Ecophysiological Studies on *Harmonia axyridis*, Pallas (Coleoptera: Coccinellidae)

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Dedication

TO MY PARENTS, FOR THEIR UNCONDITIONAL SUPPORT. I AM HONOURED TO HAVE YOU IN MY LIFE. TO MY SISTER DR. HANAN FOR HER KNOWLEDGE, WISDOM AND GUIDANCE, WITHOUT THEM I WOULD NOT ACHIEVED MY DREAMS.

Declaration

I declare that all the work presented in this thesis is my own work and any material interpreted as the work of others, is fully cited and referenced

Signature	**Jenan Al-Harbi**
Name of Supervisor	**Prof. Simon. R. Leather**

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Abstract

Harmonia axyridis has been used as an efficient biological control agent worldwide but is causing concern because of adverse impact on native species biodiversity predominantly through intra-guild predation and competition. There is thus a need to understand the ecological, biological and physiological factors that primarily contributed to the accelerated invasiveness and establishment of this species over a wide range of agricultural systems. The main focus of this project was to investigate various life-history traits and their interaction with external environments cues. The first part of this project was designed to investigate various biological aspects of this ladybird under controlled environmental measures and fixed prey availability. First, it demonstrated the sexual body size-dimorphism and highlighted which traits showed greater dimorphism than others between sexes. In addition, the reproductive performance and the association between the body size and the life-time fecundity, egg hatchability and non-viable eggs was investigated. Several hypotheses were revaluated by this study by direct experiments. The developmental responses of the juveniles were performed under similar controlled measures by monitoring both the stage weight and duration in order to abstract the intra-specific variations among females as well as the significance of each larval stage relative to the final adult size. This species has gained additional advantage due to the presence of the 5th instar reflecting an elevation in fitness and the evolution of the predation and competitive proficiency. Although the genetic and evolutionary characteristics of this ladybird were only covered theoretically, a number of experiments has revealed the intra-specific variability among female body mass, nutrient allocation to reproduction strategies, and growth patterns that might be partially constrained by genetic background. Another important aspect of this project was to emphasize the influence of mating choice and frequency on lifespan, egg hatchability and viability of singly

mated, multiply mated as well as virgin females. It was shown that *H. axyridis* is able to be fertile throughout their life-span from a single mating without significant costs on either longevity or life time fecundity. Nevertheless, multiple mating was found to impose some constraints on the proportion of fertile eggs. These reproductive features may help to understand the success of population establishment and persistence under constantly fluctuating environment. A novel method was described in this study in which the sex of pupae could be easily identified prior to adult emergence based on pupal pattern dimorphism when pupae were reared in the laboratory or directly collected from the field. This simple method is highly effective and could be implemented in several studies without manipulating adults although care must be taken when inspecting the patterns of colouration. Behavioural characteristics of this species that facilitated intra-guild dominancy and interference competitions were also investigated by simple methods. This was performed by testing the avoidance responses mediated by olfactory organs and semio-chemicals towards the presence of tracks of con- and hetero-specific-larvae and adults as well as con-specific adults. The results suggest that *H. axyridis* has a highly specialized sense organ that functions efficiently in selecting suitable patches and tends to refrain larvae or adults from foraging in contaminated patches by certain species. In the second part of this project, the characteristics of developmental and behavioural traits were also investigated by subjecting successive larval instars to a brief period of food manipulation protocols. This unravelled the compensation patterns, and short- and long-term trade-offs on life-history traits across generations. The results suggest that *H. axyridis* is able to exhibit phenotypic plasticity in several traits that are important for successful adaptation and colonizing a new habitat. In summary, the results of this study showed that *H. axyridis* is a highly competitive species owing to their reproductive tactics and unique immediate strategies in regulating reproductive performance and in optimizing off-spring fitness after food restoration. Females were able to optimize their fecundity after one mating and possess evolved defence strategies to avoid being attacked by other species. Nevertheless, the results of this study may contribute in understanding the evolution of life-history traits in this species and could be incorporated in intra- and inter-specific comparative studies. In conclusion, the study predicts that this species will continue to spread and become more widely distributed with more ecological consequences if effective control measures and protocols are not incorporated almost immediately.

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Chapter 1

General Introduction

Like many other exotic species, *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) has become a very well known insect, not only for its potential as an efficient biological control agent (Brown et al., 2008a), but also because of its notorious invasiveness (Soares et al., 2008). This ladybird has quickly become invasive, threatening biodiversity, ecosystem integrity (Olden et al., 2004), agriculture, fisheries (Pimentel et al., 2001), public health (Ruiz et al., 2000) and with a massive potential impact on the future of the global community (Burgiel et al., 2006). Unfortunately, institutions and scientists currently lack sufficient scientific knowledge regarding the invasion process and its associated factors (Williamson, 2006). Ecologists have been trying to predict and mitigate the common characteristics of this highly invasive species and its impacts on both native biodiversity and human communities (Mooney and Drake, 1989, Hengeveld, 1989, Kareiva, 1996), yet predicting the causes and the outcome of invasions remains a daunting task (Brown, 1989, Pimm, 1991, Lodge, 1993). This is partially due to the difficulty involved in determining the important details of an introduction event - such as its date, the number of organisms involved and the locations affected (Yates et al., 2011).

Harmonia axyridis has exceptional capacities and features, thus allowing it to be successful in any new habitat. Some of these characteristics include phenotypic adaptive plasticity and dispersal ability (Soares et al., 2008). In particular, phenotypic plasticity has been seen as a potentially important factor in successfully colonising diverse habitats (Maron et al., 2004), since it ultimately enhances trait function and maximises fitness (Sultan, 2003). Several studies have stressed the biological characteristics that make *H. axyridis* a strong invader; among them, the increased developmental rate, fecundity and fertility are regarded as important factors for successful establishment (Sakai et al., 2001, Marco et al., 2002, Shea and Chesson, 2002, Lanzoni et al., 2004). Focus has often been given to fitness-related phenotypic plasticity, thought to be associated with the ability to colonise (Stearns, 1992, Soares et al., 2008). Although considerable research has been conducted into plasticity, little consensus exists regarding the association between plasticity and colonisation (Grill et al., 1997). Considering the wide range of published data on phenotypic plasticity, little attention appears to have been given to adaptive plasticity (Gotthard and Nylin, 1995). Furthermore, confusion exists regarding the terminology that should be applied to plasticity-whether adaptive or adaptation. This confusion may be one reason why the focus of many studies is not as clear as it could be (Gotthard and Nylin, 1995). This particular issue will be dealt with in detail later in this thesis. Researchers have considered invasions from a number of different aspects, including the characteristics of invaders, the characteristics of the invaded habitat, resources and natural enemies (Shea and Chesson, 2002). As these levels are independent, this study selected the biological characteristics of the invader for investigation, since that is the main element that initiates the problem of invasion.

Harmonia axyridis is considered an excellent candidate for studying the association between phenotypic plasticity and successful colonisation. The successful spread of this species after encountering new habitats reflects the great genetic variability underlying its life history traits, allowing them to overcome and withstand unpredictable resources. Evaluating some fitness-related traits (such as developmental, morphological and behavioural characteristics), as well as their plastic responses towards stressed conditions would be of great importance in partially explaining the invasion process and colonising success.

This ladybird species represents an ideal opportunity for greater international co-operation between the scientists that have been studying this species in its native range in Asia and those studying it in Europe, America and Africa, where it is an invasive exotic (Mori et al., 2005). Invasive species also offer excellent opportunities to study basic processes in population biology. For example, life-history studies and demographic models may be valuable when examining the introduction of invasive species and identifying those life history stages at which management and control will be most effective (Sakai et al., 2001).

1.1 Invasion - processes and impacts

Acceptance of the biological invasion phenomenon started becoming much more widespread in the early 1900s (Richardson and Pysek, 2007). In 1958 Charles S. Elton published a book on “The Ecology of Invasions by Animals and Plants”, a text that has since become the generally accepted starting point for considering the importance of biological invasions.

'Biological invasion' is defined as the study of the human-mediated introduction of organisms to areas outside their potential range (Mack et al., 2000, Pimentel et al., 2000, Davis, 2006). These organisms range from fungi to plants, nematodes and mammals, including exotic pests used for biological control purposes (Venette and Carey, 1998). Richardson and Pysek (2006) noted that this branch of science deals with all aspects of the released organisms, particularly their ability to establish, naturalise and interact with native species in their new location. Furthermore, consideration should be made of the trade-offs that result from their presence, with reference to the human value system (Richardson and Van Wilgen, 2004, Pysek et al., 2006, Richardson, 2006).

The invasion process is a natural phenomenon that includes several sequential processes - namely, importation, introduction, establishment, geographical expansion and integration of a species into a new region (Vermeij, 1996, Shigesada and Kawasaki, 1997). The establishment of non-native species is the second highest threat to worldwide biodiversity, after habitat destruction, generating substantial costs, both in terms of the control of such species and in lost conservation values and ecosystem functionality (Pimentel et al., 2000, Pimentel et al., 2002). Effects on the ecosystem can include changes in the availability of resources, such as water and nutrients, altered trophic structure and altered disturbance regimes (Vitousek, 1990, Holdsworth et al., 2007). The invasion of the earthworm, *Lumbricus rubellus* (Hoffmeister, 1843) (Haplotaxida: Lumbricidae), contributed to a dramatic shift in the herbaceous plant community from forb to oak sedge, *Carex pensylvanica* (Lamarck, 1792) (Poales: Cyperaceae), dominance (Hale et al., 2005).

Understanding the factors that accelerate biological invasions has become of major interest over the past few decades, following the recent rise in human activities, which has greatly accelerated the invasion rate (Mack et al., 2000). It has been hypothesised that human perturbations to native communities can reduce the adaptation of native species to the current environment by reducing their competitive ability, thus resulting in better-adapted exotic species (Mack et al., 2000, Keane and Crawley, 2002). Building a clear picture of the characteristics of release attempts and considering the variable pathways of introduction is crucial to predicting the success of establishment (Kolar and Lodge, 2001) and may help to enhance controlling and regulation measures (Pysek et al., 2011). It is widely accepted that most exotic species are spread throughout the world by a combination of intentional and unintentional means (McAusland and Costello, 2004). Intentional introductions include imports of a variety of agricultural crops, ornamental plants and animals, as well as the release of biological control agents. Unintentional introductions include those in relation to transport of commodities, people and shipping containers, or introductions that occur due to contaminated objects (McAusland and Costello, 2004). Pysek et al. (2011) noted that introducing exotic species deliberately for biological control purposes may result in easier naturalisation and faster invasion, in comparison with unintentional means, given that the introduced species have been selected previously for their survival ability in the environment into which they are being introduced (Lonsdale, 1994, Smith, 1999). Ensrink (1999) noted that repeated introduction for species that are marketed over a long period of time have a greater probability of spreading and becoming established. The number of attempts and the number of individuals introduced are thought to increase the probability of a species becoming established (Kolar and Lodge, 2001).

The initial phase of invasion - the introduction or release stage - is considered the longest and most critical stage (Venette and Carey, 1998). It is difficult to detect biological invasions at this point, as the introduced population may remain at low densities for a long period of time without being detected, while it adapts to the newly invaded habitat (Grevstad, 1999, Carey, 1996). For instance, the western corn rootworm, *Diabrotica virgifera virgifera* (LeConte, 1868) (Coleoptera: Chrysomelidae), which is native to Mexico and the east coast of North America (Branson and Krysan, 1981), has been resident in the western half of the Great Plains since at least 1867 (Krysan et al.,1983). In 1992 an infestation of western corn rootworm was found within a small maize field near Belgrade Airport (Baca, 1993). By 2007 the presence of this insect pest had been confirmed in 20 European countries (Gray et al., 2009). It is still expanding in central and eastern Europe at a rate of 100km year^{-1} (Miller et al., 2005, Ciosi et al., 2008). The Asian ladybird beetle, *Harmonia axyridis*, was first brought into Europe in 1982 (Brown et al., 2008a). It failed to become established, however, until the

1990s, when it started to spread into Germany and Belgium (Lombaert et al., 2007). Soares et al. (2008) explained that the lag phase in establishment success is dependent on the number of repeated introductions. Crawley (1989) stressed the importance of chance and timing, which may differ between cases and processes and may explain the long time taken by some species to establish and spread. Marco et al. (2002) presented a set of factors detrimental to the invasion process, such as species biological traits and invasibility. The latter determines the susceptibility to invasion, depending on the interaction between invaders, non-invaders and the local habitat.

Multiple introductions are a common feature of biological invasions (Handley et al., 2011). Lavergne and Molofsky (2007) noted that repeated introductions of a single species may have high adaptive potential, resulting in a rapid selection of genotypes and higher colonisation ability and phenotypic plasticity. Such species will be able to evolve in response, with an increasing impact on native communities. The first direct evidence that multiple introductions are primarily responsible for providing most of the evolutionary potential of an invasive population came from the freshwater snail the red-rimmed melania, *Melanoides tuberculata* (Müller, 1774) (Gastropoda (class): Thiaridae) which demonstrated an accumulation of variants and generation of the novel variant combination amplified by interbreeding (Facon et al., 2008). The brown anole, *Anolis sagrei* (Duméril & Bibron, 1837) (Squamata: Polychrotidae), a small, diurnal lizard native to the Caribbean, was first established in South Florida, but took more than 30 years to spread northward (Williamson, 1996, Lee, 1985). The lizard's expansion probably resulted from a combination of northward movement following early introductions in southern Florida, and additional introduction attempts. Genetic analyses have indicated that at least eight introductions have occurred in Florida from across this lizard’s native range and that blending genetic variation from different geographic source populations resulted in substantially more genetic variation than native populations (Koble et al., 2004). It has been reported that most of the separate outbreaks of the western corn rootworm, *D. virgifera,* resulted from repeated trans-Atlantic introductions (Miller et al., 2005, Ciosi et al., 2008).

Despite the importance of multiple introductions, particularly when populations are genetically divergent (Facon et al., 2003, Kolbe et al., 2004) exact explanation for the high genetic diversity in invasive species is disputed (Lavergne and Molofsky, 2007). Indeed, in some cases the high genetic diversity may not be an essential component of invasion success (Dlugosch and Parker, 2008). For instance, Eales et al. (2010) noted that the high genetic diversity in the invasive population of anole lizards, *Anolis cristatellus* **(**Duméril & Bibron, 1837) (Squamata: Iguanidae), was a consequence of a single introduction event; therefore, other contributing factors may have been responsible for increasing the number of genotypes, such as the reproduction rate.

When introducing an exotic species, the decision will reflect several aspects, such as social customs, the incentive effects of existing institutions, human interference, trade rules and regulations, relative prices and wealth (Mack et al., 2000, Perrings et al., 2000). Globalisation is also recognised as playing a substantial role in increasing the impact of invasive species on the ecosystem (Perrings et al., 2005), with the development of a system of world trade increasing the number of new invasive species being introduced to ecosystems. The risk of invasion is known to have a negative consequence on economic activity and human welfare (Perrings et al., 2002). The economic implications of these impacts have been intensively studied (Perrings et al., 2005), but more work is still needed (Perrings et al., 2002).

Carey (1996) noted that not all invasions will have adverse effects. Even when the invasion is potentially severe, the range of expansion and the impact will vary in response to host and climate suitability. Many deliberately or accidentally introduced species fail to become established in their new habitat (Roy and Wajnberg, 2008). Of those species that do become established, many have negligible effects and some are even considered beneficial or desirable, such as those introduced with agriculture food crops and livestock (Williamson, 1999). Pimentel et al. (2000) and Pimentel et al. (2004) noted that in the United States 90% of crops are introduced and only a small fraction become invasive, affecting native species and ecosystem levels negatively (Pimentel et al., 2000). Simberloff (1989) stressed the importance of habitat modifications for the success or failure of insect invasion. For example, conversion of land to agricultural use in Hawaii may decrease the suitability of the habitat for native birds, while increasing the suitability for introduced species (Williamson and Fitter, 1996). Holdsworth et al. (2007) stressed that the impact of an invasive population varies with species, population size, recipient community and ecosystem level. A number of studies have presented examples on the suitability of recipient habitat, biotic resistance and life-history traits in influencing the genetic variability, degree of expansion and adaptation ability of introduced species (Soares et al., 2008, Handely et al., 2011).

Despite the growth in models for understanding the causes and ecological impacts of invasion, improved modelling frameworks are still required in order for the responses of invasive species towards global changes to be understood. For example, global climatic warming is predicted to continue by up to 5.8°C this century (IPCC, 2001). Several studies have indicated that some species have responded to the warming and shifted their distributions (Walther et al., 2002, Parmesan and Yohe, 2003). When tracking climatic changes in those species, an evolutionary increase in dispersal ability was revealed in the established population. This characteristic may have significant consequences, altering the relative costs and benefits of dispersal ability versus reproduction (Niemela and Spence, 1991, Hanski et al., 2002, Thomas et al., 2001). Such trade-offs have been shown in many insect species, particularly wing dimorphic species (Zera and Denno, 1997). Likewise, a recent study by Hughes et al. (2012) demonstrated that increased dispersal is associated with reduced investment in reproduction in the invasive populations of the speckled wood butterfly, *Pararge aegeri* (Linnaeus, 1758) (Nymphalidae: Lepidoptera). This helped to increase their potential colonisation ability into areas beyond their native ranges. According to Shine (2012), in an invasive population, both mating patterns and genetic diversity undergo divergent selection in their interaction with the determinants of dispersal rate. Thus, the direct relationship between dispersal, gene flow and population structure has already become established in most invasive species (Heimpel and Asplen, 2011).

Global climatic changes have already elevated CO_2 and other elements, such as nitrogen and phosphorous; it is therefore important to understand the likelihood of invader adaptation success in this context (Richardson and Pysek, 2008). The increase in the magnitude of these compounds plays a significant role in influencing insect population dynamics and predator-prey and plant-insect interactions (Fagan et al., 2002, Woods et al., 2003). Likewise, nitrogen and phosphorus have lately been proven to have major consequences for herbivore success, consumer-driven nutrient cycling and the fate of primary production in ecosystems (Fagan et al., 2002).

Predictions and ecological theory in respect of successful introductions of insect species are still imprecise (Borges et al., 2006), with much research focusing on the establishment stages, but neglecting other stages, such as the spread phase (Duncan et al., 2003). The probability of a species spreading and expanding depends on both species-specific traits, such as the dispersal capacity, and on the environmental conditions of the targeted habitat (Sakai et al., 2001). Venette (1997) noted that the species that are more likely to be invasive and become established are those that are characterised by high growth rates, rapid maturation and reproduction and a greater dispersal ability across a wide range of environmental conditions. For example, the widespread distribution of the ash whitefly, *Siphoninus phillyreae* (Halliday, 1835) (Hemiptera: Aleyrodidae), in California is due to its ability to reproduce and develop at wide temperature ranges (between 10°C and 30°C) and to its capability to maintain itself on several hosts (Venette, 1997).

Another factor that has long been studied is the genetic make-up of the released species (Rejmanek, 1996ab). It was reported previously by Moyle and Light (1996ab) that invasions are most likely to succeed in sites that are highly disturbed or have a mild climate. A disturbed climate often reduces the number of established predators or/and increases the availability of resources per individual. For example, the mild climate and rapid urbanisation of southern and coastal California make these regions vulnerable to invasion by exotic vascular plants. Furthermore, the survival rate of *H. axyridis* overwintering inside human houses has been found to be greater than that of individuals remaining outside during winter (McClure, 1987, Labrie et al., 2008).

To date, humans have altered the modern environment in several ways that affect biodiversity, of which habitat destruction and alteration are the most noteworthy. Both have likewise substantially affected populations by changing the entire structure of local habitat assemblages, with subsequent downstream effects on ecosystem functionality (Rickman and Connor, 2003, Stefanescu et al., 2004). A study has revealed that the current motors of global change will have a negative effect on Mediterranean Catalan butterfly assemblages by transforming land use and fragmenting the landscape into a less suitable matrix for butterflies. Loss of diversity over the coming years is predictable, due to the negative correlation between species richness and temperature, posing a serious threat to biodiversity (Stefanescu et al., 2004).

The replacement of natural habitats by urbanisation has caused the disappearance of some insect species. For example, in San Francisco, only 57% of the native butterfly species have been seen in the past 10 years, as a consequence of continuous urbanisation (Hafernik and Reinhard, 1995). Furthermore, it has been reported that the abundance of *Dryseriocrania auricyanea* (Walsingham, 1882) (Lepidoptera: Eriocraniidae) has been found to be lower at highly urbanised sites (Rickman and Connor, 2003). Replacement of natural habitats by urban land use may alter food availability or create a microclimate, leading to changes in habitat quality and serious impacts on the diversity of native insects. Altered water or nutrient regimes may affect the abundance of folivorous insects (Connor, 1988, McQuate and Connor, 1990). The effects of the introduction of species as biological control agents can often be subtle initially, with more pronounced impacts accumulating slowly through time, causing substantial changes to system functioning (Mack et al., 2000, Goulson, 2003). These intentional or unintentional interferences have substantive impacts on insect populations, promoting changes to life-history characteristics and the extension of some species (Chown and Terblanche, 2007, Chown et al., 2009).

The *climate-matching hypothesis* states that greater invasion success often occurs when species are released into areas with a similar climate to that of their natural origin (Greathead, 1971, Williamson, 1996, Mack et al., 2000). Samways et al. (1999) however, have argued that there is always a problem in accurately predicting whether a species will become established based on the climate. The authors claimed that climate is not always the limiting factor in predicting invasion; other determinants, such as a localised response to microclimate, host availability, occurrence of natural enemies and hibernation sites also play a major role (Samways et al., 1999). Richardson and Pysek (2008) explained that predicting an accurate potential range of invasion is problematic, since most invasive species have had insufficient time in their new range, thus sampling the invisible habitats at this stage would be unreliable. In conclusion, to best understand invasion mechanisms, the approach should integrate climatic suitability models and other relevant factors, such as predation, host suitability and behavioural plasticity (Samways et al., 1999).

Kolar and Lodge (2001) have noted that in order to achieve the best prediction, several quantitative methods have long been used, although they have only been applied to the species for which their certainty was approved. Based on this, the authors suggested that these quantitative approaches should be applied more widely to allow patterns of invading species to be predicted with greater success. They have argued that the probability of bird establishment increases with the number of individuals released and the number of release events; and that the probability of plant invasiveness increases if the species already has a history of invasion. Richardson and Pysek (2006) argued that invasion is context-specific, and that generalisation and theories are therefore often not beneficial. Although methods have been developed to quantify expansion, a high-quality computing database, such as the DAISIE database (Daisie, 2008), is needed instead to improve models predicting distributions, and hence to lead to new progress in understanding invasion success (Richardson and Pysek, 2008). Although interest in preventing and controlling such biological invasions has led to an increase in the number of studies related to invasion over the past few decades, satisfactory explanations of differential introduction success remain elusive (Richards et al., 2006).

Several hypotheses have been proposed to explain the success or failure of introduced species in recipient communities. One is the *biotic resistance hypothesis*, which posits that the strong biotic interactions between native and introduced species often limit the latter's impact (Maron and Vilà, 2001). Alternatively, the *enemy release hypothesis* suggests that only some naturalised species have a large impact (Keane and Crawley, 2002, Mitchell and Power, 2003, Colautti et al., 2004), due to liberation from their co-evolved predators, pathogens and herbivores (Maron and Vilà, 2001). The success of some species in invasion, such as *H. axyridis*, may be partly linked to this hypothesis, which states that “the potential invader is successful because of the inefficacy of natural enemies in the new area” (Keane and Crawley, 2002). Therefore, the introduced species will be successful in expansion as it experiences a decrease in regulation by natural enemies (Keane and Crawley, 2002). This implies that natural enemies are important regulators of native populations, have a greater influence on native than on exotic species and are able to capitalise based on a reduction in enemy regulation. The extent to which this hypothesis can be applied varies between species, particularly when dealing with community disturbance (Shea and Chesson, 2002).

One mechanism that predisposes a species to successful establishment and colonisation is its phenotypic plasticity (Kaufman and Smouse, 2001, Yeh and Price, 2004, Richards et al., 2006, Ghalambor et al., 2007). This is considered one of species' intrinsic responses, alongside evolutionary adaptation to the varying environment (Lee et al., 2007). Many studies have also suggested that the evolution of plasticity may be beneficial in novel sites after colonisation or migration (Agrawal, 2001, Yeh and Price, 2004). In general, rapid evolutionary change appears to be common in invasive species (Sakai et al., 2001, Lee, 2002, Bossdorf et al., 2005), and rapid evolution of plasticity could play an important role in explaining their success (Richards et al., 2005). It has been proposed in this context that, after the release of an exotic species, it rapidly evolves to be less defensive but more competitive, thereby increasing the species vigour in introduced populations (Bossdorf et al., 2005).

Populations vary in their phenotypic plasticity responses, with some having greater plasticity than others (Carroll et al., 2001ab). Furthermore, other populations may act as part of a complex strategy as a result of the influence by both phenotypic plasticity and evolutionary selection (Lee, 2002). Price (2006) argued that although plasticity has a significant role in some cases, natural selection will act in the absence of plastic responses, which makes it difficult to ascribe a definite role to plasticity in these cases. Adaptive phenotypic plasticity confers responses that ultimately maximises fitness; such responses may comprise physiological, morphological and reproductive adjustments, thus enhancing the trait of coping well in diverse environments (Gotthard and Nylin, 1995, Sultan, 2000, 2003). Therefore, invasion can more appropriately be described by adaptive plasticity than phenotypic plasticity. The former helps genotypes of invaders to acclimatise to the diverse environment, reducing the necessity for local adaptation (Sultan, 2003) and leading to phenotypic tolerance in fitness, an important trait for successful invasion (Alpert and Simms, 2002, Chun et al., 2007). For example, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), originated from East Africa and has become established in many countries worldwide (Baliraine et al., 2004, Malacrida et al., 2007).

Colonisation of a new environment often affects the development, physiology, behaviour and morphological traits (such as body size) of invader species, as a consequence of phenotypic adaptive plasticity (Gienapp et al., 2008). Waddington (1953) has argued, for example, that the appearance of novel wing patterns in *Drosophila* after experiencing heat shock were favoured by selection and later became genetically assimilated. West-Eberhard (2003) stressed that the loss of genetic assimilation or evolution related to plasticity after successful colonisation of a novel environment should also be taken into account.

A number of studies have revealed that appropriate plastic responses to environmental variation allow organisms to express advantageous phenotypes in a broader range of environments, thus enhancing the ecological niches (Donohue et al., 2001). Nevertheless, these selective responses may represent modifications in novel sites after colonisation or migration have altered community patterns and expanded evolutionary potential of species (Agrawal, 2001). Studies have previously addressed the role of plasticity in invasions, noting that the levels of adaptive phenotypic plasticity increased in invasive species, in comparison with biological control species, such as *H. axyridis* (Lombaert et al., 2007).

Invasion biologists refer to phenotypic plasticity in two distinct ways when attempting to explain invasions. First, invasive species may be more plastic than non-invasive or native ones (McDowell, 2002). Second, populations in the introduced range of an invasive species may evolve greater plasticity than populations in the native range (Kaufman and Smouse, 2001, Parker et al., 2003). Widely successful invaders are characterised by high levels of genetic variance, high outcrossing rates and the creation of novel genotypes independent of the number introduced (Parker et al., 2003). A successful invader often has a high growth rate, captures limited resources more efficiently and is able to adjust its physiology according to spatial and temporal changes in recourse activity, particularly in a chronically disturbed habitat (Durand and Goldstein, 2001). The invasive Australian tree fern, *Sphaeropteris* *cooper* (Tryon, 1970) (Cyantheales: Cyatheaceae), has been compared with the native Hawaiian tree fern, *Cibotium menziesii* (Hook & Arn., 1844) (Cyantheales: Dicksoniaceae) - the former appeared to be more efficient at capturing and utilising light than the latter, particularly in high-light environments such as those associated with high levels of disturbance (Durand and Goldstein, 2001).

The arrival of an invasive species can initiate a rapid change in genotype frequencies, until the challenge exerted results in adaptation (Stockwell et al., 2003, Buswell et al., 2011). Likewise, the intense selection exerted by an invader may cause a reduction in the population size of the native species, resulting in extension in some cases (Lee et al., 2007). Stockwell et al. (2003) have noted that intensively managed species may reduce their fitness in native habitats. For some invaders, the continued active selection for stable expression of a trait under new environmental conditions may result in a genetically invariant response, leading to a less-plastic genotype after colonisation (Pigliucci and Murren, 2003, West-Eberhard, 2003). Richards et al. (2006) noted that plasticity is beneficial only in the initial stage of an invasion; at later stages it comprises more cost than benefit (VanTienderen, 1991, DeWitt, 1998). They alternatively suggested that the progressive reduction in plasticity in a selected trait after colonisation is due to a random accumulation of non-lethal mutations.

Despite ongoing research in this field, the relative importance and fitness trade-offs of invasive species need more investigation, since some plastic responses may involve costs that constrain their evolution (Weinig and Delph, 2001). High levels of phenotypic plasticity may enhance colonisation success (Brown et al., 2011), but environmentally induced flexibility may sometimes reduce, rather than enhance, fitness (Yeh and Price, 2004, Richards et al., 2006, Ghalambor et al., 2007). Some traits may benefit from flexibility whereas others do not. For example, cane toads, *Rhinella marina* (Linnaeus, 1758) (Anura: Bufonidae), native to Central and South America were introduced to north-eastern Australia in 1935 (Shine, 2010). The toad showed adaptive behaviour plasticity that facilitated its colonisation in the new range, reflecting significant evolutionary changes in growth rates (Brown et al., 2011). Analyses of progeny from adult toads showed a reduction in immunocompetence, with a high susceptibility to bacterial infection (Brown et al., 2008a), as well as a lower metabolic investment in response to a standardised immune challenge (Llewellyn, 2009). Richards et al. (2006) have reviewed most of the conceptual and empirical progress detailed in literature with regard to plasticity and evolution. Their review demonstrates a lack of clarity when describing that some species are more plastic than others. The authors based their view on the fact that every invasion success is species-specific and has its own scenario that could lead to different predictions and methodology. Identifying conditions that allow for a successful invasion has become a crucial research area (Cote et al., 2010). One of the characteristics that predispose a species to becoming a successful invader is its dispersal ability (Bubb et al., 2006). In terms of dispersal and spread, high dispersal rate and long-distance dispersal are likely to be key traits for successful invasion (Shine, 2010, 2012). The greater movement and dispersal by the crayfish, *Pacifastacus leniusculus* (Dana, 1852) (Decapoda: Astacidae), recorded by Cote et al. (2010) found evidence supporting the hypothesis that invaders are better dispersers than displaced native species.

Dispersal is fundamental to invasion success, since it influences the genetic makeup, the demographic structure and the ability of species to adapt to a new environment (Brown et al., 2011, Handley et al., 2011). Few studies have however, concentrated on the dispersal role in biological invasions, except in the context of biological control (Heimpel and Asplen, 2011). Travis and Dytha (2002) found that during range expansion, selection favours individuals with a higher propensity for dispersal, resulting in a more rapid spread than that expected by the evolutionary theory assumption. Rapid dispersal can evolve non-adaptively, by spatial sorting of genes within the invading species (Shine et al., 2011). High levels of variation may occur in dispersal-relevant morphological traits, such as seed shape, wing size and musculature, leg length and foot size and movement types, as detailed by Shine et al. (2011). Thus, traits that influence rates of dispersal are likely to evolve faster, facilitating rapid expansion. The range of species expansion may be enhanced by more evolved phenotypic traits, such as locomotors and dispersal ability, high reproductive rate and rapid population growth rate (Cassey, 2001, Thomas et al., 2001). Cassey (2002) noted that the strong significant variations in the successful establishment of introduced land bird species could be explained by strong selective pressure on the life history and ecological traits, including increased habitat generalism, lack of migratory tendency and sexual monochromatism.

A growing literature provides examples of dispersal facilitating traits accumulating at expanding range edges. Recolonisation of metapopulations of insular voles, *Microtus agrestis* (Linnaeus, 1761) (Rodentia: Cricetidae), on islands in the Stockholm archipelago in Sweden, showed larger body size and had longer feet than expected for their body size, when compared with voles from the mainland (Forsman et al., 2010). Likewise, speckled wood butterflies, *Pararge aegeria* (Linnaeus, 1758) (Lepidoptera: Nymphalidae), had colonised many areas in north and east England, and in the south. In the colonising populations, the body was larger in size and had a longer thorax with broader wings than conspecifics in more central parts of the species' range (Hill et al., 1999). Two species of bush crickets showed distinct changes in dispersal with expanding ranges. Colonised populations at the range margin showed increased the length of wing compared with the longer-established populations in the range (Simmons and Thomas, 2004). Cane toads, *Bufo marinus* (Linnaeus,

1758) (Anura: Bufonidae), in Australia evolved a faster dispersal during their invasion than conspecifics from long-colonised areas. This was accompanied by an increase in locomotor activity levels and relative leg length (Llewellyn et al., 2010). The above examples indicate that individuals' colonisation success is highly related to evolution in flight morphology. This feature could be used to predict the dispersal rate and may be an important determinant of range expansion that is likely to affect responses to the changing climate.

The two main known dispersal patterns are long- and short-distance pathways. Long-distance dispersal, facilitated by either wind or human activities, can accelerate the rate of range expansion (Shigesada et al., 1995, Ciosi et al., 2011). A combination of both short-diffusion, and long-distance dispersal - known as stratified dispersal - may be considered as the common feature of invasions (Heimpel and Asplen, 2011). Patterns of the invasion of the corn-crop pest western corn rootworm, *Diabrotica virgifera virgifera* (LeConte, 1868) (Coleoptera: Chrysomelidae), in its largest area of expansion in central and southeastern Europe was mainly by stratified dispersal, involving both continuous diffusion and discontinuous long-distance dispersal (Ciosi et al., 2011). Stratified dispersal has been described in several species of invasive insect, such as the firethorn leaf miner, *Phyllonorycter leucographella* (Zeller, 1850) (Lepidoptera: Gracillariidae), which colonised Great Britain in the 1980s (Nash et al., 1995). It has been demonstrated that the emerald ash borer beetle, *Agrilus planipennis* (Fairmaire, 1888) (Coleoptera: Buprestidae), has spread across North America - in Ontario, Michigan and Ohio - through a combination of diffusive range extension involving local flights, and long-distance dispersal associated with human activities (Muirhead et al., 2006). The leaf miner moth, *Cameraria ohridella* (Deschka & Dimić, 1986) (Lepidoptera: Gracillariidae), which invaded most of central and western Europe, has spread by both short-distance and long-distance dispersal (Gilbert et al., 2004).

Saastamoinen and Hanski (2008) studied the flight ability of the Glanville fritillary butterfly, *Melitaea cinxia* (Linnaeus, 1758) (Lepidoptera: Nymphalidae), and demonstrated that dispersal and flying ability is heritable and associated with variation in the allele at a PGI gene encoding for phosphoglucose isomerase. This enzyme is known as a 'temperature sensitive glycolytic enzyme' (Haag et al., 2005). The study revealed a fitness advantage to the heterozygous genotype in low temperatures, in which those heterozygous butterflies move longer distances at lower ambient temperatures than homozygous individuals (Saastamoinen and Hanski, 2008, Niitepold et al., 2008). Generally, this will be an essential starting point for understanding the factors underlying enhanced dispersal ability during invasions (Handley et al., 2011).

Ladybirds are generally observed as active flyers (Brown et al., 2011). For example, *H. axyridis* is characterised by a high dispersal capacity. Following its spread from Asia, it has become established in at least 38 countries in its introduced range - three in North America, six in South America, 26 in Europe and three in Africa (Osawa, 2000, Roy and Wajnberg, 2008, Brown et al., 2011). The harlequin beetle is capable of actively dispersing over long distances to overwintering sites (Hodek and Honek, 1996, Osawa, 2000).

Determining the probability of long- and short-distance dispersal is important for estimating population dynamics and essential for predictive models (Urban et al., 2008, Handley et al., 2011). *Harmonia axyridis* has an estimated spread rate of 442 km year^{-1} in North America (McCorquodale, 1998) and a maximum spread rate of approximately 200 km year^{-1} in Europe (Brown et al., 2011). More detailed calculations for Great Britain reveal a northerly spread rate of 105 km year^{-1} and a rather faster westerly spread rate of 145 km year^{-1}. In South Africa, *H. axyridis* spread at a rate of approximately 500 km year^{-1}, as calculated by Stals

(2010). *Harmonia axyridis* demonstrates two types of migratory behaviour - autumn and spring migration to and from overwintering sites, and summer migrations during food scarcity. The former migrations are directional, away from the afternoon sunlight and heading northeast. The latter migrations are random (Nalepa et al., 2005, Poutsma et al., 2008). The pattern of dispersal is species-specific - for example, the flight of female mosquitoes, *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) and *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) is influenced by several factors, such as oviposition, site availability, climate and blood source (Alves Honório et al., 2003). It was found that the females dispersed at least 800m in a dengue endemic area within a six-day period. If such females are infected, there will be a high potential for the virus to spread rapidly (Alves Honório et al., 2003).

The goal of invasive species research is to increase the understanding of invasion success, and hence the ability to predict and prevent invasions at an early stage. Although interest in preventing and controlling such biological invasions has led to an explosion of scientific studies over the past few decades, satisfactory explanations of differential introduction success remain elusive (Richards et al., 2006). Venette and Carey (1998) noted that more attention should be directed towards designing public policies in order to prevent invasions and further infestation, particularly for potential pests that are not yet established and whose biology is rarely described.

Williamson (1999) has stated that despite the versatile method for controlling and preventing new invasion, eradication is usually impossible when it comes to insects. The rapidly developing field of applied entomology has initiated an eradication programme based on trapping adult insects only, by using pheromones or semiochemical attractants. Other life-stages such as eggs or larvae, which are known for their aggressiveness and voracity, should also be studied. Likewise, several and diversified mechanical, biological and chemical methods have been used within eradication programmes, including mechanical removal, the construction of barriers, pesticides, sex pheromones and habitat management (all explained in detail in Gherardi and Angiolini, 2004). Most of the methods described are species-specific and, despite their benefits, have highly pronounced limitations; hence generalisation is impossible.

Despite these problems, there have been some cases of successful eradication. First, the removal of introduced rabbits from Pacific islands off Mexico and the United States resulted in the recovery of two declining native succulent species, *Dudleya linearis* (Britton & Rose, 1903) (Saxifragales: Crassulaceae) and *Dudleya traskiae* (Rose, 1942) (Saxifragales: Crassulaceae) (Clark and Halvorson, 1987, Zavaleta et al., 2001). Second, the successful removal of the exotic little red fire ant, *Wasmannia auropunctata* (Roger, 1863) (Hymenoptera: Formicidae), from Santa Fe Island in the Galapagos resulted in an increase in the population of several native ant species (Abedrabbo, 1994). However, other cases might not result in the desired level of successful eradication or recovery of native species.

A study has shown that species eradication can lead to unexpected changes to other ecosystem components, known as secondary effects. These effects include the further substantial elimination of native species and their functional role (Zavaleta et al., 2001). For instance, in a Mediterranean desert ecosystem, high soil salinity caused by the invasive ice plant, *Mesembryanthemum crystallinum* (Linnaeus, 1753) (Caryophyllales: Aizoaceace) makes it difficult for salt-sensitive native species to recover and re-establish (El-Ghareeb, 1991). There is however, a limitation on the quantitative data available regarding such ecosystem impacts. Without such quantitative data collection, pre-eradication assessment and sufficient planning, it is difficult to establish effective methodologies. Successful eradications may therefore result in adverse impacts (Innes and Barker, 1999, Blossey et al., 2001). Blossey (1999) further noted that there is an urgent need to collect and publish quantitative evidence for the ecosystem impacts of non-indigenous species, in order to guide management decisions.

1.2 Invasive species

According to the Convention on Biological Diversity (CBD), invasive alien species (IAS) are those that have been introduced, then become established, naturalized and expanded outside of their native range, causing significant harm to native species and ecosystem assemblages (Perrings et al., 2002). Likewise, Williamson and Fitter (1996) conferred the term 'invasive species' only upon those non-native populations that have become self-sustaining and have negative biological or economic impacts. These alien species are of a major concern in conservation biology, as well as to agriculture and human health (Mack et al., 2000, Mooney and Cleland, 2001, Koch, 2003). Invasive species are recognized as major drivers of biodiversity loss (Roy et al., 2011), causing significant environmental damage and leading to changes in the structure, diversity and composition of communities, as well as alteration of ecosystem-level processes and services (i.e., the biological availability of nitrogen) (O'Dowd et al., 2003, Whitney and Gabler, 2008). The combined annual cost of invasive species for six large nations has been estimated as US$335 billion, as reported by Pimentel et al. (2000). At the species level, the direct effects of invasive species occur through predation, competition and pathogen and parasite transmission to native individuals, often leading to population decline and displacement (Loehle, 2003).

Invasions frequently comprise rapid evolutionary events, resulting in populations that are genetically dynamic, both temporally and spatially (Reznick and Ghalambor, 2001). A 'rapid evolutionary change' was defined by Hairston et al. (2005) as "genetic change occurring rapidly enough to have a measurable impact on simultaneous ecological change". Tsutsui et al. (2000) called attention to the fact that genetic characteristics of invasive populations have profound impacts on their capacity for range expansions. They further stressed the influences of natural selection and genetic drift in altering the genetic structures of invading populations in ways that modify their tolerance capability or behaviour. Multiple evidence supports the importance of genetic attributes for invasion success, such as additive genetic variance or genomic rearrangements, which has a great role in evolutionary adaptation in response to environmental change (Reznick, et al., 1997, Prevosti et al., 1988, Carroll et al., 2001). For instance, Pappert et al. (2000) reported that the high levels of additive genetic variance within the population of *Pueraria lobata* (Maesen, 1985) (Fabales: Fabaceae) facilitated its invasive capability in south-eastern United States.

There are several species of insect that have become invasive after being used as a biological agent, such as the Japanese beetle, *Popillia japonica* (Newman, 1841) (Coleoptera: Scarabaeidae), which was introduced to the United States in 1911 from Japan. This species spread over the eastern United States and became a major pest, affecting crops, soybeans, apples and peaches, and defoliating over 250 tree species (Elton, 1958). The rice water weevil, *Lissorhoptrus oryzophilus* (Kuschel, 1952) (Coleoptera: Curculionidae), which was detected in Japan in Tokoname near Nagoya City in 1976. Ten years later, this weevil had extended over all the Japanese islands and had become the common insect pest of rice crops (Sato et al., 2005). In another example, the flatworm, *Platydemus manokwari* (De Beauchamp, 1962) (Tricladida: Geoplanidae), which is native to China, was introduced to control populations of another invasive species, the giant African snail, *Achatina fulica* (Férussac, 1821) (Gastropoda: Achatinidae) (McNeely, 1999). Although *P. manokwari* had a successful role as an efficient biological control agent, it is now considered a significant threat to native snail fauna (Chibaa and Roy, 2011).

1.3 *Harmonia axyridis* as model of invasive species

One of the worst invasive alien species in North America and Western Europe, is the Asian multicoloured ladybeetle *Harmonia axyridis,* the harlequin ladybird. *Harmonia axyridis* is a Palearctic species that is native to continental, temperate and subtropical parts of east and central Asia (Bazzocchi et al., 2004). This beetle occurs in Korea, Japan, the Bonin Islands, China, the Himalayas, Formosa and Siberia (Dobzhansky, 1933). *Harmonia axyridis* was first introduced as a classical biological control agent in 1916 in the United States, but it was not until the 1980s that it became established there and regarded as an invasive alien species (Koch et al., 2003). The first records for the establishment of this beetle were in North America in 1988 and in South America in 1998 (Chapin and Brou, 1991). Within five to six years, it had spread across the east coast of North America (Koch et al., 2006). Since the beginning of the 20th century this species has been sold commercially as a classical biological control agent across different parts of the world (Katsoyannos et al., 1997).

In Europe, between the 1980s and 1990s, *H. axyridis* was widely used as a biological control for aphids and coccids (Trouve et al., 1997, Adriaens et al., 2003, Brown et al., 2008ab). This beetle is now established in Europe - across France, Holland, Germany and Luxembourg (Brown et al., 2008ab). *Harmonia axyridis* threatens the diversity of native aphidophagous species through direct intraguild competition and by voracious predation (Majerus et al., 2006). In a risk assessment of 31 exotic natural enemies of pest species used in biological control in Europe, *H. axyridis* had the second highest environmental risk index (Mochizuki, 2010). This risk index was estimated based on its polyphagous ability to establish, its dispersal activity and by negative effects on native species.

Three categories of hazards caused by this invasive ladybird have been reported (Koch, 2006). First, it was found that some people have suffered from an allergic reaction to the yellow defensive chemicals secreted from the femoro-tibial joints when the ladybirds were aggravated (Yarbrough et al., 1999, Magnan et al., 2002). There have also been a few reported cases of people showing hyper-allergic symptoms manifesting as allergic rhino-conjunctivitis (Yarbrough et al., 1999, Albright et al., 2006). This ladybird often overwinters at high densities in or on buildings and is regarded as a human nuisance (Kovach, 2004). Second, it was noticed that when large numbers of *H. axyridis* were harvested and crushed with grape crops, their alkaloid defensive chemicals seriously tainted the vintage (Pickering et al., 2004). For instance, in the United States, this ladybird is considered as a pest species because it adversely affects the wine and fruit-growing industries (Kovach, 2004, Koch et al., 2004). Finally, *H. axyridis* have been found to affect biodiversity by influencing many native species assemblages, such as hetero-specific ladybirds, non-pest aphids and various herbivorous insects (Koch and Galvan, 2008).

The negative impacts of *H. axyridis* on native species provide opportunity for many studies focusing on assessing the role of intraguild predation (Roy and Wajnberg, 2008, Pell et al., 2008), as well as cannibalism, which is considered the determinant role in maintaining the dominance of *H. axyridis* and the displacement of other aphidophagous and native species (Osawa, 2011). *Harmonia axyridis* is a coccinellid that has induced hundreds of studies over many decades (Sloggett, 2005). In recent years, *H. axyridis* has become a very popular insect among biological control practitioners and scientists, not for its potential to be an efficient biological control agent, but due to its invasiveness. *Harmonia axyridis* became an invasive species affecting the dynamics and composition of various guilds (Soares et al., 2008). A review of 24 studies on the impact of *H. axyridis* on competitors, showed 15 cases where a negative impact on native species by intraguild predation or competition occurred (Lucas et al., 2007). Therefore, *H. axyridis* is a suitable biological model to test phenotypic traits related to fitness, because the diversity in level of phenotypic plasticity has already been described (Lombaert et al., 2007). Results from phenotypic plasticity studies indicate that plasticity could play an important role in invasion by allowing the invasive species to express advantageous phenotypes in a broader ecological niche (Sultan, 2001, Richards et al., 2005).

One feature that has been frequently mentioned is that *H. axyridis* is a strong intra-guild predator, interfering with other ladybirds' foraging activity and oviposition behaviour (Agarwala et al., 2003, Felix and Soares, 2004, Alhmedi et al., 2010). The dramatic decline of *Adalia bipunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) following the arrival of *H. axyridis* is of particular note (Facon et al., 2008). This species is now near the threshold of extinction, in both North America (Harmon et al., 2007) and Europe (Brown et al., 2011). Adriaenes et al. (2008) argued that *H. axyridis* appears to pose a threat to biodiversity and ecosystem integrity by displacing the native ladybirds with which it shares a high niche overlap, such as *A. bipunctata*. Facon et al. (2008) noted that some species, such as *Halyzia sedecimguttata* (Linnaeus, 1758) (Coleoptera: Coccinellidae), a mychophagous ladybird that is not in competition with *H. axyridis*, have undergone a dramatic increase in distribution in Britain (Roy et al., 2011) and Belgium, indicating a recent shift in habitat preference (Roy et al., 2011). The shift reflects, to some extent, the predation activity of *H. axyridis* on the immature stages of the *Halyzia* ladybird (Facon et al., 2008). In another example, the establishment of *H. axyridis* in natural agricultural communities in Michigan has drastically reduced the native ladybird populations of the orange-spotted ladybird, *Brachiacantha ursine* (Fabricius, 1787) (Coleoptera: Coccinellidae), the spotless ladybird, *Cycloneda munda* (Say, 1835) (Coleoptera: Coccinellidae), and the two stabbed ladybird, *Chilocorus stigma* (Say, 1835) (Coleoptera: Coccinellidae) (Colunga-Gracia and Gage, 1998). Various functional traits that have made *H. axyridis* an effective biological control agent have further implicated it as an intra-guild predator with a significant impact on ecological diversity (Snyder and Evans, 2006, Majerus et al., 2006). Biological features are known to be one of the determinants of a successful invader - these include dispersal ability, aggressiveness, various defence strategies and exploitative or interference competition (Lucas et al., 1998, Yasuda et al., 2000, Yasuda et al., 2001, Pell et al., 2008), as well as colonisation efficiency (Osawa, 2000, With et al., 2002). Another important feature is the higher conversion efficiency of prey into biomass, which results in the gain of a higher energy reserve and greater competition ability (Labrie et al., 2006). This feature is already present in the ladybird *H. axyridis*. Considered to be highly polyphagous, it possesses a high predation efficiency (Osawa, 2000, Labrie et al., 2006) and is able to consume a wide range of food, including fruit, fungi, eggs and pollen (Ferran et al., 1997, Berkvens et al., 2010b).

Cannibalism is another functional trait that appears to play an important role in influencing the population dynamics of *H. axyridis* (Osawa, 1993). Wagner (1999) showed that cannibalism is heritable and varies between lineages. It was noticed that cannibalism and predation by *H. axyridis* occurred even when prey was abundant (Majerus et al., 2006). Adults and larvae of *H. axyridis* find eggs of many insect species acceptable as prey (Cottrell, 2007). There is evidence suggesting that larvae of *H. axyridis* can successfully complete their developmental stages on a range of various foods, including the eggs, larvae, and pupae of many insects and con-specifics (Tedders and Schaefer, 1994, Koch, 2003).

One further advantage is attributed to its eurytopic ability to exploit resources in various agricultural habitats, including crops of corn, soybean and wheat (Colunga-Garcia and Gage, 1998). This definitely reflects the capability of this ladybird to invade a wider range of ecosystems (Majerus et al., 2006, Roy et al., 2006). The greater foraging efficiency often facilitates its ability to cope with various types of competition. Larvae and adults of this species have been found to have a greater predation ability and foraging efficiency when compared with indigenous species (Yasuda and Ohnuma, 1999, Yasuda et al., 2001, Lanzoni et al., 2004, Labrie et al., 2006). The foraging efficiency in *H. axyridis* was found significantly greater than indigenous species such as *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae), *Coleomegilla maculata* (De Geer, 1775) (Coleoptera: Coccinellidae), *Hippodamia variegata* (Goeze, 1777) (Coleoptera: Coccinellidae) and *Adalia bipunctata* (Coleoptera: Coccinellidae) (Yasuda et al., 2001, Lucas et al., 2002, Lanzoni et al., 2004, Labrie et al., 2006).

Furthermore, the wide natural latitudinal ranges of *H. axyridis* from Asia-extending to the cold temperate regions of Canada-allows it to breed in both warm and cool climates (Koch, 2003). In Oregon, it was recognised that *H. axyridis* is well adapted to both cold winter temperatures below freezing and summer temperatures up to 30°C (LaMana and Miller, 1996). This wide climatic tolerance and potential adaptability reflect its competitive advantage over other native ladybirds, as well as over other predators (Labrie et al., 2008). Another key factor is its physiological ability to withstand unfavourable environmental conditions. In this context, Sakurai et al. (1992) reported that *H. axyridis* shows two types of diapause-hibernation and aestivation (i.e., facultative dormancy). The aestivation may not occur when food is available. This feature may add to the success of *H. axyridis* in invasion in comparison to other species.

A number of morphological features of *H. axyridis* beetles have been reported as promoting the invasion ability of the species into new habitats (Michaud, 2002ab) by providing a protection from predation (Yasuda et al., 2001). One of these features is the relatively large body size (Soares et al., 2001, Osawa, 2000, Michaud 2002a, Labrie et al., 2006). *H. axyridis*' adult body size is often larger than that of most Coccinellidae species, such as *A. bipunctata* (Mills, 1981) and *Hippodamia quinquesignata* (Kirby, 1837) (Coleoptera: Coccinellidae) (Kaddou, 1960). Individuals of *H. axyridis* collected from fields showed that adult body size was significantly different for each colour pattern and between the sexes. In non-melanic form, the variation of size between sexes was 30.3% of median size in males, and 35.2% in females. The size variation in the melanic type was 21.7% in males and 28.5% in females (Seo, 2008). Majerus (1994) noted that the large size of *H. axyridis* undoubtedly contributes to its success as a strong intra-guild predator, despite the few reports that *C. septempunctata* larvae have successfully managed to attack larvae of *H. axyridis* (Pell et al., 2008). Evidence suggests that *H. axyridis* larvae are more resistant to reciprocal attacks, attributed to their higher attack rates and greater escape ability (Yasuda et al., 2001). This ladybird is highly equipped with effective defence mechanisms, including chemical and physical cues (Agarwala and Dixon, 1992, Hemptinne and Dixon, 2000). For instance, the presence of spines on the back of third and fourth larval instars provides physical protection (Ware and Majerus, 2008). Furthermore, the existence of surface semio-chemicals on eggs of *H. axyridis* and pupae (Felix and Soares, 2004) make these stages less vulnerable to predation by other aphidophages (Sato and Dixon, 2004, Alam et al., 2002).

Reproductive performance and the number and size of offspring are considered as further significant traits of this ladybird compared with others. A laboratory study showed that, during its lifespan, a single female can lay between 703 and 3,800 eggs (Stathas, 2001). Mignault et al. (2006) noted that the fecundity of *H. axyridis* reared on soybean aphids was about 2,008 eggs per female - significantly higher than indigenous *Coleomegilla maculata* (De Geer, 1775) (Coleoptera: Coccinellidae) (390 eggs/female), *A. bipunctata* (720.2 eggs/female) in Canada and the invasive *Propylea quatuordecimpunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae). The rapid rate of ovarian development and oosorption process in the predatory *H. axyridis* has acted to optimise the potential reproductive output compared with other ladybirds (Osawa, 2005). A key factor in the invasion process is juvenile growth and development, as safe conditions in this vulnerable stage promote high population growth in the new environment (Marco et al., 2002). It was demonstrated that second larval instars of *H. axyridis* developed in a shorter time compared to the native species *C. maculata* and *H. axyridis* reached the fourth instar more rapidly than *C. maculata* (Labrie et al., 2006). The adults of this ladybird become active early in the season (eg, in April) and both larvae and pupae are commonly documented in November (Majerus et al., 2006).

Another feature that is believed to have a significant role in *H. axyridis*'s invasion successes is the colour polymorphism. This feature is hereditary with great plasticity (Dobzhansky, 1933, Komai et al., 1951, West-Eberhard, 1989, Soares et al., 2003). Other life history traits, such as elytral patterns, body shape and size may also show phenotypic variability towards adverse conditions (Dobzhansky, 1933, Soares et al., 2003). The pattern of colouration of the pronota and elytra of adult *H. axyridis* has been shown to have a genetic basis, controlled by both a multi-allelic gene and genetic variation in reaction norms. This allows different families to respond differently in response to environmental factors (Felix and Soares, 2004, Michie et al., 2010). There are three main colour forms of adult *H. axyridis* in Europe-*Forma succinea*, *Forma spectabilis* and *Forma conspicua* (Dobzhansky, 1933).

Strong evidence has suggested a link between environmental factors and colour patterns in this coccinellid, or within a form (Majerus, 1998). The degree of melanisation is often inversely correlated with temperature (Abbas et al., 1988, Majerus, 1998). For instance, the lower the temperature experienced by the final larval instar, the darker the pupa (Majerus, 1994, 1998). Despite the genetic basis, colour polymorphism was also shown to vary seasonally and geographically (Osawa and Nishida, 1992, Soares et al., 2003). Research by Osawa and Nishida (1992) revealed a decrease in the frequency of the non-melanic phenotype from summer compared with spring, in relation to non-random mating among different phenotypes.

It appears that the ladybird *H. axyridis* had different responses in relative fitness between melanics and non-melanics in response to climate (Soares et al., 2003). Melanic forms of *H. axyridis* showed a greater fitness in response to climate fluctuations, which would be an advantage over non-melanic elytra. During cold days, melanic forms are therefore able to attain higher body temperatures than non-melanic forms (Koch et al., 2006). Furthermore, it was noted that the pattern of elytral colouration indicates a signal of chemical defence in *H. axyridis* (Bezzerides et al., 2007). This was based on the positive significant correlation that exists between the degree of melanin and alkaloid content (i.e., it is considered a defensive chemical) (Bezzerides et al., 2007). In addition to the above, the elytral phenotypes showed variation in response to suitability of host plants and aphid quality and quantity (Komai and Hosino, 1951, Soares et al., 2005). Soares et al. (2005) noted that *H. axyridis* showed considerable intra-specific variation in elytral pattern, and the related genotype property confers that pattern might reflect a specific ecological attribute. Therefore, the above features support the contention that *H. axyridis* possess a high degree of phenotypic adaptive plasticity for a number of life-history traits. Having that degree of plasticity allows this ladybird to be an efficient competitor and promotes its establishment and colonisation of a wide range of ecosystems (Kock, 2003).

Entomopathogenic fungi are common natural enemies of insects and other arthropods worldwide (Roy et al., 2006). Several field and laboratory studies have reported the susceptibility of various ladybirds to *Beauveria bassiana* ((Bals.-Criv.) Vuill., 1912) (Ascomycota: Hypocreales) (Cottrell and Shapiro Ilan, 2003, Roy et al., 2008), but not *H. axyridis*. Cottrell and Shapiro Ilan (2003) demonstrated that *H. axyridis* was extremely resistant to *B. bassiana* infection, even after using various isolates. Furthermore, laboratory and field studies have revealed low levels of successful parasitism of adult *H. axyridis* by *Dinocampus coccinellae* (Schrank, 1802) (Hymenoptera: Braconidae) (Hoogendoorn and Heimpel, 2002, Firlej et al., 2005). In another study conducted by Smith and Krischik (2000), it was revealed that *H. axyridis* showed a great resistance to biorational pesticides, such as soap, oil and azadiractin. Although the impact of fungicides and conventional insecticides on *H. axyridis* appears to be minimal (Smith and Krischik, 2000, Michaud, 2001, Musser and Shelton, 2003), examples to the contrary do exist - for example, *H. axyridis* was found susceptible to certain herbicides such as glufosinate-ammonium (Ahn et al., 2001). The susceptibility of *H. axyridis* to various herbicides or other pesticides, however, varies depending on the developmental stage. Adults are often less susceptible than immature stages, due to the former's ability to fly (Ahn et al., 2001, Michaud, 2002ab).

In conclusion, all of the above features make *H. axyridis* a highly competitive insect and facilitate its invasion success and establishment in a wide range of ecosystems. Clearly, the ability of *H. axyridis* to invade native ranges worldwide (Roy et al., 2008) is potentially related to fitness; if the colonising ability of this ladybird is associated with plasticity, there is a need for rigorous research data to clarify better the degree of plasticity in fitness-related character traits (Grill et al., 1997, Lanzoni et al., 2004). Moreover, there is growing evidence that integrating both genetic and ecological factors is crucial in understanding the biology of invasive species, since the two are explicitly linked and together may help to explain the invader strategy in successful establishment (Sax et al., 2007, Handley et al., 2011). Gaining such information on the invasion processes may help in establishing management and prevention policies against future invasion (Handley et al., 2011).

Successful invasion has been referred to as a phenomenon known as 'bridgehead', which often results in several secondary invasions stemming from already established invasive populations, as was the case with *H. axyridis* (Estoup and Guillemaud, 2010). For instance, Lombaert et al. (2010) clarified that the invasive population in eastern North America has acted as a source population for colonists invading Europe, South Africa and South America. In such cases, the introduced species may lead to evolutionary shift and several outbreaks, regardless of the number of introduction events (Estoup and Guillemaud, 2010). Indeed, the invading populations may also be capable of invoking potential evolutionary changes in native species (Filchak, et al., 2000). Therefore, incorporating evolutionary and molecular genetics should be of a high priority when revealing the characteristics that determine invasion success and preventing subsequent spread (Tsutsui, et al., 2000, Lee, 2002, Estoup and Guillemaud, 2010). Lee (2002) stressed the importance of genetic constituents and natural selection over having a broad physiological tolerance or plasticity. He emphasised that a genomic approach was essential for determining invasion mechanisms, such as analysis of gene expression, gene interactions, and genomic rearrangements.

The question must be addressed of whether other ladybirds will become worldwide invasive species at some stage. Soares et al. (2008) claimed that it is possible that other aphidophagous coccinellids may reveal similar phenotypic responses and be as competitive as *H. axyridis* if they are released extensively and repeatedly. His postulation was based on several comparisons collected from various sources. For example, comparative studies showed significant differences among pre-adult development times, with *H. axyridis* havong the longest time when compared with: *A. bipunctata*; the black-spotted lady beetles, *H. variegata* (Lanzoni et al., 2004), the 14-spot ladybird, *Propylea quatuordecimpunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) (Mignault et al., 2006), and the ashy grey ladybird beetle, *Olla v-nigrum* (Mulsant, 1866) (Coleoptera: Coccinellidae) (Michaud and Olsen, 2004).

In a study by Lanzoni et al. (2004) it was revealed that the intrinsic rate of increase, net reproductive rate and mean generation time were higher for *H. variegata* than for *H. axyridis.* Regarding the high fecundity in *H. axyridis* mentioned previously, some other studies showed that the fecundity of *H. axyridis* was slightly higher, similar or lower than indigenous species (Bazzochi et al., 2004). For instance, contradicting the finding of Mignault et al. (2006), Bazzochi et al. (2004) found that the mean fecundity of *H. axyridis* was 783.8 eggs per female, which was slightly higher than *A. bipunctata* (720.2 eggs/female); the difference was, however, not significant. Another fact was revealed when two native species - *C. maculata* and *O. v-nigrum* ladybirds - were starved, both attacked *H. axyridis* eggs. Nevertheless, most aphidophagus species are also known to be good competitors in their habitat range, such as *C. septempunctata* (Evans, 2004, Alyokhin and Sewell, 2004).

Finally, it is not only *H. axyridis* that has been found able to complete its lifecycle on supplementary food such as pollen - other native species such as the convergent lady beetle, *Hippodamia convergens* (Guérin-Méneville, 1842) (Coleoptera: Coccinellidae), and the unspotted ladybird *Cycloneda sanguinea* (Linnaeus, 1763) (Coleoptera: Coccinellidae) could do the same (Finlayson et al., 2010). Therefore, all the above imply that the biological traits of *H. axyridis* may partly explain its invasiveness, but there are definitely other contributing factors. According to Soares (2008), some aphidophagous coccinellids are already invasive or have great potential to invade, but their role has not been revealed. Most attention has been directed towards studying the bio-ecological attributes of *H. axyridis*, as it has been released in many more locations than any other coccinellids. Furthermore, *H. axyridis* is extensively recognised and has been of great concern to the public, due to its nuisance overwintering behaviour inside and on buildings.

1.4 Spread of *Harmonia axyridis* in the United Kingdom

The multicoloured Asian ladybird *H. axyridis* is indigenous to Asia and was introduced to combat aphids and coccid species in orchards in Europe and North America (Adriaens et al., 2003, Koch, 2003, Koch et al., 2004). It was established first in North America in 1988, as well as in Canada (Chapin and Brou, 1991). In mainland Europe, *H. axyridis* was first marketed as a bio-control agent in 1982 (Brown et al., 2008a). Since the late 1990s, *H.*

axyridis has has established and expanded its range rapidly, reproducing populations in the wild in at least 13 western European countries (Brown et al., 2008ab, Lombaert et al., 2007, Poutsma et al., 2008, Bidinger et al., 2012). Commercial releases as a classical biological control agent were made in European countries from 1993 onwards**,** including in France, Belgium, the Netherlands and Italy (Katsoyannos et al., 1997, Iperti and Bertand, 2001), but it has never been sold officially in Germany, the United Kingdom and Switzerland (Brown et al., 2008b). The spread and distribution of *H. axyridis* in Europe has been reviewed by Brown et al. (2008b). At present, it is abundant in a large area of Europe, including southern France, Denmark, the United Kingdom and Czech Republic. The abundance of this ladybird increased rapidly from 2004 to 2010 and is predicted to continue in the same manner if it is not controlled (Majerus et al., 2006). *Harmonia axyridis* tends to form very large overwintering aggregations, often on or in buildings (Adriaens et al., 2003). An analysis of land cover revealed that *H. axyridis* is more frequently aggregated in urbanised landscapes than in semi-natural landscapes (Adriaens et al., 2003).

There is no evidence indicating that *H. axyridis* was established in the United Kingdom before 2004 (Majerus and Roy, 2005). Evidence suggests that *H. axyridis* arrived into the United Kingdom by multiple routes in that year (Majerus et al., 2006). Likewise, Brown et al. (2008b) assumed that the expansion of *H. axyridis* in the United Kingdom originated from various means, such as by imported flowers from the Netherlands and in packing cases from Canada. Recently, Lombaert et al. (2010) have retraced the routes of all five worldwide *H. axyridis* invasion events. They noted that the invasive population of Eastern and western North American possibly originated from the Asian range through two different unrelated introductions. In Europe, there is clear evidence of admixture between the eastern North American and European strains that were released as biocontrol agents. Lately, Turgeon et al.

(2011) reviewed the above assumptions and noted similar findings. In fact, admixture and hybridisation and their consequences on the adaptive potential of invasive populations have been explicitly investigated by many scientists (Facon, et al., 2011).

'Hybridisation' is described as the process of interbreeding between genetically differentiated lineages (Rieseberg et al., 2003, Mallet, 2005). This plays a critical role in a variety of evolutionary processes, ranging from local adaptation to speciation (Rieseberg et al., 2003, Mallet, 2005). For invasion biology, hybridisation may comprise a potential stimulus in the invasiveness evolution (Lavergne and Molofsky, 2007, Ryan et al., 2009). 'Admixture' is a hybridisation process that may involve a recombinant formed from two genetically differentiated individuals, leading to phenotypes with greater fitness. In fact, admixture may result in a heritable phenotypic variation with a long-lasting response to selection that eventually facilitates invasiveness (Facon et al., 2005, 2008, Lavergne and Molofsky, 2007, Kolbe et al., 2007). The positive influence of the admixture was revealed when Facon et al. (2011) tested a hybridised strain taken from both the invasive French population and the biological control strain. This experiment showed that the hybrid strain had a shorter development time, a larger size and a higher genetic variance for survival under stressed conditions. Apparently, the presence of a substantial genetic differentiation within a population could partially explain the several cases of invasion and the persistence of this beetle worldwide. Despite the above findings, they noted that there is a possibility of out-breeding depression, due to recombination disrupting co-adapted gene complexes or meiotic problems that might be expressed in future generations of admixed *H. axyridis* individuals. This finding was in accordance with Burke and Arnold (2001).

On the arrival of *H. axyridis* to the United Kingdom, there was extensive national and local media coverage, alongside public involvement in detecting it through a dedicated website (http://www.harlequin-survey.org). The arrival of the harlequin ladybird has had a negative impact on up to 1,000 species in the United Kingdom, including non-target Homoptera, and members of aphidophagous and coccidophagous species (Majerus et al., 2006). A study by Brown et al. (2011) assessed the changes in native ladybird species assemblages, including *A. bipunctata*, *C. septempunctata*, and *P. quattuordecimpunctata*, across arboreal habitats that encompassed the invasion phase of *H. axyridis* in eastern England over three years after the establishment of *H. axyridis* in the country. There was an increase in *H. axyridis* population ranges from 0.1% to 40% of the total ladybirds sampled, accompanied by a decline in the number of native aphidophagous ladybirds from a mean of 19.7 in year 1, to 10.2 in year 3. This reduction was caused by intensive competition and intra-guild predation dominated by *H. axyridis*.

Exceptions may occur to the contrary, since not all the native species had been affected by the establishment of *H. axyridis*. Roy et al. (2008) noted that the distribution and abundance of *C. septumpunctata* appeared to be stable and unaffected across Europe, due to non-overlapping habitats. Nonetheless, the ladybird *C. septumpunctata* itself is a strong competitor that is thought to have caused a dramatic decline in native species in the United States and Canada (Harmon et al., 2007). In conclusion, the increasing dominance of *H. axyridis* has disrupted native ladybird communities remarkably and reduced the diversity and the resilience of the aphidophagous guild (Winter et al., 2009). The impact, as shown above, is substantial; therefore, more laboratory studies should be designed that focus on the influence of *H. axyridis* on fitness-related traits of indigenous coccinellids.

There are three main elytral colour patterns of adult *H. axyridis* that have been verified in the United Kingdom - the non-melanic form, *F. succinea*, and the melanic type, which includes both *F. spectabilis* and *F. conspicua*. The apparent difference in the pattern of elytral coloration forms suggesting a genetically distinct population (Dobzhansky, 1933, Brown et al., 2008ab). In the United Kingdom, *H. axyridis* is bivoltine with a spring peak in larval records in weeks 22-25 (i.e. late May to late June) and an autumn peak most frequently in weeks 42-43 (i.e. mid to late October) (Brown et al., 2011). The existence of two generations passing through the lifecycle within a year, and the resultant high number of offspring, helps to explain the rapid dispersal of this ladybird in the United Kingdom (Brown et al., 2008ab). Adaptation to winter temperatures below freezing and to summer high temperature up to 30˚C in different areas would definitely lead to the same adaptive response in response to UK weather (LaMana and Miller, 1998, Majerus et al., 2006).

The mean activity period calculated from *H. axyridis* was 30.75 (range 26 to 34) weeks per year (based on the difference between the beginning and end of overwintering in buildings). A strong correlation was found between the length of this period and the mean annual temperature in Britain, which possibly implies a degree of phenotypic adaptation (Brown et al., 2011). A recent field study by Berkvens et al. (2010a) tested the variation in cold hardiness of overwintering populations of *H. axyridis* in Belgium for both an indoor and an outdoor hibernaculum. The results revealed that individuals of *H. axyridis* failed to tolerate winter outdoor temperatures, ranging from -17.1˚C to -16.3˚C, while the indoor population possessed a higher tolerance response, withstanding temperature ranges from -18.5˚C to -13.2˚C. The strong cold-hardiness may enable the species to become established over a large range of the Europe continent. *Harmonia axyridis* populations have also become a threat to the endemic British fauna (Majerus et al., 2006). As mentioned above, *H. axyridis* is very common in urban habitats and it increasingly uses semi-natural habitats, with its larvae recorded foraging over 50 plant families (Brown et al., 2010).

Following the rapid increase in the negative impact of exotics species worldwide (Howarth, 1991, Mack et al., 2000), ecologists have been working to identify the key factors that could explain why some introduced species have turned into invasive while others did not (Williamson, 2006). The mechanisms behind successful colonisation and establishment are essential to helping to prevent further invasion and improving release programmes for biological control agents, since some countries are still adopting exotic species that have no apparent influence on non-targeted species or the ecosystem (Mori et al., 2005). Environmental factors, as well as the degree of plasticity in life-history traits, are thought to be the main factors for successful invasion and establishment (Shea and Chesson, 2002, Marco et al., 2002). These factors might, however, only be partly responsible for determining the invasion in some cases.

Despite what has been mentioned above, Mack et al. (2011) emphasised the fact that the common perception that such exotic species always have a drastic impact on biodiversity is invalid in some cases, and is not always supported by enough data. They further argued that the claim made by Wilcove and others in 1998 that "invaders are the second greatest threat to the survival of threatened or endangered species after habitat destruction" was supported by little evidence. Thus, they encouraged researchers to take care when quoting a particular finding. A recent work by Gardener et al. (2011) suggested that invaders do not always lead to a major extinction in most native species.

The effect of the introduced non-native species varies with time and between species (Mack et al., 2011), independent of nativity or the species' origin (Mack et al., 2011). A study by Lee (2002) suggested that the success of invasion depends mainly on the ability of introduced species to respond to natural selection. For example, the lack of success in the establishment of *H. axyridis* in the islands of the Azores could be a result of the effect of species saturation and the competitive exclusion of *H. axyridis* by other previously established species. The absence of cold weather (which is essential for the induction of diapause although not obligatory diapause) also played a significant role in suppressing the establishment of *H. axyridis* (Lee, 2002).

1.5 Regulations and prevention measures

In an ongoing effort to evaluate the impact of invasion, ecologists have recently started to examine the proximate factors that could depress invasion success. A methodology for risk assessment has been developed as a basis for the regulation of the import and release of exotic natural enemies (Van Lenteren et al., 2003). Out of these attempts, some scientists have encouraged the use of indigenous species rather than exotic agents, since any risk would be normally reversible (Follett and Duan, 2000). Fortunately, there is a growing recognition among the public, policy-makers and the business community urgently to address the problem of the invasive alien species (Van Lenteren et al., 2003, Burgiel et al., 2006, Ehlers, 2011).

Despite considerable efforts, however, there is evidence to suggest that the expansion of invasive alien species is still progressing, with its direct relationship with global trade making control impossible (Burgiel et al., 2006). Due to the absence of protective measures and regulations, the increased movement of goods through growing global trade has led to the inevitable introductions of various invasive alien species (Ruiz and Carlton, 2003). Generally, preventing the introduction of invasive alien species is recognised as the best effective method for keeping the risk of invasion low, since eradicating attempts have been shown to be less successful and highly expensive (McNeely et al., 2001). It is important to estimate the risk and benefits carefully when considering a regulating rule, as any kind of exaggeration of the risk may cause trade-off effects (Ehlers, 2011). Graham and Wiener (1995) declared that the first step before developing any regulation policy must be to take into account the cost and benefits, in order to estimate the magnitude of the potential problem. Public and society concerns have been raised towards over-regulation of biological control agents, which may keep older riskier methods in place instead, thus resulting in more adverse consequences (Graham and Wiener, 1995, Burgiel et al., 2006, Loomans, 2007). Since 1992, more and more countries have put legislation in place concerning those biological introductions that threaten biological diversity. This has also increased the international interest in risk assessment as a legislative tool (Loomans, 2007).

Countries such as Australia, New Zealand, Canada and the United States already have many years' experience in terms of regulating the import and release of exotic invertebrate biological control agents (IBCAs) and in analysing the associated risks (Sheppard et al., 2003). They have successfully implemented the classical biological control programmes (Coulson et al., 2000, Sheppard et al., 2003), such as the International Plant Protection Convention (Sheppard et al., 2003). Despite that, these regulation programmes have not been well coordinated internationally, because they depend upon a complex intersection of environmental, trade and transportation agreements. In addition, there have been concerns that differing requirements could lead to conflicts, in particular between trade and environmental agreements (Burgiel et al., 2006). Although there have been few reported negative effects linked to the import and release of IBCAs, 20 countries worldwide have already implemented and revised their regulatory framework for the release of biological control agents, in others regulation is currently being implemented, and in some countries no regulation yet exists (Sheppard et al., 2003, Van Lenteren et al., 2006).

European countries have experienced an increase in the establishment of exotic invertebrate and plant pest species (Bigler, 2001). The use of IBCAs in Europe is not covered by a directive equivalent to that which regulates biocontrol with microorganisms or the genetic modification of crop plants (Bigler, 2001). When the legislation and the administration for regulation of IBCAs existed, it fell under the responsibility of the national plant quarantine service and focused mainly on preventing introduced IBCAs from becoming agricultural pests (Wapshere, 1974, Waage, 1997). Interest in implementing classical biological control programmes has been growing throughout Europe, aiming to avoid the use of chemical controls, which can be harmful, whether to animal or human health, plant health or biodiversity conservation (Waage, 1997, Sheppard et al., 2006). Some European countries have already established their own well-organised programme for regulating the introduction of exotic IBCAs (Bigler, 2001, Bigler et al., 2005, Loomans, 2007).

About 90 species of IBCAs are widely used and commercialised across Europe at present (EPPO, 2002), and many more are under investigation for future release. Europe leads the world in this activity and national regulatory societies have an obligation to facilitate international trade in an efficient regulated way (Loomans, 2007). There has been a growing intention in recent years to introduce a unified scheme and regulatory procedures across all European member countries. Yet, despite these initiatives, large differences still exist in the degree of implementation of regulatory procedures of IBCAs across Europe (Waage, 1997, Bigler et al., 2005). A survey of 20 European countries, conducted in 2004, revealed that despite all countries under investigation having national legislation in place, an active regulatory process had only been implemented to some degree in nine countries (Austria, the Czech Republic, Denmark, Hungary, Norway, Slovenia, Sweden, Switzerland and the United Kingdom). Five countries are still working on the design and implementation of a regulatory system (Finland, Germany, Netherlands, Slovenia and Spain) and six countries have no regulation implemented yet, and are unlikely to do so in the foreseeable future (Belgium, France, Greece, Italy, Poland and Portugal) (Bigler et al., 2005).

The Community Agriculture Policy (FAO) Code of Conduct as the International Standard for Phytosanitary Measures (ISPM) No. 3 under the IPPC was published in 1996 (Bigler et al. 2005). Also known as the "Code of Conduct for the Import and Release of Exotic Biological Control Agents", this was the turning point for several activities related to the release of biological control agents in Europe (Bigler et al., 2005). The FAO Code of Conduct has brought about important changes in the regulation of IBCAs in western and developing countries, but these were still largely non-legislative instruments and were not compulsory (Kairo et al., 2003). In Europe, ISPM No. 3 has never been fully implemented (Loomans, 2007).

Shortly after the code’s publication, several international organisations developed guidelines on the implementation of regulation for IBCAs and data needed for environmental risk assessment (Bigler et al., 2005, 2006). The European and Mediterranean Plant Protection Organisation (EPPO) together with CABI Bioscience organised a workshop in Europe (EPPO, 1997). One of the main outcomes of the workshop was the proposal for harmonising guidance for importation practice between European countries, detailing the criteria recommended for importation for different purposes - such as for research or classical and commercial biocontrol (http://www.eppo.org/STANDARDS/standards.htm). Several conferences and research stemmed from the publication of the FAO code, including the Evaluating Environmental Risks of Biological Control Introductions into Europe (ERBIC) research project, executed from 1998 to 2002 and funded by the European Union (Bigler et al., 2005, 2006). Another promising activity was initiated in 1999 by the OECD (Organisation for Economic Co-operation and Development), aiming to harmonise the regulation of IBCAs. As a consequence, the International Biocontrol Manufacturer Association (IBMA) proposed to the International Organisation for Biological Control (IOBC/WPRS) that it coordinates harmonisation among the European regulatory authorities (http://www.iobc-wprs.org). The IOBC/WPRS appointed a commission in 2003 with several objectives, including homogenisation and regulation of IBCAs, based on the FAO Code of Conduct and the EPPO standards (Bigler et al., 2005).

Recently, Bale (2011) listed precisely the problems surrounding the fragmented pattern of regulation between European countries - for example, there is a variation in collected scientific information required for release licences, the methods of environmental risk assessment for different species of IBCAs needs further formatting, the European Plant Protection Organisation Positive List needs a continuous updating and revision, expert advice on environmental risk assessment data and more communication are needed between IBCA regulators, and the international leadership must be able to provide provision to coordinate regulatory and environmental risk assessment related issues with IBCA-based biocontrol in Europe.

In conclusion, at present there is no coordinated system of regulation for IBCAs across Europe. Biological control agents are instead regulated according to the nature of the agent, the mode of action (microbial or macrobial), and the purpose for which it is used (Loomans, 2007). It is clear that the legislation for the regulation of IBCAs varied among European countries and in some countries the law has not yet been authorised. Uncoordinated regulations among European countries may have impacts and cause serious ecological and economical crises. Yet, there is no obvious contribution from governments, ministries and scientific institutions towards this important issue (Bigler et al., 2005). Since the establishment of *H. axyridis*, many European countries stopped releasing coccinellids based on documented non-target risks.

The EU commission declared the need for a proposal in order to organise the importation policies of exotic species. Subsequently, the Regulation for Biological Control Agents (REBECA), founded in 2006, has taken initiatives to develop several guidelines for harmonised methodologies for risk assessment and subsequent implementation of regulation procedures. The regulation procedures were applied, however, at national level (Bigler et al., 2005). This and other examples demonstrate that Europe urgently needs a harmonised regulation of biological control agents, which will prevent import and release of unsafe organisms, but which will not put an unnecessary burden on biological control (Bigler et al., 2005). Hunt et al. (2007) conducted a research and comparison between the different systems adopted by New Zealand, Australia, Canada and the United States. The component of their work determined the best recommendations for incorporation into a workable regulatory framework to suit the needs of Europe. There is therefore a need to improve understanding of the processes underlying their success or failure in order to collaborate with these organisations, so that the best policy for controlling and preventing future invasion may be found.

1.6 Life-history theory

Life-history theory seeks to understand the factors that produce variation in life histories found both among and within species (Roff, 2007). It deals with how natural selection shapes an organism's development, reproduction and death; thus, it is a theory of fitness (Nylin and Gotthard, 1998). Two basic considerations form the foundation of life-history theory. First, there is some definable measure of fitness that is maximised by natural selection. Second, there are trade-offs that determine the possible set of life-history trait interactions (Roff, 2007). According to Stearns (1976), life-history theory assumes that the environment is frequently fluctuating either predictably or stochastically over time and resources often become limited. Organisms must therefore adjust their resource acquisition and their allocation patterns for various life history traits in order to compensate for unpredicted environmental conditions (Boggs, 2009).

The decision and degree of resource allocation are likely to result in different relationships between life-history traits and demographic rates, depending on the prey or food availability (Plaistow et al., 2006). Resource allocation for holometabolous insects is highly complex in comparison with that of hemimetabolous insects. The allocation in the former is a three-stage process corresponding to life stage, namely, maternal and larval-derived nutrients, pupal stage and adult. The patterns of allocation at each stage are highly dependent on the external nutritional status (Boggs, 2009). Furthermore, the nutritional requirements are variable according to the life-history trait and can change according to age or allocation duration (Giron and Casas, 2003). For example, the butterfly *Speyeria mormonia* (Boisduval, 1869) (Lepidoptera: Nymphalidae) needs to be fed nitrogen during the larval stage, whereas nectar mixed with carbohydrates is necessary during the adult stage (Boggs and Ross, 1993). Providing nectar only at the adult stage constrains the potential total fecundity (Boggs and Ross, 1993). Thus, multiple types of nutrients must be considered simultaneously in some cases and at specific stages of the lifecycle (Jervis et al., 2008). Nutrient allocation and partitioning towards multiple traits can therefore generate trade-offs between several traits, particularly when food is the limiting factor (Roff, 2002). Likewise, it has been reported that the dependent trade-off between life history traits may differ between low and high food abundance and the type of trade-off is important in determining the population dynamic patterns (Benton et al., 2006, Plaistow et al., 2006).

Classic trade-offs have been widely documented between growth, reproduction and survival, which may vary through time and among individuals (Fischer et al., 2006). In addition, some trade-offs occur at a smaller trait scale, such as those reported between egg number and size (Smith and Fretwell, 1974). Thus, in order to understand the context of pest life-history traits, the interaction between fitness-related traits, adaptive evolution and population dynamics must be considered (Nylin and Gotthard, 1998, Nylin, 2001). Several studies have focused on physiological trade-offs, life-history evolution and genomics, ignoring the cross-connections between such areas (Lee et al., 2008). Roff (2007) noted that evolution proceeds due to variations in fitness, and that life-history traits directly contribute to enhancing this fitness. He further stressed that both evolution and genomics-based approaches are essential when explaining several trade-offs. Furthermore, the related resource allocation will always contribute to the future development of life-history theory. Roff (1992) and Stearns (1992) summarised the classical life-history theory, which is based on an optimisation model that always attempts to maximise several measures of fitness under certain constraints (Stearns, 1977, Lewontin, 1979). By the 1990s, scientists had achieved a plausible explanation for the evolution of life-history traits away from optimality, as reviewed by Stearns (2000). In agreement, Lande (1982) stressed that not all organisms or organs are expected to function at their optimal level during their lifespan, and therefore any results based on optimality would be unreliable.

'Evolutionary theory' predicts that in "populations subjected to rapid extinction, recolonisation dynamics and natural selection should favour rapid evolution of traits enhancing dispersal and recolonisation ability" (Forsman et al., 2012, Hanski, 2001, Roff and Fairbairn, 2001). For example, the rate of dispersal and emigration of metapopulations of field voles *Microtus agrestis* (Linnaeus, 1761) (Rodentidae: Cricetidae) significantly increased when subjected to extinction and recolonisation (Roff and Fairbairn, 2001). The evolutionary increase in dispersal ability occurring in newly established populations is a partial consequence of trade-offs between dispersal and reproductive activities. Such evolutionary trade-offs were found to have a significant role in affecting the expansion rate in invasive species (Hill et al., 1999, Hughes et al., 2003). Furthermore, Reznick and Ghalambor (2001) demonstrated an association between adaptive phenotypic evolution, which may result in significant trade-offs, and the process of colonising a novel habitat. Trade-offs have played a definite role in the development of life-history theory (Stearns, 1989) and have been the subject of controversy (Reznick et al., 1986, Bell, 1986). The most obvious life-history trade-offs are those related to reproduction and future survival (Williams, 1966ab). Measuring the costs of reproduction on other traits was proven to be impractical, because both phenotypic and genetic correlations must be determined in advance in the natural environmental range to enable one to draw a conclusion (Stearns, 1989). The trade-offs between growth and reproduction in invasive plant species and how such trade-offs relate to population demographics have been studied and reviewed intensively (Lambrinos, 2004, Colautti et al., 2010ab). Invasive insects, however, have not received the attention commensurate with their importance.

Of fundamental importance to invasion and the spread of an organism are the temporal patterns of reproduction and survival, which are considered the main make-up of an individual's lifecycle (Juliano and Lounibos, 2005). The trade-offs in life-history traits of invasive species are expected to participate in accelerating invasiveness. According to Bell (1980), in many instances the trade-offs that manifest as costs of reproduction are reduction in adult longevity and future reproductive potential. In addition, some trade-offs are likely to occur within the reproductive output, such as the negative relationships between fecundity and investment per offspring (Bell, 1980, Roff, 2002). For example, large females of the recolonised population of the Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae), from North America (i.e., associated with the extreme winter climate) died sooner after their last oviposition when compared with the southern population (Leisenham et al., 2008). Nevertheless, there were also pronounced inter-populational variations in the survival and reproductive schedules of the North American population. Reznick (1985) noted that it is highly important to consider the trade-offs resulting from reproductive traits, because they are essential in predicting the optimal life-history mode in a given environment.

Range expansion may greatly affect the evolution of life-history traits, owing to the varying selection pressures (Burton et al., 2010). Dispersal and reproduction traits that favour expansion are often selected over other traits, such as competitive ability (Burton et al., 2010). A study was conducted to compare the reproductive potentials of the large invasive lady beetle, *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae), with four smaller native North American species that have been displaced from alfalfa fields in Utah (Kajita and Evans, 2010). The results revealed that the rapid predominance of the invasive population of *C. septempunctata* was mainly due to the greater investment in reproduction and reproductive rate. This has great implications for understanding both the rate of spread of invasive species and the range of native species' displacement in response to climate change. Nylin (2001) noted that there are only a few studies that deal with various life-history traits and trade-offs associated with colonising a novel or heterogeneous environment. Additional studies are therefore required to demonstrate how individuals allocate resources in response to fluctuating environmental conditions and the presence of natural enemies, in order to understand the drivers of population dynamics in invasive species (Nylin, 2001). Similarly, Forbes et al. (2010) claimed that better knowledge of the interaction between life-history traits in a specific environment can be used to optimize methodologies for prevention and control strategies.

1.6.1 Life-history traits

Nylin (2001) noted that 'life-history traits' can be best defined as those that quantitatively describe the transitions between different aspects of a species' lifecycle. These traits typically include the different aspects of reproduction, development, maintenance and survival. Thus, they are traits that contribute to the overall fitness of an organism (Tatar et al., 1993, Nylin, 2001). The most commonly recognised life-history traits are age and size at maturity, reproductive investment, reproductive period, ageing (Stearns, 2000) and those traits that determine demographic features of a population (Engelen and Santos, 2009). Other long-recognised life-history traits include adaptational strategies to diapause, parthenogenesis, hermaphroditism, protandry, migration and longevity (Cole, 1954, Tatar et al., 1993). All of the above-described traits are those that primarily ensure persistence and survival of a population under uncertain environmental conditions (Cole, 1954, Stearns, 1976, Nylin, 2001).

Life-history traits are often highly variable among geographic populations, both intra- and inter-specifically (Stearns, 1977, Partridge and Harvey, 1988). Several factors contribute to the divergence in life-history traits, such as random genetic drift or resource variability, which may evoke an increase in predation risk as well as competition (Mousseau and Roff, 1987, Spence, 1989). For example, regarding intraspecific variability, a comparison between two strains of seed beetle, *Callosobruchus maculates* (Fabricius, 1775) (Coleoptera: Bruchidae), exhibited significant variations in life-history traits, particularly fecundity, oviposition period, and egg and body sizes (Messina, 1991). Stearns (2000) suggested that to predict the evolution of a particular life-history trait, it is sufficient to consider its impact on both mortality rates and fecundity, since these two traits are the predominate ones that determine population dynamics and intrinsic growth.

Scientists are particularly interested in life-history traits, because these are the traits on which natural selection acts in order to maximise the fitness of an organism (Nylin, 2001). In other words, life-history traits are under strong directional selection and are expected to evolve rapidly unless opposed by physiological, genetic and or phylogenetic constraints (Nylin, 2001). In this regard, it has been recognised that phenotypic plasticity as a life-history trait is a very important factor in facilitating colonisation, as colonists must adapt and tolerate the new range of environmental conditions, but that other traits are also required for establishment (Sakai et al., 2001). Invasion theory has attempted to identify which life-history traits best explain the successful establishment and colonisation of a novel range (Engelen and Santos, 2009), whether reproduction mode, growth rate, sexual maturity, phenotypic plasticity, tolerance patterns or others (Baker, 1974). Predicting which traits are mainly associated with invasive species is, however, difficult since such traits may be altered as the invasion process proceeds (Engelen and Santos, 2009).

The importance of lag phases or slow population growth following introduction is a controversial subject. The extent of lag phases is dependent mainly on the aspects of the lifecycle of a species (Kowarik, 1995) and may be affected by the pattern of growth of a population (Parker, 2004). Thus, it is necessary to understand population characteristics in order to predict which species is the probable invader (Engelen and Santos, 2009, Kolar and Lodge, 2001). From the numerous models of population dynamics that have emerged, it has become clear that equal impacts on different life-history traits do not have equal consequences on population dynamics, either within a species or among species, depending on the species' lifecycle (Forbes, et al., 2010). The demographic importance of life-history traits can be defined as either the absolute 'sensitivity' or relative 'plasticity' change in population growth rate (De Kroon et al., 2000). Forbes et al. (2010) demonstrated that life-history traits that have a high sensitivity to chemical toxicants tend to have a low elasticity, meaning that changes in them have relatively less impact on population growth rate, in comparison with other traits. For example, it was found in their proposed model that fecundity was more sensitive to chemical stress and less elastic when compared with adult or juvenile survival. The above approach may provide guidance in selecting the most sensitive traits when estimating impacts on population dynamics. Likewise, Nylin (2001) pointed out the importance of having more studies investigating life-history traits, particularly those that would eventually describe which types of insects are most likely to become serious pests.

1.6.2 Phenotypic Plasticity

Many scientists have defined 'phenotypic plasticity' and most of these definitions are virtually identical (Whitman and Agrawal, 2009). Phenotypic plasticity refers to the capacity of a single genotype to consecutively express a range of discrete phenotypes as a function of unexpected environmental conditions during ontogeny (Shapiro, 1976, West-Eberhart, 1989, Nijhout, 1999, Debat and David, 2001, Fordyce, 2006). The set of phenotypes produced in a specific set of environments is referred to as 'the norm of reaction' of the genotype (Stearns and Koella, 1986). The direction and degree of responses to environmental factors is genetically variable and known to respond to natural selection (Gupta and Lewontin, 1982). Plasticity is by itself a trait subjected to natural selection and evolutionary change (Williams, 1966ab, Stearns, 1983, Sultan, 1987). Thus, it is a mistake to consider phenotypic plasticity a non-genetic phenomenon (West-Eberhard, 1989).

The concept of phenotypic plasticity was first applied to morphological traits (Schlichting and Pigliucci, 1998). It is important, however, to realise that phenotypic plasticity is not just restricted to morphology. Organisms may reveal plasticity in physiological, behavioural and developmental aspects of their life-history traits (Agarwala, 2007). As all phenotypic traits result from the joint effects of biochemical and physiological processes, changes in any phenotypic trait are virtually always labelled as a consequence of physiological influences exclusively (Whitman and Agrawal, 2009). Examples of phenotypic plasticity are many, such as, alternative reproductive tactics that have been described in many insect orders, in which individuals switch facultatively between different behaviours to acquire mates (Gadgil, 1972, Thornhill and Alcock, 1983, Shuster and Wade, 2003), as well as foraging, feeding or provisioning behaviour (Moczek, 1998, Moczek, 1999). Some patterns of phenotypic plasticity expression are mediated by the joint contributions of genetic and environmental factors, such as the regulation of appendage growth and development (Abouheif and Wray, 2002), or the expression of seasonal morphs in butterflies that encompass different behavioural plasticity (Brakefield and Reitsma, 1991).

Using the optimality model is, however, sometimes incorrect when it comes to phenotypic plasticity for several reasons (Sultan, 1995). First, not all phenotypic plasticity is adaptive - some traits are plastic due to the constraints imposed by the biochemistry, physiology or developmental features of an organism. Second, the degree and expression of plasticity show variable reversibility, for instance, biochemical and physiological responses can be reversed over a short period of time, whereas developmental and morphological responses tend to be irreversible, or in some cases take a very long time. Third, the pattern and degree of plasticity are trait-specific, depending on a particular set of environmental cues (Pigliucci et al., 2006). Parker and Smith (1990) noted that optimisation is not a universal rule; it helps to improve understanding of adaptations, rather than producing optimal solutions. Thus, it is important to distinguish between the specific and general model - the former is applied to collect quantitative data on a target species, but the latter provides qualitative range and solutions for a number of common biological problems (Parker and Smith, 1990).

In the absence of the environment cues, a given phenotype may sometimes disappear from a population and be replaced by an alternate phenotype, which becomes the subject of selection (West-Eberhard, 1989, West-Eberhard, 2003). Waddington (1953) introduced the word 'assimilation' to refer to this phenomenon. In such cases, the evolution and developmental switch mechanisms are thought to play a central role in mediating the diversification of alternative phenotypes (Nijhout, 1999). An explicit example is those developmental switch mechanisms in insects that are regulated by hormonal activities, such as switching for determining the timing of pupation in butterflies (Nijhout, 1976), or the expression of alternative male phenotypes in horned beetles (Moczek and Nijhout, 2002ab). The occurrence of a phenotype with altered or decreased responsiveness to environmental conditions (i.e., self regulatory mechanism) is also known as 'canalisation' (Waddington, 1956, Etges, 1989) or developmental homeostasis (i.e., a flat fitness reaction norm resulted from absolute adaptive phenotypic plasticity) (Waddington, 1956, Richards et al., 2006). Canalisation, which is commonly used in the evolution of phenotypic plasticity studies, generally results from selection in favour of reaction norms that counteract environmental cues (Siegal and Bergman, 2002). Alternatively, as viewed by Wilkins (1986), it is the stabilisation of developmental pathways by multiple genetic factors within the genome.

Conover and Schultz (1995) noted that plasticity is often under conflicting selective pressures, depending on the environmental conditions. If the selection is upwards in bad environments, it is known as 'counter-gradient selection'. For example, Levins (1969ab) described the counter-gradient variation in body size of *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae) with respect to altitude and temperature gradients. Flies from a cold high-altitude site were found to be larger in size than those from a warmer low-altitude set. The downwards selection in good environments is usually known as 'antagonistic selection' (Falconer, 1990). When antagonistic selection occurs in both directions, it is known as 'stabilising selection' (Falconer, 1990, Bradford and Roff, 1995), and the reverse of antagonistic selection is synergistic or co-gradient selection (Falconer, 1990). This subject is explained explicitly in Trussell (2000).

An accurate description of population dynamics requires knowledge of how phenotypic plasticity works (Beckerman et al., 2002, Benton et al., 2006), as it plays an important role in describing the origin and subsequent diversification of both morphological and behavioural traits (West-Eberhard, 1989). Some authors have used inconsistent terms when pointing out the different aspects of phenotypic plasticity, such as adaptive and non-adaptive, active and passive, reversible and irreversible, etc. (Nylin and Gotthard, 1998). Moczek (2010) argued, ultimately, all of the different terms that have been used to describe phenotypic plasticity belong to the same fundamental property of an organism, its ability to respond to environmental cues by changing the expression of a phenotype. For instance, it was noted that 'beneficial plasticity' should include all such changes related to adaptive phenotypes - such as, acclimatisation, diapause, life-history shifts, dispersal (Mousseau and Roff, 1987, West-Eberhard, 2003, Lyytinen et al., 2004, Schmid-Hempel, 2005), seasonal colour and wing polyphenisms in butterflies (Brakefield and Larsen, 1984, Nylin et al., 2005), plasticity in reproductive performance (Wessels et al., 2010), reproductive phases in aphids (Le Trionnaire, 2008), alternative male morphologies in horned beetles (Moczek and Emlen, 2000, Moczek and Nagy, 2005), and anti-predator mediated plasticity (Dixon and Agarwala, 1999). 'Non-adaptive plasticity' includes various susceptibilities to potential abiotic factors, as well as manipulation in hosts that have been parasitised (Roy et al., 2006, Kenyon and Hunter 2007, Poinar and Yanoviak, 2008).

Adaptive plasticity is of interest to evolutionists as it represents the production of diversity through adaptive responses whose environmental sensitivity is subjected to natural selection (West-Eberhard, 1989). An organism has the ability to compare its intrinsic conditions relative to extrinsic cues by altering its bio-physiological regulatory mechanisms (Juliano, 2004). Adaptive behavioural plasticity evolves more readily than adaptive morphological plasticity, because of the availability of more potential factors regulating the expression of an immediate response (Carson, 1978). Thus, it is expected that behaviour patterns must be established first, followed by morphological improvement, which usually takes a long time (Plotkin, 1988ab). In addition, numerous studies have shown that both the nature and the magnitude of plastic responses can evolve independently of other phenotypes, leading to evolutionary diversification between populations and species. For example, families of cabbage-white butterflies, *Pieris rapae* (Linnaeus, 1758) (Lepidoptera: Pieridae), showed a heritable variation for adjusting host-plant preferences (Snell-Rood and Papaj, 2009). Fordyce and Muizon (2001) noted that plasticity carries numerous costs and trade-offs and some have argued that it is almost impossible to ever estimate the total ratio of costs to benefits.

All biological processes exhibit some degree of plasticity response, in that their function is influenced by external conditions (Mousseau and Roff, 1987, West-Eberhard, 2003). Importantly, such responses involve adjustments on not just one but many levels of biological organisation (Gilbert, 2001, West-Eberhard, 2003). Organisms are not infinitely plastic, but are limited in their responses towards a variable environment (Moczek, 2010). Even in the most extreme cases, such as polyphenism's expression of alternative phenotypes, it is often restricted to two morphs only (West-Eberhard, 2003). Despite the effect of environmental input, some cases, such as the physiological or reproductive functions, typically face regulatory mechanisms or constraints that may act to limit the degree of their plasticity expression (Ricklefs and Wikelski, 2002, Hatle et al., 2004). For example, the fixed allocation patterns in insects for reproduction, which suggests their reproductive plasticity, is greatly constrained by regulatory mechanisms activated by meeting the minimal nutritional threshold (Ricklefs and Wikelski, 2002). This, however, cannot necessarily be the general rule (Wessels et al., 2010). Stearns and Koella (1986) suggested that in order to make predictions or even to interpret observed patterns and generalise on other organisms, models of fitness are needed. In addition, discerning adaptive plasticity from non-adaptive ones is sometimes a difficult issue and may need a thorough evaluation of many life-history traits for different species (Reznick et al., 1990, Ghalambor, 2007).

Phenotypic plasticity has long been suggested to facilitate biological invasions (Daehler, 2003, Pysek and Richardson, 2007). Ghalambor et al. (2007) pointed out that persistence and colonising in a variable environment, whether temporal or spatial, is likely to be facilitated by phenotypic plasticity. It is more appropriate to consider adaptive phenotypic plasticity when describing the invader strategies in a novel range, because this pattern of plasticity may lead to a predisposition to enhance physiological and reproductive functions and maximise individual fitness, unlike phenotypic plasticity (Sultan, 2003). A growing number of studies have now shown that adaptive plasticity allows for the acclimation of invasive genotypes to varying environments and buffers the existing genetic variation from selection, leading to phenotypic homeostasis or increasing the degree of tolerance (West-Eberhard, 1989, Rejmanek, 2000, Alpert and Simms, 2002, Sultan, 2003, Boggs, 2009). On the introduction of target species, both short- and long-term phenotypic plasticity responses can be accounted for as important mechanisms that enhance individual chances of survival (Chown and Terblanche, 2007, Whitman and Ananthakrishnan, 2009), and have been demonstrated to be significant components of field fitness in insects (Kristensen et al., 2008). For example, Nyamukondiwa et al. (2010) compared the widely distributed Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), with the narrowly distributed congener, *Ceratitis rosa* (Karsch, 1887) (Diptera: Tephritidae). The comparisons revealed that both species differed in the time-course of plastic responses to cold hardening.

It was addressed previously that during invasiveness there is an increase in the levels of plasticity in invasive species (Lombaert et al., 2007), since they are expected to predispose adaptation to the wide range of environments encountered (West-Eberhard, 2003, Richardson and Pysek, 2006, Chown et al., 2007, Slabber et al., 2007). It was reported that after the target species have been released, they tend to evolve higher levels of adaptive phenotypic plasticity gradually (Richards et al., 2006, Van Kleunen et al., 2008), which can result in remarkable quantitative genetic variations between native and invasive species (Facon et al., 2006, Lee et al., 2002) (explained explicitly in the above section on invasion), For instance, a study demonstrated that an invasive population of *H. axyridis* displayed significantly different plastic responses to temperature variations and showed a higher survival rate when compared with the population used as biological control (Lombaert et al., 2007). In another related example, the invasive *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) showed rapid adaptive evolution of the photoperiodic response during invasion and range expansion across latitude in the United States. The above results emphasise the importance of photoperiodism as a basic key for frequent adaptation to spatial and temporal environmental variation and climatic warming. In addition, it basically elucidates the underlying physiological bases of photoperiod in regulating seasonal development that could be incorporated in invasive-related studies (Bradshaw and Holzapfel, 2001ab, Bradshaw and Holzapfel, 2006).

Richards et al. (2006) noted that generalising the plasticity term for invasive species remains limited. It is therefore premature to draw any firm conclusions based on the above results or any other similar emerged findings, since plastic responses may differ according to external cues and be trait-specific. On another level, it has been reported that the trait to be measured and the environments to be tested must be chosen with caution when attempting to detect the variation in the adaptive phenotypic plasticity among invading populations (Richards et al., 2006). Determining the plasticity of a specific trait can be accomplished by several approaches, rather than simply quantifying degree of phenotypic plasticity in invasive species (Pigliucci, 2001, Richards et al., 2006, Hulme, 2008). Additional approaches must be taken in order to better understand the role of phenotypic plasticity in geographic distribution, colonisation success and persistence in the introduced range. These issues must incorporate measures of invasive species' performance and behaviour, rather than crude differences in individual traits across an environmental gradient (i.e., plasticity is often a comparative rather than an absolute measure, unlike many other life-history traits) (Hulme, 2008). Richards et al. (2006) stressed that both the evolutionary and ecological aspects of the invader species should be integrated, since they have led to highly fruitful conclusions when interpreting the plasticity in invasive species.

1.7 Body size

One of the most important life history characters of an organism is body size which is considered to be a key determinant of organism's morphology, physiology, behaviour, and eventually fitness (Kingsolver and Huey, 2008). Body size is one of the characters of organisms that varies continuously due to natural selection, resource acquisition and rearing conditions (Chown and Gaston, 2010). The variation in body size has long been of central attention to macroecologists (Brown, 1989, Olson et al., 2009). Many empirical approaches have been adopted to understand the distribution of organisms at large spatial and temporal scales (Blackburn and Gaston, 2003, Smith et al., 2004, Chown and Gaston, 2010). The evolution and the distribution of body size is not the subject of the present study and it is well presented in large and growing literatures such as the review by Blackburn and Gaston (2003), Kingsolver and Pfennig (2004), Mirth and Riddiford (2007), Clauset and Erwin (2008) and Chown and Gaston (2010). It was noted that without some comprehension and consideration of the physiological and biochemical basis that determine body size in insects and life-history consequences of size variation, the large-scale patterns of body size variation cannot be understood (Mirth and Riddiford, 2007, Chown and Gaston, 2010). Therefore, the following sections were assigned to highlight some of the important regulatory mechanisms and hypotheses underlying the evolved variations in body size.

Several studies have focused on the regulatory mechanisms determining body size in holometabolous insects (Nijhout, 2003, Emlen and Allen, 2004, Nijhout et al., 2006, Mirth and Riddiford, 2007) such as *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae) (Nijhout, 2003) and the tobacco hornworm, *Manduca sexta* (Linnaeus, 1763)

(Lepidoptera: Sphingidae) (Safranek and Riddiford, 1975). The regulatory mechanisms in these two models were of controversy. Although the physiological mechanisms in those insects were presumably generalized in their most significant aspects, the extent to which they operated at the cellular level has not yet been fully verified for generalization on other species (Nijhout, 2003, Parker and Johnston 2006). The most significant developmental stage that has a great influence on the final body size in Lepidopterans (i.e., that is applied to Coleopterans as well) is the larval mass at which the juvenile hormone (JH) secretion ceases (Nijhout and Williams, 1974ab) due to the action of JH esterase secretion (Roe et al., 1993, Browder et al., 2001). The weight at this stage is referred to as the critical weight (Nijhout et al., 2006). Once the JH falls below a certain threshold, the prothoracic gland (PG) will start secreting prothoracicotrophic hormone (PTTH) and ecdysteriods that are already inhabited by JH (Nijhout and Williams, 1974b). The hormone PTTH is secreted during a photoperiodic gate which is controlled by a photoperiodic clock (Nijhout et al., 2006). The subsequent increases in ecdysteroid levels will ultimately cause the cessation of feeding, and initiation of pre-pupal stage (Nijhout and Williams, 1974ab, Browder et al., 2001).The hormonal regulation of growth cessation in other holometabolous insects are likely similar to those of the lepidopteran (Chown and Gaston, 2010). This is not however, the case in *D. melanogaster* (Edgar, 2006). Mirth and Riddiford (2007) believe that the sequence of endocrine events leading up to critical weight is much better defined in *M. sexta* than they are in *D. melanogaster*.

The critical weight has been the focus of many studies particularly in *M. sexta* as reviewed by Nijhout (2003). The critical weight defined as the minimal weight in which further feeding and growth are not required for a time period to metamorphosis and pupation (Nijhout and Williams, 1974a). Others defined the critical weight as the minimal size at which transient starvation no longer delays metamorphosis (Beadle et al., 1938). The time period between attaining the critical weight and the secretion of PTTH (i. e., full clearance of JH) is of considerable significance in the context of adult size, and is referred to as the interval to the cessation of growth (ICG) (Davidowitz et al., 2004) or the terminal growth period (TGP) (Shingleton et al., 2007). D'Amico et al. (2001) summarized the three important physiological bases that play a major role in regulating body size in *M. sexta*, which are the critical weight, TGP and rate of growth. The quantitative change in these three factors was shown to account for over 95% of the evolutionary change in body size (D'Amico et al. 2001).

Body size regulation is a function of a species-specific critical weight (Nijhout and Williams, 1974ab, Nijhout, 2003, Mirth and Riddiford, 2007). The question that should be addressed in this contetxt is how does an insect assess their critical size achievement and stop growing? Studies on the fruit fly *D. melanogaster* revealed that insulin and Target of Rapamycin (TOR) pathways are the main factor regulating nutrition-dependent growth rates (Oldham and Hafen, 2003, Mirth and Riddiford, 2007). It was noted that TOR responds to ATP and amino acids levels in the haemolymph and is highly sensitive to oxygen levels (Harrison et al., 2006). Insulin-signalling pathways play the key mediator between available nutrients and the growth of internal organs such as the imaginal disc (Nijhout, 2003). An additional support evidence that the growth modulation is mediated by insulin-signalling pathways comes from experiments on the lepidopteron, *Precis coenia* (Hübner, 1822) (Lepidoptera: Nymphalidae) (Miner et al., 2000, Nijhout and Grunert, 2002). Basically, there are three mechanisms identified to regulate the critical size including insulin/insulin-like growth factor signalling (IIS) (Brogiolo et al., 1998, Rulifson et al., 2002, Shingleton et al., 2005), the production of PTTH by neurosecretory cells in the brain, and growth of the imaginal disc (Caldwell et al., 2005, McBrayer et al., 2007, Rewitz et al., 2009). There are a number of checkpoints for size assessment that determine whether the larva is fully prepared to proceed to the subsequent stage, or when to stop growing in order to enter metamorphosis on time first, the metamorphosis threshold size (Nijhout, 1975, Zhou et al., 2004). In *M. sexta*, failure to cross this threshold results in a supernumerary larval instar (Nijhout, 1975). Second, the minimal viable weight is defined as the minimal weight or the amount of body stored nutrients that are sufficient for a larva to survive metamorphosis period (Nijhout, 1975). Third, surpassing the critical weight that guarantees the entry into metamorphosis can no longer be delayed by starvation (Nijhout, 2003, Nijhout and Williams, 1974ab). It was noted in *Drosophila* starvation of larvae after attaining their critical weight accelerated the onset of pupation (Beadle et al., 1938, Mirth and Riddiford, 2005). Starved larvae of *Manduca* at post-critical weight on the other hand, had no effect on metamorphosis initiation time (Nijhout and Williams, 1974ab).

It was noted that after attaining the critical weight and larvae stopped feeding, the imaginal discs continue to grow until the beginning of the pupal stage. Thereafter, the higher levels of ecdysteroids titre cause the imaginal discs to stop growing and differentiate into their respective adult structures (Champlin and Truman, 1998ab). Thus, the critical weight has an important role in controlling the final size of larval instars since it has a linear relation with the initial larval weight. In addition, it marks the initiation of important physiological and morphological processes (Davidowitz et al., 2003).Thus, the size that a larva attains at the time of metamorphosis defines the final adult body size eventually. Nijhout et al. (2006) noted that the initial mass of each larval instar is a constant multiple of that of the previous instar which is more generally known as Dyar’s constant or rule and the weight gain at the last instar has a great influence on the final body size. Premature metamorphosis in response to starvation conditions has also been reported in some insects. Food depletion has been shown to accelerate time to metamorphosis in the dung beetle, *Onthophagus taurus* (Schreber, 1759) (Coleoptera, Scarabaeidae) (Shafiei et al., 2001) and in the tree hole mosquito, *Aedes triseriatus* (Say, 1823) (Diptera: Culicidae) (Carpenter, 1983). The role of hormonal activities during starvation is explained in detailed by (Truman and Riddiford, 1974, Gilbert, 2002). More remains however, to be understood about how critical weight is assessed and how adult size is finally determined during starvation in particular (Edgar, 2006, Mirth and Riddiford, 2007, Shingleton et al., 2008).

Ecological factors such as seasonal variation result in intraspecific variations in body size (Chown and Gaston, 2010). It was reported that nutrition condition, population density as well as temperature determine the rate and duration of larval growth (Mirth and Riddiford, 2007). Increased temperature or larval density, or decreased nutritional quality or quantity often results in smaller adult size (Lazebnyi et al., 1996). The resultant smaller body size is presumed to have important consequences on the life-history attributes such as longevity, reproductive rate and resource use (Partridge and Farquhar, 1983, Dixon and Hemptinne, 2001, Khazaeli et al., 2005). For example, temperature was found to have a great influence on the developmental rate and body size in some species such as *Drosophila* spp. (Kari and Huey, 2000), beetles and butterflies (Ernsting and Isaaks, 1997). Other ecological factors such as changes in vegetation structure, transformation of landscapes caused by invasive alien species have substantial impacts on assemblage body size distributions (Steenkamp and Chown, 1996, Coetzeeet al., 2007). Basically, large species are usually lost from the assemblage, probably as a consequence of flight impairment by dense vegetation and a change in resource availability (Steenkamp and Chown, 1996, Coetzee et al., 2007). In contrast, habitat fragmentation and extinction risk has no direct effect on size-related variation in population abundance and growth rate (Davies et al., 2000). For instance, a study showed no relationship between body sizes in ground-dwelling beetles as a consequence of fragmentation (Davies et al., 2000). Nijhout et al. (2006) noted it is important to consider adult feeding habit, nutritional quantity and quality when studying correlation between body size and life- history allometry since they may result in additional weight gain even though adult linear dimensions are fixed.

Many life-history theories consider body size to be a continuously changing trait depending on several of factors functioning at different life stages and their integration (Roff, 2002, Bede et al., 2007). The relationship between body size and life-history characters has been long recognized since both share the same regulatory mechanisms including genetic, physiological and developmental mechanisms (Stearns, 1992). Literarily, any change in body size will have consequences on other life-history traits, and vice-versa in order to bias fitness ultimately (Stearns, 1992, Roff, 2002). The benefit of having a large body size has been widely studied (Honek, 1993, Parker and Simmons, 1996, Rivero and West, 2002). It plays an important function in maximizing reproduction, fecundity and longevity (Gross, 1981, Stearns and Koella, 1986, Roff, 1988). In most organisms, fecundity selection in females and sexual selection in males are the major evolutionary selective forces for larger body size (Honek, 1993, Blanckenhorn, 2000). These benefits may be affected by resource acquisition and allocation such as lipid reserve (Leather, 1988, Kemp and Alcock, 2003). Adults may suffer size-dependent costs such as costs of fast growth or longer developmental period (Roff, 1980), decreased reproductive success due to late reproduction (Blanckenhorn, 2000), or it could be disadvantage due to the high absolute energy requirements for maintenance (Blanckenhorn, 2005). Large sizes may incur energetic costs particularly under limited resources (Reim et al., 2006). Life-history costs of large size may be difficult to estimate because they only become detectable under stress (Reim et al., 2006, Teuschl et al., 2007). Despite the above disadvantages as a consequence of having a large body size, there are well recognized benefits (Reim et al., 2006) for instance, possessing high energy efficiency for foraging, mating success, off-spring provision and competition (Reim et al., 2006). The benefits of small size selection in poor conditions however, are attributed to reduced costs of growth, maintenance energy and reproduction as well as an increase in competition ability (Blanckenhorn, 2000, Blanckenhorn, 2005). The cost of having large or small bodies was discussed in detail in Blanckenhorn (2000) and (2005). Small individuals may survive and reproduce better under food stress because they need less nutrients to sustain themselves (Blanckenhorn et al., 1994) such as the milkweed bug, *Oncopeltus fasciatus* (Dallas, 1852) (Hemiptera: Lygaeidae) (Dingle, 1992). Solbreck et al. (1989) found that body size and mass are typically strongly correlated in the black and red bug, *Lygaeus equestris* (Linnaeus, 1758) (Hemiptera: Lygaeidae) (Solbreck et al., 1989), but this correlation may not always be the general case, and there are situations when it is important to decide which is best used as a potential fitness correlate (Nylin and Gotthard, 1998). In contrast, Ohgushi (1996) pointed out that larger insects may also survive better in the absence of food such as during hibernation or drought conditions particularly if body size is correlated with nutrient reserves (Ohgushi, 1996) such as in African dung beetle, *Scarabaeus zambesianus* (Peringuey, 1901) (Coleoptera: Scarabaeidae) (Chown et al., 1995). A key conclusion is that when dealing with insects, accepting that females with large bodies will be able maximize other traits such as reproductive output compared to small sized females might not be correct, unless a detailed knowledge of other factor affecting this fecundity is known (Leather, 1988).

Knowledge of the distribution of body sizes among taxa might help Nijhout et al. (2006) to explain the ways in which resources are partitioned among species and to understand the composition of animal assemblages (Pagel et al., 1991, Blackburn and Gaston, 1994). Blackburn and Gaston (1994) noted that at large scale body size distribution of a wide range of plants, herbivores, and carnivores are right skewed. The size diversity curves for ladybirds are not all right skewed and vary significantly in shape both with-in and between biogeographic regions (Hall and Raffaelli, 1993, Begon et al., 1996, Dixon and Hemptinne, 2001). For instance, the form of the body size distributions of predatory ladybird beetles differs among countries with that for Japan significantly right skewed and that for Central Europe significantly left skewed (Dixon and Hemptinne, 2001). It was argued whether to explain the variation in body size distribution based on physiological or ecological constraints. In the case of predatory ladybirds the shape of their body size distribution curves is determined by the nature and the relative abundance of their prey, that is, by ecological rather than physiological constraints (Dixon and Hemptinne, 2001). Witting (1998) suggested more attention should be given to looking for the generality in terms of ecological constraints. It was noted, in spite of the numerous studies on the factors influencing the final adult size in insects, body size frequency distributions have been poorly documented (Loder et al., 1997). They further suggested that there are many factors limiting the interpretation of body size frequency distributions that have not been yet considered, such as sample size, class size and class width.

There are two patterns of spatial variation in body size associated with latitudinal and altitudinal inclines (Chown and Gaston, 1999, Blanckenhorn and Demont, 2004, Dillon et al., 2006). It was reported that the body size increases with both latitude and altitude and this phenomenon is often referred to as Bergmann’s rule (Chown and Gaston, 1999, Blanckenhorn and Demont, 2004). The proximate underlying factors associated with this phenomenon remain controversial (Chown and Gaston, 2010). In this regard, several hypotheses have been addressed in order to understand this phenomenon such as the influence of changes in temperature, variation in oxygen level and the ability of an individual to resist, tolerate and grow under various types of stress (Chown and Gaston, 2010). Despite the complexity of this debate, latitudinal and altitudinal gradients were reported to have also an impact on body size variation with an established genetic basis. It should be noted however, that that Bergman’s rule is not always the general case since some species have shown a negative correlation between body size and latitude (Mousseau, 1997). Walters and Hassall (2006) noted plasticity response in adult size is mainly determined by the relative difference between the minimum temperature thresholds for growth and development rates. Chown (2001) demonstrated despite the apparent plasticity there is a considerable phylogenetic constraint in the evolution of these responses to environmental cues.

Seasonal variation in the temperature, photoperiod and resources variables, in temperate regions, have shown pronounced influences on the evolution of growth rate and patterns, the optimal age and size at maturation and body size (Gotthard, 2004, Davidowitz and Nijhout, 2004). Environmental cues may also constrain the potential increase in growth rate or developmental period in a way that does not always result in an increase in body size depending on insect species (Esperk et al., 2007). Etilé and Despland (2008) reported that the constraints on the relationship between final size and age at maturity vary between species. There might be compensation for body size attained by increasing the rate of growth, this might be however, result in a reduction in body mass (Strobbe and Stoks, 2004). Therefore, it is important to discriminate well between body size and mass when testing life history responses under stress and when analysing some of the models’ predictions.

Environmental abiotic factors can induce wide morphological, physiological and behavioral modifications through natural selection (Dobzhansky et al., 1977, Ricklefs, 1990). Body parameters including size, shape and surface area are important characters in regulating body temperature which plays a substantial role in determining feeding activity in ladybeetles (Willmer, 1982, Hodek and Honek, 1996). The forms of colouration, melaninc or non-melanic phenotypes, are likely to be important regulator for body temperature and lady beetle activity (Brakefield and Willmer, 1985, De Jong et al., 1996). Melanic phenotypes of the ladybird *A. bipunctata* showed lower reflectance than non-melanic phenotypes that would result in higher temperature excess, faster warming-up, faster walking speed and less mean time to activity (Brakefield and Willmer, 1985, De Jong et al., 1996). Melanic phenotypes of *H. axyridis* occur especially in boreal forests, likely reflecting their adaptive advantage in colder climates (Koch et al., 2006). A previous study has evaluated the differences in the light-coloured *aulica* and dark-coloured *nigra* phenotypes of *H. axyridis*. It was revealed that the optimum temperature for *nigra* adults was higher than for *aulica* phenotype at low ambient temperature resulting in increased activity, predation, developmental rate, reproduction and eventually fitness (Brakefield, 1984, Stewart and Dixon, 1989). In addition, larval body size and shape varied between the two phenotypes (Soares et al., 2003). A study on *D. melanogaster* showed a variation in body size between the laboratory reared adults and the wild type collected from the field (David et al., 1997). A field study on the same species showed that various changes in body size within the wild type adults occurred due to food availability, temperature and oxygen tension variations (Frazier et al., 2001). For example, they noted that high temperature reduced oxygen delivery to the tissue relative to tissue needs and resulted in smaller body sizes.

Phenotypic plasticity may enhance colonizing ability. Indeed, phenotypic plasticity in development time and adult size is apparent in invasive populations of *H. axyridis* (Grill et al., 1997). Invasive species are often characterised by having large body size (Roy et al., 2001, Roy et al., 2002, Brown and Sibly, 2006). Although the factors that determine the size of organisms need more investigation, their size is ultimately associated with other life attributes such as reproduction, longevity and resource use (Harvey and Pagel, 1991, Dixon and Hemptinne, 2001). Large body size may also promote success of invasive species (Roy et al., 2002). The link between large body size and greater fecundity has been suggested as a possible key factor promoting invasion particularly in lady beetles (Stewart et al., 1991ab, Dixon, 2000). The invasive success of *C. septempunctata* (Evans, 2000, Hodek and Michaud, 2008, Kajita and Evans, 2010) and *H. axyridis* (Koch, 2003, Roy et al., 2006) may be linked to their large size, which in turn is associated with high potential reproductive rate and dispersal ability that promote their rapid spread through new geographic regions. Brown and Sibly (2006) noted that the reproductive advantage was expressed in the larger number of ovarioles, the greater numbers of eggs produced, and the greater volume of eggs laid per day by invasive females compared with the smaller females of native species. As discussed earlier the body size in the invasive slug, *Arion lusitanicus* (Mabille, 1868) (Gastropoda: Pulmonata) played a significant role in its dispersal and colonisation of cereal crops (Honek and Martinkova, 2011). Genetically based polymorphism may be also important in enabling invasive species to exploit multiple habitats and micro-niches (Soares et al., 2005, Michie et al., 2010). Different phenotypes may differ also in several characteristics such as voracity, longevity, and reproductive capacity (Soares et al., 2001). Polymorphism of the indigenous populations of species *H. axyridis* occurs also in Europe, and may promote its invasion and establishment (Majerus et al., 2006, Brown et al., 2008 ab, Adriaens et al., 2008, Evans et al., 2011).

1.8 Allometry and relative growth

The term 'allometry' was coined in 1936 (Huxley and Tessier, 1936), and is applied to the phenomenon of relative growth (Huxley and Tessier, 1936). Allometry literally means "different measure" and originally referred to the scaling relationship between the size of a body's appendages - such as the head, thorax, abdomen, antennae, wings and legs - and the body as a whole during the developmental stages (Bonduriansky and Day, 2003, Shingleton, 2010). More recently, the term has taken on a broader meaning and is used to refer to the allometric relationships that can be described for almost any co-varying biological measurements, such as those between the size or function of one trait and the size of another trait or the body as a whole (Shingleton et al., 2008) - for example, morphological traits (i.e., the relationship between brain size and body size in humans), physiological traits (i.e., the relationship between metabolic rate and body size) or ecological traits (i.e., the relationship between wing size and flight performance).

The allometric relationship is usually modelled using the allometric equation ($y=bx^a$). A log transformation of this equation produces a simple linear equation ($\log y = a \log x + \log b$) where x and y are the two measured traits, respectively (Huxley and Tessier, 1936, Huxley, 1924), $\log b$ is the intercept of the line on the y-axis and a is the slope of the line, also referred to as the 'allometric coefficient' (Huxley and Tessier, 1936, Huxley, 1924). If the relative size of the two traits remains constant irrespective of absolute size, the allometric coefficient will be around 1, a condition usually called 'isometry' (Shingleton et al., 2008). In other cases, when the organ is growing at a faster rate compared with the body as a whole, the slope may be less than 1 - this is called 'positive allometry' or 'hyper-allometry'. When the organ has a slower growth rate than the body as a whole, it is called a 'negative allometry' or 'hypo-allometry' (Emlen and Nijhout, 2000).

Differences in the relative growth of body parts (i.e., allometric growth), rather than the absolute growth, can account for much intraspecific variation and diversity among taxa (Nijhout and Wheeler, 1996, Shingleton, 2010). To date, there are three different phenomena of allometry - ontogenetic, phylogenetic and individual or static allometry (Cock, 1966). These three levels are usually tightly interrelated (Rieppel, 1990). Any evolutionary change depends on the heritable static variation of morphological traits in various life-history stages, as produced by ontogenetic variation (Rieppel, 1990, Stearns, 1992).

When allometry is measured in different individuals at the same developmental stage within a population or conspecific individuals, the relationship is called 'static allometry' (Shingleton et al., 2007) or referred to as the 'scaling relationship' (Huxley, 1932, D'Arcy Thompson, 1942, Emlen and Nijhout, 2000). In static allometry, variation in growth may occur due to genetic or environmental factors, or the interaction between the two (Via, 1984). In most cases, variation in static allometry of traits is a consequence of the nutritional status experienced during development, therefore these variations are important components for the evolution of phenotypic diversity (Shingleton et al., 2007, 2008).

Nutrient quality or quantity is considered a major regulator of body and organ size in animals (Oldham et al., 2000) and so the mechanisms that control the developmental response to nutrition probably determine many of the static allometries observed in nature (Shingleton et al., 2008). It was hypothesised by Shingleton et al. (2008) that in order to maintain correct allometric scaling, sensitivity to any alteration in nutrition must vary among and within organs during development time. It was then suggested by Shingleton et al. (2008) that the variable insulin-sensitivity, duration of TGP and attaining the critical weight may all eventually influence different aspects of an organ's static allometry in holometabolous insects.

The majority of allometric relationships are straight lines; there may be, however, sigmoidal or discontinuous allometries (in which case they are referred to as non-linear allometry or complex allometry) (Nijhout and Wheeler, 1996), depending on the measured trait, the unit of measurement or the species (Emlen and Nijhout, 2000). Complex allometry is associated with the polyphenism phenomenon, whereby individuals develop into two or more morphs, depending on the environmental cues or their genetic makeup (Eberhard and Gutierrez, 1991, Emlen and Nijhout, 2000). Examples of polyphenism include the alternative mating tactics or dimorphisms in horn size displayed by many species of beetle (Knell, 2009). Some allometric relationships, such as the curvilinear and discontinuous allometries, may be explained in part by interactions among imaginal tissues of metamorphosing holometabolous insects (Wheeler, 1991, Emlen, 1994, Nijhout and Wheeler, 1996), particularly during competition for limited resources (Nijhout and Wheeler, 1996, Knell et al., 2004). One further allometric concept is 'ontogenetic allometry', which is defined as the growth trajectory of an organ relative to body size during the growth of a single individual; when the allometry is measured between organs across species, the relationship is referred as evolutionary or phylogenetic allometry (Stern and Emlen, 1999).

Shingleton (2010) pointed out that the study of allometry is very important, since it highlights the functional mechanisms that generate scaling relationships, how they impact ecology and how they respond to and influence evolution. The distinction between imaginal and larval tissues highlights one important fact in understanding allometry - the implication that different body parts have a variable growth pattern (Nijhout and Wheeler, 1996). The allometric relationship in holometabolous insects occurs due to the fact that the imaginal discs for appendages grow at a relatively slow rate during larval developmental stages and then undergo a brief period of accelerated growth just before metamorphosis, during the pre-pupal and pupal periods, precisely at the time when food intake has ceased (Williams, 1980, Nijhout and Wheeler, 1996). Different types of imaginal tissues grow at variable times and rates (Huxley, 1932, Wilson, 1953, Williams, 1980, Nijhout and Wheeler, 1996). More complex allometrics and growth models are described by Nijhout and Wheeler (1996) and Stern and Emlen (1999).

There are many examples illustrating the scaling relationships of traits growing independently, such as in the male rhinoceros beetle, which has long horns and disproportionately small eyes (Kawano, 1995). Comparative morphological studies on allometry of genitalia emphasise the importance of two existing hypotheses based on sexual selection - the 'good genes hypothesis' and the 'weapon hypothesis' (Eberhard et al., 1998). These two hypotheses were tested on 20 species of insects belonging to the Coleoptera, Diptera, Hymenoptera, Hemiptera, Dermaptera and Odonata and spiders. The results revealed that the allometric slopes of male genitalia tended to be consistently lower than 1 and lower than the slopes of non-genital parts. Consequently, an alternative hypothesis was proposed - namely the 'one-size-fits-all hypothesis' (Eberhard et al., 1998, Eberhard, 2009). This hypothesis proposed that for the male sexual trait, allometric relationships between genitalia and body size usually have slopes lower than 1, compared with the other body parts that have no role in reproduction, such as the pronota (Eberhard et al., 1998, Bernstein and Bernstein, 2002, Tatsuta et al., 2007). Thus, within a species, sexual selection favours males with genitalia of standard sizes. Several other studies have supported the theory detailed by Eberhard et al. (1998) and similarly detected negative allometry for male genital size, (Wheeler et al., 1993, Schmitz et al., 2000, Tatsuta et al., 2001).

Tatsuta et al. (2007) presented a study on the sexually dimorphic stag beetle, *Prosopocoilus inclinatus* (Motschulsky, 1857) (Coleoptera: Lucanidae). It was revealed that all the genital parts of the males scaled to body size with a slope of less than 1, except the male intromittent organ, which showed smaller variability than the other body parts. This is consistent with the prediction by Eberhard et al. (1998) that individuals with intermediate genital size are favourably selected by stabilising sexual selection. This appears to play an important role in maintaining certain intermediate standardised size of genitalia appropriate for the most typical size of the opposite sex in many insects (Eberhard et al., 1998, Bernstein and Bernstein, 2002). Some exceptions might, however, exist - for example, the male intromittent organ (as mentioned above) and female genitalia exhibited large variability, which may account for rapid diversification of genital morphology, even in closely related populations in beetle species (Eberhard et al., 1998, Bernstein and Bernstein, 2002). It has been postulated that positive allometry sometimes found in a part of genitalia is associated with postcopulatory sexual selection (Lupold, et al., 2004, Hosken et al., 2005). These facts lead to a contradiction in terms of developmental stability and rapid diversification of genital traits intraspecifically. Further comparative studies on phenotypic variation in the allometric relationship are needed to contribute to distinguishing this inconclusive evidence.

Likewise, Ohno et al. (2003) tested the contrast between genital and somatic traits for males of the moth, *Ostrinia latipennis* (Warren, 1892) (Lepidoptera: Hepialidae). In their study they examined allometry of five genital and 11 somatic traits for each of three local populations of *O. latipennis*. Fourteen out of the 15 allometric slopes for genital traits showed significantly negative allometry, whereas none of the 33 somatic traits showed the same negative allometry obtained from genital traits. These results revealed that the size of male genitalia in *O. latipennis* is more stable than the size of somatic traits when scaled with changes in body size. This study supports the hypothesis put forward by Eberhard et al. (1998).

Green et al. (1992) argued that positive allometry may often occur in characteristics used in mating choice or those that display competitive ability. They disagreed with the evidence presented by Eberhard et al. (1998) that insect and spider genitalia tend to be negatively allometric. They based their arguments on the fact that the methodology used by Eberhard et al. (1998) - an analysis of linear ordinary least squares (OLS) regression of log (length of body part) on log (length of body-size indicator) - was inappropriate and, therefore, revealed non-compelling and inconclusive results. Green et al. (1992) reanalysed a similar allometric relationship using different regression techniques, such as the two model II regression methods - major axis (MA) and reduced major axis (RMA) - which they claimed may be more appropriate in different cases and provide valuable alternatives to OLS. Others have supported the analytical method used by Green et al., since both MA and RMA are known to be standard techniques in allometry studies (Boag, 1984, LaBarbera, 1989, Herrera, 1992, Cane, 1993, Simmons and Scheepers, 1996, Silva, 1998), although there is debate regarding their relative merits (Ricker, 1984, McArdle, 1988, Jolicoeur, 1990, Sokal and Rohlf, 1995). Stern and Emlen (1999) suggested that future studies of the developmental basis of allometry must focus on individuals growing in natural populations, rather than under 'standardised' laboratory conditions. This could highlight the actual and raw variations in scaling relationships resulting from growth under varied conditions.

1.9 Sexual size dimorphism

One manifestation of sexual selection is sexual dimorphism - defined as morphological differences between sexually mature males and females of plants and animals (Fairbairn, 1997, Colgoni and Vamosi, 2006). Such dimorphism is caused by independent, sex-specific selection on morphological traits (Price, 1984, Andersson, 1994, Blanckenhorn, 2000). The sexual size dimorphism (SSD) phenomenon is a common widespread dimorphism among different groups of animals (Shine, 1989, Fairbairn, 1997, Badyaev, 2002, Blanckenhorn, 2005).

According to reports, male-biased SSD is predominant in birds and mammals (Cabana et al., 1982, Isaac, 2005), whereas in the majority of insects and other invertebrates, as well as many fish and amphibians, the reverse is true (Shine, 1979). In most invertebrates and poikilothermic vertebrates, females are the larger sexes (Shine, 1994, Teder and Tammaru, 2005). Invertebrate males usually tend to be smaller in size than females (Wiklund and Karlsson, 1988). Female-biased SSD has been recorded in several groups of invertebrates, including aphids (Dixon, 1987, 1998), damselflies (Anholt, 1997), flies (Nunney, 1996), ladybird beetles (Dixon, 2000), butterflies (Wiklund and Karlsson, 1988, Nylin et al., 1993), midges (Neems et al., 1990), wasps (Coelho, 1997), spiders (Vollrath and Parker, 1992) and waterstriders (Preziosi and Fairbrain, 1997). Several studies have referred to protandry as the easiest common explanation for female-biased SSD - i.e., males develop faster than females, resulting in the males being smaller. In contrast, Dixon (2000) noted that both males and females often take the same developmental period, thus implying that the existence of other limiting factors influences the smaller size of males in most of invertebrate species.

Darwin (1871) pointed out the importance of two types of selective forces that may operate during reproduction - inter-sexual selection, resulting from a preferential choice by one sex for individuals of the opposite sex, and intra-sexual selection, resulting from competition within one sex for individuals of the opposite sex. In general, SSD is caused by variations in the sexual differences in selective pressures on adult body size. For example, fecundity selection (a positive correlation between fecundity and female body size) (Stillwell and Davidowitz, 2010) has been viewed to be the main force leading to female-biased SSD, whereas sexual selection is generally stronger on males than females (Andersson, 1994) and has often been considered in cases of male-biased size dimorphism (Darwin, 1871, Trivers, 1972) in insects (Tseng and Rowe, 1999, Teder, 2005).

Rensch's rule has been widely accepted in predicting SSD: the ratio between the size of the larger sex and the size of the smaller sex increases with size and is positively correlated with mean body size (hype-allometry) in taxa in which males are the larger sex, and negatively correlated with mean body size (hypoallometry) in taxa in which females are the larger sex (Fairbairn, 1997, Abouheif and Fairbairn, 1997). The generality of Rensch's rule has been questioned (Selander, 1966, Reiss, 1986), since it is not applicable on statistical analysis of all taxa (Abouheif and Fairbairn, 1997). It has been suggested that allometry consistent with Rensch's rule may be more common in taxa in which males are the larger sex (Earhart and Johnson, 1970, Head, 1995). This means that male body size varies or diverges more over evolutionary time than female body size, irrespective of which sex is larger (Fairbairn, 1997, Abouheif and Fairbairn, 1997).

The direction and magnitude of SSD varies considerably among taxa and among species, due to variability in the sources of selection that act to create SSD (Stillwell and Davidowitz, 2010). The magnitude of SSD changes considerably only among populations within species (Blanckenhorn et al., 2006, 2007, Stillwell et al., 2007ab). In this context, several hypotheses have been proposed to explain the different selection forces on the sexes in dimorphic species, including sexual selection, fecundity selection and ecological niche divergence (Fairn et al., 2007).

As explained above, the variation among species in the magnitude of sexual selection that favours large size in males is due to female preferences or male-male competition (Stillwell and Davidowitz, 2010). Furthermore, increased fecundity in large females' selection (a positive correlation between female size and fecundity) is generally thought to be the major cause leading to female-biased SSD (Honek, 1993, Andersson, 1994, Blanckenhorn, 2000), according to the "Big Mother" hypothesis (Greenwood and Adams, 1987).

Despite the fact that female-biased size dimorphism is the more common pattern of SSD, especially in invertebrates (Teder and Tammaru, 2005), there are arthropod species in which males are the larger sex (Wiklund and Forsberg, 1991, Juliano, 1992). Large male size could also be favoured by fecundity selection via males' contribution to female fecundity. It has been reported that large males tend to contribute more biomass to egg production than small males, such as in *Stator limbatus* (Horn, 1873) (Coleoptera: Bruchidae) (Fox et al., 1994ab). Mating with large males would be favoured if those males are capable of providing larger spermatophores or ejaculates that may result in larger eggs (in a greater number) being laid (Thornhill, 1976, Thornhill and Alcock, 1983, Ridley 1988, Andersson, 1994). Despite the above, few studies have revealed a direct benefit from mating with large males (Savalli and Fox, 1998).

Blanckenhorn et al. (1995) introduced the "Mate or Eat" hypothesis to clarify the differences in body size between sexes. In a similar context, Yasuda et al. (2002) showed that female larvae of the ladybird *A. bipunctata*, for example, had consumed more food and grown at higher relative growth rate when compared with the larvae of males, particularly at late stages in their development. In addition, it was revealed that after hatching, small-sized males accomplished more successful mating when compared with large-sized males when food was limited. The greater mating success by smaller males supported the above "Mate or Eat" hypothesis, which predicts that when food is limited, smaller males spend less time in feeding and have a greater mating frequency when compared with large males.

Other important factors that play important roles in influencing SSD are differentiation in reproductive roles, mating rates, reproductive success and parental investment. These factors may lead directly to morphological differentiation of the reproductive organs resulting in significant SSD (Lloyd and Webb, 1977). Such selection is frequently associated with hyperallometric growth of organs or structures, particularly in males, as indicated above (Trivers, 1972, Andersen, 1994, Winquist and Lemon, 1994, Fairbairn, 1997). The significance of parental investment in controlling the process of sexual selection has been deliberated by Trivers (1972). Likewise, Thornhill (1976) described the various types of nutritional parental investment in different insect groups associated with behavioural and physiological mechanisms that affect sexual bias.

Wiens (2001) demonstrated that sexually dimorphic characters may be predetermined by one of three mechanisms – the lineage remains sexually dimorphic (i.e., stasis); the lineage becomes monomorphic and loses the dimorphic character or ornament; or a gain of the dimorphic character by the non-ornamented sex, where the lineage becomes monomorphic and both sexes possess the conspicuous ornament. The above was discussed in detail in Oliver and Antónia Monteiro (2010).

Darwin (1871) proposed a mechanism for the origin of secondary sexual characters. He stressed that in order to understand diversity in such traits, it is important to investigate the related potential fitness costs. Such costs play a significant role in the sex-limited expression that is the hallmark of sexually selected traits and are essential for explaining diversity in sexual traits (Houslay and Bussiére, 2012). The elaborate morphologies of sexually selected ornaments are ubiquitous across the animal kingdom (Andersson, 1994).

Many studies have revealed a positive correlation between the size of sexually selected ornaments and some aspect of reproductive fitness (Hingle et al., 2001). Therefore, shape can provide additional insights into the morphological differences between individuals and the potential limits on sexual trait exaggeration (Worthington et al., 2012), e.g. the stalk-eyed fly, *Teleopsis dalmanni* (Wiedemann, 1830) (Diptera: Diopsidae) exhibited patterns of sexual shape dimorphism. A significant difference in head shape was demonstrated between the sexes, with males exhibiting smaller eye bulbs, thinner stalks and smaller heads compared with females. Several studies have predicted that when females bear ornaments as a result of correlated selection, they are expected to be closer to the optimum set by natural selection (Haas, 1976, Lande, 1980, Anholt, 1997).

The sexual difference in external morphology ranges from totally undifferentiated to conspicuous dimorphism in both size and shape in beetle species (Kawano, 2006). Several studies have shown that the magnitude of SSD varied substantially among populations intra-specifically (Blanckenhorn et al., 2007, Stillwell et al., 2007ab). Although most of these variations in size and shape are partly based on genetics, as indicated above, some exist due to other important pressures, such as phenotypic plasticity (Fairbairn, 2005, Stillwell et al., 2010). The influence of phenotypic plasticity on SSD is not yet clear, as both sexes share the same genes controlling their growth and developmental processes (Badyaev, 2002). Sexual selection was believed to reduce population fitness, owing to its association with direct fitness costs. Sexual selection causes diversion of resource allocation away from egg production towards costly external morphological traits - i.e., ornaments that do not add to population growth (Agrawal, 2001, Siller, 2001).

Body size measurements were quantified in the mammal yellow-pine chipmunk, *Tamias amoenus* (Allen, 1890) (Rodentia: Sciuridae) found in the Kananaskis Valley, which is commonly known to have female-biased SSD. It was revealed that females were significantly heavier and had a larger overall structural body size and skeletal tissue (Schulte-Hostedde and Millar, 2000). It was also observed that larger females produce larger offspring (in a greater number) and provided superior maternal care (more or better quality milk) in comparison with smaller mothers (Ralls, 1976, Myers and Master, 1983, Dobson and Michener, 1995). Another prediction stated that in such contexts female-biased SSD may also be the result of selection for smaller males, as smaller-sized males may be faster and more active (Trombulak, 1989). They can spend a longer period searching for mates and less time in feeding (Blanckenhorn et al., 1995).

Another example is female bias in the parasitoid wasp, where most females take a longer time to develop. Sex-specific differences in the effect of size on reproductive success are attributed as the significant factor responsible for SSD in parasitoids. It is commonly known that fertilized eggs laid by most parasitoid wasps, such as *Microplitis mediator* (Haliday, 1834) (Hymnoptera: Braconidae), develop into females and unfertilized haploids give rise to males. This process gives adult females the ability to potentially control the sex of future offspring by using hosts of varying quality (Harvey and Strand, 2003).

Many studies focus on SSD in the adult stage, often ignoring juvenile development (Badyaev et al., 2001, Badyaev, 2002). Esperk and Tammaru (2006) stressed that it may not be possible to fully know the mechanisms underlying the evolution of SSD without understanding the details of developmental stages. This is because of the different growth strategies leading to SSD, rather than adult SSD itself (Badyaev, 2002). It was reported that there are three basic processes that may lead to SSD -individuals of the larger sex could be larger in size at hatching time, have faster instantaneous growth rate or take a longer time to develop, known as sexual bimaturation (Mackey, 1978, Ernsting and Isaaks, 2002, Yasuda and Dixon, 2002). Some species with female-biased SSD, however, attain their final body size through a longer developmental period with no sex-related differences in growth rates, as noticed by many studies, including Mackey (1978), Lederhouse et al. (1982), Nylin et al. (1993), Brakefield and Mazzotta (1995), DeBlock and Stoks (2003) and Mikolajewski et al. (2005). Other studies revealed that in some sexually dimorphic insects, growth rates but not larval periods differ between the sexes (Telang et al., 2001, Yasuda and Dixon, 2002). Exceptions may exist in which both options are used simultaneously - i.e., a prolonged larval period and higher growth rates occurring together, eventually resulting in the female being the larger sex (Bradshaw and Holzapfel, 1996, Ernsting and Isaaks, 2002). Thus, both mechanisms (growth rate and larval period length) are available in insects and are not universally used options for formation of SSD among insects.

Developmental constraints may play an important role in forming patterns of SSD (Maynard et al., 1985, Schluter et al., 1991, Badyaev, 2002). In arthropods, in particular, the evolution of SSD may be limited by developmental and related physiological constraints on growth at each larval stage. SSD could, however, be accomplished if the larvae of the larger sex pass through a greater number of larval instars (Esperk et al., 2007). Several other hypotheses have been suggested to explain the differences in selection pressures on the sexes in dimorphic species, including ecological niche divergence (Fairn et al., 2007). For instance, the variation in optimal morphology for the different niches may lead to SSD (Slatkin, 1984, Shine, 1989). In addition, Stillwell et al. (2010) noted that the fluctuation in source conditions resulted in a variety of sources of selection favouring small size in both sexes. Likewise, it has been reported that the magnitude of sexual selection varied under limited resources in which selection favours small sizes in both sexes (Stillwell and Dividowitz, 2010). Furthermore, sexual selection may even favour small males, due to an occasional rapid developmental period compared with females - e.g. selection for protandry (Singer, 1982, Bulmer, 1983), efficient searching for a mate (Fagerstrom and Wiklund, 1982), greater agility during courtship and copulation (Andersson and Norberg, 1981, McLachlan, 1987) or female preferences for smaller males (Petrie, 1983, Steele and Partridge, 1988, Andersson, 1994), as previously mentioned in *T. amoenus*. Savalli and Fox (1998) noticed that female preference for large males is less frequently considered an explanation for SSD.

Most beetle species are indistinctively dimorphic, where the male and female can be notable only by minor microscopic morphological characteristics (Kawano, 2006). In contrast, Fairn et al. (2007) noted the opposite when quantifying SSD in the whirligig beetle, *Dineutus nigrior* (Roberts, 1895) (Coleoptera: Gyrinidae). Females possess significantly larger body mass shape and size, in terms of both width and length. Fecundity selection was used to explain the likely cause of body size dimorphism in this beetle (Andersson, 1994). Males were characterized by a larger tarsal size, profemora and protibiae, which may confer an advantage in grasping the female (Aiken and Khan, 1992, Bergsten et al., 2001, Schulte-Hostedde and Alarie, 2006). This suggests the existence of differential selection of different aspects of body size, rather than overall body size (Fairn et al., 2007). Although many of these intra-specific variations in SSD are based partly on genetics, some of these variations may occur as a consequence of sexual differences in phenotypic plasticity in relation to body size (Fairbairn, 2005, Stillwell et al., 2010). How such sexual differences in body size plasticity are created in response to environmental variability - particularly in insects - is mystifying, as males and females have similar genes for the control growth and development (Badyaev, 2002, Stillwell et al., 2010).

Many environmental and ecological variables prompt plasticity in body size and other traits of ectothermic animals (Stillwell et al., 2007a, Teder et al., 2008, Blanckenhorn, 2009). Diet (both quantity and quality) and temperature are very important factors in inducing plasticity in life-history traits and developmental processes (Davidowitz et al., 2004, Stillwell et al., 2007b). Teder and Tammaru (2005) noticed that intra-specific variation in SSD is likely to increase, particularly when the growth rates of males and females vary in their sensitivity to environmental conditions. For example, Bonduriansky (2007) noticed that in the Australian fly *Telostylinus angusticollis* (Berg, 1947) (Diptera: Neriidae), males showed more sensitivity towards diet quality compared with the opposite sex. On the low-quality diet, the size of males and females, however, were nearly identical. When both sexes were kept on a high-quality diet, the former showed a considerably larger body size compared with the latter.

Temperature, on the other hand, varied in its consistency in influencing the sex-specific plasticity of insect body size (Stillwell et al., 2010). For example, Stillwell and Fox (2007) found temperature-induced differential variability in SSD between both sexes of the seed-feeding beetle, *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae). It was noted that males exhibited more sensitivity towards rearing temperature than females (Stillwell and Fox, 2007). In contrast, another study performed on the same species revealed that temperature failed to produce sex-specific plasticity in body size (Stillwell et al., 2010). Despite a recent increase in interest in investigating differences in body size plasticity between the sexes, the mechanisms that lead to sex variation in plasticity remain largely unknown. Understanding the ultimate evolutionary, ecological, developmental and physiological aspects generating these patterns is highly essential to evaluating the evolution of intra-specific variation in SSD in several taxa (Esperk et al., 2007, Stillwell and Fox, 2007, Stillwell et al., 2010).

A study was performed to test the physiological aspects of development, including body size regulators - such as growth rate and critical weight - in generating sex-specific plasticity. Larvae of the hawk moth, *M. sexta*, were reared at different diet qualities and temperatures until the adult stage (Stillwell and Davidowitz, 2010). It was revealed that the larval mass at late stages did not display sex-specific plasticity in response to either to diet or temperature. In contrast, mechanisms that control body size - such as growth rate and critical weight - exhibited sex-specific plasticity in response to both diet and temperatures. This suggests that the sexes exhibit differential sensitivity to the physiological factors that determine body size to environmental variations. Teder and Tammaru (2005) reported that females of other species, however, were found to be more sensitive to environmental cues compared with males and, after condition restoration, females have a tendency to increase in size more than males.

Female-biased sex has been reported in several coleopteran species (Dixon, 2000), particularly in seed-feeding beetles, *Callosobruchus maculatus* (Fabricius, 1775) and *Stator limbatus* (Horn, 1873) (Coleoptera: Chrysomelidae) (Stillwell and Fox, 2005, Teder and Tammaru, 2005). Stillwell and Davidowitz (2010) noted that although intra-specific variations in SSD are partly genetically controlled, some of these variations may happen due to phenotypic plasticity, which occurs in response to ecological and environmental factors (Fairbairn, 2005), as mentioned above. Furthermore, Blanckenhorn et al. (2006) noted that females tend to be larger at higher latitudes, whereas males were larger at lower latitudes. Stillwell et al. (2010) reported that it is extremely difficult to discriminate between the phenotypic plasticity that arises in response to ecological and environmental conditions from evolutionary responses to climatic or other variables that co-vary with latitude or altitude.

Invasive species often exhibit geographical variations in life-history traits, which may allow them to successfully establish and colonize different environments. For instance, geographical variations in body size and SSD of invasive bullfrogs in southwestern China, *Rana catesbeiana* (Shaw, 1802) (Anura: Ranidae), were tested by sampling two breeding populations inhabiting two sites at low and high altitudes (Xuan et al., 2010). It was revealed that both populations displayed a remarkable significant SSD, with females being the larger sex. There was a significant reduction in the mean body size of both sexes and growth rate with increasing altitude, with this reduction found to be more pronounced in females in particular. A similar trend was also noticed in the average age of females at high altitude. This implies that the introduced bullfrogs exhibit geographical variation in morphology in the invaded areas, likely in response to climatic changes (Ashton, 2002, Campbell and Echternacht, 2003, Morrison et al., 2004).

Likewise, the Cuban tree frog, *Osteopilus septentrionalis* (Duméril & Bibron, 1841) (Anura: Hylidae), which is native to Cuba, has been introduced and become established in several sub-tropical and tropical locations, including Florida (Barbour, 1931, Meshaka, 2001, Lindsay and Cooper, 2008). It was found that significant decreases in female bias and SSD were exhibited with increasing latitude. This trend was predicted to be driven by a reduction in growth rates, increased time to maturity and reduction in longevity, all of which may be tied to climatic variation and are indicative of invasion success (McGarrity and Johnson, 2009).

Studies investigating inter-specific variation in SSD typically assume that the degree of sexual difference in body size does not vary within species. Datasets for 158 insect species were studied by Teder and Tammaru (2005). Each dataset contained the weight of adults or pupae of both sexes for two or more different subsets reared at different conditions during immature development. It was revealed that there was a variation in the SSD exhibited at the intra-specific level in insects. The results predicted that environmental conditions may effectively influence the degree, but not the direction of SSD within species. It was found that within species, female body size appeared to be more sensitive to environmental conditions than male size, which contradicted the above prediction reported by Bonduriansky (2007) in which the opposite was true. Thus, across different insect orders, sexual differences in size tended to increase with increasing body size in species with female-biased SSD (Teder and Tammaru, 2005).

Badyaev (2002) stressed that the underlying growth patterns within a species that may lead to variation in SSD remains poorly understood. Further studies are required to increase the knowledge covering the evolutionary mechanisms behind intra- and inter-species patterns of SSD (Shine, 1990, Stamps, 1993, Badyaev, 2002, Oliver and Antónia Monteiro, 2011)**.**

1.10 Reproductive performance

1.10.1 Ovariole number

The typical insect ovary has a modular arrangement, with the ovariole as its fundamental modular unit (Hodin, 2009). The ovariole number correlates with potential fecundity and reproductive output (Stewart et al., 1991a, Hodin and Riddiford, 2000). In general, an increased ovariole number appears to correlate with total potential reproductive output, but other physiological characteristics of the ovary may affect the rate and timing of egg production, as well as the rate of oocyte maturation (Hodin, 2009). All ovarioles mature eggs simultaneously, thus the maximum ovariole number correlates positively with potential reproductive output (Cohet and David, 1978, Stewart et al., 1991ab, Hodin, 2009).

Ovariole number is an important trait for understanding life history strategies, both between different populations and within a species, since it influences resource acquisition and nutritional allocation (Hodin and Riddiford, 2000). In addition, there is substantial difference across broad and narrow taxonomic groups of insects in the degree to which the rates of oocyte maturation and the rate of egg production are phenotypically plastic (Hodin, 2009). Several aspects of the pre-adult environment, such as larval nutrition, have a direct influence on ovary size and number, thus determining the reproductive capacity (Flatt and Heyland, 2011).

Nonetheless, given sufficient adult nutrition, the ovariole number sets the upper limit for reproductive capacity (Dixon, 2000). For example, a positive relationship between the number of ovaries and fecundity is present within and amongst the Diptera species (Honek, 1993). In *D.* melanogaster, the maximum daily rate of egg production was positively correlated with the total number of ovarioles (Wayne et al., 2006). As demonstrated by Flatt and Heyland (2011) for *D. melanogaster*, larval feeding affects the ovariole number by altering the rate of differentiation of the terminal filament cells, located at the anterior tip of the ovariole. This modification usually occurs at the wondering stage when larvae have ceased feeding. The latter implies that ovariole number is mainly determined by endocrine signals secreted from the fat bodies or the prothoracic gland (Mirth et al., 2005). In addition, Orgogozo et al. (2006) suggested that the genetic variation in insulin singling pathways was the main factor that underlies the variation in optimal ovariole number between two related species - *Drosophila simulans* (Sturtevant, 1919) (Diptera: Drosophilidae) and *Drosophila sechellia* (Diptera: Drosophilidae). The latter species, *D. sechellia*, possesses a remarkably low number of ovarioles compared with the former when reared under similar environmental conditions. Orgogozo et al. (2006) pointed out that the locus of insulin receptors located on Chromosome 3 showed genetic variations that led to that great variation. In addition, Tu and Tatar (2003) hypothesised that mutation in the insulin singling pathway may also lead to such variation in ovariole number.

Since there is an abundant genetically fixed variation in ovariole number, it is highly sensitive to the larval environment and is species-specific (Hodin, 2009). Several aspects of the pre-adult environment, such as food quality and accessibility, temperature and crowding play a large role in influencing the development and maturation of ovarioles (Rhamhalinghan, 1986, Grenier et al., 1994, Hodin and Riddiford, 2000, Tu and Tatar, 2003). In higher food quality and abundant nutrients assimilated in uncrowded conditions led to an increase in the ovariole number (Hodin, 2009) such as in the *D. melanogaster* (Robertson, 1957).

As stated above, ovariole number shows both phenotypic plasticity and inter-specific and inter-population variations (Hodin and Riddiford, 2000). Despite this, some species seem to refute this expectation. For example, ovariole numbers are uniform in females belonging to the Lepidoptera species - an adult female has eight ovarioles, suggesting tight genetic influences (Swevers and Latrou, 2003). In both the sycamore aphid, *Drepanosiphum platanoidis* (Schrank, 1801) (Hemiptera: Drepanosiphidae), and the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus, 1758) (Hemiptera: Aphididae), ovariole number was found to be constant across generations, despite constant changes in ecological conditions (Leather and Wellings, 1981). Several comparative studies were performed between different species to investigate the relationship between the potential number of ovarioles and the reproductive output, such as those studies conducted between dipteran *Episyrphus balteatus* (De Geer, 1776) (Diptera: Syrphidae), the predaceous aphidophagous *A. bipunctata* and lacewing species including *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae), the Neotrophical green lacewing, *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae), and the West-Palaearctic lacewing, *Chrysoperla mediterranea* (Hölzel, 1972) (Chrysopidae: Chrysopidae) (Stewart et al., 1991a b, Zheng et al., 1993a b, Carvalho et al., 1996).

Stewart et al. (1991b) demonstrated that the ovariole number and the rate of egg production in the hoverfly *E. balteatus* (Diptera: Syrphidae) are much greater than the similarly sized predaceous ladybird, *A. bipunctata.* Compared with the latter, the daily rate of egg production recorded in *E. balteatus* was between 1 to 2 eggs per ovariole per day. In *A. bipunctata*, the daily egg production, however, was only 0.5 eggs per ovariole per day (Stewart et al., 1991b, Branquart and Hemptinne, 2000). It has been suggested that the functional number of ovarioles affected reproductive output as well as lifetime fecundity. The record showed that the average ovariole number in *A. bipunctata* was 46 ovarioles, whereas in *E. balteatus* the average recorded was 80 ovarioles (Branquart and Hemptinne, 2000). Generally, ovariole number can be a good indicator for the daily egg production and to estimate lifetime fecundity in Coccinellidae, Drosopilidae, Tachinidae and Ichnenumonidae, (Price, 1975, Stewart et al., 1991ab).

Hodek et al. (2012) demonstrated that the ovarioles in Coccinellidae are of a meriostic telotrophic type, characterised by the presence of trophic tissue as well as oogonia and oocytes in the distal germarium. A single oocyte is connected to the germarium by a cytoplasmic nutritive cord extending to the trophic core. The total number of ovarioles in female coccinellids is controlled either genetically (Hodin and Riddiford, 2000) or by the experienced nutrient availability during juveniles' development (Honek, 1993), such as in *H. axyridis* and *A. bipunctata* (Ware and Majerus, 2008). Both factors are likely to control the number of ovarioles in the grasshopper *Romalea microptera* (Houttuyn, 1813) (Orthoptera: Romaleidae) (Taylor and Whitman, 2010).

Scaling of body size with many fitness-limiting traits, such as ovariole number, displays a wide variation between species (Bellinger et al., 1987, Karlsson and Wickman, 1990, Arnaud et al., 2005). Such variation in scaling has also been attributed to the basic genetic control and major influences of environmental cues (Venesky and Parris, 2009). Hodek et al. (2012) argued that there is a significant positive correlation between ovariole number and female body size, particularly in relation to body length. Fitt (1990) also noted that in both *Dacus tryoni* (Froggatt, 1897) and *Dacus jarvisi* (Tryon, 1927). (Diptera: Tephritidae), there was a positive correlation between ovariole number and body size (as measured by wing length). In addition, Dixon and Guo (1993) demonstrated a positive relationship between the number of ovarioles and body weight in *C. septumpunctata*. Moreover, the potential fecundity (expressed as reproductive biomass), the number of ovarioles and the abdomen volume all scale isometrically with female body size in the species *E. balteatus* (Branquart and Hemptinne, 2000).

Honek (1993) investigated intra-specific variations between female body size and potential fecundity (determined by the ovariole number), particularly in Coleopterans and found a positive relationship between these two phenotypic traits with a common slope that closes to unity. In addition, a study was conducted to investigate the pattern of reproductive strategies for eight species of iteroparous beetles (i.e., those in which females produce more than one generation during their lifespan) belonging to the genus *Tribolium* (Coleoptera: Tenebrionidae) under similar conditions (Arnaud et al., 2005). Several life-history traits - including egg mass, adult mass, developmental time and fecundity - were investigated and comparison was made. The results revealed an absence of common reproductive strategies among these different species. Larger and smaller *Tribolium* species adopted different reproductive tactics. Small-sized females were less fecund and produced smaller eggs in comparison with large-sized females. In addition, univarate analysis showed strong disparities between the species for most of the traits investigated and only a few correlations appeared to be significant between tested traits (Arnaud et al., 2005). This was not unexpected, because the absence of significant intraspecific allometry is often reported in iteroparous organisms (Fox and Czesak, 2000). For instance, no such linear relationship was found between the number of ovarioles and body length or female body weight in the ladybird *H. axyridis* (Osawa, 2005). In contrast, Hodin (2009) suggested that, with regards to the interspecific allometric relationship, a strong association is always revealed. Such association as the one found between fecundity and body size is always expressed strongly owing to the ecological, phylogenetic influences and due to the phenotypic non-adaptive plasticity that generated from differential feeding habit. Similar observations were also noted by Stewart et al. (1991ab), Honek (1993), Gasser et al. (2000) and Tu and Tatar (2003).

It was suggested that within species the number of ovarioles is positively correlated with body size, particularly when the actual body size is small, such as in small flies (Bennettova and Fraenkel, 1981). Others suggested that the relationship between female body weight or size and the ovariole number may vary according to the maturation and developmental stages - such as in aphids where the total number of ovarioles is often set early in the nymphal stage

- but the potential fecundity is a consequence of the conditions experienced during development (Leather and Wellings, 1981, Llewellyn and Brown, 1985).

The number of ovarioles varies intra-specifically. It was shown that a female of *C. septumpunctata* with a 24mg body weight contained 80 ovarioles, but a female weighing 31mg contained 139 ovarioles (Rhamhalinghan, 1985, 1986). Having a high number of ovarioles does not always reflect a greater fecundity. It has been noted that although females of *C. septumpunctata* possess approximately 139 ovarioles, each ovariole comprises only three follicles. In another species, *Stethorus pusillus* (Herbst, 1797) (Coleoptera: Coccinellidae), the total ovariole number recorded was 4, but each ovariole contained between four and eight follicles (Hodek et al., 2012) Thus, attributes such as body capacity, lifetime fecundity and reproductive biomass are the main factors in determining the reproductive performance, rather than the actual number of ovarioles. These attributes display a wide range of variation and are strongly correlated with female size. This relationship between reproductive investment and female body size is not unanticipated, since larger females have proportionally more stored nutrients to allocate for reproduction compared with smaller sizes (Reiss, 1985, Sibly and Calow, 1986, Roff, 1992, Dixon and Guo, 1993).

The suitability of different diets for larval development, and the influence of food quality and quantity on adult reproduction have been well studied (Majerus, 1994, Evans et al., 1999, Dixon, 2000). The number of the ovarioles in several insect species was studied under different diet regimes. For example, female adults of the two-spot ladybird, *A. bipunctata* showed a reduction in the number of ovarioles when larvae were reared on a low quantity of cowpea aphids, *Aphis craccivora* (Koch, 1854) (Hemiptera: Aphididae), compared with those females that were reared on a low quantity of pea aphids, *Acyrthosiphon pisum* (Harris, 1776)

(Hemiptera: Aphididae) (Ferrer et al., 2008). It was revealed that exposure to suboptimal food quantity not only reduced the number of ovarioles, but also altered the oosorption process (i.e., resorption of oocyte) and the maturation of ovarioles. Comparable results were obtained from a study in which the predaceous ladybird *C. septumpunctata* was reared on a low quantity of aphids (Osawa, 2005). It has also been reported that food shortage during larval development may prompt the emergence of small-sized females with a low number of ovarioles, which in turn might lead to low fecundity (Scott and Barlow, 1984, Dixon and Guo, 1993, Zheng et al., 1993ab). Other studies have tested the effects of mixed versus monotypic diets in generalist predators - such as carabid beetles (Jorgensen and Toft, 1997, Fawki and Toft, 2005, Toft, 2005) and spiders (Oelbermann and Scheu, 2002, Allard and Yeargan, 2005) - on reproductive performance and fecundity.

Environmental factors, such as temperature, have an effect on determining ovariole number (Sanburg and Larsen, 1973, Hodin and Riddiford, 2000). For example, exposure to different temperatures caused a reduction in ovariole number in *Drosophila kikkawai* (Burla, 1954) (Diptera: Drosophilidae) (Karan et al., 1998). It was suggested that the effects of temperature on ovariole number were in essence a non-adaptive biophysical plastic response, with the optimum temperature merely representing the metabolic optimum for the molecules involved in terminal filament formation (Karan et al., 1999, 2000, Gibert et al., 2004, Wayne et al., 2005). Nevertheless, the ovariole number might also differ in *D. kikkawai* among latitudinal clines (Karan et al., 1998). Factors such as larval crowding as mentioned formerly in *D. melanogaster* reduced food availability, leading to a reduction in ovariole number in their hatchlings (Robertson, 1957).

Gilbert (1990) predicted that since specialist species encounter suitable oviposition sites at a lower rate on average than generalists, they would as a consequence lay fewer and larger eggs than generalists. The generalist *Dacus tryoni* and the specialist *D. jarvisi* are the main tephritid pests of cultivated fruits in Australia. In contrast to the prediction by Gilbert (1990), it was noticed that there was a broad positive relationship between the breadth of the natural host range and the ovariole number and recorded potential fecundity in *Dacus* species (Fitt, 1990). Comparative measurements for clutch size, egg size and ovariole number and the influence of body size on these parameters were made for both species in the laboratory. It was revealed that the number of ovaries in the polyphagous species *D. tryoni* ranged between 35-40 ovaries (38 ovarioles/ovary), in comparison with the specialist species *D. jarvisi*, which ranged between 8-20 ovaries (27 ovarioles/ovary). The number of ovarioles had apparently affected the rate of egg production. The generalist was less fecund and produced small clutches with three to four eggs per clutch, compared with *D. jarvisi* which produced large clutches with relatively more eggs (10-15 eggs/clutch). This further supports the fact that having a large number of ovaries does not imply greater fecundity.

Hodin (2009) noted that the rate of oogenesis may sometimes be inversely related to the number of ovarioles and developing oocytes. These, and other trade-offs, suggest that ovariole number may be shaped by natural selection. In addition, the variation in the optimal number of ovarioles could be one of the features of variable environmental cues. Therefore, natural selection should encourage plasticity for this trait among insect populations inhabiting newly fluctuating environments (Hodin, 2009). For example, Leather and Wellings (1981) noticed that the slope of the line relating ovariole number and body size decreases seasonally (Llewellyn and Brown, 1985). Others suggested that a lack of conformity between field and laboratory results must be expected when comparing such relationship, owing to the constant variability in food availability and changing environmental conditions (Dixon and Guo, 1993, Honek et al., 2007).

According to Stewart et al. (1991a), the alternate activity of ovarioles in egg production might also affect the probable association between body size and potential fecundity. It is not only body parameters that could be incorporated in assessing the potential fecundity and reproductive output. For example, Gilbert (1984) suggested that the pupal weight of the small white butterfly *Pieris rapae* **(**Linnaeus, 1758) (Lepidoptera: Pieridae) could be used as a measure of potential fecundity, since both have a positive significant correlation. Despite the above, Stewart et al. (1991a) noted that the reproductive capacity might be constrained by the body design itself for most species, irrespective of their size, particularly in Coccinellidae. Therefore, they suggested that large aphidophagous species might have the same relative reproductive capacity as small species in terms of reproductive biomass and reproductive rate. For instance, doubling the weight of an adult might then result in doubling the reproductive performance. In conclusion, the allocation of resources to reproduction in aphidophagous coccinellids appears to be independent of size (Stewart et al., 1991b).

In accordance, both predaceous ladybird - *Anatis ocellata* (Linnaeus, 1758) (Coleoptera: Coccinellidae), which is the largest of the ladybird species and found mainly associated with coniferous trees, and the relatively small species *Adalia 10-punctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae), which is equally specialised and found mainly associated with oak trees - were investigated to compare the relative reproductive performance and the number of ovarioles relative to their body size and type of habitat. It was found that *A. ocellata* developed a higher number of ovarioles - 56 in comparison with the 20 ovarioles of *A. 10-punctata*. Fewer eggs were laid by the larger ladybird than by the smaller one, but the allocation to reproduction did not vary significantly. This supported the suggestion of Stewart et al. (1991a) that all coccinellids, irrespective of the habitat that each occupies, tend to assign the same proportion of nutrients to reproduction, and either lay a few large or many small eggs. This further contradicts the general proposition that stated that the maximum clutch size laid by a female ladybird is limited by the number of ovarioles within an ovary and capacity (Ware et al., 2007, Ware and Majerus, 2008).

This scenario is, however, entirely different with butterflies. A study performed on different butterflies contradicted the above findings and showed that large females allocated a greater proportion of their body weight to the abdomen at eclosion when compared with small females (Karlsson, 1987, Wickman and Karlsson, 1989). Fischer et al. (2003ab) noted that in butterflies there was a very weak correlation between female mass and fecundity. In accordance with the above, it was shown that larger females of the red flour beetle *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) invested more in reproduction compared with smaller ones relative to their body size (Arnaud et al., 2005). In addition, larger females laid eggs that were approximately 3.5 times larger, but the developmental time for eggs did not vary significantly when compared with the eggs laid by smaller females. It is likely that there is a minimum egg size that is necessary to maintain egg viability (Arnaud et al., 2005).

Schmalhausen (1949) and Matsuda (1987) proposed that most cases of adaptive plasticity may underline non-adaptive physiological responses that were eventually shaped by natural selection leading to either continuous or discontinuous adaptive plasticity. This proposal seemed applicable for many cases of insect reproductive plasticity. Therefore, as a conclusion, insect reproductive plasticity is relevant to ecological and evolutionary, physiological and ontogenetic foundations. These backgrounds interact with insect ecology to mould the evolution of insect reproduction and determine its plasticity (Schmalhausen, 1949, Matsuda, 1987, Hodin, 2009).

1.10.2 Egg size

Egg size is a function of adult weight since egg size multiplied by egg number is directly proportional to the adult weight (Wickman and Karlsson, 1989). Stewart et al. (1991ab) noted that egg size is determined by the ratio of body weight to ovariole number such that large species with a greater number of ovarioles per unit body mass may lay small eggs and *vice versa*. A similar association was reported for aphids (Dixon, 1987), *Palpada mexicana* (Macquart, 1847) (Diptera: Syrphidae) (Gilbert, 1990) and speckled wood butterfly, *Pararge aegeria* (Linnaeus, 1758) (Lepidoptera: Nymphalidae) and the wall brown butterfly, *Lasiommata megera* (Linnaeus, 1767) (Lepidoptera: Nymphalidae) (Wickman and Karlsson, 1990). Therefore, for those species, reproductive activity increases in direct proportion to body weight. For example, it in butterflies (Lepidoptera: Papilionoidea, Hesperioidea), fecundity is highly correlated to adult body size, and there is evidence for trade-off between egg size and number relative to adult size across species (Garcia-Barros, 1998).

Egg size is an important life-history trait because it affects both maternal and offspring fitness including initial offspring size (Fleming and Gross, 1990). Egg size remained constant over a broad range of body sizes in many insects' species as noted by (Fitt, 1990) such as in eight species of *Dacus* (Diptera: Tephritidae) (Fitt, 1990) the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann, 1830) (Diptera: Calliphoridae) (Webber, 1955), *D. melanogaster* (Roff, 1981) and *Brachinus lateralis* (Boulenger, 1900) (Coleoptera: Carabidae) (Juliano, 1985). In aphidophagous coccinellids, egg size is independent of clutch size within a species however, interspecifically clutch size is directly proportional to ovariole number (Stewart et al., 1991ab). Life-history theory assumes that limiting resources lead to trade-offs between different structures and activities (Berrigan, 1991, Messina and Fox, 2001). Given a fixed amount of resources obtainable for reproduction, there must be a trade-off between the number and size of offspring leading to a balance between selection for large eggs and high number of eggs (Bernardo, 1996, Messina and Fox, 2001). In addition, despite the many reports regarding intra-specific trade-off between egg size and number in Arthropods, no highly significant correlations were observed in any species (Fischer and Fiedler, 2001ab, Fox and Czesak, 2000). Stearns (1992) noted that the trade-off between size and number of offspring is largely noticeable in semelparous species (capital breeders) on the other hand in iteroparous species (income breeders) it is much harder to identify such relationships (Stearns, 1992, Fox and Czesak, 2000).

Stewart et al., (1991a) noted that the trade-off between egg size and number is manifest as species differences and habitat characteristics in which the species occupy. For instance, in butterflies, body size increases in relation to the mean annual temperature of the species geographic range, whereas the size decreases in relation to any elevation in aridity (Garcia-Barros, 1998). These changes are likely to affect the relationship between fecundity and body size. In addition, Stewart et al., (1991a) stressed that the variability in the size of egg does not always account for enhanced larval growth conditions favouring small offspring and *vice versa*. In coccinellids there is a lower limit for egg size constrained by the size of the newly hatched larva that is able to capture aphid prey easily. Thus, larval capture efficiency is largely a function of size (Dixon, 1959, Wratten, 1973, Mills, 1979). Thus aphidophagus ladybirds have relatively similar sized eggs of approximately 0.115 mg (Stewart et al., 1991ab).

Despite the plasticity in ovariole number of aphidophagus ladybirds, the number of ovarioles imposes more constraints on the body-egg-size relationship (Stewart et al., 1991ab). In addition, the suitability of oviposition sites may also influence the rate of egg laying by ovipositing females. Females of the monandrous butterfly, *P. aegeri* usually prefer to lay larger eggs on good quality host plants selectively (Gibbs et al., 2005). Gibbs et al. (2005) suggested that the population of this species is adapted for a flexible oviposition strategy, ruled by external clues such as plant variety and the appearance of males. This strategy promotes the trade-off between egg size and number particularly during unfavourable conditions.

Likewise, the developmental time of eggs might also be, in essence one of the characteristic of reproduction that has been shown to control egg size (Stewart et al., 1991ab). The developmental time is known as the function of the ratio of adult size to egg weight (Stewart et al., 1991b). For instance, the development of a large-sized progeny from a large egg would take a generally faster time to hatch into a larger-sized adult (Stewart et al., 1991ab). Nevertheless, it was revealed that adults develop from larger eggs will possess higher survivorship compared with the progeny developing from smaller eggs (Stewart et al., 1991ab). Therefore, for a particular species and every population, there is an optimal balance between three main reproductive traits, the size of eggs, the potential fecundity and the developmental time (Fleming and Gross, 1990, Fox, 1994 ab). For instance, *Tribolium audax* (Triplehorn, 1968) (Coleoptera: Tenebrionidae) is a small beetle known to oviposit large sized-eggs (Garcia-Barros, 1998). Garcia-Barros (1998) suggested that the total developmental time to adulthood was very short despite the large eggs due to the physiological constraints which could reflect a recent adaptation to the environment. It may be an advantage of having shorter developmental time in order to reduce pupal cannibalism. Another purely correlative explanation for such behaviour is that producing small eggs instead of a few large ones is likely to decrease female fitness in this species due to the increase in offspring developmental time (Park et al., 1965, 1970). A comparative study on another species showed that, large butterflies (Papilionoidea) tend to have larger eggs with longer larval developmental period resulting in a large sized-adult. In conclusion, the vast difference in body size and habitat type preference among different species and population encourage the inter- and intra-specific variations notably in the association between egg size and number as well as larval developmental time (Garcia-Barros, 1998). Wilbur (1977) noted that for each species egg characteristics may signify an optimal egg size at which sustainable larvae can be produced and this is likely determine the degree of reproductive investments toward producing many eggs.

The phenotype of an individual is usually influenced by genetic make-up, environmental conditions and the phenotype of the parents and their environment (Mousseau and Fox, 1998). The parental effects for instance are often consequences of variation in propagule size or the clutch size (Fox et al., 1997). A recent study showed that the variable offspring could be attributed to maternal age, female body size and survival cost of reproduction (Kindsvater, 2010, 2011). In some cases, such as in highly fecund insects, size constraints may not have an influence on the size of laid clutch (Marshall et al., 2010). Sometimes, old females experiencing better habitat conditions are able to produce larger and many offspring (Plaistow et al., 2007). The size of an egg may also be constrained by physical restriction according to the shape of the ovipositor (Fitt, 1990, Bernardo, 1996). In addition, it was noted great variability in offspring are the consequences of the association between parental effects, physiological and ecological factors (Brown and Shine, 2009). Therefore, carefulness should be considered when inferring the outcome of offspring-size from different mothers that are reared under the same conditions (Marshall et al., 2010). Kindsvater et al. (2011) stressed that maternal investment strategies in reproduction and the related survival costs have great part in influencing the relationship between offspring size and number.

1.11 Food stress and fitness

Resources are frequently heterogeneous in time and space (Stearns, 1992). Therefore, animals often alter their developmental and reproductive performances according to the existing conditions, in order to maximise their fitness (Agarwala et al., 2008). Resource competition is generally considered to be the key factor shaping the structure of insect communities and constraining the growth of populations (McPeek, 1996, Morris et al., 2005). Limited food potentially affects inter- and intra-specific competition, cannibalism and intra-guild predation in particular circumstances (Wise, 2006). If food scarcity is seasonal and predictable, insects deal with it by engaging in diapause-mediated responses, such as dormancy or seasonal migration (Tauber and Tauber, 1993, Phoofolo et al., 2008).

Life-history traits such as larval growth and fecundity are often greatly influenced seasonally and in sporadic habitats (Agarwala et al., 2001). In addition, it is often assumed that larval food stress reduces lifetime fitness, regardless of the conditions subsequently experienced by adults. Several hypotheses have been postulated for testing the effect of starvation on lifetime fitness. The 'environment-matching hypothesis' states that the plastic developmental response towards poor nutrition often results in a phenotype that is better adapted to future starvation than one developed in high food abundance (Monaghan, 2008). In contrast, the 'silver spoon hypothesis' states that favourable juvenile growth conditions lead to higher adult fitness and animals tend to mature at a larger body size (Rowe and Ludwig, 1991, Honek, 1993). Dmitriew and Rowe (2011) noted that contrary to the former hypothesis, food stress during the larval period of *H. axyridis* did not result in adults that were better suited to continuing food stress. In addition, the reproductive rate was invariably lower in females of *H. axyridis* reared during larval stages at low food, regardless of whether the adults were well fed or food stressed. Nevertheless, most of the adults lost more body weight during a period of starvation in the adult stage compared with the weight they lost when they were starved as larvae. The variation in weight was due to the accelerated growth that took place when larvae were food stressed. Furthermore, accelerated growth often leads to an increased risk of starvation during subsequent periods of food stress (Dmitriew and Rowe, 2011).

Honek (1993) noted that at food deprivation, the fecundity and body size relationship varies according to size of females. Several studies have recorded that fecundity decreases when food is limited, depending on maternal size, such as in the ladybird beetle, *H. axyridis* (Hodek and Honek, 1996, Grill et al., 1997, Obrycki et al., 1998). The large amount of energy expended to maintain a large body size over reproduction may be the main reason (Reznick, 1985). Another study has revealed that the fecundity of small-sized females of the web spider, *Dolomedes trion* (Walckenaer, 1837) (Araneae: Pisauridae) was unaffected by food availability, in comparison with the large-sized females, which failed to attain their potential fecundity under similar conditions (Spence et al., 1996). Several studies have mentioned that larvae reared on limited food tend to develop into smaller adults, such as the larvae of the aphidophagous predator, *Menochilus sexmaculatus* (Fabricius, 1781) (Coleoptera: Coccinellidae) (Ng, 1991).

Food stress during the larval development of the predatory ladybird *C. septempunctata* resulted in significant variations in the rate of egg production, as well as clutch size (Dixon and Guo, 1993). In addition, several studies on aphidophagous beetles have revealed that at limited food levels, larvae tended to develop at a slower rate (Kaddou, 1960, Smith, 1965, Ng, 1991, Agarwala et al., 2001) and the adult survivorship was reduced. Other life-history traits, such as the pre-reproductive period, reproductive period, and longevity, were also influenced by the limited food, as females spent more energy on basic physiological process and body maintenance (Agarwala, et al., 2001, Agarwala, 2007). For example, in unpredictable environments, females of waterstrider, *Gerris thoracicus* (Schummel, 1832) (Heteroptera: Gerridae) tended to reduce their reproduction during food stress, so that they maintained long reproductive lifespans (Kaitala, 1991). Likewise, female coccinellids often decrease their rate of oviposition when aphids are unavailable so that their offspring will not be forced to develop under low food availability (Banks, 1955, Evans and Dixon, 1986).

Despite the reduction in fecundity and clutch size, the average egg size tends not to change under severe food limitation (Dixon and Guo, 1993). When larvae and adults of *C. septumpunctata* were exposed to food stress, there was a large variation in clutch production rate and in clutch size (Dixon and Guo, 1993). The average egg size, however, was not significantly affected by the absence or the range of the aphid (Dixon and Guo, 1993). Phoofolo et al. (2008) noted that a reduction in the percentage of weight loss in response to starvation was likely to depend on the species, the length of food deprivation and the age when larvae are starved. In one such example, larvae in their fourth stadium belonging to three ladybird species, *Coleomegilla maculate* (De Geer, 1775) (Coleoptera: Coccinellidae), *Hippodamia convergens* (Guérin-Méneville, 1842) (Coleoptera: Coccinellidae) and *H. axyridis,* - were starved for variable periods of time. The results revealed that larvae did not vary in their starvation resistance (Phoofolo et al., 2008).

Hoffmann and Parsons (1991) noted that the differences in resource quality are considered important for determining competitive consequences among species that belong to the same trophic guild. Likewise, Omkar and Srivastava (2003) noted that food quality may affect the length of the oviposition period in aphidophagous ladybirds. Evans and Gunther (2005) observed that the link between food consumption and reproduction is not always straightforward in polyphagous predators, since their reproductive output is mainly linked to consumption of a specific prey. For example, the polyphagous predator, *H. axyridis* laid more clutches when they were placed on a diet of pea aphid, *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae) compared with a diet comprising larvae of the alfalfa weevil, *Hypera postica* (Gyllenhal, 1813) (Coleoptera: Curculionoidea) (Evans and Gunther, 2005). Likewise, larvae of *C. septempunctata* reared on the mustard aphid, *Lipaphis erysimi* (Kaltenbach, 1843) (Homoptera: Aphididae) and another set of larvae were kept on an artificial diet consisting of yolk, sucrose, honey, casein and protein hydrolyzate (Sarwar and Saqip, 2010). It was revealed that the predator larvae completed their development at a normal rate from egg to adult in 20.6 days when reared on aphid prey, but in 29 days when reared on the artificial diet. Artificial foods can be a good substitute for natural prey at aphid prey scarcity, but it may not plausible to expect the same reproductive activity and developmental rate (Sarwar and Saqip, 2010).

In conclusion, phenotypic plasticity in reproductive behaviour is an important life-history trait, particularly during varying food conditions (Kaitala, 1991). A long reproductive period is scarified when conditions are favourable and maintained with an increase in the number of generations. At unpredictable varying environmental conditions, any significant changes in fecundity, however, will be conveyed as costs in other life-history traits, such as longevity (Kaitala, 1991). In addition, the above sections demonstrate how species are able to cope and resist acute food stress and survive new climatic conditions (Phoofolo et al., 2008).

Cannibalism is another adaptive behaviour that occurs in response to any changes in timing of oviposition or food availability (Osawa, 1989). It has been widely recognised that in populations of arthropods, sibling cannibalism provides stability and persistence when food is available (Fox, 1975, Polis, 1981, Osawa, 1992). Under severe food stress, sibling cannibalism for both fertile and infertile eggs often occurs, causing intense competition over food and resulting in a rapid decrease in larval survival rate (Osawa, 1992).

Reduction in metabolic rate has been known to be an important response of stress resistance (Barros et al., 1991). It has been suggested that a reduction in metabolic rate is an important physiological mechanism that occurs in order to conserve metabolites, such as carbohydrates and fats (Hoffmann and Parsons, 1989). Hoffmann and Parsons (1989) reported that increased tolerance to a range of environmental stresses will be associated with a reduction in the metabolic rate in many organisms, which might affect resource allocation as well as correlations between life-history traits. Food stress during both development and growth affect the relative resource allocation and the constituents of adult phenotypes (Gotthard et al., 1994). Therefore, the adult phenotypes - including body composition, allocation decision and the resultant allomeric relationships - are manipulated to match the expected environmental conditions (Gotthard et al., 1994, Scharf et al., 2009). For instance, semi-starvation of the butterfly, *Speyeria mormonia* (Boisduval, 1869) (Lepidoptera: Nymphalidae) resulted in many costs associated with body maintenance, such as a significant alternation in the allometric relationship between body mass and wing length in females. This may result in changes to the flight performance and related costs. In addition, a significant decrease in the potential fecundity has been noted, accompanied by a reduction in females' body mass and the relative fat content (Boggs and Freeman, 2005).

Sibley and Calow (1989) suggested that the types of stress that may lead to a great diversity in phenotypic responses were dependent on the species. For example, *D. melanogaster* showed a reduction in metabolic rate during starvation, but not towards desiccation (Djawdan et al., 1998). Another study showed that adults of *H. axyridis* displayed different phenotypes when reared at different temperatures. Lombaert et al. (2007) stressed that caution must be taken when selecting environmental parameters that will be implemented for assessing phenotypic plasticity variations under stress conditions.

One of the most studied trade-offs associated with food deprivation is that between reproduction and longevity, as reported in several species of insects (Dixon and Kundu, 1997, Omkar and Mishra, 2005, Agarwala et al., 2008). When food is scarce, female longevity in the waterstrider, *Gerris buenoi* (Kirkaldy, 1911) (Hemiptera: Gerridae) nearly doubled (Rowe and Schudder, 1990). In contrast, Blanckenhorn et al. (1995) reported that longevity for both sexes of the water strider *Aquarius remigis* (Say, 1832) (Hemiptera: Gerridae) increased with moderate food supply. The association between the level of food and longevity is not that simple, particularly when food is supply at moderate and higher levels (Blanckenhorn, 1994). Likewise, Omkar and Srivastava (2003) suggested that the trade-off may only become apparent when organisms are food stressed, such in the predaceous ladybird, *Adalia bipunctata*. Recently, Kindsvater et al. (2011) stressed that the costs of reproduction can better predict various types of correlations between females longevity and fecundity when considering age, size and nutritional conditions.

When food is abundant, fecundity is positively correlated with female body weight in many taxa (Beck and Connor, 1992, Honek, 1993, Preziosi and Fairbairn, 1996). It has been revealed that during limited food conditions, the influence on fecundity depends predominantly on the size of the females (Spence et al., 1996). For instance, the fecundity of the small web spider, *Dolomedes trion* (Walckenaer, 1837) (Araneae: Pisauridae) was not influenced by the availability of food; in contrast, large-sized females could not accomplish their prospective reproductive activities when they were deprived of food (Spence et al., 1996).

Another important factor that has been found to affect the relationship between female reproduction rate and fecundity is the lifetime period of a species - that is, whether it is a short- or long-lived individual (Rana et al., 2002, Agarwala et al., 2008). A positive correlation was found between fecundity and longevity for *H. axyridis* females that had a shorter lifespan compared with those that lived relatively longer (Rana et al., 2002, Agarwala et al., 2008). In addition, during limited food conditions, larvae of the predaceous ladybird *H. axyridis* tend to develop slower relative to the control. Surviving adults of females developed into smaller individuals, and their pre-reproductive period was considerably prolonged. In addition, the reproductive period and fecundity were reduced, but longevity increased significantly. The relationship between longevity and lifetime fecundity of females of *H. axyridis* varies intra-specifically, according to the type of lifespan and is determined by the body size (Agarwala et al., 2008).

It is broadly recognized that any increase in reproductive effort tends to reduce the longevity of insects, even when food is normally available (Stearns, 1992, Blanckenhorn, 1994, Blanckenhorn et al., 1995, Dixon and Kundu, 1997). This association might not be, however, apparent in some species (Ohgushi, 1996), particularly in females that are characterised by a sperm-limited fecundity (Omkar and Mishra, 2005). For such cases, multiple mating allows those females to express their full reproductive capacity, which rarely influences their longevity (Arnqvist et al., 2005). Furthermore, the environmental conditions experienced by parents have a significant influence on their offspring in some cases (Taborsky, 2006). The potential effect of the maternal adaptive phenotypes can extend through generations and modify the selective balance for traits that possess a potential influence on offspring life-fitness (Rossiter, 1991, Mousseau and Dinglt, 1991).

Furthermore, the ovarian physiological status could have a negative effect on longevity and mortality rate (Carey et al. 2002). A physiologically young ovary might demonstrate a decrease in mortality rate, compared with an old ovary that performs ordinarily during full access to food. Dixon (2000) noted that the reproductive output is often determined according to the individual age and senescence, as well as food quantity and quality (Omkar and Pervez, 2003). Old females are less efficient at assimilating food into eggs, leading to a decline in fitness components, including fecundity and longevity (Partridge, 1987, Dixon and Agarwala, 2002). Dixon and Agarwala (2002) explained that the role of fecundity is well-shaped by senescence, in that reproductive output is customarily maximised at early stages of adult life and then tends to decline with increasing age. Similar observations were also reported by Omkar et al. (2006) and Omkar and Prevez (2003). Not all age-related declines in reproductive activities are elicited, however, by senescence; injuries and the related incidence of damaged organs tend to occur more in older females (Crudgington and Siva-Jothy, 2000).

Generally, reproduction incurs a cost for both sexes (Dixon, 2000). The cost of reproduction on females, however, has been more recognised (Carin, 1991, Tatar et al., 1993, Paukku and Kotiaho, 2005). Receiving harmful effects from male ejaculate and parental provisional activities constitutes the major costs on reproduction capacity (Hunt et al., 2002, Kotiaho and Simmons, 2003). A study has showed that in the three species of horn-dimorphic dung beetles - *Onthophagus taurus* (Schreber, 1759), *Onthophagus binodis* (Thunberg, 1818) and *Onthophagus australis* (Guérin-Méneville, 1830) (Coleoptera: Scarabaeidae) - male mating success was affected by the courtship rate. The courtship rate was condition dependent, such that males in poor conditions had lower courtship rates compared with those that had been manipulated to be in good food conditions (Kotiaho, 2001). Kotiaho (2001) noted that courtship rates and sexual activities generally were capable of significantly reducing the longevity of males.

As the trade-off between fecundity and longevity appears to be a rather complex area and might be influenced by ecological conditions, future studies to understand life-history responses of organisms should embrace evaluations of a varying degree of food limitations and mating behaviour, both intra- and inter-specifically (Mohammed and Van Emden, 1989, Helden et al., 1994). Environment-induced variability in life-history traits plays a key role in determining population dynamics, particularly during the invasion of exotic species (Obrycki et al., 1998). The occurrence of small-sized predatory ladybirds in the field is highly common (Obrycki et al., 1998). This backs up the prediction that ladybird beetles that are able to live and survive in habitats with limited food and harsh environmental conditions often live in habitats that are limited by food (Dixon, 2000). Therefore, if some aphidophagous beetles manage to overcome and survive these unpredictable conditions, they assert their efficiency as potential competitive predators and their capability to become a successful invader (Obrycki et al., 1998).

1.12 Competition and oviposition deterrence pheromones

The 'optimal oviposition theory' predicts that prey quality affects female ladybirds' oviposition preferences (Charnov and Stephens, 1988). It has been found that patch quality may be assessed by aphid species, aphid density (Dixon, 1997, Kalushkov and Hodek, 2004) and the presence of intra- or inter-specific competitors (Burgio et al., 2002). Females of several ladybird species often refrain from laying their eggs in prey patches or host-bearing broods (eggs and larvae), or sites that have already been marked by con- or hetero-specific adults or larval tracks (Oliver et al., 2006, Magro et al., 2007). Such behaviour acts to diminish the level of competition among their offspring and other hetero-specific brood. The avoidance of contaminated hosts is usually mediated by cues or signals associated with a brood, such as marking pheromones (Nufio and Papaj, 2001), e.g. females of *H. axyridis* avoided oviposition close to the desirable aphid colonies that were already marked with oviposition-deterring pheromones (Yasuda et al., 2000).

Semio-chemicals are a mixture of hydrophobic alkanes (Omkar, 2004). It has been reported that some alkaloids exist also on the elytra, which aids in mate recognition and allocation (Hemptinne et al., 2000). Some of these alkaloids are found on the surface of the egg shell as a defence, contributing to a reduction in intra-guild predation, as in *A. bipunctata* and *C. septempunctata* (Agarwala and Yasuda, 2001, Hemptinne et al., 2001). Oviposition-deterring pheromones have been identified among various insect taxa, including in Coleoptera, Diptera, Hymenoptera, Lepidoptera and Neuroptera (Prokopy, 1981ab, Van Lenteren, 1981, Roitberg and Prokopy, 1987, Landolt and Averill, 1999). Prokopy (1981a) noted that the behavioural consequences after recognition of the oviposition-deterring pheromones involve a lessening in the time consumed by females on previously exploited resources, accompanied by a decrease in oviposition activities. Nufio and Papaj (2001) demonstrated stereotypical behaviours of females directly following the oviposition event, such as dragging the ovipositor on the host resource while depositing a clear liquid.

Females of aphidophagous, phytophagous and parasitoid insects usually perceive oviposition-deterring semio-chemicals, through several kinds of sense organs, such as the olfactory chemo-receptors (Rûzicka, 2003). Three types of chemo-receptors have been recognised by Jourdan et al. (1995) - gustatory, mechanoreceptors and olfactory receptors. The total number of these receptors is usually high in polyphagous species compared with oligophagous or monophagous and they are fewer in coccidophagus coccinellids (Jourdan et al., 1995). These receptors differ in their purpose and structure between sexes. For instance, the antennae of a predatory beetle, *Trogossita japonica* (Reitter, 1875) (Coleoptera: Trogossitidae), exhibited sexual dimorphism in structure, types and quantity of receptors, signifying their differential functions in males and females (Usha Rani and Nakamuta, 2001). Parasitic and phytophagous insects are known to deposit chemical signals subsequent to egg oviposition that alter the behaviour of conspecific individuals, who accordingly avoid laying future eggs into formerly exploited host resources (Nufio and Papaj, 2001). These signals have been named oviposition-deterring pheromones, marking pheromones or epideictic pheromones (Prokopy, 1981ab, Roitberg and Prokopy, 1987, Nufio and Papaj, 2001). In phytophagous insects, chemical or physical changes in hosts - including the release of plant compounds associated with oviposition activities or destruction of plant tissue by larvae or adults - will aid in assessing the conspecific brood (Fitt, 1984, Landolt, 1993, Heard, 1995). Similarly, oviposition by entomophagous parasitoids may trigger changes in a host's haemolymph composition that may be used to distinguish unparasitized from parasitized hosts (Fisher and Ganesalingam, 1970, Vinson and Iwatsch, 1980, Ferkovich et al., 1983).

Most studies have considered physical and chemical interference in larvae and not that of adults (Doumbia et al., 1998). For example, Hemptinne et al. (1992) studied the oviposition avoidance of coccidophagous ladybirds in the presence of con-specific larvae. Likewise, Rûzicka (2003) studied the ability of the aphidophagous coccinellids, *Cycloneda limbifer* (Casey, 1899) (Coleoptera: Coccinellidae) and *Ceratomegilla undecimnotata* (Schneider, 1792) (Coleoptera: Coccinellidae) to discriminate between those sites contaminated with oviposition-deterring larval track and clean ones. To date, few studies have examined the effects of the physical interference of con-specific adults on the reproductive performance of the aphidophagous, *Propylea dissecta* (Mulsant, 1850) (Coleoptera: Coccinellidae) (Mishra and Omkar, 2007). More knowledge on the action of pheromone chemistry will be of great assistance in constructing hypotheses on cross-recognition and even to distinguish between instances in which insects utilize cues associated with brood presence. In addition, it will help in evaluating intra-guild competition and the displacement of native species chiefly during invasion (Nufio and Papaj, 2001).

1.13 Thesis objectives

The predaceous ladybeetle *Harmonia axyridis* was considered as an excellent candidate to be studied in this project because of its various unique functional traits. It is extremely important to have a good representation of each developmental life-stage of this insect, since it has been categorised as “the most invasive ladybird in the world” (Tedders and Schaefer, 1994, Kuroda and Miura, 2003). Because ecologists often fail to detect the likely successful invaders prior to establishment (Snyder et al., 2004), there is a necessity to apprehend the underlying biological, physiological and morphological features in order to give a satisfactory interpretation for that success (Keane and Crawley, 2002, Shea and Chesson, 2002). This project focused on evaluating the various life-history traits of this predaceous beetle. Its fundamental focus was to understand the reproductive tactics and developmental parameters, in particular. Several investigations were conducted to estimate the above by addressing the following: (1) intra-specific variations in body size between and within genders; (2) the significant variations in developmental aspects and growth rate among developing juveniles; (3) the reproductive capacity of females measured under a controlled diet and reproductive plasticity in response to manipulated diet regimes; (4) pupal colour polymorphism evaluated at field conditions and under controlled laboratory conditions; (5) the effect of mating rate manipulations on the reproductive performance, egg hatchability and longevity; and (6) behavioural strategies of *H. axyridis* adults in evading the presence of con-specific and hetero-specific larval and adult tracks.

The objectives of each chapter are as follows. Chapter 2 has been designated to giving a broad description and thorough background into various life-history traits of *H. axyridis*. Body size and parts' allometric relationships are described, as well as sexual size dimorphism. Chapter 3 sets out to gain a detailed understanding of the reproductive capacity of *H. axyridis* reared on one of its essential diets and maintained at constant laboratory conditions. The reproductive potentials have been evaluated by determining life-time fecundity, fertility, egg and clutch size parameters, and oviposition period, as well as counting the number of ovarioles and testicular filaments. Investigations in Chapter 4 focus on the study of pre-imaginal development and immature survival under a controlled diet regime as well as laboratory conditions. The key aim was to assess the presence of intra-specific inconsistency and variability in respect to various developmental and growth qualities, such as the two estimates of the critical weight (maximum and minimum), total growth rate, length of larval period and total developmental period. Chapter 5 focuses on an inspection of pupal sexual dimorphism in *H. axyridis*, based on external surface pigmentation, under constant controlled laboratory parameters. This chapter aims to find a reliable consistent technique for sex identification based on pupal pigmentation prior to adult emergence. The objective of Chapter 6 is to estimate the influence of manipulating the rate of mating on reproductive output and longevity of females of *H. axyridis*. Females were exposed to different mating frequencies in order to measure the influences on life-time fecundity, egg hatchability and longevity. In addition, this will give an overview on the way this beetle manages to overcome variability of environmental conditions.

Chapter 7 evaluates various responses to a short-term period of limited diet at different stages of immature development. Short- and long-time costs on lifetime fitness of adults and their offspring has been assessed through measuring the constituents of fitness, including longevity, body weight, sex ratio, fecundity, fertility and percentage of mortality. Finally, Chapter 8 describes the avoidance approaches of *H. axyridis* adults in the existence of con- and hetero-specific adults and larval tracks. The significance of olfactory sense represented by antennae was tested by complete amputation for both sexes. The consequent impact of their absence on life-history traits such as body weight and longevity was determined. This may benefit in evaluating the role of sense organs and receptors in modelling the behaviour of females in optimising oviposition sites.

Finally, it is hoped that the present laboratory studies will provide a comprehensive background on physiological, morphological and behavioural-related qualities of the predaceous beetle *H. axyridis* that are often associated with its developmental features and reproductive performance. The results will help to clarify the reasons attributed to its ferocious invasion and persistence and the on-going successful establishment over wide areas.

Chapter 2

Sexual Size Dimorphism and Variability in Life History Traits of *Harmonia axyridis* Reared on a Constant Diet

2.1 Introduction

Sexual size dimorphism (SSD) is well defined as the morphological differentiation of sexually mature males and females (Blanckenhorn, 2005). It is a widespread phenomenon occurring widely across the animal kingdom (Badyaev, 2002, Blanckenhorn, 2005) and even among populations of a single species (Pearson et al., 2002, Krause et al., 2003). The direction and degree of SSD is highly variable among populations and within taxa (Stillwell and Davidowitz, 2010). It was reported that SSD tends to decrease with body size in species where females are the larger size sex and increases with body size where males are the larger individuals (Blanckenhorn, 2005). Male body size varies more among populations and species, or evolutionarily deviates more rapidly than female body size, irrespective of which sex is larger (Abouheif and Fairbairn, 1997).

This phenomenon of SSD was designated as Rensch's rule by Abouheif and Fairbairn (1997) and Fairbairn (1997). Fairbairn and Preziosi (1994) were the first to posit that sexual selection for large male size was regarded as the primary force driving this allometric conformation in SSD. Subsequently, extra evidence for this proposition was found (Abouheif and Fairbairn 1997, Fairbairn, 1997, Székely et al., 2000, 2004, Kraushaar and Blanckenhorn, 2002). Much of the variation in SSD is partly genetically based and has been viewed as resulting from sexual differences in selective pressures on adult body size (Stillwell and Fox, 2007, 2009). Fecundity selection has been suggested to be the main force generating female-biased SSD (Teder and Tammaru, 2005), sexual selection has however, often been elicited in cases of male-biased size dimorphism (Isaac, 2005). Abouheif and Fairbairn (1997) stated that Rensch's rule predicts that SSD (size of the larger sex/size of the smaller sex) will be correlated positively with mean body size (hyperallometry) in taxa in which males are the larger sex and negatively correlated with mean body size (hypoallometry) in taxa in which females are the larger sex.

Female-biased sexual dimorphism has been recorded in most invertebrates and poikilothermic vertebrates. For instance, female-biased sexual dimorphism was reported at between 72-95% in insect species (Shine, 1979, Arak, 1988, Teder and Tammaru, 2005), such as aphids (Braendle et al., 2006), butterflies (Arikawa et al., 2005), parasitoids (Harvey, 2003) and ladybird beetles (Dixon, 2000, Yasuda and Dixon, 2002). An exception is the Odonata, of which only 27% of species show female-biased SSD and the remaining species, particularly the damselflies (Zygoptera), displayed male-biased or no SSD.

Male-biased SSD predominates only among birds (Weatherhead and Teather, 1994) and mammals (Lindenfors and Tullberg, 1998, Székely et al., 2000) where males are found to be much the larger of the species and may weigh two to eight times as much as females (Cabana et al., 1982, Bjorklund, 1990, Székely et al., 2000); for instance, in the order Primates, the fin-footed mammal, the pinniped, which belongs to order Carnivora and to the families, Odobenidae, Otariidae and Phocidae. Furthermore, in ungulates, the mean ratio of the average mass of males to females approached 2.98 (Weckerly, 1998), with some males weighing up to eight times as much as the females (Fairbairn, 1997). In addition, in, the Hawaiian monk seal, *Monachus schauinslandi* (Matschie, 1905) (Carnivora: Phocidae)

(Ralls, 1977, Weckerly, 1998), the majority is male-biased, but the rest is female-biased. Despite the predominance of male-biased sexual dimorphism, exceptions exist such as the yellow-pine chipmunk, *Tamias amoenus* (Allen, 1890) (Rodentia: Sciuridae) from Kanansakis Valley, in which the females are significantly heavier (10-20%), have a 4% longer body and a skull 0.9% longer and 2.2% wider than male chipmunks, and the overall size of skeletal tissue or structural parts is larger (Kenagy and Barnes, 1988, Levenson, 1990, Schulte-Hostedde and Millar, 2000). In mammals, possessing a larger body size (or the opposite) can incur substantial advantages for both sexes. Larger females can deliver a greater number or larger-sized offspring and provide more nutritious milk. Large-sized mothers are likely to provide better maternal care than smaller mothers (Ralls, 1976). Smaller males are capable of spending more time searching for a mate and less time on feeding because of lower absolute energy requirements (Blanckenhorn, 2000). Nevertheless, smaller males may have great mobility and be more agile (Trombulak, 1989).

In insects, there are three key ways to attain larger body size: laying large offspring, a longer developmental period, and having a faster growth rate. These approaches are likely to incur costs that entail substantial trade-offs (Roff, 1980, 1992, Nylin and Gotthard, 1998). For instance, laying large offspring may lead to an increase in parental investment, as described above. The longer developmental period is likely to increase the cumulative mortality rate due to predation and the faster growth rate may raise the metabolic demand as well as the foraging behaviour, resulting in an increase in the rate of predation (Roff, 1992, Wiklund and Karlsson, 1988, Nylin and Gotthard 1998, Gotthard, 2000). While SSD has been extensively studied (Dunn et al., 2001, Karubian and Swaddle, 2001, Blanckenhorn, 2005) the proximate mechanisms generating it have received considerably less attention predominantly in the invertebrates (Badyaev, 2002). For example, sex variability in body size plasticity is still under discussion: patterns of heritability are analogous between sexes because both share identical genes controlling the growth rate and developmental features (Lande, 1980, Reeve and Fairbairn, 2001). The variation between males and females in the ideal phenotype that is favoured by selection can be determined by the evolution of differential gene expression in the two sexes (Ellegren and Porsch, 2007). Sex-biased genes show unusually rapid sequence evolution, are frequently labile in their form of expression, and are non-randomly distributed in the genome (Ellegren and Porsch, 2007). Genes that are male-biased in their expression have on average more intra- and interspecific divergence in expression than those with female-biased expression. This was verified when it was recorded that the divergence among amino acid sequences in the male reproductive tract of *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae) and gene expression evolve more rapidly than in a female (Meiklejohn et al., 2003).

Intraspecific body size variations are arbitrated by differences in organism growth and development characteristics mediated by endocrine regulators operating at molecular, cellular and physiological levels (Badyaev, 2002, Nijhout, 2003). Growth to different sizes and display of sex-explicating plasticity in response to these factors is as yet poorly understood, mainly for invertebrates such as insects (Stillwell et al., 2010). The macroecological forms of body size variation among insects and their probable underlying mechanisms remain poorly recognised, unlike groups such as birds and mammals that have been intensively studied (Chown and Gaston, 2010). Many variables such as environmental factors (Teder et al., 2008) and diet quality and quantity (Stillwell and Fox, 2007) have been typically viewed as important elements inducing plasticity in body size and related life-history traits (Davidowitz et al., 2004). In summary, size difference is not just limited to the significance of the interactions concerning the genotype and environmental elements, but also relates to robust phylogenetic determinants (Smith et al., 2004).

There is extensive evidence among a number of taxa that both growth rate and development time can vary between the sexes and that this variation is usually associated with SSD (Shine, 1994, Teather and Weatherhead, 1994, Badyaev et al., 2001, 2002). In insects, the relationship between development time and SSD has been inferred either from the perspective of protandry, earlier male emergence, (Wiklund and Fagerström, 1977, Fagerström and Wiklund, 1982), or in the context of developmental constraints that presumably determine the correlation between body size and development time (Roff, 1992, Wiklund and Karlsson, 1988, Fairbairn, 1990). In both the above cases, SSD is thought to be associated with the variation of development time between sexes in which the larger sex develops for a proportionately longer time (Fairbairn 1990, Wiklund et al., 1991, Teather and Weatherhead, 1994). A study by Blanckenhorn et al. (2007) revealed that SSD is likely to be strongest in groups such as Lepidoptera, Hymenoptera, and Araneae, as well as mammals and birds in which quicker male development is of selective advantage. In some insects groups, however, the development time of males was equal to, or longer than, that of females. It was noted that on average, males are smaller than females in most of the taxa studied. This implies that faster growth rates of females in the majority of the investigated groups of arthropods species are highly important than development time differences in proximately mediating SSD (Blanckenhorn et al., 2007). In conclusion, in arthropods, for example in insects, the variation in SSD occurring at the adult stage entails that both males and females vary considerably in final body size, growth rate, developmental period and survival (Fairbairn 1990, Wiklund et al., 1991, Teather and Weatherhead, 1994, Blanckenhorn et al., 2007).

Allometry designates the changes in the relative dimensions of parts of the body that are correlated with changes in overall size. Allometric growth is described using the conventional formula: $y = bx^{\alpha}$ (Huxley and Teissier, 1936), where x and y are the two measured traits, $\log b$ is the intercept of the line on the y-axis and a is the slope of the line.

Allometry and its patterns have been defined in detail in the general introduction above. To date, four different concepts of allometry have been detailed: "Interspecific Allometry" refers to similar phenomena among related species; "Intraspecific Allometry" refers to variations in shape and developmental stage between individuals of the same species; "Ontogenetic Allometry" reveals any alterations in relative trait size through individual development; and "Evolutionary Allometry" refers to the character covariation among organisms between mean trait size and mean body size from several evolutionary lineages sharing a common ancestor within a single ontogenetic stage (Gould (1966) references phylogenetic allometry – that is, constant differential growth ratios in lineages) (Shea, 1985, Klingenberg and Zimmermann,1992ab, Arevalo and Heeb, 2005, Blanckenhorn et al., 2007). The first and second categories are commonly characterised as "Static Allometry" and the third and the fourth are known as "Dynamic" or "Truly Temporal" (Gould, 1966). Allometric growth, which is identified as the differences in relative growth rates among body parts, also accounts for intraspecific diversity perceived within taxa (Nijhout and Wheeler, 1996, Gayon, 2000). In holometabolous insects such as the coleopterans, the appendages of the adult do not grow constantly and/or synchronously as the larval body grows, but instead, just before the start of metamorphosis process the appendages undergo a period of accelerated growth (Huxley, 1931). This makes it problematic to infer patterns of relative growth rates and types of allometric relationships (Wilson, 1953, Nijhout and Wheeler, 1996). In fact, there are several remarkable consequences associated with allometry. First, the allometric relationship is not a direct outcome of the relative growth particularly when correlating body appendages with the overall body size. Second, certain sexual and numerous non-sexual traits, such as the ornaments and weapons, always exhibit positive allometric relationships (e.g., the horn length in the male dung beetle, *Onthophagus acuminatus* (Harold, 1880) (Coleoptera: Scarabaeidae) (Emlen, 1994) and the horn and wing in the giant rhinoceros beetle, *Chalcosoma caucasus* (Fabricius 1801) (Coleoptera: Scarabaeidae) (Kawano, 1995)). This has driven Kodric-Brown (2006) to construct a model presenting a universal pattern of positive allometry exhibited in such traits and generalised it. Third, some traits grow rapidly only just before metamorphosis altering the relative growth and the nutrient allocation (Emlen, 1994, Kawano, 1995).

One method of measuring the patterns of a trait or a character covariation is the allometry (Cock, 1966, Gould, 1966), as well as the multivariate generalisations that are proposed by Hopkins (1966). In these two approaches only one single factor or principle character is considered for allometric variations and the data point will be plotted and concentrated along straight line of log-transformed measurements; the variations can be described according to the direction of that line as negative or positive correlations (Gayon, 2000). These approaches do not give a full description for biological data sets, as they disregard measurement errors (Gibson et al., 1984, Bookstein, 1989). The history of the concept of allometry and its related problems are well detailed in Gayon (2000).

Limited studies have been carried out on several insect species to examine SSD directly after egg hatching, however, none of those have revealed any noteworthy variations in hatching size between the sexes (Esperk et al., 2007). In addition, most of the studies on SSD have focused on investigating large body sizes due to easier handling. Few studies, however, have examined sexual selection in small-sized species (Kasumovic and Andrade, 2009). Thus, this has narrowed our understanding of SSD patterns and its diversity among taxa (Stillwell et al., 2010).

The general aim of this study was to determine the validity of Rensch's rule by quantitatively assessing various allometric measures for SSD in *H. axyridis*. The allometric trends were quantified using regression method and log–log transformation. As mentioned in the general introduction, the proposed Rensch's rule, which is more common in taxa in which males are the larger sex. Several studies have suggested that allometry consistent with Rensch's rule occurs in response to sexual selection in relation to male size, in which 66% of taxa demonstrated male-biased SSD and only 48% of taxa demonstrated female-biased SSD (Clutton-Brock et al., 1977, Leutenegger, 1978, Webster, 1992, Fairbairn and Preziosi, 1994). Sexual selection is expected to occur more often in association with male-biased size ratios (Fairbairn and Preziosi, 1994). Allometry inconsistent with Rensch's rule has been documented in taxa in which females are the larger sex (Earhart and Johnson, 1970, Vollrath and Parker, 1992, Head, 1995).

Understanding the evolution and the maintenance of SSD involves first measuring and quantifying size dimorphism to verify that SSD actually exists. Various methods of measuring SSD have been used in the past in numerous studies among various taxa (Lovich and Gibbons, 1992, Ranta et al., 1994) but the results were imprecise. For instance, investigation of SSD and intraspecific variations in body size of mammals has focused only on differences in body mass (Sauer and Slade, 1987, 1989, Boonstra et al., 1993, Yoccoz and Mesnager, 1998). Interpreting intraspecific variation in body mass can be problematic, particularly when size dimorphism is substantially small, because body mass varies for two main reasons. First, it may reflect the size of skeletal tissue or structural body size, thus individuals with large body are still heavy (Dobson, 1992). Second, body mass may reflect internal body conditions in which heavy individuals may have higher stored metabolic tissues, such as fat (Dobson, 1992). Thus, the best descriptive method for evaluating SSD is through measuring structural body size including the length or the width of the body and wing, the length of the hind and the fore legs, the head and the pronotum and others. For instance, using the length of the hind foot in red squirrels, *Sciurus vulgaris* (Linnaeus, 1758) (Rodentia: Sciurus) (Wauters and Dhondt, 1989), and body length in Rodentia: Cricetidae, such as, *Microtus oregoni* (Bachman, 1839), *Microtus townsendii* (Bachman, 1839), *Clethrionomys californicus* (Merriam , 1890) and chipmunks *Tamias amoenus* (Allen, 1890) (Heske and Ostfeld, 1990, Levenson, 1990).

Biological invasions are having major ecosystem impacts on indigenous species and are thought to be the second foremost cause of natural biodiversity loss (Pimentel et al., 2000). There is a great need to understand the association between life-history traits of invasive species and the criteria of the invasive process (Labrie et al., 2006). This knowledge is essential to develop a better understanding of inter-specific competition within native species (Lanzoni et al., 2004). Ecologists often lack the capability to identify potential invaders and their ways of establishment (Snyder et al., 2004). Therefore, the underlying morphological, behavioural and ecological mechanisms explaining invasion success need to be evaluated carefully by incorporating many studies and experiments (Shea and Chesson, 2002, Kimberling, 2004).

Harmonia axyridis is a strong intra-guild predator displaying competitive interactions with native species (Felix and Soares, 2004, Snyder et al., 2004). This ladybird beetle dominates the aphidophagous guild in numerous agricultural ecosystems to some extent owing to its great fecundity, polyphagous feeding habit, aggressiveness, fast mobility, efficient predation, low susceptibility to infection, elaborate searching tactics and rapid larval development (Osawa, 2000, Michaud, 2002a b, Koch, 2003, Cottrell and Shapiro Ilan, 2003, Felix and Soares, 2004, Lanzoni et al., 2004). Among the factors that potentially contribute to the powerful dominancy of *H. axyridis* over native species is the possession of a large body size. By extension, large invasive species may gain a greater significant reproductive advantage over small native species (Tilman, 1999, Kajita and Evans, 2010). Despite the benefits body size offers as a superior intraguild species, the adverse impact of this beetle on agricultural habitats has been continuing to rise. *Harmonia axyridis*' population has been increasing and spreading dramatically every year harming non-target species and reducing crop production. In addition, the role of this beetle as a household invader especially with its aggressive behaviour could be an additional destructive aspect of invasiveness (Koch and Galvan, 2008).

Does the invasive species exhibit specific life-history traits that encourage its successful invasion and spread? It was revealed that *H. axyridis* is a suitable model to test this prediction, as its invasion has been far from instantaneous and its population is expected to show high adaptive phenotypic plasticity. The objectives of this study were to evaluate some of the structural traits of the beetle *H. axyridis* in particular, body and appendage size at hatching. In addition, it aimed to describe sexual dimorphism in overall body size and body parts. Allometric analyses were also performed in order to determine which structural traits best explain SSD in *H. axyridis* and to find out which trait is the species-specific and could be used for interspecific comparison with other species for a particular study. In this context, the study hypothesised that larvae reared under similar diet supply and environmental conditions involving temperature, humidity and lighting would still display SSD in overall body and body appendages at adult emergence.

In order to accomplish the above, the study evaluated the SSD in fresh body weight, and most of the structural body parameters - including body length and body width and length of right tibia, scutum width, wing length and wing width - as well as longevity. The study employed an analytic method to analyse the dimorphism index for each of the above-listed parameters to evaluate the relative sex differences between males and females that infer the variation in growth and developmental time. The study also re-evaluated the general validity of Rensch's rule on previously analysed species, which so far has been documented only in very few taxa (Abouheif and Fairbairn, 1997). Specifically, dragonflies (Odonata) (Johansson et al., 2005) and waterstriders (Hemiptera: Gerridae) (Andersen, 1997) were found to follow Rensch's rule. In contrast, tephritid fruit flies (Diptera: Tephritidae) (Sivinski and Dodson, 1992), caddisflies (Trichoptera: Hydropsychidae) (Jannot and Kerans 2003) autumn spider, *Metallina segmentata* (Clerck, 1757) (Araneae: Tetragnathidae) (Prenter et al., 1999) and the ladybird beetle *H. axyridis* (Coleoptera: Coccinellidae) (Dixon, 2000) did not follow Rensch's rule.

2.2 Materials and methods

2.2.1 *Harmonia axyridis* maintenance

Adult *H. axyridis* beetles were collected from naturally infested fields in Silwood Park South-east England, U.K. (National Grid References 41/944691). Sexing of beetles was performed based on the labrum and pro-sternum pigmentation (McCornack et al., 2007). Beetles were kept as pairs in a 9cm Petri-dish and were supplied daily with 2cm² tissue moistened with a 10% honey solution. A folded filter paper (42.5mm, Whatman) was also kept in each Petri-dish to create an oviposition substrate. The culture was maintained according to Kajita and Evans (2010) in a controlled temperature room at 20±1 ˚C, 70-75% RH, and a photoperiod of

L16: D8 under fluorescent lamps following the method proposed by Stewarts et al. (1992 a, b). The daily food provided was live adult pea aphids, *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae). Subsequently, all the clutches laid by each pair were kept separately in 9cm Petri-dishes and maintained in the same laboratory conditions until larvae started to emerge. Larvae were considered to have emerged when all legs were completely detached from their egg shell.

2.2.2 Aphid culture maintenance

The food provided for the ladybirds was a mixture of the pink (JP1) and the green (LL01) strains of pea aphid *A. pisum*. This particular aphid species is widely regarded as a suitable prey for *H. axyridis* and other species of ladybirds such as *A. bipunctata* (Dixon and Guo, 1993, Evans and Gunter, 2005). The aphid culture was maintained on broad bean plants *Vicia faba* (Linnaeus, 1753) (Fabales: Fabaceae) variety Aquadulce Claudia under the same conditions following Agarwala et al. (2008) and Kajita and Evans (2010).

2.2.3 Laboratory feeding

The aphids offered to each predator larvae depended on the developmental stage of the coccinellid according to the method detailed by Agarwala et al. (2008) and Kajita and Evans (2010), with a few modifications. The first instar larva was fed 15 first instar aphids, the second instar larva was fed 15 second instar aphids, the third instar larva was fed 16 second and third instar aphids of equal portions, the fourth instar larva was fed 16 fourth instar and adult aphids of equal portions, the fifth instar larva and pre-pupal stage and adult were fed 16 adults of roughly equal sizes. The number and weight of aphids provided daily to each stage was evaluated and kept standardized in order to ensure that approximately the same aphid biomass was provided to all individuals equally.

2.2.4 Experimental procedures

100 newly hatched larvae were collected randomly from the above monitored clutches. Each larva was reared individually in a 9cm Petri-dish and was supplied with prey as detailed previously. Adult beetles were collected randomly from different clutches after ecdysis. They were sexed and paired into 30 couples. Each pair was supplied daily with aphids (16/adult), 2cm^2 piece of moistened tissue paper with 10% honey solution and a folded filter paper. After mating, the clutches were collected and maintained separately in a 9cm Petri-dish. In order to prevent cannibalism, monitoring of those clutches was done every 30 min and the freshly emerged larvae removed. The thirty couples used in this study were reared until they died. Subsequent to their death male and female bodies were dissected directly to record the total number of testicular filaments and the ovarioles respectively, with the aid of the stereomicroscope (Schott, Leica MS25).

2.2.4.1 Determination of body part parameters

Variations in body parameters within and between genders were investigated. After adult death, maximum linear measures including hind width of scutum, body length, body width, right hind tibia length, wing length and wing width were recorded for the examined 30 pairs. The measurements were calibrated to 0.065 mm with an eyepiece micrometre attached to a stereomicroscope (Schott, Leica MS25). Subsequently, the measurements of body size were divided into three classes of equivalent intervals, large, medium and small, in order to be analysed further following the method detailed by Dixon and Hemptinne (2001), in which they had only considered one body measurement (body length of different insect species).

2.2.4.2 Determination of adult fresh body weight

The adult weights used were the mean values of the fresh adult body weight recorded three times throughout the experiment (i.e., after emergence, after first mating and at the end of the oviposition period). All weights were determined to a precision of ±0.02mg using a balance (Precisa, 125A).

2.3 Data analysis

Mean fresh body weight for males and females was considered as the independent variable in all data analyses. The body size and parts measurements (including body width, body length, scutum width, right tibia length, wing width and wing length), as well as longevity, were regarded as dependent variables. The Shapiro-Wilk test was performed on each variable to check for normality. Log-transformation and squared-root-transformation were applied to normalise data distribution. For the parameters that failed to be normalised, non-parametric analyses were applied using a generalised linear model with Poisson errors. Any overdispersion was removed using Quasipoisson modelling.

Assessments of SSD depend on what body size parameter is used. Much of life-history theory treats size as a varying trait dependent on a range of factors functioning at different stages in an individual’s life (Roff, 1981, Bede et al., 2007). This suggests that estimates of body mass may fluctuate greatly throughout life in many insects according to the external ecological factors and food status. Overall, body mass will provide a poor assessment of size and SSD mainly in the field collected samples. Some morphological size measures are less variable throughout adult lifespan and may be unresponsive to any changes either in the nutritional conditions or environmental factors, including the body length and scutum or pronotum width. Patterns of SSD may differ according to the measured traits of an individual (Väisänen and Heliövaara, 1989). As a consequence, multivariate traits analysis is highly recommended for interpreting intra-specific SSD recorded in particular between sexes and individuals belongs to the same species (Fairn et al., 2007).

The study intended to calculate the SSD index for all the above listed traits following Teder and Tammaru (2005). Teder and Tammaru (2005) demonstrated that the SSD index was expressed as the ratio of mean fresh body weight of females over mean weight of males. In view of that, if the females were the larger sex, this index exceeded unity whereas if males were the larger then this index remained below unity (Teder and Tammaru, 2005). Likewise, in the present study, SSD index was calculated by dividing the measured female body variable traits over that of males and results were interpreted similar to Teder and Tammaru (2005).

Fisher's test was applied to check for significance between body classes because the obtained SSD values were less than 5. Two tailed t-tests were used to test for variability in trait values recorded for male and female groups, as well as for each individual class using the model (i.e., t. test (response variable ~ sex as factorial variable)). These variables included all the above listed variables as well as the average number of the male testicular filaments and female ovarioles. Wilcoxon Rank Sum was used when the errors were not normally distributed using the same above model. Multivariate analysis of variance (MANOVA) was used to determine which of the size variables could be used to make a distinction between males and females.

A matrix of Pearson's Correlation tests was implemented to assess any negative or positive correlation that might exist between the measured response variables and the correlation coefficient was recorded for each test. Nevertheless, all the response variables were subjected to allometric analysis to examine how they scaled with body weight. Allometric relationship is often defined by a power function ($y=ax^b$) (Huxley, 1931) in which b and a are derived from the equation ($\log y = b \log x + \log a$) (Bales, 1996). To accurately investigate the above, linear regression analysis was performed. Therefore, each of the transformed and the other normally distributed variables was regressed against log-fresh body weight. Regression analysis was applied for both sex groups as well as individuals belonging to every class and some variables were regressed and plotted accordingly. As a final point, testing for the variability within each sex group and between classes of each sex were further performed by using the sex type and class type as independent variables using One-Way ANOVA (response variable ~ sex or class (categorical)). A similar interaction was applied for each class independently. All statistical analyses were carried out using the statistical programme 'R' version 2.8.1 (Ihaka and Gentleman, 1996).

2.4 Results

In the present study SSD was revealed among several variables. The mean size of most measured traits was found to be significantly greater in females compared with males except that recorded for the right tibia length. Irrespective to the classes, the mean size of right tibia length recorded in males was 2.6 mm (in comparison with 1.65 mm for females) (Table1).

The calculated dimorphism indices for all traits in male and female groups are presented in Figure 2.1 and Table 2.1 and the sexual index for every class is shown in Table 2.2. Females grouped collectively regardless of their classes revealed greater dimorphism indices than the male group excluding the index calculated for tibia length. Further comparisons between all mean variables for male and female groups revealed that the female group was significantly bigger than the male group regarding all traits except the mean right tibia length and the mean length of the wings. Statistical comparisons were thus performed between measured traits in male and female classes, small, medium and large, respectively. The component of analysis showed that all the measured traits were significantly bigger in the large-sized class of females compared with the respective class for males. The other traits measured for the small- and medium-size classes of females were also significantly bigger, excluding the mean wing length, when compared with the respective classes of males. In addition, the index estimates for each trait belonging to each class were added up collectively for male and females and the average was taken. Comparison between the averages of indices calculated for every class irrespective to the sex was performed using Fisher's test and revealed that none of the comparisons were significantly different, $\chi^2 = 0.096$, P=n.s. between the small and medium class irrespective of the sex $\chi^2 = 0.01$, P=n.s. between small and large class indices and $\chi^2 = 0.01$, P=n.s. between medium and large class indices. The purpose of conducting the above was to inspect the intraspecific variations within *H. axyridis* species regardless of the interaction of the sex. The interpretation was based on multivariate analysis of multicomponent traits; body width, scutum width, body length or mean fresh body weight offered a good understanding of how the body size of females is significantly larger than that of males. The results showed that females were the bigger sex (Wilks lambda=0.3806, $F_{(3,56)}$= 30.37, P<0.0001). Similar results were attained when body length was replaced by mean fresh body weight (Wilks lambda= 0.427, $F_{(3,56)}$= 30.37, P<0.0001).

Females varied significantly between the three classes after comparing mean fresh body weight (F=52.8, df= 27, RSE=3.5, P<0.0001) (Fig. 2.2), wing width (F=3.81, df=27, RSE= 0.39, P<0.05) (Fig. 2.3), wing length (F=15.82, df= 27, RSE= 0.64, P<0.0001), body width (F=29, df= 27, RSE= 0.43, P<0.0001) (Fig. 2.4) and total ovariole number (F= 76.4, df= 27, residual deviance = 0.09, P<0.0001) (Fig. 2.5). Other traits such as length of right hind tibia, scutum width and body length did not display significant variations between the tested classes (F= 0.58 df= 29, RSE= 0.13, P=0.56), (F= 0.14, df= 29, RSE= 0.34, P=0.86) and (F= 1.13, df=29, RSE= 0.81, P=0.28), respectively.

Table 2.1. Means (±SE) and ranges for morphological and life history traits of male and female *H. axyridis*. The results of independent Wilcoxon.test and t-test for variation between sexes are also provided.

	Female				Male								
Morphological Trait	n	Mean	±SE	Range	n	Mean	±SE	Range	*t*-value	*w*-value	P	df	Dimorphism index
Fresh body weight (mg)	30	32.31	1.37	27.63	30	26.26	0.95	18.24	3.62		<0.0001	51.9	1.23
Body length (mm)	30	6.58	0.08	1.82	30	6.2	0.06	1.8		672	<0.01		1.06
Body width (mm)	30	5.67	0.05	1.04	30	4.82	0.07	1.7		857.5	<0.0001		1.17
Right tibia length (mm)	30	1.65	0.02	0.58	30	2.6	0.02	0.45	1.45		0.15	55.6	0.63
Scutum width (mm)	30	3.06	0.03	0.65	30	2.95	0.028	0.65		599.5	<0.05		1.03
Wing length (mm)	30	9.43	0.19	3.64	30	9.24	0.14	2.99		527	0.25		1.02
Wing width (mm)	30	3.2	0.08	1.62	30	2.98	0.05	1.43		593	<0.05		1.07
Longevity (days)	30	36.73	1.7	30	30	33.36	1.44	28		542	<0.001		1.1

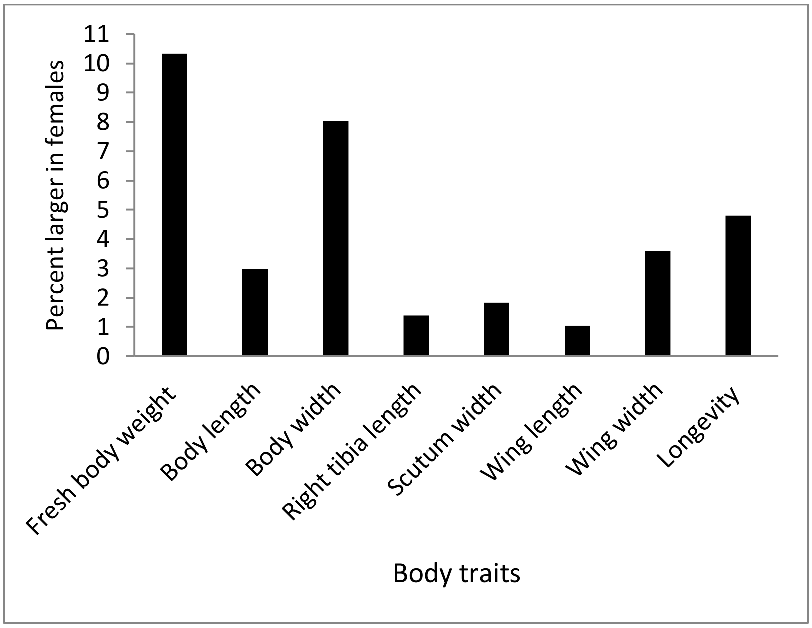

Fig.2.1. Index of dimorphism for measured body traits. Trait means ranged from 1.01-10.32%, larger in females than in males.

Table 2.2. Means (±SE) and ranges for morphological and life history traits of male and female *H. axyridis*. The results of independent Wilcoxon.test and t-test for variation between sexes within each class are provided.

Class		Female			Male							
Small	Life-history trait	n	Mean	±SE	n	Mean	±SE	t-value	w-value	P	df	Dimorphism index
	Fresh body weight (mg)	10	24.46	1.059	10	19.82	1.006	3.18		<0.01	17.95	1.23
	Body length (mm)	10	6.74	0.189	10	6.11	0.12	2.85		<0.05	16.16	1.1
	Body width (mm)	10	5.38	0.098	10	4.45	0.06	8.17		<0.001	17	1.2
	Right tibia length (mm)	10	1.69	0.043	10	1.62	0.018	0.73		0.473	17.16	1.04
	Scutum width (mm)	10	3.08	0.065	10	2.92	0.051	1.84		0.088	13.11	1.05
	Wing length (mm)	10	8.99	0.3	10	9.03	0.26	-0.0841		0.934	17.57	0.99
	Wing width (mm)	10	3.07	0.17	10	2.71	0.09	1.83		0.083	14.35	1.13
	Longevity (days)	10	36.2	2.44	10	34.6	2.86	0.54		0.59	17.35	1.04
Medium												
	Fresh body weight (mg)	10	31.9	0.55	10	27.94	0.37	5.98		<0.0001	15.75	1.4
	Body length (mm)	10	6.43	0.12	10	6.39	0.063	0.3		0.77	13.37	1.006
	Body width (mm)	10	5.73	0.032	10	4.8	0.016		100	<0.001		1.19
	Right tibia length (mm)	10	1.62	0.038	10	1.57	0.048	0.73		0.47	17.16	1.03
	Scutum width (mm)	10	3.04	0.053	10	2.93	0.026	2.06		0.1	13.66	1.03
	Wing length (mm)	10	9.45	0.18	10	9.65	0.043	-0.087		0.4	15.73	0.98
	Wing width (mm)	10	3.17	0.094	10	2.95	0.051	2.06		0.058	14	1.07
	Longevity (days)	10	33.4	3.24	10	35	2.54	-0.54		0.6	17.2	0.95
Large												
	Fresh body weight (mg)	10	40.57	1.5	10	31.02	0.53	6.54		<0.0001	12.67	1.3
	Body length (mm)	10	6.58	0.069	10	6.37	0.074	2.13		<0.05	18	1.03
	Body width (mm)	10	5.89	0.029	10	5.23	0.099		99.5	<0.001		1.12
	Right tibia length (mm)	10	1.65	0.045	10	1.633	0.029	0.35		0.72	15.48	1.01
	Scutum width (mm)	10	3.07	0.065	10	3.013	0.06	0.65		0.52	18	1.01
	Wing length (mm)	10	10.66	2.91	10	10.553	1.01		90	<0.01		1.01
	Wing width (mm)	10	3.53	0.1	10	2.953	0.043	5.6		<0.0001	13	1.19
	Longevity (days)	10	40.6	0.092	10	30.5	2.034	3		<0.05	16.08	1.33

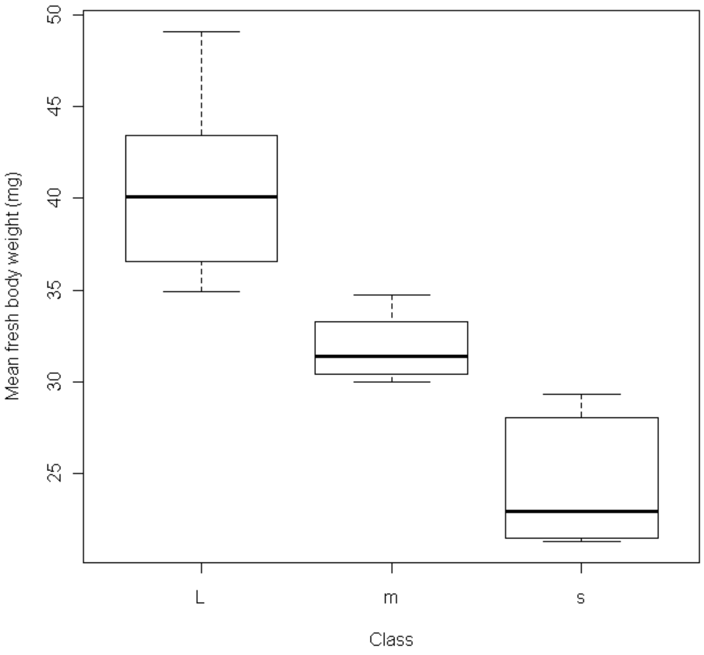

Fig.2.2. Variability in mean fresh body weight between female size classes. Data were transformed for statistical analysis (F=52.8, df= 27, RSE=3.508, P<0.0001).

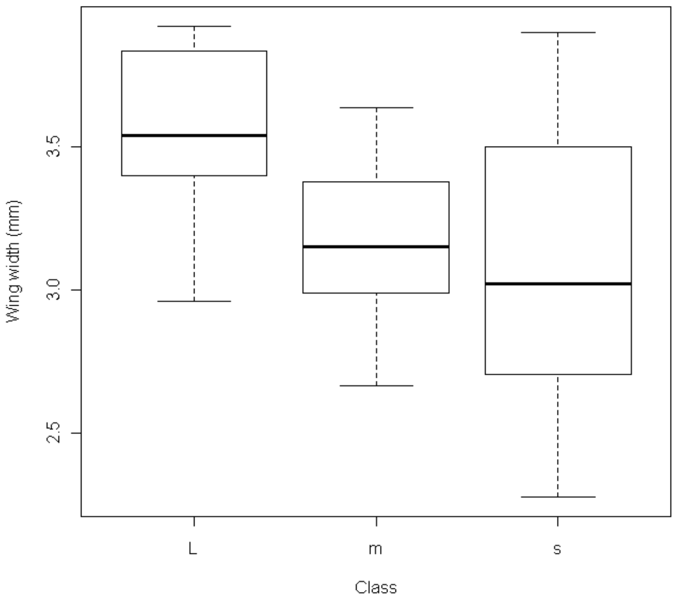

Fig.2.3. Variability in wing width between female size classes. Data were transformed for statistical analysis (F=3.814, df= 27, RSE= 0.39, P<0.05).

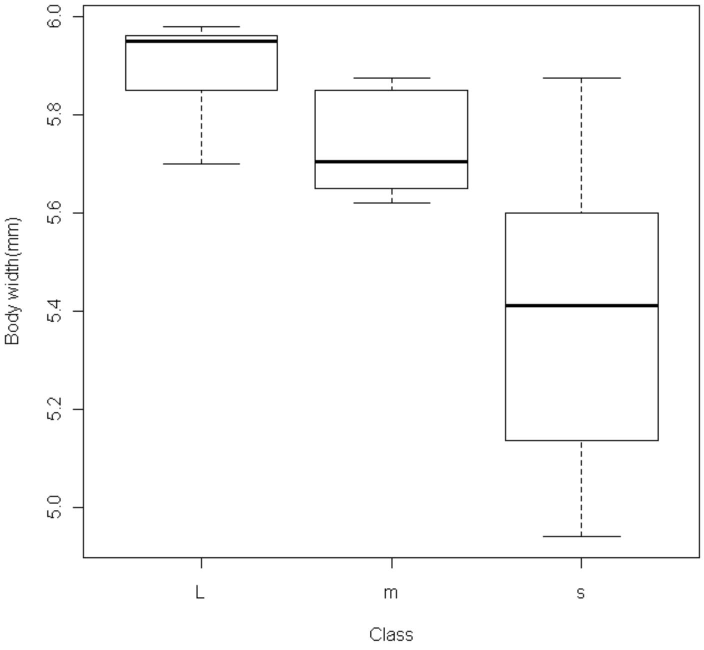

Fig.2.4. Variability in body width between female size classes. Data were transformed for statistical analysis (F=29, df= 27, RSE= 0.436, P<0.0001).

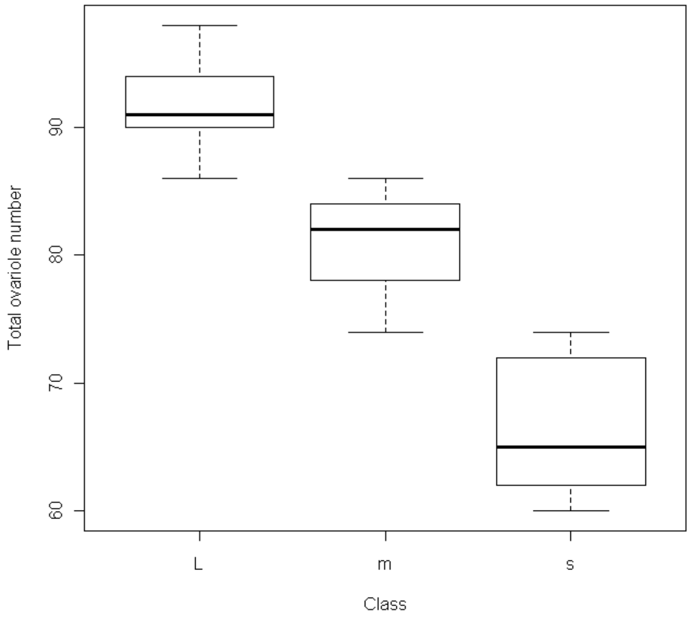

Fig.2.5. Variability in total count of ovarioles between female size classes. Data were transformed for statistical analysis (F=76.4, df= 27, residual deviance= 0.09, P<0.0001).

Five measured traits out of eight showed significant variability between the three classes of females, that is, approximately 62% of the overall measured traits. Likewise, among the male category, only half of the eight measured traits accounted for 50% overall significant variability between the three classes. These traits are body length variable (F= 11.45, df= 27, RSE= 0.28 P<0.0001) (Fig. 2.6), testicular follicle number (F=4.12, df=27, residual deviance= 0.1, P<0.0001) (Fig. 2.7), wing width (F=4.12, df= 29, residual deviance= 0.59, P<0.05) and fresh body weight (F=60.98, df= 27, residual deviance= 5.88, P<0.0001) (Fig. 2.8). Therefore, females displayed 12% extra variability among different body sizes compared with that of the males.

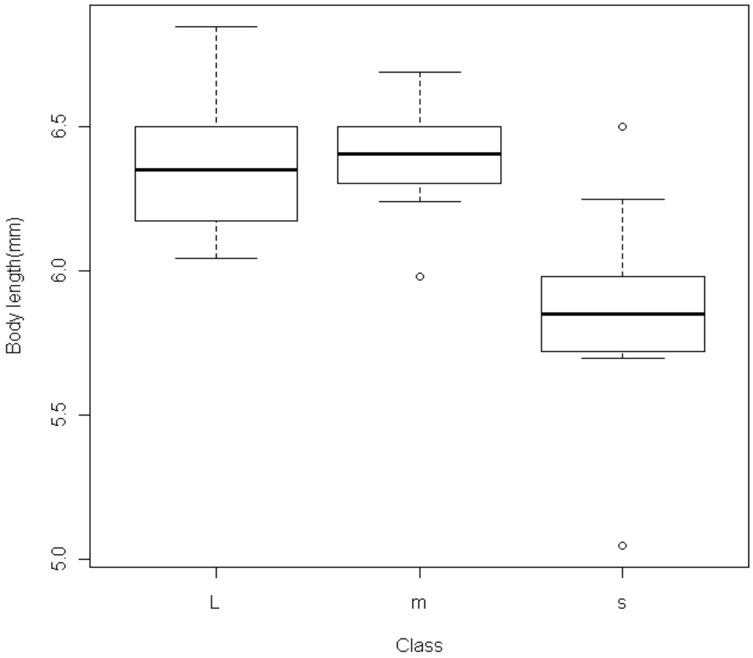

Fig.2.6. Variability in body length between male size classes. Data were transformed for statistical analysis (F= 11.45, df= 27, RSE= 0.281, P<0.0001).

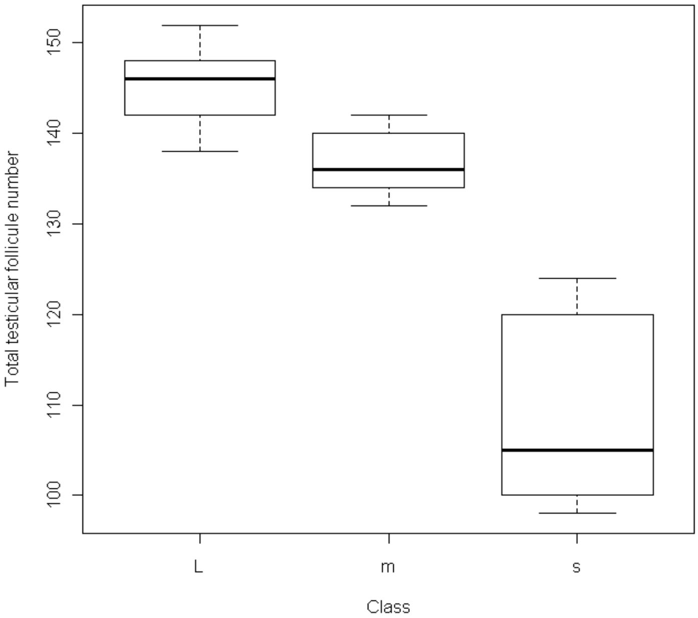

Fig.2.7. Variability in total count of testicular follicles between male size classes. Data were transformed for statistical analysis (F=4.12, df=27, residual deviance= 0.097, P<0.0001).

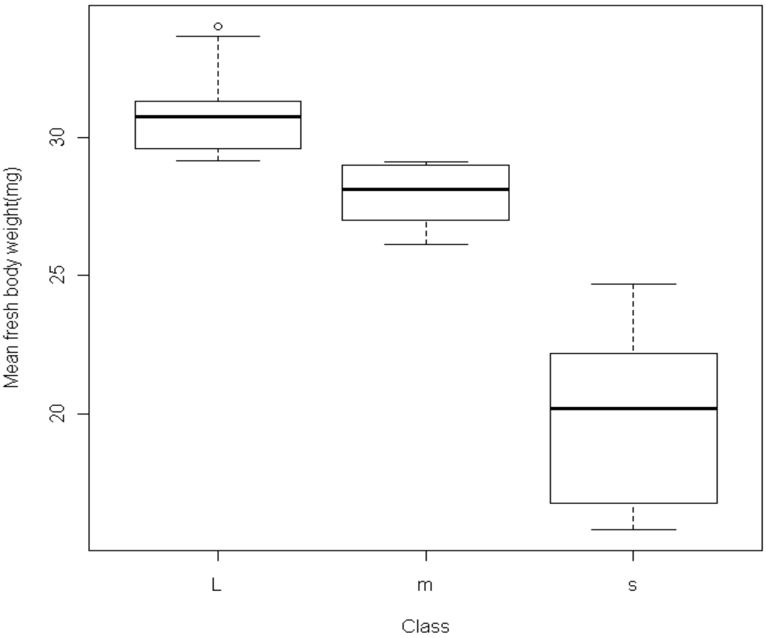

Fig.2.8. Variability in the mean fresh body weight between male size classes. Data were transformed for statistical analysis (F=60.98, df= 27, residual deviance= 5.88, P<0.0001).

In addition, correlation tests were performed between all body parameters of male and female categories independently, as well as for the variables categorised for each class independently. The purpose of this test was to assess any relationship between body part variables that might reveal their interactive association particularly when food and external conditions are fluctuating (i.e., their plasticity and occurrence of trade-offs). The results for the correlation tests are presented in Table 2.3.

It was noticed that in males, highly significant correlations existed, chiefly between the number of testicular filaments (gonads) and body width (R=0.81, P<0.0001, t=8, df= 28) (Fig. 2.9). A very weak link was revealed between the former and body length (R=0.7, P<0.0001, t=5, df= 28). After applying glm with Poisson error model to assess the dependency of gonads on body weight of males, a highly significant linear relationship was obtained (F=53, RSE=0.03, P<0.0001) (Fig. 2.10). These results infer that possession of a higher number of testicular follicles requires a substantially wider body. In addition, the weight of these testicular filaments may have the direct effect of further increasing the fresh body weight in males. Consistent findings were obtained for females correlating the total ovariole number with body width (R=0.71, P<0.0001, t=5.38, df= 28) (Fig. 2.11) as well as with the fresh body weight of these dissected females (RSE=0.042, F=313.8, df= 29, P<0.0001) (Fig. 2.12). Body length of the females was negatively correlated with ovariole number (R=0.7, P=0.34, t= -95, df= 28). This indicates that having longer body length is not indicative of a high ovariole number or greater lifetime fecundity.

Allometric relationships between body size measures and fresh body weight were measured following the formula (log-transformed fresh body weight as an independent variable against log-transformed body measures as dependent variables). In males, a highly significant linear relationship was found between the fresh body weight and body length, as well as the former with body width. The slope estimates revealed a weak inclination for positive allometry (slope=0.19, P<0.0001) and (slope=0.3, P<0.0001) (Fig. 2.13) respectively. In contrast, females have a tendency to have non-significant negative weak allometry with respect to body length (slope=-0.044, P=0.55) and highly significant allometric relationship with body width (slope=0.16, P<0.0001) (Fig. 2.14). Neither of the intercept estimates of body width of females (0.27) or males (0.51) were likely to overlap. More allometric estimates assessed for other body variables in both sexes are presented in Table 2.4.

Table 2.3. Correlation estimates between various body part traits in males and females where each class has n=10. A correlation coefficient is measured between -1 and 1. A positive coefficient value indicates that if one variable increases, the other increases also whereas, a negative coefficient indicates that as one variable increases, the other decreases.

Class		Female			Male							
Small	Life-history trait	n	Mean	±SE	n	Mean	±SE	t-value	w-value	P	df	Dimorphism index
	Fresh body weight (mg)	10	24.46	1.059	10	19.82	1.006	3.18		<0.01	17.95	1.23
	Body length (mm)	10	6.74	0.189	10	6.11	0.12	2.85		<0.05	16.16	1.1
	Body width (mm)	10	5.38	0.098	10	4.45	0.06	8.17		<0.001	17	1.2
	Right tibia length (mm)	10	1.69	0.043	10	1.62	0.018	0.73		0.473	17.16	1.04
	Scutum width (mm)	10	3.08	0.065	10	2.92	0.051	1.84		0.088	13.11	1.05
	Wing length (mm)	10	8.99	0.3	10	9.03	0.26	-0.0841		0.934	17.57	0.99
	Wing width (mm)	10	3.07	0.17	10	2.71	0.09	1.83		0.083	14.35	1.13
	Longevity (days)	10	36.2	2.44	10	34.6	2.86	0.54		0.59	17.35	1.04
Medium												
	Fresh body weight (mg)	10	31.9	0.55	10	27.94	0.37	5.98		<0.0001	15.75	1.4
	Body length (mm)	10	6.43	0.12	10	6.39	0.063	0.3		0.77	13.37	1.006
	Body width (mm)	10	5.73	0.032	10	4.8	0.016		100	<0.001		1.19
	Right tibia length (mm)	10	1.62	0.038	10	1.57	0.048	0.73		0.47	17.16	1.03
	Scutum width (mm)	10	3.04	0.053	10	2.93	0.026	2.06		0.1	13.66	1.03
	Wing length (mm)	10	9.45	0.18	10	9.65	0.043	-0.087		0.4	15.73	0.98
	Wing width (mm)	10	3.17	0.094	10	2.95	0.051	2.06		0.058	14	1.07
	Longevity(days)	10	33.4	3.24	10	35	2.54	-0.54		0.6	17.2	0.95
Large												
	Fresh body weight (mg)	10	40.57	1.5	10	31.02	0.53	6.54		<0.0001	12.67	1.3
	Body length (mm)	10	6.58	0.069	10	6.37	0.074	2.13		<0.05	18	1.03
	Body width (mm)	10	5.89	0.029	10	5.23	0.099		99.5	<0.001		1.12
	Right tibia length (mm)	10	1.65	0.045	10	1.633	0.029	0.35		0.72	15.48	1.01
	Scutum width (mm)	10	3.07	0.065	10	3.013	0.06	0.65		0.52	18	1.01
	Wing length (mm)	10	10.66	2.91	10	10.553	1.01		90	<0.01		1.01
	Wing width (mm)	10	3.53	0.1	10	2.953	0.043	5.6		<0.0001	13	1.19
	Longevity(days)	10	40.6	0.092	10	30.5	2.034	3		<0.05	16.08	1.33

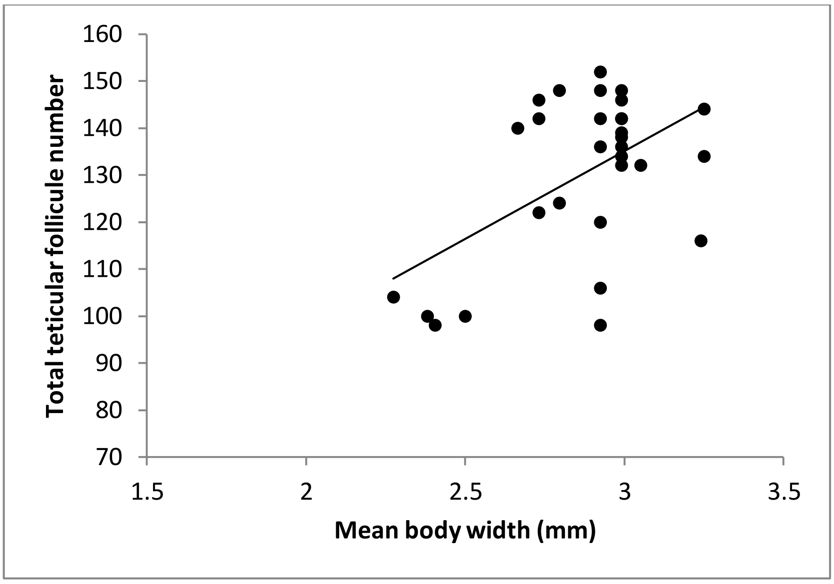

Fig.2.9. Relationship between the measured body width and the total number of testicular follicles in males (n=30, correlation coefficient= 0.81, P< 0.0001).

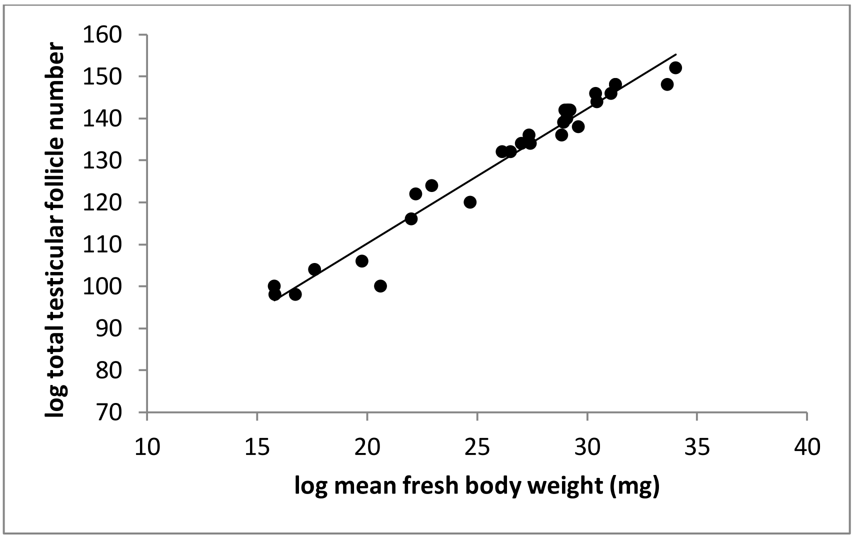

Fig.2.10. Relationship between logged mean fresh body weight against logged total testicular number in males (n=30, y=3.21x + 45.99, R^2=0.95).

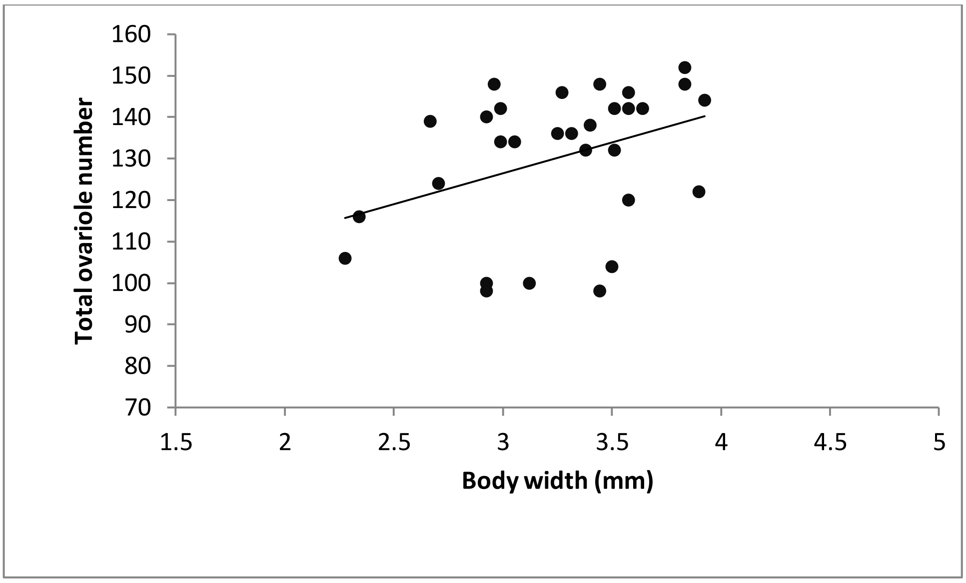

Fig.2.11. Relationship between the measured body width and the total count of ovariole number in females (n=30, correlation coefficient= 0.71, P<0.0001)

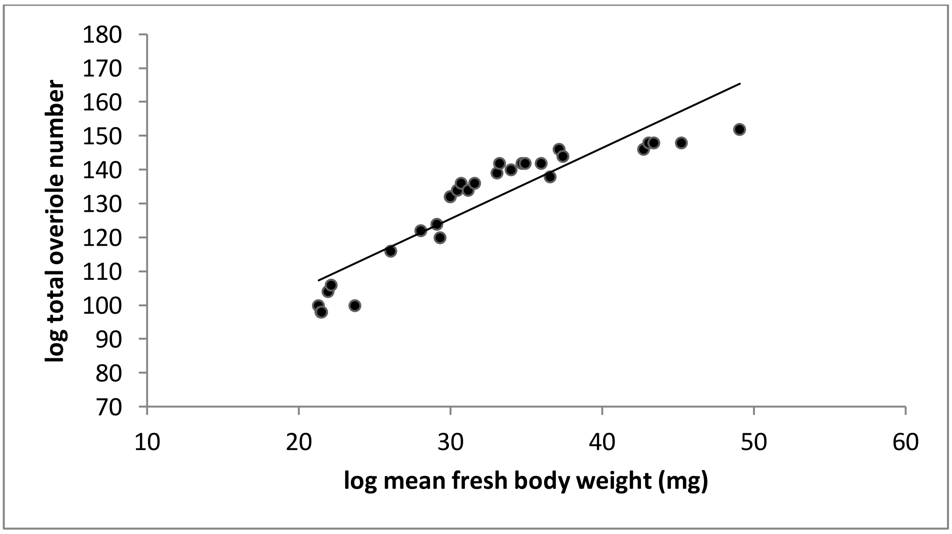

Fig.2.12. Relationship between logged mean fresh body weight against logged total ovariole number in females (n=30, y=2.09x + 62.62, R^2=0.83).

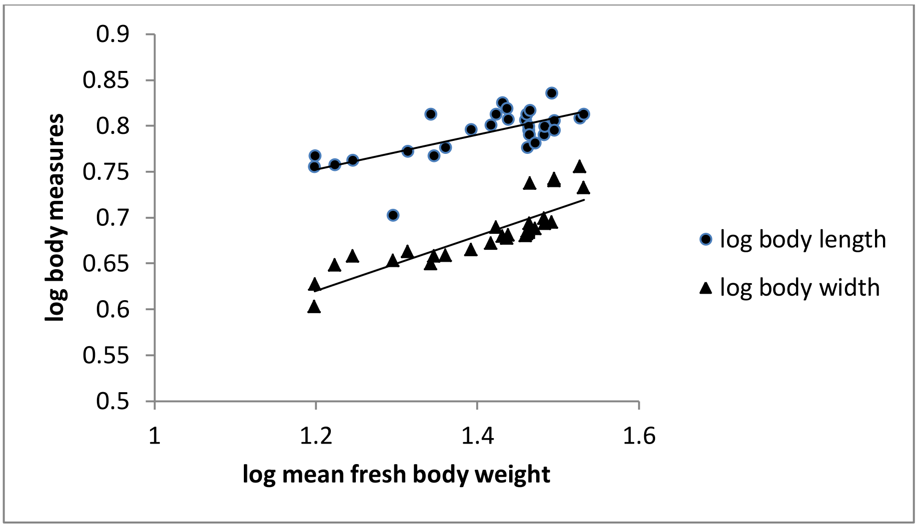

Fig.2.13. Relationship between log-transformed fresh body weight regressed against both logged body width and body length for males, n=30. For body width (y=0.3x + 0.26, R^2=0.72) and for body length (y=0.19x + 0.52, R^2=00.52).

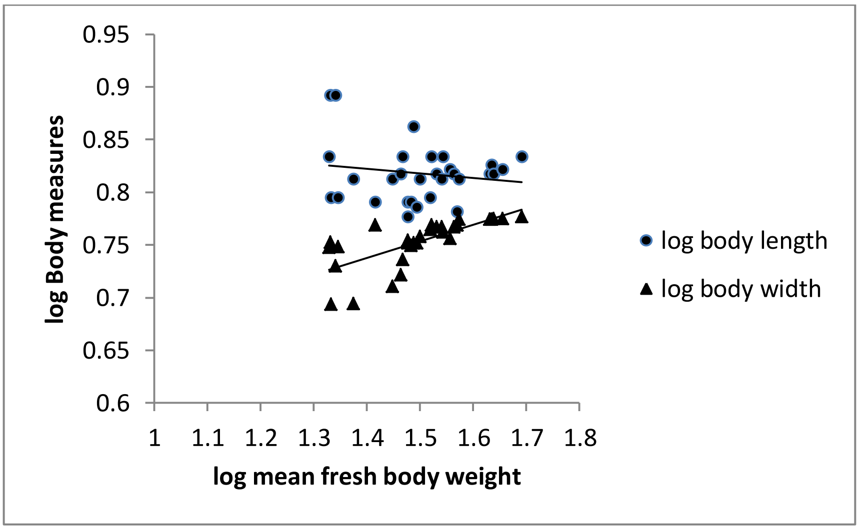

Fig.2.14. Relationship between log-transformed fresh body weight regressed against both logged body width and body length for females, n=30. For body width (y=0.15x + 0.51, R^2=0.5) and for body length there was a negative allometry represented by negative slope (y=-0.043x + 0.88, R^2=0.026).

Table 2.4. Allometric slope estimates of log-transformed body part measures against log-transformed mean fresh body weight for both sexes.

Body part measures	Male, n=30	Female, n=30
	Slope	*Slope*
Body length	0.19	-0.043
Body width	0.29	0.15
Wing length	0.22	0.3
Wing width	0.23	0.26
Hind tibia length	-0.015	0.63
Scutum width	0.05	0.01

2.3 Discussion

The present study investigated SSD in the beetle *H. axyridis*. The study was started by initially measuring and analysing various morphological and structural traits of both sexes based on these measurements. The analysis revealed that *H. axyridis* exhibited sexual size dimorphism in most of its body structures, chiefly in fresh body weight and body width, which both showed stronger strength of significance. Likewise, female-biased SSD was also reported by Ueno (2003) for *H. axyridis* and in several other invertebrate species (Gotoh, 1982, Dixon, 2000, Teder and Tammaru, 2005). Females of *H. axyridis* were significantly larger than males with respect to most body size components, except the length of the hind tibia and the wing length in the small and medium classes. Approximately 87% of the overall measured traits, regardless of the sex (as indicated in Table 1) would be applicable traits for describing body dimorphism, excluding the hind tibia length. Sutum width was found to be comparably similar in both sexes, thus it could be implemented specifically in studies dealing with inter-specific comparisons. In the current study, the pattern of SSD was found to vary and was inconsistent with Rensch's rule, which states that where the "females are the larger sex, increasing size is coupled with decreasing SSD (hypoallometry), whereas in species where males are the larger sex, SSD increases with increasing mean size (hyperallometry)" (Dale, 2007, Herczeg et al., 2010). The present study showed this to be false when it comes to *H. axyridis*, the female was found to be the biggest sex and SSD was likely to increase concomitant with female size or with measurements of body structures. The broad view and the validity of Rensch's rule have not been established yet (Abouheif and Fairbairn, 1997, Stephens, 2009), but have been widely recognised on the whole in taxa in which males are the larger sex (Webster, 1992, Fairbairn and Preziosi, 1994, Johansson, 2005, Fairbairn et al., 2007). In contrast, allometry that is inconsistent with Rensch's rule has been stated in a quantity of taxa in which females have proven to be the larger sex (Vollrath and Parker, 1992, Head, 1995). In a well-matched study, it was found that the role of body size in determining fitness is superior in males compared with females (Ueno, 1994), but this is cannot be applied for body weight, due to its association with fitness, any trait that directly shapes fitness is regarded as less heritable (Mousseau and Roff, 1987). Teder and Tammaru (2005) noted that the application of Rensch's rule to interpret intra-specific SSD is still debatable due to the disparity among underlying mechanisms associated with its evolution.

Among the three size classes, the dimorphism index for body weight irrespective of sex index was slightly greater compared to other body variables. The degree of dimorphism index showed great variability across three size classes. Female biased dimorphism is not commonly found in beetles, in some insects there are few peculiar male traits that are likely to have evolved through sexual selection and may alter the degree of SSD towards male-biasness (Thornhill and Alcock, 1984). This could occur when the sexually selected morphological traits begin to greatly enlarge as a result of hyperallometric growth, such as the increase in head length, several parts of prothoracic leg of *Dineutus nigrior* (Roberts, 1895) (Coleoptera: Gyrinidae) (Fairn et al., 2007), the long stalks of compound eyes and the antler-like projections from the head of *Cyrtodiopsis whitei* (Curran, 1936) (Diptera: Diopsidae) (Burkhardt, 1988, Panhuis and Wilkinson, 1999).

It has been noted that SSD varies within and among taxa (Reiss, 1986, Arak, 1988, Fairbairn, 1990). It is not uncommon for SSD to range from female-biased to male-biased within a species or a single family or even a specific genus (Fairbairn, 1990, Shine, 1994). The variations of SSD provide essential data for judging hypotheses and to examine quantitative comparative data. For example, highlighting the effect of selection on reproductive traits in particular, those are associated with sexual selection in males and prenatal investments (Sivinski and Dodson, 1992, Head, 1995, Forsman and Shine, 1995). In the present study, a similar observation was noted when various traits exhibited significant variability between each sex group and within *H. axyridis* regardless of the sex.

There are quite a few challenging matters associated with assessing SSD. Several comparative evolutionary analyses of SSD revealed a strong statistical association between SSD and body measurements (Fairbairn, 1990). Therefore, the influence of body size allometry is often removed statistically before testing starts on the adaptive divergence of SSD (Fairbairn, 1990, Oakes, 1992). Some scientists regarded the allometric scaling as being a functional constraint on determining SSD (Stearns, 1983, 1984). Thus, it was suggested that the significant influence of the allometry on the size be neglected, instead of including it in statistical analyses, or vice versa (Smith et al., 2004). In this trend, despite the numerous recommended allometric hypothetical approaches (Leutenegger, 1978, Shine, 1979), none have yet been found to deliver a wide-range interpretation for allometry (Cox et al., 2003, Blanckenhorn et al., 2007, Dale, 2007). Moreover, choosing the suitable traits for inter- and intra-specific comparative studies must be well thought-out. For example, the present study revealed that using body length measure for assessing the allometry was not the best technique. Body length was found to be an unreliable indicator for allometry estimates since it varied significantly between sexes. The trait of body length showed a significant correlation with fresh body weight ($P<0.0001$) of males, but it was non-significantly correlated with the latter in females ($P=0.39$). Obtaining a slightly negative linear direct relationship between fresh body weight of females and the respective body length implies quite a few suggestions; heavy females may not often possess a long body. Body weight may be influenced by the mass of internal organs, the stored fat and the status of their reproductive period. Therefore, the longer female is not often more highly fecund than the shorter one and the former might be heavy due to fat deposition. In agreement, Leather (1988) noted that body size as well as body weight in females are often weakly correlated and can be easily influenced by other factors.

The advantages of possessing a large body size in females have been extensively investigated and are acknowledged to include customary components of individual fitness such as greater life-time fecundity, reproductive and copulation success (Dixon, 2000), access to a mate (Osawa and Nishida, 1992), foraging and searching for a suitable habitat (Rivero, 2005), as well as faster growth and substantial viability (Blanckenhorn, 2005). Positive correlations between body size of females and their offspring number has been reported in a number of insect species (Thornhill and Alcock, 1984, Osawa, 2002) however, defining such a relationship is not often a clear task (Leather, 1988). The analyses obtained by the present study also support the positive association between body weight or body width and ovariole number in females, which represents the potential fecundity. According to Blanckenhorn et al. (2007), large individuals are more advantaged over smaller ones, due to their high energy use efficiency. In contrast, being large is not always valuable, due to the excessive increase in energy requirement (Blanckenhorn, 2005, Blanckenhorn et al., 2007). Nonetheless, some insects with large-sized bodies often suffer from extreme mortality when conditions are stressed or adults might be more vulnerable to frost due to their longer developmental period (Teuschl et al., 2007). Reim et al. (2006) reported that it is not easy to assess various life-history traits of large bodies under ordinary conditions and the traits tend to become more predictable when an individual is stressed.

The aim of this study was not to investigate the evolution in SSD but to quantify the estimates of SSD in *H. axyridis* and its distinctive role in promoting and escalating competitive and invasive capabilities. Invasive species often display variations in life-history traits that may permit them to invade diverse environments successfully (Xuan et al., 2010). Numerous successful invaders exhibit a high degree of phenotypic flexibility or plasticity in life-history traits, permitting them to tolerate fluctuating environments and evolve self-sustaining populations (Lockwood 1999, Rosecchi et al., 2001, Sakai et al., 2001, Bohn et al., 2004, Xuan et al., 2010). Body size and SSD have been viewed as essential evolutionary measurable factors in many species (Ashton, 2002, Campbell and Echternacht, 2003, Morrison et al., 2004). Any changeability between these two traits may deduce the role of morphological plasticity as well as environmental and ecological factors such as food status (Travis, 1994).

Likewise, the degree of intraspecific variations varies significantly within a species. For example, the idiobiont ectoparasitoid, *Dendrocerus carpenter* (Curtis, 1829) **(**Hymenoptera: Megaspilidae**)** displayed an 1.8-fold difference between minimum and maximum samples with minimum and maximum in SSD (Otto and Mackauer, 1998). In contrast, Ueno et al. (1999) noted that the *H. axyridis* reared at different environmental temperatures did not exhibit any variation in SSD across samples despite an 1.2-fold difference between samples in female sizes. The latter is diametrically opposed to the results obtained by the present study, in which significant intraspecific variations in SSD and among the dimorphism index in *H. axyridis* were seen. The variation between the two studies could be a result of different rearing conditions. Teder and Tammaru (2005) suggested that it was implausible to suggest that environmental conditions influence the direction of sexual dimorphism in body size. In contrast, the effects of environmental conditions on the degree of sexual differences are highly common and distinguishable.

According to Brown and Sibly (2006), insects with a large body size and associated high absolute fecundity may be favoured by natural selection particularly in environments where food is highly abundant. A similar proposition was stated by Dixon (2007). For *H. axyridis*, large-sized bodies in females may confer competitive advantages and favour intraguild predation, which may lead to an increase in the potential impacts on native species, leading to their displacement and a decrease in their abundance (Cottrell and Yeargan, 1998, Cottrell, 2004, Yasuda et al., 2004). *Harmonia axyridis* is known by its large adult body size and high voracity and high reproductive rate (Evans et al., 2011, Dixon, 2007, Adriaens et al., 2008). Adult body size features are likely consequences of the composite interaction of genotypes with environmental conditions experienced during immature stages (Evans et al., 2011). Therefore, the great variability in body size of *H. axyridis* may reveal the possession of high genetic pool variations for body size (Evans, 2000, Lombaert et al., 2007, Turgeon et al., 2011). As such, individuals with these diverse genotypes have the capability to succeed in growing, developing, and reproducing under extraordinarily diverse conditions. Nevertheless, their offspring may attain also a highly diverse adult size depending on the food status and habitat nature (Hodek and Honek, 1996).

Harmonia axyridis is usually considered to be generalist and polyphagous (Brown et al., 2011) with a high degree of ecological flexibility. It can reproduce successfully under adverse conditions (Dixon, 2000, Brown et al., 2011). Such features are consistent with the hypothesis that the invader's success derives from being a generalist with much 'ecological flexibility' with respect to the conditions under which it succeeds in reproduction (Evans, 2000). Lombaert et al. (2007) demonstrated that the invasive species of *H. axyridis* displayed a variety of phenotypic plasticity in several traits, such as fecundity, survival rate, egg hatchability, sex ratio and developmental period, and exhibited a significantly higher fitness towards diverse environments. The features that are usually connected with reproductive performance may act as a critical factor contributing to the rapid rise and abundance of this successful invader (Lombaert et al., 2007). It was noted that high fecundity associated with large body size has been suggested as the main key factor promoting prevalence of invasive ladybeetles, such as *H. axyridis* in its native and invaded habitats (Kock, 2003, Roy et al., 2006). Kajita and Evans (2010) showed that the ladybird beetle, *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) which is also intensive successful invader, had a high fecundity linked with its large body size. This fact supports the broad view proposed by Evans (2000) and may often be an important factor encouraging the dominancy and the competitive capacity of *C. septempunctata* over native species in resource-rich environments. Generally, the invader is distinctive in having a particularly large variation in body size among other native individuals (i.e., in having relatively high proportions of both unusually large and small individuals). The present study revealed that males are usually smaller than females, despite the occasional occurrence of large males. These males are characterised by fast mobility, great foraging behaviour and mate searching. In fact, these behaviours were facilitated by the presence of the long hind tibia compared to females. Similar features regarding the small-sized males are conformed previously by Dixon (2000) and Reim et al. (2006)

In summary, the result of the present study demonstrated the importance of considering various body size dimensions when trying to analyse SSD as well as intra-specific allometric variations. In insects in particular, the presence of variability should not be disregarded during comparative life-history studies, even under controlled conditions. The high variability in *H. axyridis* noticed in adult body parts undeniably reflects the possession of a diverse genetic pool. Therefore, the greater measured variability the more generalist is the insects and subsequently superior ability to survive under highly diverse conditions. Nevertheless, the present study gave an overview on sexual dimorphism in *H. axyridis* which might have a direct relevance for understanding some of the qualities of *H. axyridis* that account in part for its success as a competitor and invader as well as highlighting its ability to establish over a wide ecological range.

Chapter 3

Relationships between Body Size and Fecundity of *Harmonia axyridis* Reared on a Fixed Diet: Intra-Species Variability

3.1 Introduction

Egg size is an important component of fitness, because it influences larval survival and female fertility (Holloway et al., 1987). Egg size in ladybirds is often independent of clutch size and displays little variation within species (as compared with clutch size, which differs greatly between different species) (Dixon and Agarwala, 2002). For coccinellid beetles, clutch size is directly associated with prey quantity and quality (Agarwala and Bardhanroy, 1999) and might be affected by oviposition site characteristics (Carter et al., 1984). In contrast, the size and the number of eggs are to some extent constrained by the ovariole number (Agarwala and Dixon, 1993, Dixon and Guo, 1993) and by other influences imposed by female body size, anatomy or morphology (Fox and Czesak, 2000). This could explain why aphidophagous ladybird beetles often oviposit their eggs in clusters in the vicinity of food or prey (Dixon, 2000, Timms and Leather, 2007). It has been suggested that egg size is probably governed by the size at which the first instar larvae are sufficiently big enough to be able to capture mobile prey, as well as by the length of their developmental period (Stewart et al., 1991ab). In addition, in some species, there is an ecological significance to geographical and seasonal differences in egg size, for example, in the sole flatfish *Solea Solea* (Linnaeus, 1758) (Pleuronectifomes: Soleidae) (Rijnsdorp and Vingerhoed, 1994). Mashiko (1992) noted that these seasonal variations have genetic control bases that interact with phenotypic plasticity.

For a given reproductive output, there are several trade-offs associated with egg and clutch sizes (Stewart et al., 1991a). For example, an ovipositing female may increase the number of eggs or offspring but reduce their size, or *vice versa* (Parker and Begon, 1986, Holloway et al., 1987). In practice, a female that is endowed with a large amount of resources could intensify its reproductive performance and lay both large eggs and big clutches (Parker and Begon, 1986). Much interest has been concentrated on the reproductive tactics of coccinellids (Bell and Bohm, 1975, Stewart et al., 1991b). In this context, it has been noted that the proportion of body mass assigned for the reproductive process is typically constant for a wide range of species with variable body sizes (Dixon, 2007, Honek et al., 2007). Therefore, females with a large body size devote the same proportion of their biomass to reproduction as those with a smaller body size (Gordon, 1989). The effective acquisition and allocation of nutrition are chiefly governed by the capability to transform most nutrients into mass (Karlsson and Wickman, 1990) and by body design (Stewart et al., 1991b).

Several attempts have been made to evaluate the body size-fecundity association (Honek 1993); however, generalisation of this relationship has been unsuccessful. Leather (1988) reported that because of the small reliability of body size under natural conditions, it is not easy to predict the association between body size and fecundity. Despite the importance of egg size as a significant evolutionary and ecological trait, there are a several limitations in understanding the factors governing its features (Fischer et al., 2002).

One of the greatest survival qualities in coccinellids is their reproductive strategy (Hodek, 1967, Osawa, 2005). Possession of high fecundity and large body size might be one of the key factors subsidising the predominance of invasive species like the ladybird *H. axyridis* (Brown and Sibly, 2006). Coccinellid ladybeetles have specialised ovaries and vitellogenic mechanisms that empower them to produce a relatively greater egg mass (Eckelbarger, 1994). Stewart et al. (1991b) noted that in coccinellids the actual number of eggs per clutch equals half the ovariole number; this implies the staggering of egg production between ovarioles. This process reveals stability and consistency in egg oviposition throughout the reproductive period. It was noted that the ovarian specialisation in ladybeetles varies among generalist and specialist species, mainly under stressed conditions (Ferrer et al., 2008). For example, a study revealed that the generalist *Adalia bipunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) had faster ovarian dynamics than the specialist *Adalia decempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae), in terms of oosorption induction. Thus, the ovarian performance of predatory ladybirds is closely linked to their feeding practice. One more important reproductive quality in coccinellids is the production of trophic eggs or non-developing eggs (Bell and Bohm, 1975, Perry and Roitberg, 2005). Trophic egg production is regarded as maternal adaptive behaviour adopted for larval provisioning, providing extra nutrition to the freshly hatched larvae (Perry and Roitberg, 2005).

Numerous studies have reported that possession of great fecundity and pronounced reproductive output might incur expenses comprising adverse effects, such as a decline in longevity accompanied by accelerated senescence (Partridge, 1987, Paukku and Kotiaho, 2005), elevated predation (Calow, 1979) and an increase in parental care (Hunt et al., 2002). Females of *H. axyridis* are renowned for their high reproductive potentials compared with other aphidophagous species (Bazzocchi et al., 2004, Majerus et al., 2006, Pell et al., 2008). *Harmonia axyridis* females are capable of producing between 1,642 and 3,819 eggs/female throughout their entire lifespan, at a rate of 25 eggs/day (Koch, 2003, Osawa, 2005). Apart from egg production, the ovaries of *H. axyridis* serve as an energy storage system to give preferentiality to reproductive success under poor conditions. This could be accomplished by the development of asymmetrical ovaries (Osawa, 2005, Kajita and Evans, 2009). Nevertheless, females of *H. axyridis* possess adaptive ovipositional strategies, permitting them to endure and cope with heterogeneous and fluctuating resources (Osawa, 2005). Such ovipositional tactics may include immediate ovarian development in favourable conditions and fast oosorption (i.e., follicular cells stop producing yolk protein precursors and reabsorption of ooplasm occurs through phagocytic activity) during prey insufficiency (Eliopoulos et al., 2003, Osawa, 2005). The latter is a vital mechanism in which the nutrients are transferred from oocytes to the somatic tissue, particularly under poor conditions (Papaj, 2000, Eliopoulos et al., 2003, Kajita and Evans, 2009).

A previous study demonstrated that ladybirds reared on constant diet might not show a significant variability in the size of an egg or a clutch (Dixon and Guo, 1993). Coccinellids lay their eggs in clutches in which the size is mainly reliant on the number of ovarioles (Stewart et al., 1991a). In addition, the proportion of the body that is made up of gonads is equal for all coccinellids. Thus, any variation in ovariole number could be corrected by dividing adult body weight by total ovariole number (Stewart et al., 1991a). Subsequently, the relationship between body weight and reproductive biomass could be dealt with as a direct association, doubling up of body weight would result in a doubling of the reproductive biomass. Furthermore, Sibly and Calow (1986) suggested that in iteroparous species that reproduce more than once in a lifetime, reproductive growth rate should scale with body weight and the allometric coefficient *b* will be equal to or less than 0.75. In semelparous species that reproduce only once in a lifetime, however, the reproductive mass should scale with body weight and the allometric coefficient will be equal to or less than one. In a similar context, (Holloway et al., 1987) observed that for 16 iteroparous species, the allometric coefficient was approximately 0.45. This value authenticated Sibly and Calow’s prior prediction.

In view of the above, this study aimed to examine the reproductive performance of *H. axyridis* females reared on a constant diet of pea aphids. Investigations were performed mainly to reveal the intraspecific variability between lifetime fecundity, reproductive period and longevity, and to expose the allometric association with body parameters. Additional investigations were carried out to assess intraspecific variations among eggs belonging to the same and different clutches produced by the same female. Extra assessment was conducted in a similar manner, but this time between eggs belonging to different clutches laid by different females. *Harmonia axyridis* is known to be an iteroparous species, laying eggs at several times throughout its lifetime. This ladybird was, however, not considered with other ladybirds in the study by Holloway et al. (1987). The current study decided to test the above proposition on this beetle. The results of this study might shed light on the oviposition strategies in *H. axyridis* that might contribute to explaining the rapid increase in its population over native species. Furthermore, estimates of its reproductive output and how they scale with body parameters may deliver some theoretical basis on allometry and body fitness as parts of life-history studies.

3.2 Materials and Methods

Freshly emerged adults of *H. axyridis* beetles were selected from the *Harmonia* culture that were originally collected from naturally infested fields in Silwood Park, South-east England, United Kingdom (National Grid References 41/944691). The adults were sexed and assigned into 30 couples based on the labrum and prosternum pigmentation (McCornack et al., 2007).

The pairs were weighed and kept in 9cm Petri-dishes in a controlled temperature room at 20±1℃, 70-75% RH and a photoperiod of L16:D8 under fluorescent lamps. Each pair was supplied with damp tissue paper and a piece of corrugated filter paper (42.5mm, Whatman) to create an oviposition substrate. The daily food provided was 16 live adults of the pea aphid, *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae) maintained on broad bean plants *Vicia faba* (Linnaeus, 1753) (Fabales: Fabaceae, variety Aquadulce Claudia) under the same conditions as above and following the feeding method of Dixon and Guo (1993). The aphid and ladybird cultures were maintained according to Kajita and Evans (2010) and Agarwala et al. (2008). Subsequently, all the clutches laid by each pair were kept separately in 9cm Petri-dishes and maintained in the same laboratory conditions until larvae started to emerge. The couples were maintained under the above laboratory conditions until they died.

3.2.1 Determination of lifetime fecundity, daily fecundity and daily fertility

Lifetime fecundity (F) was determined by counting the total number of eggs laid during the oviposition period and mean total fecundity was subsequently estimated for the species. This was done following Stewart et al. (1991ab). Eggs were counted with the aid of a stereomicroscope (Schott, Leica MS25). Because not all of the eggs laid were viable, the daily fertility (number of hatched larvae=hatchability) and the proportion of non-viable eggs (trophic eggs), cannibalised eggs and eggs with incompletely developed larvae were also determined for each female.

3.2.2 Determination of weight and volume of an egg and a clutch

The weight of a single clutch laid by each female was measured to a precision of ±0.0002mg using a balance (Precisa, 125A). The weight of a single egg in each clutch was then determined by dividing the weight of the single clutch by the egg clutch size (egg number). The mean egg weight and the mean clutch weight were then determined for each female. The mean volume of an egg was calculated by measuring the width and the length of three randomly selected eggs from a single clutch to a precision of 0.041mm with the aid of an ocular micrometre attached to the stereomicroscope (Schott, Leica MS25). The measurements were then used to calculate the volume of the egg by applying the prolate spheroid formula (volume = $4/3 \times \text{pi} \times A^2 \times B$), following Osawa and Ohashi (2008) and Kajita and Evans (2010), where A is the half the width and B is half the length (Web of CalcEnstein). Subsequently, the mean egg volume was determined for each clutch, and then the volume of a single clutch was determined by multiplying the number of eggs in a clutch by the mean volume of an egg. This practice was applied to all clutches laid by a single female.

3.2.3 Determination of reproductive traits

The oviposition period, that is, the period between the first and last day of oviposition, was recorded for each female. Reproductive biomass (R) was determined for each female by multiplying the lifetime fecundity (F) by egg weight (E). Reproductive growth (G) was defined by Holloway et al. (1987) as the average daily output of reproductive material during adult life. Thus, the reproductive growth for each female was calculated by multiplying lifetime fecundity (F) first by the mean weight of an egg (E) and then dividing the answer by adult longevity (L). Reproductive rate (Rr) was calculated by dividing the lifetime fecundity over the total reproductive period. Upon adult death, male and female bodies were dissected in order to count the total number of testicular follicles and ovarioles, respectively. This was performed with the aid of the stereomicroscope (Schott, Leica MS25).

3.2.4 Adult body size measures

Body sizes (width and body length) were measured and then calibrated to a precision of ±0.065mm with an eyepiece micrometre attached to a stereomicroscope (Schott, Leica MS25). The mean fresh body weight of a single female was estimated by measuring the wet weight three times through the adult's life, after emergence, before first oviposition and at the end of the reproductive period, using a balance (Percisa, 125A) with a precision of ±0.0002mg. Finally, the mean fresh body weights for all couples were separated into three classes of equivalent intervals (large, medium and small) for further analysis.

3.3 Data analysis

All data were tested for normality prior to applying the parametric analysis and those that failed were transformed. One-way ANOVA and GLM model with Poisson errors (glm (y~x, Poisson)) was performed to analyse the variation between females in terms of lifetime fecundity and egg and clutch parameters. Over-dispersion was compensated for by refitting the model using Quasipoisson. The proportion of hatched larvae, trophic eggs, eggs with incompletely developed larvae and cannibalised eggs were arc-sine transformed, then dealt with as continuous data. Most of the allometric analysis was performed using either log-linear regression or GLM-linear model with Poisson errors for data that were not normal distributed data, such as reproductive biomass and reproductive rate. The logarithm of reproductive growth (EF/L) was regressed against log body weight (W) using linear regression. The significant difference between the slope obtained from that regression model and the slope reported by Sibly and Calow for iteroparous species (0.75) was compared using Chi-square test. The relationship between mean clutch weight against mean body fresh weight (W) / total ovariole number (Ov) = (W/Ov) was analysed using GLM-model with Poisson. All statistical analyses were carried out using the statistical programme 'R' version 2.8.1 (Ihaka and Gentleman, 1996).

3.4 Results

3.4.1 Reproductive output

The reproductive parameters of the 30 females of *H. axyridis* are presented in Table 3.1 and estimates of body size measurements for the 30 couples classified into three classes are presented in Table 3.2 as well.

Table 3.1. Reproductive parameters of 30 females of *H. axyridis* reared on the constant diet of pea aphid.

Class		Female			Male		
Small	Life history traits	n	Mean	±SE	n	Mean	±SE
	Fresh body weight (mg)	10	24.46	1.059	10	19.82	1.006
	Body length (mm)	10	6.74	0.189	10	6.11	0.12
	Body width (mm)	10	5.38	0.098	10	4.45	0.06
	Right tibia length (mm)	10	1.69	0.043	10	1.62	0.018
	Scutum length (mm)	10	3.08	0.065	10	2.92	0.051
	Wing length (mm)	10	8.99	0.3	10	9.03	0.26
	Wing width (mm)	10	3.07	0.17	10	2.71	0.09
	Longevity (days)	10	36.2	2.44	10	34.6	2.86
	Gonad	10	66.4	1.68	10	108.8	3.33
Medium							
	Fresh body weight (mg)	10	31.9	0.55	10	27.94	0.37
	Body length (mm)	10	6.43	0.12	10	6.39	0.063
	Body width (mm)	10	5.73	0.032	10	4.8	0.016
	Right tibia length (mm)	10	1.62	0.038	10	1.57	0.048
	Scutum length (mm)	10	3.04	0.053	10	2.93	0.026
	Wing length (mm)	10	9.45	0.18	10	9.65	0.043
	Wing width (mm)	10	3.17	0.094	10	2.95	0.051
	Longevity (days)	10	33.4	3.24	10	35	2.54
	Gonad	10	80.8	1.2	10	136.7	1.21
Large							
	Fresh body weight (mg)	10	40.57	1.5	10	31.02	0.53
	Body length (mm)	10	6.58	0.069	10	6.37	0.074
	Body width (mm)	10	5.89	0.029	10	5.23	0.099
	Right tibia length (mm)	10	1.65	0.045	10	1.633	0.029
	Scutum length (mm)	10	3.07	0.065	10	3.013	0.06
	Wing length (mm)	10	10.66	2.91	10	10.553	1.01
	Wing width (mm)	10	3.53	0.1	10	2.953	0.043
	Longevity (days)	10	40.6	0.092	10	30.5	2.034
	Gonad	10	91.6	1.14	10	145.4	1.26

Table 3.2. Mean estimates of body size parameters including longevity and the total number of gonads in males and females of *H. axyridis*.

Female	No of oviposited clutches	Oviposition period (days)	Lifetime fecundity	Mean proportion of trophic egg±SE	Mean proportion of cannibalized egg±SE	Mean proportion of unhatched larvae±SE	Mean proportion of hatched larvae±SE	Mean clutch size±SE
1	4	7	73	0.75 ±0.47	2±1.22	2±1.15	13±8.77	18.25±9.6
2	2	3	31	15.5±11.5	0	0	0	15.5±11.5
3	5	13	106	1.8±0.58	1.2±0.58	0.6±0.24	18±2.48	21.2±3
4	4	7	115	1.5±0.28	2.75±1.37	0.75±0.47	23±6.28	28±8
5	6	16	115	4.16±1.37	1.83±0.6	1.16±0.47	11.6±2.32	19.16±2.03
6	4	12	109	0.75±0.47	4±0.91	1.5±0.28	20.5±3.47	27.25±4.6
7	3	6	181	10.66±7.055	3.66±1.85	19.6±16.6	26.33±12.6	60.33±13.04
8	5	5	140	1±0.44	3.2±0.96	0.6±0.6	23.2±4.72	28±6.22
9	5	12	139	3.2±1.01	2.2±0.96	0.8±0.48	20.4±3.7	27.8±3
10	6	9	106	5.5±2.57	0	8.83±5.72	0.5±0.34	17.66±3.48
11	7	7	155	1.28±0.64	0.85±0.4	1.28±1	18.71±2.5	22.14±1.95
12	2	3	69	1.5±1.5	2.5±2.5	0.5±0.5	30±12	34.5±15.5
13	8	13	191	2.12±0.69	2.12±0.87	1±0.68	18±3.53	23.8±4.26
14	3	5	53	0.66±0.33	0	0.66±0.66	16.33±6.7	17.66±7.33
15	6	7	159	5.6±1.87	1.33±0.8	2.5±0.67	17.16±2.31	26.5±3.73
16	4	7	130	3.25±2.28	1.75±1.18	1.25±0.94	26.25±3.1	32.5±5.18
17	7	12	152	0.71±0.42	2.57±1.06	0.71±0.42	17.57±2.51	21.71±2.02
18	7	12	146	0.85±0.4	1±0.57	0.85±0.85	18.14±5.35	20.85±6
19	6	15	203	3±1.84	5.6±1.58	1.5±0.56	23.83±4.51	33.83±7.34
20	9	15	244	0.57±0.29	1.85±0.82	0.42±0.42	31.87±2.18	34.85±3
21	5	18	183	0.66±0.23	0.77±0.27	0.66±0.55	18.22±2.62	20.33±2.84
22	5	8	145	1±0.44	0.6±0.24	1.2±0.73	26.6±4.77	29±5.38
23	4	4	60	2±1.14	0.8±0.58	0.4±0.4	8.8±2.53	12±3.61
24	3	7	61	2.33±1.2	1±1	0.66±0.66	16.33±2.02	20.33±4.05
25	4	6	107	0	1.75±0.85	0.25±0.25	24.5±3.6	26.75±3.7
26	5	6	114	2.4±1.91	1.8±1.11	0.4±0.4	17.8±5.45	22.8±6.71
27	2	4	87	0	2.5±2.5	5±5	36±9	43.5±1.5
28	4	9	115	1.75±0.85	2.5±0.64	1±0.57	22.75±4.3	28.75±4.11
29	6	11	191	0.83±0.47	1.83±0.94	1±0.68	28.33±5.57	31.83±6.33
30	4	9	157	2.75±0.75	1.75±1.18	3.25±0.47	31±5.72	39.25±3.68
n=30	4.83±0.31	9±0.74	127.9±9					

3.4.1.1 Daily egg clutch size and weight

Neither mean clutch size laid by a single female nor the mean clutch weight varied significantly between the three classes (F=0.13, RSE=0.34, df=27, P=0.87) (Fig. 1) and (F=2.08, Residual deviance=0.55, df=27, P=0.14), respectively. When considering the total number of clutches laid by a single female instead of their mean with their own weights, no variability was revealed either (F=1.5, RSE=1.33, df=146, P=0.22) and (F=1, RSE=1.6, df=147, P=0.31), respectively. Allometrical analysis revealed that the mean clutch weight had no direct relationship with the mean fresh body weight (F=2.43, Residual deviance=0.32, df=28, P=0.12), with body length (F=1.17, Residual deviance=0.08, df=28, P=0.28) or with body width (F=1.48, Residual deviance=0.054, df= 28, P=0.23). Similar non-significant trends were also observed between the mean clutch size and the total number of ovarioles (F=1.21, RSE=0.32, df=28, P=0.27). Thus, ovariole number could not reflect the actual clutch size.

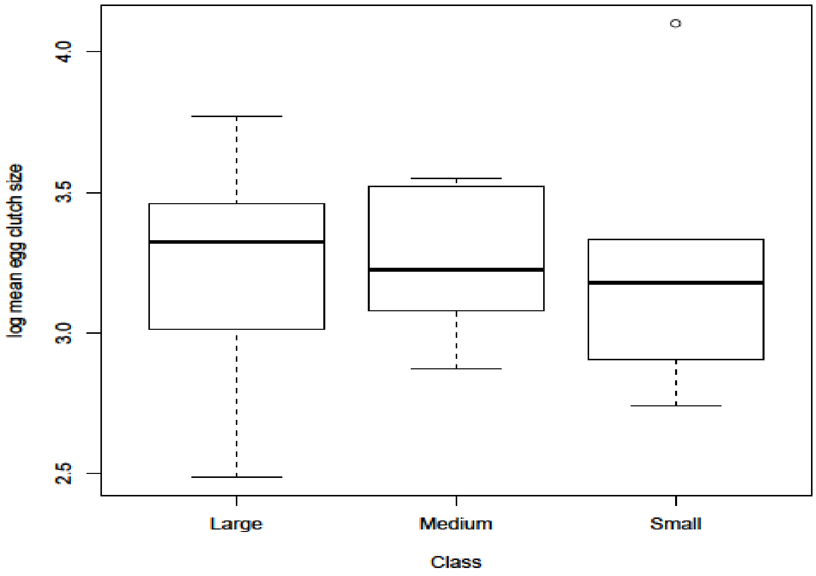

Fig.3.1. Variability in mean egg clutch size among body weight classes of females. Data were transformed for statistical analysis (F=0.08, P=0.92).

Lifetime fecundity of the 30 females is presented in Figure 3.2. Generally, at the onset of the reproductive period, the clutches comprised a relatively large number of eggs, and the number of eggs started to decline gradually towards the end of this period. A similar trend was observed in terms of the number of hatched larvae. Some eggs contained larvae but those larvae were not able to hatch. Those eggs were counted with the aid of a stereomicroscope (Fig.3.2). The mean number of clutches was directly related to the mean reproductive period (F=57.21, df=28, RSE=0.57, P<0.0001) (Fig. 3.4). The reproductive period was indirectly, but not significantly, related to both mean clutch size (F=0.344, RSE=0.5, df=28, P=0.56, slope=-0.1) and to the number of clutches (F=2.22, RSE=0.383, df=28, P=1.46, slope=-0.31). The above was found to be considerably convincing, since both the egg clutch size and the number of clutches was likely to decrease towards the end of oviposition or reproductive period, as shown in Figure 3.2.

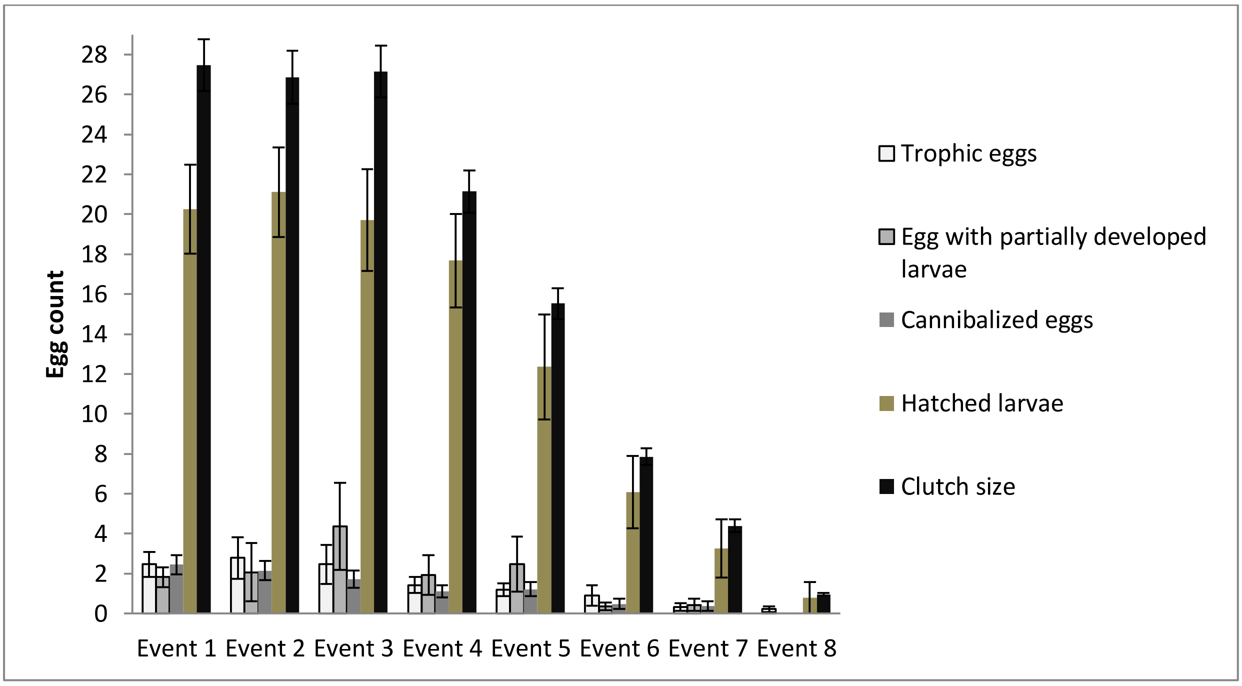

Fig.3.2. Life-time fecundity, and other reproductive output of 30 females during their oviposition period (n=30, mean± SE). Each event represents an oviposition.

Fig.3.3. Incomplete developed unhatched larva inside an egg.

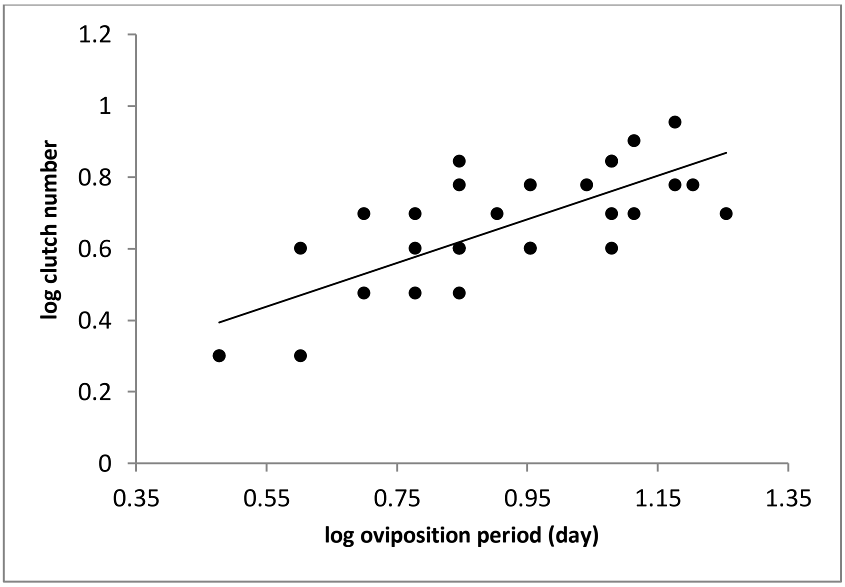

Fig.3.4. Relationship between log number of clutch against log oviposition reproductive period ($y=0.6x+0.1$, $R^2=0.57$). Data were transformed for statistical analysis ($F=57.28$, $P<0.0001$).

3.4.1.2 Egg parameters

Mean estimates of egg and clutch parameters are presented in Table 3.2. The weight of an egg laid per each event or oviposition did not differ significantly between different females (F=0.53, Residual deviance=0.04 on df=147, P=0.46). The volume of an egg, however, exhibited significant variability among females (F=7.14, Residual deviance=5, df=147, P<0.0001). Similarly, since a single clutch volume was estimated from an egg volume, it was found that the clutch volume varied significantly among females (F= 5.59, RSE= 1.7, df=146, P<0.05). There was a significant direct relationship between mean egg volume and female mean fresh body weight (F=4.53, RSE=0.083, df=28, P<0.05) (Fig. 3.5) and likewise with female body width (F=4.24, Residual deviance= 0.28, df= 28, P<0.05). The mean weight of an egg, on the other hand, showed no significant relationship with either the mean female fresh body weight (F=0.91, RSE=0.0042, df=28, P=0.34) or with mean female body width (F=1.92, RSE=0.0042, df= 28, P=0.17). The mean volume of an egg, however, was significantly related to the total number of gonads (ovariole number) (F=4.5, RSE=0.083, P<0.05) (Fig. 3.6). There was no significant direct relationship between the mean volume of an egg and the mean weight of a clutch from which they hatched (F=0.88, Residual deviance=1.58, df=146, P=0.3). This implies the occurrence of a trade-off between the egg number and their volume. Nevertheless, the egg clutch size was directly related to the number of eggs of which it consisted (F=21.65, Residual deviance=1.24, df = 147, P<0.0001).

Table 3.3. Mean egg and clutch parameters estimated from 30 females (mean±SE).

Female	No of oviposited clutches	Oviposition period (days)	Lifetime fecundity	Mean proportion of trophic egg±SE	Mean proportion of cannibalized egg±SE	Mean proportion of unhatched larvae±SE	Mean proportion of hatched larvae±SE	Mean clutch size±SE
1	4	7	73	0.75 ±0.47	2±1.22	2±1.15	13±8.77	18.25±9.6
2	2	3	31	15.5±11.5	0	0	0	15.5±11.5
3	5	13	106	1.8±0.58	1.2±0.58	0.6±0.24	18±2.48	21.2±3
4	4	7	115	1.5±0.28	2.75±1.37	0.75±0.47	23±6.28	28±8
5	6	16	115	4.16±1.37	1.83±0.6	1.16±0.47	11.6±2.32	19.16±2.03
6	4	12	109	0.75±0.47	4±0.91	1.5±0.28	20.5±3.47	27.25±4.6
7	3	6	181	10.66±7.055	3.66±1.85	19.6±16.6	26.33±12.6	60.33±13.04
8	5	5	140	1±0.44	3.2±0.96	0.6±0.6	23.2±4.72	28±6.22
9	5	12	139	3.2±1.01	2.2±0.96	0.8±0.48	20.4±3.7	27.8±3
10	6	9	106	5.5±2.57	0	8.83±5.72	0.5±0.34	17.66±3.48
11	7	7	155	1.28±0.64	0.85±0.4	1.28±1	18.71±2.5	22.14±1.95
12	2	3	69	1.5±1.5	2.5±2.5	0.5±0.5	30±12	34.5±15.5
13	8	13	191	2.12±0.69	2.12±0.87	1±0.68	18±3.53	23.8±4.26
14	3	5	53	0.66±0.33	0	0.66±0.66	16.33±6.7	17.66±7.33
15	6	7	159	5.6±1.87	1.33±0.8	2.5±0.67	17.16±2.31	26.5±3.73
16	4	7	130	3.25±2.28	1.75±1.18	1.25±0.94	26.25±3.1	32.5±5.18
17	7	12	152	0.71±0.42	2.57±1.06	0.71±0.42	17.57±2.51	21.71±2.02
18	7	12	146	0.85±0.4	1±0.57	0.85±0.85	18.14±5.35	20.85±6
19	6	15	203	3±1.84	5.6±1.58	1.5±0.56	23.83±4.51	33.83±7.34
20	9	15	244	0.57±0.29	1.85±0.82	0.42±0.42	31.87±2.18	34.85±3
21	5	18	183	0.66±0.23	0.77±0.27	0.66±0.55	18.22±2.62	20.33±2.84
22	5	8	145	1±0.44	0.6±0.24	1.2±0.73	26.6±4.77	29±5.38
23	4	4	60	2±1.14	0.8±0.58	0.4±0.4	8.8±2.53	12±3.61
24	3	7	61	2.33±1.2	1±1	0.66±0.66	16.33±2.02	20.33±4.05
25	4	6	107	0	1.75±0.85	0.25±0.25	24.5±3.6	26.75±3.7
26	5	6	114	2.4±1.91	1.8±1.11	0.4±0.4	17.8±5.45	22.8±6.71
27	2	4	87	0	2.5±2.5	5±5	36±9	43.5±1.5
28	4	9	115	1.75±0.85	2.5±0.64	1±0.57	22.75±4.3	28.75±4.11
29	6	11	191	0.83±0.47	1.83±0.94	1±0.68	28.33±5.57	31.83±6.33
30	4	9	157	2.75±0.75	1.75±1.18	3.25±0.47	31±5.72	39.25±3.68
n=30	4.83±0.31	9±0.74	127.9±9					

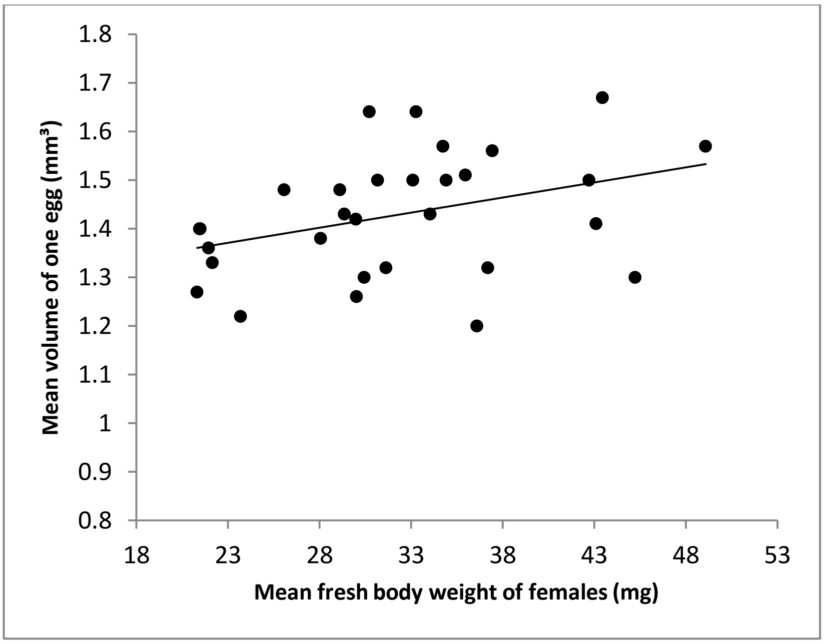

Fig.3.5. Relationship between mean volume of an egg and mean female fresh body weight ($y=0.006x+1.3$, $R^2=0.13$). Data were transformed for statistical analysis ($F=4.53$, $P<0.05$).

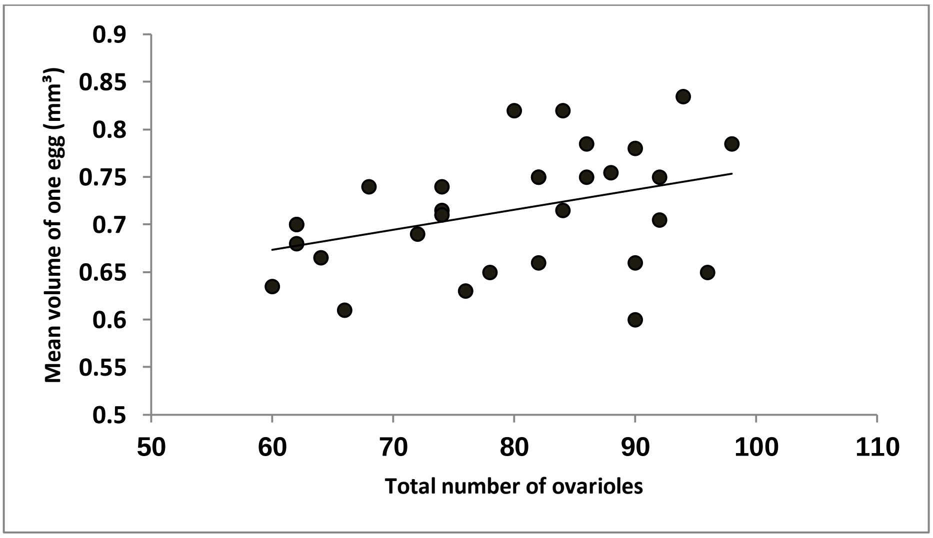

Fig.3.6. Relationship between mean volume of an egg against total number of ovarioles ($y=0.0021x+0.54$, $R^2=0.14$).

3.4.1.3 Total fecundity

The results revealed the absence of variability between females that belong to the three classes, even when reared under constant controlled measures (F=1.07, Residual deviance=5.51, P=0.354). There was a significant positive relationship between lifetime fecundity and the total number of gonads (t =2.07, Residual deviance=4.78, df=28, P<0.05) (Fig.3.7). Thus, females with more ovarioles were inclined to lay more eggs compared with those that possessed fewer ovarioles. No direct relationship was exhibited between the lifetime fecundity and the mean fresh body weight of females (F=1.8, Residual deviance=5.59, df=28, P=0.19). In contrast, the body length of females had a positive direct relationship with lifetime fecundity (F= 5.41, Residual deviance=4.98, df=28, P<0.05). As the study expected, longevity was found to be negatively related to both lifetime fecundity and mean clutch size – (F=0.22, slope=-18, P=0.63) and (F=0.24, slope=-0.17, P=0.62), respectively. This implies that investing in reproduction appeared to have a cost on longevity of females.

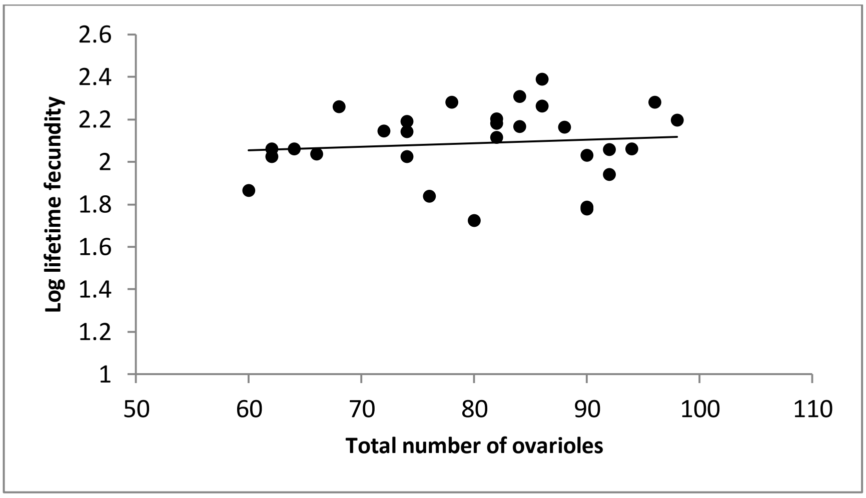

Fig. 3.7. Relationship between total number of ovarioles and log lifetime fecundity of females ($y=0.001x+2$, $R^2=0.01$). Data were transformed for statistical analysis (t-value=2.07, P<0.05).

3.4.1.3 Egg viability

Since all the larvae were removed directly after their emergence, the number of different types of eggs did not influence the total number of hatched larvae. For example, the number of trophic eggs produced was unrelated to the number of hatched larvae (z-value=-0.98, Residual deviance=0.54, df=26, P=0.32). The incubation period was found to be one of the determinants of potential fertility or the success of larval hatching, since both were found to be indirectly related to this period (F=21, Residual deviance=0.35, df=27, P<0.0001, slope=-2.4). This implies that the longer the incubation period, the higher the likelihood of having more infertile eggs. The latter was supported by the fact that the incubation period was directly related to the proportion of eggs with unhatched larvae, as well as to the proportion of trophic eggs – (F=53.5, Residual deviance=0.07, df=27, P<0.0001) (Fig.3.8) and (F=12.4, Residual deviance=0.55, df= 26, P<0.001), respectively.

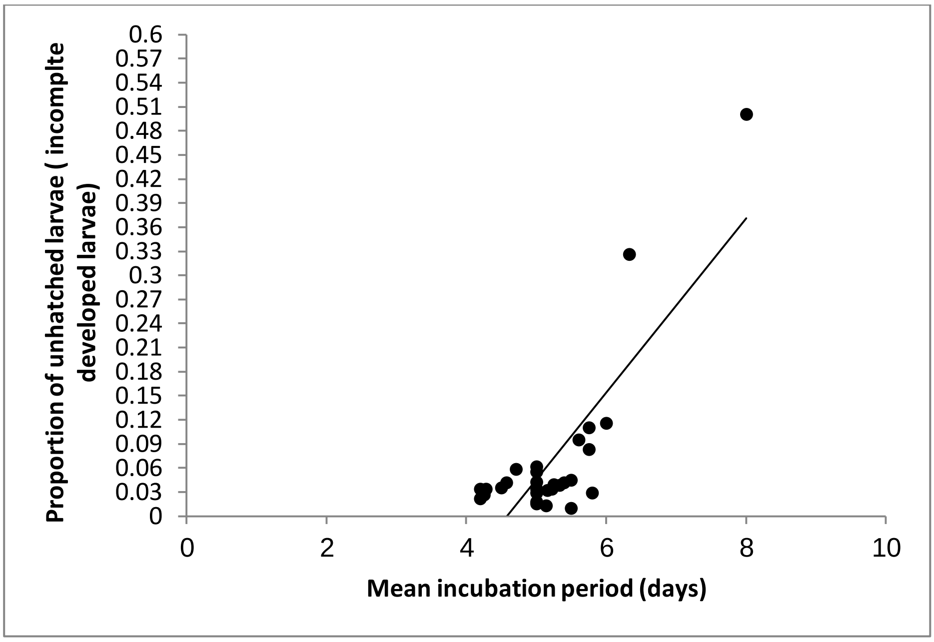

Fig.3.8. Relationship between the proportion of hatched larvae and the incubation period ($y=0.108x +0.5$, $R^2=0.66$). Data were transformed for statistical analysis (F=21, P<0.001).

There was a direct linear relationship between the number of hatched larvae and the size of the clutch from which they had hatched (F=330, RSE=5.82, df=138, $P<0.0001$). This could prove the absence of the cost in terms of the number of both eggs with unhatched larvae and trophic eggs relative to large clutch size. The size of a clutch was directly correlated with eggs having unhatched larvae (F=19.96, Residual deviance=19.68, df= 63, $P<0.0001$) and with the number of trophic eggs (F=5, Residual deviance=61, df= 90, $P<0.05$). Eventually, this led to the proposition that the size of a clutch might not represent the potential fecundity, as larger clutches may possess a relatively high number of infertile eggs or eggs with unhatched larvae that are expected to reduce the fertility of a female. The above was verified when the lifetime fecundity was found to be directly related to the proportion of trophic eggs and non-hatched larvae – (F=5.002, Residual deviance=61.6, df= 90, $P<0.05$) and (F=20.67, Residual deviance=19.6, df= 62, $P<0.0001$), respectively. In light of this, it could be assumed that high fecundity does not always reflect high fertility.

3.3.1.5 Reproductive biomass and growth rate

Using the formula indicated in the method section, the mean reproductive biomass was calculated, when using the mean weight of an egg it was 0.4±0.004 egg/mg and when using the mean weight of one clutch it was 3.3±0.43 egg/mg. Both of the above calculated values had a linear relationship with the mean fresh weight of females – (F=4.8, Residual deviance=0.4, df= 28, $P<0.05$) (Fig. 3.9) and (F=4.22, RSE=5.24, df=28, $P<0.05$), respectively. This seemed to support the statement by Stewart et al. (1991a) that doubling adult body fresh weight would possibly result in doubling the reproductive biomass or egg production.

The number of the ovarioles varied significantly between females (F=4, RSE=10.7, df=28, P<0.05). Therefore, the variation in ovariole number could be corrected following Stewart et al. (1991a) by dividing the mean fresh body weight (W) by the total ovariole number (Ov) = (W/Ov). Regression analysis subsequently performed between the mean weight of a single egg (E) against (W/Ov) revealed no direct relationship between the two (F=0.91, Residual deviance=0.004, df=28, P=0.34). The results were inconsistent with the statements of Stewart et al. (1991a). Accordingly, this study suggested modifying their proposition by considering the mean clutch weight instead of an egg weight in the formula. As expected, regressing the mean weight of a single clutch (CW) against the (W/Ov) revealed a significant direct relationship (F=4.74, Residual deviance=0.02, df=28, P<0.05) (Fig. 3.10).

The mean reproductive rate for the 30 females was found to be 15.58±1.06 eggs/day (maximum=30.16 eggs/day, minimum=7.18 eggs/day). Log-log regression analysis showed that the reproductive rate had a linear relationship with mean fresh body weight (F=4.8, Residual deviance=0.34, df=28, P<0.05) (Fig. 3.11). The interaction between the mean fresh body weight and the total number of ovarioles also showed a significant positive relationship with reproductive rate (F=5.94, Residual deviance= 1.47, df=14, P<0.05).

Fitting the reproductive growth against the adult fresh body weight revealed no significant direct relationship (F=2.1, RSE=0.23 df=28, P=0.16). The obtained slope estimate from the analysis was 0.83, which was found to be not significantly greater (X^2=0.0041, P=1) than the 0.75 slope recorded for iteroparous species by Sibly and Calow (1986). The concealed relationship between the reproductive growth rate and fresh body weight was likely to be due to the influences of other reproductive factors or morphological aspects of *H. axyridis*. Regarding males' contribution in reproductive success, the number of testicular follicles were regressed against the total fecundity and the results revealed a significant direct relationship (t=2.07, Residual deviance =4.7, df= 28, P<0.05) (Fig. 3.12).

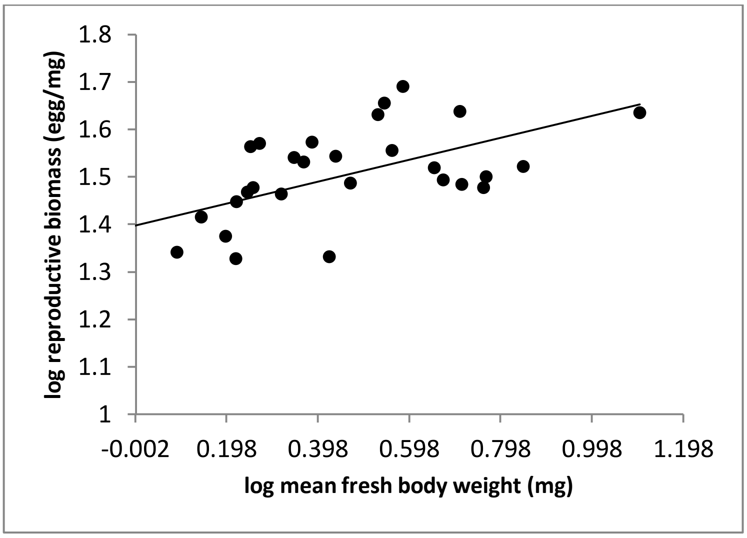

Fig.3.9. Relationship between log mean fresh body weight of females against log reproductive biomass (y=0.23x+1.4, R^2=0.36). Data were transformed for statistical analysis (F=4.22, P<0.05).

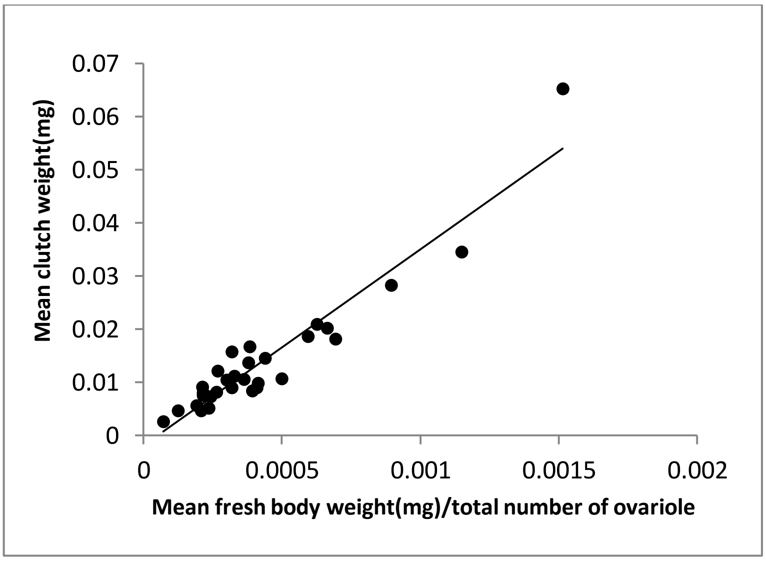

Fig.3.10. Relationship between mean clutch weight plotted against mean fresh weight divided by the total number of ovarioles (y=37x-0.002, R^2=0.9). Data were transformed for statistical analysis (F=4.74, P<0.05).

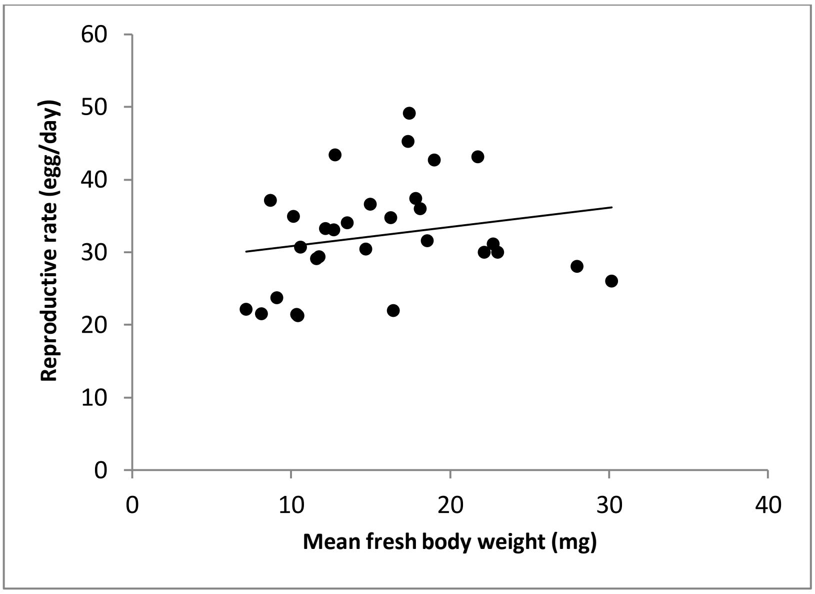

Fig.3.11. Relationship between mean female fresh body weight and reproductive biomass estimates between ($y=0.26x+28.14$, $R^2=0.0.043$). Data were transformed for statistical analysis ($F=4.74$, $P<0.05$).

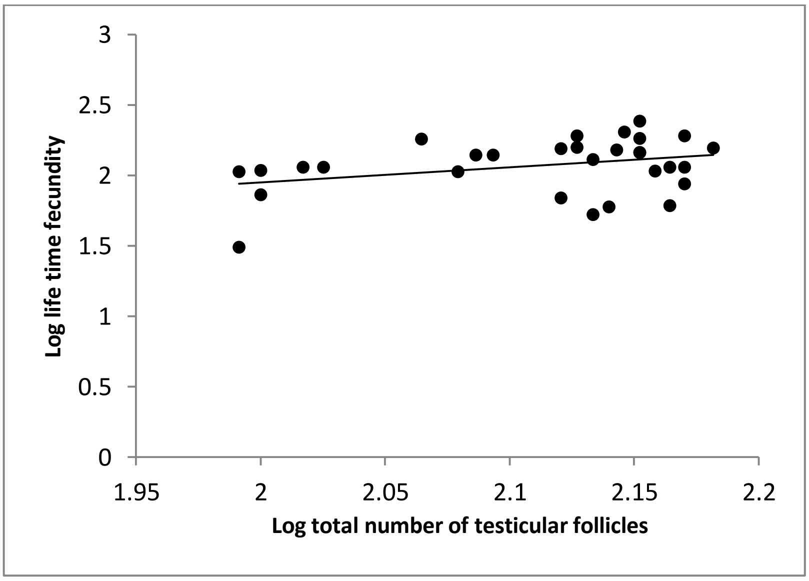

Fig.3.12. Relationship between log total testicular follicles and mean fresh body weight ($y=1.08x+0.21$, $R^2=0.11$). Data were transformed for statistical analysis ($t=2.07$, df= 28, $P<0.05$).

3.5 Discussion

Invasive species are often characterised by having large body size (Roy et al., 2001, 2002, Brown and Sibly, 2006). A larger body size usually promotes greater fecundity, such as in the predatory coccinellids (Stewart et al. 1991a, Dixon, 2000). For example, *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) is possesses a large body size and has become extremely successful and well established throughout North America in recent years (Maredia et al., 1992, Hesler and Kieckhefer, 2008). It has been reported that large body size is always concomitant with high fecundity. Brown and Sibly (2006) proposed that the association between large body size and the lifetime fecundity may be preferred by natural selection, particularly when conditions are favourable. By extension, this association had been recognised as a possible key factor promoting the predominance of invasive species, such as the multicoloured beetle *H. axyridis* (Koch, 2003, Roy et al., 2006) over smaller local species, especially in resource-rich environments (Tilman, 1999).

The present study was carried out mainly to investigate the reproductive capacity of the coccinellid *H. axyridis* maintained under a controlled feeding protocol and rearing conditions, in order to reveal the connection between the reproductive performances relative to body size of females (which has been ultimately proposed by many studies as the key factor for successful invasion).

Eventually, it was recognised that this predator exhibits distinct reproductive strategies that are determined by a combination of factors, including the body size parameters, number of ovarioles and food availability. In addition, the reproductive performances increased and then levelled off at a certain number of clutches, due to genetic influences. Hodek and Honek (1996) noted that there is a limitation on the reproductive output that is genetically fixed even when food is plentiful and environmental conditions are favourable. In addition, despite the regular prey availability in this study, there seemed to be an upper limit to the egg production rate, linked to body morphology, in conjunction with the female strategies in assessing their external environment. Physical constraints were also recognised by others and the maximum egg size was found to be controlled by the nature of the ovipositor (Fitt, 1990).

This study estimated egg volume using the prolate spheroid method. Accordingly, it was revealed that the volume and weight of an egg must be dealt with as separable independent egg parameters. The volume of a single egg appeared as the most reliable measure used to assess the clutch parameters, based on several aspects. One of these aspects was the strong positive relationship between egg volume and body weight and body width, as well as the number of ovarioles. These distinguishable relationships are exceptional and could be incorporated to predict the performance of other species with equivalent qualities to *H. axyridis*. Nevertheless, the study revealed the existence of a significant direct relationship between total life fecundity, trophic eggs and fertility. Similar observations were previously noted by Omkar et al. (2006). Clearly, the possession of high fecundity did not always convey great fertility and a high rate of hatchability. In some cases, high fecundity reflects the exquisite provisional strategy manifested in producing deliberately large-sized clutches comprising a relatively high number of non-viable eggs in spite of prey availability. This phenomenon is an adaptive behaviour known as "nursery" behaviour and is performed in order to provide extra food for the newly hatched larvae, besides reducing sibling predation and egg cannibalism (Omkar and Mishra, 2005).

Variability in egg size interspecifically was recognised to be subject to genetic influences and environmental factors, but intraspecific disparity was frequently ascribed to food status, or in some cases to the parental age (Fox, 1993ab). Dixon and Guo (1993) noted that the variability in egg size was more likely to be due to starvation and season. This study noted the absence of intraspecific variability in terms of lifetime fecundity. Therefore, on the whole, all large-, medium- and small-sized females allocated the same amount of resources for reproduction. The study suggested that the presence of intraspecific variations were not extremely pronounced because the rearing conditions were well controlled. Although some studies asserted the presence of positive intraspecific variation in some species of insect, the lack of such variations was also confirmed frequently in others (Fitt, 1990, Honek et al., 2008). The current study at least affirmed the presence of intraspecific variability, particularly in the clutch and egg volume. In fact, variability within these two parameters (though to a greater extent egg volume) has been reported in several studies on ladybirds including *H. axyridis* (Dixon and Guo, 1993, Honek et al., 2008). Stewart et al. (1991a) confirmed that the weight of an egg must be regarded as a species characteristic, since it merely differs between small- and large-sized clutches. This is in agreement with the results revealed by the current study, as no variability was found among egg weights taken from different clutches. Therefore, this study reinforces the theory that egg weight could be regarded as a species-specific characteristic.

Lifetime fecundity was not corelatd with fresh body weight, as was reported by Leather (1988). In his work, it was noted that such a relationship existed, but it may be controlled by factors including environmental and physiological constraints. In view of that, the present study delivered suggestions that could accentuate this relationship. For instance, it was expected that using fresh body weight without considering the changes (particularly during growth and reproduction) might be a crucial issue. This is in agreement with Dixon and Agarwala (2002), in which there was about 20-40% rise in the body weight of *H. axyridis* adult after emergence. By the same token, Dixon and Guo (1993) reported that the weight of an adult female coccinellid was reliant on the stage of their gonads. This implies that the process of egg formation during the reproductive period might lead to a notable increase in female body weight. A previous study had demonstrated that the ovaries of freshly emerged female of *H. axyridis* did not contain any eggs (Dixon and Agarwala, 2002). A similar observation was noted in this study after dissecting newly emerged females as part of a preliminary investigation before the onset of the study. Therefore, it is of great importance to incorporate the body weight of a female at different times during living period by calculating the average. It appears that the average weight might have a great impact in appraising the probable fecundity and body weight relationship.

Another supportive proposal built on statistical analysis was the significance of using the total number of clutches and total fecundity (i.e., all eggs in each clutch per female), rather than using their means. Taking that proposition into account would result in a better explanation of the reproductive performance, particularly when comparing individuals and populations reared under uniform conditions. For instance, intraspecific analysis conducted between females revealed that the event of oviposition and the number of clutches in each event along the reproductive period failed to explain the lack of variability in lifetime fecundity between females. All appeared to have relatively similar lifetime fecundity. Furthermore, following the oviposition events for each female illustrated why small-sized females had lifetime fecundity almost reaching that of medium- or large-sized females. Identifying these variations was not straightforward, because most of them were concealed by each individual female’s oviposition strategies. Some females had a tendency to lay large clutch sizes at slow rates (i.e., intermittent oviposition) and others laid smaller clutches at higher rates (i.e., non-intermittent oviposition) during their reproductive period. In both circumstances, none of them implied high fecundity. To better illustrate this, among females of the small class, one female laid only three clutches but yielded a lifetime fecundity of 181 eggs, whereas a large sized-female produced five egg clutches but laid only a total of 183 eggs during her lifetime. In the former, only 43.64% managed to hatch into larvae and the remaining 50.27% were non-viable eggs. The latter female, however, had 90% hatched larvae and only 6.5% were considered non-viable eggs.

The great weight possessed by large females may not always be a positive indicator of high lifetime fecundity. The stored fat tissues may sometimes act as a constraint confining egg development. For example, females of *Coccinella trifasciata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) had a mean body weight of 22.5±5mg and their median number of ovarioles was 49. In comparison, females of *Calvia 14-guttata* (Linnaeus,1758) (Coleoptera: Coccinellidae) had a lower mean body weight (16.9±2mg), but similar median ovariole number (Stewart et al., 1991b). In the same study *Propylea 14-punctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) had a median ovariole number of 24 and *A. bipunctata* (Coleoptera: Coccinellidae) had a median ovariole number of 46. The latter produced the same mass of eggs per day as the former. Another comparative study revealed that although large-sized females of *C. septempunctata* laid large clutches compared with the small-sized *Coccinella trasnversoguttata* (Linnaeus, 1758) (Coleoptera: Coccinellidae), both responded to prey deprivation in a similar fashion, by stabilising body weight and reducing the oviposition rate (Kajita and Evans, 2009). As concluded from previous studies, larger and smaller species were likely to embrace significantly diverse reproductive strategies (Arnaud et al., 2005).

The present study summarised that the degree of intraspecific dissimilarities in reproductive qualities when measured under controlled conditions reflected the influences of a number of factors, such as the characteristics of an ovary, male fertility and genetic aspects. These factors may act synergistically in determining the reproductive effort, regardless of body size measurements. Furthermore, variations probably occurred when comparing laboratory results with field studies (Phoofolo et al., 2009). In addition, intraspecific variations are widely recognised among iteroparous species (Stearns, 1992, Fox and Czesak, 2000, Arnaud et al., 2005).

In conclusion, despite the widespread data on intraspecific variations, complications inevitably arise during investigation and data analysis (Bernardo, 1996, Fischer and Fiedler, 2001ab). The study revealed that the mean clutch size of *H. axyridis* was 26.86±1.75 eggs, which was very close to the value of 27.4 eggs reported by Stewart et al. (1991b) and 29.5±12.10 eggs noted by Stathas (2001). The mean lifetime fecundity of *H. axyridis* recorded by the present study was 127.9±9 eggs, which was found to be significantly different to the 1,641.6±420.95 eggs previously recorded for this beetle when it was reared on *Aphis fabae* (Scopoli,1763) (Hemiptera: Aphididae) (Stathas, 2001). Abdel-Salam and Abdel-Bakey (2001) demonstrated that when *H. axyridis* was reared on *A. pisum* the average number of eggs produced per female was 163.9±144 eggs. In comparison, a study conducted by McClure (1987) obtained a result of 718.7 eggs per female, after rearing *H. axyridis* on the same aphid species (*A. fabae*) but at a different temperature (27^0C). Schanderl et al. (1988) reported that the lifetime fecundity of *H. axyridis* was 522 eggs per female when reared on the eggs of *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae). Clearly, the dissimilarities observed among the reproductive output of *H. axyridis* could be attributed to the rearing conditions, feeding regime and possibly to genetic origin.

It was noted that the triangular association between total fecundity, egg parameters and body traits could be shaped by several factors, such as longevity, parental age and prey quality (Stearns, 1992, Omkar et al., 2006, Mishra and Omkar, 2006). Several hypotheses have been put forward regarding the deterioration of fecundity with age (Dixon and Agarwala, 2002). The declines in reproduction and prey searching ability throughout adult life may be a consequence of senescence (Mishra and Omkar, 2006). In this study, the effect of senescence on female feeding ability was unclear, because most of the tested females were capable of catching their prey effectively even at later stages in their lives. The study expected, however, that in field studies and under adverse environments, such a relationship would be more prominent due to the higher vitality requirement for prey searching. In a similar context, Dixon (2000) reported that when oviposition is not of an intermittent type, the trade-off between longevity and fecundity would be excessive. He pointed out those females of *H. axyridis* followed distinctive strategies to achieve a sense of balance between early and late age fitness for successful survival. This was in agreement with Omkar et al. (2006), who reported a linear relationship between longevity and the intermittent type of ovipoistion, suggesting that intermittent oviposition often resulted in a delay phase that tended to increase the life expectancy of ladybirds. In this study, the oviposition pattern of *H. axyridis* was found to be intermittent, but had an indirect relationship with longevity. The study therefore assumed that the fecundity-longevity trade-off might be likely to be significant when dealing with variable diet quantity or quality and different temperature ranges.

The present study showed that reproductive biomass and rate were positively allied with the body size in *H. axyridis*. This association delivered a resilient reproductive advantage over other predators. This advantage is credited to the large body size and the high number of ovarioles. Both resulted in the achievement of a high rate of egg production. It was previously assumed by Dixon and Guo (1993) that species with a large body size should be highly fecund and lay larger clutches. This could be achieved either by having a high number of ovarioles or through alternate egg production between the left and the right ovaries (Dixon, 2000). The role of the relationship between both body size and ovariole number in determining efficient reproductive output was also reported by Stewart et al. (1991b).

Large body size, however, is not always preferred by natural selection, particularly during food scarcity (Obrycki et al., 1998, Brown and Sibly, 2006, Dixon, 2007). Being excessively large makes some insects more susceptible to predation by several larger predators, such as birds (Holmes et al., 1979). Nevertheless, as noted by this study previously, the number of ovarioles occasionally imposed a constraint on the maximum number of produced eggs (Stewart et al., 1991a). This was validated when several aphidophagous ladybirds had the almost the same rate of egg production despite significant variations in their body size (Mishra and Omkar, 2004, Omkar and Mishra, 2005). Furthermore, in the present study it was found that the median ovariole number for *H. axyridis* was 82 ovarioles and the mean clutch size produced by one ovariole was approximately 26.86±1.72 eggs. Stewart et al. (1991 a) noted that the clutch size produced daily by a female of *H. axyridis* must be equal to half the number of the total ovarioles in that female. In this study, the mean total ovariole number was 79.6 ovarioles and half of this number was 39.8 ovarioles. The clutch size (26.86±1.72 eggs) was found to be less than half of the ovariole number (39.8 ovarioles), but 26.86±1.72 eggs was the production of one ovary and the study expected this number to be doubled when both sides, left and right ovaries, worked simultaneously. As noted previously by Stewart et al. (1991a), in coccinellids and other insects egg production alternates between ovarioles in order to allow for continuous production. This explained how the reproductive performance promoted the inevitable colonisation and establishment of *H. axyridis* worldwide.

It could be concluded that the allometric relationship between reproductive attributes and body size was found particularly in reproductive rate and biomass, however, it could be masked by individual reproductive strategies, as well as rearing conditions. Individual strategy in oviposition pattern and maternal provision might be expected to change during stressful conditions, such as food deprivation. The variable strategies in the individual performance of *H. axyridis* highlight the association between the phenotypic plasticity and ability to colonise new habitats in variable conditions. Two main trade-offs were noticed under constant feeding regime, first, the longevity-fecundity relationship, and second, an individual egg volume and clutch size that might possibly influence the reproductive output and body size relationship. Although the influence of a male's gonads on female fecundity was found to be significant, more investigations are required to highlight further a male's contribution.

The successful invasion of *H. axyridis* in many countries has been attributed to its reproductive tactics, which is regarded as one of the most important aspects of its life-history strategies. This reproductive performance is unique and associated with large body size as well as high fecundity. Nonetheless, being able to provide extra food for the hatchlings without apparent cost was another distinctive capability that would further help in increasing the survival rate of the immature stages when invading a new unpredictable range of habitat; or when facing intra-guild competition with native species over food scarcity. The alternate functions of the ovary could provide an extra advantage over other species, facilitating the consistent availability of this species around the time that it may have become a dominator, as well as a threat to other species. In addition to all of these features, further investigations are needed to highlight the importance of the male potency role in fertilising that high number of eggs within a short period of time and the associated trade-offs.

Chapter 4

Factors Affecting Variation in Developmental Attributes of *Harmonia axyridis* Larvae Reared on Constant Diet

4.1 Introduction

Body size and developmental time are important determining factors of an organism's fitness, as they arbitrate the basic biological aspects, such as mating success, reproduction and competition (Roff, 1992, Stearns, 1992, Brown and Sibly, 2006, Phoofolo et al., 2009). Among insects, the regulation of body size and growth period is an important issue in developmental biology (Nijhout, 2003). Growth in holometabolous insects mainly ends with two stages, pupation and metamorphosis (Chapman, 1998). Therefore, body size regulation requires an understanding of the mechanisms regulating the termination of the growth period (Davidowitz et al., 2004). The rate of growth, however, cannot explicitly determine body size, because the latter is a product of the interaction between growth rate and growth duration (Atkinson, 1994, Gotthard, 2001, Stern, 2001, Davidowitz et al., 2004).

It is often assumed that attained final adult body size features are influenced by performance during early stages of development and frequently adjusted by both genetic and environmental factors, functioning at both cellular and organismal levels (Nijhout, 2003). Even with the longstanding awareness of the ecology and evolution of body size plasticity (Stearns, 2000), little is known about the developmental and physiological mechanisms that eventually determine body size, or the mechanisms leading to the great diversity of sizes (Stern, 2001, Mirth and Riddiford, 2007). Via and Lande (1985) and Higgins and Rankin

(1996) noted that both genetic and environmental factors were found to induce significant variations in the age and size at which an individual matures. In addition, growth conditions experienced in larval development were revealed to exert fitness costs on body size and its parts (Dmitriew et al., 2009). The most frequently noticed costs are the additional growth in structural size that precedes the gains in body mass (Dmitriew, 2005, Boggs and Freeman, 2005). This extra structural growth might result in composite effects on body allometry (Frankino et al., 2005), could increase the length of the developmental period (Davidowitz et al., 2003), raise pre-reproductive mortality (Metcalfe and Monaghan, 2001) or reduce size at maturity. A reduction in body size has been found to be accompanied by a decrease in reproductive performances, such as lifetime fecundity and mating success, and a reduction in longevity in the order Odonata (encompassing dragonflies and damselflies) (Sokolovska, 2000). Whether the final accomplished body size is mainly determined by initial larval size, nutritional status, environmental factors or has strong genetic determinants, is a question that still has no definitive answer and must be investigated further.

Numerous studies have reported that size control in *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae) is often governed by regulation of growth rate and control over growth termination (Mirth and Riddiford, 2007). In contrast, in the tobacco hornworm, *Manduca sexta* (Linnaeus, 1763) (Lepidoptera: Sphingidae), the physiological bases of body size evolution are determined by several factors, including initial size, growth rate of the last instar, critical weight estimates and time delay between achieving the critical weight and metamorphosis hormonal secretion (D'Amico et al., 2001, Davidowitz et al., 2003). The hormonal regulations over the growth characteristics and the critical weight have already been explained thoroughly in the general introduction (Chapter 1) and more details have been reported elsewhere, in particular by Davidowitz and Nijhout (2004).

One of the important key factors in regulating the growth of larvae is that, in the absence of juvenile hormone, there are two pulses of ecdysteroid hormone secretion. The first minor pulse prompts the shift from larval to pupal stage, and the second much larger pulse induces the moulting of pupa (Nijhout, 1999). D'Amico et al. (2001) investigated the physiological basis of body size development in *M. sexta* and noted that achieving the critical weight or size is one of the critical stages at which development becomes committed to metamorphosis in this species. This is because the critical weight always leads to alterations in hormonal levels that make the respective stage ready for an advance in growth (Berreur et al., 1979, Nijhout, 1981, Riddiford, 1993ab). There are many definitions recognised for critical weight. For some insects, the critical weight is the critical stage occurring during the course of the last larval stage period before metamorphosis; in others the mean critical weight is the weight at which 50% or 54% of larvae are able to pupate (Robertson, 1963, Royes and Robertson, 1964, Bakker, 1969, Davidowitz et al., 2004).

Misconceptions have arisen over the years regarding the exact definition of the critical weight. For example, Jones et al. (1981) examined the critical weight in the cabbage looper caterpillar *Trichoplusia ni* (Hübner, 1800-1803) (Lepidoptera: Noctuidae). Their weight classes were, however, defined at the time of the moult to the last instar (and not during the last instar). Woodring (1983), Ochieng Odero (1990), and De Moed et al. (1999) all defined the critical weight as the weight at which 50% of the larvae were able to pupate. Davidowitz and Nijhout (2004) noted this is not a measure of critical weight, as originally defined by Nijhout and Williams (1974ab), but described the minimal viable weight instead. In holometabolous insects such as *D. melanogaster* and *M. sexta*, development after attaining the critical weight is often determined by a post-critical weight period that extends until the commencement of pupation (Beadle et al., 1938, Bakker, 1961, Robertson, 1963, Nijhout and

Williams, 1974ab). As yet, there is no model species that could be used to describe the hormonal regulation during development. For example, in *M. sexta*, the critical weight is reliant on specific size-related characteristics (Nijhout and Williams, 1974ab, Blakley and Goodner, 1978, Nijhout, 1979). In contrast, in *D. melanogaster* the critical weight occurs directly after accomplishing the second moult and the developmental rate is no longer affected by the available food level (Beadle et al., 1938, Bakker, 1961, Robertson, 1963). In general, the larval developmental period is determined by the time needed to reach the critical weight.

Despite longstanding interest in studying the development of insect bodies and how an organism interprets the diverse environmental signals that may lead to plasticity of body size (Stern, 2001), little is known about the physiological mechanisms that govern larval development and their growth rate. Further details about the hormonal regulations of critical stage and size have been explained in the general introduction. This study will therefore not repeat these theories, but will attempt to apply the previously stated findings. In addition, the study will assess and determine the critical weight estimates and developmental features of *H. axyridis* larvae through direct simple methods.

For genetically similar insects, the variation in body size can be attributed to nutrient assimilation and allocation, mainly during the fourth instar stage (Nijhout, 2006, Berner and Blanckenhorn, 2007). This fact however, needs further assessment, particularly when bearing in mind the environmental and genetic interactions with body size characteristics, as well as the sex type. It has been noted that the larval developmental period (excluding pupal period) and the commencement of pupation are the main determinants of post-embryonic development among holometabolous insects (Dixon, 2000, Omkar et al., 2004). Few studies have made an effort to determine the regulation of larval developmental duration or the timing of pupation (Nijhout, 1981). According to Riddiford (1996), the main cues that holometabolous insects use to regulate the developmental processes were found to be unclear. Regulation processes among some holometabolous insects rely on a pre-programmed critical size threshold and on their capability of assessing their own sizes (Nijhout, 1981). Many insects assess their body weight through their stored fat tissues, which act as a detector for nutrient availability and control larval development by initiating insulin regulating signals (Nijhout, 1981). Pupal stage initiation is, however, a very crucial process and cannot be evaluated easily using the critical size threshold, as there is a limitation to the level of successful pupation that is bound to attain the minimum threshold weight (Shafiei et al., 2001). The latter is entirely different from the critical weight threshold. The critical weight threshold is the weight essential to regulate the metamorphosis process, whereas the minimum threshold weight is the weight of stored fat that is sufficient to guarantee metamorphosis survival of developing larvae (Shafiei et al., 2001). Further explanations were summarised in Chapter 1.

Some holometabolous insects, if deprived of nutrients before attaining their critical threshold size, tend to extend their larval period in order to allocate additional food resources (Halffter and Edmonds, 1983) or would undergo multiple moults into gradually smaller supernumerary instars (Blakley, 1981). It has been reported that when some insects encountered low food availability, they were able to terminate their larval developmental period ahead of time and initiate their pupation period, ultimately leading to a smaller body size (Moczek, 1998). Thus, juvenile developmental plasticity has an important role in the determining the success of lifecycle phases (Bernardo, 1993). Not all developmental aspects are plastic, some are canalised (i.e., controlled genetically) and their consequences are highly variable (Via and

Lande, 1985). It was noted that canalised development is favoured when environmental variations are unpredictable and take place at generation level; in contrast, developmental plasticity is favoured over a small spatial scale and when cues are reliable and consistent (Stearns and Koella, 1986, Gomulkiewicz and Kirkpatrick, 1992).

Several studies have shown that the size and age at maturity are often governed by three developmental factors, changes in size at ecdysis, intermoult interval and number of instars (Beck, 1971, Stearns and Koella, 1986, Higgins and Rankin, 1996). Therefore, any genetic or plastic variability will definitely result in maturity being reached at an unexpected different size or age (Higgins and Rankin, 1996). During reproduction, the size and age are highly important. These two life-history traits are correlated with female fitness in many invertebrates and ectothermic vertebrates. Any noteworthy alteration concerning them might therefore directly affect potential female fecundity and possibly increase pre-reproductive mortality (Beck, 1971, Stearns and Koella, 1986, Higgins and Rankin, 1996). For instance, in iteroparous annual organisms, an early female maturation would have more opportunities for reproduction (Suter, 1990); their juveniles, however, would be frequently at risk of predation (Berven and Gill, 1983).

In the past, extensive studies have concentrated primarily on the role of genetic variations, ignoring the importance of the presence of developmental plasticity over ecological and evolutionary timescales (Dingle et al., 1980, Gomulkiewicz and Kirkpatrick, 1992). Without detailed knowledge of how an organism assesses its size and weight with respect to environmental, physiological and biochemical factors, plasticity in size and weight cannot be utterly understood (Nijhout, 1975, Higgins and Rankin, 1996). Despite the widespread data on insect life-history traits, few dealt with plasticity in growth and developmental facets of larvae (Nijhout, 1975, Nijhout and Wheeler, 1996). More investigations are needed in order to illustrate the connection between developmental factors and plasticity in insects, particularly those that have become highly invasive, like *H. axyridis*. Without such details, it would not be easy to set apart plasticity from genetic variability and their interactive roles in modelling the final adult size (Higgins and Rankin, 1996).

In addition, attempts to demonstrate insect growth rates in relation to physiologically based allometry are few; this is unfortunate because insects have been used as scientific models in several life-history studies (Tammaru et al., 2002, Gotthard et al., 2007). Most growth curve models postulated that larvae grow exponentially and the allometric coefficient is equivalent to one (D'Amico et al., 2001, Berger et al., 2006). In contrast, several studies have showed that the allometric coefficient at all times is less than the unity (Tammaru and Esperk, 2007). In addition, there are noticeably few studies on growth curves of larvae, which might partly be due to the methodological difficulty in monitoring simultaneously many larval instars during the course of an experiment (Esperk and Tammaru, 2004).

Several studies have revealed that environmental factors, such as temperature range, might affect the critical weight during development. Thus, recognising how environmental variation influences the hormonal cascade could aid in understanding the physiological control of phenotypic plasticity over body size and developmental time (Davidowitz et al., 2004). For example, increased temperatures during larval development in *D. melanogaster* led to a reduction in the final adult size and the developmental time, accompanied by an increase in larval growth rate (Ray, 1960, Partridge et al., 1994ab, De Moed et al., 1998). Despite the above, the way in which growth and development act together in response to specific temperature is still incomprehensible. Food level is another factor that has been found to a greatly affect the weight-independent mortality, but has no influence on the critical weight once it has been attained (De Moed et al., 1999).

In conclusion, the ‘critical weight’ is operationally defined as the minimal weight at which further feeding and growth are not requisite for a normal time course to metamorphosis and pupation (Nijhout and Williams, 1974ab). It determines the way age and size at maturity respond to environmental variation (Bernardo, 1993), and is therefore important in life-history evolution. In addition, the critical size is an important determinant for enabling the evolution of body size; it may in turn act as a constraint on the evolution of plasticity of body size itself (Davidowitz, et al., 2003). In addition, genetic variation in mean critical weight has ecological and evolutionary implications.

Many biological and environmental factors contribute to increasing the invasiveness of several species (Marco et al., 2002). Life-history aspects such as growth rate, fecundity and fertility are the key determinants for fitness in predaceous ladybirds (Agarwala et al., 2008), probbaly due to their successful invasion and establishment over a wild range of habitats (Marco et al., 2002, Lanzoni et al., 2004). Knowledge of various life-history traits is essential to develop a better understanding of intra-guild interactions between invasive and native species (Lanzoni et al., 2004). It has been noted that the most important factor in invasion is the immature juvenile stages (Marco et al., 2002, Labrie et al., 2006). These immature stages appear to have high agility with a great capability to attack their prey. They are voracious and polyphagous, although the total number of aphids consumed depends on the larval and species of aphid, ranging from 90 to 370 aphids (Hukusima and Kamei, 1970). These larvae are able to survive on pollen grains, nectar and fungus exclusively (Hukusima and Itoh, 1976, Lamana and Miller, 1996) and cannibalised eggs (Osawa, 1993), as well as their siblings

(Dixon, 2000) and other insect species (Lucas et al., 1997, Michaud, 2002ab). As they grow to the third and fourth stage, they become very strong and aggressive, due to the presence of the spine (scoli) on the dorsal side of their bodies (Sasaji, 1971, He et al., 1994, Kock, 2003). These structures provide protection against attacks by other larvae (Sasaji, 1971, He et al., 1994, Kock, 2003). In addition, they are equipped with deterrent larval semiochemicals that are secreted to avoid interface competitions (Obata, 1986, Yasuda et al., 2000, Verheggen et al., 2007). Remarkably, larvae of coccinellids are able to adjust their developmental period and growth rate according to exterior cues, such as nutrients or temperature range (Osawa, 2000, With et al., 2002). By so doing, they tend to decrease the percentage of mortality when conditions are unpredictable (Lanzoni et al., 2004). Although some scientists have referred to their foraging activities as random searching (Kawai, 1976, Kock, 2003), others have reported that larvae and adults start foraging by extensive searching followed by an intensive search once their prey has been located, as is the case for *H. axyridis* (Ettifouri and Ferran, 1993). Lambin et al. (1996) noted that larvae of *H. axyridis* showed both long- and short-distance visual perception (Lambin et al., 1996). The number of eggs laid by coccinellid ladybirds is extremely high, in some species such as *H. axyridis* it may reach up to 3,819 eggs (Hukusima and Kamei, 1970, Koch, 2003). This can give rise to thousands of larvae that consume everything in their path in order to complete their development, particularly during poor resources. Thus, during invasion, the larval stages are the most intensive and competent, due to their extraordinary number and great voraciousness. It has been noted that larvae of *H. axyridis* are considered as the key factor in population dynamics (Osawa, 1993); they remain longer at the site and fight aggressively to survive over other species (Osawa, 1992, 1993, Joseph et al., 1999, Michaud, 2003a). Therefore, understanding the biological processes and developmental stages of the invasive habits of *H. axyridis* is essential in order to understand the underlying factors that promote invasion success (Shea and Chesson, 2002, Kimberling, 2004, Snyder et al., 2004).

The multi-coloured Asian ladybeetle, *Harmonia axyridis* (Coleoptera: Coccinellidae), has been released in many areas as a classical biological control agent against aphids (Roy and Wajnberg, 2008). This coccinellid appeared to be a very strong intraguild predator compared with other coccinellid species, and some studies have assumed that it has been displacing competitively native species, such as *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) (Saito et al., 2005, Roy and Wajnberg, 2008). The adults and larvae of *H. axyridis* are polyphagous (Lucas et al., 1998). Aphids are, however, considered to be their preferred and essential prey, because they ensure complete and fast pre-imaginal development, low mortality and a larger body size (Dixon, 2000, Sloggett and Majerus, 2000). Aphids vary widely, both spatially and temporally, in nature (Dixon, 1958, 2000). One of the characteristics that allow this ladybird to dominate the aphidophagous guild is their rapid juvenile development compared with other indigenous species (Lanzoni et al., 2004). In order to appraise how the *H. axyridis* ladybeetle was able to respond developmentally to transient or unpredictable prey resources, this study was conducted to investigate three important facets during larval development, namely, developmental period, critical weight and growth rate -for larvae reared on a fixed diet under constant controlled conditions. Stage assessments and intra-specific allometric analysis were performed to find out which of them had the greatest influence in determining the final adult size. This study aimed to help to draw attention to which juvenile stage best explains the main cause for the invasiveness of *H. axyridis*. Variability within developmental stages (weight and duration) was studied explicitly. It was noted by Stearns (1995) that the less variable the phenotype is, the stronger the degree of canalisation. According to Stearns (1995) the study was able to point out the most canalised qualities during larval development. Several criteria of the developmental process were considered in this study, including estimates of critical weight (both minimum and maximum), actual critical weight, relative growth rate for each stage, total growth rate, total developmental period and total larval period, as well as the sex ratio and body size parameters of adults. The study did not consider alternative food, as it aimed to focus on a more simplified food regime in order to help to assess the developmental responses to the availability of essential food.

4.2 Materials and Methods

Adults of *H. axyridis* were selected from a ladybird culture originally collected from naturally infested fields in Silwood Park, South-east England, United Kingdom (National Grid References 41/944691). The adults were sexed into 30 couples based on the labrum and pro-sternum pigmentation (McCornack et al., 2007). Each couple was placed in a 9cm diameter Petri-dish and each individual supplied with 16 live adults of the pea aphid, *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae), maintained on broad bean plants *Vicia faba* (Linnaeus, 1753) (Fabales: Fabaceae, variety Aquadulce Claudia). The couples were kept in a constant temperature room maintained at 20±1℃, 70-75% RH and a photoperiod of L16: D8. Afterward, 10 larvae were collected randomly from different clutches laid by each oviposited female. Each larva was reared individually in a 9cm diameter Petri-dish under similar conditions as the above and was provided with aphids according to the method in Chapter 2 until adult eclosion. Larvae that died and others that could not transform into adults were excluded from this study. Only five larvae out of the 10 monitored larvae per female were assigned for analysis.

4.2.1 Determination of developmental weight and duration of successive instars

Larval weight was taken upon egg hatching (initial weight), after first moult, second moult, third moult, fourth moult, at the onset of pre-pupal stadium, after pupation and finally after adult emergence. The presence of exuviae was noted and examined to determine the larval instar. The weights were measured and the mean weight for each instar stage was determined independently. Daily weight was taken after the fourth moult up to the onset of the pre-pupal stage. These weights were assigned to determine the estimates of the critical weights discussed later. The duration of development (Dd) for each instar period was expressed as the time from moulting into each respective instar up to the succeeding moult, or in the case of last instar, up to the initiation of the pre-pupal stage. The sequential duration for each instar was determined for each larva and then the mean duration period for each instar was estimated for each sex.

4.2.2 Determination of duration larval development period and the total developmental period

The newly emerged adults were sexed and the sex ratio was calculated. Subsequently, the total duration of the larval developmental period was expressed as the duration from egg hatching up to the initiation of pupation. This duration was determined for each larva, and the mean was estimated. Subsequent to adult emergence and sexing, the total larval period was determined for each gender independently. The total developmental period, on the other hand, was defined as the time after successful egg hatching up to successful adult emergence. This period was measured for each larva and from that the mean was estimated. Similar to the above, the total developmental period was also determined for each gender separately.

4.2.3 Determination of the estimates of critical weight

Estimates of critical weight were determined according to Phoofolo et al. (2009). They noticed that, first, the mean minimum viable weight often takes place 1-2 days after the fourth moult. The mean minimum critical weight is usually attained 3-5 days and the mean maximum critical weight occurs after 5-9 days, after accomplishing the fourth moult. All respective weights were estimated from Figure 4.6, following Phoofolo et al. (2009). This study suggested an alternative method for calculating the estimates of the critical weight. First, the mean minimum critical weight suggested by this study was calculated directly after larvae attained the fourth moult and the mean maximum critical weight was calculated directly when larvae started to cease feeding. The two estimates of the critical weight, the minimum and the maximum, were calculated separately for each gender. In addition, the mean actual critical weight could be estimated according to Davidowitz et al. (2004), in which 50% of the peak larval weight was noted to be equivalent to the critical weight, as in *M. sexta*.

4.2.4 Determination of total growth rate and the relative growth rates

The total growth rate and relative growth rate for each larval stadium were calculated following Leather and Dixon (1984) and Tammaru and Esperk (2007). The total growth rate was calculated by deducting adult weight from the initial larval weight then dividing it by the total developmental period. The relative growth rate of an instar was calculated by subtracting the weight at the end of the instar from the weight at the end of the preceding instar, divided by the duration of the respective instar period. Both the total and the relative growth rates were also determined for each gender independently.

4.2.5 Determination of larval growth rate index

The index of larval growth rate (G) was calculated following Sibly and Calow (1985), with a slight amendment through which G = (log W - log E)/D, where (W) was the body weight, (E) was an initial larval weight and (D) was the total developmental period. Subsequently, the larval growth rate index was determined for each sex independently.

4.3 Data analysis

All the data were tested for normality using the Shapiro test prior to conducting statistical analyses. Transformation models were used to normalise data distribution. Data that failed to be transformed to normal distribution were subjected to the appropriate non-parametric analysis, such as the generalized linear model (GLM) with Poisson errors and Wilcoxon rank sum test. Sex dimorphism in body size was explained by three estimates, the variations in adult weight, growth rate and duration of developmental period, according to Stillwell et al. (2010). To investigate the variability within each instar in terms of weight and duration (as response variables), each instar was analysed separately using one-way ANOVA analysis by fitting them into the models (response~larva) and (response~sex). Simple linear regression was performed to test the existence of any direct relationship between developmental parameters after data had been transformed. Chi-square test was used to test the significant variation between the relative growth rates of each instar and to compare the estimates of critical weight (both the minimum and the maximum). Student t-test was used to compare the means of different body measurements, as well as total growth rate, total larval period, total developmental period and growth index between the two sexes. All statistical analyses were carried out using the statistical programme 'R' version 2.8.1 (Ihaka and Gentleman, 1996).

4.4 Results

4.4.1 Variability in post-moult weight of each instar

Larvae of *H. axyridis* went through five consecutive instars, instead of the four usually found in most ladybird and insect species. The mean weight of immature stages of each sex type and their body measurements are provided in Table (4.1). There was no significant difference between developing larvae with respect to their initial weight (F=0.039, Residual deviance=4.17, df=119, P=0.84), weight after third moult (F=2.61, RSE=0.75, df=118, P=1.08) and adult weight (F=0.042, RSE=0.33, df=118, P=0.83). The other instars were found to be significantly variable in terms of weight, mostly after the first moult (F=4.86 RSE=20.8, df=118, P<0.05), second moult (F=6.21, RSE=0.51, df=118, P<0.05), fourth moult (F=8.47, RSE=5.07, df=118, P<0.001), at the commencement of pre-pupal period (F=22.53, RSE=5.54, df=118, P<0.0001) and at initiation of pupation (F=66.42, RSE=5.8, df=118, P<0.0001). The mean weight of immature stages tended to increase progressively as they grew to the consequent stages until they reached their peak weight (31.57±0.55 mg) which was recorded exactly at the pre-pupal stage (Fig.4.1)., There was a 7.12% drop in the mean pre-pupal weight as larvae grew towards the pupal stage, followed by another 38.72% drop in mean weight after adult ecdysis (Fig. 4.1). In general, the growth pattern of instars had five main phases, which were found to be separated by four inflection points. The first inflection point occurred at the beginning of the third instar stadium, subsequent to the second moult. The second inflection point happened at the fourth moult and at the onset of the fifth instar. The third inflection point was at the onset of the pre-pupal stage and the last one at the beginning of the pupation. The first phase was characterised by a slow growth rate followed by a second phase that showed a gradual increase in weight towards the second inflection point as time progressed. During the third phase there was a sudden accelerated rate of growth that reached a peak exactly at the onset of pre-pupal period. The fourth phase started with slight decrease in weight that progressed towards the last inflection point followed by a fifth phase characterised by a rapid drop in weight as the pupae was transferring into complete adult tissue. Comparisons between the stages in relation to the mean weights of males and females revealed that females had 10.7% more weight compared with males, built up at pupation, and 4.8% more after adult ecdysis (Fig. 4.2).

The mean weight of the second instars was related directly to the mean weight of the fifth instars (F=24.33, Residual deviance=13.5, df=118, $P<0.0001$) (Fig. 4.3). There was an indirect significant relationship between the mean weight of adults (regardless of the sex) and the mean weight of the third instars (F=6.2, Residual deviance=16, df=118, $P<0.05$, slope=-0.024). Likewise, the mean weight of the second instars had a linear negative relationship with the mean weight of the fourth instars (F=4.2, Residual deviance=26, df=118, $P<0.05$, slope=-0.023), and with the mean weight of the fifth instars (F=5.52, Residual deviance=13.16 df=118, $P<0.05$, slope=-0.015). The mean weight of adults was found to have a positive linear relationships with the mean weight of the third instars (F=6.24, Residual deviance=16, df=118, $P<0.05$), as well as with mean weight of pupae (F=3.04, Residual deviance=55.6, df=118, $P<0.05$, slope=0.0113). In contrast, the mean weight of the third instars had an indirect linear relationship with the mean weight of the fourth instars (F=4.6, Residual deviance=26.08, df=118, $P<0.05$, slope=-0.024) and with the mean weight of the fifth instars (F=5.5, Residual deviance=13.16, df=118, $P<0.05$, slope=-0.025). The mean weights of the second (Fig. 4.4), third, fourth and fifth instars had a direct linear relationship with the mean weight of pupae – (F=19, Residual deviance=14.03, df=118, $P<0.0001$, (F=14.45, Residual deviance=15, df=118, $P<0.0001$), (F=6.86, Residual deviance=25.61, df=118, $P<0.05$) and (F=13.4 Residual deviance=4.97, df=118, $P<0.0001$), respectively. The mean weight of pupae that gave rise to females was 29.25±0.82mg and of pupae that ecdysed into males was 23.6±0.81mg. After comparing the former with the latter, a significant difference was revealed ($t=4.85$, $df=105$, $P<0.0001$). The estimated sex ratio in *H. axyridis* was female-biased in which 66.6% out of the total turned to be females and 33.3% males.

Table 4.1. Mean weight (mg) of 120 larvae during each instar and the attained adult size parameters.

Sex	Initial weight of 1st instar	After 1st moult	After 2nd moult	After 3rd moult	After 4th moult	At pre-pupation	After pupation	Adult	Body length(mm)	Body width(mm)	Scutum width(mm)	Right tibia length (mm)	Total number of Gonads
Female (n=80)	0.18±0.008	1.46±0.2	2.93±0.18	8.8±0.51	16.5±0.63	31.94±0.67	29.25±0.82	12.67±0.46	6.24±0.043	5.24±0.04	3±0.029	1.58±0.01	35.33±0.61
Male (n=40)	0.21±0.103	1.27±0.11	2.88±0.24	8.79±0.69	16.22±0.69	30.85±0.94	23.6±0.81	10.93±0.55	5.86±0.06	4.96±0.06	2.89±0.03	1.53±0.016	47.87±1.25
Mean (120±SE)	0.19±0.007	1.400±0.14	2.92±0.14	8.79±0.41	16.42±0.47	31.57±0.55	27.37±0.66	12.09±0.36	6.11±0.03	5.18±0.03	2.95±0.02	1.5670.01	39.51±0.8

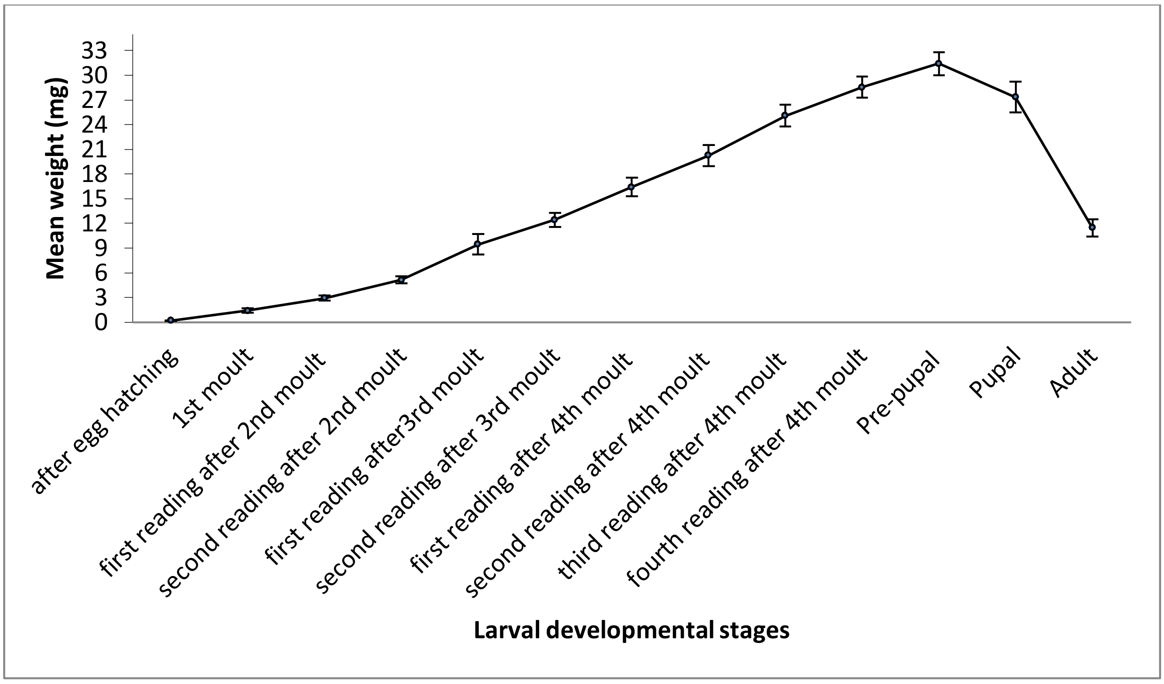

Fig. 4.1. Mean weight during each instar recorded during developmental period. The mean was estimated from 120 developing larvae ±SE.

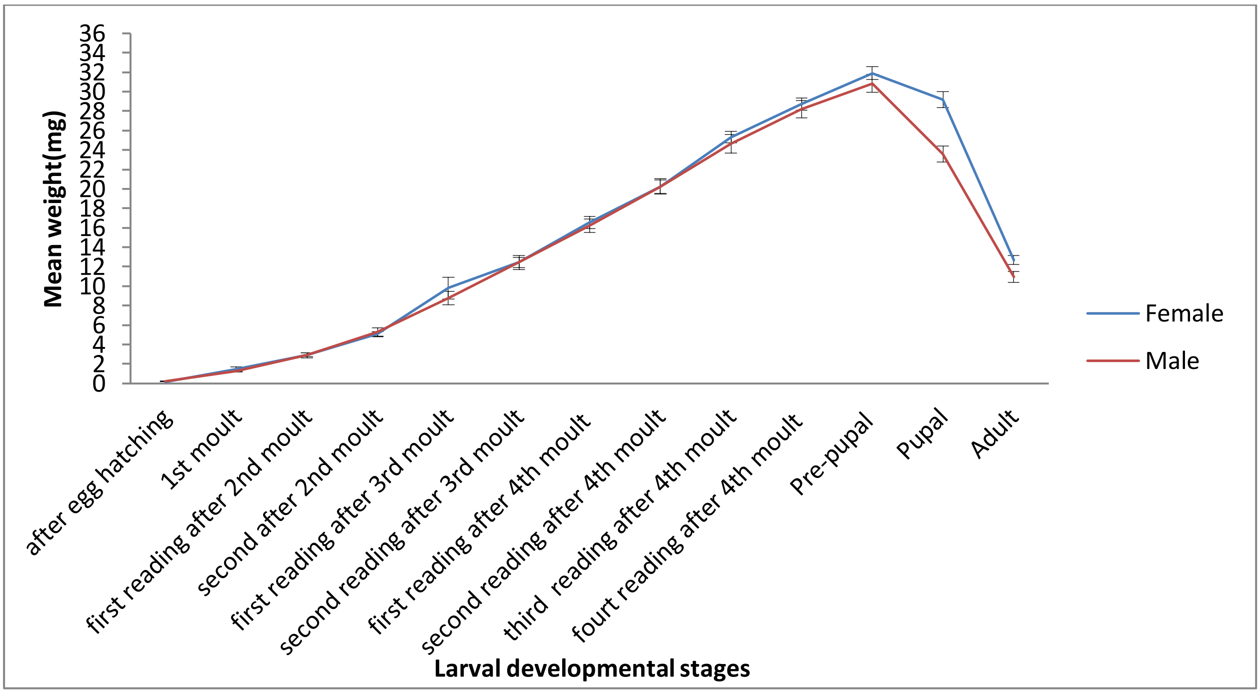

Fig.4.2. Mean weight during each instar recorded during larval developmental period for males and females. The mean was estimated from 80 females and 60 males ±SE.

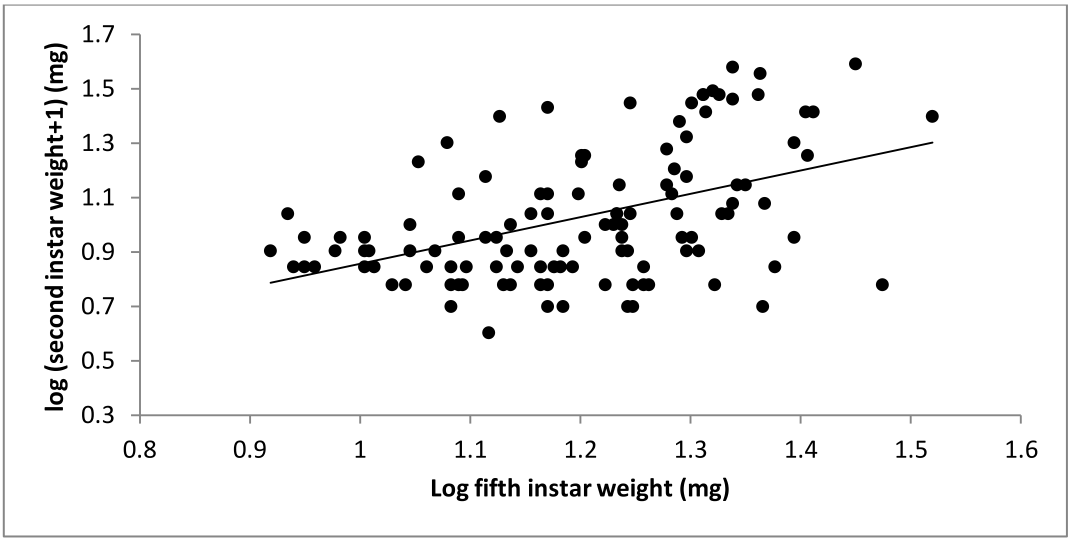

Fig. 4.3. Relationship between log (weight of 2nd instar +1) against log weight of 5th instar, (y=0.85x-0.003, R²= 0.21). Data were transformed for statistical analysis (F=24.33, P<0.0001).

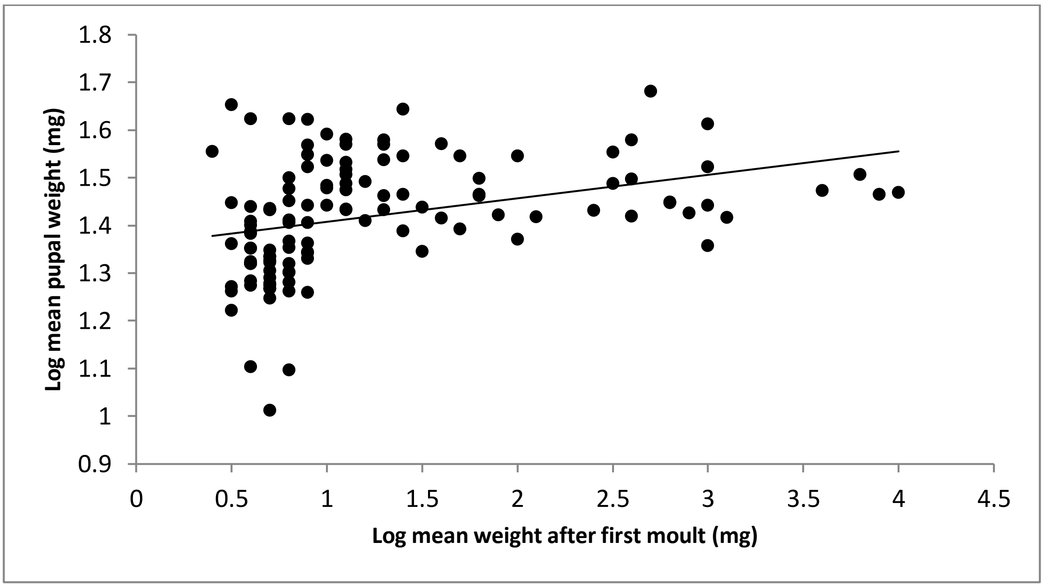

Fig.4.4. Relationship between the mean weight of the 2nd instar against log weight of pupae (y=0.05x+1.35, R²=0.12). Data were transformed for statistical analysis (F=14.03, P<0.0001).

4.4.2 Developmental durations and larval weight

Developmental durations for each instar stage are presented in Table 4.2. Among instars, the means duration of the second instar (F=9.8, Residual deviance=3.6, df= 118, P<0.001), third instar (F=5.8, RSE=2.7, df= 118, P<0.05), fifth instar (F=17.73, Residual deviance=0.52, df=118, P<0.0001) and pre-pupal stage (F=12.75, Residual deviance= 2.02, df=118, P<0.0001) differed significantly between males and females. The mean total larval developmental period recorded for all larvae, irrespective of sex, was 27.9±0.11 days and the mean total developmental period was 36.58±0.16 days. The mean total larval period for the 120 larvae did not show any variability when sex was disregarded (F=0.24, Residual deviance=0.21, df=118, P=0.62). Likewise, the mean total developmental period did not show any variability between developing larvae irrespective of sex (F=0.34, Residual deviance=2.8, df=118, P=0.55). The mean total developmental period did not differ significantly between larvae that gave rise to males or females (F=0.01, Residual deviance=0.28, df=118, P=0.91). The mean larval developmental period showed no significant variability between larvae that turned out to be males and females (F=0.3, Residual deviance=0.22, df=118, P=58). The mean total growth rate of all larvae was found to vary significantly between males and females (t=2.3, df= 82.7, P<0.05), in which females had 12.66±0.016mg/day, whereas males had 10.92±0.56mg/day. Thus, females had a higher growth rate compared with males. Despite the fact both males and females had a relatively similar mean total developmental period, females were found to be significantly heavier than males (t=2.3, df= 82.69, P<0.05). Likewise, the growth index was 1.10±0.016 in females and 1.03±0.02 in males, and both were found to be significantly different (t=2.37, df=82.7, P<0.05). The mean total growth rate had a direct linear relationship with the mean total developmental period, but the relationship was not significant (F=0.1, Residual deviance=0.32, df= 118, P=0.74, slope=0.0003). The mean total growth rate, however, had an indirect linear relationship with the mean total larval period (F=5.13, Residual deviance=0.21, df=118, $P<0.05$, slope=-0.062) (Fig. 4.5).

Table 4.2. Mean duration of successive instars during developmental period. Developmental parameters are also detailed.

Sex	Incubation period (day)	Duration after 1st moult (day)	Duration after 2nd moult (day)	Duration after 3rd moult (day)	Duration after 4th moult (day)	Duration of pre-pupal stage (day)	Duration of pupal stage (day)	Total developmental period (day)	Total growth rate (mg/day)	Total larval period (day)	Growth index
Female (80±SE)	5.87±0.08	2.86±0.06	5.73±0.079	4.07±0.084	8.85±0.07	4.02±0.07	8.67±0.18	36.58±0.21	12.66±0.46	27.9±0.144	1.1±0.16
Male (40±SE)	6±0.12	2.86±0.068	5.73±0.08	4.07±0.08	8.85±0.07	4.07±0.1	8.57±0.22	36.6±0.26	10.92±0.55	28.02±0.18	1.1±0.02
Mean (120±SE)	5.91±0.06	2.85±0.054	5.74±0.06	4.09±0.07	8.86±0.06	4.04±0.06	8.64±0.14	36.58±0.16	12.08±0.36	27.94±0.11	1.07±0.013

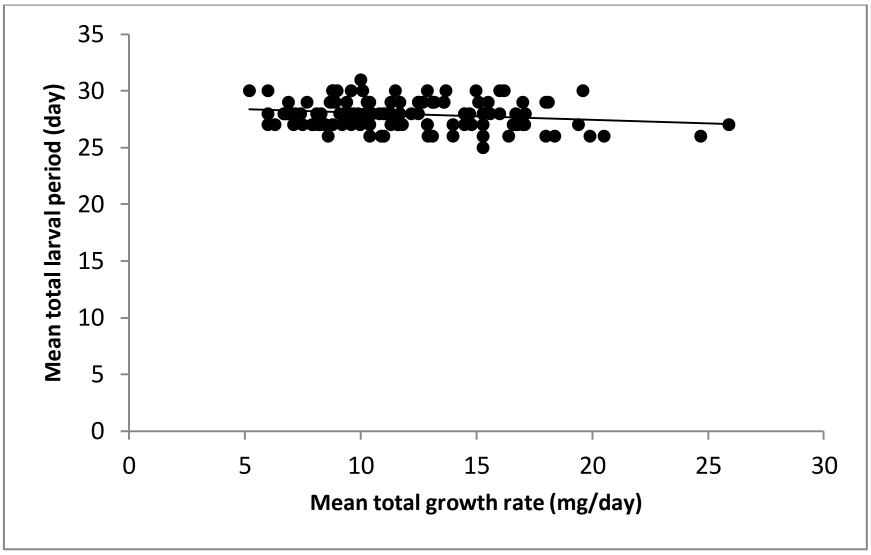

Fig.4.5. Relationship between mean total growth rate against mean total larval period ($y=-0.062x+28.69$, $R^2=0.039$). Data were transformed for statistical analysis ($F=5.13$, $P<0.05$).

The mean incubation period of the 120 larvae had a significant indirect relationship with the mean duration of the second instar ($F=27.63$, Residual deviance=1.17, df=118, $P<0.0001$, slope=-0.08). Similar negative significant trends were also revealed between the mean duration of the pupal period, the mean duration of the third instar stadium ($F=8.5$, Residual deviance=1.25, df=118, $P<0.001$, slope=-0.021) and the mean duration of the fifth instar ($F=17.28$, Residual deviance=0.52, df=118, $P<0.0001$, slope=-0.003). Regarding the relationship between the duration of instars and their stage weight, it was revealed that the mean duration of the fourth instar stage had an indirect significant relationship with the mean weight at the pre-pupal stage ($F=13.83$, Residual deviance=2.5, df=118, $P<0.0001$, slope=-0.09). The mean duration of the fifth instar was indirectly related to the mean initial weight of the first instars ($F=27.7$, Residual deviance=3.23, df=118, $P<0.0001$, slope=-0.12).

The mean relative growth rate recorded during the first instar was 1.33±0.14mg/day, the second instar was 2.67±0.14mg/day, the third instar 8.05±0.39mg/day, the fourth instar 15.41±0.44mg/day and the fifth instar 27.4±0.55mg/day (Fig.4.6). The mean weight gain during the first instar stage was significantly less than that of the third instar ($\chi^2=4.8$, df=1, P<0.05) or the fourth instar ($\chi^2=11.8$, df=1, P<0.0001). For the second instar, the mean weight gain was also significantly lower than the fourth instar ($\chi^2=9$, df=1, P<0.001). Finally, the mean weight gain of the latter stage was found to be significantly higher than the third instar ($\chi^2=5.4$, df=1, P<0.05). Only the mean weight gain of the first, fourth and fifth instar periods varied significantly among larvae -(F= 6.09, RSE=16.32, df=118, P<0.05), (F= 8.34, RSE=4.74, df=118, P<0.01) and (F= 19.52, RSE=5.63, df=118, P<0.000), respectively. None of the instars showed any significant variability in the main weight gain when sex was considered – (F= 0.06, RSE=17.16, df=118, P=0.8) for the first instar, (F= 0.0003, RSE=17.5, df=118, P=0.98) for the second instar, (F= 0.002, RSE=0.76, df=118, P=0.96) for the third instar, (F= 0.093, RSE=4.9, df=118, P=0.78) for the fourth instar and (F=0.73, RSE=6.06, df=118, P=0.39) for the fifth instar, respectively.

Only the mean weight of the second instar had a linear indirect relationship with the mean total growth rate (F= 7.4, RSE=16.48, df=118, P<0.01, slope=-0.026) (Fig. 4.7). Another linear direct relationship was observed between the mean duration of the third instar and the mean total larval period (F= 5.7, RSE=28.64, df=118, P<0.05) (Fig. 4.8). Thus the mean duration of the third instar was one of the determinants of the length of the mean total larval period.

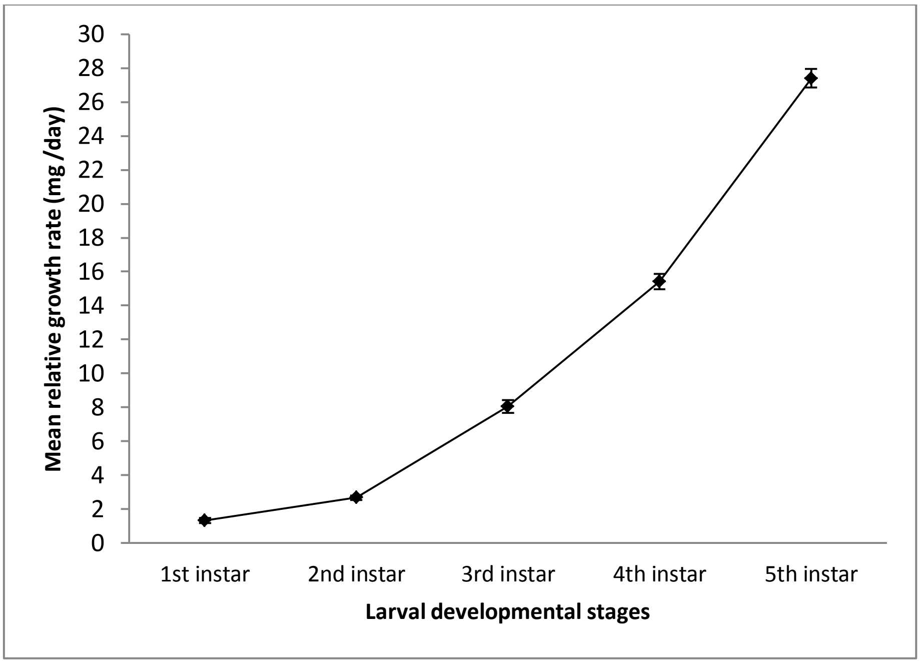

Fig.4.6. Mean relative growth rate estimated during respective instar (mean± SE, n=120).

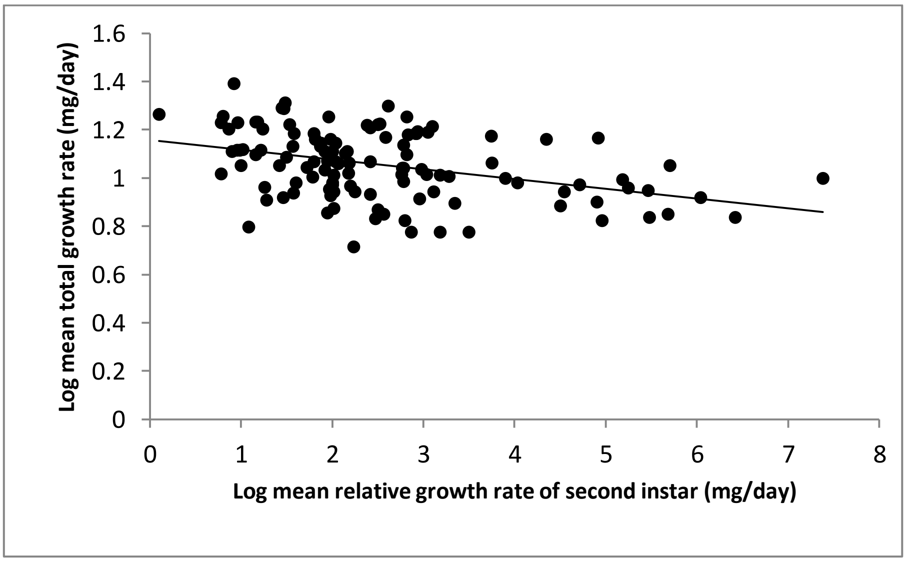

Fig.4.7. Relationship between log mean weight gain of 2nd instar and logged mean total growth rate ($y=-0.04x+1.15$, $R^2=0.15$). Data were transformed for statistical analysis ($F= 7.4$, $P<0.01$).

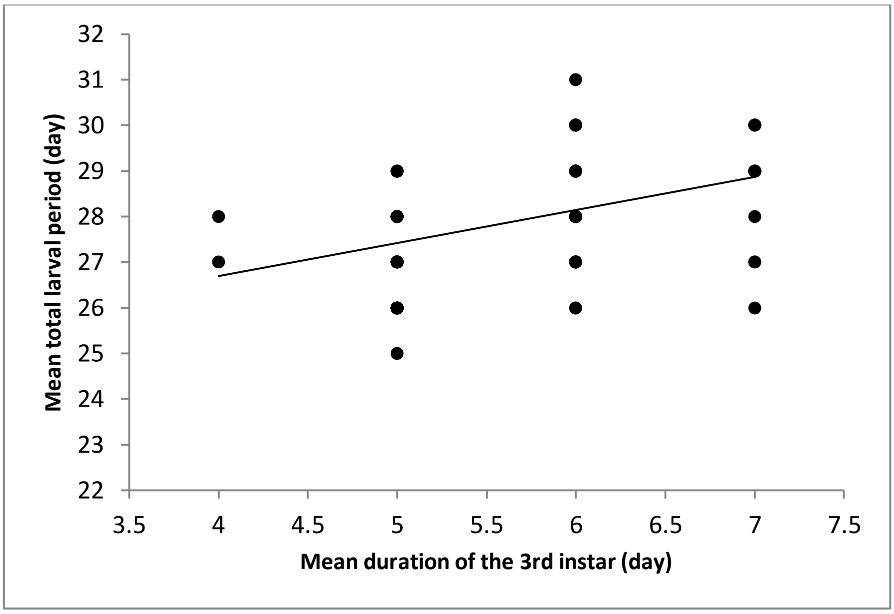

Fig.4.8. Relationship between duration of the 3^{rd} instar and the total larval period ($y=0.72x+23.8$, $R^2=0.175$). Data were transformed for statistical analysis ($F= 5.7$, $P<0.05$).

4.4.3 Critical weight

Changes in weight during the fifth instar are shown in Figures 4.1 and 4.9. The mean maximum estimate of critical weight for the 120 larvae was 31.57±0.55mg, with 16.42±0.47mg the minimum estimate. These two values were read directly from Figures 4.1 and 4.9, in which the former corresponds to the pre-pupal weight (the peak weight) and the latter represents the weight measured directly after the fourth moult. When sex type was considered, the minimum estimate of mean critical body weight was 16.52±0.63mg for females and 16.22±0.69mg for males. There was no difference revealed between the mean minimum critical weight for males and females ($t=0.31$, $df=97.57$, $P=0.75$) (Fig. 4.2). This absence of difference was also revealed between opposing sexes in terms of the mean maximum estimate of critical weight ($t=0.48$, $df=76$, $P=0.63$), for which females achieved 31.94±0.67mg and males 30.85±0.94mg. Regardless of the sex, there was no linear relationship revealed between mean minimum and mean maximum estimates of critical weight with the mean total growth rate (F=0.015, df=118, Residual deviance=0.33, P=9) or with the mean total developmental period (F=0.14, df=118, Residual deviance=10.9, P=0.7). Only the mean minimum estimate of critical weight had a significant direct relationship with mean total larval period (F=7.3, df=118, Residual deviance=0.2, P<0.001).

The changes in weight during the fifth instar were recorded at three consecutive intervals throughout nine days (at the fourth moult, after two days, after four days, after six days and after nine days) (Fig. 4.9). The ninth day is the day of the onset of the pre-pupal stage. From Figure 4.9, according to the method proposed by Phoofolo et al. (2009), the mean maximum estimate of critical weight can been see to occur exactly six days after the fourth moult and was approximately 28.55±0.47mg. The mean minimum estimate of critical weight was 25.24±0.51mg, recorded on the second day, just after the fourth moult had been accomplished. Finally the minimum viable weight (20.24±0.53mg) was found likely to occur 1-2 days after the fourth moult. (25.08±0.51mg) occurred after four days, the minimum estimates of the critical weight occurred after two days (20.25±0.53mg), the maximum estimate of the critical weight was 28.55±0.5mg and the peak weight was 31.5766±0.55mg and the minimum viable weight was 16.42±0.47mg. Despite that, hormonal analysis incorporation would be useful in that case to verify these results.

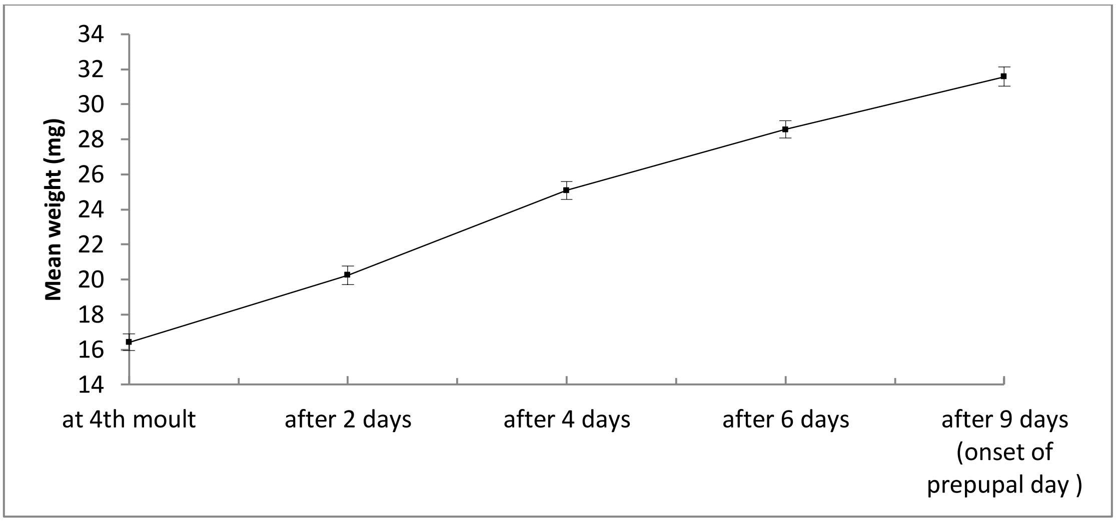

Fig.4.9. Changes in mean weight (mg) during the 5th instar until the onset of pre-pupation period (mean±SE, n=120).

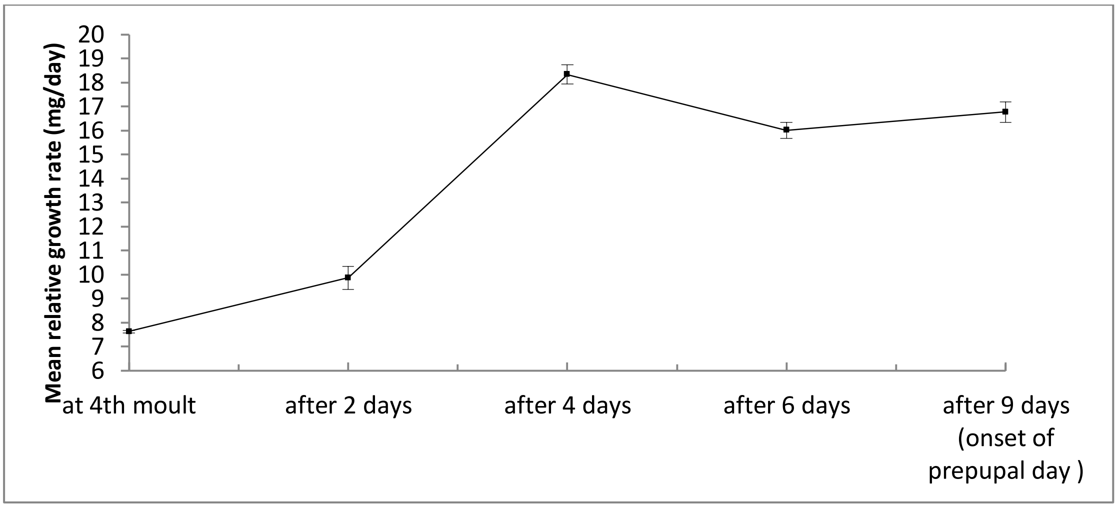

Fig.4.10. Mean relative growth rate (mg/d) recorded at successive intervals during 5th instar period, (mean±SE, n=120).

4.5 Discussion

Insect growth from instar to instar is typically exponential, in that most growth occurs in the last larval instar (Davidowitz and Nijhout, 2004). Phoofolo et al. (2009) noted that for *H. axyridis* the growth after attaining the fourth moult is in two phases, with one inflection point separating the two phases. The initial phase was characterised by a slow initial growth rate that progressed into its maximum with time. In the second phase, the growth rate began to decline and sometimes approached zero and became flat. This type of growth has been widely described in many insects (Teuschl et al., 2007). In this study, the growth period found after attaining the fourth moult was increasing rapidly in accelerated trend. In *M. sexta,* 90% of mass accumulation occurs in the fifth (and final) instar (Davidowitz et al., 2004). Davidowitz et al. (2004) noted that during the final instar of *M. sexta*, the growth becomes approximately linear after the first day. A relatively analogous trend was also noted in this study, but after 4 days from attaining the critical weight as seen in Figure 4.10. The pupal mass is 54% on average in *M. sexta* and the moth had 25% of the peak larval mass. As with other insects, adults do not grow and therefore peak larval mass largely determines the size of the adult moth (Davidowitz et al., 2004). In *H. axyridis*, larvae did grow after attaining the peak mass, therefore the pupal weight calculated in this study (using a method similar to the above) was 46.55% on average of the larval peak weight and the adult weight was 26.70% of the larval peak weight.

Environmental conditions and genetic factors are both considered to be the main factors prompting variability in life-history traits (Sebens, 1987, Stern, 2001, Davidowitz et al., 2003). Awareness of the fundamental factors inducing disparity in body size and its structures is without doubt essential (Stern, 2001). In most species, the ruling of the physiological and biochemical processes during the course of juveniles' development, in particular, the late instars and their interfaces with the external cues, play a substantial rule in defining the final body size (Osawa, 1992, Ueno, 1994, Berner and Blanckenhorn, 2007). The performance of larvae during growth, along with the onset of pupation are obligatory parts of growth in holometabolous insects (Nijhout and Williams, 1974 ab). In light of these particulars, the study planned to analyse the alterations in larval stage weight and duration during the course of developmental period upheld at controlled measures. This raised some questions, such as, what involvement did the weight of each stage have on the accomplishment of subsequent stages? Which of the larval stages would determine the possible success of pupation and the attainment of the requisite adult weight? Which larval stage or duration would be able to modify the developmental responses against food scarcity?

To answer two of the above questions (those that revolve around the contribution of each stage to the success of the following one), the present study showed that the consistent success in the progress of stages development of this ladybird was ruled by a combination of two main fundamentals, the duration of an instar and the weight gained during that duration. In terms of an instar's weight, the weight gained during the first, second, third and fourth instars had a strong propensity to influence the achieved pupal weight. More notably, it was revealed that the greater the weight of the first instar, the shorter the fifth instar period would be. Thus, the initial weight of the first instar governed to a great extent the length of the fifth instar period. Nonetheless, in terms of instar duration, the pre-pupal period was found to be negatively influenced by the period length of the fourth and the fifth instar. Thus, having short a fourth or fifth instar period implies that the larvae still would have required additional time to allocate and assimilate resources along with hormonal reorganisation for the next stage. On account of this, the length of the pre-pupal stage would be prolonged in order to accomplish these tasks, which might result in an extra-long developmental period. The above was confirmed statistically, as indirect relationships were revealed between the total developmental period and these two final instars.

The above findings imply that the post-larval developmental period that occurs at the initiation of the pre-pupal stage appears to be flexible in *H. axyridis*, rather than being of a determinant type. Furthermore, the study showed that the weight during the fourth and fifth instars was one of the important determinants of final adult weight. This was also verified statistically, the adult weight was found to be indirectly related to the weight obtained at both fourth and fifth instars and directly related to the weight at pupation. Thus, the second proposed question could be answered, the final instar weight is the stage that definitively governed the pupal weight. Nevertheless, a direct linear relationship was revealed between the duration of the third instar period and the pupal weight. Similar findings were reported by Nijhout (2003) and Phoofolo et al. (2009), namely, that the achieved pupal and final adult body characteristics are often influenced by the performance of the last instar.

The contribution of all instars to the progress of larval development has been further verified in this study. According to several previous studies, it been had emphasised that the weight of larvae during the fourth instar and pre-pupal stage are the most critical weights, disregarding the involvement and the input of preceding instars. For instance, Phoofolo et al. (2009) reported that in predatory coccinellids, *H. axyridis* and *Hippodamia convergens* (Guérin-Méneville, 1842) (Coleoptera: Coccinellidae), the final instar (i.e., the fourth instar) was the only stage found to determine the adult body size. In their study, three different species of coccinellids were examined and the results revealed that for *H. axyridis*, 99% of larvae that had succeeded in reaching pupation were predominantly based on the weight of fourth instar.

Likewise, Phoofolo et al. (2009) and Dmitriew et al. (2009) reported the influence of the fourth instar on the final pupal and adult weight.

In order to begin offering a proper explanation for each question, it is crucial to mention that this study revealed that the ladybeetle *H. axyridis* had five consecutive instars, and not four instars as for other coccinellids and some insect species. This concurs with the finding reported by Labrie et al. (2006). A few other coccinellid species had previously been reported to have five larval instars, Coleomegilla *maculata* (De Geer, 1775), *Chilocorus bipustulatus* (Linnaeus, 1758) and *Callicaria superba* (Mulsant, 1853) (all Coleoptera: Coccinellidae) (Warren and Tadic, 1967ab, Majerus, 1994). Generally, when a fifth larval instar was accomplished, the feasible postulation was that the juveniles had been experiencing relatively low food quality or quantity during development. In this study, the rearing conditions were entirely different, food was supplied on regular basis at a similar quantity and the external conditions (temperature, humidity and day length) were controlled during the course of the experiment. The accuracy of the controlled protocol used in this study was validated, since variability among total larval period and developmental period was absent. This extra instar was found to be well-equipped through similar physical, behavioural and morphological features as in the fourth instar, reflecting the fundamental underlying genetic variability and the potential evolution of reproductive performance in this invasive species. Generally, the importance of the fifth instar was promoted by several developmental aspects, for example, the mean weight achieved by developing larvae at the fifth instar was found to be significantly higher than the weight of other stages ($\chi^2=8.48$, df=3, $P<0.05$). Another issue was the length of the fifth instar period, it was found to be the longest in comparison with other instars' durations. Unexpectedly, there was a significant variability in terms of weight among developing larvae during this stage, along with a higher relative growth than that of other stages. Nevertheless, despite the voracity and the aggressiveness of most of the instars observed by this study, the fifth instar was categorised by elaborate morphological features complemented with behavioural characteristics, including the presence of the longer spikes, elevated intensity of voraciousness and aggressiveness compared with preceding instars. This coincides with the observation in which larvae of *H. axyridis* at their fourth instar had a high predatory efficiency accompanied by increased voracity and greater foraging activities, associated with a longer searching period compared with other predators (Lucas et al., 1998, Dixon, 2000, Labrie et al., 2006). Therefore, it could be expected that the above features might be exaggerated, since the fifth instar larvae are heavier in weight and bigger in size compared with the fourth instar. Presumably, possessing these extra developmental and behavioural phenotypes at the last instar might emphasise its unique role during a period of food deprivation or changes in environmental conditions. The study thus answered the last question, in relation to which stage may have a distinguished rule during starvation or adverse environmental conditions. Besides emphasizing the significance of the fifth instar in this study, the contributions of all other instars and their potential influences on each other, including the incubation period, were also evaluated and its role was stressed. More research, however, is needed to provide the best explanation for accelerating the phenomenon of the fifth instar.

It has been reported that the critical weight naturally occurs at about 54% of peak larval mass, depending on the species (Davidowitz et al., 2004). The critical weight is not an inert trait of a species, since it can be subjected to evolution (D’Amico et al., 2001, Davidowitz et al., 2003). In the present study the mean minimum critical weight of *H. axyridis* was 20.24±0.53mg, which was very similar to the 19.3mg reported by Phoofolo et al. (2009) for the same species. The maximum critical weight reported in the study by Phoofolo et al.

(2009) was 21.5mg, which was lower than the value found by this study (28.55±0.5mg). Unquestionably, the differences could be attributed to the rearing conditions and the prey species used in both studies. Phoofolo et al. (2009) used the greenbug, *Schizaphis graminum* (Rondani, 1852) (Hemiptera: Aphididae), which might vary in nutritional value from the pea aphid that was used by this study. This was reinforced further by Omkar et al. (2005), who noted that the quality of consumed prey has a major influence on development, growth and survival of immature larval stages, especially if the predators are either generalist or specialist ladybirds. Furthermore, Dixon (2000) and Omkar and Srivastava (2003) reported that the developmental period of larvae might vary according to the aphid species.

A great variation was found between the method suggested by this study compared with the one proposed by Phoofolo et al. (2009). This study tended to calculate the actual critical weight by taking 50% of the peak larval weight, according to Davidowitz et al. (2003). It was found that the peak larval weight was approximately 31.57mg, 50% of which would be 15.75mg. Ultimately, the discrepancy in the values of the critical weight and its estimates varied according the method used and could not be entirely trustworthy without incorporating hormonal analysis. Stillwell and Davidowitz (2010) suggested a formula for calculating the critical weight of *M. sexta*. This formula is highly reliable and could be easily used to calculate the critical weight based on hormonal measurement (critical weight = peak mass- (individual growth rate x ICG (i.e., interval to the cessation of growth)). Through this method, the minimum estimate of the critical weight should be directly after the fourth moult. The minimum estimate of the critical weight suggested by this study might not be definitely accurate, because this made the minimal viable weight occur before the fourth moult. Therefore, the weight after the fourth moult must be the minimal viable weight (16.42±0.47mg), which was found to be less than the minimum viable weight

(20.24±0.53mg) calculated by Phoofolo et al. (2009). In spite of that, it had been noted previously that the minimum estimate of the critical weight sometimes became equal to the minimum viable weight or might not exist (Shingleton, 2010). This study came to the definite conclusion that the timing of the minimal viable weight must be found 1-2 days after fourth moult, as suggested by Phoofolo et al. (2009). Since the study did not measure the weight after one day from the fourth moult, the minimal valuable weight could be regarded as 20.24±0.53mg.

It was noted that during development the minimum estimate of the critical weight usually occurred after attaining the minimum viable weight in 3-5 days (Davidowitz et al., 2004). Therefore, the minimum estimate of the critical weight was 24.08±0.51mg (from Fig. 9.4). The ‘maximum critical weight’, as proposed by this study, was defined as the peak weight at which no more feeding by larvae was commenced. This was also noted by Nijhout and Williams (1974a), who defined it as the minimal weight at which further feeding and growth are not required for a normal time course to metamorphosis and pupation. The propositions put forward by this study, together with those of Nijhout and Williams (1974a), imply that the maximum critical weight must equal the peak weight (31.57±0.55mg). Others, however, have stated that on attaining the critical weight, extra growth is committed by the imaginal disc in order to differentiate into adult tissue (Davidowitz and Nijhout, 2004). As noted before, the focus of this study is the critical weight and finding out the approximate value that could be used to define it in the ladybeetle *H. axyridis*. In order to indirectly evaluate the critical weight it would be best to look at the relative growth rates and the gained weights at different intervals after the fourth moult.

It was shown by this study that the mean relative growth rate at the fourth moult was 7.62±0.06 mg/day and the gained weight was 11.34%. After two days, the relative growth rate was 9.86±0.48 mg/day and the percentage weight gain was 18.87% (Fig. 4.10). After four days, the mean relative growth rate was 18.34±0.4 mg/day and the percentage weight gain was 19.3%. The mean relative growth rate after six days was 16.01±0.33 mg/day and percentage weight gain was 12.14%. The mean relative growth rate at the ninth day was 16.77±0.43mg/day and the percentage weight gain was 9.56%. This implies that the actual mean critical weight for *H. axyridis* occurred approaching the sixth day, because before the ninth day, the relative growth was at its maximum rate and the percentage of the weight gain had also reached its highest value (19.3%) compared with other days (excluding the weight gain after two days).

Davidowitz and Nijhout (2004) noted that the critical weight usually occurs before attaining the peak weight, because larvae can grow and achieve greater weight after attaining the critical weight before the secretion of the prothoracicotropic hormone (PTTH). Thus, this study predicted that the critical weight occurred in that case close to 4-5 days and was 25.08±0.51mg, based on the great relative growth rate and weight gain and not the actual weight. In summary, for *H. axyridis,* the critical weight

The critical weight during larval development accounted to a large degree for the variation in body size (Dmitriew et al., 2009). Davidowitz et al. (2005) noted that the larger critical weights resulted in larger peak larval sizes and longer development times. Davidowitz et al. (2003) defined ‘critical weight’ as the weight at which 50% of larvae are able to pupate. In contrast, Nijhout and Williams (1974ab) objected and noted that this definition describes the minimum viable size that signifies the minimal larval weight needed for successful pupation.

In agreement, Davidowitz et al. (2003) noted that the minimal larval weight is always less than the actual critical weight and, unlike the critical weight, has never been linked with any biological procedures. Phoofolo et al. (2009) stressed that the minimum viable weight is the weight at which larvae can endure metamorphosis. They also demonstrated that the relative growth rate at the minimum viable weight is often smaller than the relative growth rate at the critical weight or any other stages. This was also proved by this study, since the relative growth rate of the insects when attaining the critical weight was found to be the highest in comparison to other measurements (18.34±0.39mg/day), and the relative growth rate during the minimal viable weight was the lowest compared to other measurements (7.62±0.06mg/day).

The importance of the critical weight has been a dominant subject in developmental biology. For example, Brogiolo et al. (2001) noted that the critical weight functions at the level of the whole organism, rather than just imposing regulation at cellular or organ level. This study proved that the relative growth rate at the minimum viable weight was found to be smaller than the relative growth rate calculated for the minimum critical weight and the maximum critical weight. This is validated by the proposition by Phoopolo et al. (2009) that the minimum viable weight is entirely different from the minimum critical weight, not just in *M. sexta* but also in *H. axyridis* ladybirds. Calculating the actual critical weight is very problematic. Although the study had managed to give a full picture of what might be the respective weight values attained over the entire last instar period, calculating the actual number needs an appropriate hormonal examination.

The effects of temperature and nutritional quality on growth rate and body size are well recognised. Little has been recognised, however, with respect to the physiological mechanisms by which an organism interprets the variability in food and environmental conditions into reaction norms of body size or development time (Davidowitz and Nijhout, 2004). Davidowitz et al. (2003) and Davidowitz et al. (2004), from their work on *M. sexta*, illustrated that the critical weight is highly sensitive to diet quality, but not to temperature. Thermal reaction norms of body size and developmental time are the functions of three main fundamentals, the growth rate, the critical weight and the interval to cessation of growth (ICG). These three factors often interact with temperature. For instance, when temperature is high, the final body size will be smaller, as the higher growth rate results in a lessening of the ICG period and reduces the body weight. Similarly, at higher temperatures, the developmental period becomes shorter due to the greater growth rate, which in turn leads to a drop in the time required to attain the critical weight.

The contribution of other instars was investigated thoroughly and the results showed, for example, that the duration of the second instar and the weight achieved at this stage were the only parameters found to have a direct relationship with the total growth rate, the pupal weight and the adult weight. This was expected, since the role of the second instar had been recognised previously by Labrie et al. (2006). In addition, the study revealed that the second instar was the stage that took place directly after first moult at which the first inflection point fell. In addition, the weight at the second instar was also labelled the minimum viable weight. Another important stage was the third instar weight, which had a direct influence on the final stages of larval development, such as the fourth instar weight, pre-pupal and pupal weights.

Previous studies have indicated that the more variable the phenotype, the stronger the degree of plasticity (Nylin and Gothard, 1998, Ayrinhac et al., 2004). The variability among developing larvae in terms of any developmental parameters was proven by this study, reflecting the possession of a definite degree of plasticity in the ladybird *H. axyridis*. Nevertheless, the direct and indirect significant linear relationships revealed by statistical analysis between larval weights and durations also implied selective regulatory roles. The plasticity revealed in the fourth stadium was investigated intensively by numerous studies. For example, Phoofolo et al. (2009) noted that the fourth stadium could be titled the assessment developmental stage, because larvae assess their body weight during this period. Therefore, when accomplishing the critical weight, all the concomitant biological processes would be initiated, leading to successful pupation.

It was shown that under prolonged nutritional stress, larvae of *H. axyridis* increased the length of their fourth stadium and spent a much longer time in reaching their threshold weight for successful pupation (Giles et al., 2000, Phoofolo et al., 2009). Under food scarcity, the type of larval developmental response depends mainly on the species and whether it has a determinant or a flexible developmental period. For instance, when the moth *Orgyia antiqua* (Linnaeus, 1758) (Lepidoptera: Lymantriidae) was starved at early larval stages, there was a 5-10% rise in the total larval period (Esperk and Tammaru, 2010). If food deprivation occurs before attaining the critical weight, many insects tend to extend their last larval stadium beyond normal lengths (Nijhout et al., 2006). On the contrary, a reduction in the fourth stadium period was observed in larvae of the dung beetle *Onthophagus taurus* (Schreber, 1759) (Coleoptera: Scarabaeinae), instead of an extension (Shafiei et al., 2001). Other larvae might respond by ceasing their growth without altering their developmental period (Bradshaw and Johnson, 1995). This study revealed that *H. axyridis* showed a great variability in total growth rate during larval development, irrespective of the sex type. In addition, an indirect relationship was revealed between total growth rate and total larval period, but such relationship was not significant. The patterns of relationship of life-history traits might be modified and revealed better when insects were subjected to stress conditions.

The present study revealed that *H. axyridis* had a female-biased sex ratio. This was expected, since female-biasness had also been recognised in several ladybird species, according to Majerus et al. (2000) and Dixon (2000). The pattern of sex ratio has been ascribed to male-killing bacteria (Hurst and Jiggins, 2000), as well as to prey quality, and both appeared significantly to alter the ratio towards more female-biasness (Omkar and Srivastava, 2003, Srivastava and Omkar, 2004). For example, prey quality affected the survival rate and the growth index significantly in the ladybird *Micraspis disolor* (Fabricius, 1798) (Coleoptera: Coccinellidae) (Omkar et al., 2005). A similar remark on the effect of food quality on sex ratio was made by Omkar and Srivastava (2003). Other studies have detailed another reason for female-biasness in three coccinellids – *Scymnus apetzi* (Mulsant, 1846), *Scymnus subvillosus* (Goeze, 1777) and *Exochomus nigromaculatus* (Goeze, 1777) (all Coleoptera: Coccinellidae), feeding on *Hyalopterus pruni* (Geoffroy, 1762) (Homoptera: Aphididae), where it was noted that females tended to survive on all types of aphid species in comparison with males, thus the percentage of female survival would go beyond that of males, leading to female-biasness (Atlihan and Kadydan, 2002, James, 2004). In addition, the present study was able to predict the sex type of a developing larva prior to the adult stage. It was revealed that the initial weight of larvae, as well as the pupal weight, had a direct highly significant relationship with the sex type. The pupae of a substantial weight tended to give rise to female adults, whereas the pupae of a lighter weight gave rise to male adults. Therefore, the pupal weight could possibly be used to predict the sex prior to adult emergence.

A female-biased body size dimorphism was also revealed by this study. The simplest explanation that could be given in this case is the protandry phenomenon, in which males usually grow faster than females (Yasuda and Dixon, 2002). Yasuda and Dixon (2002) noted that males had a shorter developmental time compared with females and the trade-off that would be expected is a smaller body size in males. In contrast, sexual dimorphism in total developmental period and total larval period was not revealed by this study. This study showed that total growth rate, however, differed significantly between the sexes, females had the higher growth rate, but this had no direct relationship with body weight; and females had a greater body size in comparison with that of males. The lack of differences in developmental time between sexes was documented previously in coleopterans (Blanckenhorn et al., 2007). They demonstrated that the developmental time was the same in males and females, despite the bigger size of the tested females. This implied that there was an additional regulator for the variation in body size, the total growth rate. The total growth rate in their study was found to be significantly greater in females compared with males. They suggested that the variation in the total growth rate was more important than the developmental time in estimating SSD.

Individual fitness is usually concomitant with large body size, greater lifetime fecundity, faster growth rate and shorter developmental period (Phoofolo et al., 2009). All of the above traits are interrelated and are traded-off against each other and against other life-history traits (Nylin and Gotthard, 1998). It had previously been noted that when large females managed to attain, as a minimum, one of the above traits, other life-history characteristics would be negatively affected (Roff, 1992, Gotthard, 2000, Phoofolo et al., 2009). For instance, having a faster developmental rate might incur a greater possibility of high mortality, as well as a substantial risk of predation.

The current study demonstrated that between the sexes, the final body weight varied in its relationship with the total growth rate. Such divergence between males and females in order to attain the final body size was mainly ascribed to the variation in total growth rate rather than the total developmental period. The females in this study were characterised by having a higher growth rate, as well as heavier bodies. This is in agreement with the fact that greater relative fitness is frequently associated with individuals that have a faster growth rate, because it might lead to a higher survival rate at adulthood (Phoofolo et al., 2009). The present study revealed that there was a trade-off between attaining heavier bodies and total developmental period revealed by this study. Fairbairn and Preziosi (1994) suggested that the variation between sexes could be attributed to several aspects in relation to selection for rapid development and early maturation of males, gonads in males often develop earlier than females (Dixon, 2000) and females are required to attain a greater size to be more fecund (Fairbairn, 1990).

It was observed in this study that the weight of the pre-pupae was significantly greater than the weight of pupae, which was attributed to the physiological and biochemical processes taking place during metamorphosis. Isikber and Copland (2001) reported that the reduction in pre-pupal weight could be ascribed to the water loss as a metabolic cost during this process. For example, in the Japanese beetle *Popillia japonica* (Newman, 1841) (Coleoptera: Scarabaeidae), there was a great loss of weight during ecdysis (Ludwig, 1931). Changes in weight were referred mainly to the alternation in body water content (Gray, 1946). The marked reduction in body weight subsequent to pupation revealed by this study was probably due to the shedding of the heavy exoskeleton and to the biochemical changes needed to build up the adult tissues.

Although the present investigation did not cover physiological processes during development, it is quite plausible that these processes were partly hormonally regulated, analogous to those reported in many insects (Davidowitz et al., 2003, Nijhout, 2003). It has been noted that both moulting and metamorphosis in insects are controlled by both prothoracicotropic and juvenile hormones (Nijhout, 2003). The former principally control the accurate timing of moulting. Ecdysteroid hormones are often governed by size, temperature and photoperiod (Wigglesworth, 1934, Riddiford et al., 2003). The latter is present during larval development regulating larval growth and the progression to the subsequent stages.

In summary, for invasive species, growth, development and survival of immature stages are considered as the key factors defining the attainment of an invasion process. Food availability and temperature range together play a substantial role in modifying the characteristics of larval development weight, duration and growth rate. Although this study did not address the underlying genetic factors that regulate the developmental process, investigation of variability among development parameters revealed that there were some developmental canalised phenotypic traits often maintained among the population, such as the initial larval weight, the third adult weight and the minimum as well as maximum critical weights, despite the controlling protocol of rearing conditions during the course of investigations. The significant variability in some traits stated above of developing larvae specified some effective underlying genetic factors. Several instar weights were considered as a crucial element for determining success of larval development. For example, the weight at the final instar initiated numerous biological and physiological processes leading to successful pupation and affecting the subsequent adult size. Likewise, the weight of the initial instar, pre-pupae and pupae were found to impressively contribute in determining the sex of an adult. Nevertheless, the contribution of other instars should not be discounted or mistreated, as each instar has its own noteworthy role in accomplishing successful development. The unique developmental characteristics, coupled with the distinguished behavioural and morphological features, were engaged in enhancing larval growth rate and increasing predatory proficiencies of the ladybird *H. axyridis*. All the above had substantially attributed to the unpredictable successful invasion of this ladybird. Thus, *H. axyridis* seemed to perform several trade-offs during its developmental patterns, even under controlled measures that could explicate its sustainability when conditions are adverse and against any variable environment.

Chapter 5

A Novel Method for Identifying the Sex of Pupae of *Harmonia axyridis*

5.1 Introduction

The multicolored Asian ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae) has become a popular study model due to its potential adverse impact on non-target species and intraguild composition (Michaud, 2002ab, Cottrell, 2004, Koch, 2006, Roy and Wajnberg, 2008). This predator is native to eastern Asia, but has recently invaded North America, Europe, and South America (Koch, 2003). This lady bird is considered a strong intra-guild predator and potential competitor that poses a significant risk to the diversity of native ladybird species and the ecosystem (Cottrell, 2004, Snyder et al., 2004, Majerus et al., 2006). *Harmonia axyridis* is becoming a pest of fruit production (Koch et al., 2004), and can occasionally be a nuisance to home owners (Huelsman et al., 2002). Therefore, research conducted on *H. axyridis* could benefit from an easier non-disruptive sexing technique for this ladybird.

External morphological characters have been used to determine the sex of *H. axyridis* in the laboratory (Majerus, 1994) as well as during field studies (McCornack et al., 2007). Numerous methods have been used to determine the sex of this beetle such as to identify the shape of the distal margin of the fifth abdominal sternite (McCornack et al., 2007). This method however, was found to some extent challenging, as it often requires adult manipulation and the aid of a microscope. Sex determination based on the pigmentation of labrum and prosternum was an alternative manageable method proposed by McCornack et al. (2007) in which males of *H. axyridis* are usually characterized by light labrum pigmentation compared with females (Galvan et al., 2008). The necessity of a new method to determine the sex of coccinellids rather than the customary means has become important requisite particularly for ecological and genetic studies (Majerus, 1994). This study tested the probability of using the pupal pigmentation to predict the sex of the adults.

Phenotypic plasticity in pupal colouration was described some time ago (Hazel, 1977, 1987). This type of plasticity was known as environmental cued polymorphism (Hazel and West, 1996) in which two key environmentally factors are involved, the texture and the colouration (background) of pupation site (Smith, 1980, Starnecker and Hazel, 1999). Several other environmental elements are known to govern polymorphism of pupal colouration such as light (Smith, 1980), temperature (Hiraga, 2006), humidity (Majerus, 1998) and diet quantity and quality (Grill and Moore, 1998). The time of day at which larvae pupate also influences the final pupal colouration (Smith, 1980). Out of all these factors, temperature is one of the vital cues affecting development, maturation and survival rate of insects (Kemp and Bosch, 2005, Sgolastra et al., 2010). For instance, in the fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) the optimum temperature for pupal development ranges between 20-25ºC with 75-90% RH (Langley et al., 1972). Furthermore, the coloration of the eyes of pupa of *Bactrocera philippinensis* (Drew & Hancock, 1994) (Diptera: Tephritidae) are very sensitive to temperature and are used as a reliable indicator for pupal maturation stage (Resilva et al., 2007). A latest study revealed that the duration of the pupal stage of the fruit fly, *Anastrepha oblique* (Macquart, 1835) (Diptera: Tephritidae) reduced with increasing temperature reaching 30ºC (Telles-Romero et al., 2011). The effect of temperature on pupal colouration has been studied extensively in quite a lot of insect species (Smith, 1980). For example, the rate of recurrence of brown pupae in the lepidopteran, *Byasa alcinous* (Fruhstorfer, 1901) (Lepidoptera: Papilionidae) was considerably higher at low temperature (25ºC) compared with high temperature that often induced yellow pupae (Yamamoto et al., 2011). Pupae of the painted lady butterfly, *Vanessa cardui* (Linnaeus, 1758) (Lepidoptera: Nymohalidae) exhibited dark pupae when larvae were kept at 16ºC, whereas those that were reared at 32ºC developed into white pupae regardless of photoperiod length. Likewise, it was noted that the temperature experienced during the immature juvenile stages of the small copper butterfly, *Lycaena phlaeas daimio* (Seitz, 1909) (Lepidoptera: Lucaenidae) has an detrimental effect in facilitate the variation in final pupal coloration regardless of the background or the photoperiod length (Usui et al., 2004). In conclusion, pupal colouration might respond or react in a different way to any variation in temperature level (Smith, 1978, 1980).

Several studies revealed that there is an association between developmental time of immature stages, final adult size (Nylin and Gothard, 1998) and the melanization process (Majerus, 1998, Bezzerides et al., 2007) in which all might act together to determine an individual's fitness. Windig (1999) noted that there is often a trade-off between growth rate and obtaining a larger body size which are interrelated with the costly melanization process. The latter usually requires many proteins to be entirely accomplished. Therefore, the melanization in general may have an important influence on the survival of immature stages and individuals' fitness.

Hormonal control is accounted further for the variations in pupal colouration in *Papilio polytes* (Linnaeus, 1758) and *Papilio demoleus* (Linnaeus, 1758) butterflies belong to the family Papilionidae. The “green factor” or hormone was observed secreted in the larval head throughout the pre-pupal stage leading to green pupal cuticle (Smith, 1978). In the absence of this hormone, brown coloured pupae were formed. It was noted that there was a greater possibility that the pupal colorations depended on the texture of the background. Green pupae often formed amongst green vegetation and brown ones on brown stems (Smith, 1978). The influences of endocrine control on the development of brown colored pupae was also reported by Smith et al. (1988) and Smith (1978).

Furthermore, the genetic background of pupal polymorphism was also have conspicuous influences on the pupal phenotype plasticity (Hazel, 1977). He reported that pupal dimorphism in the American swallowtail or parsnip swallowtail butterfly, *Papilio polyxenes* (Fabricius, 1775) (Lepidoptera: Papilionidae) was sustained in nature by stabilizing selections. Stabilizing selection is a type of natural selection tends to favour genotype combinations that produce an intermediate phenotype (i.e., selected against extreme variations) (Charnov, 1989, Gould, 2002). Although the pupal colouration was known to be mainly influenced by environmental cues as well as based on the slight input of genetic factors, exemptions might exist such as the case of the the pain tiger or African monarch butterfly, *Danaus chryssippus* (Linnaeus, 1758) (Lepidoptera: Nymphalidae) mostly which is widespread in Asia and Africa. The pupal phenotypes in this butterfly were found to be entirely genetically controlled (Smith et al., 1988).

Several studies revealed that photoperiod physical characteristics (West, 1972) and wave lengths of light to which pupae were exposed played a significant influential role in determining the phenotype of the developing pupae (Gardiner, 1974). For example, larvae of the swallowtail butterfly, *Byasa alcinous* (Klug, 1836) (Lepidoptera: Papilionidae) reared under short-day conditions particularly at 18ºC and 60±5% RH developed into light yellow pupae instead of brown (Yamamoto et al., 2011). Warm temperatures and long days were often described to increase the production of brown pupae and short photoperiods tended to increase the occurrence of green pupae (Sims and Shapiro, 1983). Marshal et al. (2005) reported that the pupation site preferences with the matching background or texture interacted together in order to determine the final pupal colouration. For instance, it was found that dark pupae of *Araschnia levana* (Linnaeus, 1758) (Lepidoptera: Nymphalidae) that is common throughout the lowlands of central and eastern Europe and peacock butterfly, *Inachis io* (Linnaeus, 1758) (Lepidoptera: Nymphalidae) found in Europe and temperate Asia as far east as Japan, are often formed against lighter back ground and *vice versa* (Windig, 1999). Geographic factors such as latitude and altitude might also play an important role in affecting the duration of the pupal stage and the patterns of colouration (Van Dyck, 1998, Hashimoto et al., 2008).

In this study, during laboratory investigations, it was noticed that there was a substantial variation in pupal pigmentation with individuals tending to be either dark brown or lighter orange morphs under a constant temperature, 21 ºC (Fig.5.1). The darker pupae developed into males while the lighter gave rise to females. A question was raised in such case, why pupae that had been reared under constant temperature would show noticeable variations in the form of pupal pigmentation? Could the pupal pigmentation be implemented as a new method for sex identification in *H axyridis?* If yes, this could be of a great benefit for identifying sex ratio preceding to conducting a study. The above led to the premise that there is a pupal colour dimorphism in *H. axyridis* between sexes. Accordingly, this study hypothesized a new method for sex identification prior to an adult stage based on pupal pigmentations.

Fig.5.1. Pigmentation patterns of the cuticle of male (dark/ brown) and female (light/ orange) pupae.

5.2 Methods and materials

For this particular study, 168 larvae were reared individually in a 9 cm Petri-dish and were kept in a controlled temperature room at 20±1˚C, 70-75% RH, and a photoperiod of L16:D8 under fluorescent lamps. Upon pupation the pupae were weighed and sexed according to the above hypothesized method by this study, in which females were assigned from lighter or orange pupae (group A) and the darker or brown pupae were considered as males (group B). The pupae that were found difficult to categorize into A and B, were assigned into third group called (group C). Once adult emerged, they were sexed according to the degree of pigmentation on the prosternum following McCornack et al. (2007) method. Subsequently, the percentages of sex type that was predicted correctly for the three groups were calculated. Image-J software (Abramoff et al., 2004) was used to assess the variations in density of pigmentation on the external surface of the pupae assigned for group A and B. In order to validate the hypothesized method and to find out if it could be appropriate for pupae developing in the field, 124 pupae were collected arbitrarily from naturally infested fields in Silwood Park South-east England, U.K. (National Grid References 41/944691). The collected pupae from the field were assigned by the above similar hypothesized protocol but this time they were sexed by different people.

5.3 Data analysis

All data were expressed as proportions and they were arc-sine transformed to be normalized. Mean comparisons were performed using Student .t. Test and Chi-square test. On data that failed to be transformed Wilcoxon Rank Sum Test was applied. All statistical analyses were carried out using the statistical programme ‘R’ version 2.8.1 (Ihaka and Gentleman, 1996).

5.4 Results

For laboratory reared pupae that belonged to group A (orange) 84.46 % were sexed correctly as females. For group B (brown) 96.92% were sexed correctly as males (Table 5.1). The inclusive percentage of correctly sexed pupae was 89.28%. Both values of successfully and unsuccessfully attempts of sex determination differed significantly ($X^2=61.76$, $P<0.0001$). The mean weight of pupae reared in the laboratory also displayed weight dimorphism in which, dark/brown pupae, that developed into males, were significantly lighter in weight and not heavier than the lighter/orange pupae, that gave rise to females, ($W=35.5$, $P<0.0001$). A similar trend in weight was revealed between dark/brown pupae that were correctly sexed as males and lighter/orange pupae that gave rise to males ($W=235$, $P<0.01$). The difference between the weight of dark/brown pupae that developed into both males and females was significant (W=37, P<0.05). There was no significant difference concerning the mean weight of light/orange and dark/brown pupae that ecdysed exclusively into females (W=89, P=0.9669).

Table 5.1. Estimated mean weights of field and laboratory reared pupae and their predicted sexes.

	Laboratory reared pupae				
	Light pupae (A)		Dark pupae (B)		
	Male	Female	Male	Female	
Total No of Pupae =168	16	87	63	2	
Mean pupal weight(mg)±SE	35.4±0.012	26.9±0.002	20±0.014	24±0.005	
	Field collected pupae				
	Light pupae		Dark pupae		Pupae failed to sex(C)
	Male	Female	Male	Female	
Total No of pupae=124	36	29	47	6	6
Mean pupal weight(mg) ±SE	37.5±0.0008	48±0.11	33.1±0.0007	33.4±0.0031	39±0.0017

Pupae collected from the field however, revealed completely different results in which both dark/brown and light/orange pupae gave rise to both males and females. In addition, dark/brown pupae resulted into more males than females (Table 5.1). The intensity of pigmentation on field pupae, in particular the light/orange pupae, was considerably less in comparison with the laboratory reared pupae (Fig.5.2). This was regarded as the key factor that probably influenced the judgment for sex identification since 42 (33.87%) out of total

124 pupae were sexed inaccurately. Field collected dark/brown pupae developed into 11.32 % females and 88.6% males whereas; light pupae gave rise to about 44.61% females and 55.38% males. In overall 64.4% of pupae collected from the field were sexed successfully. Despite the 64.4% is not that high but the statistical comparison between the percentage of the correctly sexed pupae that were reared in the laboratory with those collected from the field revealed no significant difference ($X^2=0.09$, $P=0.763$). Similar comparison was performed between the unsuccessfully sexed pupae from both the field and the laboratory, showed non-significant differences ($X^2=0.18$, $P=0.667$). This infers that the hypothesized method for sex determination could be without doubt appropriate to field studies.

Fig. 5.2. Pigmentation on the pupal cuticle of the field collected pupae.

Image-J software was used to validate the variation in melanin pigmentation between dark and light pupae. Thirty light and another 30 dark laboratory reared pupae were assigned to this test. The aim of this test was to measure only the area of the dark pigmentation on the external surface or the cuticle of the assigned pupae using image-J software. All the required set up procedures were completed according to the provided instructions in the manuals in advance. The accuracy of the measurements depends mainly on selecting the appropriate threshold level, get rid of the noise and choose properly the area of interest. The results revealed noticeable variations in the intensity of the total black patches or areas over all the cuticle of both types of pupae. The measured mean of the total black areas on male pupa (dark) was about 37808.87± 925.3 μm² and it was 22328.8 ± 284.1μm² for the female pupae (light). The difference between measured means was significant (W=0, P<0.0001). This verified and supported the phenotypic variations in pupal coloration between genders.

5.5 Discussion

It was reported previously that the environment experienced during immature development had substantial influence on defining the intensity or /and the degree of pupal pigmentation (Yamamoto et al., 2011). The present study revealed that pupae that had been reared in the laboratory under a photoperiod of L16:D8 exhibited two distinctive phenotypes, dark brown and orange colorations. In addition, in the field pupae that were collected in summer (late June) the intensity of coloration was relatively lighter compared with laboratory reared pupae. Sims (2007) suggested that the pupal sensitivity to the length of photoperiod is determined on whether the larvae are in diapause phase or are not. He noted that the overriding effect of short photoperiods had produced brown diapause pupae irrespective of their background whereas, long photoperiods lead to either green or brown pupae depending on the features of the pupation site. In comparison with the present study, there were several other elements contributed in defining the pupal phenotype plasticity such as the background. The influences of the background together with the texture and quality of the substrate where larvae decide to pupate have been recognized widely (Sims and Shapiro, 1983, Hazel and West, 1996). Throughout the present study, transparent Petri-dishes were used to rear the larvae that were kept on a white shelf as a background. Regarding the above rearing elements it was uncertain whether the intensity of light in these small containers (Petri-dishes) or their smooth texture jointly with the white background had act together to determine the resulted pupal pigmentation.

It was reported previously that light stimuli were regarded as an influential regulator for the patterns of pupal coloration, in particular the yellow and blue spectral wavelengths (Smith, 1980). It was noted that the yellow and blue wavelengths were important determinants for yellow and brown pupal colouration in the butterflies that belong to order Lepitoptera and the family Pieridae, for example, the small cabbage white butterfly, *Pieris rapae* (Linnaeus, 1758), the green-veined white butterfly, *Pieris napi* (Linnaeus, 1758) and the white cabbage butterfly, *Pieris brassicae* (Linnaeus, 1758) In the same study it was debated that yellow light wavelength resembled the green foliage that stimulated the production of yellow pupae instead of brown coloured pupae. This could be incorporated in conjunction with other factors to clarify the finding by the present study the dominance of the light orange coloured pupae in the field. It was noticed by this study that field pupae were located in association with the stinging nettle plant, *Urtica doica* (Linnaeus, 1753) (Rosales: Urticaceae) which is a dioecious herbaceous perennial plant characterized by dark green colouration and rough texture. Several questions must be raised in that sense; did the preference of green background selected by these field larvae have a determinant role in giving rise to lighter coloured pupae? Did the high summer temperature influence the production of the melanisation rate in the field pupae? These questions must be well regarded in future work.

In a similar context, it was reported that throughout winter the dark brown pupa colouration was extremely common that might hold a cryptic purpose such as the swallowtail, *Papilio machaon* (Linnaeus, 1758) (Lepidoptera: Papilionidae) (Wiklund, 1975). Nevertheless, the intensity of the pupal colouration may as well play a significant part in heat absorbance particularly during winter weather (Legrand et al., 2004). Thus, the darker coloured pupae, the more is the heat gain and absorbance. Thus, the high field temperature could explain why the field pupae in the present study had a lighter colour in comparison with laboratory reared pupae. It is apparent that field pupae that were developed during a hot summer tended to reduce their cuticle pigmentation in order to lessen the heat absorbance that might have a great role in affecting the on-going developmental process. Thus, larvae experiencing high temperature during their development often compensate by producing less melanin that was regarded as a high costly process (Usui et al., 2004). Temperature-dependent regulation of pupal coloration was reported previously among insects species such as the common copper butterfly, *Lycaena phlaeas* (Linnaeus, 1761) (Lepidoptera: Lycaenidae) (Usui et al., 2004).

The present study showed that the size of the pupae played an important role in determining the intensity of colouration. Laboratory reared pupae were found to be smaller in size compared with field collected pupae because on small-sized pupae the colour would appear stronger and darker. The difference in pupal size was verified when the mean weight of pupae reared in the laboratory and those collected from the field were compared irrespective of their sex ($W=1130$, $P<0.0001$). Smith (1980) reported that the cessation of the hormonal secretion regulating pupal pigmentation might result in lighter coloured pupae. Hormonal termination could be as consequence of increasing temperature (Eiji, 1959, Smith, 1978). More importantly, under high temperature there is often a trade-off between growth rate and the rate of melanin production (Windig, 1999). Since females are characterized by greater growth rate compared with males (Blackenhorn et al., 2007), the trade-off would be more pronounced particularly due to the high costly melanin process production. This fact in combination with other factors could explain the lighter coloured pupae mostly developed into females rather than males. The present study also revealed that both pupal weight and size could be regarded an essential key indicators for predicting the sex since heavy pupae either dark or light coloured gave rise to females and *vice versa*.

In conclusion, the hypothesized sexing method based on pupal colouration could help to isolate males from females at earlier stages preceding to adult emergence. Nevertheless, the efficiency of this method could be intensified by incorporating further investigations to elucidate some of the above raised questions. Considering the effect of the background colouration and texture as well as the wavelengths on pupal coloration could be of great asset. The capability of *H. axyridis* larvae to select precisely their pupal site according to the surrounding environmental conditions as well as the substrate features could be a vital key factor in explaining their survival success throughout hash winter and during adverse environmental factors. It is well recognized that *H. axyridis* ladybeetle has been considered as an aggressive competitor and a strong predator displacing many native species as well as affecting the intraguild stability. The above finding by this study could be added to the advantages that accelerate the dominancy and establishment of this species. Although the percentage of the successfully sexed pupae gathered from the field was not high, incorporating more investigations could aid to add additional benefits to increase the accuracy of the hypothesized method. Laboratory investigations however, may employ this method with no trouble since the percentage of its success was relatively high. A study by Mclnnis et al. (2004) reported the first genetic sexing system for the melon fly, *Bactrocera cucurbitae* (Coquillett, 1849) (Diptera: Tephritidae) based on pupal colour. Thus, sexing based on the pupal coloration and predicting the ratio of sex is a progressing new topic that required more examinations. For example, there is a scope for further work on rearing larvae at different temperatures and photoperiods in the laboratory. In addition, there is a great necessity to assess the connection between temperature and photoperiods on regulating the resulting adult sex ratio.

Chapter 6

Effects of Single versus Multiple Mates on Fecundity and Egg Hatchability of the Female Asian Ladybird *Harmonia axyridis* Reared on Constant Diet

6.1 Introduction

Synthetic sex pheromones, namely, volatile organic chemicals that are designed to mimic natural sex pheromones, are presently used for controlling and monitoring population dynamics, as well as for inhibiting mating, either by trapping males or disrupting mating behaviour (Torres-Vila et al., 2002). The latter is now widely used and is becoming a more favoured method than the use of conventional insecticides, as it is environmentally friendly and has proven to be highly effective (Fadamiro et al., 1999).

Insect fecundity is influenced by numerous factors, including adult body weight (Leather et al., 1983, Leather, 1988), mating rate (McCauley and O'Donnell, 1984), habitat (such as the choice of host plant) (Leather et al., 1985), environmental factors (Obata, 1988) and the age of sexual maturity (Fox, 1993ab). Among insects, mating behaviour patterns and the associated reproductive performances maximise the chance of future generation survival (Arnqvist et al., 2005), as well as helping to realise the ecological situations promoting this process (Per-Olof and Rutwoski, 1999). Therefore, studies on mating patterns and the associated behaviour will help to provide valuable information for deducing several biological and evolutionary-related matters (Omkar et al., 2005).

Little is known about mating behaviour in ladybirds, despite their wide use in biological control programmes and their potential for disrupting the composition of intraguild systems (Dixon, 2000, Omkar and Singh, 2010). It is well known that females of several species are polyandrous - that is, they often reproduce with multiple males during their lifetime (Arnqvist et al., 2005). Polyandrous mating can be either sequential or simultaneous, depending on the species (Arnqvist et al., 2005). Polyandrous mating is a widespread reproductive strategy of natural insect populations (Zeh, 1997, Arnqvist and Nilsson, 2000). Several factors increase the selection of polyandry over other types of mating - the selection of males to enhance their fitness may cause female polyandry, improve genetic compatibility, intensify sperm competition and increase offspring survival success (Archer and Elgar, 1999, Stutt and Siva-Jothy, 2001, Campbell, 2005). Reynolds (1996) hypothesised that polyandrous behaviour could be explained based on whether it involves nutritional material, such as receiving a nuptial gift that is nutritious, or through male donation or genetic benefits.

The costs and benefits of mating behaviour are well documented (Arnqvist, 1989). The benefits of re-mating have been found to exceed the costs, since female fitness may increase greatly with a higher mating rate (Arnqvist and Nilsson, 2000). The benefits gained by females that mate more than once are poorly understood (Archer and Elgar, 1999), but can be categorised into direct and indirect fitness benefits (Ridley, 1988). Direct benefits include, principally, a higher female reproductive potential (Zeh, 1997), parental care and nuptial feeding (Thornhill, 1976, Walker, 1980). These propositions imply that females may seek out multiple copulations, but not necessarily multiple partners (Archer and Elgar, 1999). The indirect effects encompass genetic diversity and compatibility, offspring with greater genetic sustainability and effective sperm fertilisation (Zeh, 1997). For example, multiple mating significantly enhanced sperm competition in the moth *Atteva punctella* (Fabricius, 1798) (Lepidoptera: Yponomeutidae) (Taylor, 1967, Walker, 1980). Extra benefits result from polyandrous females also seeking copulations with different partners, rather than just having multiple copulations (Archer and Elgar, 1999). Therefore, female mating choice is one of the influential mechanisms known to boost reproductive success and offspring fitness, such as in females of *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae) (Partridge, 1980).

Cryptic female choice is a post-copulatory sexual selection phenomenon (Ben-Ari, 2000), and plays a key role in maximising fertilisation. As the female mates with several males, she can choose which male's sperm will be used to fertilise her eggs. This may be accomplished through the female sensing the size of the spermatophore or nuptial gift deposited in them by the multiple males and selectively storing them by choosing the largest size. In so doing, she is able to manipulate the paternity role by choosing the best sperm to fertilise her eggs according to their phenotypes. This phenomenon has been observed in the female moth *Utetheisa ornatrix* (Linnaeus, 1758) (Lepidoptera: Arctiidae), the field cricket *Gryllus bimaculatus* (De Geer, 1773) (Orthoptera: Gryllidae) and the female hanging fly or scorpionfly, *Hylobittacus apicalis* (Hagen, 1861) (Mecoptera: Bittacidae) (Holland and Rice, 1997, Ben-Ari, 2000).

Sperm transfer during mating plays an important role in determining mating patterns (Singh and Prevez, 2006). There are two basic mechanisms for sperm transference in ladybirds - either through direct sperm transfer with no spermatophore involvement, as in the case of

Cheilomenes sexmaculatus (Fabricius, 1781) (Coleoptera: Coccinellidae) (Omkar, 2004), or indirectly through the presence of a spermatophore, as in many species of *Chilocorus* (Coleoptera: Coccinellidae) and *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) (Obata and Hidaka, 1987). Several studies note that an interruption during copulation can result in more infertile eggs in *H. axyridis* (Obata and Hidaka, 1987, Osawa, 1994), whereas, re-mating and uninterrupted copulation were shown to increase potential fecundity, as reported in the yellow swallowtail butterfly, *Papilio xuthus* (Linnaeus, 1767) (Lepidoptera: Paplionidae) (Watanabe, 2002). It has been reported that during multiple mating, males derive factors that prompt oogenesis (Herman, 1977) and greatly enhance egg maturation. These factors are found in a proteinaceous compound that is secreted by the accessory gland of male (or in some cases, found in seminal fluid) and either have a transient effect or (in some species) cause long-term suppression of receptivity, such as in moths and butterflies (Boggs, 1990, Wedell, 2005). In both cases, these factors represent male investment in reproduction.

It has been reported that some insect species show considerable variability in female mating frequency, ranging from monandrous species that mate only once, to females mating several times in their lifetime (Wedell, 2002ab). The degree of polyandry also varies within species, with some females only mating once and others mating multiple times. Monandrous species may sometimes, however, engage in extra pair-copulations to compensate for low fertility (Wetton and Parkin, 1991) or to confer a genetic advantage to females (Gray, 1997). For example, the European corn borer, *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera: Crambidae), was classified as a monandrous species (Fadamiro and Baker, 1999), but 10% of laboratory-reared females mated twice (Showers et al., 1974). It has also been reported that mated females of *O. nubilalis* were less likely to be mated with again when males had the choice (Jiménez-Pérez and Wang, 2004).

Several debates have centred on the importance of male investment, authors have argued over whether male ejaculate is considered as a parental investment (Parker and Simmons, 1996) or as a mating effort (Perry and Rowe, 2008). Mating effort arguably cannot be regarded as a parental investment, due to the low amount of nutrient content needed to fertilise all the eggs (Simmons, 1990). As mentioned above, males sometimes provide considerable valuable nutrients to females through spermatophores or nuptial gifts (Gwynne, 1988, Simmons, 1990). Both of these types of nutrients may have pronounced effects on fecundity and offspring fitness (Tregenza and Wedell, 1998). It has also been noted that females that have received a large spermatophore were able to lay larger-sized eggs (Fischer et al., 2009). Nevertheless, the effects of multiple mating on the relationship between fecundity, fitness and costs have been debated constantly (Arnqvist et al., 2005, Campbell, 2005). It has been suggested that the relationship between the number of matings and the resulting fecundity could be positive (Jiménez-Pérez and Wang, 2003, Campbell, 2005); neutral, as in the windmill butterfly, *Atrophaneura alcinous* (Klug, 1836) (Lepidoptera: Papilionidae) (Kawagoe et al., 2001), or negative, as in the female leaf beetles, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) (Orsetti and Rutowski, 2003).

Several studies, such as the one by Savalli and Fox (1999), have revealed that multiple mating will increase the rate of egg production. Others have noted that the influence of multiple mating on egg production may be untraceable when other factors, such as maternal age, are involved (Fox, 1993ab). For instance, in one study, females of the beetle *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Bruchidae) that had received large ejaculates laid large clutch sizes (Savalli and Fox, 1999), but in another study, they oviposited small clutches (Eady et al., 2000). The inconsistency between the above findings is not unexpected in *C. maculatus* species for a number of different reasons. First, Eady at al. (2000) had worked on dehydrated females. Second, females of this species are known to receive large male ejaculates, which may counteract the benefits, resulting in a delay in mating and a reduction in sperm competition and may make the ejaculate itself unprofitable (Eady et al., 2000).

In the majority of insects, mating may comprise significant direct or indirect costs for females (Wing, 1988). Previous work has revealed that when costs become highly significant, females are expected to maximise fitness by decreasing their mating rate (Holland and Rice, 1998). Furthermore, when it comes to male costs, it has been assumed that males should utilise ejaculatory strategies that maximise their reproductive success. The latter could be performed by altering sperm number or spermatophore size according to the female's age, body size or mating number and type (Dewsbury, 1982, Lewis, 2004). Several possible costs of multiple mating have been described, such as an increased threat of predation and damage during copulation (Savalli and Fox, 1999). For example, females were found to have been injured internally by the spiny genitalia of males (Crudgington and Siva-Jothy, 2000). Others have reported that males sometimes transfer parasites and seminal fluid containing chemical toxic materials during mating (Daly, 1978).

In addition, exposure to high mating frequency may significantly decrease the longevity of females in some species (Partridge et al., 1987, Fowler and Partridge, 1989) and increase adult female lifespan in others, such as in bruchid beetles, *C. maculatus* (Fox, 1993ab). Other investigations have showed that neither the rate of mating nor the received spermatophore size had an effect on longevity of females, such as in the ladybird, *Adalia bipunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) (Perry and Rowe, 2008). Nevertheless, situations arise in which the expected relationship between female fitness and mating rate might be more complicated (Arnqvist et al., 2005). It has been suggested that there is an intermediary optimal mating rate and that the observed mating rate often exceeds the optimum, resulting in a slightly positive effect on longevity (Arnqvist and Nilsson, 2000). In fact, repeated mating (as noted by Ridley (1988)) has been shown to enhance the fecundity and longevity of species, depending on whether the female was already characterised by great fecundity as well as longevity.

An understanding of why females mate with multiple males is fundamental for sexual selection studies (Gowaty, 1994). When females of the harlequin beetle riding pseudoscorpion, *Cordylochernes scorpioides* (Linnaeus, 1758) (Pseudoscorpiones: Chemetidae), were constrained with one male each, significantly fewer offspring were produced that were associated with high mortality rate (Zeh, 1997). Others have argued that the reduction in reproduction success may be caused by the insufficient sperm quantity received from single males (Ridley, 1988). Nevertheless, polyandrous behaviour often provides females with post-copulatory mechanisms that act to reduce the risk from genetically incompatible sperms (Zeh and Zeh, 1996). It could be concluded that the costs and benefits of multiple mating may greatly influence several aspects of an organism's activities and could affect the implementation of an effective procedure for pest control management (Sadek, 2001).

Sexual activities in predaceous ladybirds have been studied intensively, due to the accelerated invasion process of exotic species and their adverse impact on ecosystems (Singh and Prevez, 2006). Random mating has not been observed in certain ladybird species, such *H. axyridis* and *A. bipunctata* (Hodek and Ceryngier, 2000). In *H. axyridis,* male mobility, elytral colour and body size were involved in affecting female mate selection (Osawa and Nishida, 1992). In *A. bipunctata*, however, the colour of the elytra and the pronotum of males were found to be very important in females' choice of mate (Majerus et al., 1982). Mating in *H. axyridis* varied frequently between melanic and non-melanic forms, with seasonal variation. Females were found to mate with melanic males during summer and with non-melanic males during spring. Thus, females showed a genetic preference towards the quality of elytron phenotype (Wang et al., 2009).

Females of *H. axyridis* are known to undergo multiple matings during their lifetime (Osawa, 1994), making it a model system for evolutionary ecological studies (Joseph et al., 1999). Mating refusal has also been recorded in females of *H. axyridis* under starvation conditions (Obata, 1988). Prey availability could arguably be a major environmental cue in influencing mating receptivity in *H. axyridis* (Obata, 1988). It has been reported that mating receptivity in females of *H. axyridis* is reduced during starvation and under unfavourable conditions, thus the choice in mating is very important for this ladybird beetle. Fox (1993ab) noted this might be not the case in other species, such as the starved females of *C. maculatus*, as when they mated with multiple mates they lived longer than singly mated starved females. Thus, the species type plays an additional role in affecting the consequences of multiple mating and its associated costs.

Evidence of the effect of multiple mating on females' fertility and fecundity is still remarkably poor and the need for more well-designed experiments is required (Ridley, 1988). It has previously been reported that the lack of this kind of study was as a result of the inherent difficulties in rearing the beetles in the laboratory with sufficient food supply and frequent monitoring (Obata and Hidaka, 1987). Since *H. axyridis* dominates many guilds and is considered a strong polyphagous predator, as well as an aggressive competitor (Roy, 2008), it is essential to address the significance of mating rate on their survival and fitness. It has become the most common worldwide invader, dislocating many native species and affecting the stability of many native communities and crop production. Mating in females of *H. axyridis* is of the polyandrous type (Osawa, 1994). Accordingly, a study was commenced to evaluate the major costs and benefits of multiple mating on the lifetime fitness of females. The experiments were planned to determine the effect of variable exposure periods to a single male on egg hatchability, trophic egg production, lifetime fecundity, mortality and female longevity. In addition, the reproductive performance of virgin females that had never mated was also investigated, in order to understand the capability of this species to survive without mating, particularly during invasion.

6.2 Materials and methods

Freshly emerged two-day old adult virgin males and females of *H. axyridis* were collected from an established laboratory culture maintained at a constant temperature of 20±1˚C, 70-75% RH and a photoperiod of L16:D8. In order to identify the influence of mating number on the reproductive output of females, four treatments were prepared, following (to a large extent) the method detailed by Siswanto et al. (2009). The first treatment had 12 virgin females that were kept separately in 9cm Petri-dishes. A new set of 24 virgin adults of opposite sexes were paired, each in a 9cm Petri-dish, and were observed until the end of the first mating. Thereafter, they were kept in pairs for five days, following which each female was separated from its partner in a new Petri-dish and assigned for five days of treatment.

Another set of 12 couples of virgin males and females were each kept in 9cm Petri-dishes until the end of the first mating. Females were then separated from their mates in new Petri-dishes before the second mating. Females from the latter were assigned as one-mated treatment. For the control (continuous exposure to mates), 12 virgin pairs were coupled in 9cm Petri-dishes and monitored until they died. All the adults were used only once during the course of the experiment and were not reused in any other sets. During the experiment, each of the adults was provided daily with 16 live adults of the pea aphid, *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae), maintained on broad bean plants, *Vicia faba* (Linnaeus, 1753) (Fabales: Fabaceae), variety Aquadulce Claudia. Monitoring of the experiment was continued until all the ladybeetles had died.

6.3 Data analysis

All data were tested for normality prior to applying parametric analysis, and the one that failed was transformed. One-way analysis of the variance ANOVA or general linear model (GLM) with post hoc Tukey's multiple range tests were performed to analyse the variation between females, in terms of lifetime fecundity and clutch parameters. The data on percentage fertility, trophic eggs, eggs with incomplete developed larvae and cannibalised eggs were subjected to arcsine square root transformation analysis and then dealt with as continuous data. Most of the allometric analysis was performed using either log-linear regression; for data that were not normally distributed, the Glm-linear model with Poisson errors data was applied. Reproductive biomass was determined by multiplying egg weight by lifetime fecundity, reproductive growth was calculated by dividing reproductive biomass over longevity and the reproductive rate was determined by dividing total fecundity over reproductive period. All statistical analyses were carried out using the statistical programme 'R' version 2.8.1 (Ihaka and Gentleman, 1996).

6.4Results

6.4.1 Variability in clutch parameters between treatments

Clutch size and weight, egg fertility and others parameters are presented in Table 6.1. Mating patterns in all treatments had a significant influence on mean daily clutch size (F=16.84, RSE=8.32, P<0.0001). The mean clutch size laid by virgin females was 5.86±1.8 eggs which was significantly less than the control (t=-4.9, ESE=-2.12, P<0.0001), the once mated treatment (t=1.14, ESE=-22.45, P<0.0001) and the five-days mated treatment (t=-0.78, ESE=-15.89, P<0.0001). For both once mated and five-days mated treatments, the mean clutch size did not significantly differ from the control, that is, (t=0.81, diff=0.18, P=0.42) and (t=-0.2, diff=-0.2, P=0.46) respectively. The proportion of trophic (non-viable) eggs was significantly greater in virgin and in five-days mated females, compared with that of the control, namely, (t=7.33, diff=0.56, P<0.0001) and (t=3.14, diff=0.17, P<0.01), respectively. Once mated females, on the other hand, did not show a significant difference in the proportion of deformed eggs when compared with the control (t=1.68, diff=0.09, P=0.09). The proportion of incompletely developed larvae was found to be significantly higher in the five-days mated treatment relative to the control (t=2.9, diff=0.76, P<0.01). The proportion of hatched larvae obtained from the five-days mated treatment was 0.57, which was significantly less than the control (t=-2.37, diff=-8.01, P<0.05). Finally, there was no significant difference in clutch weight among the three treatments in comparison with the control (F=1.36, diff=0.014, P=0.25).

Table 6.1. The reproductive output for the four treatments (mean±SE, n=12).

Treatments	Once mated females	Five-days mated females	unmated females	control
Mean total fecundity (eggs)	188.83±25.27	107±33.38	3.82±1.46	22.38±3.19
Mean clutch size (eggs)	25.46±1.6	21.83±1.7	5.86±1.8	23.8±2.02
Proportion of non-viable eggs	0.17	0.29	1	0.042
Proportion of egg with non- developed larvae	0.048	0.092	0	0.035
Proportion of cannibalized eggs	0.044	0.033	0	0.044
Proportion of hatched larvae	0.73	0.58	0	0.878
Mean clutch weight (mg)	0.0094±0.0008	0.0074±0.0006	0.0078±0.0064	0.00067±0.0005
Pre-reproductive period (days)	11.33±0.7	8.33±0.6	13.09±2.8	7.48±0.18
Reproductive period (days)	29.33±0.82	16.16±1.85	1.08±0.35	15.63±0.7
Longevity (days)	42.75±1.11	31.27±2.64	31±2.22	32.29±0.5
Reproductive biomass (egg No x mg)	0.14±0.018	0.21±0.05	0.12±0.013	0.15±0.028
Reproductive rate (egg No/day)	6.3±0.78	4.7±0.67	3±0.0.58	7.11±1.41
Reproductive growth (reproductive biomass/ Longevity) (egg x mg/days)	0.003±0.0004	0.006±0.0015	0.0045±0.0005	0.005±0.001
	n=12	n=12	n=12	n=12

6.4.2 Impact of exposure to male on females on fecundity and fertility

Daily clutch size and the related fertility of females, assigned for the four treatments, are presented in Figures 6.1 and 6.2 respectively. Overall, fecundity and fertility tended to have similar trends, with high values at the beginning of the reproductive period followed by a gradual decline towards the end of that period. Singly-mated females started their reproductive period by laying the highest largest clutch sizes compared with other treatments. This behaviour was continued until the end of their reproductive period. The general trend of fertility curve for once mated treatment started at the least value compared with five-days multiple mated females and the control treatment; subsequently, it increased suddenly, reaching approximately 23.58±7.28 days. The fertility curve of singly mated females stayed higher towards the end the reproductive period compared with other treatments. The drop seen in the fertility curve of singly mated females was due to the fact that one of the females had laid only six eggs in its fourth clutch. The control and five-days mated treatments both showed a noticeable reduction in fecundity accompanied by a decrease in fertility at early stages of reproductive period compared with singly-mated females. It was obvious that the presence of males had a substantial influence on the female lifetime fecundity.

Total lifetime fecundity varied significantly between the four treatments ($F=12.188$, $P<0.0001$) (Fig. 6.3). Virgin females had the lowest fecundity compared with the control ($t=-4.12$, $ESE=-7.31$, $P<0.01$), once mated females ($diff=-10.59$, $P<0.0001$) and five-days mated females ($diff=-5.8$, $P<0.05$). Females that mated once were significantly more fecund than the control ($t=2.06$, $ESE=-3.27$, $P<0.05$) and the five-days mated females ($diff=4.76$, $P<0.05$). The fecundity of five-days mated females, however, did not differ significantly from the control ($t =-0.93$, $ESE=-1.48$, $P=0.35$).

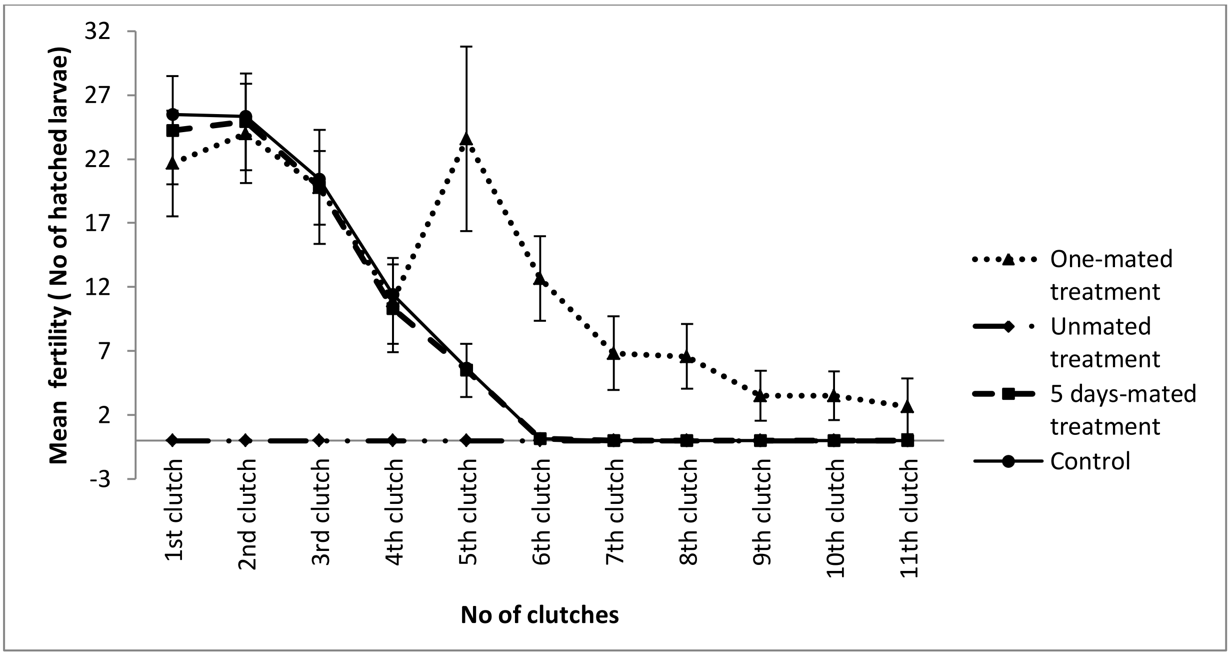

Fig.6.1. Mean clutch size throughout the reproductive period obtained from the four treatments plotted against the clutch number.

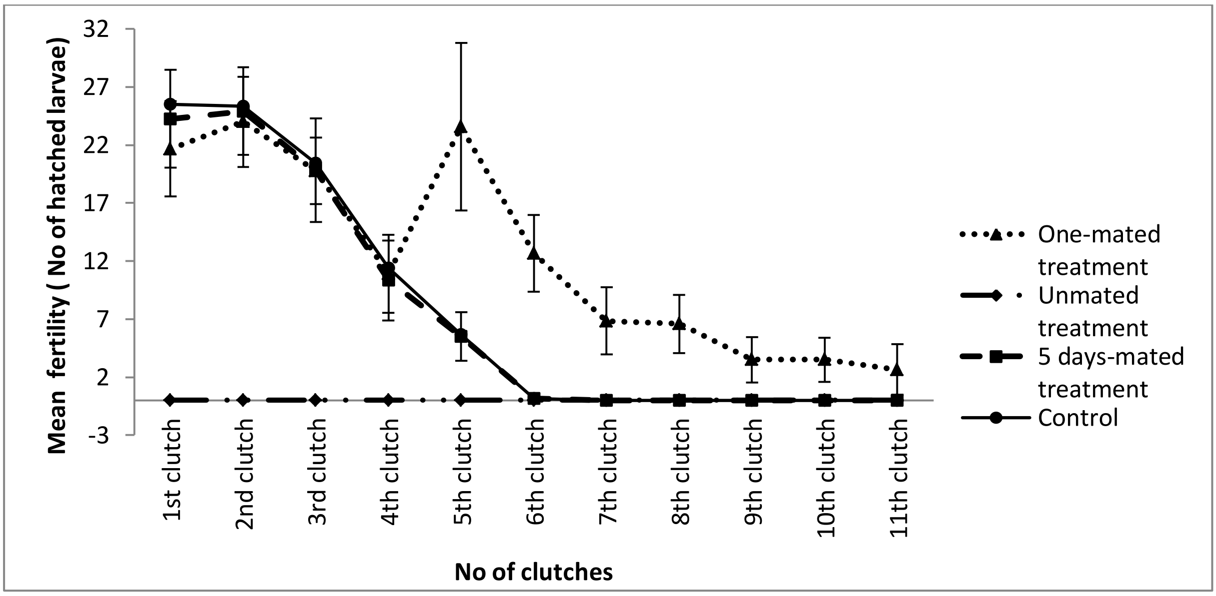

Fig.6.2. Mean fertility as number of hatched eggs calculated for each clutch obtained from the four treatments plotted against the clutch number.

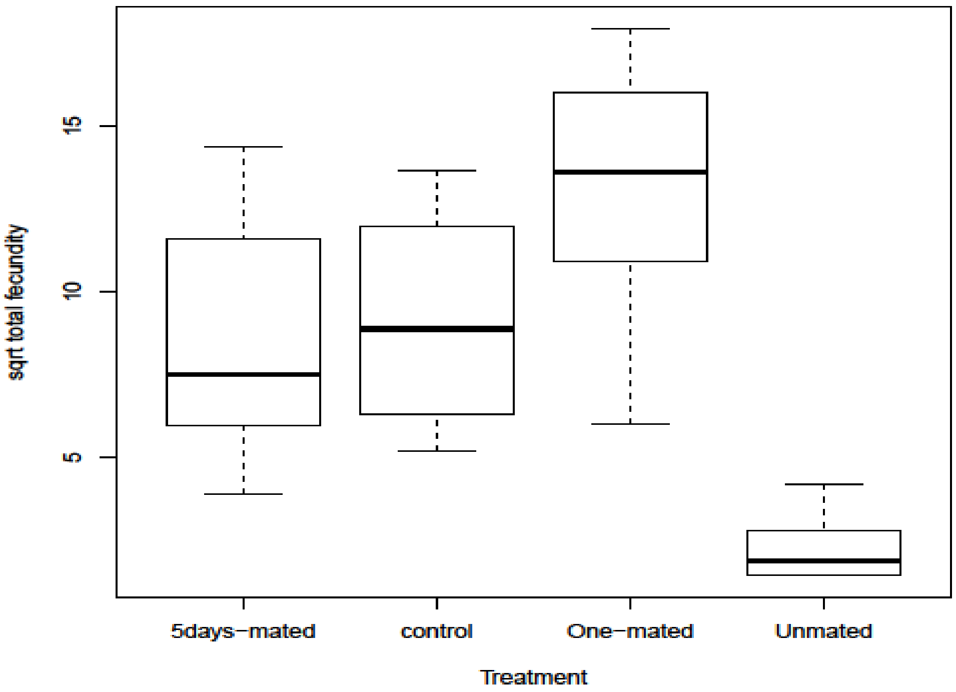

Fig.6.3. Variability in the life-time fecundity obtained from the four treatments. Data were transformed for statistical analysis (F=12.188, P<0.0001).

There was no relationship between lifetime fecundity and the proportion of fertility in all treatments (F=2.003, P=0.1). A similar non-linear relationship was obtained between the proportion of fertility and clutch size for all treatments (F=2, P=0.11). Only females of the control had a significant negative relationship between their proportion of trophic (deformed) eggs and lifetime fecundity (t=-4.67, ESE=-3, P<0.0001), as well as between the former and the clutch size (t=-4.03, ESE=-2.66, P<0.001). The results revealed a significant relationship between the clutch size and the proportion of eggs with incompletely developed larvae for all treatments (F=2.75, RSE= 0.733, P=0.05). The interaction between the clutch size with the proportion fertility assessed for all treatments resulted in a significant effect (F=25.36, P<0.0001). Virgin females, singly-mated females and five-days mated females were less fertile compared with the control, (diff=-0.65, P<0.0001), (diff=-0.36, P<0.0001) and (diff=-

0.2, P<0.001), respectively. Virgin females had a significantly lower fertility, compared with females that had mated only once (diff=-0.29, P<0.001) and with five-days mated females (diff=-0.38, P<0.0001).

6.4.3 Variability in reproductive period and longevity

The reproductive period varied significantly between the four treatments (F=100.57, P<0.0001) (Fig. 6.4). For unmated virgin females, the mean reproductive period duration was 1.08±0.35 days, which was significantly shorter than the control (diff=-1.78, P<0.0001), whereas in once mated females, the mean of that period was 29.33±0.82 days, significantly longer than the control (diff=0.72, P<0.0001). The duration of the reproductive period in the five-days mated females did not vary from the control (diff=0.09, P=0.88). The interaction effect of the independent variables (the reproductive period) and the fertility against the type of treatments revealed a significant effect (F=69.08, P<0.0001) in which control had the longest reproductive period (compared with virgin females (diff=-0.57, P<0.0001), once mated females (diff=-0.44, P<0.0001) and five-days mated females (diff=-0.28, P<0.0001)). The interaction of the reproductive period with the proportion of trophic eggs of the four treatments was also significant (F=14.8, P<0.01). Virgin females had significantly higher values of trophic eggs than that of the control (diff=0.41, P<0.001) or singly mated females (diff= 0.28, P<0.05). In addition, in relation to the pre-reproductive period for five-days mated females, the fertility was significantly greater compared with the once mated treatment (diff=-0.15, P<0.05) and virgin females (diff=-0.28, P<0.0001), when considering the effect of the reproductive period. The effect of the reproductive period on the proportion of deformed (trophic) eggs laid by virgin females was significantly higher than the control (diff=0.41, P<0.001).

When considering the effect of the pre-reproductive period instead of the total reproductive period, it was revealed that fertility was significantly less for five-days mated females compared with the control treatment (diff=-0.2, P<0.01), and similarly the fertility of the virgin females was significantly less than the control, since they produced mostly trophic eggs (diff=-0.7, P<0.0001). When deformed (trophic) eggs laid by virgin females, five-times mated females, control treatment and once mated females were analysed against the effect of pre-reproductive period, a significant effect was exhibited (F=9.42, P<0.0001). Virgin females laid significantly fewer trophic eggs compared with the control treatment (diff=0.32, P<0.0001), with singly-mated females (diff=0.4, P<0.0001) and five-days mated females (diff=0.28, P<0.0001) after regarding the effect of the pre-reproductive period.

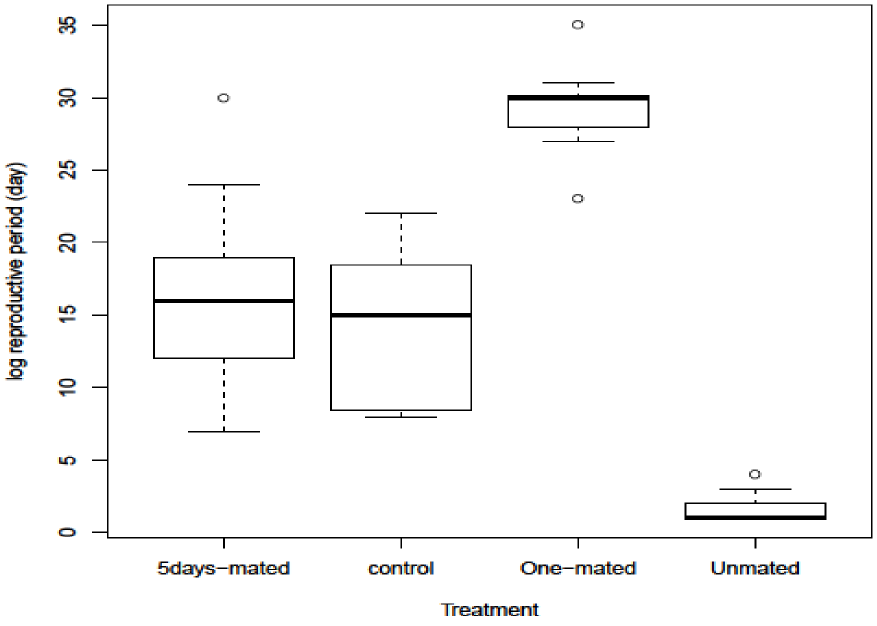

Fig.6.4. Variability in the duration of reproductive period of females assigned for the 4 treatments. Data were transformed for statistical analysis (F=100.57, P<0.0001).

The presence of males had a significant impact on female longevity. Longevity of females in all treatments varied significantly (F=8.91, P<0.0001). Singly mated females lived longer compared with the control (diff=10.8, P<0.01) and with those with multiple mates (diff=11.41, P<0.001). Longevity was, however, significantly shorter in virgin females in comparison with the control (diff=-11.75, P<0.001). The relationship between longevity and fecundity was examined and the results indicated as significant interaction (F=6.04, P<0.0001). Only females that mated for five days affected the lifetime fecundity-longevity relationship which was significantly less than the control (t=2.2, diff=1.37 P<0.05). The study investigated if the different forms of laid eggs had a direct influence on longevity. The results revealed that only the proportion of eggs containing non-developed larvae laid by the control treatment had a linear negative relationship with longevity (t= -2.15, dif=-5.5, P<0.05). This means that any increase in the quantity of this type of eggs will reduce female longevity. The proportion of deformed (trophic) eggs calculated for all treatments in general tended to increase with increasing longevity (F=11.21, dif=0.9, P<0.0001). Finally, reproductive rates for all treatments showed no significant variability when they were compared statistically (F= 2.08, dif=0.61, P=0.11) and a similar trend was observed when comparing the reproductive growth and reproductive biomass of variable treatments - that is, (F=1.32, dif=0.024, P=0.27) and (F=1.24, dif=0.1, P=0.3) (Fig. 6.5) respectively.

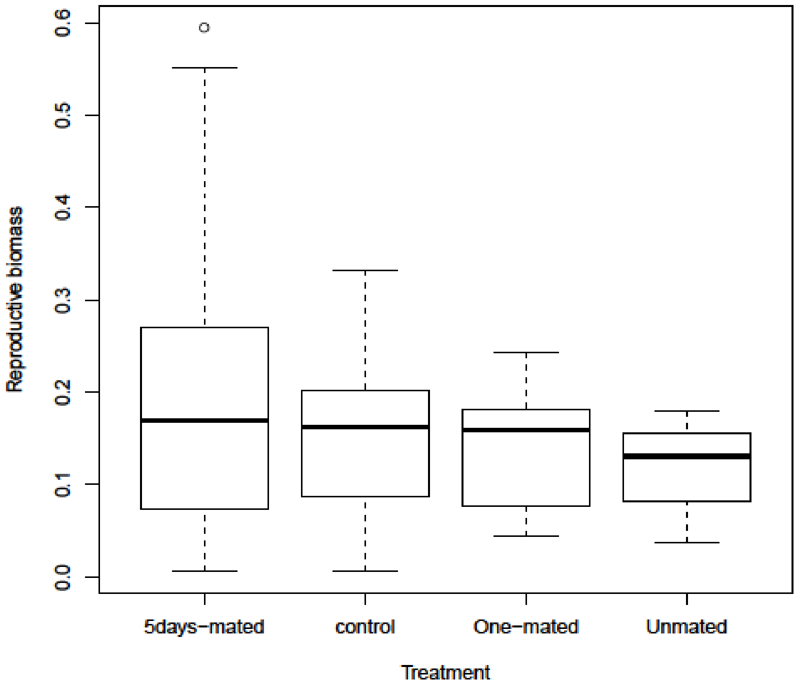

Fig.6.5. Variability in reproductive biomass between different treatments. Data were transformed for statistical analysis (F=1.24, P=0.3).

6.5 Discussion

One feature of reproduction that has attracted a lot of attention in insect taxa is the number of matings, that is, polyandry. The degree of polyandry is a vital variable in understanding post-mating behaviour, pre-fertilisation and mating competition (Eberhard, 1996). As previously noted, multiple mating is highly advantageous, since it decreases the risk of mating with an infertile partner (Archer and Elgar, 1999) and it might significantly increase egg production and fertility (Omkar and Ahmad, 2005).

The evolution of polyandry demonstrates either direct benefits or indirect genetic benefits. Direct benefits comprise the fact that females may look for multiple copulations but not necessarily multiple partners, while the indirect benefits suggest that females will search for multiple copulations with different partners (Archer and Elgar, 1999). In part, polyandry explains the level of sexual conflicts of awareness with male over-mating (Chapman, 2006), for example, the form of gene flow and level of diversity (Zeh, 1997). The advantage of having genetically diverse offspring is important for the evolution of sexual reproduction (Stearns, 1989) and the advantage of having multiple mates is regarded as a crucial strategy to maximise the diversity as well as the fitness of offspring (Walker, 1980). Williams (1975) noted that mating with multiple partners slightly affected offspring diversity.

Low fecundity among singly mated females is a common phenomenon that many failed to explain (Ridley, 1988). The present study revealed that virgin females that mated only once in their lifetime with virgin males were more reproductive and fertile. In addition, singly mated females accomplished the greatest fecundity compared with the control treatment and the other treatments. In addition, *H. axyridis* females that mated only once were able to oviposit over a longer reproductive period in comparison with those in the control. Nonetheless, the proportion of fertility stayed throughout the reproductive period significantly higher than the control. On the other hand, singly mated females of the hide beetle, *Dermestes maculatus* (De Geer, 1774) (Coleoptera: Dermestidae), failed to lay a high proportion of fertile eggs during their reproductive period (Archer and Elgar, 1999).

Attaining high fecundity and fertility after one mating imply that females of *H. axyridis* are able to store viable sperm without any reduction in the efficiency of fertilisation process throughout the reproductive period - approximately 29.33±0.82 days. This indicated that the longevity of the sperm was greater after one mating and it was reduced significantly after several matings, as demonstrated in relation to the five-days treatment and the control.

Analogous results were noted by Arnqvist (1989), in which multiple mating was found to be relatively costly to female water striders, *Gerris odontogaster* (Zetterstedt, 1828) (Hemiptera: Gerridae), which needed to mate every tenth day in order to receive sufficient viable sperm (Arnqvist, 1989). In the present study, the low fecundity and fertility of five-days mated females could be clarified according to Tseng et al. (2007). It was explained that in the female beetle *C. maculates,* re-mating every so often stimulated female oviposition but did not increase the egg maturation rate. It was further reasoned that the essential proteins for egg maturation might be replenished and needed some time to be transported by the circulatory system to their targeted organ or tissue. Electrophoretic studies demonstrated that in the groundnut beetle, *Caryedon serratus* (Olivier, 1790) (Coleoptera: Bruchidae), it took about one day for such proteins to move to the genital tracts (Boucher and Huignard, 1987).

In the current study there was a decline in clutch size accompanied by reduction in fertility, particularly subsequent to the first and the second-laid clutches towards the end of the reproductive period. This trend was noticed in all treatments without exception. Similarly, a previous study on *C. maculates* noticed the same observations (Wilson et al., 1999). In addition, it was also reported that females of cowpea weevil, *Callosobruchus subinnotus* (Fabricius, 1775) (Coleoptera: Bruchidae), which had experienced four matings laid fewer eggs compared with females that mated twice or three times (Mbata, 1997). It was debated that male ejaculates might have both stimulatory and nutritive roles in the above cases, given that the extent to which oviposition stimulation was potentially limited by the number of oocytes in the ovaries at eclosion and by the maturation rate of occytes (Wilson et al., 1999). Likewise, the fecundity of singly mated females of the cat flea, *Ctenocephalides felis* (Bouché, 1835) (Siphonaptera: Pulicidae) was greater relative to multiple-mated females as the quantity of sperm was very low and was not capable of fertilising all of the matured eggs formed after multiple mating (Hsu and Wu, 2000).

The male contribution may vary over time in response to its age or as a function of mating history (Savalli and Fox, 1999). Lewis (2004) reported that male paternity success was found to deteriorate across multiple matings, owing to the depletion of sperm or the accessory gland products, or possibly due to a drop and weakening in the coplulatory courtship behaviour (Edvardsson and Arnqvist, 2000). In contrast, Simmons (1996) noted that male mating history does not always influence sperm production, since he could not find any similar effects on male sperm precedence in his study.

The present results revealed that, although multiple matings were undoubtedly advantageous to the females in some ways, there was a reduction in the reproductive output towards the end of the reproductive period in all assigned treatments. This indicates that there is a potential reproductive output with parental contribution that was limited by many factors, such as the constant amount of aphid supply. Although the number of aphids given to the adults throughout the study was considered the best for the optimal reproductive activity for this ladybird (Agarwala et al., 2008, Kajita et al., 2010), from time to time body maintenance requirements may exceed that quantity. As a result, the study predicted some alternation in the reproductive performance curve if those couples have unlimited access to their prey.

Furthermore, there was no positive linear relationship revealed between fecundity and the number of matings in the present study. Seemingly, multiple mating in *H. axyridis* might interfere simultaneously with egg fertilisation process. This is in accordance with previously reported information, in that the products of male accessory glands might reduce the mobility of sperm deposited previously in the female's spermatheca (Smid et al., 1997). In addition, it was noted that mating with the same partner over a long period might also restrict egg production (Lewis, 2004). The latter showed that repetitive copulations with similar females did not increase male insemination success or the quantity of eggs produced and their viability. Others have argued that repeated copulation might affect the storage capacity of the sperm in spermatheca, resulting in fewer fertilised eggs (Simmons, 1996).

In this study, there was no evidence showing a reduction in fecundity or variability in five-days mated females of *H. axyridis,* when compared with the control. This indicated that females of *H. axyridis* are capable of surviving with only one single mating and accomplishing a reasonable potential reproductive output. This agrees with the results reported previously on *C. maculates* by Ofuya (1995). He showed that virgin females mated with the same males successively resulting in a trend in the direction of lower fecundity and viability, when compared with singly mated females.

In this study, the age of couples and mating history appeared to have a negative influence on offspring production, which was observable at the late stages of the reproductive period. Fecundity and egg viability often depend on the reproductive history of the males and females (Ofuya, 1995). Fox (1993ab) claimed that the influence of maternal age was greater in comparison with the effects of multiple mating on egg size and number. Likewise, Moore and Singer (1987) declared that maternal age had several contradicting influences on the offspring size, it may have a negative influence on egg size, may positively increase egg size (Kasule, 1991) or have no apparent influence (Marshall, 1990). Others have proposed that the influence of maternal age on egg size characteristics may be adaptive, particularly as clutch size is known to be constrained by morphological, physiological and ecological elements (Begon and Parker, 1986).

It has previously been reported that female longevity might be reduced as a consequence of multiple mating (Arnqvist and Nilsson, 2000). The trade-off between the rate of mating and longevity appeared explicitly in the present study. Singly mated females lived longer than five-day mated females and the control. In contrast, virgin females that had never mated had the lowest longevity of all. This implied that there should be a balance between the number of matings and the trade-off favouring females' fitness. This was supported further by Arnqvist and Nilsson (2000) who argued that "the balance between the fitness cost and benefits of polyandry should limit the potential for the evolution of extreme levels of polyandry". In extremely polyandrous species, such as the leaf beetle, *Chrysochus cobaltinus* (LeConte, 1857) (Coleoptera: Chrysomelidae), there was no influence of multiple matings on longevity (Schwartz and Peterson, 2006). Apparently, polyandrous behaviour in *H. axyridis* seemed likewise to have a substantial trade-off on female reproductive success and longevity. This was concurrent with what was noted formerly, namely, although females may achieve benefits from multiple matings, this behaviour might also impose great costs on female fitness, as frequent mating requires investment in both time and energy (Schwartz and Peterson, 2006). Finally, the results indicated that there was no sign that increasing the time paired would reduce the longevity, since the control was found to have the greater longevity compared with five-day mated females. This is logical, since there is no general role for the fecundity-longevity-body weight relationship, particularly if females have access to a food source (Leather, 1988).

It can be concluded that multiple mating is beneficial to some extent. It could, however, result in a reduction in egg production rate and egg viability due to the extra energy required. The decrease in reproductive performance was expressed specifically when females were constrained by exposure to a single male or by having delayed mating, like the case of virgin females. Thus, having a mate choice might improve the component of reproductive output and the future offspring fitness, as likewise noted by Partridge (1980). There should be, however, an intermediate level of polyandry that offsets the cost and benefits in order to maximise female fitness. Nevertheless, the results suggested that one mating might be sufficient for lifetime fertility in *H. axyridis* females, this can be considered as an advantageous strategy, promoting their reproductive success compared to other ladybird species. Although the study had highlighted some of the costs and benefits of mating rate, other related concerns remained elusive and quantifying them is more difficult. They must be recorded under conditions similar to which the beetle is adapted. Furthermore, in polyandrous species such as *H. axyridis*, detailed knowledge of sperm transfer, sperm longevity and the number and storage process in spermathecae must be determined in order to fully understand a female's receptivity pattern and to provide an insight into the dynamics of paternal success worldwide. At the very least, the study highlighted some of the factors that promote the success of *H. axyridis* in surviving adverse environments, namely, having a peculiar reproductive strategy. The reproductive organ has the capability of reserving the sperm gained from a single mating, with one mating able to provide lifetime fertility. This could be added to the many abilities demonstrated by this ladybird that make it capable of sustaining and surviving longer in different habitats effortlessly.

Chapter 7

The Influence of Conspecific and Heterospecific Tracks on the Defensive Behaviour of *Harmonia axyridis*

7.1 Introduction

Because of the temporary nature of aphid colonies and other kind of prey, ladybirds often face competition for inadequate food and risk intraguild predation (Agarwala and Dixon, 1993). The latter is a widespread interaction taking place at diverse trophic levels within a community that plays a vital part in affecting the distribution, abundance and evolution of the interacting species (Polis et al., 1989). It further helps in understanding the community structure of the potential competitors (Snyder and Ives, 2003). Intraguild predators acquire benefits from feeding and displacing other competitors occurring at the same trophic level, like the behaviour of invasive species (Polis, et al., 1989, Holt and Polis, 1997). Usually, intraguild interaction encompasses both predation and the competition process. Many important issues in community ecology revolve around interference competition and predation (Snyder and Ives, 2003). Species that compete may also be constrained in predator-prey interactions, which comprise the mixture of competitions and predations classified as intraguild predation (Holt and Polis, 1997). Theoretically, there are two types of predators, top and intermediate predators. The intermediate species, which is the prey, should be superior at exploitative competition for communal resources. The top species, however, is the predator that consumes the intermediate. If this system is in place, then intraguild predation is regarded as stable (Walls and Williams, 2001). Intraguild interactions may be classified as synergistic and are used to describe a lower rate of prey consumption. These interactions modify normal prey behaviour, making it more susceptible to predator attack. They also include the non-additive or antagonistic predation used when fewer of the prey are killed by the combined actions of different predators (Tapia et al., 2010). The antagonistic/synergistic interfaces on co-occurrence of predators depend principally on the parameter and degree of feeding niche diversity (Huxel, 2007).

Intraguild predators are regarded as a major risk for co-existent ladybirds (Cottrel and Yeargan, 1998, Schellhorn and Andow, 2005), specifically because their eggs or juveniles are highly susceptible to cannibalism (Lucas et al., 1998). Therefore, interacting predators exhibit several behaviours, including the avoidance response towards larval tracks triggered by oviposition-deterring pheromones or semiochemicals (Meisner et al., 2011b). Semiochemicals are those that arbitrate interactions between organisms. They are divided into allelochemicals and pheromones, depending on whether the interactions are interspecific or intraspecific, respectively (Flint and Doane, 2009). Allelochemicals are subdivided into two groups, allomones and kairomones. The former is favourable to the receiver but not the emitter. The latter are significant to individuals of a species different from the source species and are favourable to both emitter and receiver (synomones) (Sonenshine, 2004, Flint and Doane, 2009). Pheromones, in contrast, are used intraspecifically and may be further classified based on the interaction mediated, such as alarm, aggregation, or sex pheromones (Sonenshine, 1985, 2004, Flint and Doane, 2009).

Ladybirds are common intraguild predators and are usually characterised by their bright colour and the ability to secrete a distasteful yellow droplet of haemolymph from their joints when disturbed. These droplets are bitter and repellent to many insects and birds (Marples, 1993). Oviposition avoidance in response to these semiochemicals has been described in most aphidophagous coccinellid predators (Hemptinne and Dixon, 2000, Yasuda et al., 2000). The effect of these semiochemicals is concentration-dependent and often associated with larval track of hetero- or conspecifics, or both (Yasuda et al., 2000). The presence of hetero- or conspecific faeces may also trigger a similar response (Doumbia et al., 1998). The nature and effects of these substances vary both intra- and interspecifically. For example, the larval tracks of coccinellids are chloroform soluble only, but the larval tracks of lacewings (Neuroptera: Chrysopidae and Hemerobiidae) are soluble in both chloroform and water (Doumbia et al., 1998). The semiochemicals present in ladybird larval tracks contain alkanes that deter females from laying eggs (Yasuda et al., 2000, Hemptinne et al., 2001). In contrast, it has been noted that the presence of semiochemicals did not affect the searching time spent by female beetles (Ruzicka, 2001).

Female coccinellids usually refrain from laying eggs in areas where the population density of prey is low (Dixon, 1959) or in the existence of conspecific larvae (Hemptinne et al., 1992), hetero-specific tracks (Oliver et al., 2006) or conspecific larval tracks (Doumbia et al., 1998). The ability to discriminate between simultaneously provided clean paper strips and ones contaminated by oviposition-deterring larval tracks has been reported in at least two aphidophagous ladybird species, *Cycloneda limbifer* **(**Casey, 1899) and *Ceratomegilla undecimnotata* (Schneider, 1792) (Coleoptera: Coccinelliade) (Ruzicka, 2003). The avoidance of oviposition in the presence of conspecific larvae is an adaptive behaviour towards reducing the risk of egg predation, but may vary according to the larval track species (Ruzicka, 2001). The deterrent semiochemicals consist of a mixture of alkanes, similar to those found on the elytra of an adult or on the surface of eggs of some species (Hemptinne et al., 2000, Pervez and Gupta, 2004) or to the adhesion substances present on the tarsal segment (Geiselhardt et al., 2009). Females of *C. limbifer and C. undecimnotata* have been shown to use tactile chemoreceptors present on their maxillary palpi to detect oviposition deterrent tracks of conspecific larvae (Ruzicka, 2003).

Alkanes are organic compounds that are common in nature, such as those covering the surface of plants used to stimulate the oviposition of specialist herbivores (Ferguson and Mudd, 1997). The chemical nature of these alkanes was first determined in the ladybird *Adalia bipunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) (Hemptinne et al., 2001). These alkanes are usually composed of saturated hydrocarbon chains that are stable and non-reactive in nature - they have little biological activity and cannot be oxidised easily (Hemptinne et al., 2001, Sloggett and Davis, 2010). In fact, the way in which these chemicals intervene with recognition behaviours has not been fully identified (Hemptinne et al., 2001).

Ladybird faecal pellets also contain such semiochemicals (Doumbia et al., 1998). Insect faeces contain volatile info-chemicals derived from their diet (Agelopoulos et al., 1995). Faeces in insects are usually used as a defence mechanism, as a chemical deterrent compound or as a physical barrier in a chemically mediated defense against predators (known as a faecal shield), such as in the tortoise beetle *Chelymorpha reimoseri* (Spaeth, 1928) (Coleoptera: Chrysomelidae) (Bottcher et al., 2009). Volatile faeces from herbivorous insects are known to prevent predators and conspecifics from oviposition close to their habitat (Hilker and Klein, 1989). In addition, faeces of carnivorous insects contain protein metabolites that are recognised to be effective oviposition deterrents (Grostal and Dicke, 2000). *Harmonia axyridis* (Pallas, 1773) is known to be a strong intraguild predator (Alhmedi et al., 2010) and faecal cues of this predator were found to influence their risk assessment regarding predation by heterospecifics, as well as by conspecifics (Agarwala et al., 2003). Faecal cues of *H.*

axyridis are capable of lessening the rate of feeding behaviour and oviposition of both conspecific and heterospecific ladybirds (Agarwala et al., 2003).

Ladybird predators usually orientate their movement towards their prey using olfactory cues that lead them to areas of high prey density (Seagraves, 2009). The sense of balance between both olfactory and visual cues influences the oviposition decisions of the ladybird predators. *Harmonia axyridis* uses both olfactory and visual cues to trace its prey. In contrast, other coccinellids, such as *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) and *Pharoscymnus numidicus* (Pic, 1900) (Coleoptera: Biphyllidae), depend on random searching to locate their prey (Banks, 1957, Kehat, 1968, Obata, 1986). For example, the foraging behaviour of adult *C. septempunctata* starts first with extensive random searching followed by intensive pattern of searching, assisted to a degree by volatiles produced by aphids (Al-Abassi et al., 2000).

Alarm pheromones, such as the aphid alarm pheromone (*E*)-β-farnesene (EβF) and the plant volatile pheromones (-)-β-caryophyllene, also approximate the cues that attract some species of coccinellids (Seagraves, 2009). It has been noted that these two semiochemicals - (*E*)-β-farnesene (EβF) and (-)-β-caryophyllene, were able to stimulate antennal activity in both sexes of *H. axyridis* (Verheggen et al., 2007). The antennae of *H. axyridis* possess specific neuronal receptors that allow the perception behaviour to be more elaborate than that of other ladybirds *(*Al-Abassi et al., 2000, Hemptinne et al., 2000). Their receptors have a high specificity for (-)-β-caryophyllene (Al-Abassi et al., 2000).

Antennae are important sensory organs in insects (Srivastava and Omkar, 2003). They play an important role in courtship behaviour and in sex differentiation (Khadka et al., 2011).

Little information, however, is available regarding this sensory organ in coccinellids (Srivastava and Omkar, 2003). Studies on morphological features of the antennae in *C. septempunctata* revealed great variation between sexes (Faucheux, 1985). Srivastava and Omkar (2003) showed, however, that the sensory apparatus of the antennae of *C. septempunctata* did not vary greatly between sexes with respect to their gross morphology, as both consist of eleven annuli. They found that most of the variation instead existed in their ultra-structure. Similar observations were also reported by Hemptinne and Dixon (2000) for the ladybird *A. bipunctata.* Even though some species of ladybirds often orientate their movement using their olfactory organs (Dixon, 2000, Verheggen et al., 2007, Seagraves, 2009), they also exploit pheromone production with great versatility. This behaviour depends on the species and environmental cues (Hemptinne and Dixon, 2000). An additional feature is the *sensillae basiconica,* located on the shaft of the antennae of coccinellids. This sense structure is regarded as the most generalist sensillae, exhibiting a tactile chemoreceptive role compared with others (Broeckling and Salom, 2003ab).

A fundamental concern in community ecology is how a species of prey is sustained in assemblages with its predators (Hunter and Price, 1992). The answer to this question has wider consequences in terms of understanding what will impact on community composition and species richness. Many introduced species are involved in intraguild predation, in which they compete for shared resources with native species. Previous studies have predominantly focused on the effects of larval tracks on oviposition, competitive interference and survival, but the faecal deposit response, associated with avoidance behaviour, towards these tracks is less well studied. Accordingly, the present study aimed to investigate the presence of biotic factors, such as faecal cues, of conspecific and heterospecific larvae and adults, as well as the physical presence of conspecific and heterospecific adults on the evading behaviour of adults of *H. axyridis.* The influence of the presence of tracks of larvae and adults of the two-spot ladybird *A. bipunctata*, as well as adult tracks of *C. septempunctata* were well considered in this study. In addition, the physical presence of con- and heterospecific adults of *C. septempunctata* was also examined. Olfactory organs allow insects to forage optimally by more efficiently finding and using favourable food sources. Even though olfactory organs has been studied in honey bees, fruit flies and parasitoids (Vet and Dicke, 1992, Dukas, 2008ab) as well as insect herbivores and parasitoids, there is less attention on the polyphagous predators (Glinwood et al., 2011). The significance of the antennae was investigated subsequent to antennal amputation and evaluation of their influences on life-history traits was performed. The study aimed to validate that, in *H. axyridis*, prey location is not performed by random searching; instead, their sensory cues are definitely involved in adjusting searching and foraging behaviour. The results could demonstrate how this predator has been capable of successfully avoiding the risk of predation and was able to survive in competitive habitats, starting actually to dominate many intraguild assemblages worldwide. Hall (2011) noted that invasive species that consume resources more than native competitors may be able to spread and establish where invasion by pure competitors would fail, and that invasion speed increases with increasing levels of intraguild predation. Notably, the ladybird *H. axyridis* has been considered the most invasive species; therefore, using the above factors to predict the invasion speed of already introduced intraguild predator is quite challenging, but indispensable for quantifying the adverse impacts on community structure and assists for developing an applicable approaches for controlling and reducing the risk of persistent invasion. In addition, knowing the association between behavioural and other aspects of life-history traits in this ladybird, such as body weight and longevity, are essential, so that they may be incorporated in future comparative researches for better understanding of the evolution of life-history trait.

7.2 Materials and methods

Adults and final larval instars of *H. axyridis* and *A. bipunctata* were collected from the laboratory culture maintained under a controlled temperature of 20±1℃, 70-75% RH, and a photoperiod of L16:D8 under fluorescent lamps. Both adults and larvae were provided daily with 16 live adults of the pea aphid, *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae) that had been maintained on broad bean plants *Vicia faba* (Linnaeus, 1753) (Fabales: Fabaceae, variety Aquadulce Claudia). Adults of the seven-spot ladybird, *C. septempunctata,* were collected from naturally infested fields in Silwood Park, South-east England, United Kingdom (National Grid References 41/944691). All adults were sexed and weighed prior to the onset of this study and were all maintained under the above conditions.

7.2.1 Experiment procedures

7.2.1.1 Part I

Tracks were prepared by placing 4mm Whatman filter paper in the bottom of 9cm Petri-dishes and keeping 10 individuals of two-day-old adults of *H. axyridis* and 10 individuals of 5th instar larvae in them each separately for 24 hours, together with 2cm² moistened tissue paper with 10% glucose solution and 16 live adults of pea aphid. Subsequently, larvae were removed and 12 adults of *H. axyridis* (six males and six females) were kept individually with the filter papers contaminated with larval tracks. For the control, eight males and another eight females were used - each one was placed in a clean Petri-dish. Another 20 adults of *H. axyridis* (10 males and 10 females) were each placed in a Petri-dish enclosing filter paper contaminated with adult tracks, after removing the adult. The control was prepared by keeping 16 adults of *H. axyridis* (eight males and eight females) each in an uncontaminated clean Petri-dish. All the dishes were provided with 16 adults of pea aphid and moistened tissue with glucose solution then left for 24 hours, under similar conditions as stated above.

The effect of the larval and adult tracks collected from the *A. bipunctata* was performed corresponding to the above protocol, the number of *H. axyridis* adults assigned for this experiment was 12 adults of unspecified sex used for inspecting the adult tracks; a similar number was used for the larval tracks as well as the control. To detect the influence of the adult tracks collected from *C. septempuncta* on *H. axyridis* adults, 18 adults (10 males and eight females) of the latter species were used for the treatment and another different 22 adults (12 males and 10 females) were assigned for the control. As above, the lady beetles of the control were kept individually in a clean Petri-dish. Furthermore, the influence of the physical presence of the adult of *C. septempuncta* in a Petri-dish containing an adult of *H. axyridis* was also investigated. This was performed by placing an adult of *C. septempuncta* covered with an inverted smaller 3.5cm Petri-dish in the centre of the 9cm Petri-dish. At the exterior of the smaller Petri-dish an adult of *H. axyridis* was placed after being weighed and sexed. The latter was supplied with food as above and left for 24 hours. For that treatment, twelve adults with unspecified sex of *H. axyridis* were used and another 12 adults as the control were each kept in a clean Petri-dish. In all treatments, the number of deposits of both kinds were counted, with the black being the normal kind of faeces and the yellow droplets being secreted only as a distress response.

7.2.1.2 Part II

Forty-eight freshly emerged adults of *H. axyridis* were sexed and weighed. Twelve males and 12 females had their antennae amputated fully. After that, each adult was kept individually in a 9cm Petri-dish and provided with daily food as above. The presented adult aphids were weighed daily in order to control the biomass distribution. A set of 12 males and another set of 12 females were used as controls. The adults of the control had their antennae intact.

7.3 Data analysis

All data were tested for normality prior to applying parametric analysis. Those that failed were transformed. One-way analysis of the variance ANOVA or general linear model (GLM) was performed to analyse the variation between type of secretion and treatment or sex. Student t. test or Wilcoxon rank sum test were used to compare the means of different types of secretion - yellow and black. All statistical analyses were carried out using the statistical programme ‘R’ version 2.8.1 (Ihaka and Gentleman, 1996).

7.4 Results

7.4.1 The influence of conspecific larval track

The response of *H. axyridis* towards different treatments and their controls are presented in Table 7.1. There was no significant difference in either yellow droplet secretion (F=2.5, P=0.14) or in the number of black faecal deposits (F=0.2, P=0.65) between the treatment and respective control when the sex of the ladybirds was disregarded. The number of yellow droplets did not differ between males and females (F=0.023, P=0.88) or vary from the number of black faeces (F=1.011, P=0.32). There was no response towards the presence of the larval tracks when comparing the males of the treatment with the control, either in terms of yellow count (F=0.48, P=0.51) or of black faecal count (F=0.26, P=0.62). Similar results were acquired when comparing the number of black faeces deposited in the presence of larval tracks by the females of the treatment with the females of the control (F=0.0007, P=1). Females in the control treatment produced significantly more yellow droplets than females that were exposed to contaminated filter paper with larval tracks (F= 1.656e+32, P<0.0001).

Table 7.1. The responses of *H. axyridis* adults towards the presence of heterospecific and conspecific larval and adult tracks and adult of conspecifics as well as heterospecifics.

Treatment		Sex of *Harmonia* adults	Yellow extract No	Black faeces No	Body weight (mg)
Conspecific larval track	Treatment	Male(n=6)	1.33±0.23	1.5±0.4	35.86±2.6
		Female(n=6)	1	2±0.4	40.3±4.3
	Control	Male(n=8)	2.2±0.63	1.75±0.31	30.24±1.7
		Female(n=8)	2	2±0.28	29.57±2
Conspecific adult tracks	Treatment	Male(n=10)	2.5±0.17	1.6±0.25	28.5±0.9
		Female(n=10)	1.12±0.11	1.14±0.11	26.72±1.1
	Control	Male(n=8)	2.2±0.63	1.75±0.31	30.24±1.7
		Female(n=8)	2	2±0.28	29.57±2
Conspecific adult	Treatment	n=6	1.6±0.36	1.5±0.23	26.21±1.3
	Control	n=6	1	1.5±0.28	29.4±11.8
Heterospecific adult track of *A. bipunctata*	Treatment	n=12	1.66 ±0.33	4 ±0.37	26.61 ±1.7
	Control	n=12	1	2.09 ±2	32.11 ±1.9
Heterospecific larval track of *A.bipunctata*	Treatment	n=12	2.66 ± 0.54	1.5 ±0.15	31.28 ±2.7
	Control	n=12	1	2.09 ±2	32.11 ±1.9
Heterospecific adult track of *C. septempunctata*	Treatment	Male(n=10	1	3.9±1	30.61±1.8
		Female(n=8)	2±0.25	4.66±1.13	29.28±2.95
	Control	Male(n=12)	3±0.45	2.75±0.46	30.14±2.5
		Female(n=10)	1.6±0.17	4±0.77	30.7±1.7
Heterospecific adult of *C. septempunctata*	Treatment	n=12	2.1±0.46	3±0.28	28.21±2
	Control	n=12	2.57±0.4	4.54±1.11	28.43±2

7.4.2 The influence of conspecific adult tracks and adults

When the sex of the beetles was excluded, the production of both yellow secretion and black faeces did not show significant differences from the control in response to the adult tracks - (F=0.52, P=0.47) and (F=2.84, P=0.1) respectively. In contrast, considering the sex as a categorical variable had a great influence. For example, the number of yellow droplets secreted by control females was significantly greater than that of the treatment females (F=17.18, P<0.01). Similar results were gained for the black faecal number, in which control females produced a significantly greater number than the treatment females (F=6.65, P<0.05). Males did not show a significant difference in terms of black faecal count (F=0.016, P=1) or in yellow droplet secretion (F=1.19, P=0.3) when both secretions of the treatments were compared with those of the control. The presence of a conspecific adult did not in any way affect the production of either yellow droplets (F=0.85, P=0.39) or black faeces (F= 9.997e-31, P=1) after comparing both treatments with the control.

7.4.3 The influence of heterospecific larval and adult tracks of *A. bipunctata*

Adults of *H. axyridis* showed variable responses towards the larval tracks of *A. bipunctata*. The number of yellow droplets of the treatment varied significantly from the control (F=6.35, P<0.05), but the black faecal count of the treatment did not (F=3, P=0.1). Similarly, adults of *H. axyridis* produced a higher number of black faeces compared with the control in response to adult tracks (F=17.18, P<0 .0001). In contrast, the count of yellow secretion did not differ significantly between the control and the treatment in which the adults were exposed to adult tracks (F=2, P=0.18).

7.4.4 The influence of hetero-specific tracks and the presence of adults of *C. septempunctata*

The presence of the adults of *C. septempunctata* had no influence on the behaviour of *H. axyridis* and both yellow secretion and black faeces did not vary in comparison with the control - (F=2, P=0.18) and (F=0.14, P=0.7), respectively. Males of *H. axyridis,* on the other hand, responded to the presence of the heterospecific adult tracks by secreting significantly more yellow droplets compared with the control (F=14, 04, P<0.001). The number of the black faeces, however, was the same as the control (F=0.65, P=0.42). Females did not respond at all to the presence of the *C. septempunctata* adult tracks, the number of yellow droplets and black faeces did not differ from the control (F=0.8, P=0.4) and (F=0.08, P=0.78) respectively.

7.4.5 The influence of the amputated antennae on longevity of adults *H. axyridis*

The mean longevity of females that had had their antennae amputated was 25.16±3.4 days (min. =8 and max. =44), and for males with amputated antennae was 35.91±3.33 days (min. =14 and max. =51). For males in the control, the mean longevity was 67±1.44 days (min. =59 and max. =74) and 70.5±1.6 days (min. =62 and max. =77) for the females of the control. Females with amputated antennae had significantly lower longevity compared with normal females in the control (W=144, P<0.0001). Similarly, males with amputated antennae had lower longevity relative to their control (W=144, P<0.0001). The absence of antennae had a strong influence on females compared with males, as females showed a significant lower longevity compared with their respective males that had had their antennae amputated (t=-2.23, P<0.05).

7.4.6 The influence of antennae amputation on body weight

Females with no antennae died sooner compared with their control and compared with males with amputated antennae. The effect of the absence of both antennae on body weight of females and males was measured in 26 successive trials, from the initial weight before amputating the antennae until death. The results are presented in Fig. 7.1 and 7.2. Initially, mean body weight in females increased slightly after amputating the antenna, followed by a gradual decline that led to their death (Fig. 7.1). The mean weight of females belonging to the treatment recorded straight after their death did not differ significantly from the control (t=1.64, P=0.1173). This could be related to the low number of individuals that had survived and were incorporated in calculating that mean. For the control, females did not show any evidence of variability in their mean body weight during their lifespan, only at late stages when they were about to die. The lack of variability in weight was due to the controlled feeding process, in which the aphids were provided daily with 16 adults and their weight ranged between 1.1-1.37mg. In contrast, males with amputated antennae exhibited a slight increase in body weight, attaining a peak of 27±2mg (Fig. 7.1). The weight at that peak did not show a significant difference when compared with the control (t=-1.37, P=0.1836), which had a mean of 23.8±1mg.

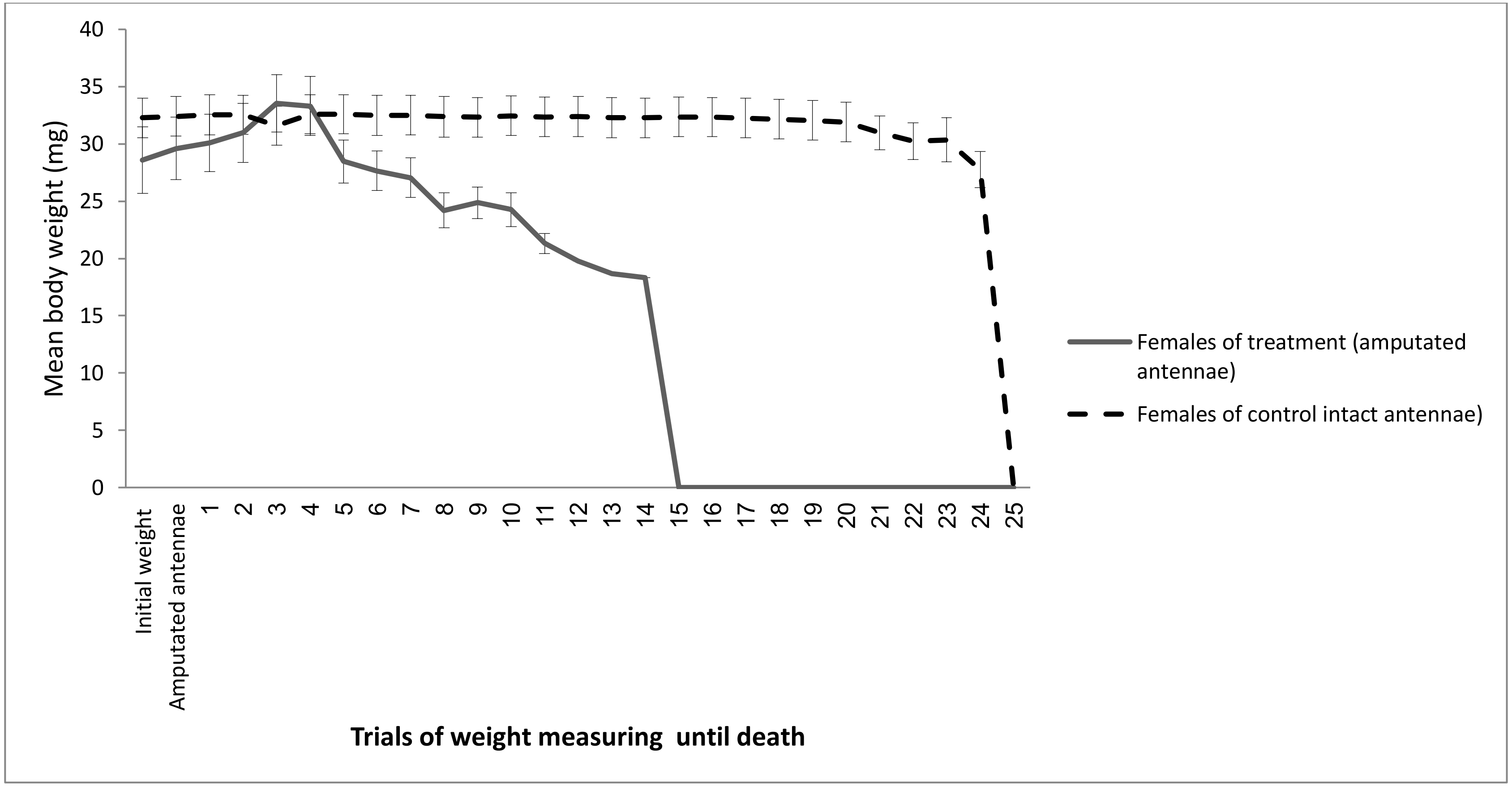

Fig.7.1. Changes in mean body weight calculated over 25 trails for both females having their antennae amputated and intact females (mean±SE, n=12 for treatment & n=12 for control).

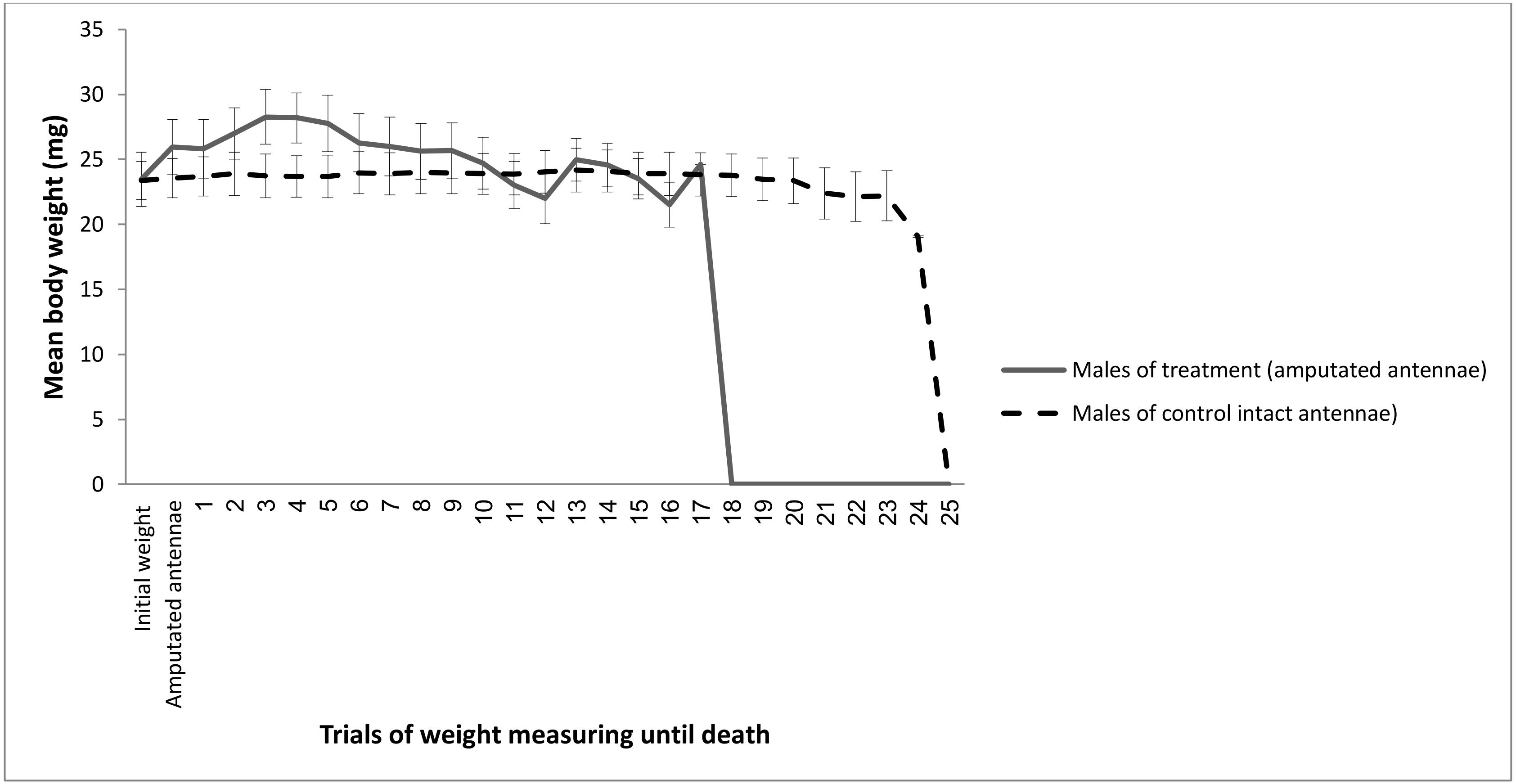

Fig.7.2. Changes in mean body weight calculated over 25 trails for both males having their antennae amputated and intact males (mean±SE, n=12 for treatment & n=12 for control).

7.5 Discussion

This study has shown that adults of *H. axyridis* exhibited variable responses towards conspecific and heterospecific species, in some cases depending on the sex of the adults. The presence of conspecific larval tracks had a negative influence on the behavioural response of *H. axyridis* adults, during which they did not discharge the yellow secretion throughout the investigated period. If this negative response happens in the field, it would cause the conspecific larvae to persist in the area and to be cannibalised by others, due to their inability to detect the existence of conspecifics. In contrast, it was reported that the scavenging larvae may use either the conspecific adult tracks or larval tracks for avoiding competition and predation (Lucas et al., 1998, Meisner et al., 2011a). Females of the control treatment, however, reacted first by secreting significantly more yellow droplets in order to mark their territory. Females belonging to the treatment had a different response, because the contaminated paper with the larval track was placed first, then the adult was hosted in the Petri-dish. The cautious defensive response displayed by the control females once they had been placed in the Petri-dishes reflected the anticipated field competition. In addition, it was proposed that larger ladybirds such as *H. axyridis* or *C. septempunctata* often try to evade intraspecific competitions, but not interspecific ones, due to the higher likelihood of them prevailing over small species (Agarwala, 2003).

Doumbia et al. (1998) and Yasuda et al. (2000) stated that females usually refrain oviposition and evade encountering conspecific larvae by responding negatively to their oviposition-deterring pheromone. This could explain why females in this study secreted a significantly higher number of yellow droplets when they were hosted with conspecific adult tracks. Nevertheless, those females additionally intensified their response by depositing a significant number of black faeces. These responses were not displayed by adult males, suggesting that males of *H. axyridis* are more competitive and hostile than females, due to their smaller body size.

The current study revealed that females of *H. axyridis* did not avoid filter papers contaminated with *C. septempunctata* adult tracks. The lack of response reveals the degree of the aggressiveness and hostility between the two species, with *H. axyridis* being the more aggressive species. *Harmonia axyridis* is a very voracious and violent predator and has been cited as a reason for the displacement of many native competitors (Sato and Dixon, 2004). It was noted that although *H. axyridis* from time to time preys on *C. septempunctata*, the latter does not pose a significant threat to the former (Yasuda et al., 2001). This could explain the lack of response towards heterospecific adult tracks. In Japan, *H. axyridis* and *C. septempunctata* generally coexist and possess an analogous number of juvenile developmental stages (Sato and Dixon, 2004). The most logical explanation is that *H. axyridis* has smaller larvae compared with *C. spetempunctata* when it coexists in the same habitat. This could be responsible for the defensive chemicals possessed by *H. axyridis* against predation by other ladybirds, such as *C. septempunctata* (Sato and Dixon, 2004). A previous study has shown that adults of *H. axyridis* are rarely preyed on and that this could only be performed by conspecific larvae (Hough-Goldstein et al., 1996). In addition, Agarwala et al. (2003) noticed that the eggs and larvae of *H. axyridis* are protected by deterrent chemicals, preventing their predation by larvae and adults of heterospecific ladybird *Propylea japonica* (Thunberg, 1780) (Coleoptera: Coccinellidae). This backs the existence of the defensive chemicals on larvae and eggs of *H. axyridis*, making them unpalatable to other coccinellids (Hough-Goldstein et al., 1996). Furthermore, when both males of *H. axyridis* and *C. septempunctata* coexist, males of former display less aggressive behaviour compared with the latter (Agarwala et al., 2003, Meisner et al., 2011ab). Nevertheless, since *C. septempunctata* is the only species that is larger than the Harlequin in Britain (Meisner et al., 2011ab), this might give a reasonable explanation for the unresponsive behaviour by the males in this study.

Generally, cannibalism and intraguild predation depend on the relative size and the developmental stage of the prey and predators (Hemptinne et al., 2011). Coccinellids are common within aphidophagous guilds and small species are more likely to be preyed upon by larger species (Lucas et al., 1998). In species of ladybirds that have overlapping habitat preferences, small species might be protected chemically from predation, A. *bipunctata* was found to be toxic to larger species such as *C. septempunctata and H. axyridis* (Meisner et al., 2011ab). All *A. bipunctata*, all the developmental stages of juveniles of *A. bipunctata*, are protected chemically (Karlsson and Losman, 1972, Holloway et al., 1993, Lognay et al., 1996). Regardless, it has been found that *A. bipunctata* eggs are not well protected chemically from predation by *H. axyridis* (Sato and Dixon, 2004). This was also revealed by the present study, in which *H. axyridis* exhibited a positive response towards both larval and adult tracks of *A. bipunctata.* In addition, in terms of body size, it is implausible that *H. axyridis* would be preyed upon by *A. bipunctata* - the percentage of survival in the latter was extremely low when it had been reared with the ladybeetles *C. septempunctata* and *H. axyridis* (Brown, 2003).

The results revealed that there was no interface between conspecific adults of *H. axyridis* of either sex. In some ways, the lack of intraspecific competition was not on the whole surprising, but did conflict, however, with the earlier results recorded from the conspecific larval track test. One possible reason is that *H. axyridis* is regarded as a territorial and defensive species that could mark its territory by discharging its repellent tracks as soon as it lands on a new site. Since, during the course of the test, both adults and their tracks were kept physically isolated, there was no question of one intruding on the other's territory. This is not expected to be same when evaluating the oviposition behaviour response of *H. axyridis* ladybird when in coexistence with conspecific adults.

It has been noted that females of *H. axyridis* consumed significantly less prey and consequently laid fewer clutches of eggs in response to the presence of faeces of conspecifics, compared with the existence of the faecal cues of heterospecific species, such as the predator ladybird *Propylea japonica* (Thunberg, 1780) ((Coleoptera: Coccinellidae) (Agarwala et al., 2003). In contrast, females of aphidophagous coccinellids *C. limbifer* laid significantly larger batches of eggs on paper strips contaminated with conspecific larval tracks, when compared with clean papers (Ruzicka, 2003). The opposing influences of conspecific larval tracks on the oviposition behaviour must be regarded, but further investigation is required, particularly in relation to *H. axyridis*.

Tactile stimuli receptors present on the antennal shaft, as well as visual stimuli, are important prompts in the presence of natural enemies (Kunert and Weisser, 2005). This study showed that amputating the antennae appeared to have a greater effect on adult females than on males. It is well known that females are in charge for prey quality assessment and scavenging for suitable oviposition sites (Dixon, 2000). The predatory arthropods, particularly the ladybird beetles use chemical information in host foraging in the form of volatile cues released by plants in response to feeding by their herbivore prey (Heil, 2008, Dicke, 2009) therefore, they learn how to associate odours with the prey occurrence (Dukas, 2008ab, Glinwood et al., 2011). In addition, the volatile odours blends may notify the foraging predators about the identity and quality of the host plant and herbivore, since each varies widely (Leitner et al., 2005) even between the same prey on different genotypes of the same plant species (Degen et al., 2004). This study showed that the lack of the antennae significantly hindered the metabolic activity. This reaction tended to reduce longevity. The influence of amputating the antennae was also investigated by Kunert and Weisser (2005), but was performed on different species. In the Kunert and Weisser (2005) study, the antennae and the maxillary palpi were ablated in females of *C. limbifer and C. undecimnotata*; as a result, those females failed to discriminate between clean and contaminated papers with larval tracks and laid significantly larger clutch sizes on both types of papers. Thus, the response of not having the sense organ, the antennae, has been found to vary between insect species, as shown above. Therefore, having a significant decrease in the longevity after amputating the antennae in *H. axyridis* would be the only resort in order to avoid unpredictable circumstances. In addition, it was noted that an odour cue is temporally paired with a conditional stimulus such as feeding or contact with the prey or its products (Papaj and Prokopy, 1989, Vet and Dicke, 1992). For a predator that often forages in a range of resources varying in quality and quantity temporally and spatially, the olfactory associative learning of odour cues may intensify the efficiency with which the most current favourable prey is found and exploited (Glinwood et al., 2011).

The effect of the absence of this sensory organ on males was not unanticipated, as it had been noted previously that the pattern of field activity of males is completely different from that of females (Hemptinne, 1996). In addition, it has been reported that the degree of the antennal sensitivity in females of *H. axyridis* species is greater than that of males. This suggests further sexual dimorphism in antennal anatomy (i.e., types of sensory receptors) as well as physiology (i.e., interface with external cues and stimuli conduction) (Verheggen et al., 2007). To further validate the present study's findings, Verheggen et al. (2007) found that females were highly attracted towards (E)-β-farnesene (EβF), whereas males were more attracted to (-)-β-caryophyllene. Thus, being unable to assess the surroundings, despite the presence of food and other senses (including visual and tactile stimuli), demonstrates the high specificity of antennae in females of *H. axyridis*. In addition, it was noted that since females are the ones that choose favourable oviposition sites for emerged larvae, the hypothesis that "odour learning ability" differs between the sexes such as in *C. septempunctata* (Glinwood, et al., 2011). In contrast, Hodek et al. (2012) recently noted the frequent absence of the aphids prey and the need to find an alternative food evidently worked against the evolution of the gustatory senses in the aphidophagous and other predaceous ladybirds. This indicates the inability to discriminate between toxic and suitable prey. That is why the non-contact sensory organs, both visual and olfactory appeared to be the most important organs and have opened the research towards which is called "the learn response in coccinellids" as indicated above as the olfactory associative learning of odour cues (Zhu and Park, 2005, Mondor and Warren, 2000). In this study, it was noticed that after antennal removal completely from the base, the ladybeetles lost control on movement and started to move from side to side in a horizontal plane without turning their heads or bodies in addition to the deviated from forward movement. Other of the lady beetles moved around themselves in circular motions, avoided forward movement after antennal ablation. Similarly, the cricket, *Cryllus bimaculatus* (De Geer, 1773) (Orthroptera: Gryllidae) uses sensory clues from the antennae and cerci to sense the roll angle around the body long axis relative to an upright posture (Horn and Bischof, 1983, Horn and foller, 1985). Thus, when the antennae were ablated at the scape, it elicited head counter- roll. Another example, is the stick insect, *Carausius morosus* (Sinety, 1901) (Diapheromeridae: Phasmatodea) that showed negative geotaxis an orientation behaviour depending partially on the information collected by the antennae (Dean, 1991, Simpson, 2005). The above emphasized the importance of the antennae in self-orientation and forward movement (Dean, 1991, Simpson, 2005).

It could be concluded from the above results that the possession of effective physical and chemical defence strategies could be added to the functional traits that make *H. axyridis* such a strong intraguild predator. The behavioural response of *H. axyridis* towards others' tracks may play an important role in being a strong competitor dominating intraguild interactions. Nevertheless, the importance of the antenna as a sensory organ was shown, through its role in influencing several activities, such as self-orientation, and prey allocation, prey selection and ability of feeding. The antennae might influence a female’s assessment decision relative to an unsecured environment. Notably, the lack of antennae made the ladybeetle lose its ability to discriminate between good and bad prey. This led to a significant reduction in feeding ability accompanied by a lowering of the metabolic rate. As a final consequence, the body failed to maintain itself and to support the on-going physiological processes, resulting in rapid death. Therefore, there is a need for a better understanding of the behavioural mechanisms and avoidance responses, such as the one presented in this study, in order to highlight better the intraguild interaction in the presence of this ladybird. Further studies are required to investigate the contaminated tracks of con- and heterospecifics on the oviposition behaviour of *H. axyridis.* The effect of partial and complete antennae amputation, as well as the maxillary palpi, on mating and oviposition behaviours will be considered in future studies. It could be concluded that *H. axyridis* ladybirds had a sexual dimorphism in antennae, with differential function and morphology. This fact is important in going some way towards explaining the on-going success of invasion and establishment of *H. axyridis*, ascribed to the highly sensitive sense organs, elaborate avoidance tactics towards other con- and heterospecific species (in order to increase survival rate) and aggressive completive ability for resources. Hall (2011) noted that if the benefits from consuming competitors prevail over the loss of shared resources to competitors, invasion continues faster than invasion in the absence of competitors.

Chapter 8

Responses of Immature Larval Instars of *Harmonia axyridis* to Short-Term Diet Regimes: A study on Trade-offs

8.1 Introduction

Phenotypic traits may develop through different ontogenetic trajectories that are influenced by an individual's previous experimental environment (Taborsky, 2006). A wide range of responses to fluctuations in growth conditions or environmental conditions have been recognised. Growth conditions experienced during early development might thereby have pronounced costs on life-history traits, manifested in reduced body size at maturity as well as delayed maturation, a decrease in lifetime fecundity and a pattern of aging (Metcalfe and Monaghan, 2001, Day and Rowe, 2002, Taborsky, 2006). In some situations, any delay in maturation elevates pre-reproductive mortality and reduces individual fitness (Stearns, 1992, Blanckenhorn, 2000). In addition, poor environments could lead to the lowering of energy reserves, resulting in reduced competitive capability (Ali et al., 2003) and mating efficiency (Sokolovska, 2000). A deficiency of nutrients during early development is principally important in this context, because this can have unexpected long-term consequences besides the more instant and obvious effects on early growth rates (Metcalfe and Monaghan, 2001).

Nutritional conditions during key periods of development are essential in determining the subsequent life-history trajectory of an organism (Metcalfe and Monaghan, 2001). Food is heterogeneous in space and time and is the chief requirement for growth and reproduction in an organism (Agarwala and Bhowmik, 2011). This is true for coccinellids that frequently forage in an unstable aphid population (Honek, 1991). Dixon et al. (1993) reported that the potential decline in aphid quality or quantity triggered wing development and reduced the size of gonads in some cases. In agreement, Day and Rowe (2002) noted that resource restriction during individual development might lead to a number of changes in life-history traits, regarded as an adaptive phenotype response. Nonetheless, experiencing sub-optimal growth conditions often results in alteration in the relative allocation of resources to all body parts, causing dramatic changes in the relationships between structural parts and the overall body size (Boggs and Freeman, 2005, Dmitriew and Rowe, 2005, Stoks et al., 2006). For instance, when damselflies experienced low food quantity, an extra growth in structural size occurred, affecting the degree of allometry with overall body size (Strobbe and Stoks, 2004, Dmitriew and Rowe, 2005). Negative environmental influences can also be much more subtle and challenging to recognise, mainly if they do not appear until later in life. For example, very small variations in the prenatal hormonal environment can have a substantial effect on adult reproductive behaviour (Clark and Galef, 1995, Qvarnström and Price, 2001).

It has been reported that when an insect’s population experienced food deprivation, immature stages tended to reduce maintenance costs and invest more in dispersal phenotypes (Blanckenhorn, 2000, Reim et al., 2006). The investment in dispersal traits might come at a cost to resource allocation for reproduction (Braendle et al., 2006). This in particular reflects the way in which insects might adapt to an anticipated variable environment (Boggs and Freeman, 2005, Stoks et al., 2006). Despite the above, in some cases, the response might be entirely different, particularly if the conditions experienced are predictable and likely to be encountered by emerged adults (Boggs and Freeman, 2005, Stoks et al., 2006). In a similar context, Gotthard and Nylin (1995) claimed that most organisms are able to complete their development and become fully matured as growth conditions improve. Metcalfe and

Monaghan (2001), however, stressed that even if an organism seems to improve from the food scarcity when resources subsequently restore, nutritional insufficiencies experienced during early development can have intensive, pervasive and long-lasting effects on the adult individual, and even on its future offspring.

Trade-offs play a crucial role in the evolution and development of life-history traits (Charnov and Krebs, 1974, Bell, 1980). Usually, trade-offs exist when an advantageous change in one trait accompanies a new change in other traits (Stearns, 1989). In light of the costs arising from food depletion, one might expect that accelerated growth might be selected in terms of a period of rapid growth, mainly when succeeding conditions are re-established (Metcalfe and Monaghan, 2001). Regardless, accelerated or compensatory growth may result in immediate or long-term fitness costs (Ali et al., 2003) that might spread across generations (Huck et al., 1987). Compensatory growth is considered as an adaptive mechanism, defined as "[a] phase of accelerated growth when favourable conditions are restored after a period of growth depression" (Ali et al., 2003). This kind of growth could diminish the costs and result in complete or partial compensation, depending on the timing, magnitude and duration of the growth restriction period (Mangel and Munch, 2005, Robby et al., 2006). In the case of partial or incomplete compensation, it can be difficult to delineate whether the downstream fitness cost is an extra cost or a penalty of alterations in body size (Dmitriew and Rowe, 2007). Metcalfe and Monaghan (2001) reported that immediate costs as a result of compensatory growth are not always apparent, but they may include a negative effect on future offspring quality and fitness (Block and Robby, 2005, Stoks et al., 2006). The degree and intensity of costs are chiefly governed by the pattern of growth. Gotthard (2001) noticed that individuals that grow faster may display a greater weight loss after malnourishment compared with slower developing individuals (Gotthard, 2001). This occurs because the weight of the former is usually accomplished under greater metabolic rates, resulting in a remarkable reduction in energy reserves (Stoks et al., 2006). Furthermore, behavioural, physiological and anatomical expenses might also result from rapid growth, but have been somewhat ignored (Stoks et al., 2006). For example, Gotthard (2000) suggested that there are a variety of behavioural costs, such as the intensification in searching behaviour.

Partridge (1987) commented that the methods used to measure several types of trade-offs have been subject to controversy. There are three levels of approaches implemented to evaluate the trade-offs, costs of a genetic basis, the intermediate (physiological) approach and phenotypic costs (Williams, 1966a b, Calow, 1979). Knowledge of the three levels is highly important in order to understand how each cost works (Stearns, 1989). The impact of environmental fluctuation on the genetic and phenotypic trade-offs is well established (Gebhardt and Stearns, 1988). Nonetheless, not all the trade-offs have received the courtesy relative to their significance, particularly intra-individual trade-offs (Stearns, 1989). Measuring the trade-offs of reproduction has proven to be problematic, because both genetic and phenotypic associations must be carefully recognised in advance before identification of actual costs. Thus, extra consideration must be given to the role of the endocrine system associated with both phenotypic and genetic aspects in determining the basis of several costs.

One vital reason for arthropod success is the presence of specialised ovaries with elaborated vitellogenic apparatus, which enable them to lay high numbers of eggs (Eckelbarger, 1994). Koch (2003) reported that a female of *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinelldae) often produces a clutch of 10-50 eggs every 1-2 days over a period of 1-3 months. In addition, the development of their ovaries shows its specific strategies that allow the ovaries to become capable of coping with food prey fluctuation (Papaj, 2000). Over a wide range of insect taxa, food scarcity plays an essential role in inducing both oosorption and trophic egg production (Perez Mendoza et al., 2004). The production of trophic eggs is considered an important part of a mother's provisional strategy to ensure nutrient availability for developing juveniles (Perry and Roitberg, 2005).

Accelerated or compensatory growth is another potential method used by most ladybirds to overcome stressful situations. Accelerated or compensatory growth is a physiological mechanism regulating body size in response to food limitation (Davidowitz et al., 2003). Compensatory growth is well documented across a wide range of taxa (Metcalfe and Monaghan, 2001, Tammaru et al., 2004, Dmitriew and Rowe, 2007). Accelerated growth is expected to be selected for when conditions are subsequently restored (Stoks et al., 2006). Thereafter, the growth response might either result (as mentioned above) in full or partial compensation for body size or stored resources, depending on the severity and length of growth constraint (Dmitriew and Rowe, 2007). In some cases, behavioural and physiological compensations might complement each other, resulting in complete compensation (House, 1965). Dmitriew and Rowe (2007) stressed that full or nearly full-size compensation appears to be the custom in some insects, such as in the damselfly species (Stoks et al., 2006). Ali et al. (2003) noted that many animals are able to compensate after food stress by accelerating their growth rates above the normal levels of non-stressed individuals.

Growth rates are often kept below the physiological maximum level, signifying that rapid growth can be highly costly (Arendt, 1997, Mangel and Stamps, 2001). Both accelerated growth rate and growth experienced during early developmental stages may lead to a significant rise in mortality rate (Honek, 1993, Sokolovska, 2000).

The cost of compensation may have short- or long-term effects (Arendt and Wilson, 2000, Mangel and Stamps, 2001). Short-term costs may hinder structural development (Arendt and Wilson, 2000), reduce body size and maintenance (Morgan et al., 2000) and increase predation risk, due to elevated foraging activity (Gotthard, 2000). Long-term costs may include reduced longevity. For example, the wild brown trout *Salmo trutta* (Linnaeus, 1758) (Salmoniformes: Salmonidae) had high winter mortality subsequent to a compensatory growth (Johnsson and Bohlin, 2006). Furthermore, all deprived groups of trout *S. trutta* displayed an increase in body length (Johnsson and Bohlin, 2006). Accelerated growth may result in major weight loss (Dmitriew et al., 2009). For example, when the ladybirds *Coleomegilla maculata* (De Geer, 1775) (Coleoptera: Coccinellidae), *Hippodamia convergens* **(**Guérin-Méneville, 1842) (Coleoptera: Coccinellidae) and *H. axyridis* were deprived of food for varying times during the fourth instar, they revealed variable drops in weight and a reduction in adult size (Dmitriew et al., 2009, Phoofolo et al., 2009). Food restriction in the Atlantic salmon, *Salmo salar* (Linnaeus, 1758) (Salmoniformes: Salmonidae) resulted in a smaller sized body, lower rate of maturation and decrease in stored lipids (Morgan and Metcalfe, 2001). Likewise, when larvae of the butterfly *Pararge aegeria* (Linnaeus, 1758) (Lepidoptera: Nymphalidae) were starved of food, they showed a relatively high compensatory growth rate accompanied by excessive weight loss (Gotthard, 1994). The reduction in body weight of starved insects might exist due to the alteration in the proportional allocation of resources such as lipids to precise components of body parts (Zhao and Zera, 2002). It was reported that the stored lipid is often reduced, particularly in individuals characterised by improved early-age fecundity or in juveniles with a high rate of growth (Zera and Harshman, 2001). The above suggest that the evolution of many life-history traits requires interchanging lipid metabolism in order to increase energy reserves. This may, however, have an adverse impact on fitness components, such as reproduction (Zhao and Zera, 2002).

Stevens et al. (1999) noted that exposing larval caddis flies to stress altered the scaling relationships between various body part traits and overall body size. The degree of costs depended on whether the stressed species was of a short- or long-lived type (Stevens et al., 2000). They further demonstrated that the thorax size in the long-lived species was well-preserved compared with the short-lived species. Another study showed that food deprivation during juvenile stages of *H. axyridis* resulted in increased mass and size of head and pronotum and that when reared on low food quantity, they displayed a phenotype favouring dispersal, manifested in increasing the wing area (Dmitriew and Rowe, 2007). Similarly, the wing area was found to be relatively greater in starved adults compared with unstarved ones (Dmitriew et al., 2009). Regardless of the above, the compensatory mechanism remains hard to explain in some cases, such as in the damselfly *Ischnura verticalis* (Say, 1839) (Odonata: Coenagrionidae). This species showed a complete compensation in structural size, but partial compensation in body mass after food deprivation (Dmitriew and Rowe, 2005, 2007). In contrast, a later study performed on the same species did not reveal any effects on the wing length or body size parameters after a period of food restriction (Dmitriew et al., 2009).

The connection between the observed changes in the body allometry and fitness is not always clear when it arises from food stress. Some traits may be positively affected and others negatively influenced (Ali et al., 2003). In conclusion, the degree of compensation exhibited may vary among species (Srygley and Oliveira, 2001) and according to the life stage (Dmitriew and Rowe, 2011). Therefore, stronger compensatory growth will be expected if the final size is entirely associated with fitness and minimal costs (Stevens et al., 2000).

In ladybirds, accelerated or compensatory growth is used by most to eliminate any differences in weight or size of individuals that would suffer starvation later in life (Dmitriew and Rowe, 2007). The connection between the observed costs in life-history traits and related fitness is not always obvious, implying difficulties in assessing them (Ali et al., 2003). Little work has been done on this issue and further studies are required to identify the various patterns of costs related to compensatory growth. Despite the large quantity of data on compensatory responses, the basis of this mechanism remains uncertain (DeBlock et al., 2008), particularly among arthropods (Tammaru et al., 2004).

Harmonia axyridis is an aphidophagous predatory ladybeetle that was introduced to many countries as a biological control for aphid pests, and has since become established as an invasive species worldwide (Koch, 2003). One of the characteristics that has promoted *H. axyridis* over other species and accelerated its invasion and establishment is its highly efficient reproductive performance. This ladybird is highly fecund and usually reveals an adapted provisional behaviour, manifested in producing trophic eggs and eggs with partially developed larvae as extra food for emerging juveniles (Koch, 2003, Evans and Gunther, 2005). *Harmonia axyridis* undergoes strong selection to cope with rapid changes in prey resources and environmental changes during juvenile development and adult growth (Agarwala and Bardhanroy, 1999, Helden et al., 1994).

In this ladybird, most life-history traits (e.g., fecundity and longevity) depend predominantly on diet quantity and quality (Evans and Gunther, 2005). Habitat suitability for reproduction and development are discussed in detail by Osawa (2005). The question remains as to how this ladybird has managed efficiently across heterogeneous habitats and become a successful invader to the detriment of many native species. Therefore, the present study was commenced to directly and indirectly assess the composite traits associated with fitness after a period of early resource restriction in the ladybird *H. axyridis*.

8.2 Aims

The experiment was designed to test the effect of a brief period of food manipulation on the life-history traits of pre- and post-adult periods, as well as across generations. The general aim of this study was to evaluate the phenotypic responses manifested in short- and long-terms trade-offs, since Gotthard (2000) and many others had stressed the great difficulty in identifying such trade-offs. In order to achieve the above, the study looked at the effect of early food scarcity at successive instars and the subsequent alterations in life-history traits, particularly adult fitness. This was performed by analysing the weight and duration of successive instars after the stressful period, adult fresh body weight, sex ratio, percentage of mortality and the reproductive parameters of the emerged adults, including lifetime fecundity, fertility, proportion of trophic eggs, total reproductive period, reproductive biomass and longevity. The study also investigated the relationships between three important characteristics, fresh body weight, lifetime fecundity and longevity. In addition, the effects on the first generation reared under constant conditions were measured to unravel any long-term costs associated with their mothers being stressed.

8.3 Materials and methods

Adults of *H. axyridis* were collected from a culture maintained in a controlled temperature room at 20±1ºC, 70-75% RH and a photoperiod of L16:D8 under fluorescent lamps. They were sexed, paired and allowed to mate separately in 9cm Petri-dishes. Each couple was provided daily with 32 pea aphids, *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae). The couples were maintained under the above similar conditions until eggs were laid. Upon egg hatching, five sets of larvae were assigned for this experiment. Larvae belonging to the first set were starved at their second stadium, the second set of larvae was starved at their third stadium, a third set of larvae were starved at their fourth stadium, and the fourth set of larvae was starved at their fifth stadium. Larvae belonging to each set were placed individually in 9cm Petri-dishes and allocated to one of the four feeding treatments: treatment A (provided with only water for two days, normal feeding then resumed), treatment B (deprived of water and aphids for two days then fed as normal), treatment C (aphids fed only without water throughout their lifetime) and finally the control, in which all larvae were provided with 32 aphids and water daily. The aphids offered to each larva were dependent on the developmental stage according to Agarwala et al. (2008) and the method mentioned in Chapter 2. All larvae were checked and monitored until adult emergence under similar controlled conditions, as described above. The weight after each moult and the stage duration were recorded for each larva in order to calculate the total developmental period, total larval period and total growth rate. Fresh body weight, sex ratio and the percentage of mortality were determined for each treatment. Subsequently, adult females were paired with unstarved males and each couple was placed individually in a 9cm Petri-dish maintained under similar above laboratory conditions. Couples were fed 32 adult aphids daily and supplied with a 2cm² piece of moistened tissue paper with a 10% honey solution. A folded filter paper (42.5mm, Whatman) was also kept in each Petri-dish to create an oviposition substrate. Couples were monitored until their death. Longevity, lifetime fecundity, egg hatchability, number of trophic eggs, reproductive period and pre-oviposition periods were determined for each female. Freshly hatched larvae from different clutches obtained exclusively from the set that were starved at the fifth instar were collected and reared individually under the above laboratory conditions and supplied with aphids daily (according to the method detailed by Agarwala et al. (2008) stated above) until adult emergence. As above, life-history traits, including developmental parameters such as total developmental period, total larval period, total growth rate and final adult weight, were determined for the progeny in order to commence comparison with their mothers. The few females obtained from the experiments in which larvae were starved at their second stadium and those at their third stadium were dissected to investigate the status of their ovarioles and compare them with the dissected ovarioles obtained from the control.

8.4 Data analysis

All data were subjected to the Shapiro-Wilks test to check for normality. Log- and squared-root transformations were used to normalise data distribution. Testing for the differences between treatments in terms of instar weights and durations was performed using one-way ANOVA. Tukey-HSD test was used in association with ANOVA to reveal the variations between the tested variables. For the parameters that failed to be normalised using the above transformation, non-parametric analyses were applied using a generalised linear model with Poisson errors. Any overdispersion was removed using Quasipoisson modelling. Student t-test for unpaired data was applied to check the variations between mean total developmental period, total growth rate and fresh adult weight of females and their progeny. Wilcoxon Rank

Sum was used when the errors were not normally distributed. Pearson's correlation test was performed to estimate any correlation between different response variables, such as lifetime fecundity, fresh adult weight, longevity and others for each treatment. All statistical analyses were carried out using the statistical programme 'R' version 2.8.1 (Ihaka and Gentleman, 1996).

8.5 Results

8.5.1 Immature developmental stages

8.5.1.1 Second larval instar

Changes in the weights of developing larvae across stages (instars) and the related developmental parameters are presented in Table 8.1. All the treatments had a significant influence on larvae weights directly at the termination of the stressful period (F=45.73, df=146, RSE=0.283, P<0.0001). The mean weight of larvae analysed immediately at the end of treatment courses A, B and C was significantly different compared with the control – (difference =0.7, P<0.0001), (difference=0.61, P<0.0001) and (difference=-0.31, P<0.0001), respectively. Larval weight after the fourth moult varied significantly between the treatments (F=6.4, df=87, RSE=0.515, P<0.0001). The mean weight at the onset of the fifth instar (after the fourth moult) of larvae assigned to treatment B was 21.12±0.76mg, which was significantly greater (difference=-0.67, P<0.01) than the 15.41±0.45mg obtained from the control. The mean weight of pupae varied significantly between treatments (F=32.23, df=74, RSE=5.168, P<0.0001). The mean weight at the commencement of the pupal stage was 21.74±1mg for treatment A, 21.01±0.77mg for treatment B and 29.13±0.63mg for treatment C, all significantly higher than the control:– the difference was -6.1 (P<0.05), -5.43 (P<0.05) and 13.55 (P<0.0001), respectively. Upon adult emergence, the mean weight of adults in treatment A was slightly greater but not significantly so, than the control (difference=-0.32, P=0.58); likewise, for those that were obtained from treatment B, the mean weight was a little more than the control but not significantly so (difference=-0.4, P=0.25). For treatment C, however, the mean weight of adults was significantly greater than the control (F=4.346, df=63, RSE=0.526, P<0.01), (difference=0.5, P<0.01). The above results implied that all larvae were capable of successively compensating, as the final adult body weight was found to be to some extent similar to the mean weight of the control larvae (or was considerably more in the case of treatment C).

Table 8.1. Changes in larval performance as measured by the weight (mg) and the related developmental parameters for successive instars of *H. axyridis* ladybirds experiencing different treatments (A, B, C and the control).

			Treatments		
Larval stage	Developmental stage weight(mg)	Treatment A (n=52)	Treatment B (n=32)	Treatment C (N=32)	Control (n=64)
2nd larva stadium	After egg hatching	0.23±0.01	0.44±0.02	0.16±0.01	0.3±0.01
	After 1st moult	0.52±0.025	1.28±0.13	0.65±0.04	1.25±0.14
	After 2d starvation	0.27±0.01	0.38±0.025	0.91±0.06	1.79±0.16
	After 2nd moult	1.6±0.08	4.7±0.3	1.5±0.068	4.85±0.3
	After 3rd moult	4.21±0.2	12.76±0.6	5.038±0.21	8.69±0.5
	After 4th moult	13.59±0.31	21.12±0.76	15.1±1	15.41±0.45
	Pre-pupal weight	22.6±0.71	23.23±0.55	31.33±1	19.7±0.7
	Pupal weight	21.74±1	21.01±0.77	29.13±0.63	15.58±0.72
	Adult weight	13.3±1.09	13.52±1	15±1.2	8.89±0.47
3rd larva stadium	Developmental stage weight(mg)	Treatment A (n=47)	Treatment B (n=51)	Treatment C (N=27)	Control (n=32)
	After egg hatching	NA	NA	NA	NA
	After 2nd moult	1.3±0.068	1.15±0.58	0.83±0.036	2.78±0.18
	After 2d starvation	1.16±0.07	0.9±0.04	1.16±0.05	4.55±0.26
	After 3rd moult	4.68±0.34	3.25±0.11	3.35±0.2	7±0.26
	After 4th moult	10.55±0.36	11.6±0.48	10.62±0.38	11.8±0.28
	Pre-pupal weight	21.8±1	22.12±0.87	26.03±1.125	19.14±0.61

Table 8.1(cont.)

	Pupal weight	20±0.74	24.67±0.55	24.81±0.66	16.2±0.6
	Adult weight	10.87±0.47	14.4±0.65	21.02±0.82	10.33±0.72
4th larval stadium	Developmental stage weight(mg)	Treatment A (n=59)	Treatment B (n=36)	Treatment C (N=27)	Control (n=32)
	After egg hatching	0.13±0.007	0.3±0.036	0.21±0.016	0.16±0.012
	After 1st moult	0.87±0.032	1.08±0.068	0.78±0.062	0.84±0.04
	After 2nd moult	1.36±0.032	1.65±0.077	1.7±0.17	2.06±0.1
	After 3rd moult	5.7±0.26	5.48±0.48	5±0.23	3.8±0.14
	After 2d starvation	4.61±0.22	5.37±1.2	8.6±0.3	6.85±0.37
	After 4th moult	12.2±0.52	11.73±0.45	12.62±0.61	15±0.46
	Pre-pupal weight	26±0.36	27.72±0.41	28±0.57	23.2±0.61
	Pupal weight	21.2±0.48	24.27±0.4	25.04±0.84	20.1±0.55
	Adult weight	10.4±0.46	13.6±0.66	15.53±0.7	17±0.45
	Developmental stage weight(mg)	Treatment A (n=28)	Treatment B (n=30)	Treatment C (N=30)	Control (n=27)
5th larval stadium	After egg hatching	0.37±0.025	0.45±0.02	0.17±0.01	0.33±0.03
	After 1st moult	0.85±0.06	1±0.05	1.34±0.05	0.66±0.038
	After 2nd moult	1.54±0.07	1.48±0.03	1.82±0.07	1.48±0.08
	After 3rd moult	5.72±0.28	5.68±0.022	5.34±0.32	5.83±0.26
	After 4th moult	11.27±0.22	11.33±0.4	12.26±0.46	16.16±0.56
	After 2d starvation	8.07±0.48	10.12±0.27	20.89±0.66	22.81±0.52
	Pre-pupal weight	24.31±0.76	23.12±0.6	27.81±0.57	25.61±0.6

Table 8.1(cont.)

	Pupal weight	20.75±0.68	19.42±0.45	24.55±0.55	22.76±0.6
	Adult weight	17.33±0.5	16.58±0.62	20.4±0.6	18.68±0.68
	Developmental stage weight(mg)	Treatment A (n=4)	Treatment B (n=10)	Treatment C (N=9)	Control (n=23)
Off-spring from 4th instar	After egg hatching	0.22±0.024	0.26±0.02	0.122±0.014	0.33±0.03
	After 1st moult	1.2 ±0.17	0.58±0.02	0.55±0.1	0.66±0.038
	After 2nd moult	2.82±0.23	2±0.24	4.63±0.4	1.5±0.08
	After 3rd moult	5.6±0.28	7.45±0.55	15.46±0.54	5.73±0.26
	After 4th moult	12.1±0.8	19.37±0.77	10.34±0.68	16.16±0.56
	Pre-pupal weight	21.75±1.25	21.9±0.42	18.52±0.67	26.04±0.59
	Pupal weight	16.674±0.83	16.5±0.76	15.18±1.13	22.25±0.6
	Adult weight	11.77±0.43	11±0.4	11.18±1	18.68±0.45

The total developmental period varied significantly between the four treatments (F=1.857e+05, df=62, RSE=0.315, P<0.0001). Comparing the mean total developmental period of larvae assigned for the three treatments (A, B and C) versus the control revealed that treatment C possessed the longest period (39.88±0.7 days) compared with the control (difference=0.28, P<0.0001), followed by 34.2±1.35 days for treatment A, which was also found to be significantly longer than the control (difference=-0.13, P<0.0001) (Table 8.2). Larvae subjected to treatment B, on the other hand, had the shortest period in comparison with the control (difference=0.15, P<0.0001), with treatment A (difference=-0.28, P<0.0001) and with treatment B (difference=0.43, P<0.0001). In addition, the mean total growth rate varied between treatments A, B and C (F=2.67, df=62, RSE=0.146, P=0.05). The mean total growth rate was slightly greater in treatments A and B compared with the control (difference=-0.06, P=0.77) and (difference=-0.16, P=0.046), respectively. Larvae subjected to treatment C grew faster than the control, since they accomplished a mean total growth rate of approximately 0.37±0.04mg/day. This was found to be slightly more than the control, which undertook 0.29±0.021mg/day, but the difference between them was not significant (difference=0.05, P=0.53).

The reproductive parameters of females that managed to survive the later application of the treatments are presented in Table 8.3. All of the treatments had a significant influence on the lifetime fecundity of females (F=8.8, df=38, RSE=21.8, P<0.001). The mean lifetime fecundity of the control was 64.13±4 eggs, which was significantly greater than that of females in treatment B (18.5±2.72 eggs (difference=45.6, P<0.01); females collected from treatment C, however, were found to be more fecund than the control but the difference was not significant (difference=12.1, P=0.41). Likewise, females obtained from treatment A had a lower lifetime fecundity compared with the control but the difference was not significant (difference=28.13, P=0.1). Treatment did not affect the proportion of trophic egg production (F=2, df=36, RSE=0.5, P=0.14). The mean proportion of trophic eggs produced by females obtained from treatments A, B and C were found to be comparably less than the control, but the differences were not significant (difference=-0.36, P=0.54), (difference=-0.55, P=1) and (difference=0.26, P=0.47), respectively.

The mean longevity of females varied between treatments (F=32.29, df=38, RSE=0.13, P=0.001). The mean longevity differed significantly between the treatments, females in treatment A lived for approximately 24.75±1.75 days and females in treatment B had a mean longevity of 20.54±0.6 days. Both were found to be comparably less than the control (39.68 ±1.22 days), (difference=0.45, P<0.0001) and (difference=0.62, P<0.0001), respectively. Females in treatment C had a slightly lower mean longevity, but the difference was not significant compared with the control (difference=-0.05, P=0.66).

There was no correlation between lifetime fecundity and longevity in treatment A (correlation coefficient=0.42, P=0.57), treatment B (correlation coefficient=-0.47, P=0.52) or treatment C (correlation coefficient=0.25, P=0.43). The percentage of mortality for treatment A was 90%, for treatment B was 78.12% and for the control was 62.5%; the lowest recorded percentage was that for treatment C (50%). The estimated sex ratio as presented in Table 8.2 leaned towards female-biasness for treatments A, B and C, compared with the control which revealed an equal ratio.

Table 8.2. Mean total developmental duration, total growth rate and survivorship for the instars experiencing treatments A, B, C and the control.

			Treatments		
Larval stage	Developmental stage weight(mg)	Treatment A (n=52)	Treatment B (n=32)	Treatment C (N=32)	Control (n=64)
2nd larva stadium	After egg hatching	0.23±0.01	0.44±0.02	0.16±0.01	0.3±0.01
	After 1st moult	0.52±0.025	1.28±0.13	0.65±0.04	1.25±0.14
	After 2d starvation	0.27±0.01	0.38±0.025	0.91±0.06	1.79±0.16
	After 2nd moult	1.6±0.08	4.7±0.3	1.5±0.068	4.85±0.3
	After 3rd moult	4.21±0.2	12.76±0.6	5.038±0.21	8.69±0.5
	After 4th moult	13.59±0.31	21.12±0.76	15.1±1	15.41±0.45
	Pre-pupal weight	22.6±0.71	23.23±0.55	31.33±1	19.7±0.7
	Pupal weight	21.74±1	21.01±0.77	29.13±0.63	15.58±0.72
	Adult weight	13.3±1.09	13.52±1	15±1.2	8.89±0.47
3rd larva stadium	Developmental stage weight(mg)	Treatment A (n=47)	Treatment B (n=51)	Treatment C (N=27)	Control (n=32)
	After egg hatching	NA	NA	NA	NA
	After 2nd moult	1.3±0.068	1.15±0.58	0.83±0.036	2.78±0.18
	After 2d starvation	1.16±0.07	0.9±0.04	1.16±0.05	4.55±0.26
	After 3rd moult	4.68±0.34	3.25±0.11	3.35±0.2	7±0.26
	After 4th moult	10.55±0.36	11.6±0.48	10.62±0.38	11.8±0.28
	Pre-pupal weight	21.8±1	22.12±0.87	26.03±1.125	19.14±0.61

Table 8.2 (cont.)

	Pupal weight	20±0.74	24.67±0.55	24.81±0.66	16.2±0.6
	Adult weight	10.87±0.47	14.4±0.65	21.02±0.82	10.33±0.72
4th larval stadium	Developmental stage weight(mg)	Treatment A (n=59)	Treatment B (n=36)	Treatment C (N=27)	Control (n=32)
	After egg hatching	0.13±0.007	0.3±0.036	0.21±0.016	0.16±0.012
	After 1st moult	0.87±0.032	1.08±0.068	0.78±0.062	0.84±0.04
	After 2nd moult	1.36±0.032	1.65±0.077	1.7±0.17	2.06±0.1
	After 3rd moult	5.7±0.26	5.48±0.48	5±0.23	3.8±0.14
	After 2d starvation	4.61±0.22	5.37±1.2	8.6±0.3	6.85±0.37
	After 4th moult	12.2±0.52	11.73±0.45	12.62±0.61	15±0.46
	Pre-pupal weight	26±0.36	27.72±0.41	28±0.57	23.2±0.61
	Pupal weight	21.2±0.48	24.27±0.4	25.04±0.84	20.1±0.55
	Adult weight	10.4±0.46	13.6±0.66	15.53±0.7	17±0.45
	Developmental stage weight(mg)	Treatment A (n=28)	Treatment B (n=30)	Treatment C (N=30)	Control (n=27)
5th larval stadium	After egg hatching	0.37±0.025	0.45±0.02	0.17±0.01	0.33±0.03
	After 1st moult	0.85±0.06	1±0.05	1.34±0.05	0.66±0.038
	After 2nd moult	1.54±0.07	1.48±0.03	1.82±0.07	1.48±0.08
	After 3rd moult	5.72±0.28	5.68±0.022	5.34±0.32	5.83±0.26
	After 4th moult	11.27±0.22	11.33±0.4	12.26±0.46	16.16±0.56
	After 2d starvation	8.07±0.48	10.12±0.27	20.89±0.66	22.81±0.52
	Pre-pupal weight	24.31±0.76	23.12±0.6	27.81±0.57	25.61±0.6

Table 8.2(cont.)

	Pupal weight	20.75±0.68	19.42±0.45	24.55±0.55	22.76±0.6
	Adult weight	17.33±0.5	16.58±0.62	20.4±0.6	18.68±0.68
	Developmental stage weight(mg)	Treatment A (n=4)	Treatment B (n=10)	Treatment C (N=9)	Control (n=23)
Off-spring from 4th instar	After egg hatching	0.22±0.024	0.26±0.02	0.122±0.014	0.33±0.03
	After 1st moult	1.2 ±0.17	0.58±0.02	0.55±0.1	0.66±0.038
	After 2nd moult	2.82±0.23	2±0.24	4.63±0.4	1.5±0.08
	After 3rd moult	5.6±0.28	7.45±0.55	15.46±0.54	5.73±0.26
	After 4th moult	12.1±0.8	19.37±0.77	10.34±0.68	16.16±0.56
	Pre-pupal weight	21.75±1.25	21.9±0.42	18.52±0.67	26.04±0.59
	Pupal weight	16.674±0.83	16.5±0.76	15.18±1.13	22.25±0.6
	Adult weight	11.77±0.43	11±0.4	11.18±1	18.68±0.45

Table 8.3. Reproductive performances for females obtained from treatments A, B, C and the control (i.e., the number of samples were very small depending on the % of survived females).

Larval stage	Developmental stage weight(mg)	Treatments Treatment A (n=52)	Treatment B (n=32)	Treatment C (N=32)	Control (n=64)
2nd larva stadium	After egg hatching	0.23±0.01	0.44±0.02	0.16±0.01	0.3±0.01
	After 1st moult	0.52±0.025	1.28±0.13	0.65±0.04	1.25±0.14
	After 2d starvation	0.27±0.01	0.38±0.025	0.91±0.06	1.79±0.16
	After 2nd moult	1.6±0.08	4.7±0.3	1.5±0.068	4.85±0.3
	After 3rd moult	4.21±0.2	12.76±0.6	5.038±0.21	8.69±0.5
	After 4th moult	13.59±0.31	21.12±0.76	15.1±1	15.41±0.45
	Pre-pupal weight	22.6±0.71	23.23±0.55	31.33±1	19.7±0.7
	Pupal weight	21.74±1	21.01±0.77	29.13±0.63	15.58±0.72
	Adult weight	13.3±1.09	13.52±1	15±1.2	8.89±0.47
3rd larva stadium	Developmental stage weight(mg)	Treatment A (n=47)	Treatment B (n=51)	Treatment C (N=27)	Control (n=32)
	After egg hatching	NA	NA	NA	NA
	After 2nd moult	1.3±0.068	1.15±0.58	0.83±0.036	2.78±0.18
	After 2d starvation	1.16±0.07	0.9±0.04	1.16±0.05	4.55±0.26
	After 3rd moult	4.68±0.34	3.25±0.11	3.35±0.2	7±0.26
	After 4th moult	10.55±0.36	11.6±0.48	10.62±0.38	11.8±0.28
	Pre-pupal weight	21.8±1	22.12±0.87	26.03±1.125	19.14±0.61

Table 8.3 (cont.)

	Pupal weight	20±0.74	24.67±0.55	24.81±0.66	16.2±0.6
	Adult weight	10.87±0.47	14.4±0.65	21.02±0.82	10.33±0.72
4th larval stadium	Developmental stage weight(mg)	Treatment A (n=59)	Treatment B (n=36)	Treatment C (N=27)	Control (n=32)
	After egg hatching	0.13±0.007	0.3±0.036	0.21±0.016	0.16±0.012
	After 1st moult	0.87±0.032	1.08±0.068	0.78±0.062	0.84±0.04
	After 2nd moult	1.36±0.032	1.65±0.077	1.7±0.17	2.06±0.1
	After 3rd moult	5.7±0.26	5.48±0.48	5±0.23	3.8±0.14
	After 2d starvation	4.61±0.22	5.37±1.2	8.6±0.3	6.85±0.37
	After 4th moult	12.2±0.52	11.73±0.45	12.62±0.61	15±0.46
	Pre-pupal weight	26±0.36	27.72±0.41	28±0.57	23.2±0.61
	Pupal weight	21.2±0.48	24.27±0.4	25.04±0.84	20.1±0.55
	Adult weight	10.4±0.46	13.6±0.66	15.53±0.7	17±0.45
	Developmental stage weight(mg)	Treatment A (n=28)	Treatment B (n=30)	Treatment C (N=30)	Control (n=27)
5th larval stadium	After egg hatching	0.37±0.025	0.45±0.02	0.17±0.01	0.33±0.03
	After 1st moult	0.85±0.06	1±0.05	1.34±0.05	0.66±0.038
	After 2nd moult	1.54±0.07	1.48±0.03	1.82±0.07	1.48±0.08
	After 3rd moult	5.72±0.28	5.68±0.022	5.34±0.32	5.83±0.26
	After 4th moult	11.27±0.22	11.33±0.4	12.26±0.46	16.16±0.56
	After 2d starvation	8.07±0.48	10.12±0.27	20.89±0.66	22.81±0.52
	Pre-pupal weight	24.31±0.76	23.12±0.6	27.81±0.57	25.61±0.6

Table 8.3 (cont.)

	Pupal weight	20.75±0.68	19.42±0.45	24.55±0.55	22.76±0.6
	Adult weight	17.33±0.5	16.58±0.62	20.4±0.6	18.68±0.68
	Developmental stage weight(mg)	Treatment A (n=4)	Treatment B (n=10)	Treatment C (N=9)	Control (n=23)
Off-spring from 4th instar	After egg hatching	0.22±0.024	0.26±0.02	0.122±0.014	0.33±0.03
	After 1st moult	1.2 ±0.17	0.58±0.02	0.55±0.1	0.66±0.038
	After 2nd moult	2.82±0.23	2±0.24	4.63±0.4	1.5±0.08
	After 3rd moult	5.6±0.28	7.45±0.55	15.46±0.54	5.73±0.26
	After 4th moult	12.1±0.8	19.37±0.77	10.34±0.68	16.16±0.56
	Pre-pupal weight	21.75±1.25	21.9±0.42	18.52±0.67	26.04±0.59
	Pupal weight	16.674±0.83	16.5±0.76	15.18±1.13	22.25±0.6
	Adult weight	11.77±0.43	11±0.4	11.18±1	18.68±0.45

8.5.1.2 Third larval instar

Mean weight during each larval stage and associated developmental parameters are presented in Table 8.1. All the treatments (A, B and C) impacted the weight significantly (F=141.5, df=120, RSE=0.206, P<0.0001). The mean weight of larvae assigned for treatment A was 1.16±0.07mg, for treatment B was 0.9±0.04mg, for treatment C was 1.16±0.05mg and for the control was 4.55±0.26mg. All of the former three weights were found to be significantly less than the control, (difference=-1.03, P <0.0001), (difference=-1.02, P <0.0001) and (difference=-1, P <0.0001), respectively. Treatments A, B and C had a significant influence on the weight of larvae at the onset of the fourth stadium (after the third moult) (F=24.3, df=82, RSE=1.61, P<0.0001). The mean weight of larvae exposed to treatments A, B and C was significantly less than the control, (difference=-3.7, P<0.0001), (difference=-2.32, P<0.0001) and (difference=-3.6, P<0.0001), respectively. Only after the fourth moult did the weight of the larvae start to increase to values that did not significantly differ from the control (i.e., treatment A (difference=-0.15, P=0.46), treatment B (difference=-1.2, P=0.99) and treatment C (difference=-1.1, P=0.42)). As larvae grew and reached the onset of the pre-pupal stage, they started to get heavier than the control (F=4.88, df=63, RSE=5.54, P<0.01), particularly those that belonged to treatment C. The pre-pupal weight of treatment C was 26.03±1.1mg, whereas that of those that belonged to the control was 19.14±0.61mg, which is significantly less (difference=6.88, P<0.01). Unexpectedly, pupae of the control group were found to be lighter in weight than treatment A (difference=5.203, P<0.0001), treatment B (difference=3.81, P<0.05) or treatment C (difference=6, P<0.0001), since all the treatments had significant influences on the pupal weight (F=14.8, df=51, RSE=4, P<0.0001). Finally, the emerged adults from treatments B and C were found to be heavier compared with the control (difference=4, t-value=2.24, P<0.05) (difference=15, t-value=6.45=P< 0.0001).

Adults obtained from treatment A were slightly heavier, but not significantly different from the control (difference=4.07, t-value, 0.27, P=0.13).

The total developmental period varied significantly between the treatments (F=29.03, df=37, RSE=1.73, P<0.0001), as shown in Table 8.2. The mean duration of the total developmental period for treatment C did not vary from the control (difference=-0.4, P=0.92), whereas, the equivalent period was found to be shorter for treatment A (difference=-6.5, P<0.0001) and treatment B (difference=-4, P<0.0001), in comparison with the control. Likewise, the treatments had significant influences on the total growth rate of larvae in general (F=13.63, df=37, RSE=0.11, P<0.0001). The mean total growth rate of larvae belonging to treatments C and B was found to be significantly greater than the control – (difference=0.27, P<0.0001) and (difference=0.14, P<0.05), respectively. Larvae of treatment A also obtained a greater total growth rate, but did not differ significantly from the control (difference=0.063, P=0.63).

Regarding the longevity, treatments A, B and C affected the longevity significantly (F=108.71, df=18, RSE=0.032, P<0.0001). Larvae from treatments A and B lived for a significantly shorter time than the control, (difference=-0.33, P<0.0001) and (difference=-0.42, P<0.0001), respectively. In addition, adults of treatment C also had a slightly shorter lifespan, but did not differ significantly when compared with the control (difference=-0.04, P=0.3). The percentage mortality was 87% in treatment A, 82.35% in treatment B, 55.55% in treatment C and 60% for the control. The sex ratio, as provided in Table 8.1, varied completely between all the treatments and tended to be male-biased in treatment B and in the control, whereas treatments A and C revealed a female-biased sex ratio.

The reproductive parameters for females obtained from all treatments are presented in Table 8.3. The length of the reproductive period for all the tested females was significantly affected by the treatments (F=52.18, df=18, RSE=2.38, P<0.0001). The longest mean reproductive period was found to belong to females collected from treatment C (20.57±1.08 days) and the shortest period was recorded for treatment A (2±1days). Both were found to differ significantly from the control, (difference=8.28, P<0.0001) and (difference=-10.28, P<0.001), respectively. The mean length of the reproductive period for treatment B was also found to be significantly shorter than the control (difference=-5.7, P<0.01).

The mean lifetime fecundity of females belonging to treatments A and B were significantly lower compared with the control, (difference=-0.27, t-value=-8.1, P<0.0001) and (difference=-0.5, t-value=-2.188, P<0.05), respectively. For treatment C, the mean lifetime fecundity was 148.14±24.23 eggs, which was slightly higher, but not significant compared with the control (difference=0.45, t-value=2.04, P=0.055). The treatments revealed significant effects on egg hatchability or females' fertility (F=37.01, df=18, RSE=0.06, P<0.0001). Females from treatment B produced 30% fertile eggs, significantly less than the control (87%) (difference=-0.45, P<0.0001). Females of treatment A were also found to be less fertile compared with the control, but that was not significant (difference=-0.31, P=0.73). Adults from treatment C laid slightly more fertile eggs than the control, but not significantly more (difference=0.04, P=0.6). The treatments had significant influences on the mean proportion of trophic eggs (F=3.51, df=18, RSE=0.1, P<0.05). The higher proportions of trophic eggs were produced by females in both treatments A and B.

8.5.1.3 Fourth larval instar

Changes in larval weight during development after experiencing various treatments are presented in Table 8.1. All the treatments had a significant impact on the larval weight, which was measured directly at the end of the stressful period (F=20.21, df=139, RSE=19.23, P<0.0001). The mean weight of larvae recorded directly after experiencing treatment A was significantly less than the control (difference=-0.34, P<0.0001); an analogous observation was revealed when comparing treatment B with the control (difference=-0.42, P<0.0001). Treatment C on the other hand, did not differ from the control significantly for the respective above weight (difference=0.22, P=0.12). The significant reduction in mean weight persisted even after accomplishing the fourth moult (fifth instar) in treatment A (difference=-0.22, P<0.01) and treatment B (difference=-0.23, P<0.01), in particular compared with the control. For treatment C, the mean weight was slightly less than the control, but not significant (t-value=-0.17, P=0.08).

In the same way, as larvae went into the pre-pupal stage, their weights varied significantly between the treatments (F=19.24, df=125, RSE=3, P<0.0001). The mean weight of the pre-pupal stage was significantly greater in treatments A, B and C compared with the control, (difference=2.74, P<0.001), (difference=4.51, P<0.0001) and (difference=5.6, P<0.0001), respectively. The treatments largely affected the pupal weight significantly (F=11.48, df=114, RSE=3.4, P<0.0001). The weight of pupae obtained from treatment A was considerably greater, but not significantly different from the control (difference=0.1, P=0.73). The weight of pupae collected from treatments B and C were to some extent less, but once more not significant compared with the control – (difference=-0.067, P=0.87) and (difference=-0.01, P=1), respectively. The treatments had a significant influence on the adult weight (F=16.72, df=102, RSE=8.2, P<0.0001). The mean weight of emerged adults from treatment C was 15.53±0.7mg, which was somewhat less but not significantly different from the control (difference=-0.1, P=0.66). The mean weight of adults obtained from treatments A and B were significantly lighter than the control, (difference=-0.49, P<0.0001) and (difference=-0.24, P<0.05), respectively.

The treatments had a significant influence on the total developmental period of larvae (F=16.42, df=102, RSE=1.33, P<0.000). The mean total developmental period for treatments A, B and C was found to be significantly shorter compared with the control, (difference=-0.1, P<0.01), (difference=-0.21, P<0.0001) and (difference=-0.14, P<0.001), respectively, as shown in Table 8.2. In the same way, the treatments significantly influenced the total growth rate (F=12.32, df=102, RSE=0.153, P<0.0001). The mean total growth rate of the control was 0.56±0.01mg/day, which was significantly greater than treatment A (0.38±0.025mg/day) (t-value=-0.17, P<0.001), but not significantly different from treatment B (0.55±0.03mg/day) (difference=-0.0001, P=1). Individuals in Treatment C had a significantly greater total growth rate than the (difference=-0.24, P<0.01). Upon comparing the mean total growth rate of treatment A with treatment C it was revealed that the latter had a significantly greater growth rate (difference=-0.22, P<0.0001). Treatment C, however, did not show a significant difference from treatment B with respect to the relative growth rate (difference=-0.04, P=0.71). Finally, larvae that were exposed to treatment B had a mean total growth rate that was significantly greater than treatment A (difference =-0.17, P<0.001).

Females that had experienced treatments A, B and C had significantly shorter longevity compared with the control, (difference=-0.32, P<0.0001), (difference=-0.3, P<0.0001) and (difference=-0.09, P<0.0001), respectively, since all the treatments had significantly affected the longevity (F=19.24, df=125, RSE=2.91, P<0.0001). To check if investing in maintaining body size had influenced the longevity, correlation tests were conducted. The results revealed an absence of correlation between these two traits in treatment A (correlation coefficient=0.3, P=0.43), treatment B (correlation coefficient=0.35, P=0.38) and treatment C (correlation coefficient=-0.5, P=0.467). Any correlation between longevity and lifetime fecundity in treatments A, B, C and the control was also absent, (correlation coefficient=-0.27, P=0.46), (Correlation coefficient=-0.3 P=0.46), (t=-0.023, P=0.82, Glm modelling) and (t=-0.43, P=0.68, Glm modelling), respectively.

The results of the reproductive output of females starved at the onset of their fourth instar are presented in Table 8.2. Unexpectedly, the results revealed that the treatments did not have any apparent significant effect on the lifetime fecundity of surviving females (F=2.26, df=31, RSE=3.23, P=0.1). Although females collected from treatment A had a mean lifetime fecundity of 124.25±26.08 eggs, this was found to be slightly higher than the control (91.33±9 egg), but not significantly so (difference=1.05, P=1). Females obtained from treatments B and C were found to be less fecund than the control, but the differences were not significant – (difference=-2.3, P=0.44) and (difference=-2.22, P=0.47), respectively. In contrast, the proportion of fertility or egg hatchability varied significantly between the treatments (F=24.73, df=10, RSE=0.08, P<0.0001). The proportion of fertile eggs produced by females of the control was shown to be significantly greater than that produced by females of treatment A (difference=-0.8, P<0.001), treatment B (difference=-0.8, P<0.01) and treatment C (difference=-0.83, P<0.05). The significant reduction in percentage of fertility was found to be associated with the high proportion of trophic eggs. The proportion of trophic eggs varied significantly between treatments (F=224.4, df=31, RSE=0.06, P<0.0001). It was found that the mean proportion of trophic eggs was greater than the control in treatments A (difference=0.77, P<0.0001), B (difference=0.85, P<0.0001) and even in C (difference=0.84, P<0.0001). Thus, laying a larger clutch was not often an indicator for relatively higher fertility. For example, females that had experienced treatment A had a mean lifetime fecundity of 124.25±26.08 eggs, accompanied by a high percentage of trophic eggs (89%). Females invested more resources into the well-recognised adaptive provisional behaviour to secure immature survival. This might, however, constitute a cost on longevity.

All the treatments influenced the pre-oviposition period significantly (F=5.51, df=31, RSE=0.34, P<0.01). There was a delay in the pre-oviposition period by females collected from different treatments. The comparisons revealed that the delay in egg production was significantly greater in treatments A and C compared with the control, (difference=0.47, P<0.05) and (difference=0.62, P<0.01), respectively. Females from treatment B showed a slight non-significant delay in the reproductive period compared with the control (difference=0.42, P=0.06). In contrast, the length of the reproductive period of all treatments did not differ significantly from the control (F=2.85, df=31, RSE=0.37, P=0.053). The length of the reproductive period in treatments A, B and C did not vary significantly from the control, (difference=0.29, P=0.4), (difference=0.13, P=0.86) and (difference=-0.22, P=0.6), respectively. The greatest mortality was found in treatment B (42.37%) and the least in treatment A (22.22%) (Table 8.2). The sex ratio was not consistent, it tended to be male-biased in treatment A and in the control, but for treatments B and C was female-biased.

8.5.1.4 Fifth larval instar

Changes in larval developmental weight in response to all treatments are presented in Table 8.1. Similar to the above stages, the mean weight of larvae recorded directly after the treatment period varied significantly (F=51, df=101, RSE=3.5, P<0.0001). The weight of larvae was less significant in treatment A (difference=-0.46, P <0.0001), in treatment B (difference=-0.61, P<0.0001) and in treatment C (difference=-0.27, P<0.0001), compared with the control. The weight at the onset of the pre-pupae varied significantly between the treatments (F=7.58, df=100, RSD=0.14, P<0.0001). The mean pre-pupal weight in treatment C was slightly greater than the control, but did not differ significantly (difference=0.08, P=0.23). The weight of pre-pupae was lighter in treatment A and in treatment B, but both were found not to be significant compared with the control, (difference=-0.05, P=0.51) and (difference=-0.1, P=0.07), respectively. Likewise, the pupal weight varied significantly between the treatments (F=12.42, df=87, P<0.0001). The mean weight of pupae obtained from treatment A was lower compared with the control, but not significantly so (difference=-2, P=0.14). For treatment B the weight was, however, significantly less than the control (difference=-3.3, P<0.01). Treatment C resulted in slightly heavier pupae, than the control but not significantly so (difference=1.8, P=0.22). Finally, adult weights varied between the treatments (F=6.72, df=79, P<0.001). The mean weight of adults was found to be higher in treatment C, but did not show any difference from the control (difference=1.71, P=0.3). Adults collected from treatments A and B were lighter relative to the control, but the differences were also found to be not significant, (difference=-1.3, P=0.49) and (difference=-2.1, P=0.13), respectively.

The mean total developmental period varied between the treatments (F=31.52, df=79, RSE=0.074, P<0.0001). The mean total developmental period of larvae of treatment C was found to be significantly longer compared with the control (difference=0.19, P<0.0001). The duration of that period for treatment A was shorter than the control, but was not significant (difference=0.01, P=0.95). Larvae assigned to treatment B took a slightly longer time to develop into adults than the control, but the difference was not significant (difference=0.023, P=0.77). In contrast, the mean total growth rates did not vary between the treatments (F=2.16, df=79, RSE=0.08, P=0.1). The mean total growth rate was greater in the control compared with treatments A (t-value=-0.044, P=0.38) and B (t-value=-0.047, P=0.33).

Mean lifetime fecundity and other reproductive parameters are presented in Table 8.3. Lifetime fecundity did not vary between the three treatments in comparison with the control (F=2.36, df=15, RSE=0.73, P=0.11). Females collected from the three treatments A, B and C had lower lifetime fecundity compared with the control, not significantly so, (difference=-1.05, P=0.12), (difference=-0.9, P=0.33) and (difference=-0.88, P=0.23), respectively. In contrast, the proportion of egg hatchability or fertility varied significantly between the treatments (F=5.26, df=8, RSE=0.66, P<0.05). The mean proportion of fertile eggs was significantly less in females collected from treatment B, since they laid 0.44±0.07 eggs compared with 0.88±0.03 eggs recorded for the control (difference=-6.8, P<0.05). Lifetime fecundity of females in treatments A and C did not differ significantly from the control, (difference=-0.34, P=0.48) and (difference=-0.28, P=0.31), respectively. In addition, the treatments had influenced the production of trophic eggs significantly (F=7.59, df=17, RSE=0.28, P<0.01). Females obtained from treatments A, B and C laid a significantly higher proportion of trophic eggs compared with the control, (difference=0.72, P<0.01), (difference=0.65, P<0.05) and (difference=0.53, P<0.05), respectively. This explains the reduction in the proportion of fertility, particularly in treatment B.

There was a significant delay in the onset of the reproductive period in treatments A (difference=-0.81, P<0.0001), B (difference=-1, P<0.0001) and C (difference=-1, P<0.0001), after comparison with the control (F=66.36, df=17, RSE=0.134, P<0.0001). The mean reproductive period varied significantly between the treatments (F=20.74, df=17, RSE=0.38, P<0.0001). The length of the reproductive period was found to be significantly longer in the control compared with treatment A (difference=-1.48, P<0.0001), treatment B (difference=-1.4, P<0.0001) and treatment C (difference=-1.4, P<0.0001). The extension in the pre-reproductive period affected the length of reproductive period, since they both showed a direct significant correlation (Correlation coefficient=0.8, P<0.01). Similarly, the longevity varied significantly between treatments (F=22.08, df=23, RSE=0.13, P<0.0001). It was found that females gathered from treatments A, B and C lived for a shorter period in comparison with the control, (difference=-15.38, P<0.0001), (difference=-15, P< 0.0001) and (difference=-9.08, P<0.01), respectively. The percentage of mortality in treatment A was 85.7%, in treatment B was 86.6%, in treatment C was 83.3% and only 37% in the control (Table 8.2). The sex ratio was male-biased in treatments B and C and the control, however, treatment A had an equal sex ratio. Apparently, the sex ratio was affected by the high percentage of death, as in the above experiments.

8.5.2 Offspring fitness

As stated in the method, females that survived the four treatments that they had experienced at their fifth instar (after the fourth moult) were coupled with a normal unstarved freshly hatched male until they produced eggs and larvae were hatched. When those larvae reached adult stage, several life-history traits were measured and compared with those of their mothers only, excluding their normal fathers.

The developmental parameters of offspring obtained from the females that had experienced treatments A, B and C at the onset of the fifth instar (after the fourth moult) and the control are presented in Tables 8.1 and 8.2. The results showed no significant differences between the mean weights of the adults obtained from the progeny whose mothers came from the control (t=-0.7, P=0.5). The mean adult weight was found to be significantly less in offspring obtained from females experiencing treatment A (t=25.71, P<0.0001), treatment B (t=9, P<0.0001) and treatment C (t=13.5, P<0.0001). Larvae of offspring obtained from females experiencing treatment A took approximately 28±0.28 days to develop into adults. This was found to be significantly shorter compared with their mothers, which took around 37.65±0.48 days (V=406, P<0.0001). Similarly, offspring obtained from treatment C also had a significantly shorter total developmental period compared with their mothers (V=136, P<0.001) and a similar shorter total developmental duration was revealed for offspring compared with their mothers from the control (t=-5.82, P<0.0001). The mean total developmental period of offspring obtained from treatment B was 40.57±2.3 days, which was significantly longer than their mothers (38.09± 0.62 days) (t=19.67, P<0.0001). It is possible that the shorter total developmental period exhibited by offspring compared with their mothers from treatment A was a result of the high total growth rate compared to their mothers (t=29.25, P<0.0001). The longer total developmental period in offspring of treatment B, on the other hand, was due to having a significantly lower growth rate compared with their mothers (t=7.3, P<0.0001). The total growth rate was found to be significantly lower in offspring compared with their mothers where they had experienced treatment C (t=15.86, P<0.0001). The total growth rate for offspring was likewise significantly greater than their mothers in the control (t=6.46, P<0.0001).

8.5.3 Longevity and trade-offs

There was a significant correlation between lifetime fecundity and mean adult weight of females that had experienced treatment C (t=4.58, P<0.01, correlation coefficient=0.82) (Fig. 8.1). Correlation tests performed between longevity and fecundity for females obtained from the third instar (after the second moult) experiment revealed that lifetime fecundity for females that experienced treatment A was not significantly correlated with longevity (t-value=0.13, P=1, correlation coefficient=0.66). For treatment C, an indirect influence on longevity was revealed with increasing fecundity, however, the association was not significant (t-value=-0.4, P=0.65, correlation coefficient=-0.2). Lifetime fecundity of females from the control revealed a similar non-significant relationship with longevity (t-value=-0.7, P=0.51). The analysis was not performed on treatment B, due to the low percentage of survival. For the fifth instar (after the fourth moult), females that had experienced treatment A revealed a significant indirect correlation between their weights and lifetime fecundity (t=-6.87, P<0.01, correlation coefficient=-1) (Fig. 8.2), but this was based on only five surviving females. Finally, for treatment C, the longevity of females showed an indirect significant correlation with their fresh body weight (t=-6, correlation coefficient=-1, P<0.001) (Fig. 8.3), as well as with lifetime fecundity, but the correlation was not significant (t=-10, correlation coefficient=-1, P<0.064).

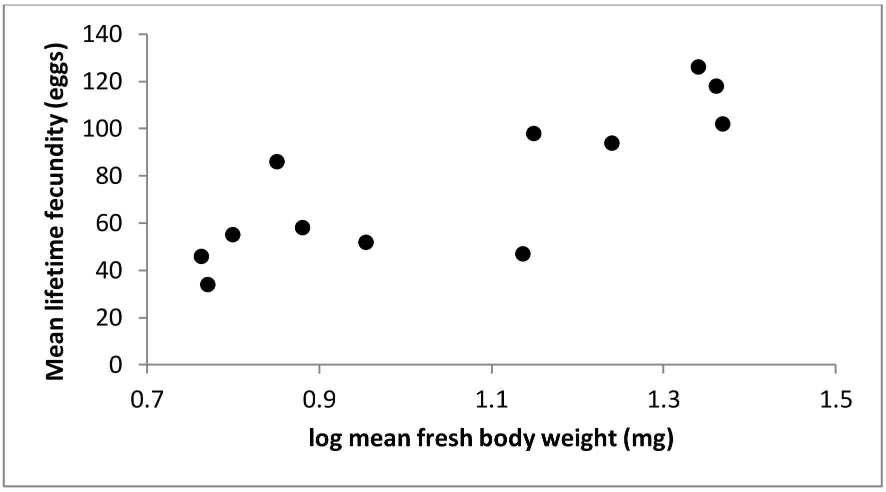

Fig. 8.1. Relationship between mean lifetime fecundity of females that experienced treatment C after their 1st moult against log mean fresh body weight (t=4.58, P<0.01, correlation coefficient, R=0.82, n=12, mean±SE). Data were transformed for statistical analysis.

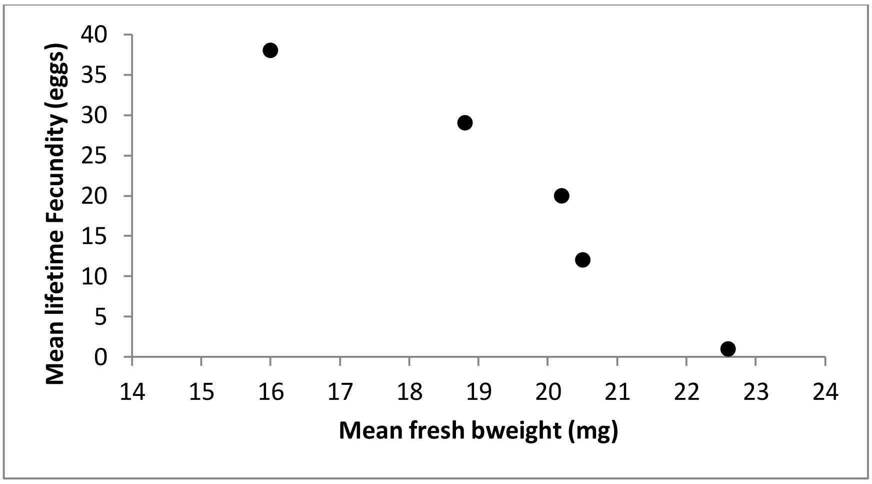

Fig.8.2. Relationship between mean lifetime fecundity and fresh body weight for females that experienced treatment A at their 5th instar (after the 4th moult) (t=-6.87, P<0.01, correlation coefficient, R =-1, n=5, mean ±SE).

Fig.8.3. Relationship between mean longevity and mean fresh body weight of females experiencing treatment C from 5th instar experiment (after 4th moult) (t=-6, P<0.001, correlation coefficient, R=-1, n=8, mean ±SE).

8.5.4 Ovary status

The ovaries were examined to investigate the effect of food and water manipulation on their general status. The typical morphology of a fully fed freshly emerged female and directly after 1-2 days of mating are presented in Figures 8.4 and 8.5, respectively. In general, the reproductive organ of a coccinellid female consists of a pair of ovaries, two lateral oviducts (which join to form a common oviduct), a copulatory sac, a pair of accessory glands and a spermatheca (Ehara, 1952, Majerus, 1994). As shown in Figure 4, for normal unstarved females, each ovary consists of elongated tubes of ovarioles that are symmetrical in shape and have a relatively similar size and number, as also observed by Obata (1988) and Osawa (2005). The shape and colour of ovaries differ according to the food availability experienced during immature development and adult stages (Osawa, 2005). A similar observation was revealed by this study when dissecting the ovaries of females that had experienced various treatments at their second and third instars. The shape of ovaries obtained from dissecting a female subjected to treatment B or A at its second stadium (after the first moult) are presented in Figures 8.6 and 8.7, respectively. For both treatments, the ovaries were found to be asymmetric in shape, pale in colour and partially developed. For the third instar (after the second moult), a female that had experienced treatment A was dissected and it was found that the ovaries had retained their ordinary morphology (Fig. 8.8). After 1-2 days mating, the ovaries had developed eggs that differed during their development stages inside the ovarioles compared with the ordinary shape, like those in the females of the control (Fig. 8.9). This implies that the performance of ovaries was unquestionably changed due to the brief exposure to stressful food conditions.

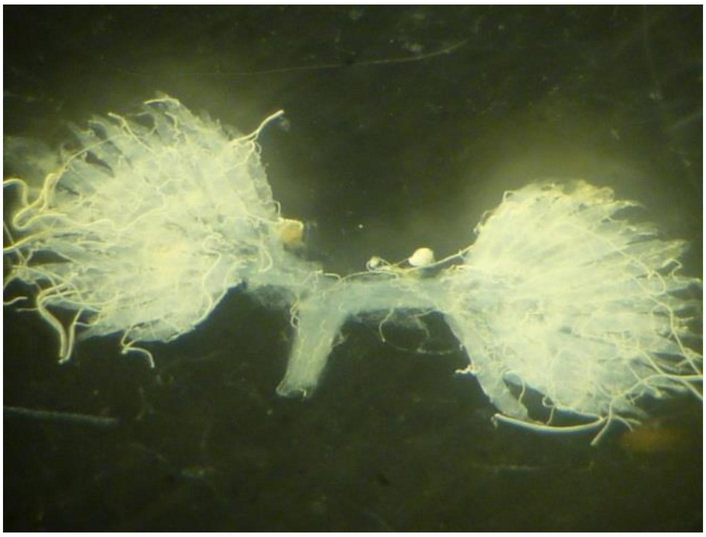

Fig.8.4. Ovaries of a fully fed newly emerged virgin female obtained from the control.

Fig.8.5. Appearance of the right and left ovaries of a fully fed female after 1-2 days of mating.

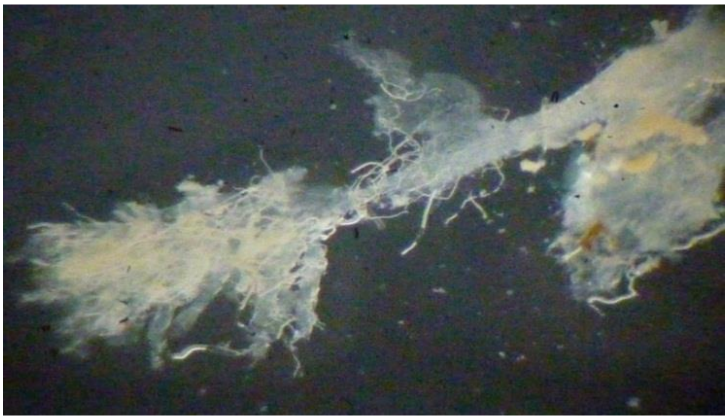

Fig.8.6. The ovaries of a female that experienced treatment B at the beginning of their 2nd instar (after the 1st moult).

Fig.8.7. Asymmetrical appearance of the left and the right ovaries of a female that experienced treatment A at the beginning of the 2nd instar (after the 1st moult).

Fig.8.8. Appearance of the ovaries of a female experienced treatment A at the beginning of the 3rd instar.

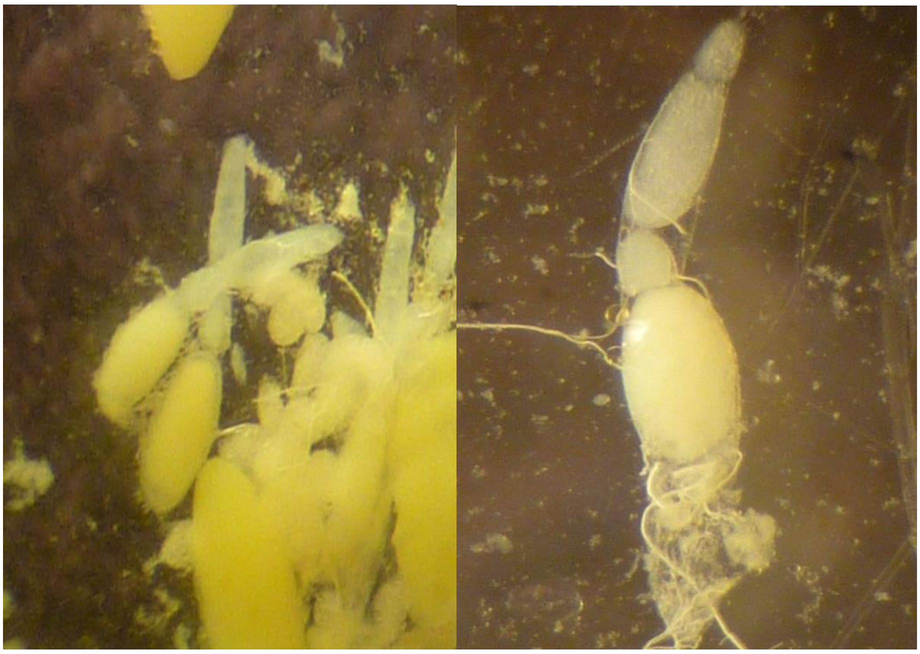

Fig.8.9. A comparison between egg development within ovarioles of normal fully fed female (on the left side) and inside ovarioles of a female that experienced treatment A at the beginning of their 3rd instar (after 2nd moult) (on the right side).

8.6 Discussion

Food quantity and quality are some of the most fundamental factors determining individual potential fitness and offspring fitness (Agarwala et al., 2008, Agarwala and Bhowmik, 2011). Any variation in food availability greatly influences life-history traits, particularly the development and reproduction performances (Agarwala et al., 2001). These two life-history traits tend to trade off with one another (Agarwala et al., 2008). Life in variable environments favours adaptive plasticity in life-history traits (Nylin and Gotthard, 1998). It is well known that timing and size at maturity will vary, depending on various ecological factors, such as resource accessibility and predation risk (Abrams and Rowe, 1996, Nylin and Gotthard, 1998). In light of the above, the study tested the effects of a transitory period of food manipulation early in the life of *H. axyridis* on both development parameters and reproductive performance. The overall results revealed that *H. axyridis* was able to compensate almost entirely for the loss in weight by the time of adult emergence. There were instantaneous costs, however, that were manifested in reduced survivorship and lifespan for most responses in this study. Mangel and Stamps (2001) stated that short-term trade-offs range from a rapid growth rate to a high percentage of mortality. In the same way, it was noted that deficiency of nutrients in the course of early development might have significant instant costs on survivorship, which is attributable to the enhanced growth rate (Gotthard, 1994, Metcalfe and Monaghan, 2001). In this study it was demonstrated that a brief period of food restriction was followed immediately by accelerated growth in most of the larvae, except for those subjected to treatments A and B during the fifth instar experiment after the fourth moult. In addition, the accelerated growth rate was highly associated with a high percentage of mortality, approaching in some cases 90% (e.g., in the second instar experiment, particularly after the first moult). Several studies have revealed that with increasing size at adult emergence, there might be an elevation in fecundity; accumulating size may, however, cause an instantaneous mortality or elevation in the possibility of mortality during the pre-reproduction period, due to a postponement in maturation (Nylin and Gotthard, 1998, Metcalfe and Monaghan, 2001).

A study conducted on *H. axyridis* by Agarwala et al. (2008) revealed that on a limited food supply of only three adult aphids per day, larvae took a longer time to develop into adults, resulting in a 40% greater mortality compared with those that had been provided with plentiful food. Similarly, larvae that were reared on a low quantity of prey took three times longer to attain maturity compared with normally fed ones (Agarwala and Bhowmik, 2011). In accordance, the present study showed that in the second instar experiment (after the first moult), larvae that had experienced either treatment A or treatment B tended to lengthen their total developmental period upon food restoration. These were the only cases in which larvae had possessed longer developmental periods compared with their control. Others that experienced the three categories of treatments A, B and C at different stadia, however, attempted to reduce that period by significantly increasing their growth rates. Total larval growth rates appeared to have an indirect influence on their likelihoods of survival. Larvae in the second instar (after the first moult) and the third instar (after the second moult) experiments were compensating entirely for adult sizes after improving the food conditions, compared with those that were subjected to treatments during the fourth instar (after the third moult) and fifth instar (after the fourth moult). Those larvae had compensated to some extent for body sizes that at last appeared significantly less than the control. Although compensatory or accelerated growth may offset some costs of the nutrient restriction period, the accelerated growth itself may be costly. For instance, some butterflies and the wood frog tadpoles, *Rana sylvatica* (LeConte, 1825) (Anura: Ranidae) tended to increase their searching rates when resources were insufficient and ultimately suffered from increased predation (Anholt and Werner, 1998, Gotthard, 2000). In addition, intrinsic costs of accelerated growth may comprise a reduction in longevity and a depressed immune function (Metcalfe and Monaghan, 2001). In another example, in the damselfly *Lestes barbarus* (Fabricius, 1798) (Odonata: Lestidae), when photoperiod was attuned to mimic late season, larvae reacted with an increase in foraging effort, as well as an accelerated growth rate at the cost of increased predation risk (Johansson et al., 2001).

A recent study revealed that larvae of *H. axyridis* that experienced low food conditions invested fat at a higher rate than protein, which is essential for egg production. They continued this behaviour even after achieving the anticipated body size (Dmitriew et al., 2009). This fact could be used to explain the reduction in lifetime fecundity for most of the survived tested females in this study. Likewise, Osawa (2005) noticed that starvation might have had a great impact on females of *H. axyridis*; even after re-feeding them, some of them were found to be unable to attain their potential fecundity and others failed entirely to oviposit. An alternative study revealed that females that had experienced low prey quantity laid about 14 times fewer eggs compared with unstarved ones (Agarwala and Bhowmik, 2011). A similar observation was further reported by this study, in particular for females that experienced treatment A during the fourth instar experiment (after the third moult). Those females were found to be significantly less fecund compared with the control. Apparently, the compensation for body weight by those females had reduced resource investment and allocation for reproduction. This was consistent with the observation by Dmitriew and Rowe (2007) on the damselflies. Honek (1993) and Sokolovska et al. (2000) reported that somatic tissue growth is limited to the larval stage, and fecundity has a tendency to be an increasing function of body size in insects. Consequently, the success of future reproduction is dependent in a large part on foraging and growth rate taking place prior to emergence (Johansson and Rowe, 1999, Johansson et al., 2001)

In this study, it was revealed those larvae that were provided with aphids only in the second instar (after the first moult) and the third instar (after the second moult) experiments had developed into larger adults compared with the control. Apparently, there might be larger nutritional benefits gained from eating only aphids. Likewise, Evans and Gunther (2005) and Nakamuta (1984ab) noticed that because of the continuous contact with the body fluids of aphids, this might stimulate a high rate of food consumption and assimilation. Having exclusively aphids as a diet appeared, however, to be highly costly, since it delayed maturation, particularly in the second instar (after the first moult) and the fourth instar (after the third moult) experiments. In addition, access to prey did not show a positive influence on either female fecundity or longevity, as those females had assigned more fat for body maintenance rather than future reproduction. In agreement, Dmitriew et al. (2009) reported that having food only at high quantity might cause stress and could be interpreted through diverse responses, based on food quality, external conditions and species. It was noted that *H. axyridis* evolved against extremely variable resources (Koch, 2003). As such, unceasing access to large amounts of food might not maximise body fitness (Dmitriew and Rowe, 2007) and has been shown to reduce both longevity and fecundity in mice (Johnston et al., 2006).

An important factor for the success of *H. axyridis* is the acquisition of a specialised reproductive system (Osawa, 2005). This system has the capacity to develop and perform in means that permit surviving and coping with discrepancies in food abundance (Eckelbarger, 1994, Papaj, 2000). Perry and Roitberg (2005) noted that females of *H. axyridis* retain plastic strategies to mitigate offspring starvation risk, these strategies can be accomplished by reevaluating the ovarian dynamics in certain ways, such as trophic egg production and the oosorption process (Perry and Roitberg, 2006). These two phenomena have been recognised largely in *H. axyridis*, in response to a number of reasons, such as nutritional insufficiency and variation in prey quality (Perry and Roitberg, 2005, Osawa, 2005). The proportion of trophic egg production varied significantly between treatments and depending on the stage at which larvae had experienced nutritional stress. Perry and Roitberg (2005) noted that trophic egg production varies according to the level of starvation risk, whether it is of the relatively great or intermediary type. Studies have verified that the percentage of trophic eggs produced under stressful conditions by a female of *H. axyridis* was 15.2% (as recorded by Kawai, 1978) or 24.5% (as recorded by Osawa, 1992). Although these values were not comparable with the maximum value revealed by this study (89%), this was not unanticipated if the background of rearing conditions was considered.

The present results revealed that there were no explicit values regarding different types of egg production, in particular for trophic eggs. It was noted in this study that trophic eggs were produced in various quantities in response to the same type of treatment, even by the control. The study concluded that the production of trophic eggs was performed principally for two purposes, first, as a provisional adaptive behaviour and second, for decreasing the risk of cannibalism by freshly emerged larvae. Production of trophic eggs in order to decrease cannibalism of viable eggs and siblings was also noticed by O'Connor (1978). The different roles for trophic eggs are discussed in depth by Perry and Roitberg (2006).

The production of trophic eggs by females of the control in this study strongly suggests that both trophic and viable eggs are alike in cost, as proposed by Banks (1956) and Osawa (1992). It has been recognised that trophic eggs have a unique shape and colour compared with viable eggs and larvae can easily differentiate between the two types (Kudo et al., 2006). In contrast, other studies showed that freshly hatched larvae were not able to distinguish between viable and non-viable (trophic) eggs (Banks, 1956, Osawa, 1992). The results of this study embraced the latter, since it was noticed during the course of the study both egg types were consumed by newly hatched siblings.

Previous studies reported that food availability has a direct effect on the length of the developmental period, reproductive period and longevity (El-Hariri, 1966, Schuder et al., 2004, Agarwala and Bhowmik, 2011). Similarly, this study showed that food manipulations resulted in a significant reduction in the length of reproductive period, particularly in the fifth instar (after the fourth moult) experiment. The study suggests that, due to food stress, the development and performance of the ovaries were extremely affected, regardless of the subsequent improved food status. This was verified after dissecting the ovaries of the starved females. Osawa (2000, 2005) also noted that in relation to the *H. axyridis* ladybird, despite the fact that the ovarian development is known to be genetically determined, its performance is greatly affected by food availability. This study revealed that even in the cases where aphids were supplied exclusively and constantly (as in treatment C and the control of the third instar (after the second moult) experiment), there was no significant direct effect either on fecundity or fertility, in spite of the long oviposition period. In agreement, it was noted that although the ovariole dynamics might recommence the process of egg production upon re-feeding, the number of viable eggs that might develop tended to be significantly less than the fully fed adults (Osawa, 2005). Therefore, the prevalent reproductive rate in this study implied that adults that experienced treatments A, B and C, particularly as in the second instar (after the first moult) and the third instar (after the second moult) experiments, invested heavily to maintain the internal state of their bodies and survival, rather than optimising their reproduction. This conclusion was based on their reproductive outputs, which were found to be sub-maximum if that of the control was taken into account as the maximum value. Therefore, it could be concluded that after experiencing stress at early instars, the main concern was to expend resources on basic physiological maintenance rather than on future reproduction. In fact, those instars did not achieve their minimum critical weight, even when they were subjected to various treatments compared with the late instars. Reznick (1985) noted that during food deprivation, a female must either expend energy on body maintenance or reproduction; if there is a reduction in energy expenditure for maintenance, subsequently the longevity becomes shorter.

In this study, lifetime fecundity was used as a quota for reproductive output, as there was a great limitation on dissecting ovaries, due to the low number of surviving adults, and counting the ovarioles prior to mating was impractical. Likewise, Leather (1988) noted that a measure of achieved fecundity is more realistic and more practical than potential fecundity, since few if any insects live long enough to achieve the latter. Since the study regarded lifetime fecundity as a measure of ovary status, it was revealed that most larvae that were re-fed after food restriction were found to be less fecund compared with the control in general. This reflects either a reduction in resource allocation to reproduction or partially developed ovarioles, as noticed after dissecting a few of the starved females. In contrast, Osawa (2005) demonstrated that the percentage of matured ovarioles per female in fully fed *H. axyridis* did not differ significantly from that of the re-fed sample. The conflict between the present study and that of Osawa was mainly due to the starvation protocols. In the latter study, adults were starved rather than larvae (as selected for starvation by this study). This confirms that the performance of the ovaries could be influenced not just by food conditions, but also by the stage at which an organism has been starved. This was further supported by the present study when the ovaries were revealed to be quite similar in morphology compared with the control. The development of the oocyte inside the ovariole, however, varied greatly.

This study anticipated that the fourth instar (after the third moult) and the fifth instar (after the fourth moult) would be more resilient to nutritional stress compared with earlier stages. In contrast, the present study revealed that the late larval stages were more susceptible to stress conditions – in particular they failed to retain the entire weight loss and were incapable of recovering as quickly as the early instar stages. For example, larvae that experienced treatments A and B at the fourth and the fifth instars tended to maintain their body weight at a lower threshold value than the control, resulting in smaller sized adults. Undertaking this process did not, however, assist them to invest more in reproduction since their lifetime fecundity was found to be lower than the control. Similarly, Kajita and Evans (2009) demonstrated that starved adults of the predatory ladybirds *Coccinella transversoguttata* (Fabricius, 1781) (Coleoptera: Coccinellidae) and *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) responded by stabilising at a lower body weight and laying fewer eggs. A similar observation was noted by Agrawala and Bhowmik (2011). Although in the present experiment adults had not been starved as in Kajita and Evans (2009), but one would expect this quality also to occur in adults that were experiencing starvation early in the immature stages.

This study also showed that the minimal viable weight was completely different from the minimum estimate of critical weight attained by the larvae in the fourth and fifth instar experiments. Davidowitz et al. (2003) noted that there is a minimal weight, known as the minimal viable size, defined as the minimal amount of resources needed for larvae to successfully pupate. This weight is also known as the lower limit of body size that is never associated with any of the physiological and morphological processes (Davidowitz et al., 2003). The second instar and third instar were starved before attaining the critical weight, but they had sufficient time to allocate and reserve an adequate amount of nutrients in order to the required critical weight. This allowed them to attain relatively the same size as the control. Their reproductive output, however, was found to be less compared with the control. The fourth instar and the fifth instar, on the other hand, had been starved before reaching the critical weight or attaining the maximum estimate of the critical weight (the peak mass). Because they were starved before these two assessment points, they had less time for recovering and allocating reserves in order maintain their bodies. Therefore, they had two options, either they accelerated their development or growth rate and transferred quickly into pre-pupae, or they extended the duration of the developmental period which might incur high percentage of death or pupae might not be able to transfer into complete adult tissues. Because of that, the fourth and fifth instars failed to achieve the aimed weight or size, resulting in adverse consequences on life-history traits, such as a reduction survival success. Therefore, it could be concluded that the influences of starvation had numerous impacts on different larval stages and the larvae at the end of the developmental course possessed a different interpretation for their responses towards food stress.

The delay in pre-reproductive period, as well as the shorter reproductive period, explained the low lifetime fecundity in most of females obtained from the fourth and fifth instar experiments. According to Singh and Pervez (2006), the pre-reproductive period is highly important for egg production and any reduction in that period might adversely influence the magnitude of fecundity and egg hatchability (fertility). The delay in reproduction recorded in females from the fourth and fifth instar experiments was a result of the greater allocation of nutrients for reproduction and to regulate the duration of the reproductive period, despite the fact that the fecundity of females obtained from the fourth instar and the fifth instar was less in comparison with the control. Another reason for the low fecundity was the short developmental time recorded for those instars. Ceryngier (2000) noted that for insects, there appears to be a trade-off between developmental time and fecundity. In addition to that, the development of the ovaries may be subjected to the conditions experienced during larval or adult stages. According to Buning (1994) and Lemos (2005), the development of ovaries often starts at late embryogenesis and proceeds throughout the larval and pupal stages. Seemingly, the development and the performance of ovaries in this study were affected significantly, in particular when larvae were starved at the fourth and fifth instar periods.

The results of this study revealed that stressing the larvae at any stage had a negative impact on longevity and the survival of adults. It seems most likely that when larvae were starved at the second and third instar, there was additional investment in body maintenance compared with the fourth and fifth instars. This was explained by the fact that the former instars had plenty of time to compensate for the weight loss. This study supported the facts that are common to many insects, if not most, there are long-term costs manifested in smaller sized adults associated with a longer developmental period, whereas short-term trade-offs appear as low fertility and a high proportion of trophic eggs (Trivers, 1972, Dmitriew and Rowe, 2007). Aside from the above, compensatory responses themselves might have substantial costs, but costs that present at the physiological and cellular levels are usually paid on a longer term (Metcalfe and Monaghan, 2001).

Reproduction is recognised to decrease longevity in insects (Dixon and Kundu, 1997, Gibbs and Van Dyck, 2010). The present results revealed that in the majority of experiments, accelerated growth rate or compensatory growth had a great impact on longevity. One of the possible consequences of deprived growth conditions is the reduction of protein turnover (Morgan et al., 2000, Dmitriew et al., 2007). The latter is regarded as a measure of the rate of protein repair and maintenance associated with ageing and cellular senescence (Dmitriew et al., 2007). Any decline in that rate would be interpreted as a decrease in longevity (Bokov et al., 2004). Trade-offs were greatly recognised between reproduction and longevity in many insect species (Dixon and Kundu, 1997), but the general relationship between resource allocation and longevity is, however, to a certain extent unresolved (Kaeberlein, 2006, Flatt and Schmidt, 2009). Several studies have shown that the correlation between fecundity and longevity might be sometimes unapparent or indirect (Blanckenhorn et al., 2002) or direct (Kaitala, 1987). For example, species with short longevity might have a direct connection between reproduction and longevity, and *vice versa*. Since *H. axyridis* is known to be a long-lived organism, the above cannot be relevant (Agarwala et al., 2008).

In this study, the trade-offs between longevity and fecundity were revealed, particularly when applying treatments A and C. The trade-offs between these two traits were also reported by Ohgushi (1996). The results showed that investing in survival that was measured by longevity had a significant cost on fecundity, as revealed by females that experienced treatment C at their fifth instar. The effect of food on fecundity is well-known in arthropods (Richardson and Baker, 1997). At high food availability, fecundity tends to increase with body weight (Honek, 1993), but when food is inadequate, the influence on fecundity depends on the weight and age of the female (Spence et al., 1996, Agarwala and Bhowmik, 2011). In this study, the relationship between fecundity and body weight was investigated and the results predicted that the relationship might be direct or positive, as in the second instar experiment when food was present (treatment C), or it might be indirect or negative in response to exclusively water, as in treatment A.

Another important trade-off revealed by this study was between longevity and body weight. Adults that experienced treatment C during their fifth larval stadium had a reduction in longevity associated with the reduction in body weight compared with the control. In agreement, Agrawala et al. (2008) reported that any reduction in body maintenance and resource preservation would have a cost on longevity. Skalski et al. (2005), and others such as Perry and Roitberg (2006) and Dmitriew et al. (2007), emphasised the significance of the degree of stress severity in revealing the trade-offs between longevity and body weight. To validate the above, a preliminary investigation performed before the commencement of this study revealed that starving the first instar larvae for more than two days led to 100% mortality. Therefore, it was decided that this study would aim to control the duration of starvation and manipulate only the type of treatments. Thus, the obtained trade-offs at different levels would be expressive and trustworthy enough to evaluate as much as those phenotypic responses that may come about under field conditions. The results at this point are consistent with Dixon and Agarwala (2002), in which the nature of the trade-offs between traits or functions could be ascribed as a triangular association linking fecundity, body weight and survivorship.

It is well established that environmental conditions are likely to influence the sex ratio and parents are under selective pressure to bias their sex ratio according to the prevailing environmental conditions (Ross et al., 2011). Most ladybird species show a 1:1 sex ratio, but in some species such as the eyed ladybird *Anatis ocellata* (Linnaeus, 1758) (Coleoptera: Coccinellidae), *Exochomus quadripustulatus* (Linnaeus, 1758) (Coleoptera: Coccinellidae), *Chilocorus renipustulatus* (Scriba, 1791) (Coleoptera; Coccinellidae) and others, the sex ratio is female-biased, reflecting the recombination of segments present at the ends of the neo-XY sex bivalent (Henderson and Albrecht, 1988). Variations in the sex ratio will be expected if this recombination is more lethal in males than females. Furthermore, the interaction between Y-linked factors and maternally inherited factors, such as the male-killing bacteria, may alter the sex ratio. The maintenance of such an excess of females in several coccinellid species reflects a selective advantage for them in natural populations of these species (Henderson and Albrecht, 1988, Omkar et al., 2005). In this study, the sex ratio varied significantly between treatments and even the control did not always reveal the female-biased ratio, as previously reported by Majerus et al. (2000). Some treatments resulted, however, in an extremely male-biased ratio, in particular when the larvae were starved at late stages at the fourth and the fifth instars. This was not unexpected, since a male-biased ratio was reported for *H. axyridis* in some areas (Heimpel and Lundgren, 2000).

Srivastava and Omkar (2004) showed that prey quality and quantity have a significant influence in altering the sex ratio. For example, more suitable food tends to alter the sex ratio towards female biasness. Another study revealed that environmental stress, such as an increase in soil pore water salinity, might affect the lepidopteran herbivore *Ascia monuste* (Linnaeus, 1764) (Lepidoptera: Pieridae), resulting in a sex ratio almost equal to 1:1, compared with the control, which demonstrated a female-biased ratio (Moon and Moon, 2010). It was reported that delayed mating in the meal bug led to a female-biased ratio (Ross et al., 2011). Although this study revealed a delay in the reproductive period, the comments made by Ross et al. (2011) could not, however, be taken into consideration, as the larvae had been starved (rather than the adults as in their study). Instead, the present study showed that the delay in reproduction, particularly in females that experienced stress at their fifth instar, gave rise to a male-biased ratio – this conclusion could be added to the study findings.

It had been noted that a period of depressed growth conditions associated with food stress had a pronounced influence on fitness and offspring phenotypes at a later stage (Metcalfe and Monaghan, 2001, Ali et al., 2003). Furthermore, even if an individual appears to recover after the subsequent nutritional improvement, the stress experienced during early development might still have profound effects on the individual itself and on its offspring (Lindström, 1999, Qvarnström and Price, 2001). Dmitriew et al. (2007) noted that poor growth conditions might delay maturity and result in small-sized bodies that may reduce the consequent fitness. This was also validated by the present study, because the females that survived from the fifth instar experiment were found to be smaller in comparison with the control and their offspring were even lighter in weight than their mothers. Small body sizes resulting as a consequence of delaying maturation were also reported by Roff (1980), Honek (1993) and Sokolovska et al. (2000). Even though all the tested juvenile stages belong to one species in this study, there appeared to be a variety of responses in response to similar treatments. In agreement, Dmitriew et al. (2007) noted that timing of the resource restriction had a pronounced role in defining the responses and the capability of an individual to compensate for that stress.

It is well known that the key factor in the invasion process is the successful development and survival of immature stages (Marco et al., 2002). How these juveniles have coped with the fluctuating environment was considered as the key promoter for conducting this study. The study pointed out some common short- and long-terms trade-offs that resulted from subjecting larvae at consecutive stages to diverse feeding regimes. The short- and long-term costs were found to be variable, depending on the age of the larvae and the stress severity. The study revealed that there is an upper limit for feeding, in which exceeding it would affect the larvae. Therefore, unceasing continuous access to diet might adversely influence the rate of egg production and may incur a great cost over longevity. This suggests that *H. axyridis* could be able to survive food deprivation or low quantity of prey without this study critically disrupting the normal performance of its biological and physiological processes. This was concluded from that fact that some survived females compensated for weight loss and others were capable of achieving fecundity that did not differ significantly from the control. Such a study on juvenile performance may possibly improve the understanding of how these larvae have been coping with habitat versatility, which plays a substantial part in invasion success. Because most species evolved in fluctuating conditions, as for *H. axyridis*, natural selection has acted on the resource allocation process, allowing effective responses and greater survival towards food scarcity (Sohal and Weindruch, 1996). This study highlighted the role of the ovaries in *H. axyridis*. They produce eggs and act simultaneously to increase the offspring survival by intensifying their provisional behaviour through trophic egg production. In addition, in some cases it was found that they reserve energy by producing asymmetrical ovaries, since developing fully developed ovaries is costly. By doing so, they contribute to increasing offspring survival. The study also demonstrated that larvae of *H. axyridis* were able to fully compensate for body size when they were starved at early stages, whereas with starvation at later stages, compensation was only partial. In most compensated cases, there was a delay in maturation and significant costs manifested in the reduction of survivorship as well as longevity and alternation of sex ratio. The types of cost and trade-offs depend primarily on whether the ladybirds have been deprived of food during their adult life or juvenile stages. Nevertheless, having variable adaptive responses towards unpredictable resources might act to enhance the survival and fitness of offspring. Further investigation must be considered to study the effect of a range of food qualities on larvae. Since the study was unable to dissect many females due to the high mortality rate, more investigations are required to examine the morphology and development of ovaries under versatile food quality and at variable temperatures. This might additionally focus on evaluating the performance of the ovaries at diverse habitats with unpredictable temperature ranges. Nevertheless, the above approaches could serve to unravel the underlying reasons of invasion success, particularly of *H. axyridis* and other invaders, and how their biology and physiology manage to withstand the different ranges of temperature. In conclusion, the results of this study support the generalisation that high lifetime fecundity associated with large body size (Evans, 2000, Hodek and Michaud, 2008) may be one important key factor promoting the dominance of introduced species such as *H. axyridis* over native competitors in resource-rich environments. Nevertheless, it revealed how *H. axyridis* possessed the distinctive ability to choose between being alive or being highly fecund, particularly when it was at a critical stage of development after food restoration.

Chapter 9

General Discussion

With increasing concern over biological invasions and their impact on ecosystems and biodiversity, there has been a drive to understand the relationship between the life-history traits of invasive species and invasion success. The study is part of the on-going efforts to measure the fitness-related phenotypes of the ladybird *H. axyridis* that favour population establishment and promote its efficacy as a successful invader. This thesis has reviewed the available research on this subject and undertaken laboratory studies to evaluate the basic biological, morphological and behavioural attributes under controlled and starved conditions. In addition, the study evaluated the phenotypic traits and the degrees of adaptive phenotypic plasticity in a response to starvation, particularly during the stressful development of larvae. The results could help to further understand the eco-physiology of this ladybird and contribute to understanding the underlying factors that have promoted its widespread invasion. This chapter aims to discuss the general findings and potential outcomes arising during the course of the overall project.

Harmonia axyridis was established in the UK at the beginning of the 21st century. Its position as an invasive species is now widely acknowledged and it is considered a suitable biological model for testing the rule of plasticity in the invasion process. *Harmonia axyridis* is often expected to show a high degree of adaptive plasticity; accordingly, it was chosen to be the model species for this study. *Harmonia axyridis* is extremely polymorphic with a wide geographic distribution. Genetic polymorphism appears to be the main strategy that it has adopted for facing different habitats at different times. *Harmonia axyridis* shows many modifications that are often known to be adaptive as a result of increasing tolerance to stressful conditions - such as body size, body shape and patterns of elytral colouration (Soares et al., 2003). It has been suggested that invasive populations should evolve toward greater phenotypic plasticity, as they come across fluctuating environments during the invasion process (Lombaert et al., 2007). The ability of a species to tolerate a wide temperature range is a critical factor in evaluating its invasion competency (Jalali et al., 2010). Since the overall evidence regarding this issue remains limited, it is, however, premature to draw general conclusions from the past information (Richards et al., 2006). Although this study was not performed at variable temperatures, assessing the phenotypic plasticities under controlled constant conditions was considered a valuable measure. The results from this study could form a reliable reference for future comparative studies, either with similar species or with other invaders.

Life-table parameters are essential when studying the general biology of an insect, since they help to determine the intrinsic rate of natural increase, regarded as the most appropriate way to describe population growth rate. Nevertheless, these tables could be incorporated to compare species performance under versatile environmental conditions (Abdel-Salam and Abdel-Baky, 2001). According to Labrie et al. (2006), some basic biological attributes of life-history traits, such as behavioural and developmental characteristics, are considered the key factors in determining competitive ability, predation efficiency, population growth rate and successful invasion. Therefore, this study was able to evaluate the reproductive rate, as well as the developmental time and other behavioural aspects of *H. axyridis* that were considered the basic measures of intrinsic growth rate. In addition, the study highlighted most intraspecific variability in life-history traits and in phenotypic plasticity under controlled measures.

The first section of this study, Chapters 2 to 7, focused on measuring various life-history traits and the associated variability under constant controlled conditions. Adults and juveniles were reared under controlled diet availability and constant temperature and humidity, with the aim of reducing the substantial influences of these variables as much as possible. This was designed so that reliable measures of these traits could be obtained and to emphasise the other relevant influences that control lifecycle attributes, such as genetic, morphological and physiological factors. Although the genetic background was not covered directly by this study, its effects were highlighted.

The designated range of temperature and the diet quantity and quality throughout the course of the experiment might be less than the optimal temperature requirement; however, this range of temperatures was documented earlier as the ideal for attaining an optimal reproductive rate, developmental period and for maintaining body weight for the ladybird *H. axyridis* (Lamana and Miller, 1998, Dixon, 2000, Stathas et al., 2001). Former studies had shown that the range of temperature that is needed to have all developmental stages accomplished at definite time is likely be within 15-30ºC (Logan et al., 1976, Roy et al., 2002, Mehrnejad and Jalali, 2004). Therefore, the temperature selected by this study was well within this range and 5±1ºC above the minimum threshold of the range stated above. A previous study that had evaluated the differences in the elytral patterns (in relation to light-coloured *aulica* and dark coloured *nigra* phenotypes of *H. axyridis*) was based on the differences in biological performances at 20°C. In addition, the range of temperature used in this study was based on several facts, for instance, the ability of *H. axyridis* to breed, survive and become accustomed to winter temperatures of below freezing and summer temperatures up to 30°C (Lamana and Miller, 1996). Obrycki and Tauber (1981) indicated that for *A. bipunctata*, which co-exists with *H. axyridis*, the optimum temperature was in the range 23-

27°C; when the temperature exceeded 26.7°C, the mortality was more than 25%. Another study on the importance of temperature revealed that the optimum rearing temperature for the spider mite predatory beetle, *Stethorus punctillum* (Weise, 1891) (Coleoptera: Coccinellidae) under laboratory conditions was 25°C (Bakr, 2009). Nonetheless, when the temperature range was selected for this study, it was taken into consideration that the estimated variability in life-history traits would not be at their optimum, but would at least be approaching their maximum standards or close to them. There was a problem in having a set of temperature ranges, due to the lack of controlled temperature rooms and manipulating and feeding hundreds of larvae at the same time was impractical. In addition, Stillwell et al. (2007ab) noted that some environmental parameters may be more appropriate than temperature to identify the phenotypic plasticity. For example, food or pre-availability may be better environmental parameters through which to test phenotypic plasticity in *H. axyridis*, since it is known to be polyphagous and encounter a taxonomically diverse range of phytophagous insects associated with various vegetation communities (Preziosi et al., 1999, Specty et al., 2003, Berkvens et al., 2008). Although generalist predators prey on diverse prey species, foraging behaviour has frequently been associated with quantitative features and has disregarded the nutrient quality of prey (Mayntz et al., 2003). Furthermore, food was noted to have a direct influence on several life-history traits, such as growth rate, reproductive period, lifetime fecundity and survival of immature stages (El-hariri, 1966). Mayntz et al. (2003) reported that both prey nutrient quality and abundance had significant but different effects on several important life-history traits, including number of instars, instar duration, development period and body weight at maturation. They specified that prey quantity was essential for growth rates, whereas prey quality had adverse effects on juvenile survivorship and female fecundity. Obrycki and Tauber (1981) reported the above in general terms, and added that in the case of ladybird coccinellids, food is usually considered the key factor determining the anticipated number of generations.

The quality and quantity of prey used in this project were based mainly on other studies that had been performed on the same species (Stewarts et al., 1992ab, Labrie et al., 2006, Agarwala et al., 2008, Kajita et al., 2010). It was found in this study that having a few aphids left uneaten sometimes reflected the sufficiency of the feeding method. The suitability of the aphid species *A. pisum* for *H. axyridis* was noted by several studies, such as Dixon (2000) and Evans and Gunther (2005).

Using body weight for comparing the successive developmental stages and adults was considered as a reliable measure, since all individuals were maintained under constant conditions. Furthermore, body mass as a measure of interaction between internal and external conditions and how insects maintain their body mass at versatile conditions has been noted in a number of studies, such as Dmitriew et al. (2009) and Phoofolo et al. (2008). Therefore, the weight for each relevant stage would reflect the situation in the field. The unreliability of body mass at unpredictable environmental conditions has been debated by Leather (1988), but it can still be used as a standard reference for intra-and inter-specific comparative studies.

Controlling measures were commenced in all the experiments except the experiment for Chapter 8. The plasticity of specific life-history traits could be accomplished by attaining the minimum requirements at which species have the ability to control environmental conditions at some level. When researchers conduct factorial experiments they often include the environmental treatments as fixed factors (Sultan, 2000, Pigliucci, 2005, Richards et al., 2006). In addition, it has been reported that the best way to measure the plasticity is by comparing the average values for a trait across different genotypes grown in each environment. Such examination of species-level plasticity can be informative in a comparative framework, assuming that these data are interpreted appropriately (Richards et al., 2006). This study did not cover the variations in genotypes; however, the intra-specific variability among life-history traits measured under controlled conditions had revealed relative influences on the genetic background as well as morphological aspects. Nevertheless, as stated above, the collected data from Chapters 2 to 7 could be used as reliable reference points for comparison with other figures recorded under entirely different environmental conditions. The last experiment, Chapter 8, focused on the study of larvae of the ladybird *H. axyridis* that had been exposed to different feeding regimes. For this experiment, the measured relative variability in life-history traits was shown to have direct associations with contrasting stress degree.

It has been noted that life-history responses, and the trade-off between fecundity and female longevity were highly dependent on the degree of food stress (Agarwala et al., 2008). Therefore, by having two distinct measurements of life-history traits - one under controlled conditions and other under food stress - would provide a thoroughly comprehensive description of the adaptive phenotypic plasticity and the associated trade-offs. The importance of the study results was discussed in the relevant chapter, but it could be suggested further that these findings would help to understand the various tactics and strategies used by the ladybird *H. axyridis* in coping with constantly fluctuating environment that have encouraged its overpoweringly invasiveness.

It has been shown that various life-history traits, such as fecundity, are highly important for effective invasion and establishment (Sakai et al., 2001, Shea and Chesson, 2002) and knowledge of such traits would help in evaluating their intraguild dominancy (which has resulted in the displacement of many native species) (Lanzonie et al., 2004). This project has investigated the reproductive performance of *H. axyridis* under both controlled and stressful conditions. One of the important biological attributes that it aimed to quantify was the proportion of trophic egg production. This behaviour is considered as a maternal adaptive provisional behaviour used to provide extra food for freshly emerged larvae and reduce cannibalism. Not all phenotypic behaviours are beneficial, only those that are of a particular importance for ecological and evolutionary aspects (Richards et al., 2006), such as trophic egg production. The study revealed that these eggs are not as costly as others had noted (Osawa, 1992, Perry and Roitberg, 2005). It turned out that these eggs were found within the majority of clutches produced, whether normally fed (as in the control) or in treatment C (stressed females during their juvenile periods); however, the proportion of this type of eggs increased with stress severity (as in treatment A and B). This was explained in detail in Chapters 6 and 8. Likewise, the production of trophic eggs often varies according to the level of starvation risk experienced by offspring, whether it is relatively high or intermediate (Perry and Roitberg 2005). With varying the levels of stress, different functions might be served by trophic eggs (Perry and Roitberg, 2006). In this study, this was also found to be true by some means. According to Perry and Roitberg (2006), *H. axyridis* was found to produce trophic eggs as a consequence of delayed mating or when manipulating the mating rates (as in Chapter 6). This was found to be consistent with Omkar et al. (2005), who stated that egg fertility increased with increasing number of matings in ladybirds. Male contribution by mating rate played an important role in determining the fertility of a female and the proportion of egg production (as also revealed by the results of Chapter 6). Perry and Roitberg (2005) noted that females of *H. axyridis* showed adaptive plasticity in laying tropic eggs and manipulated their quantity and timing according to the existence of environmental conditions. The above strategies in this maternal behaviour (trophic eggs production) explained the association between phenotypic plasticity and the colonising ability of a new habitat. Therefore, it could be concluded that this behaviour provides an important source of nutrients to avoid the risk of starvation or adverse conditions or competition when invading a new habitat. More studies are needed, such as investigating the degree of this adaptive behaviour under variable food availability and different ranges of temperatures, in order to offer valuable insight into maternal caring during the invasion process.

From the results of this study it could be argued that *H. axyridis* is equipped with reproductive strategies associated with several interacting factors, such as morphological, physiological and genetic background. These factors work simultaneously and respond to the surrounding environmental cues by optimising the function of several possible traits, such as reproductive output, internal status of the body and survivorship. Nevertheless, this ladybird has the ability to synchronise between its body maintenance and reproductive output, particularly during stressful conditions. This tactics were stated based on the capability of most of the starved instars to compensate fully or partially for body sizes and achieve fertility that was not significantly lower than the control, despite the significant trade-offs. Therefore, the strategy of females in allocating resources for oviposition and offspring provision even under stressed conditions are one of the many advantages possessed by this ladybird that have facilitated its invasion and establishment.

The main factors in the invasion process are the growth and development of juveniles into the adult stage as well as adult survival (Marco et al., 2002). When juveniles are found in great numbers, this could enhance the invasion process and may result in the displacement of native species (Marco et al., 2002). Several ecological studies are relevant when considering the growth strategy of immature juveniles and their success in reaching adulthood (Labrie et al., 2006, Phoofolo et al., 2009). The research synthesis revealed the relatively few studies that had been conducted on juvenile stages, owing to the limited potential of rearing a large number of larvae simultaneously; this makes it easier to deal with adults. This project managed to cover both adults and juvenile stages. Therefore, another objective of this study was to investigate some of the development characteristics of *H. axyridis* larvae. The existence of a high number of these larvae tends to encourage the rate of predation and accelerates the dominancy of this species over other native species (Labrie et al., 2006). As noted previously, both the growth rate and the developmental period might vary among individuals, either due to genetic constituents or variations in environmental conditions (Sebens, 1987). For insects that have similar genetic backgrounds, the differences, however, might be precisely triggered by diet availability and nutrient allocation by the final instar (Berner and Blanckenhorn, 2007). The study supported the declarations by Berner and Blanckenhorn (2007), in particular, when larvae were exposed to food stress (as described in Chapter 8), the significant variability in life-history traits during the course of the experiment was primarily due to prey manipulations. Looking back to the earlier chapters (Chapters 2 to 7), the larvae and even the pupae were reared under constant controlled conditions and fed a controlled number of aphids. Therefore, in those experiments, the synthesis was utterly different. The significant variability obtained in most of the traits was due to several factors, such as genetic influences and measurement errors. The latter was not unexpected, as taking the weight of over 100 larvae at the same time was impractical.

Another important issue regarding the larval development experiment in Chapter 3 was that every larval stadium by itself played a contributing role in determining both the successive stage and the final adult size, for example, the direct linear relationship revealed between second instar weight with the last fifth instar weight (other examples are mentioned in Chapter 3). Therefore, the synthesis of this project concluded that for individuals derived from the same genetic background and reared under constant conditions, the success of their developmental process was multi-factorial. Nevertheless, the results showed that the post-critical period, as well as the developmental period, is definitely flexible in the developing larvae of *H. axyridis* and could be adjusted according to the stress type and degree.

The study revealed the existence of the fifth larval instar, which was reported for the first time by Labrie et al. (2006). In their study, the fifth instars occurred only in 335 of the total number of ladybirds of *H. axyridis*. In addition, they used a constant temperature of 24°C (which was warmer than that of this project) and a lower relative humidity (60–75%). The food used was a mixed diet of pea aphids, *Acyrthosiphon pisum,* a liver-based artificial diet and eggs of *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae). The existence of the fifth instar by this study could be explained, as all larvae designated for this project were collected from the second generation of a culture maintained under controlled temperature, humidity and fixed diet. Keeping those larvae under predicted constant and favourable conditions until obtaining the second generation had positive influences on the pattern of development that stimulated the extra growth of this stage. Apparently, the rearing conditions had critical influences on the presence of this additional stadium. Likewise, Mayntz et al. (2003) noted that the low prey quality tended to reduce the number of instars in *H. axyridis*. This also supports the theory that the species selected by this study as a prey was appropriate. The occurrence of the fifth larval instar suggests an increase in fitness that was found to be consistent with Labrie et al. (2006). It known that this stage is characterised by an increase in voracity, aggressiveness and predation efficiency (Lucas et al., 1997, Lee and Kang, 2004). Therefore, the presence of an extra instar associated with an increase in aggression might be another crucial factor that helped to increase the predation rate and displacement of many native, even conspecific species, and accelerated the invasion of this ladybird worldwide.

Finally, in terms of sex determination, the study showed that it could be easy and reliable to identify the sex of the adult prior to emergence, either from the initial weight of the larval instar (as in Chapter 3) or from the weight of pupae (as in Chapter 5). Mayntz et al. (2003) noted that the prey availability influenced sex-ratio in *H. axyridis*; this was likewise found in this study, particularly in Chapter 8, when the prey availability was monitored for a brief period. There was no consistency in sex ratio among the emerged adults that experienced different treatments. Therefore, both prey quality and quantity may be critical elements modelling the evolution of life-history traits in generalist predators such *H. axyridis*.

Pupal colour dimorphism had already long been recognised by a number of studies (Smith, 1980, Yamamoto et al., 2011). The pattern of pupal colouration, in addition to the weight mentioned above, would be useful in identifying the sex of an adult before conducting an experiment. Identifying the sex from the colouration of the pupae is considered a novel method, introduced exclusively by this study for the first time. Chapter 7 accentuated that the antennae of *H. axyridis* is multifunctional sense organ with high specificity that interact with other sense organs to intensify the sense response towards the external cues. Nevertheless, it revealed this organ has differential function and specificity that varies between males and females, besides foraging activities, prey searching and mate recognition. Self-orientation and movement control are additional important functions that optimize foraging and maximize their encounters with the most profitable prey (Stephens and Krebs 1986).

The high aggressiveness of males over females was also revealed in Chapter 7. All of the above were considered as significant outcomes from the present project that might contribute to maximising the efficiency invasion process and the establishment of this predator over a wide range, where it started to harm the ecological integrity of an ecosystem.

Another objective of the current study was to appraise the relationship between body parameters and lifetime fecundity. It was previously reported that the link between fecundity and body parameters was not always straightforward (Leather, 1988). Similar findings were also adopted in Chapter 3. The study showed that in terms of *H. axyridis* fitness, it was always associated with having large body size and high fecundity, as also noted by Phoofolio et al. (2009). During the course of investigation, there were few small-sized females that were found to be highly fecund; however, their fecundity never exceeded the maximum fecundity of large-sized females. Unexpectedly, a direct relationship was found between the two above variables in two cases, first, when larvae were starved at early stages, particularly during the second instar and the third instar experiments, as described in Chapter 8, and second when daily egg clutch size (instead of fecundity) per female was regressed against the mean fresh body weight (as in Chapter 3). The study synthesis revealed that there are certain considerations that must first be taken into account, the total lifetime fecundity does not always give a reliable information on the body size. Second, measuring the weight of body only once through its lifespan is incorrect, as body size is not fixed, but changes according to several factors, such as the time of reproduction and food deprivation or nutrient quality. This was also noted by Dixon and Agarwala (2002) and Honek et al. (2007). It could be concluded that the direct association between female body weight and fecundity was obscured by other interacting factors, even in the presence of food, for ovariole number, where the presence of fat tissues become an obstacle for ovariole growth (as noticed by this study), male contribution might not be sufficient, and the age of the female and mating experience also played a part. Even continuous access to food might overstress the female, appearing to be costly rather than advantageous (as revealed from the starvation experiment when using treatment C in Chapter 8). It was noted that egg oviposition in the ladybeetle *H. axyridis* showed an L-shaped response to age at maturity. This may have significant costs on population dynamics in variable environments. If the females are well-fed, they would grow at a greater rate, mature early and lay a large number of eggs (Agarwala and Bhowmik, 2011). The large number of eggs may give rise to a high number of larvae being expected to undergo slow growth and suffer from interspecific competition, particularly when food is limited or under an unpredictable environment (Agarwala et al., 2008). Therefore, the association between age and fecundity towards food availability is mainly mediated by competition and is a potential mechanism for compensating density dependence in a fluctuating environment (Gotthard and Nylin, 1995).

Many factors have been addressed by Stearns (1992) and Omkar and Mishra (2005) that might have an effect on lifetime fecundity and egg viability, such as parent age, suitability of prey and environmental conditions. The deterioration in fecundity with age might be mainly due to the reduction in the efficiency of prey capturing, due to senescence. The results obtained from Chapter 3 did not show any effect of senescence on fecundity, as well as egg hatchability, most females were able to capture their prey successfully until they died, however, the association between fecundity and senescence that shapes survivorship might be entirely different from the field. In the experiment described in Chapter 3, all the couples were provided daily with sufficient prey and were confined in a small area (the Petri-dish). Therefore, the energy expenditure needed for foraging and searching for prey would be very low and might not affect their daily activities or even their longevity.

In addition, Dixon (2000) noted that the pattern of egg oviposition may shape the relationship between longevity and lifetime fecundity. This study revealed that oviposition in *H. axyridis* was intermittent, which might influence interpretation of fecundity and longevity association (as shown in Chapter 3). Continuous or daily oviposition might need more energy expenditure and additional nutrient allocation; such matters may have a negative impact on longevity. The association between longevity and fecundity needs certain factors to elicit it and make it more apparent. In this study, such relationship was found more pronounced in two particular cases, first, when females had limited access to mating with males or did not have the choice to select their mates (as shown in Chapter 6); and second, the fecundity-longevity trade-offs appeared strongly when manipulating diet (as in Chapter 8). In addition, the project proved that one mating might be enough to provide females with lifetime fertility. Females that had mated once were able to lay more fertile eggs compared with other cases. This was considered another advantage that could be added to the many revealed by this study on this ladybird.

In summary, *H. axyridis* is a very adaptable species. Being able to adapt itself to food variability through the strategy of accelerating growth rate is just one of its remarkable features. One of the main reasons for its success must surely be the physiological versatility of its specialised reproductive system. It possesses highly flexible ovarian dynamics that could perform efficiently during food stress by equilibrating the number of hatched larvae and the trophic eggs, as well as serving as an energy preserve system by producing asymmetrical ovaries (one would be completely developed and the other would be deformed). This is a clever strategy, since developing both might be highly costly when food was insufficient. This ladybird is characterised by being explicitly territorial as an adult and highly aggressive and hostile during immature stages. The project highlighted different aspects of life-history traits that are likely to quicken its efficiency as a predator, and presented a picture on how this ladybird might cope with the environment-induced variations in its life-history traits. The results of this project could be used to as an appropriate reference to describe the performance of adults and immature stages of *H. axyridis* in field conditions. It became an invasive species, affecting the dynamic and configuration of a number of guilds through direct or indirect interactions. Thus, all the above-defined developmental characteristics, coupled with behavioural features including aggressiveness and great predation efficiency, have facilitated the juvenile development, competitive success and foraging ability of this species and contributed to the invasive success of *H. axyridis.* Competitive displacement is the most severe consequence of interspecific competition following *H. axyridis* invasion, in which competition for food may result in competitive elimination of many co-existent native species. The aggressive behaviour of *H. axyridis* larvae probably accounts for the replacements recorded particularly in ladybird communities (Yasuda et al., 2001, Agarwala et al., 2003). Undoubtedly, the ongoing invasion process and establishment of this ladybird have evolved toward greater phenotypic plasticity because they encountered differing environments during the invasion process. This was confirmed when Lombaert et al. (2007) demonstrated that the invasive population of *H. axyridis* exhibited significantly greater plasticity, as well as general fitness, and displayed a significantly higher survival rate compared to non-invasive species of *H. axyridis.* Thus, the study could be incorporated into integrated pest management programmes to control the release of *H. axyridis.* Although several laboratory and field studies have attempted to examine the effects of some insecticides (including carbonyl, bifenthrin, zeta-cypermethrin, thiamethoxam and imidacloprid) that have exhibited either a toxic and/or repellent effect on *H. axyridis*, of these insecticides, only carbaryl and imidacloprid are presently labelled for use on wine grapes within seven days of harvest, the period in which *H. axyridis* normally reaches high densities

(Galvan et al., 2006). Future research should concentrate more on studying the efficacy of other insecticides that are not so labelled, but have shown high toxicity to *H. axyridis*, such as lambda-cyhalothrin and chlorpyrifos (Galvan et al., 2005, 2006). Insecticides, however, are only used when beetles are present (Galvan et al., 2006). More investigation should be focused on the genetic background and evolution of this particular species, an area that was not covered by this study, in order to come up with the best methods for stopping new invasion and encouraging the eradication of nuisance ladybirds.

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