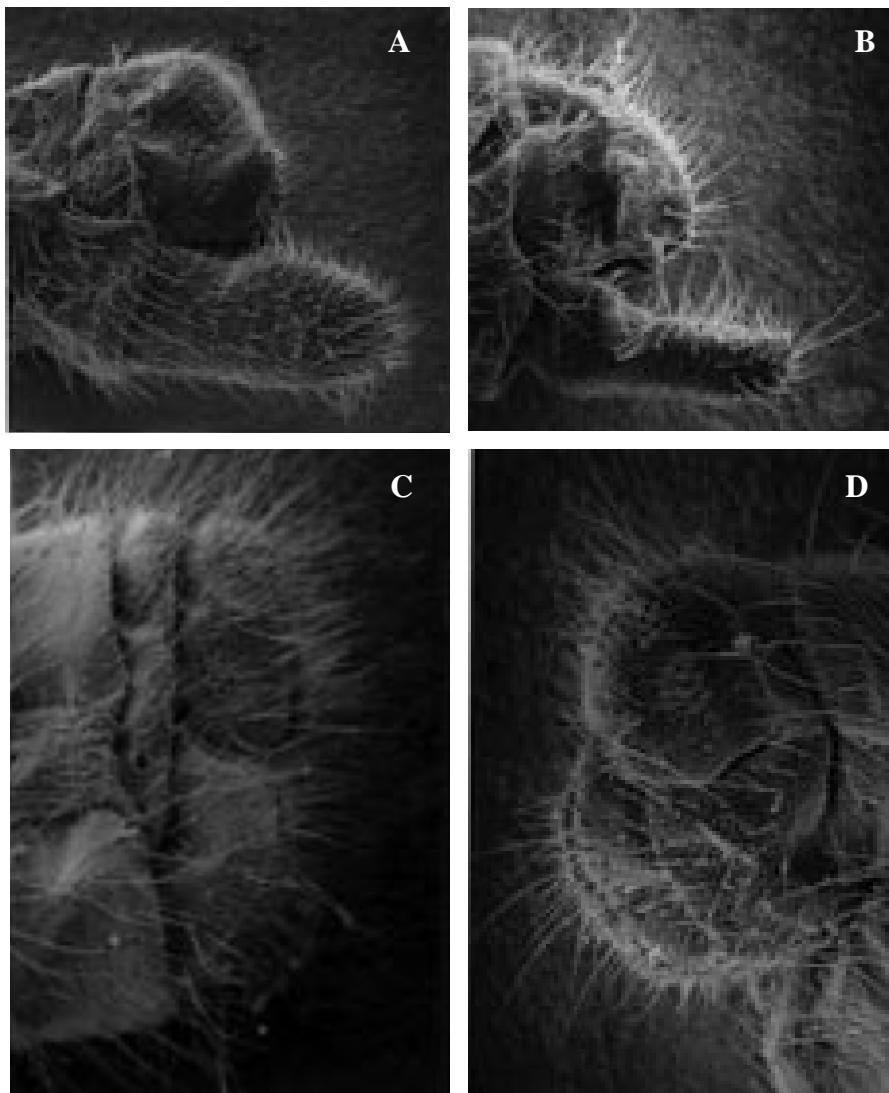
The topography of the dorsum of the head varies greatly in *M. tasmaniae* and in *M. bifasciatus* (Figure 2.3, Wise 2000). The head dorsum of *M. tasmaniae* male and female is almost smooth and that of *M. bifasciatus* is rugose with transverse ridges (Figures 2.3A-D). However, there were intermediate states, particularly in the varieties of *M. bifasciatus* (Figures 2.3C-D). In male and female of these two species,

the head dorsum has an anterior triangular area with bases for macro-setae low and smooth; while on the posterior area there lies a median longitudinal bar, which lacks macro-setae, with a pair of lateral lower fields in which the smooth base for macro-setae are raised on the tubercles (Wise 2000). In *M. tasmaniae*, these tubercles are separate (Figures 2.3A-B), while in *M. bifasciatus* they are more or less coalescent and form ridges (Figures 2.3C-D) (Wise 2000).



**Figure 2.3** Head dorsal: *M. tasmaniae* male (A) and female (B), and *M. bifasciatus* male (C) and female (D) (from Wise 2000).

The taxonomic value of certain genital structures of neuropteroid insects has been recognized (Barnard 1984). The male terminalia of *M. tasmaniae* and *M. bifasciatus* are distinct as shown in Figures 2.4A-B. While the terminalia of females of both these species are of the same pattern (Figures 2.4C-D) (Wise 2000).



**Figure 2.4** Terminalia of abdomen: (A) *M. tasmaniae* male left lateral, (B) *M. bifasciatus* male left lateral, (C) *M. tasmaniae* female left lateral and (D) *M. bifasciatus* female right lateral (from Wise 2000).

#### 2.4 Distribution of *M. tasmaniae*

In Hemerobiidae, there are around 560 described species distributed all over the world (New 1988). Hemerobiidae is one of the most spacious taxa of the Palaearctic Neuroptera (Makarkin and Monserrat 2007). There are 34 species of hemerobiids in Australia (New 1988); by comparison, Britain and Hawaiian Islands have 29 and 25 species, respectively (New 1975).

The earliest records of Hemerobiidae are the fossils of *Promegalomus anomalus* (Panfilov) from the Late Jurassic of Kazakhstan and *Mesohemerobius jeholensis* Ping from the Early Cretaceous of China (Oswald 1993b). Tertiary hemerobiids are more abundant, being found in Baltic (Krüger 1923; Weitschat and Wichard 2002) and Dominican ambers (Oswald 1999). A few compressions are found from the Eocene of Denmark (Henriksen 1922) and Germany (Illies 1941) and Oligocene of England (Jarzembowski 1980) and British Columbia (Scudder 1878). Fossils of hemerobiids are also known from the Eocene to the Holocene (Carpenter 1992).

*Micromus tasmaniae* is a widespread species that occurs throughout New Zealand and on Chatham Island (Wise 1963) and most parts of Australia (New 1997). *M. tasmaniae* is regarded as long established from Australia (Wise 1995, 2000). It was first reported in New Zealand in 1869 (Wise 1977). However, it is believed that around 1850-1852, *M. tasmaniae* settled in Auckland Island, Chatham Island and Antipodes Island in New Zealand (Wise 1971). *M. tasmaniae* has also been reported from New Hebrides (now Vanuatu) and New Caledonia (New 1975), Lord Howe Island (Lambkin and New 1989) and Norfolk Island (New 1987). *M. tasmaniae* has been recorded elsewhere in the western Pacific (Lambkin and New 1989).

Wise (1971) reported that in early 1960s, *M. tasmaniae* was abundant on Ocean Islands, in Port Ross, where hay was imported from New Zealand during World War II to keep for a small flock of sheep. This may have been a source of introduction of the *M. tasmaniae* but the abundance there may be resulted from the presence of a suitable food supply, as aphids, which also could have been introduced with the fodder (Wise 1971). However, natural dispersal agents such as wind could have played some role in the presence of *M. tasmaniae* on these islands (Wise 1971).

## 2.5 General Biology of *M. tasmaniae*

*Micromus tasmaniae* possesses four stages, the egg, larva (three instars), pupa and adult (Figure 2.5). The prepupal stage frequently referred to in the hemerobiid literature refers only to the quiescent mature third instar, which typically assumes an immobile, C-shaped state following cocoon spinning and prior to pupation (Monserrat

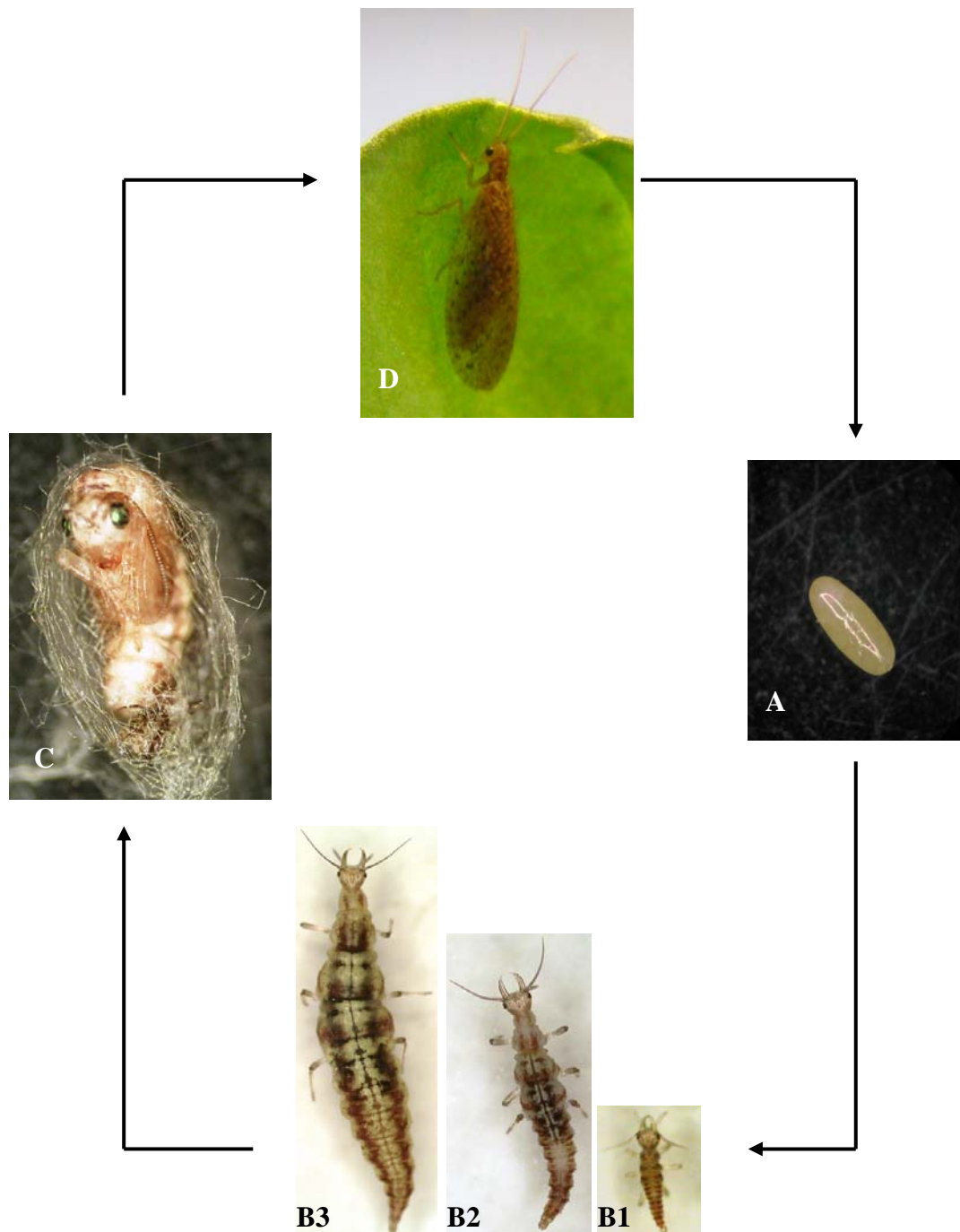
et al. 2001). The biology of *M. tasmaniae* is discussed by New (1984), and its immature stages have been described by New and Boros (1984).

### 2.5.1 Egg

*Micromus tasmaniae* females lay sessile (not stalked) eggs (Figure 2.5A) that are deposited singly near the aphid colonies. Freshly laid eggs are white to translucent pink; later the egg colour changes to pale greyish brown similar that of the developing first instar larva, which is visible through the translucent chorion (New and Boros 1983; Monserrat et al. 2001). Chorion is generally smooth except for slight irregular polygonal sculpturing immediately encircling micropyle (New and Boros 1983). Micropyle is circular and almost sessile. Greatest length of the egg is  $0.78 \pm 0.02$  mm and the width is  $0.34 \pm 0.01$  mm (New and Boros 1983). The first instar larva hatches from the egg by using a saw-like oviruptor to pierce and create a longitudinal, subpolar, rent in the egg chorion (Monserrat et al. 2001). It is a simple blade with about 20 teeth; that are more or less regular and widely spaced anteriorly (New and Boros 1983). The eclosion of the first instar thus involves escape from the prelarval cuticle and the egg chorion (Monserrat et al. 2001). The incubation period of eggs is 6 days at 20°C (Syrett and Penman 1981).

### 2.5.2 Larva

All three instars are similar in colour. However, dorsolateral bands are more pronounced in 3<sup>rd</sup> instar larva (Figure 2.5B3). The head is elongate, fusiform and does not retract into the prothorax. The pre-engorged first instar larva of *M. tasmaniae* is pale in colour with body setation sparse and simple and is easily distinguished from the later instars (Figure 2.5B1). The second instar larva is typically very similar to the third instars, except for their smaller size and fewer secondary setae (Figure 2.5B2). Third instar larva is about 7-9 mm in length (New 1984; Scott 1984). Traces of white fat body are often visible through body wall (Figure 2.5B3). The mature third instar spins a cocoon using silk produced in the Malpighian tubules, which leave the body through the anus (Monserrat et al. 2001). The larval period is 7.6 days at 20°C (Syrett and Penman 1981).



**Figure 2.5** Life cycle of *M. tasmaniae*: (A) egg, (B1) 1<sup>st</sup> instar larva, (B2) 2<sup>nd</sup> instar larva, (B3) 3<sup>rd</sup> instar larva, (C) pupa and (D) adult. All pictures were taken in the present study.

### 2.5.3 Pupa

The pupa is of light brown colour and characterised by legs and antennae that are free from the body wall (exarate) and fully functional mandibles (decticous) (New

2001a). Pupation takes place within the silken cocoon (Figure 2.5C). The cocoon is typically double-walled, with a loose outer mesh that encloses and supports a denser (opaque) inner envelope (New and Boros 1983). Prior to adult eclosion, the pupal mandibles are used to create an irregular opening in the cocoon through which the pharate adult emerges (New and Boros 1983). The pupal period is 11.3 days at 20°C (Syrett and Penman 1981).

#### **2.5.4 Adult**

The adult lacewing is 7-10 mm in length and possesses tentiform wings (Figure 2.5D). Lacewings are found all year round, but they are less active in winter due to the low temperatures (Canard 1997). At 21° C, the development of *M. tasmaniae* from eggs to adults takes about 25 days while the adult longevity is around two months (New 1984). Most of the hemerobiid adults are generalised predators; many species appear to exhibit considerable fidelity to specific habitats or plant species (Killington 1936, 1937; Monserrat and Marin 1996).

#### **2.6 Lower Threshold Temperatures for *M. tasmaniae***

Under constant temperatures, the lower threshold temperature of *M. tasmaniae* is 4.8, 5.7, 6.0 and 5.8°C for the eggs, larvae, pupae and egg-adults, respectively (Syrett and Penman 1981). The physiological time requirements for development were 99, 112, 159, and 326 day-degrees for the eggs, larvae, pupae and eggs-adults, respectively (Syrett and Penman 1981). Islam and Chapman (2001) reported that the *M. tasmaniae* larva could not survive beyond three days at 30°C. The developmental period of larvae is 25, 12, 8 and 6 days at 10, 15, 20 and 25°C, respectively (Islam and Chapman 2001).

#### **2.7 Prey Range of *M. tasmaniae***

The hemerobiids are the most important Neuroptera next to the chrysopids in controlling soft-bodied agricultural pests (Balduf 1974). *M. tasmaniae* is a generalist feeder with a wide prey range such as aphids, moth eggs and small larvae, scale insects, whiteflies, thrips, mites and mealybugs (Collyer and van Geldermalsen 1975;



Scott 1984; Australasian Biological Control Association Inc. 2002). For example, in New Zealand, *M. tasmaniae* feed on blue green lucerne aphid, spotted lucerne aphid, pea aphid and lettuce aphid (Cameron et al. 1983; Rohitha et al. 1985; Leathwick 1989; Rohitha and Penman 1986; Workman et al. 2004; Cameron et al. 2007). In Australia, *M. tasmaniae* is also found to prey on the eggs of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) (Samson and Blood 1980). *M. tasmaniae* has been reported to feed on the longtailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) (Scott 1984). However, its importance is not yet evaluated (Scott 1984).

## 2.8 Feeding and Growth of Lacewings

Tables 2.1 and 2.2 highlight the feeding potential of larval hemerobiids and chrysopids, respectively. The total number of prey eaten by larvae and adults varies from species to species. For example, a *Hemerobius pacificus* Banks larva needs 350 aphids to undergo pupation (Moznette 1915), whereas *H. nitidulus* (Fabricius) requires about 40 aphids during the larval stage and is satisfied with an average of 10 aphids per day during the adult stage (Cutright 1923). *M. tasmaniae* preferred small sized aphids over larger ones for feeding and consume 83, 45 and 27 first, second and third instar aphids, respectively, to complete larval development (Table 2.1, Leathwick 1989).

Adult hemerobiids are omnivorous; they prey not only on aphids but also on various other arthropods (Tjeder 1961; Stelzl 1990). It is found that some adult hemerobiids, for example, *Hemerobius lutescens* (Fabricius), *H. nitidulus*, *Drepanepteryx phalaenoides* (L.) and *Micromus lanasus* (Zenley) may feed on pollen and honeydew (Stelzl 1990, 1991). Many chrysopid species, for example, adults of *C. carnea*, *Chrysopa abbreviate* (Curtis), *Chrysopa oculata* (Say), and *Chrysoperla congrua* (Walker) are non-predatory in the field but feed on nectar, pollen and honeydew (New 1975; Senior and McEwen 2001).

**Table 2.1: Mean number of preys consumed by hemerobiid larvae.**

Hemerobiidae	Prey	Larval instar			Total	Country	Reference
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>			
<i>Micromus tasmaniae</i>	<i>A. pisum</i> <sup>(1)</sup>	4	8	71	83	NZ	Leathwick 1989
	<i>A. pisum</i> <sup>(2)</sup>	3	5	37	45	NZ	Leathwick 1989
	<i>A. pisum</i> <sup>(3)</sup>	2	4	21	27	NZ	Leathwick 1989
	<i>Brevicoryne brassicae</i> <sup>(4)</sup>				26	NZ	Islam and Chapman 2001
	<i>B. brassicae</i> <sup>(5)</sup>				28	NZ	Islam and Chapman 2001
	<i>B. brassicae</i> <sup>(6)</sup>				32	NZ	Islam and Chapman 2001
	<i>B. brassicae</i> <sup>(7)</sup>				35	NZ	Islam and Chapman 2001
	<i>B. brassicae</i> <sup>(8)</sup>				32	NZ	Islam and Chapman 2001
<i>Micromus posticus</i>	<i>B. brassicae</i>	10	11	20	41		Cutright 1923
<i>Hemerobius pacificus</i>	Various aphids				350	USA	Moznette 1915
<i>Hemerobius stigma</i>	<i>Adelges cooleyi</i> <sup>(a)</sup>				3000	-	Laidlaw 1936
<i>Wesmaelius subnelosus</i>	<i>Aphis fabae</i> <sup>(b)</sup>				160-190	France	Laffranque and Carnard 1975
	<i>Myzus persicae</i> <sup>(b)</sup>				57-72	France	Laffranque and Carnard 1975
<i>Hemerobius nitidulus</i>	<i>Aphis rumicis</i> <sup>(b)</sup>				80	-	Withycombe 1923

(1) Preys are 1<sup>st</sup> instar nymphs; (2) preys are 2<sup>nd</sup> instar nymphs; (3) preys are 3<sup>rd</sup> instar nymphs; (4) at 10°C; (5) at 15°C; (6) at 20°C; (7) at 25°C; (8) at 30°C; (a) preys are eggs; (b) preys are 2<sup>nd</sup> instar nymphs.

**Table 2.2: Mean number of preys consumed by chrysopid larvae.**

Chrysopidae	Prey	Larval Instar			Total	Country	Reference
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>			
<i>Chrysopa boninensis</i>	<i>Helecoverpa armigera</i> <sup>(a)</sup>	21	39	237	297	Zimbabwe	Brettell 1979
<i>Chrysopa abbreviata</i>	<i>Aphis pomi</i>				433	Bulgaria	Babrikova 1982
<i>Chrysopa oculata</i>	<i>Aphis gossypii</i>	40	74	152	266	USA	Burke and Martin 1956
	<i>Therioaphis maclata</i>				315	USA	Simpson and burkhardt 1960
<i>Chrysopa perla</i>	<i>Trialeurodes vaspোরariorum</i>				800	Bulgaria	Babrikova 1980a
<i>Chrysoperla carnea</i>	<i>Aphis gossypii</i>				208	USA	Burke and Martin 1956
	<i>A. gossypii</i> <sup>(b)</sup>				425	UK	Scopes 1969
	<i>A. gossypii</i>				487	Pakistan	Afzal and Khan 1978
	<i>Anagasta kuehniella</i>				839	France	Alrouechdi and Voegelé 1981
	<i>Panonychus citri</i>	396	891	8613	9900	USA	Fleschner 1950
	<i>T. maculata</i>				323	USA	Simpson and burkhardt 1960
	<i>Myzus persicae</i>				393	Norway	Sundby 1966
<i>Chrysoperla congrua</i>	<i>H. armigera</i> <sup>(b)</sup>				294	Zimbabwe	Brettell 1982
<i>Chrysoperla rufilabris</i>	<i>A. gossypii</i>				269	USA	Burke and Martin 1956
<i>Chrysoperla zastrowi</i>	<i>Schizaphis graminum</i>				488	SA	Barnes 1975

<sup>(a)</sup> Preys are eggs; <sup>(b)</sup> preys are 2<sup>nd</sup> instar nymphs.

Larvae of the common green lacewing, *C. carnea*, can weigh over 10 mg and other chrysopids as much as 40 mg (Principi and Canard 1984); however, *M. tasmaniae* seldom exceeds 4 mg (Leathwick 1989). Hemerobiids are generally smaller than other aphid predators (Leathwick 1989). Female *M. tasmaniae* are larger than males (Leathwick 1989), which reflects on the amount of food consumed per day (Table 2.3). Female *M. tasmaniae* are more efficient in converting prey into lacewing biomass (Leathwick 1989). It could be expected considering their greater size, and the metabolic demand of egg laying (Leathwick 1989).

**Table 2.3: Mean number of preys consumed per day by *M. tasmaniae* adults.**

Prey	Female	Male	Country	Reference
<i>A. pisum</i> <sup>(1)</sup>	12.8	5.5	NZ	Leathwick 1989
<i>A. pisum</i> <sup>(2)</sup>	10.4	3.5	NZ	Leathwick 1989

Adults were supplied with 40 aphids/day for the test period of 10 days; <sup>(1)</sup> preys were 1<sup>st</sup> instar nymphs; <sup>(2)</sup> preys were 2<sup>nd</sup> instar nymphs.

Growth in body weight begins when the first prey item is eaten by the newly hatched larva (Canard and Volkovich 2001). It continues until the third instar ceases to move and feed before spinning the cocoon (Canard and Volkovich 2001). The weight ratio can give information on larval growth (Table 2.4). The predatory efficiency also depends on the rapidity of destroying the prey population: the quicker the development, the more active the predator (Canard and Volkovich 2001). In such case the larval growth index provides a good idea of the level of predation exerted by the species (Canard and Volkovich 2001).

**Table 2.4: Cocoon weight (mg) and larval growth index of three hemerobiid species.**

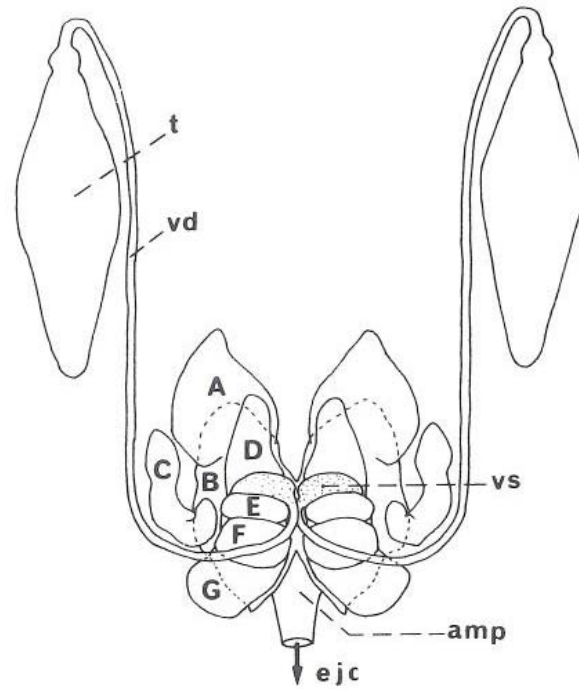
Hemerobiidae	Weight of cocoon	Growth index	Country	Reference
<i>Micromus tasmaniae</i>	3.7	53	NZ	Yadav et al. Unpublished data
<i>Eumicromus angulatus</i>	5.6	96	France	Miermont and Canard 1975
<i>Nasalala uruguayana</i>	8.5	127	Brazil	Souza 1988

## 2.9 Reproductive System of Neuroptera

Investigations of the reproductive system of Neuroptera have been reported by Newell (1918), Crampton (1918, 1920), Withycombe (1924), Killington (1936, 1937), Ferris (1940), Tjeder (1956), Principi (1954, 1956), Acker (1958, 1960), Hwang and Bickley (1961), Bitsch (1984), Walker et al. (1994), and (New 2001b). The terminology of Tjeder (1956) is accepted as the most practical for genitalic structures.

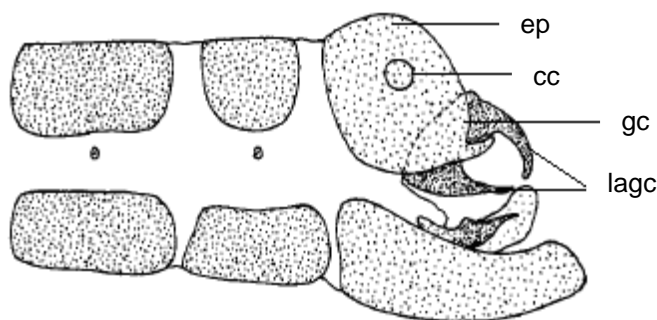
### 2.9.1 Male Reproductive System

As a rule, lacewings have immature gonads at emergence. They cannot mate and females cannot oviposit at emergence (Canard and Volkovich 2001). Gametes only appear after the pupal stage and stay in compact aggregation until the emaginal ecdysis (Philippe 1970). An example of male reproductive system in the Neuroptera is shown in Figure 2.6. The reproductive system consists of a pair of five-lobed testes, a medium-to-large pair of seminal vesicles, and three pairs of accessory glands (Walker et al. 1994). Major accessory glands are surrounded by circular and longitudinal muscle, and are lined by an epithelium (Walker et al. 1994). The paired elongate testes are situated in the 7<sup>th</sup> abdominal segment, and extended a little into the 6<sup>th</sup> (Bitsch 1984). Each testis is coated with a peritoneal sheath, lined with a follicular epithelium; the later gives off several extensions internally, dividing the testis into compartments, in which the maturation of the germ cells takes place (Bitsch 1984). The vas deferens arises from the anterior part of the testes. This duct is at first directed anteriorly, and then turns posteriorly to reach to a complex accessory gland of seven communicating sacs (A to G in Figure 2.6) (Bitsch 1984). The right and left subreniform sacs B-sacs open into a median funnel-shaped ampulla which is continuous with the ejaculatory duct. Different sacs of the accessory gland secrete various secretions forming the spermathophore (Bitsch 1984). A male will be considered as sexually mature when its gametes have reached the seminal vesicles and when all the sacs of the accessory gland are filled with various secretions (Rousset 1984).



**Figure 2.6** Male reproductive organ of a lacewing (*Chrysopa perla* L.), dorsal view. A to G = various chambers of the left accessory gland. amp = ampulla, ejc = ejaculatory duct, t = testes, vd = vas deferens and vs = vesicular seminalis (after Philippe 1972 from Bitsch 1984).

As shown in Figure 2.7, the apex of *M. tasmaniae* abdomen possesses an ectoproct with small ventral catoprocessus, and the sternite IX is long and tapers gradually (New 1988). Gonarcus excavates medially and possesses prominent posteriorly directed lateral arms (New 1988).



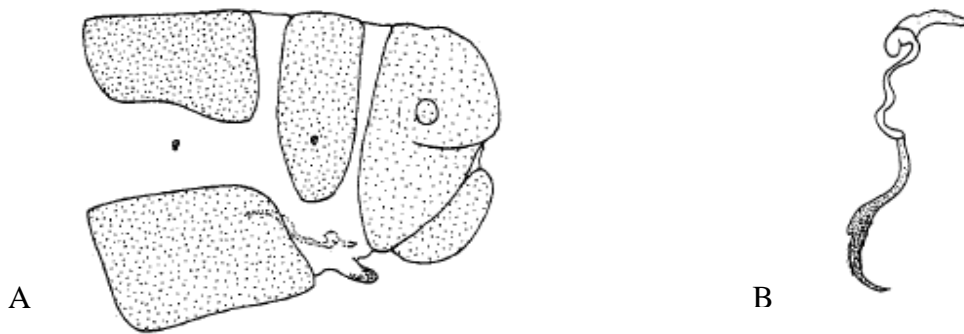
**Figure 2.7** Male apex of abdomen (lateral view), of *M. tasmaniae*. ep = epiproct, cc = cercal callus, gc = gonarcus, and lagc = lateral arms of gonarcus (from New 1988).

## 2.9.2 Female Reproductive System

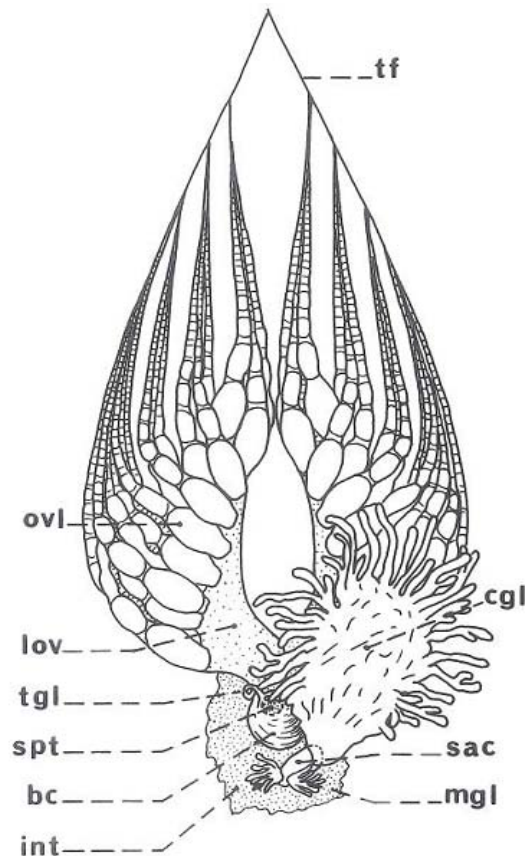
An example of female reproductive system in the Neuroptera is shown in Figure 2.9 (after Principi 1949 cited by Bitsch 1984). Each ovary consists of several ovarioles arranged in a double series along the sides of the lateral oviduct. The number of ovarioles varies from species to species and the number ranges from 8 to 12 (Bitsch 1984). The common oviduct is an ectodermal pouch receiving the two lateral oviducts (Figure 2.9). The common oviduct is located at the posterior end of the seventh segment which opens into vagina (Bitsch 1984). The median oviduct and vagina are lined with a cuticular intima, which makes it difficult to differentiate between the common oviduct and the vagina (Hwang and Brickley 1961). In *M. tasmaniae*, spermatheca is a darkly pigmented and well-sclerotised circular structure, flattened dorso-ventrally (Figure 2.8B) (Barnard 1984). The convoluted duct of the spermatheca discharges into the junction of the two lateral oviducts. More dorsally, there is a large diverticulum of the vagina which is known as the bursa copulatrix (Bitsch 1984). It is assumed that the bursa copulatrix is primarily a copulatory chamber (Hwang and Brickley 1961). During copulation, the male introduces its hypovalva into the bursa copulatrix and deposits its spermatophore there (Hwang and Brickley 1961). Bursa copulatrix bears a pair of small tubular glands at its anterodorsal end (Bitsch 1984). These pairs of accessory glands of the bursa copulatrix are pale organs, easily confused with the secretion of Malpighian tubules (Hwang and Brickley 1961). Principi (1949) found these in *Chrysopa septempunctata* Wesmael and named them as gland of bursa copulatrix (glandule della borsa copulatrice).

Most chrysopids possess a colleterial gland which, in the case of *Chrysopa oculata* (Say), is in the dorsal region of the sixth, seventh and eighth segments. The colleterial gland is slender in the middle and posteroventrally this gland opens into a small pouch which is termed as the colleterial gland reservoir (Hwang and Brickley 1961). The location and the thickness of the colleterial gland reservoir suggests that this pouch may serve to store and regulate the outflow of adhesive substance which may be used to form eggs stalks. Philippe (1972) recorded certain biochemical characteristics of the secretion from the colleterial gland. At the exit of the colleterial gland reservoir, there lies a common duct which joins the pouch with a pair of glands

which are glandular bodies located partly within the gonapophyses laterals. These paired clusters are named as ‘Scent glands’ (Hwang and Brickley 1961).



**Figure 2.8** (A) Female apex of abdomen (lateral view) and (B) spermatheca of *M. tasmaniae* (from New 1988).



**Figure 2.9** Female reproductive organs of *Chrysopa septempunctata*, dorsal view. bc = bursa copulatrix, cgl = colleterial gland, int = integument, lov = lateral oviduct, mgl = multifid gland, ovl = ovariole, sac = scullus, spt = spermatheca, tf = terminal filament and tgl = tubular gland of the bursa copulatrix (after Principi 1949 from Bitsch 1984).



## 2.10 Factors Affecting the Reproductive Fitness

Many biotic and abiotic factors may affect the reproductive potential of insects. In this section, I will review the effect of photoperiod, feeding, body weight, female multiple mating and male mating history on the reproductive output. These factors interact and affect both sexes' reproductive fitness to different degrees.

### 2.10.1 Photoperiod

Photoperiod mainly influences the insects in two ways, i.e. short-term (daily) behavioural response and long-term (seasonal) physiological response (Gillott 2005). In a broader sense photoperiod influences various processes such as the rate of development, reproduction, adult emergence, and induction of diapause (Gillott 2005).

Generally, light affects the development of many lacewing species (Canard and Principi 1984). Photoperiod reportedly affects lacewing development and diapause such as in *Chrysopa pallens* Rambur (Grimal and Canard 1990; Orlova 1998). The diapause in *C. pallens* occurs at the prepupal stage while the larval stage is a sensitive stage for diapause (Grimal and Canard 1990; Orlova 1998). It is therefore considered that *C. pallens* adjusts its diapause timing by change of larval developmental period with the photoperiod (Nakahira and Arakawa 2005).

Canard (1983) reported that if the larvae of *Nineta flava* Scopoli are reared under short-day light conditions, the adults emerge significantly earlier than if the larvae are reared under long-day regimes. Light intensity has rarely been investigated in lacewings. It appears that chrysopid larvae may react strongly to low light intensities, for example, the developmental thresholds for inducing diapauses in *Chrysopa perla* (L.) and *Chrysoperla carnea* (Stephens) are both below 5 lux (Canard and Principi 1984).

The effect of photoperiod on diapause in neuropterans has been studied by various authors (for example, Canard and Grimal 1988; Chang et al. 1995; Orlova 1998). Furthermore, overwintering mechanisms in temperate regions have been well studied in neuropterans. However, very little is known of aestivation patterns of

chrysopids in arid tropical and desert region (Canard and Principi 1984). Short days during winter may cause diapause in various life stages, such as free-living larvae, prepupae or pupae and adults (Canard and Principi 1984). Diapausing adults (e.g. *C. carnea*) are almost inactive and overwinter in dark and dry places. These adults could be found in dead leaves, litter, the underside of bark, abandoned wasp nests, the unheated parts of country houses, barns and stables (Vannier 1961; Canard and Principi 1984). Diapausing larvae may be in second and more often in third instar. Like the diapausing adults, larvae are mostly inactive; food is taken if available, for example in *Anisochrysa flavifrons* (Brauer) (Principi et al 1975). Diapausing larvae may remain in leaf litter (Babrikova 1980b) or under the bark of the tree (Canard and Principi 1984).

### 2.10.2 Feeding

In most entomophagous predators, an optimal diet for adults both in quality and quantity is necessary to achieve a higher reproductive potential (Hagen et al. 1976). Quality of imaginal feeding plays an important role both at emergence and subsequent reproduction (Rousset 1984). Insufficient larval diet may lead to formation of small cocoons, and the subsequently emerging adults are weak and small in size (Rousset 1984). Even during the adult stage, the females need a greater quality of metabolites as compared to males to regulate growth of the oocytes and oviposition (Hagen 1976; Rousset 1984).

Larvae of 'carnea-group' of insects are efficient predators with a high prey consumption and excellent searching capacity (Bond 1980). They are therefore valued as effective biological control agents (Senior and McEwen 2001). In contrast to the larval stage, the adults are not predaceous, feeding instead on nectar, pollen, and honeydew (Hagen et al. 1970; Principi and Canard 1984). The different components of an artificial food for adult green lacewing *C. carnea* affecting adult longevity and fecundity have been studied in laboratory by McEwen and Kidd (1995). Further, large scale applications of sprays containing yeast and sugar of acid-hydrolysed L-tryptophan to crops resulted in a subsequent increase in *C. carnea* adult population in the sprayed area (McEwen et al. 1994). This suggests that lacewing larvae may benefit from feeding on sugar-rich non-prey foods as an appropriate source of energy

and nutrients that can increase adult longevity and foraging activity. The applications of artificial honeydew in the field could permit *C. carnea* to complete its development even at lower prey densities. This exemplifies that *C. carnea* utilizes non-prey foods at times when high-quality prey are scanty (McEwen et al. 1993).

In *M. tasmaniae*, both larvae and adults are predators. Implications of floral resources on predation by *M. tasmaniae* were studied in New Zealand (Robinson et al. 2008; Robinson 2009). The provision of floral resources reduced the consumption of aphids suggests that *M. tasmaniae* utilized these resources even in the presence of aphids. Therefore, *M. tasmaniae* could survive under low density of aphids. Such observations suggest that floral resources could serve to maintain population of *M. tasmaniae* when prey is scanty, and lacewing may increase the oviposition when prey resources are sufficient.

### **2.10.3 Body Weight**

The understanding of relationship between body weight and reproductive potential is important for the development of mass production technique of the biological control agents. Such knowledge is also important for the development of insect control techniques (Jiménez-Pérez and Wang 2004).

Body size or weight has traditionally been considered a key determinant of an organism's ecological and physiological properties (Thornhill and Alcock 1983; Honěk 1993; Jiménez-Pérez and Wang 2004). In males, large body size or heavy weight has been used as indication of "good quality" such as having better genes and more sperm supply (Phelan and Barker 1986; Bissoondath and Wiklund 1996). Female weight is generally accepted as an index of potential fecundity, assuming a positive relationship between the number of oocytes in the ovarioles and the weight of the female (Jiménez-Pérez and Wang 2004). In females, heavy females often have more and larger eggs available for laying and are able to regenerate eggs faster when required (Cloutier et al. 2000). Fecundity in most insects varies with body size of females (Reiss 1989) and therefore, female size is usually a good forecaster of potential fecundity. Honěk (1993) showed general relationship highlighting the interspecific variation in body size and fecundity in insects. In most insect species

increases in fecundity with body weight was similar in most taxa, with only a few exceptions (Honěk 1993). The number of eggs per female could be a linear function of the space available within the mother's body, and this space should be proportional to female weight (Honěk 1993).

In general larger body weight/ size has been associated with greater longevity and extended reproductive period and therefore higher lifetime reproductive output. Thus, in a broader sense large insects have a better chance to survive either by winning food sources, displacing small individuals or due to large energy reserves (Smits et al. 2001). Even after acquiring enough resources an organism cannot grow indefinitely and some authors have pointed out the reasons (Roff 1981; Blanckenhorn 2000; Thompson and Fincke 2002). There are reports suggesting that the 'larger-the-better' hypothesis does not apply to every species as there are list of studies reporting no effect or even negative influence of body size on various fitness component (Leather and Burnand 1987; Svärd and Wiklund 1988). Furthermore, smaller males or females may be favoured in environments where risk of large-size-selective predation is high (Beck 1995) resulting in a trade-off between maximizing size and fecundity and minimizing predation (Forrest 1987; Berrigan and Charnov 1994; Smith and Buskirk 1995; Sparks 1996; Taylor et al.1998).

#### **2.10.4 Female Multiple Mating**

Mating is crucial in the insect's life that modifies its physiology and behaviour. Females can mate one or more times during their lifespan depending on their mating systems (Ridley 1988). Female reproductive fitness is a function of fecundity, fertility and longevity. Fitness of multiple mating on female has been studied in various insects largely in terms of fecundity. The effect of multiple mating on female reproductive fitness has been reviewed by many authors (e.g. Thornhill and Alcock 1983; Drummond 1984; Ridley 1990; Jennions and Petrie 2000; Gotoh and Tsuchiya 2008), and is one of the most attractive fields of study in insect mating behaviour. The number of matings is usually, but not always, correlated with egg production in many insects and mites (Kawagoe et al. 2001; Orsetti and Rutowski 2003; Campbell 2005; Gotoh and Tsuchiya 2008). Multiple mating is considered as beneficial as there are greater chances to obtain genetic variability in the offspring and

increase in fecundity; increased fecundity could impose a metabolic load on females, resulting in earlier death (Blanckenhorn et al. 2002). It is found that multiple mating in female arctiid moth, *Utetheisa ornatrix* L. (Lepidoptera: Arctiidae) brings about increases in fecundity, but not in female longevity (LaMunyon 1997).

In many insects, multiple mating could cause deleterious effect. Taylor et al. (1998) reported that the female stonefly, *Megarcys signata* Hagen (Plecoptera: Perlodidae) allowed to mate multiple times, had significantly lower total lifetime fecundity and shorter adult longevity than the female that mated once. Similarly, negative effect of multiple mating on reproduction is also reported in *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae) (Cook 1999). The reduction in female reproduction and longevity may be due to the cost of mating itself, such as physical damage by repeated copulation (Chapman et al. 1998; Arnqvist and Nilsson 2000; Yanagi and Miyatake 2003; Campbell 2005). Furthermore, multiple mating may have serious physiological and reproductive consequences for already mated female.

Several hypotheses have been proposed to explain the phenomenon of the female re-mating, and these could be divided into two basic categories: (1) material benefits and (2) genetic benefits (Reynolds 1996; Jennions and Petrie 1997; Jiménez-Pérez 2003). The material benefits of re-mating may include nutritional resources in the form of nuptial gifts or sperm replenishment. The female may remate to collect sufficient amount of sperms which could enable her to produce viable eggs throughout her life (Arnqvist and Nilsson 2000) and also to reduce the cost of long term storage (Drummond 1984). Genetic benefit of multiple mating may include the prevention of inbreeding (Keller and Reeve 1995; Edvardsson and Arnqvist 2000) and manipulating offspring paternity, like reducing the chances of fertilization by sperm genetically defective due to age (Halliday and Arnold 1987).

### **2.10.5 Male Multiple Mating**

Male's reproductive success is directly associated with the number of females the male is able to inseminate. Thus it is widely accepted that the best male strategy to maximize fitness is to acquire as many mates as possible (Trivers 1972; Thornhill and Alcock 1983). Multiple mating has certain physiological consequences for males,

because as in most Lepidoptera and Neuroptera, males transfer spermatophores to females during copulation that contain sperms and accessory gland products (Thornhill and Alcock 1983). Male mating history strongly affects female reproductive output and the most immediate explanation for the reduction in female reproductive potential may be the reduction in spermatophore size (Torres-Vila and Jennions 2005). For example, after one mating the male generally produces smaller spermatophore (Delisle and Bouchard 1995). In *Chrysoperla downesi* (Smith), males can mate up to 10 females, but after two or three matings they are unable to father progeny (Henry and Busher 1987). In *Chrysoperla plorabunda* (Fitch), males can mate 30 times and sire over 9600 offsprings during his 3.5 months reproductive life; however, after 16<sup>th</sup> mating, the males appeared to run low on sperm (Henry and Busher 1987). Patterns of mating and its effect on reproductive potential have only been studied in a few lacewing species (Henry and Busher 1987).

Multiple mating may increase the risk of predation as both male and female are exposed to natural enemies during mating (Rowe 1994; Bissoondath and Wiklund 1996). Mckean and Nunney (2001) reported that highly promiscuous males are more susceptible to bacterial infections, in turn affecting their longevity.

## CHAPTER 3 GENERAL BIOLOGY OF *MICROMUS TASMANIAE*

### 3.1 General Introduction

The usefulness of Tasmanian brown lacewing *M. tasmaniae* in the biological control of aphids in crops like lucerne, peas, lettuce and apple, etc. has been reviewed. Mass culture and periodical colonisation of natural enemies against specific pests have been successful in controlling a few pest species (Doutt and Hagen 1949; Ridgway and Jones 1969; Shands and Simpson 1972; Daane et al. 1996). In order to utilize *M. tasmaniae* in biological control of the aphids the development of effective and economical mass culture method is crucial. In New Zealand many aspects of its biology and ecology have been studied in the field (Hilson 1964; Leathwick and Winterbourn 1984; Leathwick 1989; Robinson et al. 2008; Robinson 2009). The information on various biological parameters such as developmental duration at optimum photoperiod, feeding habits, feeding potential, reproductive fitness, longevity and emergence pattern is of great importance.

This chapter describes the general methodology applied throughout this research and investigates emergence rhythm, sex maturation, mating rhythm, development, and reproductive potential of *M. tasmaniae*.

### 3.2 General Methodology

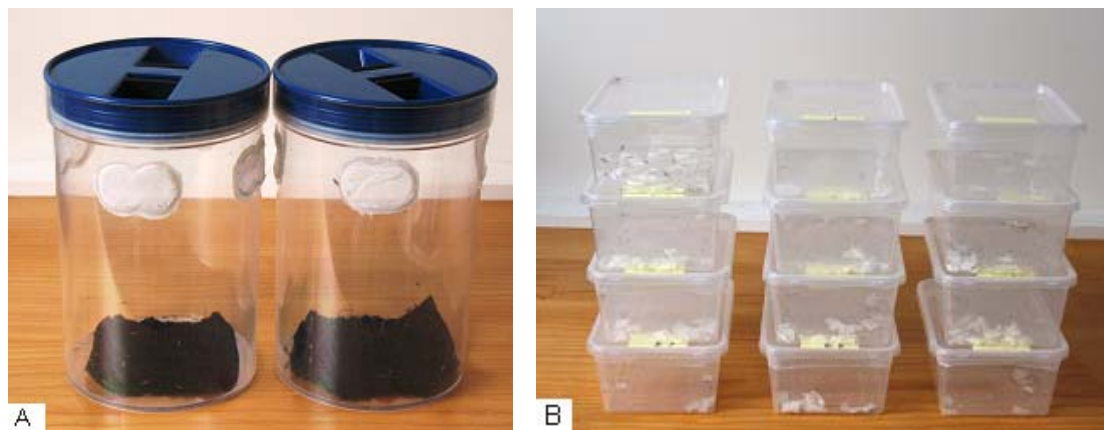
The materials, procedures, environmental conditions and definitions detailed in this section were used throughout this thesis.

#### 3.2.1 Materials

*Breeding colony*: a breeding colony of *M. tasmaniae* was obtained from a commercial insectary (Zonda Resources Ltd, Pukekohe, New Zealand) during October 2007. Thirty adults per container were housed in a plastic oviposition containers (17 cm diameter × 24 cm height), with four fine nylon mesh windows (6 cm diameter) (Figure 3.1A). They were fed with first to third instar pea aphids reared on potted broad bean plants (Figure 3.3). Laboratory and field observations on

hemerobiid in east-central Albama indicate that females oviposit mostly on fibrous materials such as cotton fibers and spidermite webbing (Miller and Cave 1987). Hence, a black cotton sheet (12 cm × 12 cm) was placed at the bottom of an oviposition container, for oviposition. The container was examined every 24 h and eggs laid on the cotton sheet were placed in a plastic boxes (17 cm length × 12 cm width × 7 cm height) for hatching (Figure 3.1B). The newly hatched larvae were further reared on pea aphids in groups of 40-50 larvae per box (Figure 3.1B) and emerged adults were used for further rearing.

Colony of pea aphid (Figure 3.3) was maintained separately in aluminium framed cages (64 cm length × 45 cm width × 40 cm height) with fine metal screen (mesh aperture = 0.25 mm) on the back and both sides and perspex on the top and front and aluminium alloy on the bottom. Broad bean plants were grown in a controlled glasshouse (Figure 3.2).



**Figure 3.1** (A) Oviposition containers; (B) larval rearing boxes used to maintain a breeding colony of *M. tasmaniae*.



**Figure 3.2** Broad bean plants were maintained throughout the study in a glasshouse.





**Figure 3.3** Breeding colony of the pea aphid.



**Figure 3.4** *M. tasmaniae* larvae reared individually in glass vials.

*Larval rearing:* newly hatched larvae obtained from the breeding colony were transferred individually to a clean glass vial (2.5 cm diameter  $\times$  8.0 cm height) with a fine nylon mesh circular window (1.2 cm diameter) on the lid (Figure 3.4). Five, 10 and 20 pea aphids were provided twice a day to 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae, respectively, until they pupated.

*Electronic scale:* the pupae and newly emerged adults were weighed individually using an electronic balance (Mettler Toledo, AG135, Switzerland) with a readability of 0.01 mg.

*Test containers:* transparent plastic containers (6.5 cm diameter × 8.3 cm height, LabServ, Auckland, New Zealand) having a lid with a double layered fine nylon mesh circular window (3 cm diameter) were used for experiments on reproductive fitness. A black cotton sheet (3 cm × 3 cm) was placed at the bottom of the plastic cylinder, for oviposition.

*Microscope:* a stereomicroscope (Leica MZ12, Germany) equipped with a micrometer eyepiece was used for measuring body size of adults.

*Photographs:* unless stated otherwise, all photographs presented in this thesis are taken by me using a digital camera (Canon Power Shot A360, China) during the course of study.

### **3.2.2 Environmental Conditions**

The colonies were maintained and experiments were carried out in bioassay rooms at  $21 \pm 1^\circ\text{C}$  and 60-70 % RH with a photoperiod of 16:8 h (light:dark, lights on at 0800hrs and off at 2400hrs). Lighting was provided by high frequency broad-spectrum biolux tubes (Osram, Germany).

### **3.2.3 Procedures**

*Egg incubation:* the black cotton sheet obtained from the above mentioned test container was placed in the bottom of a Petri dish (8.5 cm diameter × 1.3 cm height) for hatching.

*Fertility assessment:* egg fertility was determined by observing the newly hatched first instar larvae of *M. tasmaniae*.

*Sex identification:* a female adult (Figure 3.5A) could be distinguished from the male (Figure 3.5B) by the sharp abdomen tip.



**Figure 3.5** Adult *M. tasmaniae*: (A) Female and (B) Male.

### 3.2.4 Definitions

*Fecundity* is the total number of eggs laid.

*Fertility* is the total number of fertile eggs laid.

*Fertility rate* is the ratio of fertility versus fecundity.

*Female reproductive period* is the number of days during which the female laid eggs.

*Adult longevity* is the number of days for which the adult lived.

### 3.2.5 Statistical Analysis and Reported Values

All statistical analysis were set at  $P < 0.05$  and carried out using SAS STAT (SAS Institute 2006). Unless stated otherwise, all reported values are mean  $\pm$  SE.

### **3.3 Patterns of Emergence and Mating in *M. tasmaniae***

#### **3.3.1 Introduction**

Circadian clocks are endogenous timing mechanisms that control molecular, cellular, physiological, and behavioural rhythms in all organisms (Giebultowicz 2000). In insects, activities such as hatching, moulting, pupation and emergence are usually rhythmic (Saunders 1982). The study of circadian emergence rhythms elucidates the spatial and temporal distributions of abundance of individuals, which is important for interpreting adult sampling estimates (Quiring 1994). Circadian rhythms influence many aspects of insect biology, fine-tuning life functions to the temperature and light cycles associated with the solar day (Giebultowicz 2000). Furthermore, variations in circadian rhythmicity can reduce direct competition between species that shares resources, and synchronise mating activities to ensure genetic isolation of sibling species (Saunders 1982).

Although most lacewings are active principally in the evening or at night, sexual activity is often observed during daylight hours as well, especially in highly receptive individuals (Henry 1984). Yadav et al. (2008) reported that *M. tasmaniae* can feed pea aphids in both photophase and scotophase, but the circadian emergence and sexual activity rhythms are still unknown prior to this study. To provide information for the development of mass-rearing and field releasing techniques and better understanding of the biological control ecology of *M. tasmaniae*, the sex maturation, circadian patterns of emergence and mating of *M. tasmaniae* were investigated in the present study. All such data are extremely important to understand how lacewings fit into existing theories (New 1975; Henry 1984) and for implementation of biological control programmes.

#### **3.3.2 Materials and Methods**

##### **3.3.2.1 Circadian Emergence Pattern**

To observe the 24-h emergence patterns of *M. tasmaniae*, two bioassay rooms were set up (16:8 h L:D photoperiod) in which the photophase in one room was set

from 0800-2400 hours (normal-light regime) and in the other room the scotophase was between 1000-1800 hours (reverse light regime). Insects used in these experiments were mass reared (Figure 3.1) for two generations in these bioassay rooms before being used for this study and the larvae were individually reared (Figure 3.4). The emergence was observed from 458 pupae in photophase in the normal light regime and from 430 pupae in scotophase in the reverse-light regime. The emergence incidence was recorded hourly and emerged adults were sexed.

### **3.3.2.2 Sex Maturation**

Because most matings occurred during the photophase (unpublished data), all experiments on sex maturation were carried out during the photophase. To detect the sex maturation period, two treatments were set up: (1) male sex maturation – a virgin male was paired with a 3-d-old virgin female at a 8-h-interval during the photophase (i.e. 0, 8, 16, 24, 32, 40, 48, 56, 64, 72, 80 and 88 h old), until he mated and (2) female sex maturation – a virgin female was paired with a 3-d-old virgin male at a 8-h-interval during the photophase (i.e. 0, 8, 16, 24, 32, 40, 48, 56, 64, 72, 80 and 88 h old), until she mated. Each pair was kept in a glass vial and observed every 15 min during the 8 h test period. Successful mating was considered a signal of sex maturation.

### **3.3.2.3 Mating Pattern**

To determine the mating pattern of *M. tasmaniae* during the 16 h photophase, a 3-d-old virgin male and a 3-d-old virgin female were paired in a glass vial. Sixty four pairs were established for this study. These vials were observed every 15 min. Upon mating, the time at which copulation occurred and mating duration were recorded.

### **3.3.3 Statistical Analysis**

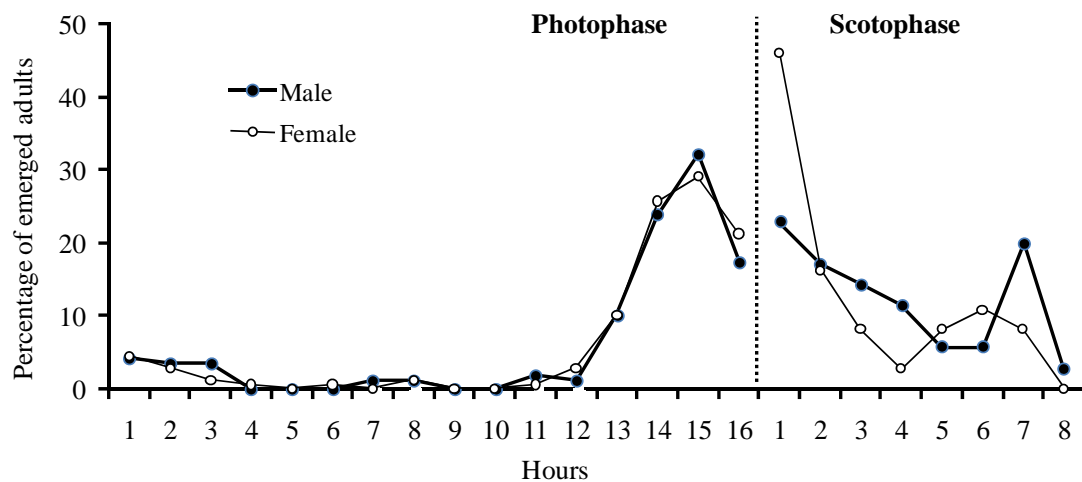
A paired t-test was used to determine the difference in emergence patterns between males and females. Data on emergence in the photophase and scotophase and sex maturation were normally distributed and analyzed using ANOVA. The mating

pattern was described using a quadratic equation:  $y = a + bx + cx^2$ , where  $y$  is the number of matings that occurred,  $x$  is the hours after lights on, and  $a$ ,  $b$  and  $c$  are the parameters of the equation. Because no mating occurred during the last three hours in the photophase, data collected in 13 hours after lights on were included for analysis (Reg Procedure).

### 3.3.4 Results

#### 3.3.4.1 Circadian Emergence Pattern

In both bioassay rooms, 100% of adults emerged with significant more individuals emerging in the photophase ( $352.5 \pm 5.5$ ) than in the scotophase ( $91.5 \pm 19.5$ ) (ANOVA:  $F = 165.95$ ;  $df = 1,2$ ;  $P < 0.01$ ). On a 24-h basis, the adult emergence peaked 3 hours before lights off (Figure 3.6). However, there was no significant difference in emergence patterns between males and females (t-test:  $t = 0.78$ ;  $P > 0.05$ ).



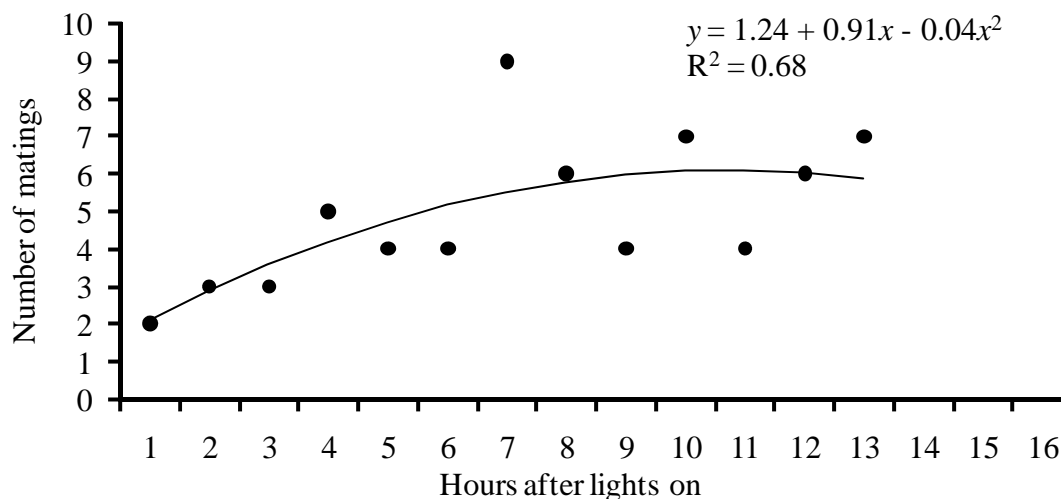
**Figure 3.6** The percentage of female or male *M. tasmaniae* emerging throughout photophase or scotophase.

#### 3.3.4.2 Sex Maturation

The period required by *M. tasmaniae* males to become sexually mature ( $46.21 \pm 1.94$  h after emergence) was significantly shorter than that of females ( $65.13 \pm 3.11$  h) (ANOVA:  $F = 26.66$ ;  $df = 1,46$ ;  $P < 0.0001$ ).

### 3.3.4.3 Mating Pattern

The quadratic model predicted that mating success significantly increased from the first to the eleventh hour after lights on, after which no further increase occurred (Reg:  $F = 4.21$ ;  $P < 0.05$ ) (Figure 3.7). No mating was observed during the last three hours in the photophase. The average mating duration was  $181.63 \pm 3.65$  minutes.



**Figure 3.7** Mating activity pattern in *M. tasmaniae* during the photophase.

### 3.3.5 Discussion

Lacewings are usually nocturnal. In green lacewing, *C. carnea*, emergence follows an endogenous circadian rhythm (Duelli 1980) and adult emergence from the cocoons occurs mainly at night, with a prominent peak in the first hour of the scotophase (Duelli 1980). However, results from this study on a 24-h basis indicate that *M. tasmaniae* adult emergence peaked 3 hours before lights off. Emergence of the adults some time before the lights off possibly coincides with more favourable conditions, such as dusk, in which adults may have the chance of safer habitat location and prey searching.

Newly emerged lacewings have immature gonads. They cannot mate and the females cannot oviposit (Canard and Principi 1984; Canard and Volkovich 2001). In certain lacewing species colour change may serve as a visual cue of sex maturation used by conspecifics before initiating courtship, for example in the *Chrysoperla*

*congrua* (Walker) (Neuroptera: Chrysopidae) (Winterson 1999). However, no such colour change was observed in *M. tasmaniae*. My results show that *M. tasmaniae* males became sexually mature earlier than females. In insects, the reproductive output of males is closely linked with the number of females they are able to inseminate (Trivers 1972; Thornhill and Alcock 1983; Torres-Vila and Jennions 2005). Therefore, early maturity in males gives them better opportunities to find and mate with as many females as possible.

In lacewings, mating takes place when the oocytes are mature in the genital tract of the virgin female and the oviposition of the inseminated female is initiated and stimulated by copulation (Rousset 1984). The females reach maturity in less than three days. In many species of Chrysopidae, the pre-oviposition period is 3-5 days (Canard and Principi 1984). The average longevity of *M. tasmaniae* female is two months and she can lay eggs until she dies (Yadav et al. unpublished data). The prolonged longevity of *M. tasmaniae* may enable their mating success in nature. Thus, the period that females required to become mature may not significantly delay or reduce the reproductive opportunity during their lifetime. Most of the mating occurred between 7 and 13 hours after lights on but no mating was observed 3 hours before lights off. Mating usually lasted for 3 hours. Thus, as nocturnal predators, this mating pattern is advantageous to both sexes in host searching and/or oviposition after successful mating.

This study has provided the foundation for better manipulation of *M. tasmaniae* for the development of mass-rearing and field releasing techniques and future studies of its reproductive behaviour. For example, newly emerged adults should be held for at least 3 days for copulation to occur before release, and they should be released at night to achieve the higher reproductive fitness and survivorship.



### 3.4 Effect of Photoperiod on Development and Reproduction of *M. tasmaniae*

#### 3.4.1 Introduction

Many factors are known to influence the capacity of predators to consume prey (Jervis and Kidd 1996). These factors can act as a stressor and often affect the predation potential and reproduction of the insect predators (Piesik 2006). Abiotic factors (e.g. photoperiod, temperature and humidity) and biotic factors (e.g. food) can interfere with the reproductive potential of the lacewings. Light exerts a major influence on the ability of almost all insects to survive and reproduce (Gillott 2005). According to Beck (1980) and Tauber et al. (1986), photoperiod may influence the development in a number of insects and growth and development are slower at short day length. Furthermore, reproductive tactics of some insects are affected by photoperiod because day length is a reliable cue indicating seasonal change in environmental suitability (Luker 2002; Piesik 2006). Photoperiod affecting lacewing development and diapause has been reported in *Chrysopa pallens* Rambur (Grimal and Canard 1990; Orlova 1998). Wang et al. (2004) reported that adult diapause in *Chrysoperla sinica* Tjeder can be controlled by photoperiod. The adult *C. sinica* is the uppermost sensitive stage for adult diapause induction; adults could develop without diapause under long-day conditions but entered diapause under short-day conditions. Furthermore, *C. sinica* adults could enter diapause only when the emerging adults were under diapause inducing short-day photoperiod (Wang et al. 2004).

Photoperiod is known to be an important factor controlling the sexual maturation of insects. In migratory locust, *Locusta migratoria* L. (Orthoptera: Acrididae), sex maturation proceeds normally at a short photoperiod, but is inhibited by a longer photoperiod (Takana 1994). Wang and Millar (2000) and He et al. (2004) reported that long photoperiod reduced the pre-mating period in Heteroptera. In bumblebees *Bombus terrestris* L. (Hymenoptera: Apidae), photoperiod along with the age significantly affects the frequency of mating and mating propensity (Kwon et al. 2006) and photoperiod has significant effect on the oviposition and colony development of *B. terrestris* (Tasei et al. 1998; Yoon et al. 2003).

Photoperiod reportedly affects lacewing development and diapause (Grimal and Canard 1990; Orlova 1998; Wang et al. 2004). However, there has been no systematic evaluation of how the photoperiod influences the foraging and reproductive potential of *M. tasmaniae*. The present study was undertaken to understand the effect of photoperiod on this predator, information of which is important for the mass production in the biological control programmes.

### **3.4.2 Materials and Methods**

#### **3.4.2.1 Effect of Photoperiod on Host Feeding and Development**

To investigate how photoperiod affected feeding, development and reproduction, four photoperiods of 24:0, 16:8, 12:12 and 0:24 h (light:dark) were established in the laboratory at  $21 \pm 1^\circ\text{C}$  and 60-70 % RH. Because lacewings are mainly nocturnal (Szentkiralyi 2001), the photoperiod of 0:24 h was set up to determine whether *M. tasmaniae* could be reared optimally without light in order to reduce energy costs during mass-rearing.

Eggs (< 12 h old) laid on a cotton sheet (20 ~ 50 eggs/sheet) from the breeding colony were maintained in a glass vial mentioned above and transferred to the tested photoperiods. There were 10 glass vials for each photoperiod. The incubating duration was recorded. Fifty newly hatched larvae were individually transferred to the glass vials for each photoperiod. Larvae were checked daily for molting to the next instar, cocoon formation, and pupation. Five, 10 and 20 pea aphids were provided twice a day to 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae, respectively (Figure 3.8).

Preliminary feeding trials involving lacewing larvae produced a wide variation in the number of aphid consumed. By reviewing the pattern of the larval development from eclosion to pupation showed that feeding rate differed. The larvae pass through a non-feeding phase before passing to the next instar larva which is also associated with loss of body weight (Leathwick 1989). As a result of this a larva can therefore feed voraciously one day and ignore food the next. Attempts to measure prey consumption by larvae could be reliable only on the basis of the number of aphids consumed per instar. Therefore, I recorded the number of aphids consumed daily by each larval

instar. Pupae were kept in the same glass vials to determine emergence under each photoperiod. The developmental duration of larvae and pupae and mortality of both larvae and pupae were recorded. The pupae and newly emerged adults were weighed individually using an electronic balance (section 3.2.1) to determine whether photoperiods had any effects on the growth of *M. tasmaniae*.



**Figure 3.8** Second instar larva of *M. tasmaniae* feeding on 2<sup>nd</sup> instar pea aphid.

#### 3.4.2.2 Effect of Photoperiod on Reproduction

To determine whether the photoperiod affected the reproduction of *M. tasmaniae*, one male and one female (< 12 h old) were paired in the test cylinder (section 3.2.1) with 6, 20, 19 and 17 pairs for 24:0, 16:8, 12:12 and 0:24 h, respectively. The paired adults were fed with 10 1<sup>st</sup> to 3<sup>rd</sup> instar nymphs of pea aphid twice a day until they died. The fecundity, fertility rate and reproductive period of each female were recorded daily.

#### 3.4.3 Statistical Analysis

A goodness-of-fit test was used to test the distribution of data before analysis. Data on *M. tasmaniae* development, female reproductive period and proportion of female progeny were not normally distributed even after transformation and thus were analysed using the non-parametric Kruskal-Wallis test (KWT) followed by Dunn's procedure for multiple comparisons. Data on body weight of pupae and adults, and female fecundity were normally distributed and analysed using ANOVA. Data on the number of aphids consumed by different instar larvae and fertility rate were subjected to square root and arcsine transformation, respectively, before ANOVA. The

Marascuilo procedure of the nonparametric analysis was used to assess the mortality of larvae and pupae with a rejection level of  $U_0 > \chi^2_{3,0.05} = 7.82$ .

### 3.4.4 Results

#### 3.4.4.1 Effect of Photoperiod on Host Feeding and Development

*Micromus tasmaniae* could complete immature development at all tested photoperiods. The number of aphids consumed by larvae increased significantly with their developmental stage at each photoperiod (ANOVA:  $F = 49.46$ ,  $df = 2,111$ ,  $P < 0.0001$  for 24:0 h;  $F = 199.65$ ,  $df = 2,81$ ,  $P < 0.0001$  for 16:8 h;  $F = 68.42$ ,  $df = 2,90$ ,  $P < 0.0001$  for 12:12 h;  $F = 98.55$ ,  $df = 2,105$ ,  $P < 0.0001$  for 0:24 h) (Table 3.1). The total number of aphids consumed by larvae was significantly higher at 24:0, 16:8 and 0:24 h than at 12:12 h (ANOVA:  $F = 12.80$ ;  $df = 3,129$ ;  $P < 0.0001$ ).

At 16:8 h, the mean number of pea aphids consumed by the larvae of *M. tasmaniae* in the photophase ( $34.07 \pm 1.44$ ) was significantly higher than that in the scotophase ( $27.14 \pm 1.29$ ) (ANOVA:  $F = 12.84$ ;  $df = 1,54$ ;  $P < 0.001$ ).

**Table 3.1: Mean number of pea aphids consumed by *M. tasmaniae* larvae at different photoperiods.**

L:D	First instar	Second instar	Third instar	Total
24:0	$11.76 \pm 0.78$ c	$16.05 \pm 1.35$ b	$28.16 \pm 1.39$ a	$55.97 \pm 1.54$ $\alpha$
16:8	$7.54 \pm 0.65$ c	$12.93 \pm 1.13$ b	$40.07 \pm 1.60$ a	$60.54 \pm 1.94$ $\alpha$
12:12	$9.45 \pm 0.38$ b	$11.06 \pm 0.82$ b	$26.27 \pm 1.58$ a	$46.73 \pm 1.43$ $\beta$
0:24	$7.14 \pm 0.40$ c	$17.94 \pm 1.71$ b	$36.54 \pm 1.79$ a	$61.63 \pm 2.05$ $\alpha$

Mean ( $\pm$  SE) followed by the same English letters in rows and Greek letters in column (i.e. Total) are not significantly differently ( $P > 0.05$ ).

*Micromus tasmaniae* developed from egg to adult significantly faster at 24:0 and 16:8 h than at 12:12 and 0:24 h (KWT:  $\chi^2 = 44.72$ ,  $P < 0.0001$ ) (Table 3.2). At each photoperiod, pupal stage was significantly longer than larval and egg stages

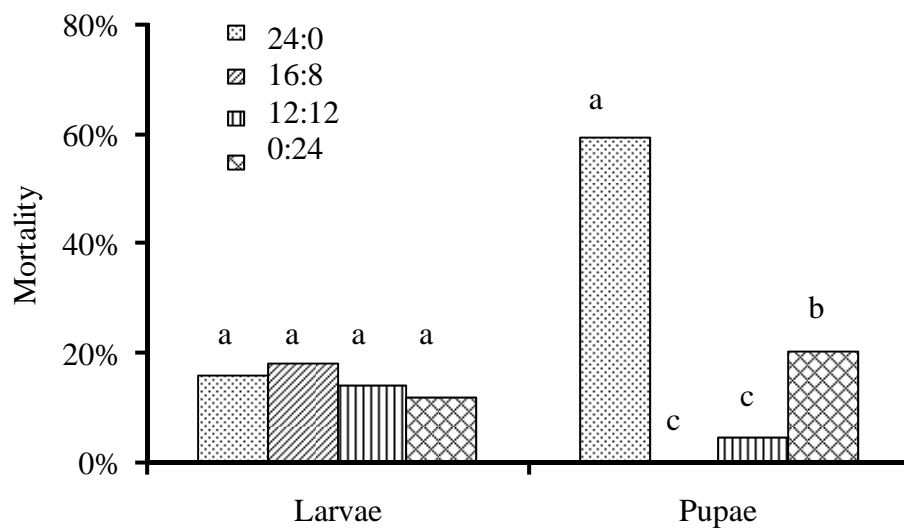
(KWT:  $\chi^2 = 38.26, 85.05, 78.83$  and  $21.57$  for 24:0, 16:8, 12:12 and 0:24 h, respectively;  $P < 0.0001$ ) (Table 3.2).

**Table 3.2: Mean developmental period (days) of *M. tasmaniae* at different photoperiods.**

L:D	Egg	Larvae	Pupae	Total
24:0	$5.57 \pm 0.14$ c	$7.93 \pm 0.07$ b	$9.86 \pm 0.18$ a	$23.36 \pm 0.23$ $\beta$
16:8	$4.84 \pm 0.08$ c	$7.06 \pm 0.09$ b	$10.87 \pm 0.12$ a	$22.77 \pm 0.13$ $\beta$
12:12	$5.24 \pm 0.08$ c	$7.83 \pm 0.12$ b	$11.28 \pm 0.13$ a	$24.34 \pm 0.20$ $\alpha$
0:24	$5.79 \pm 0.09$ b	$7.75 \pm 0.13$ a	$10.86 \pm 0.10$ a	$24.39 \pm 0.22$ $\alpha$

Means ( $\pm$  SE) followed by the same English letters in rows and Greek letters in column (i.e. Total) are not significantly different ( $P > 0.05$ ).

There was no significant difference in larval mortality when reared in different photoperiods ( $U_0 = 0.80, P > 0.05$ ); however, the mortality of pupae was significantly higher at 24:0 and 0:24 h than at 16:8 and 12:12 h ( $U_0 = 72.00, P > 0.0001$ ) (Figure 3.9).



**Figure 3.9** Mortality of larvae and pupae of *M. tasmaniae* at different photoperiods. Within the same category (Larvae or Pupae) columns with the same letters are not significantly different ( $P > 0.05$ ).

The body weight of cocoons was significantly higher at 24:0 h than at 0:24 h (ANOVA:  $F = 3.63; df = 3,60; P < 0.05$ ) (Table 3.3). Females and males were

significantly heavier at 16:8 h than at other photoperiods (ANOVA:  $F = 38.80$ ;  $df = 3,64$ ;  $P < 0.0001$  for female;  $F = 15.04$ ;  $df = 3,60$ ;  $P < 0.0001$  for male) (Table 3.3). At 16:8 and 12:12 h, females were significantly heavier than males (ANOVA:  $F = 27.28$ ;  $df = 1,28$ ;  $P < 0.0001$  for 16:8 and  $F = 19.12$ ;  $df = 1,30$ ;  $P < 0.0001$  for 12:12 h); however, no significant difference in body weight was detected between males and females at 24:0 and 0:24 h (ANOVA:  $F = 1.46$ ;  $df = 1,13$ ;  $P > 0.05$  for 24:0 h and  $F = 2.94$ ;  $df = 1,33$ ;  $P > 0.05$  for 0:24 h) (Table 3.3).

**Table 3.3: Mean body weights (mg) of cocoons and adults of *M. tasmaniae* at four photoperiods.**

L:D	Cocoon	Male	Female
24:0	$3.77 \pm 0.15 \alpha$	$1.98 \pm 0.12a \beta$	$2.20 \pm 0.19a \beta$
16:8	$3.71 \pm 0.15 \alpha\beta$	$2.42 \pm 0.09b \alpha$	$3.51 \pm 0.13a \alpha$
12:12	$3.52 \pm 0.14 \alpha\beta$	$1.61 \pm 0.08b \beta$	$2.25 \pm 0.10a \beta$
0:24	$3.16 \pm 0.11 \beta$	$1.77 \pm 0.12a \beta$	$2.03 \pm 0.10a \beta$

Means ( $\pm$  SE) followed by the same English letters in rows and Greek letters in columns are not significantly different ( $P > 0.05$ ).

#### 3.4.4.2 Effect of Photoperiod on Reproduction

All tested females laid fertilised eggs at the four photoperiods. The reproductive period of females significantly decreased with the increasing day length (KWT:  $\chi^2 = 26.71$ ,  $P < 0.0001$ ); however, the fecundity and fertility rate were significantly higher at 16:8 h than at 12:12, 24:0 and 0:24 h (ANOVA:  $F = 46.48$ ;  $56.59$  for fecundity and fertility, respectively;  $df = 3,58$ ;  $P < 0.0001$ ) (Table 3.4). The proportion of female offsprings was slightly lower than 50% with no significant difference detected between different photoperiods (KWT:  $\chi^2 = 0.41$ ,  $P > 0.05$ ).

**Table 3.4: Reproduction of *M. tasmaniae* at four photoperiods.**

L:D	Reproductive period (days)	Fecundity	Fertility (%)	Female offspring (%)
24:0	31.50 ± 2.13 c	153.20 ± 8.09 d	57.27 ± 4.08 c	43.10 ± 1.49 a
16:8	39.15 ± 0.36 bc	445.79 ± 9.53 a	75.75 ± 1.21 a	46.63 ± 9.76 a
12:12	40.05 ± 1.08 ab	352.11 ± 19.71 b	63.67 ± 1.08 b	46.28 ± 6.45 a
0:24	46.18 ± 1.15 a	252.69 ± 18.90 c	52.43 ± 1.36 c	46.79 ± 6.65 a

Means ( $\pm$  SE) followed by the same letters in columns are not significantly different ( $P > 0.05$ )

### 3.5 Discussion

As found in green lacewing, *C. carnea* (Balasubramani and Swamiappan 1994), *M. tasmaniae* of third instar larvae consumed the greatest portion of the total number of aphids. This may be due to their larger body size and better capacity in searching and capturing their prey.

In this study, *M. tasmaniae* developed significantly faster at long photoperiods (i.e. 24:0 and 16:8 h) than at short photoperiods (i.e. 12:12 and 0:24 h). According to Beck (1980) and Tauber et al. (1986), photoperiod may influence the development in a number of insects and growth and development are slower at short day length if insect diapause is induced by short photoperiods. However, my results showed that *M. tasmaniae* completed its life cycle and all females mated, oviposited and laid fertilised eggs at all tested photoperiods, suggesting that this species does not enter diapause. Canard and Volkovich (2001) also indicated that some hemerobiids have no diapause in temperate zones and life stages of cocooned pupae, reproductive adults or even eggs and active larvae can be found under short day lengths in the field, for example, in brown lacewing, *Hemerobius neadelphus* Gurney and *H. ovalis* Carpenter (Kevan and Klimaszewski 1987). The difference in development, prey consumption, mortality and reproduction under long and short photoperiods suggests that although *M. tasmaniae* does not enter diapause, photoperiod still has a significant effect on the endogenous circadian rhythms in metabolism, respiration and hormone production, which may regulate tissue synthesis and growth of insects (Beck 1980).

Although *M. tasmaniae* can complete development and reproduce under complete dark condition, the higher pupal mortality and lower fecundity and fertility suggest that such conditions are not recommended for mass-rearing of *M. tasmaniae* in the laboratory. *M. tasmaniae* reared at 16:8 h develop faster and have lower mortality, heavier adult body weight and higher reproductive output in terms of fecundity and fertility rate. Therefore, mass-rearing programmes are recommended to be carried out under 16:8 h condition. Moreover, environmental conditions leading to the production of female-biased progeny is warranted for further studies.



## **CHAPTER 4**

### **FACTORS AFFECTING REPRODUCTIVE FITNESS OF *MICROMUS TASMANIAE***

#### **4.1 General Introduction**

The effectiveness of potential biological control agents greatly depends on their reproductive fitness, i.e. fecundity, fertility, reproductive period and adult longevity. The study of the reproductive fitness of an insect helps us understand the life history and evolution of the species (Cloutier et al. 2000). Moreover, it allows the understanding of individual behaviour patterns observed in a population (Boulinier and Danchin 1997). Body size and mating strategies are the two major factors that affect the insects' reproductive fitness. This chapter investigates the impact of body weight, multiple mating in female and male mating history on the reproductive fitness of *M. tasmaniae*.

#### **4.2 Effect of Body Weight on Reproductive Fitness of *M. tasmaniae***

##### **4.2.1 Introduction**

Reproductive output is usually expressed as a function of adult mass (Hinton 1981; Leather 1988). Fitness is generally believed to be an increasing function of body size in animals (Clutton-Brock 1988; Reiss 1989), particularly in insects (Thornhill and Alcock 1983; Honěk 1993). Among insects, the relationship between body size and other reproductive traits differed significantly between males of territorial and non-territorial species (Elgar and Pierce 1988; Berrigan 1991; Sokolovska et al. 2000).

The fitness consequences of size or weight and its correlates, especially the supply of sperm or eggs and the adult longevity, are important in population dynamics and essential for understanding and modeling life history evolution and behavioural decisions (Tammaru 1998; Cloutier et al. 2000; Jiménez-Pérez and Wang 2004). It is considered axiomatic for most arthropods that the increased weight and/or size results in increased fecundity (Gilbert 1984). In some herbivorous insects, body size of

females is positively correlated with fecundity (Honěk 1993). The knowledge of relationship between body weight and reproductive performance is also important for the development of insect control techniques (Jiménez-Pérez and Wang 2004). However, body size may not always have a positive effect on reproductive success (Wall and Begon 1987; Ohgushi 1996; Tammaru et al. 1996).

Considering the importance of predatory lacewing as biological control agents, it is necessary to optimize mass production facilities of these species. The larger-the-better theory predicts that fitness is positively linearly associated with body size or weight. Size-fitness relationships are relevant to mass rearing programmes as weight is commonly monitored in laboratory insect populations and is regularly incorporated into process and product analysis of production systems as a measure of quality (Chambers and Ashley 1984). Therefore, it is important to understand the relationship between pupal weight and reproductive performance of *M. tasmaniae* by studying how and to what extent weight affected fecundity and fertility and whether it affected both sexes in a similar way.

## **4.2.2 Materials and Methods**

### **4.2.2.1 Insects and Experiment**

Insects were individually reared (Figure 3.4). Pupae were weighed and newly emerged adults were separated by sex just after emergence. All experiments were conducted under standard conditions (Section 3.2.2).

The effect of pupal weight on fecundity and fertility was studied by confining 126 breeding pairs during their lifespan in test containers (section 3.2.1). Insects were 2-d-old when used for this experiment. Twenty 1<sup>st</sup> to 3<sup>rd</sup> instar pea aphids were provided for each couple daily as food. Aphids were provided until the adults died. A complete factorial block design was used for this experiment, where each sex (factor) had three different pupal weights: light, average and heavy. A light or heavy pupa was defined as the one whose weight went below or above the standard deviation of the population. The experiment design produced nine treatments (3 female weights × 3 male weights) of breeding pairs (Table 4.1).

**Table 4.1: Number of *M. tasmaniae* breeding pairs in different body weight combinations (n = 126 pairs).**

Female class	Male class	n
Light	Light	10
Light	Average	10
Light	Heavy	10
Average	Light	14
Average	Average	26
Average	Heavy	16
Heavy	Light	12
Heavy	Average	16
Heavy	Heavy	12

To determine whether pupal weight had any effect on female reproductive potential, the fecundity, fertility, fertility rate, reproductive period, and female longevity for each female were recorded daily. The male longevity was also recorded.

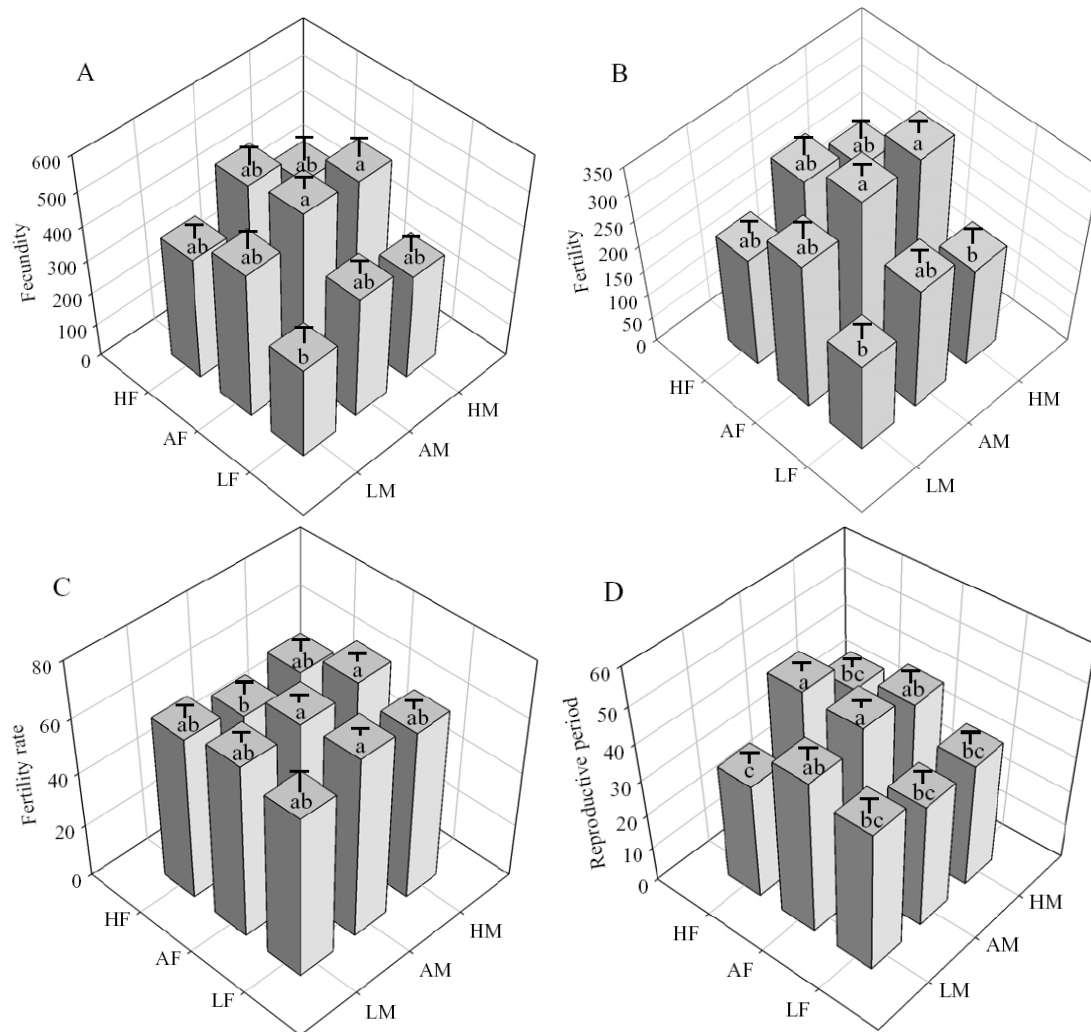
#### 4.2.3 Statistical Analysis

A t-test was used to compare the body weight between sexes. Two factor analysis of variance (ANOVA) followed by a less significant difference test (LSD) were used to analyze the influence of the pupal weight on fecundity, fertility, fertility rate, female reproductive period and female longevity. Fertility rate was arcsine transformed prior to analysis (Steel et al. 1997).

#### 4.2.4 Results

Mean female pupal weight ( $3.31 \pm 0.05$  mg) was significantly greater than male pupal weight ( $2.67 \pm 0.05$  mg) (t-test:  $F = 86.85$ ;  $df = 1,286$ ;  $P < 0.0001$ ). Females that mated with males which weighed less than 1.73 mg failed to lay eggs. For all weight categories, the mean life time fecundity, fertility, fertility rate, reproductive period (days) and female longevity (days) were  $422.62 \pm 166.81$ ,  $275.18 \pm 128.43$ ,  $62.76 \pm 1.24$ ,  $41.67 \pm 1.25$  and  $47.90 \pm 1.24$ , respectively.

Average females mated with average or heavy males had significantly higher fecundity, fertility, fertility rate and reproductive period (Figures 4.1A-D). However, body weight had no effect on longevity of males (from  $57.08 \pm 6.61$  to  $78.60 \pm 5.06$  days) and females (from  $38.33 \pm 2.92$  to  $52.75 \pm 3.53$  days) (Two Factor ANOVA:  $F = 1.52$  and  $1.68$  for male and female, respectively;  $df = 8,117$ ;  $P > 0.05$ ).



**Figure 4.1** Effect of male and female pupal weight on (A) fecundity (Two Factor ANOVA:  $F = 3.01$ ;  $df = 8,117$ ;  $P = 0.0042$ ), (B) fertility ( $F = 2.52$ ;  $df = 8,117$ ;  $P = 0.0145$ ), (C) fertility rate ( $F = 2.01$ ;  $df = 8,117$ ;  $P = 0.0451$ ), (D) reproductive period ( $F = 2.25$ ;  $df = 8,117$ ;  $P = 0.0288$ ). LM - light male, AM - average male, HM - heavy male, LF - light female, AF - average female and HF - heavy female. Columns with the same letters in each category are not significantly different ( $P > 0.05$ ).

#### 4.2.5 Discussion

Studies of reproductive performance in female insects have often focused on direct measures of gamete production such as egg size, egg number and ovary volume (Montague et al. 1981; Wickman and Karlsson 1989; Berrigan 1991). Fecundity in most insects varies with body size of the female (Reiss 1989). Female size is usually a good predictor of potential fecundity and species with no positive relationship between female size and fecundity are scarce (Slansky 1980; Boggs 1986; Johnson 1990). For example, in the lepidopteran leaf roller *Cnephasia jactatana* Walker, heavy females lay larger (Marshall 1990; Iyengar and Eisner 2002) and more eggs (Cloutier et al. 2000; García-Barros 2000). It is indicated that if fecundity is constrained only by the size of the female, a linear fecundity/weight relationship should be expected. In this study, the body weight of female *M. tasmaniae* does not affect the fecundity, fertility, fertility rate, oviposition period and female longevity. This implies that female size variation is of secondary importance in determining important fitness components of the reproductive process in the female Tasmanian lacewing (Ohgushi 1996). However, the mechanism by which the reproductive fitness of female *M. tasmaniae* is not affected by their body weight remains unclear. Similar cases have been reported in the Mormon fritillary butterfly, *Speyeria mormonia* Edwards (Boggs 1986) and the small carpenter bee, *Ceratina calcarata* Robertson (Johnson 1990). Therefore, female body weight affecting reproductive fitness may vary from species to species.

Males of many animal taxa allocate resources largely to mate acquisition and defense, contributing little more than gametes to embryo production (Fox et al. 1995). In many insects, males transfer large spermatophores or ejaculates to females during mating, and extragametic substance derived from these packages are used by the recipient female (Boggs 1981; Fox et al. 1995). Wedell (1996) showed that females of comma butterfly *Polygonia c-album* L. (Lepidoptera: Nymphalidae) may use male donations for egg production and females received larger donations live significantly longer than those received smaller donations, suggesting that females can also use males' nutrients for somatic maintenance. On the other hand, as found in an arctiid moth *Utetheisa ornatix* L., the larger spermatophores may consist of more sperm for fertilization and nutrition for reproduction and thus stimulate females to lay more eggs,

fertilize more eggs and prolong their life (Iyengar and Eisner 2002). In lacewing, males have been found to transfer spermatophores to their mates during copulation (Henry 1984; Principi 1986). In this study, average females mated with average or heavy males had significantly higher fecundity, fertility and fertility rate and longer reproductive period. These results suggest that average and heavy males transferred larger spermatophores to average females and small spermatophores to light females during mating. Thus, the average and heavy males and average females could be higher in quality.

In conclusion, the male body weight may contribute to the population growth of *M. tasmaniae*. Roff (1981) and Tanaka (1981) state that it may be advantageous for an insect to attain a certain standard (optimal) size when it reaches the adult stage. In *M. tasmaniae*, variation in temperature (Islam and Chapman 2001), larval and adult nutrition (Leathwick 1989; Robinson et al. 2008; Robinson 2009) may influence the growth and development. Moreover, male body size may also have an impact on mating behaviour, since smaller males may produce less stimulation and be less successful in maintaining a dominant position than larger ones (Jervis and Copland 1996). Thus, the results of the present study suggest that the development of any control tactics that could selectively mass produce heavy and average body weight of *M. tasmaniae* in the laboratory would further increase population growth.

### 4.3 Effect of Female Remating on Reproductive Fitness of *M. tasmaniae*

#### 4.3.1 Introduction

All biological processes directly related to reproduction usually play a role in determining fitness. In most insects, mating is one of the most important processes that determine the reproductive fitness, such as the fertility (Turner and Anderson 1983). In many insect species a female obtains enough sperm from a single mating to fertilize her full egg-load (Thornhill and Alcock 1983; Drummond 1984; He and Miyata 1997). On the other hand, many females mate more than once, either with the same male (repeated mating) or with different males (multiple mating) (Ridley 1990; Arnqvist and Nilsson 2000).

Multiple mating increases the probability of the female to mate with superior males and in turn improve her reproductive potential and longevity (Yasui 1997; Savalli and Fox 1998). Polyandry may function, in part, to facilitate females' pursuit of good genes (Walker 1980; Evans and Magurran 2000; Jennions and Petrie 2000). Female multiple mating may be essential for fertility assurance because the first copulation often does not lead to offspring production as found in red flour beetle *Tribolium castaneum* (Herbst) (Pai 2005). Alternatively, females may mate multiple times to ensure that adequate sperms are acquired (Jones 2001), avoid male harassment (Lauer et al. 1996), encourage sperm competition (Curtsinger 1991; Madsen et al. 1992), or avoid inbreeding (Hosken and Blanckenhorn 1999). Some studies have shown that female multiple mating can serve to reduce the risk that females mate accidentally with a poor quality male, thus reducing variance in reproductive success within a single environment (Watson 1991; Fox and Rauter 2003).

The effect of multiple mating on fecundity is highly dependent on the mating system of each species (Ridley 1988). Several studies have found a positive relationship between the number of matings and female fecundity (Danthanarayana and Gu 1991; Pardo et al. 1995; Oberhauser 1997; Wilson et al. 1999), while others do not show such relationship (Ono et al. 1995; Kawagoe et al. 2001), or even a negative relationship is found (Cook 1999). In some insects and phytoseiid mites,

multiple mating may result in a shorter female life span (Amano and Chant 1978; Partridge and Harvey 1988; Ridley 1988; Chapman et al. 1998; Arnqvist and Nilsson 2000; Kawagoe et al. 2001).

Lacewings have a high reproductive rate (Elkarmi et al. 1987), a relatively long adult life, and a long oviposition period (New 1975). Some extensive data are available on the egg production for chrysopids and hemerobiids (Rousset 1984; Hilson 1964; Leathwick and Winterbourn 1984). To my knowledge, however, the extent of polyandry, or the effect of multiple matings on fecundity and fertility, has not been determined for any hemerobiid. This experiment was to determine whether and how female multiple mating affected the reproductive fitness of *M. tasmaniae* in terms of fecundity, fertility, sex ratio and longevity. Such information is of great significance in gaining better understanding of mass rearing programmes for biological control.

### **4.3.2 Material and Methods**

#### **4.3.2.1 Experiment**

Insects were individually reared (Figure 3.4) and separated by sex just after emergence. Male and female adults used in this experiment were randomly selected from the individually reared insects. All experiments were conducted under standard conditions (Section 3.2.2).

To determine the effect of multiple mating on reproductive fitness of female *M. tasmaniae*, three treatments were set up: (1) once-mated female, a virgin female mated once with a virgin male; (2) twice-mated female, a virgin female mated with two virgin males in an interval of 24 hrs; and (3) permanently-paired female, a virgin female paired with a virgin male permanently. There were 19, 10, and 20 replicates for treatments (1), (2) and (3), respectively. Insects paired for the first mating were all 2 days old. The tested females were individually fed with 10 1<sup>st</sup> to 3<sup>rd</sup> instar pea aphids in treatments (1) and (2); in treatment (3), 20 aphids were provided to the couple. Aphids were provided until the females died. Test insects were housed individually in plastic containers (section 3.2.1); a black cotton sheet was placed on



the bottom for oviposition and replaced daily. The reproductive period, longevity, fecundity, and fertility were recorded. Twenty-eight virgin females were applied as control and their reproductive period, longevity and fecundity were also recorded.

### 4.3.3 Statistical Analysis

A goodness-of-fit test was used to test the distribution of data before analysis. Data on female reproductive period and longevity were not normally distributed even after transformation and thus were analyzed using the non-parametric Kruskal-Wallis test (KWT) followed by Dunn's procedure for multiple comparisons. Data on female fecundity, and fertility were normally distributed and analyzed using ANOVA.

The reproductive fitness (i.e. fecundity and fertility) of *M. tasmaniae* females at different mating conditions over times were analysed using linear regression, and an analysis of covariance (ANCOVA) was used to analyse the slopes of the those regressions.

### 4.3.4 Results

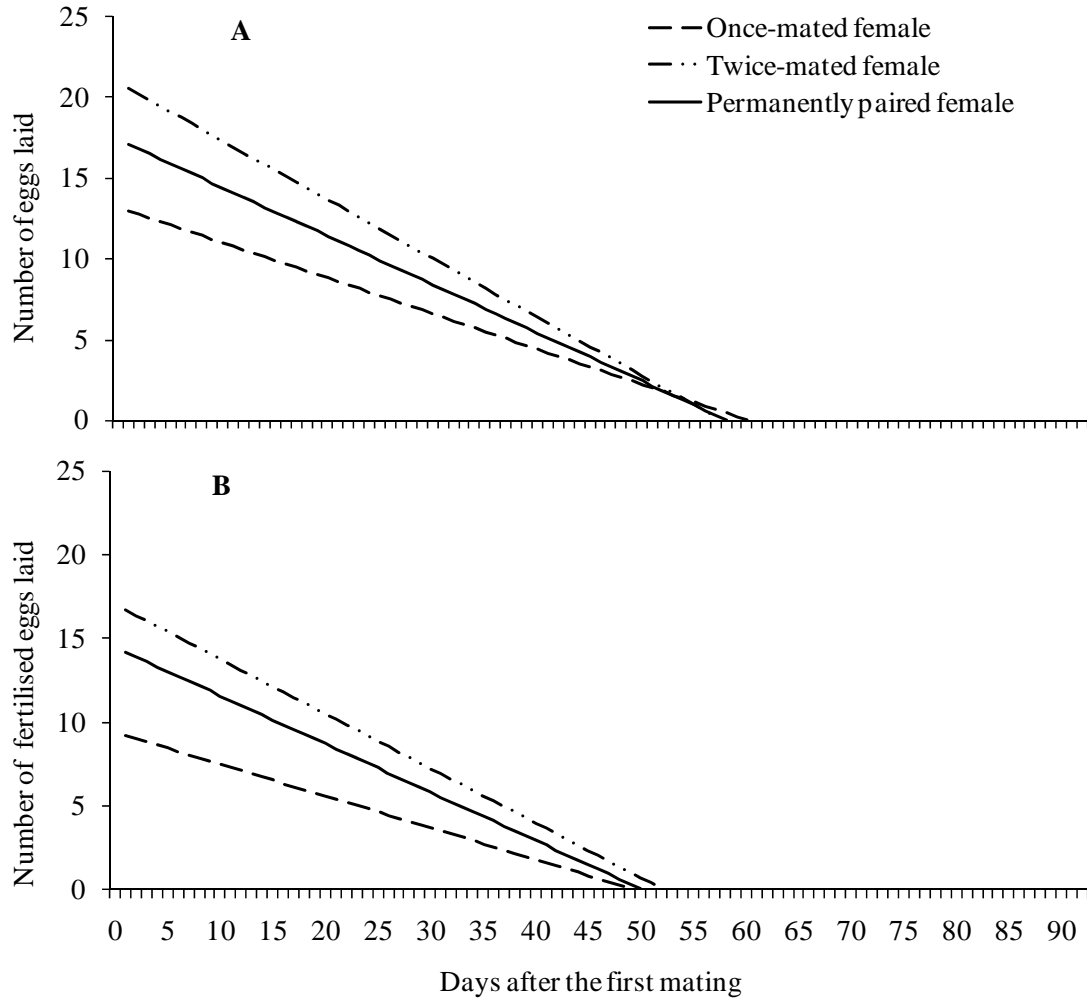
The reproductive period and longevity of virgin females were significantly longer than those of mated ones, and once-mated females had significantly longer reproductive period and longevity than multiply mated ones (KWT:  $\chi^2 = 35.25$  and  $33.17$  for reproductive period and longevity, respectively;  $P < 0.001$ ) (Table 4.2). Twice-mated females achieved significantly higher fecundity and fertility than other treatments (ANOVA:  $F = 144.57$ ;  $df = 3,73$ ;  $P < 0.001$  for fecundity;  $F = 46.04$ ;  $df = 2,46$ ;  $P < 0.001$  for fertility) (Table 4.2).

**Table 4.2: Effect of multiple mating on reproductive fitness of female *M. tasmaniae*.**

Females	Reproductive period (days)	Longevity (days)	Fecundity	Fertility
Virgin	54.07 ± 3.06a	58.93 ± 3.11a	125.29 ± 8.86 d	----
Once-mated	30.95 ± 1.71c	35.95 ± 1.95c	300.00 ± 18.21c	194.58 ± 13.89c
Twice-mated	41.90 ± 1.49b	45.70 ± 1.63ab	550.00 ± 36.01a	423.30 ± 31.24a
Permanently-paired	39.16 ± 0.38b	42.40 ± 0.38b	445.79 ± 9.53 b	339.25 ± 10.64b

Means (± SE) followed by the same letters in a column are not significantly different ( $P > 0.05$ ).

Results of linear regression indicate that females' fecundity and fertility significantly decreased over time at each mating condition (Figure 4.2). Furthermore, the decrease of fecundity and fertility was significantly faster in the twice-mated females than in permanently-paired ones, and that was significantly faster in the permanently-paired females than in once-mated ones (ANCOVA:  $F = 192.74$ ,  $df = 3,1819$ ,  $F < 0.0001$  for fecundity;  $F = 242.71$ ,  $df = 3,1819$ ,  $F < 0.0001$  for fertility) (Figure 4.2A and B).



**Figure 4.2** Reproductive fitness ( $y$ ) of *M. tasmaniae* females at different mating conditions over times: (A) fecundity (Linear regression:  $y = -0.2193x + 13.182$ ,  $R^2 = 0.1214$ ,  $F = 83.89$ ,  $df = 1,607$ ,  $P < 0.0001$  for once-mated females;  $y = -0.3632x + 20.902$ ,  $R^2 = 0.3263$ ,  $F = 178.21$ ,  $df = 1,426$ ,  $P < 0.0001$  for twice-mated females;  $y = -0.2999x + 17.401$ ,  $R^2 = 0.2210$ ,  $F = 222.76$ ,  $df = 1,785$ ,  $P < 0.0001$  for permanently-paired females) and (B) fertility (Linear regression:  $y = -0.1905x + 9.3865$ ,  $R^2 = 0.1441$ ,  $F = 102.22$ ,  $df = 1,607$ ,  $P < 0.0001$  for once-mated females;  $y = -0.3250x + 17.011$ ,  $R^2 = 0.3472$ ,  $F = 225.48$ ,  $df = 1,426$ ,  $P < 0.0001$  for twice-mated females;  $y = -0.2870x + 14.420$ ,  $R^2 = 0.2555$ ,  $F = 269.41$ ,  $df = 1,785$ ,  $P < 0.0001$  for permanently-paired females).

#### 4.3.5 Discussion

*Micromus tasmaniae* can mate up to 8 times during their life (see Section 4.4). Several mechanisms have been proposed to explain how multiple mating could lead to greater female reproduction. For example, one potential advantage of female multiple mating could be to reduce the risk of mating singly with a sterile male (Olsson and Shine 1997). However, in this study, mated females, either mated once or multiply mated, had significantly shorter reproductive period and longevity than virgin females. These may be due to the physical damage by multiple matings (Blanckenhorn et al. 2002) and energy invested in increasing egg production (Yanagi and Miyatake 2003). In the fruit fly, *Drosophila melanogaster* Meigen, a single mating significantly reduces female longevity when compared with virgin females (Kidwell and Malick 1965; Malick and Kidwell 1966). A reduced life span in multiply-mated females has also been reported in the predatory mite, *Amblyseius umbraticus* Chant (Knisley and Swift 1971).

In lacewing, males may transfer spermatophores to their mates during copulation (Henry 1984; Principi 1986). It has been reported that in several insect orders, including Neuroptera and Lepidoptera, females absorb spermatophores internally and using the lipoprotein structure for egg production and general maintenance (Boggs and Gibert 1979). For example, in the butterflies, *Danaus plexippus* (L.), *Heliconius hecale* Zuleika, and *H. erato* (L.), male-derived substances are found to be incorporated into eggs and some portion of the ejaculate enters the hemolymph and is transported throughout the body where they are metabolized (Boggs and Gibert 1979). Furthermore, postcopulatory feeding of spermatophore that protruded from the female's genital chamber has been reported in many chrysopids (Toschi 1965; Tauber 1969). Withycombe (1923) described the presence of a large, rounded spermatophore in a brown lacewing protruding dorsally from the genital opening of an inseminated female. Toschi (1965) demonstrated that in the lacewing *Nothochrysa californica* Banks, males additionally secrete a whitish-yellow collar around the female's abdomen and the female later eats the shriveled spermatophore case after its contents have been taken into her abdomen. Same habit has been noted in brown lacewing *Symphorobius pygmaeus* (Rambur) (Killington 1931) and Sisyridae (Withycombe 1923). In lacewings, female oviposition is initiated and

stimulated by copulation (Canard and Volkovich 2001). The above properties of lacewing species suggest that *M. tasmaniae* males may transfer not just sperm but also egg production stimulants and nutrients to females during copulation, resulting in the higher fecundity detected in this study.

Among the mated *M. tasmaniae* females, the longer reproductive period and longevity and higher fecundity, fertility and fertility rate of twice-mated and permanently-paired females may be because these females received twice or more spermatophores for reproduction and fertilization during their life. Many studies have reported that multiple mating has a positive effect on the reproductive fitness of insect females. For example, in the seed beetle *Stator limbatus* Horn, females that received multiple ejaculates lived longer than once-mated females (Fox 1993a, 1993b; Fox et al. 1995); in the fruit fly *D. melanogaster*, fecundity and fertility increased in multiple-mated females (Pyle and Gromko 1978); and in the codling moth *Cydia pomonella* L., females that had mated three times had a significantly higher fecundity than once-mated ones (Knight 2006). Compared to the twice-mated *M. tasmaniae* females (mated with two different males), permanently-paired females had lower fecundity and fertility. Mating in *M. tasmaniae* lasts for about three hours (see Section 3.3 and 4.4). Thus, the more frequency of matings in permanently-paired females and long mating duration may reduce the time required for them to feed and oviposit, and subsequently result in the lower fecundity and fertility.

Philippe (1971) has reported that in the green lacewing *Chrysopa perla* L., the spermatozoid stock provided by a single mating lasts an average of 24 days. Results from this study indicate that the spermatozoid stock may last up to about 50 days (Figure 4.2), because there after no fertility was detected. Among the mated *M. tasmaniae* females, the significantly faster decrease of fecundity and fertility in twice-mated females may be attributed to the higher fecundity and fertility during their early life (Figure 4.2).

It is clear from this study that a single mating does not maximize female fitness in *M. tasmaniae*. Despite the overall negative effects of multiple mating on female longevity in *M. tasmaniae*, the positive effects of remating outweigh the

negative effect. Therefore, if ejaculates provide nutrition, a multiple-mated female may be in a better nutritional state than a singly mated female.

#### **4.4 Effect of Male Mating History on Reproductive Fitness of *M. tasmaniae***

##### **4.4.1 Introduction**

Multiple mating by males is widespread and common in insects (Dewsbury 1982; Thornhill and Alcock 1983; Andersson 1994; Torres-Vila and Jennions 2004). The fitness of male insects depends on the number of progeny they father during their lifetime (Arnqvist and Nilsson 2000), which in turn depends on their capacity to acquire mates (Henry and Buser 1987) and their mates' lifetime potential fecundity (Roitberg et al. 2001). Male insemination potential refers to the number of females a male can successfully inseminate during a given period of time, and is thus related to his capacity to acquire mates (Arnqvist and Nilsson 2000; Jacob and Boivin 2004). The ultimate goal of male reproductive behaviour is the success in transmitting an individual's genes to the next generation (Henry and Buser 1987). Male fitness increases monotonically with increased mating rate (Arnqvist and Nilsson 2000).

Male insemination potential and sperm allocation vary greatly between insect species (Jacob and Boivin 2004). Steiner et al. (2007) reported that mating with sperm-depleted males did not increase female mating frequency in the granary weevil parasitoid *Lariophagus distinguendus* Först; however, the sperm-depleted males continued to mate even if they transferred only small amount of or few sperm. The *L. distinguendus* males were able to mate with up to 17 females offered in rapid succession within a 10-h test period (Steiner et al. 2007). In the egg parasitoid, *Trichogramma evanescens* Westwood, independent of their age and sperm-depletion status, males continued to mate with females until the end of their life and they quickly depleted their sperm supply after fertilizing 18 females during their lifetime (Jacob and Boivin 2004). Therefore, sperm is a limited resource in most species and females might encounter males with varying amount of sperm.

In many insects, males transfer spermatophores or ejaculates to females during mating and such extragametic substances obtained from these packages are used for

somatic maintenance and egg production by the recipient females (Boggs 1981, 1990; Fox et al. 1995). In the bollworm, *Helicoverpa armigera* Hubner, male mating history had a significant influence on female fecundity (Maolin and Chengfa 1999), i.e. the fecundity of females mated to virgin males was higher than that of females mated to previously mated males. Butlin et al. (1986) examined the effect of multiple mating on fecundity in the grasshopper *Chorthippus brunneus* Thunberg (Orthoptera: Acrididae) and reported that the male spermatophore investment increased female fecundity. However, the nutrients invested in spermatophores represent a cost to the male (Gwynne 1984) and repeated mating with females reduces the number of offspring a male can expect from a mating (Prout and Bundgaard 1977). The age of the male also affects reproductive potential of females, for example, in codling moth, *C. pomonella*, females mated with 3-day-old males have significantly lower fecundity than those mated with 1-day-old males (Knight 2006). Furthermore, the mean size of the first spermatophore transferred by males was significantly larger than that of all subsequent spermatophores (Knight 2006).

Extensive fecundity data, relating egg production to diet or age, have been published for *M. tasmaniae* (Hilson 1964; Leathwick and Winterbourn 1984; Rousset 1984; Leathwick 1989). Sexual behaviour has been extensively investigated in chrysopids, but little is known for other lacewings (Carnard and Volkovich 2001). Among hemerobiids, mating behaviour has been described only in *Symphorobius* sp. (Smith 1923; Killington 1936; Henry 1997). However, to my knowledge, the extent of polygyny, or the insemination capacity of males has not been determined for *M. tasmaniae*, information of which is useful for understanding the mating system of this species.

#### **4.4.2 Material and Methods**

Insects were individually reared (Figure 3.4) and separated by sex just after emergence. Male and female adults used in this experiment were randomly selected from the individually reared insects. All experiments were conducted under standard conditions (Section 3.2.2).

#### 4.4.2.1 Male Mating Potential

To determine the effect of the number of matings on the reproductive fitness of *M. tasmaniae*, 2-day-old virgin males and 2-day-old virgin females were randomly selected and paired individually in plastic containers for 12 h during the photophase. Twenty 1<sup>st</sup> to 3<sup>rd</sup> instar pea aphids were provided for each couple daily as food. These containers were observed every 15 min. Upon mating, the copulation duration was recorded and soon after mating termination, the female was immediately moved to another container. The male was allowed to rest for 24 h and then paired with another 2-day-old virgin female, etc. until he died. When the male and female were reared individually, ten pea aphids were provided for each of them daily. The number of females mated by each male was recorded. The time interval between successful matings and the longevity of the male were recorded. Eighteen males were used for this study.

#### 4.4.2.2 Reproductive Fitness of Females

To determine the effect of male mating history on the reproductive fitness, mated females were reared individually in above mentioned plastic containers and 10 pea aphids were provided daily. The daily fecundity, fertility and fertility rate, reproductive period and longevity of females were recorded.

#### 4.4.3 Statistical Analysis

A goodness-of-fit test was used to test the distribution of data before analysis. Data on time interval between successive matings and fertility rate were not normally distributed even after transformation and thus analysed using the non-parametric Kruskal-Wallis test (KWT) followed by Dunn's procedure for multiple comparisons. Data on mating duration, male longevity, and female fecundity, fertility, reproductive duration and longevity were normally distributed and analyzed using ANOVA followed by a Tukey's studentized range (HSD) test.



#### 4.4.4 Results

##### 4.4.4.1 Male Mating Potential

Males of *M. tasmaniae* could mate up to eight females during their life. The mean number of females that a male could mate with was  $4.67 \pm 0.40$  and a male could father  $1006.89 \pm 95.73$  offspring during his life span. The male mating history had no significant effect on mating interval (KWT:  $\chi^2 = 4.55 < \chi^2_{6,0.05} = 12.59$ ,  $P = 0.60$ ) or the mating duration (ANOVA:  $F = 1.40$ ;  $df = 7,76$ ;  $P = 0.22$ ) (Table 4.3). Longevity of mated males was  $52.4 \pm 1.7$  days.

##### 4.4.4.2 Effect of Male Mating History on Reproductive Fitness of Female

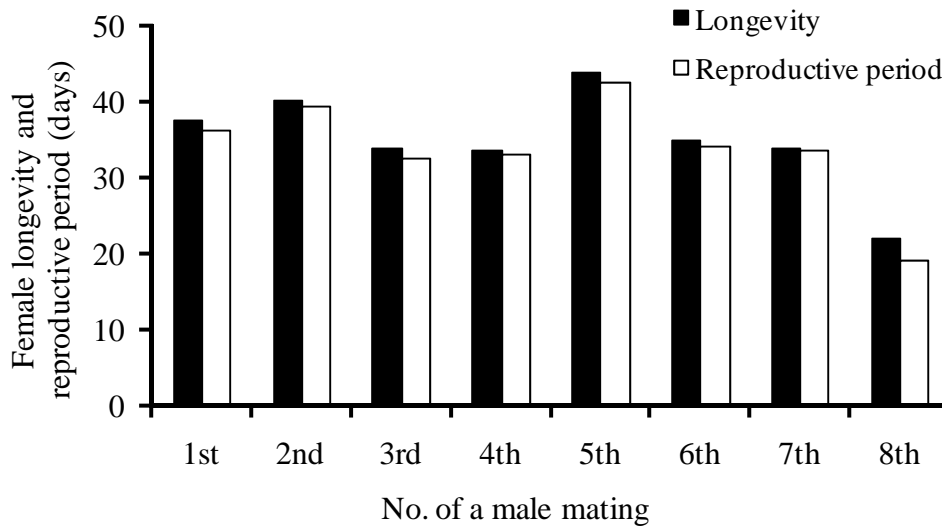
The male mating history had no significant effect on female fecundity (ANOVA:  $F = 1.46$ ;  $df = 7,75$ ;  $P = 0.19$ ). However, the fertility and fertility rate significantly decreased with an increase of the number of matings the male had achieved (ANOVA:  $F = 2.48$ ;  $df = 7,75$ ;  $P = 0.02$  for fertility; KWT:  $\chi^2 = 29.67 > \chi^2_{7,0.05} = 14.07$ ,  $P < 0.0001$  for fertility rate) (Table 4.3).

**Table 4.3: Effect of male mating history on reproductive fitness of *M. tasmaniae*.**

Male mating history	Mating interval (days)	Mating duration (min)	Fecundity	Fertility	Fertility%
First	---	$166.50 \pm 6.07a$	$320.39 \pm 17.51a$	$206.89 \pm 14.39ab$	$63.70 \pm 1.63ab$
Second	$4.06 \pm 0.96a$	$180.06 \pm 6.57a$	$404.71 \pm 42.09a$	$269.76 \pm 26.92a$	$66.95 \pm 0.92a$
Third	$2.83 \pm 0.66a$	$179.44 \pm 4.86a$	$325.00 \pm 28.37a$	$220.44 \pm 21.05ab$	$67.32 \pm 0.80a$
Fourth	$2.92 \pm 0.75a$	$179.38 \pm 3.67a$	$308.85 \pm 20.65a$	$210.77 \pm 16.79ab$	$67.77 \pm 1.28a$
Fifth	$1.67 \pm 0.42a$	$185.17 \pm 3.57a$	$393.33 \pm 56.83a$	$241.33 \pm 31.22a$	$62.22 \pm 1.74ab$
Sixth	$3.17 \pm 1.08a$	$189.00 \pm 6.20a$	$348.17 \pm 42.84a$	$181.00 \pm 19.02ab$	$52.81 \pm 2.18b$
Seventh	$2.75 \pm 1.18a$	$158.25 \pm 9.84a$	$304.25 \pm 11.58a$	$115.50 \pm 30.11b$	$38.77 \pm 10.23c$
Eighth	$1.00 \pm 0.00a$	$189.00 \pm 0.00a$	$182.00 \pm 0.00a$	$110.00 \pm 0.00b$	$60.44 \pm 0.00b$

Means ( $\pm$  SE) followed by the same letters in the columns are not significantly different ( $P > 0.05$ ).

Male mating history had no significant effect on female longevity and reproductive period (ANOVA:  $F = 1.67$  and  $1.77$  for female longevity and reproductive period;  $df = 7,75$ ;  $P > 0.05$ ) (Figure 4.3).



**Figure 4.3** Effect of male mating history on female longevity and reproductive period.

#### 4.4.5 Discussion

Male and female insects have divergent mating interests (Chapman et al. 2003; Steiner et al 2007). The ability of males to mate repeatedly has a dramatic effect on their lifetime reproductive success (Bissoondath and Wiklund 1996). A male's reproductive fitness is directly associated with the number of females he is able to inseminate; thus it is widely accepted that the best male strategy to maximize fitness is generally to acquire as many mates as possible (Trivers 1972; Thornhill and Alcock 1983). In the present study *M. tasmaniae* males could mate successfully up to eight females and a male could father more than 1000 offspring during his life span. Remating is frequently reported in male green lacewings; for example, in *Chrysoperla plorabunda* (Fitch) and *C. downesi* (Smith), a male could mate with nine and 28 different females at 24 h intervals and father average 1340 and 2220 offspring, respectively (Henry and Busher 1987).

A common notion in sexual selection literature is that multiple mating is generally a more adaptive strategy for males than for females (Thornhill and Alcock 1983; Halliday and Arnold 1987; Opp and Prokopy 2000).

The mating duration could last for about three hours. While male mating history had no effect on the mating duration, longer mating duration may be a disadvantage to insects. For example, longer copulation duration may increase the risk of being eaten by predators, physical injury, etc. (Wang et al. 2004). Similarly, there was no significant difference in the mating interval (days). However, in certain green lacewings the time interval between matings varies greatly with the success of insemination (Henry and Busher 1987). Although the rates of remating are unknown for the wild population of *M. tasmaniae*, opportunities for remating are likely to be plentiful. Thus, males are likely to have numerous opportunities to mate multiply and can maximize their fitness by mating as often as possible.

Most males from orders Neuroptera, Lepidoptera, Orthoptera, Trichoptera, Diptera, Odonata, Coleoptera, Hemiptera and Psocoptera transfer spermatophores to females during copulation (Gerber 1970). The spermatophore contains sperm and accessory gland products (Thornhill and Alcock 1983). There is a possibility that the female might absorb and use the ejaculate for nourishment and that nutrients accrued through copulation can be used for the egg production and/or somatic maintenance (Boggs and Gilbert 1979; Boggs 1990; Wiklund et al 1993). In this study, male mating history had no significant effect on longevity, reproductive period and fecundity of *M. tasmaniae* females, suggesting that these females received a similar quantity of nutrition, if any, from the spermatophores regardless of male mating history. However, there was a significant reduction in female fertility and fertility rate with the increase of the number of male matings. Henry and Busher (1987) indicated that male lacewings may suffer from irreversible sperm depletion after each mating, resulting in the reduction of fertility and fertility rate.

## CHAPTER 5

### GENERAL DISCUSSION AND CONCLUSION

#### 5.1 Introduction

In this thesis, I investigated general biology and the factors that affect the reproductive fitness of *M. tasmaniae*. Such knowledge is important for the better understanding of the reproductive biology of *M. tasmaniae*.

In this chapter, I summarise and discuss my main findings and their relevance to the reproductive fitness of *M. tasmaniae* in order to provide valuable information necessary for the improvement in the mass rearing and release technique of this biological control agent.

#### 5.2 Biological Rhythms of *M. tasmaniae*

In insects, adults' circadian activities are usually correlated with biological rhythms, such as adult emergence, mating and oviposition. On a 24-h basis, the adult emergence of *M. tasmaniae* peaked 3 hours before lights off. Emergence of the adults some time before the lights off possibly coincides with more favourable conditions, such as dusk, in which adults may have the chance of safer habitat location and prey searching.

Newly emerged lacewings can not mate and the females can not oviposit as they have immature gonads at emergence (Canard and Principi 1984; Canard and Volkovich 2001). In *M. tasmaniae*, emergence pattern is similar between both sexes; however, males become sexually mature in about two days after emergence which is 20 h earlier than females. The early maturity in males may provide better opportunities for them to find receptive females before the latter start oviposition 3 days after emergence and in turn increase reproductive fitness of both sexes. The *M. tasmaniae* females can live for about two months and lay eggs until she dies. Therefore, compared to their long longevity, the short sex maturation period of females may not significantly hamper the reproductive fitness in this species.

Mating mostly occurred between 7 and 13 hours after lights on and mating usually lasted for 3 hours. Hence, as nocturnal predators, this mating pattern may be advantageous to both sexes in host searching and/or oviposition after successful mating. This suggests that newly emerged adults should be held for at least 3 day for copulation to occur before release, and they should be released at night to achieve the higher reproductive fitness and survivorship.

### **5.3 Development and Reproduction of *M. tasmaniae* under Different Photoperiods**

Photoperiod affecting various processes such as the rate of development, reproduction, synchronised adult emergence, and induction of diapause (Gillott 2005) has been reported in many lacewing species (Canard and Principi 1984; Canard and Grimal 1988; Chang et al. 1995; Orlova 1998). The results in this study show that *M. tasmaniae* completed its life cycle and all females mated and laid viable eggs under all tested photoperiods, suggesting photoperiod does not trigger diapauses in this species.

However, difference in development, prey consumption, mortality and reproduction was observed among the tested photoperiods suggesting that photoperiod still has a significant effect on development and reproduction of *M. tasmaniae*. Individuals reared at 16:8 h (light:dark) develop faster and have lower mortality, heavier adult body weight and higher reproductive output in terms of fecundity and fertility rate. Therefore, mass-rearing programmes are recommended to be carried out at 16:8 h to obtain the higher quality of individuals and faster increase of *M. tasmaniae* population.

### **5.4 Factors Affecting the Reproductive Fitness of *M. tasmaniae***

In lacewings, males may transfer spermatophores to their mates during copulation (Killington 1931; Withycombe 1923; Henry 1984; Principi 1986). Factors such as mate body weight, female multiple mating and male mating history in relation to spermatophore transformation may significantly affect the reproductive fitness of *M. tasmaniae* in terms of fecundity, fertility and longevity. The results of reproductive

fitness are extremely important to understand how lacewings fit into existing theories of biological control (New 1975).

Body size or weight has traditionally been considered a key determinant of an organism's ecological and physiological properties (Thornhill and Alcock 1983; Honěk 1993; Jiménez-Pérez and Wang 2004). In this study, although body size had no effect on longevity of both sexes, average weight females mated with average or heavy males had significantly higher fecundity, fertility and fertility rate with longer reproductive period. It is suggested that heavier males transfer more sperm and nutrition. Therefore, in the mass-production of *M. tasmaniae* male body weight should be considered an important fitness factor.

Furthermore, multiple mating of *M. tasmaniae* females may increase their reproductive fitness. Among the mated females, twice-mated and permanently-paired females had higher fecundity, fertility and fertility rate and longer reproductive period than once-mated ones. However, mated females had shorter longevity and reproductive period than virgin ones. A reduction of mated females' longevity and reproductive period could be due to the mating cost itself, such as physical damage by repeated copulation and the cost of an increased egg production (Chapman et al. 2003; Arnqvist and Nilsson 2000; Getsuo and Tsuchiya 2008).

Male's reproductive success is directly associated with the number of females inseminated. *M. tasmaniae* males can achieve up to 8 matings and father more than 1000 offspring during his life span. Results indicate that the fertility of a male significantly decreased with the increasing number of females he mated, but male mating history had no significant effect on female longevity, reproductive period and fecundity. These results suggest that *M. tasmaniae* males may suffer from irreversible sperm depletion after each mating but the spermatophores from the same mated male may contain similar quantity of nutrition between each matings.

## **5.5 Conclusion**

In this thesis, I have reported and discussed the main findings of general biology and the factors affecting the reproductive fitness of Tasmanian lacewing *M.*

*tasmaniae*. This work has provided essential information on the reproductive fitness of this species, and a perspective of its reproductive biology. Such knowledge is important for the mass production of quality biological control agents in an insectary.

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**EFFECT OF PHOTOPERIOD ON DEVELOPMENT  
AND REPRODUCTION IN TASMANIAN LACEWING  
*MICROMUS TASMANIAE* (WALKER) (NEUROPTERA:  
HEMEROBIIDAE)**

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**ABSTRACT**

Tasmanian lacewing, *Micromus tasmaniae* (Walker), is an important predator of many aphid species. This study investigated the effect of four photoperiods on predation, development and oviposition of *M. tasmaniae* in the laboratory at  $21 \pm 1^\circ\text{C}$  and 60% RH. Results indicated that no individuals entered diapause at either immature or adult stage. At each photoperiod, late instar larvae consumed significantly more aphids than early instar larvae ( $P < 0.0001$ ). Developmental period of *M. tasmaniae* from egg to adult was significantly shorter at 24:0 and 16:8 h (light:dark) than at 12:12 and 0:24 h ( $P < 0.0001$ ). Pupal mortality was significantly lower at 16:8 and 12:12 h. The reproductive period decreased significantly with increasing day length. However, fecundity and fertility were significantly higher at 16:8 and 12:12 h ( $P < 0.0001$ ). The sex ratio was male-biased with no significant difference between photoperiods. The significance of these results in the understanding of foraging behaviour and mass rearing is discussed.

**Keywords:** Tasmanian lacewing, *Micromus tasmaniae*, *Acyrthosiphon pisum*, photoperiod, predation, development.

**INTRODUCTION**

The Tasmanian lacewing, *Micromus tasmaniae* (Walker), is an important predator of blue green lucerne aphid, *Acyrthosiphon kondoi* Shinji, and pea aphid, *A. pisum* Harris, in New Zealand (Cameron et al. 1983; Workman et al. 2004). In Australia, it preys on the eggs of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) (Samson & Blood 1980). Serological and laboratory feeding trials in New Zealand demonstrated the potential of *M. tasmaniae* as an effective predator of *A. kondoi* (Leathwick & Winterbourn 1984; Rohitha & Penman 1986). In the field, it has successfully controlled lettuce aphid, *Nasonovia ribisnigri* Mosley, in spring crops in Pukekohe, New Zealand, for 4 consecutive years (Cameron et al. 2007; Workman et al. 2004).

The hemerobiids are the most important Neuroptera next to the Chrysopidae in controlling soft-bodied agricultural pests (Balduf 1974). The high tolerance of *M. tasmaniae* to some pesticides led Rumpf et al. (1997) to believe that they have high potential in integrated pest management.

Photoperiod reportedly affects development and diapause of lacewings, such as *Chrysopa pallens* Rambur (Grimal & Canard 1990; Orlova 1998). However, there has been no systematic evaluation of how photoperiod influences the foraging and reproductive behaviour of *M. tasmaniae*. The objective of this study was to improve understanding of the biology of *M. tasmaniae*.

## MATERIALS AND METHODS

### Breeding colony

The breeding colony of *M. tasmaniae* was obtained from a commercial insectary (Zonda Resources Ltd, Pukekohe, New Zealand) during October 2007. Sixty adults were housed in a plastic container (8.5 cm diameter × 10 cm high) having a lid with a fine nylon mesh window (5 cm diameter). They were fed with 1<sup>st</sup> to 3<sup>rd</sup> instar pea aphids reared on potted broad bean plants (*Vicia faba* L). A black cotton sheet (4 cm × 4 cm) was placed at the bottom of the plastic container for oviposition (Miller & Cave 1987). The container was examined every 24 h and eggs laid on the cotton sheet were placed in a plastic box (22 cm long × 15 cm wide × 5 cm deep) for hatching. The newly hatched larvae were further reared on pea aphids in groups of 40-50 larvae in an above-mentioned plastic box and emerged adults were used for further rearing. The breeding colonies were maintained at 21 ± 1°C and 60% RH with a photoperiod of 16:8 h (light:dark).

### Development and mortality

To investigate how photoperiod affected feeding, development and reproduction, four photoperiods of 24:0, 16:8, 12:12 and 0:24 h (light:dark) were studied under the above mentioned temperature and relative humidity. Because lacewings are mainly nocturnal (Szentkiralyi 2001), the photoperiod of 0:24 h was set up to determine whether *M. tasmaniae* could be reared optimally without light in order to reduce energy costs during mass-rearing.

Eggs (< 12 h old) laid on a cotton sheet (20 ~ 50 eggs/sheet) from the breeding colony were maintained in a clean glass vial (2.5 cm diameter × 8.0 cm high) with a nylon mesh circular window (1.2 cm diameter) on the lid and transferred to the tested photoperiods. There were 10 glass vials with 200 to 500 eggs for each photoperiod. The incubating duration was recorded.

Fifty newly hatched larvae were transferred individually to above-mentioned glass vials for each photoperiod. Five, 10 and 20 pea aphids were provided twice a day to 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae, respectively, until they pupated. The number of aphids consumed daily by each larval instar was recorded. Pupae were kept in the same glass vials to determine emergence under each photoperiod. The developmental duration of larvae and pupae and mortality of both larvae and pupae were recorded. The pupae and newly emerged adults were weighed individually using an electric balance (Mettler Toledo, AG135, Switzerland) with a readability of 0.01 mg to determine whether photoperiods had an effect on the growth of *M. tasmaniae*.

### Reproductive potential

To determine whether photoperiod affected the reproduction of *M. tasmaniae*, one male and one female (< 12 h old) were paired in an above-mentioned plastic container with 6, 20, 19 and 17 pairs for 24:0, 16:8, 12:12 and 0:24 h, respectively. The paired adults were fed with 10 1<sup>st</sup> to 3<sup>rd</sup> instar nymphs of pea aphids twice a day until they died. The fecundity (number of eggs laid), fertility rate (proportion of eggs hatched) and reproductive period for each female were recorded daily.

### Statistical analysis

A goodness-of-fit test was used to test the distribution of data before analysis. Data of *M. tasmaniae* development, female reproductive period and proportion of female progeny were not normally distributed even after transformation and thus were analysed using the non-parametric Kruskal-Wallis test (KWT) followed by Dunn's procedure for multiple comparisons. Data of body weight of pupae and adults, and female fecundity were normally distributed and analysed using ANOVA. Data on the number of aphids consumed by different instar larvae and fertility rate were subjected to square root and arcsine transformation, respectively, before ANOVA. The Marascuilo procedure of the nonparametric analysis was used to assess the mortality of larvae and pupae with a rejection level of  $U_0 > \chi^2_{3,0.05} = 7.82$ .

## RESULTS

## Development and mortality

*Micromus tasmaniae* completed development at all tested photoperiods. The number of aphids consumed by larvae increased significantly with their developmental stage at each photoperiod (average  $8.97 \pm 1.06$ ,  $14.50 \pm 1.54$  and  $32.76 \pm 3.30$  for the first, second and third instar, respectively) ( $P < 0.0001$ ). The average number of aphids consumed by larvae ranged from  $46.73 \pm 1.43$  to  $61.63 \pm 2.06$  with lowest number detected at 12:12 h and highest at 0:24 h ( $P < 0.05$ ).

At each photoperiod, the developmental period was about 5, 8 and 10 days for *M. tasmaniae* eggs, larvae and pupae, respectively. The total developmental period was significantly shorter at 24:0 and 16:8 h than at 12:12 and 0:24 h (KWT:  $H = 44.72$ ,  $P < 0.0001$ ).

There was no significant difference in larval mortality when reared at different photoperiods ( $U_0 = 0.80$ ,  $P > 0.05$ ). However, the mortality of pupae was significantly higher at 24:0 and 0:24 h than at 16:8 and 12:12 h ( $U_0 = 72.00$ ,  $P > 0.0001$ ) (Table 1).

**TABLE 1: Mortality (%) of *M. tasmaniae* larvae and pupae at four photoperiods. Values followed by the same letters in rows are not significantly different ( $P > 0.05$ ).**

Light:dark	24:0	16:8	12:12	0:24
Larva	16.00 a	18.00 a	12.00 a	0.00 a
Pupa	59.25 a	0.24 c	4.65 c	20.45 b

The body weight of cocoons was significantly higher at 24:0 h than at 0:24 h ( $P < 0.05$ ) (Table 2). Females and males were significantly heavier at 16:8 h than at other photoperiods ( $P < 0.0001$ ) (Table 2). Females were significantly heavier than males at 16:8 and 12:12 h ( $P < 0.0001$ ), but not at the other two photoperiods ( $P > 0.05$ ) (Table 2).

**TABLE 2: Mean body weights (mg) of cocoons and adults of *M. tasmaniae* at four photoperiods. Means ( $\pm$ SE) followed by the same letters in columns are not significantly different ( $P > 0.05$ ).**

Light:dark	Cocoon	Male	Female
24:0	$3.77 \pm 0.15$ a	$1.98 \pm 0.12$ b	$2.20 \pm 0.19$ b
16:8	$3.71 \pm 0.15$ ab	$2.42 \pm 0.09$ a	$3.51 \pm 0.13$ a
12:12	$3.52 \pm 0.14$ ab	$1.61 \pm 0.08$ b	$2.25 \pm 0.10$ b
0:24	$3.16 \pm 0.11$ b	$1.77 \pm 0.12$ b	$2.03 \pm 0.10$ b

## Reproductive potential

All tested females laid fertilised eggs at the four photoperiods. The reproductive period of females significantly decreased with increasing day length (KWT:  $H = 26.71$ ,  $P < 0.0001$ ), but the fecundity and fertility rate were significantly higher at 16:8 and 12:12 h than at 24:0 and 0:24 h ( $P < 0.0001$ ) (Table 3). The proportion of female offspring was about 45% with no significant difference between photoperiods (KWT:  $H = 0.41$ ,  $P = 0.94$ ) (Table 3).

**TABLE 3: Reproduction of *M. tasmaniae* at four photoperiods. Means ( $\pm$ SE) followed by the same letters in columns are not significantly different ( $P>0.05$ ).**

Light:dark	Reproductive period (days)	Fecundity	Fertility (%)	Female offspring (%)
24:0	31.50 $\pm$ 2.13 c	153.20 $\pm$ 8.09 d	57.27 $\pm$ 4.08 c	43.10 $\pm$ 1.49 a
16:8	39.15 $\pm$ 0.36 bc	445.79 $\pm$ 9.53 a	75.75 $\pm$ 1.21 a	46.63 $\pm$ 9.76 a
12:12	40.05 $\pm$ 1.08 ab	352.11 $\pm$ 19.71 b	63.67 $\pm$ 1.08 b	46.28 $\pm$ 6.45 a
0:24	46.18 $\pm$ 1.15 a	252.69 $\pm$ 18.90 c	52.43 $\pm$ 1.36 c	46.79 $\pm$ 6.65 a

### DISCUSSION

As found in green lacewing, *Chrysoperla carnea* Stephens (Balasubramani & Swamiappan 1994), *M. tasmaniae* of third instar larvae consumed the greatest portion of the total number of aphids. This may be due to their larger body size and better capacity in searching and capturing their prey.

In this study, *M. tasmaniae* developed significantly faster at long photoperiods (i.e. 24:0 and 16:8 h) than at short photoperiods (i.e. 12:12 and 0:24 h). According to Beck (1980) and Tauber et al. (1986), photoperiod may influence the development in a number of insects and growth and development are slower at short day length if insect diapause is induced by short photoperiods. However, the present results showed that *M. tasmaniae* completed its life cycle and all females oviposited fertilised eggs at all tested photoperiods, suggesting this species does not enter diapause. Canard & Volkovich (2001) also indicated that some hemerobiids have no diapause in temperate zones and life stages of cocooned pupae, reproductive adults or even eggs and active larvae can be found under short day lengths in the field, for example, in *Hemerobius neadelphus* Gurney and *H. ovalis* Carpenter (Kevan & Klimaszewski 1987). The difference in development, prey consumption, mortality and reproduction under long and short photoperiods suggests that although *M. tasmaniae* does not enter diapause, photoperiod still has a significant effect on the endogenous circadian rhythms in metabolism, respiration and hormone production, which may regulate tissue synthesis and growth of insects (Beck 1980).

Although *M. tasmaniae* can complete development and reproduce under completely dark conditions, the higher pupal mortality and lower fecundity and fertility mean such conditions cannot be recommended for mass-rearing of *M. tasmaniae* in the laboratory. *Micromus tasmaniae* reared at 16:8 h develop faster and have lower mortality, heavier adult body weight and higher reproductive output in terms of fecundity and fertility rate. Therefore mass-rearing programmes are recommended to be carried out under 16:8 h light:dark. Moreover, investigation of the environmental conditions leading to the production of female-biased progeny is warranted in further studies.

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