Investigation on effects of elevated temperatures on the potential of coccinellids as natural enemies of cereal aphids in winter wheat

Untersuchungen zum Einfluss steigender Temperaturen auf das Potential der natürlichen Regulation von Coccinelliden bei Blattläusen an Weizen

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I. List of abbreviations

ANOVA	Analysis of Variances
BBCH	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundessortenamt und Chemische Industrie
d	Days
dd	Day degrees
df	Degrees of freedom
DNA	Deoxyribonucleic acid
etc.	Et cetera
ERH	Enemy release hypothesis
FCR	Food conversion ratio
h	Hour
i.e.	Id est
IGP	Intraguild predation
L	Liter
LD	Light/dark (photoperiod)
LDT	Lower development threshold
m^2	Square meter
mg	Milligram
µg	Microgram
MIC	Minimal inhibitory concentration
n	Number
p	Significance level
P	Probability of Error
PU	Predator Unit
SEM	Standard error
SET	Sum of effective temperatures
%	Percent

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IV. Abstract

In a series of preliminary experiments and three main studies we investigated the effects of elevated temperatures on native seven-spot ladybird (*Coccinella septempunctata* L.) and invasive Asian ladybird [*Harmonia axyridis* (Pallas)] as natural enemies of the grain aphid, *Sitobion avenae* (Fabricius), the most important cereal aphid in winter wheat in Germany.

Preliminary climate chamber experiments, conducted from 2006 to 2009, used two alternating night and day temperature regimes to simulate normal (mean: 18.7 °C) and elevated (3 K higher, mean: 21.7 °C) temperature conditions. In microcosm experiments both ladybird species exhibited similar predatory efficiencies against *S. avenae* under climate chamber conditions. Both species were able to reduce aphid infestation of winter wheat by up to 50%, whereby temperature only had slight effects. In a mini field experiment the two coccinellids showed a distinct dominance within an aphid predator community at both temperature regimes and the number of predators collected after three weeks of experimental period was seven times higher at elevated than at normal temperatures. When exposed to direct competition, *H. axyridis* showed higher competitive potential than *C. septempunctata*. The preliminary climate chamber laboratory experiments, which investigated the effects of elevated temperatures on life table parameters of separately treated ladybird individuals, revealed some interesting differences that indicated different responses to these changed environmental conditions.

The three main studies (2009 to 2012), which were based on the previous results and enhanced methods [e.g. more realistic daily temperature profiles; normal (T0, mean: 17.8 °C) and elevated (T3, Mean: 20.8 °C)], confirmed the preliminary findings. Both species responded similarly to elevated temperatures by increasing their consumption rates by up to 75% but differently in terms of the conversion of consumed biomass. *Coccinella septempunctata* raised its body weight and fat body contents, whereas *H. axyridis* was less affected and scarcely showed changes in life table parameters in response to higher temperatures. Specific investigations revealed that the life table parameters of adult coccinellids seem to be mainly determined by the temperatures experienced during preimaginal development. Long-term rearing of *H. axyridis* over a period of two years for up to 17 generations at normal and highly elevated temperatures (T6, mean: 23.8 °C) enabled the investigation of possible

long-term changes, such as the awakening of sleeping genes, in life table parameters and antimicrobial activity in the hemolymph of this species in response to elevated temperature conditions. No clear changes in life table parameters could be identified, but we observed a high potential of *H. axyridis* to rapidly respond to new temperature conditions unaffected by previous long-term temperature experience.

Overall, the present findings suggest that the two studied coccinellid species respond differently to elevated temperatures. *Coccinella septempunctata* seems to be more strongly affected and builds up higher weights and fat body reserves. However, different life strategies hamper conclusions about possible consequences. Further studies of these and additional influencing factors are much needed to obtain more detailed data on how elevated temperatures might influence the potential of the two ladybird species to control *S. avenae*.

1. Introduction

The Fourth Assessment Report of the United Nations Intergovernmental Panel on Climate Change (IPPC) projects mean warming of 2.3 to 5.3 °C in Northern Europe by 2080. Such an elevation would have remarkable effects on plant diseases, pests and their natural enemies. According to Jahn and Freier (2001) as well as Hullé et al. (2010) climate changes are able to directly and indirectly affect pests. They affect them directly by influencing population dynamic processes, which are considerably determined by climate variables such as temperature. Moreover, they affect them indirectly through mechanisms such as changes in the phenology of their host plants and in the population dynamics of their natural enemies. Jahn and Freier (2001) summarized the potential changes in plant diseases and pests due to elevated temperatures as follows:

- changes in geographic distribution of pests and their natural enemies,
- prolongation of the growing season,
- increased number of generations per growing season due to accelerated development,
- alteration of plant-pest synchronicity and of plant-pest-beneficial interaction,
- increased risk of invasion of migratory species.

Mild winters could additionally lead to anholocyclic hibernation and thus to earlier immigration of pests into crops at the beginning of the growing season. Based on this current knowledge, scientists expect that even small changes in temperature could significantly affect the distribution and agricultural relevance of pests and their natural enemies.

Cereal aphids are the most important pest of winter wheat in Central Germany and coccinellids are one of their most important natural enemies (Poehling et al. 2007). Therefore, it is of particular interest to know how this prey-predator system will be affected by elevated temperatures. Some scientists assume that elevated temperatures could increase the predatory potential of coccinellids (Dunn 1952, Frazer & Gilbert 1976, Triltsch et al. 1996).

Therefore, the aim of the present study was to investigate the effects of elevated temperatures on coccinellids within the tritrophic system formed by winter wheat, cereal aphids and ladybirds. In a series of climate chamber experiments conducted at Julius Kühn-Institut in Kleinmachnow, Germany we comparatively investigated the native seven-spot ladybird, C*occinella septempunctata* L., and the invasive multicolored Asian ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) feeding on the grain aphid, *Sitobion avenae* (F.) (Homoptera: Aphididae) in winter wheat.

First, this thesis will report on the preliminary climate chamber studies, which were conducted from 2006 to 2009 using alternating night and day temperatures before later adaption of the temperature regimes. These first experiments revealed interesting differences between the two ladybirds. For example, *C. septempunctata* and *H. axyridis* tended to respond similarly to elevated temperatures in terms of increased consumption rates but differently in terms of body weight and fat body accumulation. Thus, we hypothesized that fundamental differences in the responses of the two ladybirds to elevated temperatures probably exist and that these differences may considerably affect their potential as natural enemies of cereal aphids under elevated temperature conditions.

Based on these findings a further series of climate chamber investigations was conducted using enhanced methods with more realistic daily temperature profiles in the main part of this doctoral research. This enabled us to obtain more detailed data on the effects of elevated temperatures on mortality and development as well as on the consumption rate, body weight and fat body content of adult *C. septempunctata* and *H. axyridis*. These investigations were divided into three separate studies, which addressed the following issues:

a) Can previous findings on the effects of elevated temperatures on life table parameters of *C. septempunctata* and *H. axyridis* be confirmed by using new, more realistic daily temperature profiles and at which developmental period temperature mainly affects the characteristics of adult beetles of both species?
b) If both coccinellids tend to respond similarly to elevated temperatures in terms of consumption rates but differently in terms of body weights and fat body accumulation, how does their food conversion efficiency differ?
c) Which effects does long-term exposure to elevated temperatures have on coccinellids? Is it possible to detect adaption processes, such as those caused by the awakening of sleeping genes, using the example of *H. axyridis*?

2. Literature review

2.1 The tritrophic winter wheat - cereal aphid - ladybird system

2.1.1 Winter wheat and cereal aphids

Winter wheat is the most important crop in Germany with approximately 3.2 million hectares of cropping area in 2011 (de.statista.com; data source: Federal Bureau of Statistics, read on 17th November, 2012). Cereal aphids are known as its main insect pest (Oerke & Steiner 1996).

Sitobion avenae (F.), *Metopolophium dirhodum* (Walk.) and *Rhoplaisiphum padi* (L.) (Homoptera: Aphididae) are the primarily cereal aphid species in winter wheat in Central Germany. Infestation with these species can lead to yield losses by the transmission of viruses, primarily in autumn. Additionally, the aphids can hamper the storage of assimilates by sucking phloem in spring and early summer periods. The infestation of aphid produced honeydew with fungal pathogens is also able to affect yield-building processes by decreasing photoperiodic efficiency.

2.1.2 The grain aphid [*Sitobion avenae* (F.)]

Sitobion avenae particularly feeds on ears of wheat to meet its high nitrogen requirements (Freier & Wetzel 1980). The infestation of ears can result in much more massive yield losses than the infestation of leaves. Therefore, *S. avenae* is of particular agri-economical importance (Rappaport 1999). According to Carter et al. (1982), Heyer (1988) and Freier and Triltsch (1996) the population dynamics of *S. avenae* and other aphid species is considerably affected by weather conditions, cultivar choice and predatory potential (Carter et al. 1982, Freier & Triltsch, Heyer 1988). Due to their short developmental time and low developmental threshold (approximately 4 °C) cereal aphids are expected to be particularly strong affected by global warming (Harrington et al. 2007). Triltsch et al. (1996) measured the highest reproductive rates of *S. avenae* under laboratory conditions in climate chambers at 22 °C. Likewise, Dean (1974) determined that 22.5 °C was the temperature of fastest development. This temperature range clearly exceeds current temperature conditions by about 17 to 18 °C during the period of highest aphid infestation in winter wheat in Central Germany, which is from the end of June to the beginning of July as determined on mean temperatures from June 20^{th} to July 10^{th} of the years 1998 to 2006 [data source: German Meteorological Service (DWD)]. Hullé et al. (2010)

confirm that most aphids live in areas with temperatures below their optimum. Triltsch et al. (1996) revealed that an elevation of temperature from 17 to 22 °C caused a 10% increase in aphid reproduction rates in *S. avenae*. According to Poehling et al. (2007) *S. avenae* normally produces approximately eight generations per vegetation period. Yamamura and Kiritani (1998) found that a 2 °C temperature increase can result in up to five additional generations.

2.1.3 Coccinellids as natural enemies of *Sitobion avenae*

The enormous potential of the grain aphid (*S. avenae)* to increase its reproductive rate under elevated temperature conditions raises the question of whether its natural enemies benefit to an equal degree and of how the natural control of aphids could be affected by these changing temperature conditions. The most important antagonists of *S. avenae* are classified into the families of Coccinellidae (Coleoptera), Syrphidae (Diptera) and Chrysopidae (Neuroptera) as well as into the group of primary parasitoids, e.g. Aphidiidae and Aphelinidae (Hymenoptera), and fungal entomopathogenes (Entomophterales) (Ferran et al. 1987, Kreuter 1990, Freier et al. 1996, Tenhumberg & Poehling 1991).

Among them, coccinellids are one of the most important groups of antagonists (Poehling et al. 2007), whereby the native seven-spot ladybird (*C. septempunctata*) is of particular importance. According to Freier et al. (1997) *C. septempunctata* is the aphid predator with the highest feeding potential. They assigned one adult *C. septempunctata* female a Predator Unit (PU) value of 1.0 which correlates to a consumption rate of 100 aphids per day under laboratory conditions of 20 to 22 °C. The multicolored Asian ladybird, *H. axyridis*, originally native to China, Japan, Korea, Mongolia and Siberia, was first detected in Germany in 1999 (Tolasch 2002, Brown et al. 2008). In subsequent years, *H. axyridis* rapidly spread in Germany and is now qualified as an established invasive species. It primarily occurs in crops such as maize, potato and pea but is also known to colonize cereals in other countries, where it also feeds on cereal aphids (Colunga-Garcia & Gage 1998, Nault & Kennedy 2003, Snyder et al. 2004). Thus, *H. axyridis* is expected to colonize cereals in Germany as well.

The simultaneous occurrence of *C. septempunctata* and *H. axyridis* would induce a direct competition between these two species. Therefore, it is of particular interest to comparatively determine how these two species respond to elevated temperatures and how their predatory potential will be affected. Dunn (1952), Frazer &Gilbert (1976) and Lucas et al. (2002) concluded that elevated temperatures will considerably affect coccinellids and that their predatory efficiency could increase under warmer conditions. Likewise, Triltsch et al. (1996) assume that coccinellids will benefit more from elevated temperatures than cereal aphids. Climate chamber experiments conducted by Triltsch et al. (1996) showed that daily consumption rates of *C. septempunctata* increased by 200 to 400% under elevated temperature conditions (17 to 22 °C). This increase clearly exceeded the increase in aphid reproduction rates measured under the same conditions.

2.2 *Coccinella septempunctata* and *Harmonia axyridis*: A comparison

2.2.1 General knowledge

There is already an enormous body of knowledge about the two aphidophagous coccinellids *C. septempunctata* and *H. axyridis*. The evidence demonstrate amazing similarities but also very relevant differences in specific life table parameters of the two species.

For example, the eggs, larvae and pupae of both species are very similar in size. According to Freier and Gruel (1993) 5.2 to 8.00 mm is the length of *C. septempunctata* imagines, according to Kuznetsov (1997) the length of *H. axyridis* imagines is 4.9 to 8.2 mm. However, the color patterns of the two species differ considerably. *Coccinella septempunctata* usually has red elytra with seven separate black spots (Figure 1a). Deviations from this phenotype are rare (Majerus 1994).

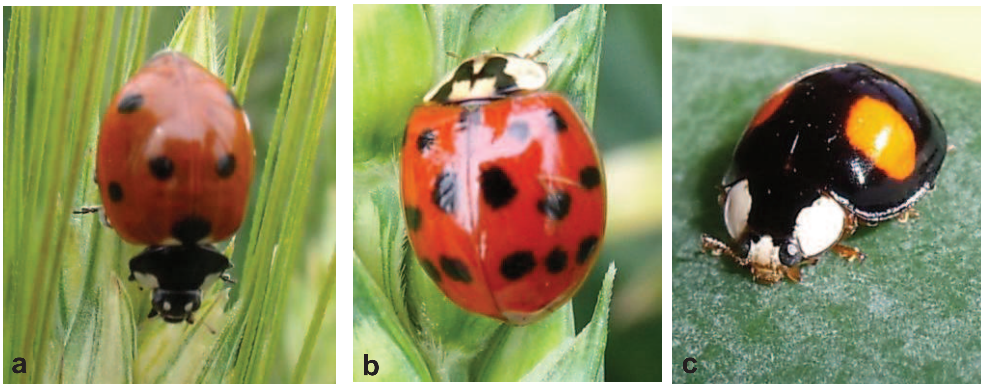

Figure 1: Phenotypes of a) *Coccinella septempunctata* adult, *Harmonia axyridis* adult of b) succinea group and c) melanic group (pictures by Sandra Krengel)

In contrast, coloration and maculation in *H. axyridis* is highly variable (Korschefsky 1932), ranging from yellow-orange to deep red elytra with zero up to 19, or also stated 21 (Berkvens et al. 2008), single black spots (succinea group, Figure 1b) or even black elytra with reddish spots (melanic group, Figure 1c) (Koch 2003, Soares et al. 2003).

Both are polyphagous coccinellids and predators of soft-skinned insects (Koch & Galvan 2008), whereby aphids are considered to be their essential prey, i.e. the type of prey required to fuel essential processes such as development and reproduction (Majerus 1994, Hodek & Honek 1996). In the case of *C. septempunctata*, aphids are stated to be their primarily prey (Majerus 1994). Scale insects (Coccoidae), adelgids (Adelgidae), mites (Acari) as well as honeydew, pollen, nectar and mildew serve as alternative prey (Majerus 1994). *Harmonia axyridis* is particularly polyphagous (Hodek & Michaud 2008). Koch (2003) compiled from literature the following list of essential and alternative prey for *H. axyridis*: spider mites (Tetranychidae), psyllids (Psyllidae), scale insects (Coccoidae), preimaginal stages of leaf beetles (Chrysomelidae), weevils (Curculionidae) and butterflies (Lepidoptera) as well as pollen and nectar. Furthermore, cannibalism within their own species and intraguild predation (IGP) towards other predators plays a decisive role in population dynamics of *H. axyridis* (Osawa 1993, Pell et al. 2008, Alhmedi et al. 2010).

A very important difference between the two coccinellid species is that they differ distinctly in terms of voltinism. *Coccinella septempunctata* usually produces one generation per year in Central Europe and is thus a univoltine species (Hodek & Honek 1996). In contrast, *H. axyridis* is able to produce up to three generations per year in these climes and is thus bi- or polyvoltine (Koch 2003).

Fertility is another remarkable difference. While the highest number of eggs per female *C. septempunctata* reared under laboratory conditions was 1600 (Hämaläinnen & Markkula 1972), *H. axyridis* can achieve oviposition rates as high as 3819 eggs per female (Hukusima & Kamei 1970).

Both species differ in hibernation behavior as well. *Coccinella septempunctata* hibernates near the ground, under stones and litter, inside little holes in the soil or in grass tussocks near their mating sites and rarely forms aggregations larger than 10 individuals (Hodek & Honek 1996). *Harmonia axyridis* forms huge aggregations and prefers to hibernate in cracks or crevices in rocks and in walls facing south-west (Koch 2003).

2.2.2 Effects of temperature

A lot of studies have been dedicated to the effects of different temperatures on life table parameters of *C. septempunctata* and *H. axyridis*. Freier and Rossberg (2001) for example, investigated which stages and processes within a coccinellid population are regulated by driving variables, of which the temperature is one of the most important (Freier & Triltsch 1996, Leslie et al. 2009, Khan & Khan 2010). This was done by using a compartment network (Freier & Rossberg 2001) (Figure 2). Such temperature-dependent processes are immigration, emigration, mortality, development, consumption and reproduction as well as derived parameters such as food conversion efficiency.

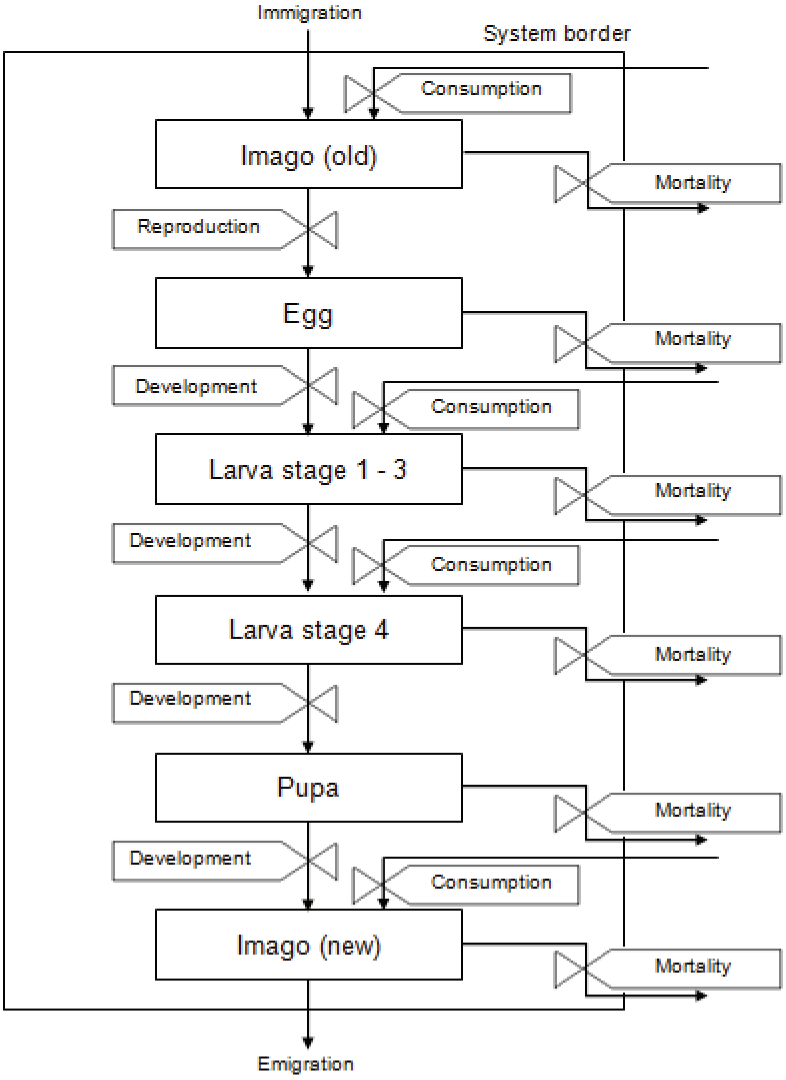

Figure 2: Compartment network representing temperature-dependent stages and processes of a coccinellid population (illustrated according to Freier & Rossberg 2001)

As most of the existing studies on temperature-dependent life table responses only investigated one of these two coccinellid species, little comparative data exist. Moreover, the use of different experimental conditions, such as different mean temperatures and temperature regimes (constant or alternating) and the study of different developmental stages, impairs the comparability of the existing data. Nevertheless, an overview of the current knowledge and understanding of the effects of elevated temperatures on mortality, development, consumption, body weight and fat body content of the two target species will be presented in the following sections.

2.2.2.1 Mortality

Mortality of coccinellids is affected by abiotic factors (e.g. temperature and humidity) as well as biotic factors (e.g. quantity of prey, cannibalism, predators and parasitoids). After summarizing the mortality data originally collected by Shands et al. (1972), Frazer (1988) and Iperti (1991), Triltsch et al. (1996) concluded that only 2 to 5 individuals of 1000 coccinellid eggs survive and reach adulthood. There is an optimal temperature range where mortality is relatively low, and two ranges of lethal temperature stress - low minimum and high maximum (Poutsma 2008). Srivasta and Omkar (2003) investigated the effects of several constant temperatures on the mortality of *C. septempunctata* larvae and found that the highest mortalities rates occur at 20 °C and the lowest at 30 °C. Katsarou et al. (2005) studied temperatures ranging from 14 to 23 °C and found that mortalities for *C. septempunctata* eggs, larvae and pupae were highest at 14 °C and lowest at 23 °C. Hodek and Honek (1996) stated that studies on the effects of low temperatures on coccinellids are still rare. It is already established that coccinellids are able to develop antifreeze chemicals to depress their freezing points. Thus, they are more or less frost resistant until their lower lethal temperature is reached. This effects is called supercooling. The external temperature that triggers supercooling is the supercooling point (Hodek & Honek 1996). The supercooling varies according to factors such as species, area of origin and cold acclimation and is therefore quite variable even within a species. Berthiaume et al. (2003) and Wantanabe (2002) reported -10 °C and -20 °C, respectively, as the lower temperature range of strongly increased mortality in *H. axyridis*.

2.2.2.2 Development

Coccinellid development, which is probably the most studied life table parameter of both ladybirds, is strongly influenced by environmental conditions such as temperature. The two species require certain minimal temperatures to start developmental processes; these temperatures are referred to as lower developmental threshold (LDT). Each developmental stage has a particular LDT. LDT values between 10.7 to 13.2 °C for overall development from egg to adult for *C. septempunctata* (Katsarou et al. 2005, Butler 1982) and from 10.5 to 11.2 °C for *H. axyridis* (Schanderl et al. 1985, LaMana & Miller 1996). The LDT is dependent on the place of population origin and day length as well as on prey quality and quantity and population density (Honek & Kocourek 1988). Thus, the values can vary even within species. Based on the specific LDT values, it is possible to calculate the thermal constant, also referred to as the sum of effective temperatures (SET), the sum of mean daily temperatures over LDT required for completion of development. Despite considerable variation, even within species, the mean SET of both species show relatively similar values of approximately 240 day degrees (dd) for *C. septempunctata* and 267 dd for *H. axyridis*, whereby much more data is available on *C. septempunctata*. Table 1 gives an overview of existing LDT and SET data on the two investigated coccinellid species.

Table 1: Sum of effective temperatures (SET) and lower developmental threshold (LDT) for total development of *Coccinella septempunctata* and *Harmonia axyridis* [data on *C. septempunctata* obtained in part from Hodek & Honek (1996)]

Coccinella septempunctata				***Harmonia axyridis***			
LDT (°C)	SET (dd)	Origin	Reference	LDT (°C)	SET (dd)	Origin	Reference
11.7	199.7	Czech Republic	Hodek 1958	11.2	267.3	USA	LaMana & Miller 1996
9.3	320.0	USA	Alan 1980	10.5	231.3	France	Schanderl et al. 1985
12.1	208.0	USA	Obrycki & Tauber 1981				
13.2	191.0	USA	Butler 1982				
10.7	281.5	Greece	Katsarou et al. 2005				

Within a favorable range elevated temperatures have a decreasing effect on developmental time (Hodek & Honek 1996). Obrycki and Tauber (1981) measured decreased developmental times (20 d less) of *C. septempunctata* [feeding on *Acyrthosiphon pisum* (Harris)] in response to elevation of constant temperatures from 18.3 to 32.2 ° C. Triltsch (1996) observed developmental times of 20.8 d at 17 °C (LD 16 h:8 h; 20/11 °C) and 15.4 d at 25 °C (LD 16:8; 28/19 °C) from egg to adult time in *C. septempunctata* feeding on *S. avenae*. Katsarou et al. (2005) reported 70.4 d at constant 14 °C and 22.2 d at constant 23 °C for egg to adult development of *C. septempunctata* feeding on *Myzus persicae nicotianae* (Blackman). These decreasing effects on developmental times were also confirmed for *H. axyridis.* Schanderl et al. (1985) measured 48 d at constant 16 °C and only 14 d at constant 28 °C for egg to adult development of *H. axyridis* feeding on *M. persicae*. Also LaMana and Miller (1995) observed that the total developmental time *of H. axyridis* (feeding on *A. pisum*) decreased from 56 to 16 days in response to an elevation of temperature conditions from 16 to 28 °C.

2.2.2.3 Food consumption

Most aphidophagous coccinellids feed on a wide range of aphid species, which are quite different terms of prey quality (Hodek & Honek 1996; Jensen et al. 2012). Numerous data on the suitability of different aphid species and the corresponding consumption rates of *C. septempunctata* and *H. axyridis* exist (Hukusima and Kamei 1970; Shands & Simpsons 1972; Lucas et al. 2002; Tsaganou et al. 2004; Katsarou et al. 2005; Soares et al. 2005; Labrie et al. 2006; Agarwala et al. 2008; Davidson & Evans 2010, etc.). Very few comparative studies of aphid consumption rates in *C. septempunctata* and *H. axyridis* are available (Lucas et al. 2002; Xue et al. 2009). Hodek and Honek (1996) stated that daily feeding rates of coccinellid larvae and adults normally increase within their favorable temperature range for survival, while total feeding rates remain more or less constant, irrespective of temperature. Regarding the effect of using alternating instead of constant temperature regimes on food consumption contrary findings can be stated. While Hodek (1957) observed higher consumption rates at alternating temperatures compared to constant temperatures, Sundby (1966) observed resulted no temperature effects.

Feeding rates are often used to measure the quality of control of a given pest (e.g. aphids) by a given predator. Comparative data on the amounts of aphids consumed by native and invasive coccinellid species are needed to quantify and compare the potential control of aphids by these species under changing conditions, such as elevated temperatures. Using alternating temperatures of 25 °C (16 h) and 15 °C (8 h), Ghanim et al. (1984) measured daily feeding rates of 86.2 up to 126.5 *S. avenae*, respectively 31.1 mg to 43.6 mg aphid biomass, for *C. septempunctata.* Freier and Triltsch (1996) as well as Hodek and Honek (1996) found that *C. septempunctata* feeding rates increase with temperature.

Regarding the comparison of the two ladybird species Lucas et al. (2002) observed that daily aphid consumption rates of approximately *Aphis citricola* van der Goot (Homoptera: Aphididae) by *H. axyridis* (35 aphids/day) were higher than those of *C. septempunctata* (25 aphids/day) at constant temperatures of 25 °C. Xue et al. (2009) measured higher voracity in *H. axyridis* than in *C. septempunctata* feeding on *Aphis glycinis* Matsumura (Homoptera: Aphididae) at a constant 26 °C.

2.2.2.4 Body weight

Studies revealed that coccinellids body weight is not only dependent on temperature but also on sex and the quality and quantity of prey. El-Hariri (1966), Zhou et al. (1995), Triltsch (1997), Hauge et al. (1998), Omkar & Srivastava (2003), Katsarou et al. (2005), Honek et al. (2008) investigated body weights of *C. septempunctata.* Body weights of *H. axyridis* were investigated e.g. by Schanderl et al. (1985), Evans and Gunther (2005) and Zhao et al. (2010). Comparative data on the effects of elevated temperatures on the body weight of these two ladybird species currently is scarce. Katsarou et al. (2005) did not observe any effect of temperature on adult weights of *C. septempunctata*. Kawauchi (1979) and Zhao et al. (2010) reported lower weights of *H. axyridis* adults reared at higher temperatures. Due to different experimental conditions, the comparability of the different studies is strongly impaired. Based on the data reported by the above authors, body weights of mature adult *C. septempunctata* and *H. axyridis* generally range from 30 to 60 mg per individual depending on sex, the quality and quantity of prey and other factors. Another weight parameter often measured is adult weight upon eclosion. Eclosion weights reflect environmental conditions during preimaginal development. Triltsch (1997) observed that eclosion weights of *C. septempunctata* adults feeding on *S. avenae* were lowest at 25 °C and highest at 17 °C, whereby the range was approximately 10 to 42 mg per individual. In the case of *H. axyridis*, Phoofolo and Obrycki (1998) measured mean eclosion weights of 34.2 mg per adult female and 30.7 mg per adult male feeding on *A. pisum* at a constant 26 °C.

2.2.2.5 Fat body content

The fat body of coccinellids stores lipids as an energy reserve for harsh conditions (e.g. diapauses periods such as hibernation) and for periods of high energy consumption rates (e.g. metamorphosis). Coccinellids are able to accumulate lipids in the fat body during the preimaginal and imaginal stages of development. Therefore, the fat body content and eclosion weight of freshly hatched imagines is a product of their preimaginal history. Beenakkers et al. (1985) concluded that the lipid content of insects differs depending on their development stage, nutritional state, sex, temperature, diapauses status, and migration activity. According to Hodek and Honek (1996) the survival of adult coccinellids largely depends on the amount of reserves they can accumulate before diapauses or hibernation. Fat body reserves are also required for migratory flights after hibernation.

Currently, the fat body content of coccinellids is a rather poorly studied parameter (Hodek & Honek 1996). In contrast to other parameters, little is known about how the fat body content of coccinellids changes in response to changing temperatures. El-Hariri (1966) found close positive correlations between the rate of fat consumed by adult coccinellids and temperature. A number of studies have investigated metabolic changes in adult coccinellids due, in particular, to hibernation or diapause (El-Hariri 1966, Hodek & Landa 1971, Kono 1980, Ali & El-Saedy 1981, Anderson 1981, Mills 1981, Shashi & Singh 1988, Zhou et el. 1995, Katsoyannos et. al 1997, Sarmento et al. 2004, Dmitriew et al. 2009). Hodek and Honek (1996) state that the fat body contents of *C. septempunctata*, *Adalia bipunctata* L. and *Propylea quatuor-decimpunctata* L. (Coleoptera: Coccinellidae) ranges from 37 to 48% of dry weight at the beginning of diapause. They also observed a decrease in fat body content of these species during the entire hibernation period (49 to 87%).

2.3 Food conversion efficiency of coccinellids

Derived parameters such as the food conversion efficiency of coccinellids are assumed to be as strongly affected by temperatures as the parameters they are calculated from. Few investigators have focused on the conversion efficiencies of various coccinellid species feeding on different aphid species (Agarwala et al. 2001, Omkar et al. 2005, Shannag & Obeidat 2006, Jalali et al. 2009, Maurice & Kumar 2011). Shannag and Obeidat (2006), who compared the voracity and food conversion efficiency of *C. septempunctata* larvae on *Aphis fabae* Scop. (Homoptera: Aphididae) reared on either partially resistant or aphid-susceptible *Vicia faba* L. cultivars, found a correlation between weight gain and consumed aphid biomass during the larval development period and observed higher conversion efficiencies in coccinellid larvae feeding on aphids reared on susceptible plants. Maurice and Kumar (2011) investigated the effect of food quantity on the body weight and development of *C. septempunctata* and *C. transversalis* (F.) larvae, and found higher food conversion efficiencies in fourth instars than in early instars of both species. Schuder et al. (2004) concluded that *Adalia bipunctata* L. larvae (Coleoptera: Coccinellidae) feeding on grain aphids (*S. avenae*) compensated for a low food supply by increasing their prey exploitation and prey-biomass conversion efficiencies. Işikber and Copland (2001) compared the food consumption and conversion efficiencies of *Scymnus levaillanti* Mulsant and *Cycloneda sanguine* L. larvae (Coleoptera: Coccinellidae) of different sizes at various temperatures. The larvae of smaller *S. levaillanti* converted consumed biomass into body mass more efficiently, presumably due to pre-oral digestion. The authors observed higher food conversion efficiencies during the entire larval development of *C. sanguine* at elevated temperatures and concluded that body size and feeding method considerably affect the conversion efficiency of coccinellids.

2.4 Long-term effects of elevated temperatures on coccinellids

The existing studies on effects of elevated temperatures on life table parameters of *C. septempunctata* and *H. axyridis* only focus on short-term effects of temperatures within specific development stages or within one ladybird generation from eggs and early instars to adult beetles. There is no data on how ladybirds respond to different temperature conditions over several generations, or on how they adapt to higher temperatures when exposed to these conditions for long periods of time. Nevertheless, there is evidence of long-term metabolic and epigenetic adaption in animals. Lucas (1991) defined metabolic programming as "*the process whereby a stimulus or insult when applied at a critical or sensitive period of development results in a long-term or permanent effect on the structure or function of the organism*". Fernandez-Twinn and Ozanne (2010) concluded that prenatal stress considerably affects traits in offspring of mammals (e.g. rats) and that epigenetic mechanisms where the initial DNA sequence remains unchanged but parts or whole chromosomes are changed in activity could be involved in these processes.

Comparable findings on insects are still not available. However, Bradshaw and Holzapfel (2001) observed genetic shifting in pitcher-plant mosquitoes (*Wyeomyia smithii* (Coquillett); Diptera: Cuclicidae), which they described as an adaptive evolutionary response to recent global warming. Bezemer et al. (1998) investigated long-term effects of elevated CO_2 concentrations and temperatures on populations of the peach potato aphid (My*zus persicae*) and its parasitoid *Aphidius matricariae* (Haliday) (Hymenoptera: Braconidae) in experiments lasting approximately nine months. Harrington et al. (1995) stated that, in temperate regions, aphids live for the greater part of the year at temperatures below their developmental optimum. They found increased aphid abundance at elevated temperatures and CO_2 concentrations and assumed that this was due to improved developmental conditions and enhanced relative growth rates under elevated temperature conditions. Bezemer et al. (1998) also observed highly increased parasitism of aphids at elevated temperatures. Therefore, they agreed with other investigators' assumptions of increased performance of aphids´ natural enemies at elevated temperatures (Miller & Gerth 1994; Freier & Triltsch 1996). Thus, long-term adaption of insects to a changed environment seems to be possible although the physiological processes involved are mostly unknown and can be quite complex.

2.5 Antimicrobial defense mechanisms in the hemolymph of coccinellids

The hemolymph of coccinellids contains substances such as alkaloids (Hodek & Honek 1996, Klausnitzer & Klausnitzer 1997, Kögel et al. 2012) which protect them from natural enemies. In times of danger ladybirds even exude this yellowish fluid. This phenomenon is called reflex bleeding (Hodek & Honek 1996). Some of its ingredients such as pyrazines are a part of their immune system (Rothschild 1961, Kögel et al. 2012). Compared to other coccinellid species, the invasive Asian ladybird is known to be less susceptible to entomopathogenic microorganisms, such as *Beauveria bassiana and Bacillus thuringiensis* ssp*. tenebrionis,* and the model organisms *Bacillus subtilis, Escherichia coli* and *Saccharomyces cerevisiae* (Roy et al. 2008, Gross et al. 2010). Gross et al. (2010) observed that the antimicrobial activity of the hemolymph of *H. axyridis* is stronger than that of *C. septempunctata*.

The enemy release hypothesis (ERH) assumes that the scarcity of natural enemies in the introduced range, compared with their native range, could be a reason for the rapid establishment of some invasive species (Keane & Crawley 2002, Torchin et al. 2002, 2003, Mitchell & Power 2003, Colautti et al. 2004).

Data on how the antimicrobial activity in *H. axyridis* responds over short-term or long-term periods to changing environmental conditions, such as increasing temperatures, do not yet exist.

3. Preliminary studies using night and day temperatures

3.1 Introduction

In the first part of this doctoral research, preliminary comparative experiments on the effects of elevated temperatures on *C. septempunctata* and *H. axyridis* and the potential of these coccinellids as natural enemies of cereal aphids in winter wheat were performed using alternating night and day temperature regimes with mean temperatures of 18.7 °C (normal; 16 h: 22 °C, 8 h: 12 °C) and 21.7 °C (elevated; 16 h: 25 °C, 8 h: 15 °C), respectively. Several experiments were conducted at Julius Kühn-Institut in Kleinmachnow, Germany from 2006 to 2009 and addressed different issues to provide a knowledge base for further studies in the second part of this doctoral research. The preliminary studies were conducted to answer the following questions:

a) Do *H. axyridis* and *C. septempunctata* feed in comparable amounts of *S. avenae* and how is their predatory potential affected by elevated temperatures?
b) Does additional drought stress positively affect the coccinellids predatory potential due to decreased aphid reproduction rates?
c) Are coccinellids the dominant members of the aphid predator community, how do elevated temperatures affect intraguild predation and what does the competition within the two ladybirds look like?
d) How do *C. septempunctata* and *H. axyridis* respond to elevated temperatures in terms of their life table parameters?

Three important preliminary experiments dealing with the predatory potential of *C. septempunctata* and *H. axyridis*, their interactions with other aphid predators and their direct competition will be presented below to explain the methods and the results gained in more detail.

3.2 Microcosm experiment on predatory potential

3.2.1 Aim

The aim of the microcosm experiment, conducted in 2008, was to comparatively investigate the potential of *C. septempunctata* and *H. axyridis* to reduce *S. avenae* infestation in winter wheat plants under normal and elevated temperature conditions.

3.2.2 Material and methods

In the experiment seven winter wheat plants of the particularly drought resistant cultivar Capo (InterSaatzucht GmbH & Co. KG, Hohenkammer, Germany) were planted and grown in Mitscherlich containers with a diameter of 20 cm and a capacity for 6 kg of potting substrate. These plants were reduced to 18 eared tillers per pot. For experimental infestation, one *S. avenae* was placed on each tiller at the beginning of flowering (BBCH 61). Each Mitscherlich container was equipped with wire scaffoldings, which was covered with mesh material to isolate the specimens from the environment (Figure 3).

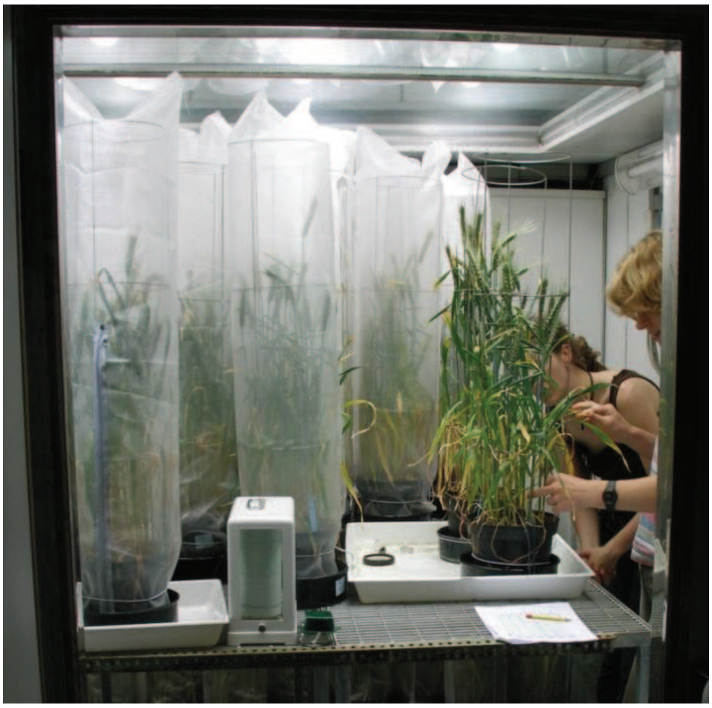

Figure 3: Counting of cereal aphid abundance in a walk-in climate chamber in the microcosm experiment in 2008

Subsequent to infestation with *S. avenae* the containers were randomly distributed in two climate chambers simulating normal (mean: 18.7 °C; 11 °C/21 °C) and elevated (mean: 21.8 °C; 14 °C/24 °C) night and day temperatures. Both climate chambers simulated a 16 h:8 h (LD) photoperiod, a relative humidity of 65% and a solar radiation of approximately 10,000 Lux. The first aphid count of aphids was conducted on 10 randomly selected tillers of each pot 5 days after the initial infestation. On the same day one male or unfertilized female *C. septempunctata* or *H. axyridis* adult was added to each pot. Each climate chamber contained n = 8 replicates of the following three treatments: 1) Sa: aphid controls (*S. avenae* alone without coccinellids), 2) Cs: one *C. septempunctata* adult + *S. avenae* and 3) Ha: one *H. axyridis* adult + *S. avenae*. After the addition of coccinellids aphid abundance was assessed (on 10 randomly selected tillers) three times in a seven-day time period. Thus the aphid infestation-reducing effect of the two ladybirds was quantified but not their aphid consumption rates. Based on these data the aphid index was calculated according to Rautapää (1966). The aphid index, expressed as aphid days per tiller, represents the area under the aphid infestation curve over the entire experimental period (Figure 4). Statistical analyses of the data were conducted using SAS 9.1 (SAS Institute Inc., Cary, North Carolina, USA). Differences between treatments within the same climate regime were tested by Tukey´s test. Differences between temperatures within the same treatment were tested by t-test. The 95% confidence interval ($p \leq 0.05$) was determined for all analyses.

3.2.3 Results

Both coccinellid species were able to reduce aphid infestation by up to 50%, whereby these differences were not significant. The experiment clearly revealed that *H. axyridis* feeds on *S. avenae* and is an effective antagonist of this cereal aphid too. Elevated temperatures caused earlier ripeness of wheat plants but had no significant effect on the predatory efficiency of the two coccinellid species, whereby slight but not significant tendencies were observed. *Coccinella septempunctata* was slightly more effective in reducing aphid infestation at normal temperatures and *H. axyridis* was slightly more effective at elevated temperatures.

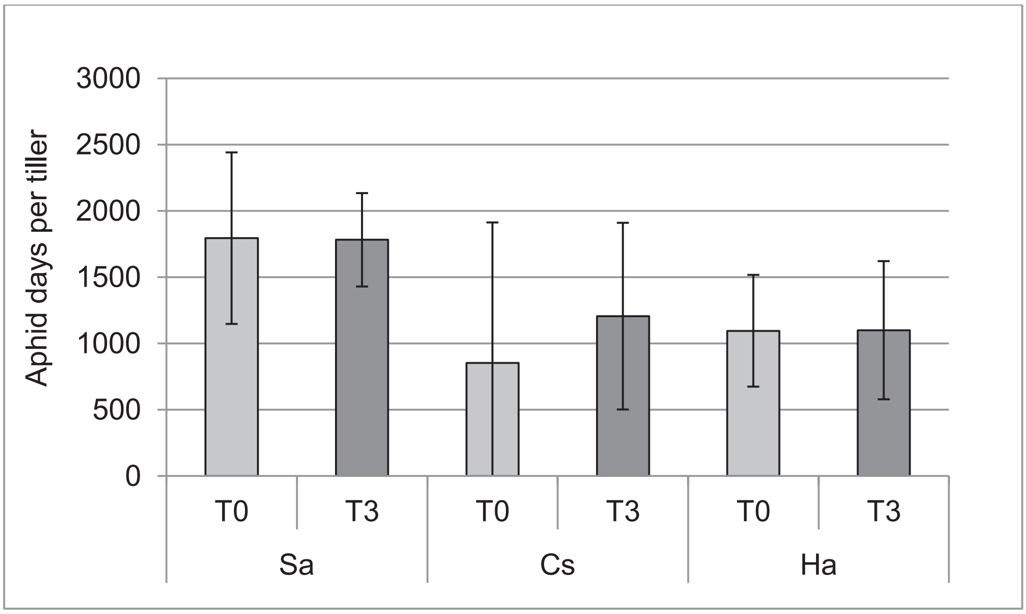

Figure 4: Aphid index (Mean and SD) of treatments Sa (aphid control), Cs (*Sitobion avenae* and *Coccinella septempunctata*) and Ha (*Sitobion avenae* and *Harmonia axyridis*) with 8 replicates each under normal (T0) and elevated (T3) temperature conditions in the microcosm experiment in 2008

3.3 Mini field experiment (“Noah´s Ark“)

3.3.1 Aim

The aim of this mini field experiment (“Noah´s Ark“) conducted in 2009, was to determine how dominant coccinellids are within the aphid predator community formed by different stages of Coccinellidae, Syrphidae, Chrysopidae and Carabidae and how their intraguild predation (IGP) is affected by elevated temperatures.

3.3.2 Material and methods

Two climate chambers were equipped with two mini fields, each 3 m² in size (Figure 5). Because of the spatial constraints it was not possible to create further replicates. Both mini fields were planted with two-leafed wheat plants (cultivar Capo, BBCH 12) to a density of 500 to 600 eared tillers per m² in the field. Plants in both climate chambers were grown under completely identical temperature conditions. The winter wheat plants were infested with 1 aphid per tiller at growth stage 51 (BBCH scale), whereby three cereal aphid species were used*: S. avenae*, *M. dirhodum* and *R. padi*. Thereafter, the whole mini field was isolated with a cover made of air-permeable mesh material (Figure 5).

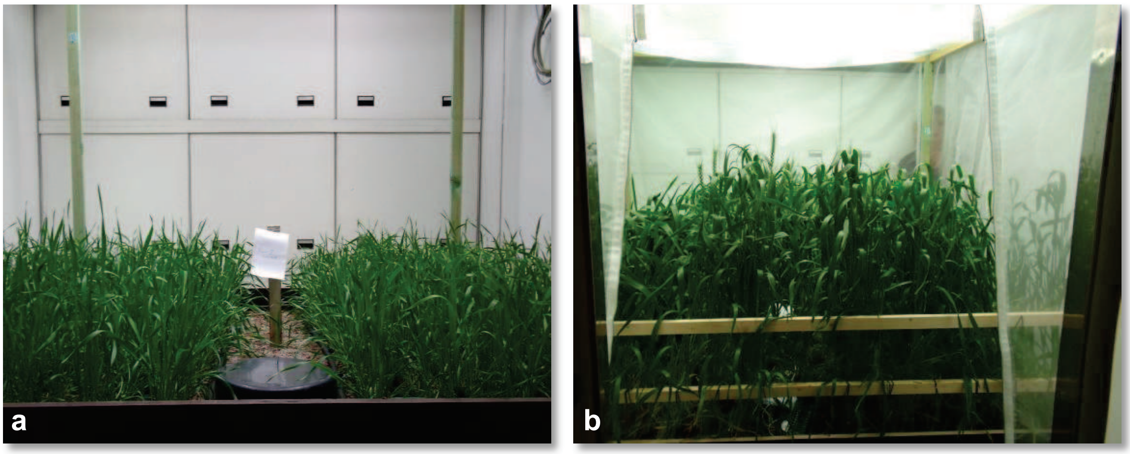

Figure 5: Mini field planted with the winter wheat cultivar Capo without (a) and with (b) isolation (pictures by Sandra Krengel)

After infesting the plants with aphids the main experimental period was started. From then on one climate chamber simulated normal night and day temperatures and the other elevated night and day temperatures, as already described above. An additional community of cereal aphid predators, composed based on results gained by Freier et al. (2007), was introduced into each mini field at the middle of the flowering period. Table 2 presents an overview of the predator species and stages used, the initial densities of the introduced individuals, and the corresponding Predator Unit (PU) values calculated according to Freier et al. (2007). Predator Units are used to characterize the feeding potential of a predator by allocating a specific value to each predator. A female *C. septempunctata* adult for instance is assigned with the highest value of 1.0 PU. At this point no values for *H. axyridis* are available. Based on the results of previous microcosm experiments, *C. septempunctata* and *H. axyridis* were assumed to reach more or less similar daily feeding rates when provided with *S. avenae*. Therefore, both ladybird species were assigned the same values. After the three-week experimental period the climate chambers were cooled down to about 8 °C to lower predator activity. This facilitated the collection of individual predators. Additionally, all plant material was harvested, immediately frozen and inspected for further predators in the laboratory.

Table 2: Predator species as well as stages, initial densities and calculated Predator Unit (PU) values used in mini field experiment “Noah´s Ark” in 2009

Predator species and stages	Predators/m²	Predators/ mini field	PU/m²
Coccinella septempunctata, adult	1	3	1,0
Harmonia axyridis, adult	1	3	1,0
Propylea quatuordecimpunctata, adult	1	3	0,5
Poecilus cupreus, adult	4	12	0,7
Coccinella septempunctata (larvae stage 1)	2	6	0,7
Harmonia axyridis (larvae stage 1)	2	6	0,7
Propylea quatuordecimpunctata (larvae stage 1)	2	6	0,5
Episyrphus balteatus (larvae stage 1)	10	30	4,6
Chrysoperla carnea (larvae stage 1)	5	15	0,7

3.3.3 Results

Despite the fact that we had no possibility to create any replicates the present experiment provided very interesting results. Elevated temperatures caused earlier ripeness of wheat plants. Plants grown at elevated temperatures reached BBCH stage 83, and those grown at the normal temperature only reached BBCH stage 77 during the three-week experimental period.

Figure 6: Predator species and stages collected in the mini field experiment “Noah´s Ark” under the elevated temperature regime (T3) in 2009

The counting of all predators collected revealed seven times higher predator abundances at elevated temperatures than at normal temperatures, whereby the coccinellids were the most dominant group in this predator community (Table 3). However, *H. axyridis* showed a strong dominance particularly at normal temperatures. Also the structure of the developmental stages clearly differed in the two climate chambers. We found considerably more highly developed stages such as late instars, pupae and imagines at elevated temperatures (Figure 6; Table 3).

Table 3: Amount of predators collected after the three-week experimental period at normal (T0) and elevated (T3) temperatures in the "Noah´s Ark" mini field experiment in 2009

Predator	Stage	T0	T3
Coccinella septempunctata	L1	1	0
	L2	4	1
	L3	1	26
	L4	1	346
	Pupae	1	292
	Imago	3	27
Harmonia axyridis	L1	21	0
	L2	28	5
	L3	57	38
	L4	79	483
	Pupae	2	227
	Imago	2	0
Propylea quatuordecimpunctata	Pupae	2	0
	Imago	1	0
Chrysoperla carnea	L3	0	0
	Pupae	4	5
	Imago	1	3
Episyrphus balteatus	Pupae	0	2
	Imago	0	4
Poecilus cupreus	Larvae	0	6
	Imago	2	8
Sum		210	1473

3.4 Microcosm experiment on coccinellid competition

3.4.1 Aim

The aim of this further microcosm experiment, conducted in 2010, was to prove the distinct dominance of *H. axyridis* over *C. septempunctata* revealed in the previous mini field experiment.

3.4.2 Material and methods

For the present experiment Mitscherlich containers (diameter: 20 cm; capacity 6 kg) were also planted with winter wheat (cultivar Capo) as described in section 3.2.2. These plants were reduced to a final number of n = 20 eared wheat tillers per pot and were then infested with one *S. avenae* per tiller at beginning of flowering. In contrast to the previous microcosm experiments, we did not add adult coccinellids but coccinellid larvae at the first stage one week after aphid infestation. This experiment also involved two climate chambers with normal and elevated night and day temperatures. Both climate chambers simulated three treatments with six replicates each (n = 6). We introduced 20 coccinellid larvae per replicate, i.e. one larva per tiller. Treatment “Cs” used 20 *C. septempunctata* larvae, treatment “Ha” 20 *H. axyridis* larvae and treatment “Cs+Ha” 10 larvae of both ladybird species, which were directly placed on the winter wheat tillers. All larvae were kept together at the two different temperatures for a total period of three weeks. At the end of this period all remaining individuals per pot were collected and counted. Statistical analysis of the data was conducted using SAS 9.1 (SAS Institute Inc., Cary, North Carolina USA). Differences between treatments within the same climate regime were tested by Tukey´s test. Differences between temperatures within the same treatment were tested by t-test. The 95% confidence interval ($p \leq 0.05$) was determined for all analyses.

3.4.3 Results

In all treatments resulted survival rates were lower than 50% of the initially introduced larvae. Temperature did not significantly affect mortality. However, *C. septempunctata* tended to have higher mortality at elevated temperatures and *H. axyridis* tended to have higher mortality at normal temperatures (Figure 7). The structure of the detected developmental stages was slightly affected by temperatures. As already observed in the previous mini field experiment, the amount of further developed stages was higher at elevated temperatures. The highest mortality was observed in “Cs+Ha” treatment, where both ladybird species were kept together. Direct competition caused distinctly lower survival rates, whereby this tendency was not significant (Figure 7). The previously measured dominance of *H. axyridis* over *C. septempunctata* was confirmed for both temperature regimes, whereby this effect was more marked at normal temperatures.

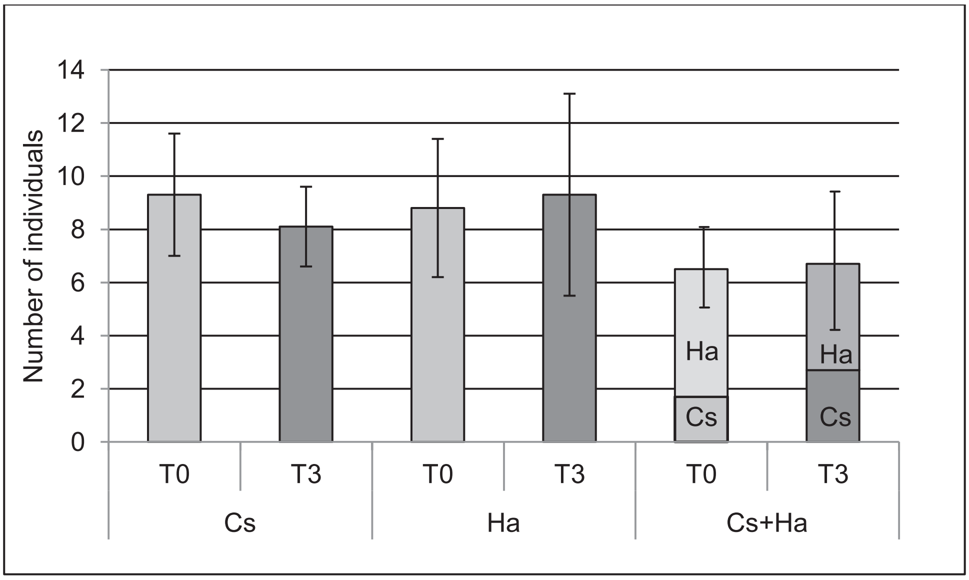

Figure 7: Number of coccinellid surviving (Mean and SD) treatment Cs (*Coccinella septempunctata* only), Ha (*Harmonia axyridis* only) and Cs+Ha (*Coccinella septempunctata* and *Harmonia axyridis* combined) at normal (T0) and elevated (T3) temperatures as determined in the microcosm experiment in 2010 (n = 6)

3.5 Discussion

The first aim of the preliminary studies was to clarify whether *H. axyridis* also feeds on *S. avenae* and if its potential to control this aphid species is comparable to that of *C. septempunctata.* First microcosm experiments, using whole winter wheat plants, cereal aphids and ladybirds, were performed to quantify the infestation-reducing effects of both coccinellid species on *S. avenae* under different temperature conditions. These primary studies were characterized by very high aphid infestation rates that clearly exceeded realistic values in the field. Thus, the methodological approach was modified to reach a moderate initial aphid abundance rate corresponding with an action threshold of approximately three aphids per tiller at flowering. These methodological improvements enabled us to gain first comparative data on the effects of elevated temperatures on the aphid infestation-reducing potential of *C. septempunctata* and *H. axyridis*. The present experiments revealed that *H. axyridis* also feeds on *S. avenae*. This confirms the findings of Colunga-Garcia and Gage (1998), Nault and Kennedy (2003) and Snyder et al. (2004), who stated that *H. axyridis* also colonizes cereals and feeds on cereal aphids in North America. Both coccinellid species were able to reduce aphid infestation by up to 50%, whereby temperature only had slight effects. In contrast to our expectations, we found no clearly favorable effect of elevated temperatures on the predatory efficiency of the two coccinellids species.

Within the next stage of primarily studies, additional drought stress was introduced in the experiments because drought stress now already affects the tritrophic system of winter wheat, cereal aphids and ladybirds, particularly in early summer periods; moreover, it s relevance is expected to get increase under the influence of climate change (Meehl et al. 2007). The aim was to determine whether additional drought stress will reduce the reproduction rates of *S. avenae* and increase the predatory potential of coccinellids, which is probable due to the strong dependency of cereal aphids on plant sap. This microcosm experiment revealed some evidence that additional drought stress reduced aphid reproduction and increased ladybird efficiency. Simpson et al. (2011) also measured decreased reproduction rates in *M. persicae* reared on plants exposed to drought stress. In contrast, White (1969) assumed that stress experienced by host plants could increase the availability of high nitrogen compounds in plants and would therefore increase the reproduction of their insect pests. Later he added that this phenomenon mainly occurs in species which prefer to infest senescent foliage (White 2009).

However, the methodological approach to the microcosm experiments had some fundamental disadvantages. The isolated environmental conditions generally enabled the aphids to reach very high abundances. This artificial aphid infestation sometimes considerably exceeded realistic values. Additionally, we introduced only one coccinellid individual per experimental pot in these first preliminary experiments. This approach totally excludes the effects of competition within different stages of the same species and between different species, which are assumed to be highly relevant too.

Based on these findings and shortcomings the subsequent climate chamber experiments included a whole predator community consisting of different developmental stages of Coccinellidae, Syrphidae, Chrysopidae and Carabidae. The primarily aim of these predator community experiments was to determine which member of this community would be the dominant species and which effects elevated temperatures could have on their intraguild predation (IGP). The mini field experiment was designed to investigate these interactions under highly realistic conditions. The initial predator densities and the stage of introduction were specified based on data gained in ten-year field studies by Freier et al. (2007) and are therefore considered to be highly realistic. Despite the fact that the experimental design did not permit further replicates and has to be classified as a case study, the mini field experiment (also referred to as “Noah´s Ark experiment”) revealed very interesting results. We observed a substantially higher number of individuals at elevated temperatures and a distinct dominance of coccinellids in both climate chambers, whereby *H. axyridis* was the most dominant species particularly under normal temperature conditions.

Koch and Galvan (2008) reported that the establishment of *H. axyridis* in newly colonized countries is associated with a decline in some native species and that various factors, such as intraguild predation, contribute to a rapid rise in the dominance of this ladybird species. Yasuda et al. (2004) stated that intraguild predation can have several benefits for a species and that *H. axyridis* larvae are particularly aggressive compared to native ladybird larvae. According to Koch (2003) *H. axyridis* generally dominates competition among individuals of the same trophic level. Moser and Obrycki (2009) also observed highest survival rates of *H. axyridis* when exposed to direct competition with *C. septempunctata* and *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae), whereby the survival rates of *C. septempunctata* and *C. maculata* decreased when paired with *H. axyridis*. According to Cottrell and Yeargan (1998), Phoofolo and Obrycki (1998), Yasuda et al. (2001), Michaud (2002), Sato et al. (2003), Kajita et al. (2006), Ware and Majerus (2008) and Moser and Obrycki (2009) *H. axyridis* is particularly adapted to competition with other coccinellids.

Considering this knowledge, a further microcosm experiment was conducted, designed to induce direct competition between the two cereal aphid predators, *C. septempunctata* and *H. axyridis*, to obtain more detailed data on the interactions of these ladybird species. Based on the fact that *H. axyridis* larvae are described as particularly aggressive and highly characterized by intraguild predation (Yasuda et al. 2004, Burgio et al. 2008), instars of both coccinellid species were used in this experiment. Ware et al. (2008) stated that intraguild predation on eggs of potential competitors by freshly hatched larvae of *H. axyridis* has obvious benefits as it can provide nutritional advantages and remove future competitors. This experiment confirmed the previously observed dominance of *H. axyridis* at both normal and elevated temperatures. The present investigation also confirmed the assumption that competition between different species remarkably affects predator performance. Direct competition between the two ladybird species resulted in considerably lower survival rates than competition within the species.

Despite these findings, there were still no comparable data on the effects of elevated temperatures on life table parameters of the two ladybird species. However, the first climate chamber laboratory experiments, which used alternating night and day temperatures, should contribute more precise data on the effects of elevated temperatures on life table parameters of *C. septempunctata* and *H. axyridis* (Krengel 2009, Krengel & Freier 2010). In these experiments, where individual ladybirds were treated separately at normal and elevated temperatures, we measured mortality, developmental time and body weight of adult beetles and found interesting differences between the responses of the two ladybirds. Both species responded to elevated temperatures by consuming higher numbers of aphids, but tended to respond differently in terms of body weight and fat body accumulation.

Before completion of the preliminary studies the temperature regimes were modified in cooperation with the Potsdam Institute for Climate Impact Research (PIK). The previously used alternating night and day temperatures did not represent the realistic range of temperatures observed during the period of highest aphid infestation in the field. In contrast to alternating or constant temperatures, daily temperature profiles fluctuate between daily minimum and maximum temperatures. New walk-in climate chambers fundamentally improved experimental conditions by simulating daily temperature profiles with one value per hour. Thus, we designed three highly realistic daily temperature profiles based on data from the German Meteorological Service (DWD) and projections of the statistical regional climate model STAR (Gerstengarbe, Werner, Orlowsky, Hauffe, PIK, Potsdam, Germany):

1) Current temperatures during the main period of aphid infestation in winter wheat (normal temperatures; T0),
2) Temperatures 3 K higher than normal (elevated temperatures; T3) and
3) Temperatures 6 K higher than normal (highly elevated temperatures; T6).

The experimental vessels were also modified for improved control of experimental conditions. These innovations were applied in the three main studies comprising the second part of this doctoral research.

4. Studies using daily temperature profiles

4.1 Introduction

Preliminary experiments in the first part of this study, revealed that the invasive species, *H. axyridis,* also feeds on *S. avenae* and that both coccinellid species, *C. septempunctata* and *H. axyridis*, have similar potentials to reduce aphid infestation in winter wheat under laboratory conditions. Temperature only slightly affected the predatory efficiency of the two ladybirds and drought stress additionally enhanced their predatory potential. Experiments on intraguild predation within an aphid predator community and competition between the two coccinellids revealed a strong dominance of the two coccinellids species, whereby *H. axyridis* was the most dominant species. The initial laboratory climate chamber experiments showed that *C. septempunctata* and *H. axyridis* tend to respond similarly to elevated temperatures in terms of development time and feeding rate, but differently in terms of weight and fat body content (Krengel 2009, Krengel & Freier 2010). They also showed that the weight of adult *H. axyridis* was significantly higher when reared at normal temperatures than at elevated temperatures, whereas that of adult *C. septempunctata* was highest when reared at elevated temperatures. Our analysis of fat body content revealed species-specific and temperature-dependent responses regarding the potential to storage lipids, but there were no clear tendencies.

Based on the preliminary findings and methodological improvements described in section 3, the second part of this doctoral research, consisted of three separate studies. Each study included several experiments, which were conducted in part in cooperation with two external working groups. Laboratory analyses of fat body content were performed by the working group of Prof. Dr. Stangl (Martin Luther University Halle, Germany). Determination of the effects of elevated temperatures on the antimicrobial activity in the hemolymph of *H. axyridis* was conducted in cooperation with the working group of Dr. Gross (Julius Kühn-Institut Dossenheim, Germany). The findings of the three separate studies have already been submitted to international peer-reviewed journals. The aims and hypotheses of these three studies are detailed below:

The first study aimed to collect comparative data on the effects of normal versus elevated daily temperature profiles on development time, consumption rate within the first 10 d of adulthood, body weight and fat body content of 10-day-old mature *C. septempunctata* and *H. axyridis* and to determine the period of greatest effects of temperature on these characteristics of adult coccinellids.

Hypothesis:
It was assumed that the temperature-dependent differences in the two coccinellid species detected in the preliminary studies could be confirmed by using daily temperature profiles and that preimaginal temperature experiences could mainly determine adult features.

This "Study on effects of elevated temperatures on life table parameters", which has already been published in the Journal "Environmental Entomology" 41 (3), pp. 676 – 687, will be presented in the following sections.

The second study aimed to perform a secondary analysis of data on food consumption rates within the first 10 d of adulthood, weight gain within the first 10 d of adulthood and fat body content of 10-day-old adults using data already collected in a set of four experiments carried out under identical experimental conditions from 2010 to 2012 to assess the food conversion efficiencies of *C. septempunctata* and *H. axyridis* beetles at normal and elevated temperatures.

Hypothesis:
Based on previous findings of similar responses of the two coccinellids to elevated temperatures in terms of consumptions rates but different responses in terms of body weight and fat body content, we assumed lower conversion efficiency of consumed biomass into body weight and fat body content by *H. axyridis,* particularly at elevated temperatures.

This study, named "Study on effects of elevated temperatures on food conversion efficiencies", is detailed in the following sections.

The third study aimed to investigate long-term effects of highly elevated temperatures on fitness parameters (e.g. body weight and fat body content) and antimicrobial defenses of adult *H. axyridis*.

Hypothesis:

We assumed that long-term experiences of elevated temperature conditions could awake sleeping genes to enable adaptations to these changes environmental conditions.

This study, named "Study on long-term effects of elevated temperatures on *H. axyridis*", is described in the following sections.

4.2 Material and methods

4.2.1 General experimental design

All experiments were carried out in TIRA BIO TB 818.1 climate chambers (TIRA Umweltsimulation, Schalkau, Germany; Figure 10) simulating normal (T0), elevated (T3) and highly elevated (T6) daily temperature profiles with constant relative humidity (65%) and a photoperiod of 16 h: 8 h (LD). The T0 regime (mean: 17.8 °C, Max: 21.8 °C, Min: 13.4 °C) represents the typical temperatures in Central Germany from the end of June to the beginning of July – the period of highest aphid infestation and predation in winter wheat in that region (Freier & Triltsch 1996) (Figure 8). The T3 regime (mean: 20.8 °C, Max: 25.5 °C, Min: 15.7 °C) simulates the current climate change scenario, assuming about 3 °C of temperature elevation by 2100 (Meehl et al. 2007) and the T6 regime (mean: 23.8 °C, Max: 29.5 °C, Min: 17.9 °C) represents highly elevated temperatures exceeding current climate change predictions by additional 3 °C (Figure 8).

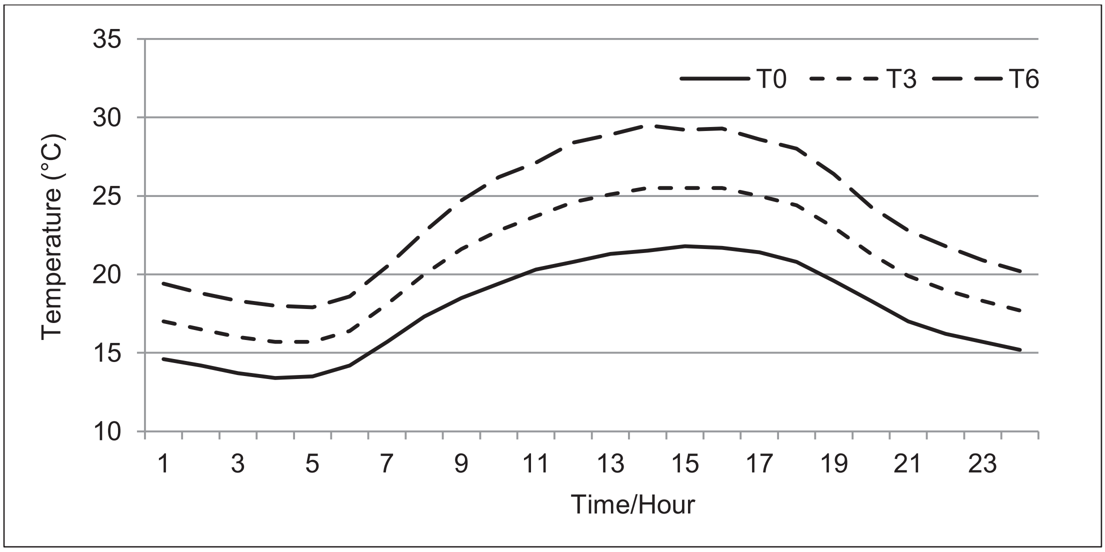

Figure 8: Daily temperature profiles T0 (normal, mean: 17.8 °C), T3 (elevated, mean: 20.8 °C) and T6 (highly elevated, mean: 23.8 °C)

The experiments used for study 1 and 2 were performed using *H. axyridis* first instars produced at Julius Kühn-Institut (Kleinmachnow, Germany) based on a strain established from beetles collected at Kleinmachnow (Germany) in autumn 2009. The first instars of *C. septempunctata* were reared from eggs laid by females produced by Katz Biotech AG (Baruth, Germany). Strains and eggs used for these experiments were stored at T0 temperatures and fed *S. avenae*.

Harmonia axyridis first instars used in study 3, which investigated long-term effects of different temperatures, originated from two different tempered *H. axyridis* strains. Therefore, two *H. axyridis* populations were established at T0 and T6 temperature regime. Both strains based on 5 fertilized female beetles collected near Berlin in autumn 2009 and hibernated by February 2010. Each generation resulted from 10 different females and males, whereby no related beetles were mated.

In all experiments larvae and adults of the coccinellids fed on cereal aphids (*S. avenae*) reared at Julius Kühn-Institut Kleinmachnow *ad libitum* during the entire test period (Figure 9), from first instar to 10-day-old adult. Directly after hatching from eggs, first instars of both coccinellid species were singly placed in aerated 14.5-cm petri dishes and distributed in the climate chambers (Figure 10). To provide fresh air, an 8-cm hole was cut in each lid and covered with polyamide mesh material (mesh pore size: 475 µm). The petri dishes were randomized by species but not by sex because sex can only be determined in adult coccinellids. The initial sample size was chosen to ensure that there would be a sufficient number of individuals in each group for statistical analysis at the end of the experiments.

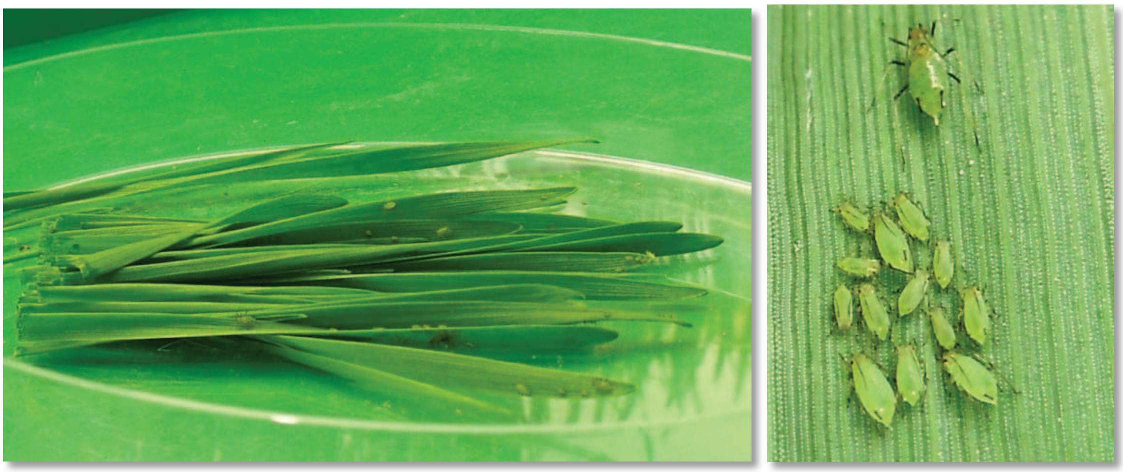

Figure 9: Wheat plants infested with *Sitobion avenae* used as prey in laboratory climate chamber experiments (pictures by Sandra Krengel)

Due to mortality, the number of replicates decreases over time, so the final number of replicates is lower than the initial number. Development time and mortality rates were recorded by daily census. Eclosion weight was determined by weighing freshly emerged adult coccinellids on an analytical scale (AX26 DeltaRange Microbalance, Mettler-Toledo GmbH, Giessen, Germany). To measure aphid consumption the adult coccinellids were provided with freshly cut wheat leaves containing a mixed population of 200 grain aphids each day. Aphids remaining from the previous day were counted prior to new feeding. Ten days after hatching, the adult ladybirds were weighed, sexed [in case of *H. axyridis* we used descriptive data of McCornack et al. (2004)] and frozen at -80 °C until measurement of fat body content. Based on studies by Hukusima and Kamei (1970) and Kawauchi (1981) regarding the duration of teneral development, we assumed that the main physiological changes would be completed within 10 d. Own unpublished analyses revealed that freshly hatched adults contain approximately 25% of fat body content compared to individuals analyzed at the 10th day of adulthood. Statistical analyses of the data were performed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina USA) with the help of Dr. Eckard Moll (Statistician at Julius Kühn-Institut, Kleinmachnow, Germany). The 0.95 confidence interval was determined ($p \leq 0.05$). The special experimental and statistical methods used in each study are listed below.

Figure 10: TIRA BIO TB 818.1 climate chambers (TIRA Umweltsimulation, Schalkau, Germany) equipped with experimental petri dishes (pictures by Sandra Krengel)

4.2.2 Analysis of fat body content

Laboratory analyses of fat body content were conducted by the working group of Prof. Dr. Stangl (Martin Luther University Halle, Germany). First, the frozen insects were freeze-dried for 24 h in a lyophilizer to determine the dry matter content. Total lipids were extracted from the dried specimens with a mixture of n-hexane and isopropanol (3:2, v/v) (Hara and Radin 1978). The individual insects were ground with a pestle in a glass vial before 1 ml of the solvent mixture was added to each. The vials were closed and mixed vigorously for 1 h. After centrifugation, aliquots of the lipid extracts were dried and the lipids were dissolved with Triton X-00/chloroform (1:1, w/w) (De Hoff et al. 1978). The triglyceride concentrations in the aliquots were analyzed using an enzymatic reagent kit (DiaSys Diagnostic Systems, Holzheim, Germany, Cat.-No. 1.5760 99 90 314) and related to the total body weights. Fat body content was expressed as content of triglycerides per mg dry weight.

4.2.3 Study on effects of elevated temperatures on life table parameters

4.2.3.1 Aim

This study was designed to study the general effects of elevated temperatures on life table parameters of *C. septempunctata* and *H. axyridis* and to determine the developmental stage where temperature mainly determines the life table parameters of adult beetles. Two separate experiments were conducted in 2010 and 2011.

Special methodological characteristics beyond the above-mentioned general methods are described below.

4.2.3.2 Experiment A

Coccinella septempunctata and *H. axyridis* were exposed to T0 or T3 temperatures during preimaginal development (first instar to pupa) and during the first 10 d of adulthood (Table 4). The experiment was started with n = 28 first instars per species. Preimaginal development time and mortality were determined by daily census. Aphid consumption rates were sampled in at least 12 specimens per group (male and female) once daily for 10 d after adult eclosion. As the experiment focused on the responses of adult coccinellids to temperature, their aphid consumption was recorded in relationship to the other life table parameters. Because daily aphid consumption fluctuates from day to day, the area under the curve was calculated based on the 10 single-day rates to generate a consumption index (C_i) using the formula:

$$C_i = \sum_{1=2}^{m} n_{i-1} \left[a_{i-1} + \frac{\left(a_i - a_{i-1}\right)}{2} \right]$$

where m is the number of days, a_i the number of aphids per day on a given running day i (i = 2,3….), and n_{i-1} the interval between day i-1 and day i.

4.2.3.3 Experiment B

To determine the stage (preimaginal or young adult) where body weight and fat body content of mature coccinellids is determined by temperature, 40 freshly hatched instars of each species (n = 40) were placed inside the two climate chambers under T0 or T3 conditions until the moment of adult emergence. After adult eclosion, each group was divided in two. The first half remained under the same temperature regime for constant temperature exposure (treatment T0-T0 or T3-T3), and the second was switched to the other temperature regime for altered temperature exposure (treatment T0-T3 or T3-T0) during preimaginal development and the first 10 d of adulthood (Table 4). Subsequently, the insects were weighed and stored.

Table 4: Temperature regimes used in experiments A and B in the study on effects of elevated temperatures on life table parameters

	Temperature exposure	
	Preimaginal stages (Instar 1 to 4; pupae)	Adult period (10 d)
Experiment A		
T0	T0	T0
T3	T3	T3
Experiment B		
T0-T0	T0	T0
T0-T3	T0	T3
T3-T3	T3	T3
T3-T0	T3	T0

4.2.3.4 Statistical analysis

The results of each climate chamber experiment (A and B) were analyzed separately. The following data sets were analyzed: developmental time from first instar to adult emergence, eclosion weight of adults, aphid consumption index during the first 10 d of adulthood, and body weight and fat body content of 10-day-old adults. The data of the following four treatment groups (species x sex) were pooled separately for each temperature regime: *C. septempunctata* x female (Cs-f); *C. septempunctata* x male (Cs-m); *H. axyridis* x female (Ha-f) and *H. axyridis* x male (Ha-m). Because developmental time (number of days) is a discrete variable, the equality of median developmental times for female and male *C. septempunctata* and *H. axyridis* reared at T0 and T3 temperatures was analyzed by Kruskal-Wallis test ($p \leq 0.05$). Differences between treatment groups (species x sex) reared at the same temperatures (T0 or T3) were analyzed by exact Wilcoxon two-sample test ($p \leq 0.05$). Differences between means for aphid consumption index, eclosion weight, body weight and fat body content of adults were analyzed using the GLIMMIX procedure, where simultaneous contrasts were performed at a significance level of 0.05 using the ADJUST = SIMULATE option of the LSMESTIMATE statement. The following two contrasts were used to test simultaneously for differences between the treatment groups (species x sex) treated under same temperature regime (T0 or T3): Cs-f versus Ha-f and Cs-m versus Ha-m. To test for differences between temperature regimes (T0 and T3) within treatment groups (species x sex), the simultaneous contrasts T0-Cs-f versus T3-Cs-f; T0-Cs-m versus T3-Cs-m; T0-Ha-f versus T3-Ha-f and T0-Ha-m versus T3-Ha-m were used. For Experiment B, the treatment groups (species x sex) were analyzed to prove the equality of mean weight and fat body content of the 10-day-old coccinellids by using the GLIMMIX procedure with the LSMESTIMATE statement and simulate test. Within the treatment groups Cs-f; Cs-m; Ha-f and Ha-m; we contrasted treatments with the same temperature conditions during preimaginal development and those treatments with the same temperatures during the imaginal period by using the simultaneous contrasts T0-T0 versus T0-T3 and T3-T0 versus T3-T3 as well as T0-T0 versus T3-T0 and T0-T3 versus T3-T3.

4.2.4 Study on effects of elevated temperatures on food conversion efficiencies

4.2.4.1 Aim

In the second study, we performed a secondary analysis of data already collected in a set of four experiments (A, B, C and D) carried out under identical experimental conditions from 2010 to 2012, as described above, to study the effects of elevated temperatures on food conversion efficiencies of *C. septempunctata* and *H. axyridis*.

4.2.4.2 Calculation of food conversion ratios

The data on adult eclosion weight (n = 388), consumption rate within the first 10 d of adulthood (n = 168), weight gain within the first 10 d of adulthood (n = 382) as well as body weight (n = 459) and fat body content of 10-day-old adults (n = 348) were pooled and re-analyzed to assess the food conversion ratios of *C. septempunctata* and *H. axyridis* beetles at normal and elevated temperatures (T0 and T3). The efficiency of the ladybirds to convert consumed aphid biomass into body weight in terms of weight gain (WG) and fat body content (FBC) was determined by calculating the food conversion ratios (FCR_{WG} and FCR_{FBC}) for 10-day-old coccinellids. The FCR relates the biomass of aphids (mg) consumed per individual during the first 10 d of adulthood to weight gain (mg) or fat body accumulation (mg Triglyceride per mg dry weight) during this period. Therefore, the number of consumed aphids per day was summarized over the 10-day period of adulthood and converted into aphid biomass, and the mean aphid weight was determined. Cereal aphids (*S. avenae*) reared on young wheat tillers and used as food for coccinellids in the experiments were brushed off the tillers and weighed three times during the experiments. The mean weight was determined to be 0.22 mg per individual (n = 268). For determination of food conversion ratios (FCR), means of aphid biomass consumed, weight gain and fat body content were calculated. Using these means, FCR was calculated based on the following formulas:

$$\mathrm{FCR_{WG}} = \text{Food conversion ratio (weight gain)} = \frac{\mathrm{A_{mg}}}{\mathrm{WG}}$$

$$\mathrm{FCR_{FBC}} = \text{Food conversion ratio (fat body content)} = \frac{\mathrm{A_{mg}}}{\mathrm{FBC}}$$

$\mathrm{A_{mg}}$ - Aphid biomass (mg) consumed during the first 10 d of adulthood (per individual)
WG - Weight gain (mg) during the first 10 d of adulthood (per individual)
FBC - Fat body content (mg triglycerides per mg dry weight) accumulated during the first 10 d of adulthood (per individual)

4.2.4.3 Statistical analysis

The species and sexes (f - female and m - male) were analyzed separately, as were the data collected at T0 (normal) and T3 (elevated) temperatures, yielding a total of eight treatment groups: T0-Csf, T0-Csm, T0-Haf, T0-Ham, T3-Csf, T3-Csm, T3-Haf and T3-Ham. Welch’s test was used to compare the mean values of two samples with unequal variances in terms of: a) differences between the effects of the same temperature treatment on ladybirds of the same sex but different species (T0-Csf vs. T0-Haf; T0-Csm vs. T0-Ham; T0-Csf vs. T3-Haf and; T3-Csm vs. T3-Ham), b) differences between the effects of the two temperature treatments on ladybirds of the same species and same sex (T0-Csf vs. T3-Csf; T0-Csm vs. T3-Csm; T0-Haf vs. T3-Haf and T0-Ham vs. T3-Ham) and c) gender differences between the effects of the same temperature treatment on ladybirds of the same species (T0-Csf vs. T0-sm; T3-Csf vs. T3-Csm; T0-Haf vs. T0-Ham and T3-Haf vs. T3-Ham).

4.2.5 Study on long-term effects of elevated temperatures on *Harmonia axyridis*

4.2.5.1 Aim

The third study aimed to investigate the effects of long-term elevated temperatures on life table parameters and antimicrobial activity of the hemolymph of *H. axyridis.*

4.2.5.2 Effects of elevated temperatures on life table parameters

Two *H. axyridis* populations have been exposed to either normal (T0) or highly elevated (T6) daily temperature profiles since February 2010 (Figure 11). Upon eclosure n = 40 first instars of the 6th (T0) and 10th (T6) generation were collected at one year (Experiment A) and n = 40 freshly emerged first instars of the10th (T0) and 17th (T6) generation were collected at two years (Experiment B) and transferred to a climate chamber with a temperature profile representing the mean of the two rearing temperatures (T3) and treated at that temperature from first instar to 10-day-old adult. Each individual was placed in single aerated 14.5-cm petri dishes and treated as already described in chapter 4.2.1.

Generational differences were due to differences in development rates of the two *H. axyridis* strains at the different temperatures. Therefore, each experiment consisted of two temperature treatments:

1) T0-T3 - Progeny of the T0 group were switched to T3;
2) T6-T3 - Progeny of the T6 group were switched to T3.

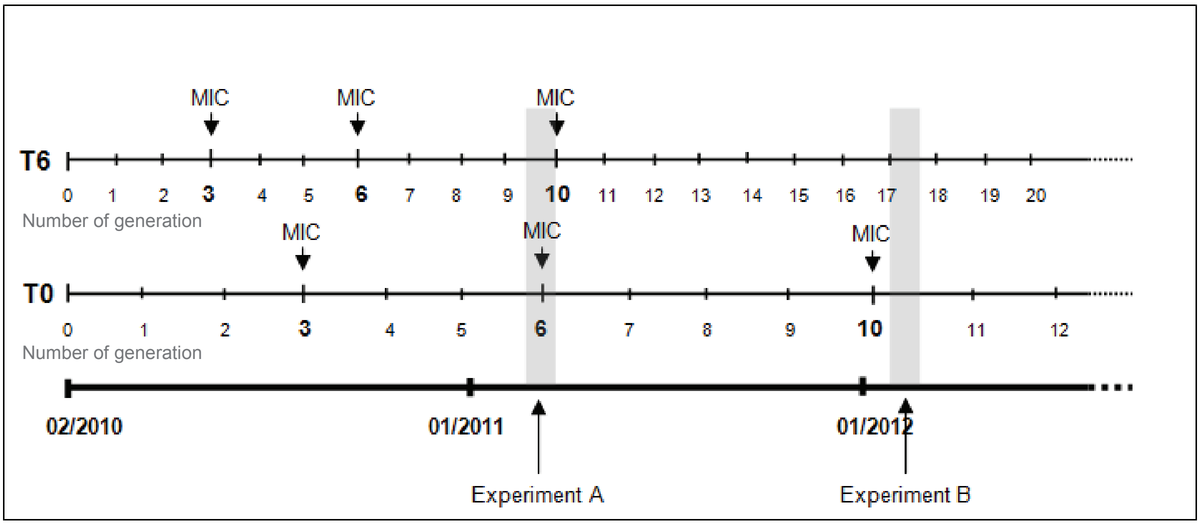

Figure 11: Chronology of generations of *Harmonia axyridis* reared at T0 and T6 temperatures, timing of Experiments A and B, and determination of the minimal inhibitory concentration (MIC)

4.2.5.3 Effects of elevated temperatures on antimicrobial activity in hemolymph

To investigate the long-term effects of elevated temperatures on antimicrobial activity of adult *H. axyridis* beetles we cooperated with the working group of Dr. Gross (Julius Kühn-Institut Dossenheim, Germany). The minimal inhibitory concentrations (MIC) against the gram-negative model organism *E. coli*, the gram-positive model organism *B. subtilis*, and the yeast *S. cerevisiae* were measured in male and female 10-day-old adults of the 3rd, 6th and 10th generation treated at T0 and T6 (Figure 11). Individuals of the respective generations of the two strains were treated at temperatures T0 and T6, respectively, from egg to 10-day-old adults and fed *S. avenae* ad libitum for the entire period. On the 10^{th} day of adulthood, the beetles were sexed and stored at approximately 8 °C until measurement of MIC within a maximum of 3 to 4 d. For determination of the MIC, hemolymph was collected with a glass microcapillary pipette from 5 to 15 males or females per generation and temperature regime by cutting one middle leg of each specimen with micro-scissors. The hemolymph obtained was pooled and 1:20 diluted in Mueller Hinton Broth (MHB) for subsequent tests with microtiter plates. All microorganisms tested originated from strains obtained from the Julius Kühn-Institut in Dossenheim, Germany. Hemolymph analysis was performed as described by Gross et al. (2010). The MIC is defined as the minimal inhibitory concentration of hemolymph or antibiotic that causes complete growth inhibition of the microorganism tested (Kögel et al. 2012).

4.1.5.4 Statistical analysis

To determine the long-term effects of the two different temperatures on life table parameters of *H. axyridis* at one and two years (Experiments A and B), we analyzed males (m) and females (f) of the two groups separately and divided them into the following four treatments: T0-T3-f; T0-T3-m; T6-T3-f and T6-T3-m. The two experiments were analyzed separately. Welch’s test was used to compare the mean values for eclosion weight of adults, body weight of 10-day-old adults, weight gain within the first 10 d of adulthood, and fat body content of 10-day-old adults of two samples in terms of a) the effects of temperature regimes T0-T3 and T6-T3 on ladybirds of same sex (T0-T3-f vs. T6-T3-f and T0-T3-m vs. T6-T3-m), b) the effects of sex on ladybirds of the same temperature treatment (T0-T3-f vs. T0-T3-m and T6-T3-f vs. T6-T3-m), and c) differences between the two Experiments A and B within the same treatment group. Because development time, measured in days, is a discrete variable, the Wilcoxon two-sample test was used to compare the median development times from first instar to adult between the groups.

Regarding the methodological approach to MIC determination, DIN Standard 58940 states that statistical analysis of MIC values is neither necessary nor possible (Kögel et al. 2012).

4.3 Results

4.3.1 Study on effects of elevated temperatures on life table parameters

4.3.1.1 Experiment A

Mortality

The mortality rate from first instar to 10-day-old adult of *H. axyridis* ranged from 3.8% to 11.1% under T3 and T0 conditions, respectively, whereas *C. septempunctata* showed no mortality from first instar to adult under either temperature regime. The observed mortality took place at first instar.

Developmental time

Developmental time from first instar to adult eclosion significantly differed among the treatment groups (species x sex) ($\chi^2 = 58.806$; df = 7; $P < 0.0001$). Under normal temperature conditions (T0), the median developmental time of *C. septempunctata* females was significant longer than that of *H. axyridis* females (Table 5). However, no other significant differences were observed.

Aphid consumption

There were no significant effects of treatment group (species x sex) on mean aphid consumption index (aphid days) during the first 10 d of adulthood under normal ($F = 0.05$; df = 21; $P = 0.8275$) and elevated temperature conditions ($F = 0.01$; df = 19; $P = 0.9278$). Also, there was no significant effect of temperature within the treatment groups (species x sex) ($F = 1.06$; df = 40; $P = 0.3749$). Only the aphid consumption index values for female and male *C. septempunctata* were significantly higher at T3 than at T0 (Table 6, 7).

Body weight

Weights of 10-day-old adult *C. septempunctata* and *H. axyridis* differed significantly between the treatment groups (species x sex) reared at T0 temperatures (F = 7.53; df = 36; P = 0.0094). Otherwise, there were no significant weight differences between the treatment groups (species x sex) reared at T3 temperatures (F = 0.43; df = 31; P = 0.5157). Additional effects of temperature on body weight of adult coccinellids were observed within the treatment groups (F = 3.03; df = 67; P = 0.0354). Mean weights of female *C. septempunctata* were significantly higher than that of female *H. axyridis* only at T3 temperatures (Table 6), whereas those of males of both species were significantly different under both temperature regimes (Table 3). Significant weight differences within the treatment groups (species x sex) reared at T0 and T3 temperatures were observed only in female *C. septempunctata* (Table 7).

Fat body content

A significant effect of treatment group (species x sex) on fat body content of 10-day-old coccinellids was observed at T0 temperatures (F = 5.52; df = 16; P = 0.0320). Conversely, there were no significant effects of treatment group (species x sex) at elevated (T3) temperatures (F = 1.74; df = 16; P = 0.2053). No significant effect of temperature could be observed within treatment groups (species x sex) (F = 2.03; df = 32; P = 0.1288). Only the fat body content of female *C. septempunctata* was significantly higher than that of female *H. axyridis* at both T0 and T3 temperatures (Table 6). Significant higher fat body contents at elevated (T3) than at normal (T0) temperatures were observed in male *C. septempunctata* (Table 7).

Table 5: Experiment A - Developmental time (in days) from first instar to adult for female and male *Coccinella septempunctata* and *Harmonia axyridis* at normal temperatures (T0) and elevated temperatures (T3)

Female									Male								
C. septempunctata				*H. axyridis*					*C. septempunctata*				*H. axyridis*				
n	Median	Q_1	Q_3	n	Median	Q_1	Q_3	P	n	Median	Q_1	Q_3	n	Median	Q_1	Q_3	P
T0																	
11	28.0	28.0	29.0	11	27.0	26.0	28.0	0.0116*	11	28.0	28.0	29.0	11	26.0	28.0	28.0	0.2101
T3																	
10	18.0	18.0	20.0	10	19.0	18.5	20.0	0.3817	9	19.0	19.0	19.0	9	19.0	18.5	21.0	0.7748

*: Significant differences (Wilcoxon-test, $p \leq 0.05$)

Table 6: Experiment A - Consumption index (aphid days), body weight (mg), and fat body content (µg triglycerides/mg dry weight) of 10-d-old female and male *Coccinella septempunctata* and *Harmonia axyridis* at T0 and T3 temperatures

	Female					Male				
	C. septempunctata		*H. axyridis*			*C. septempunctata*		*H. axyridis*		
	n	Mean (SEM)	n	Mean (SEM)	P	n	Mean (SEM)	n	Mean (SEM)	P
T0										
Consumption index (aphid days)	6	743.17 (68.33)	6	623.42 (47.38)	0.1803	6	562.83 (34.77)	7	464.14 (35.86)	0.2753
Body weight of 10-day-old adults (mg)	11	42.73 (1.79)	8	46.09 (2.21)	0.2828	11	37.55 (1.18)	10	32.23 (1.09)	0.0375*
Fat body content (µg triglyceride/mg dry weight)	5	219.62 (48.75)	5	89.69 (13.30)	0.0129*	5	129.52 (26.18)	5	137.20 (13.90)	0.9784
T3										
Consumption index (aphid days)	7	1056.71 (58.21)	8	815.00 (83.46)	0.0625	5	988.00 (106.07)	4	729.67 (57.17)	0.1795
Body weight of 10-day-old adults (mg)	11	49.07 (1.68)	12	43.79 (1.05)	0.0129*	8	40.96 (1.39)	4	33.57 (1.12)	0.0179*
Fat body content (µg triglyceride/mg dry weight)	5	249.11 (46.34)	6	51.35 (8.99)	0.0042*	5	260.19 (61.81)	4	169.36 (23.73)	0.2727

*: Significant differences (Simultaneous contrast with simulate test, p ≤ 0.05)

Table 7: Experiment A - Statistical analysis of differences in effects of temperature regimes T0 and T3 on aphid consumption index (aphid days), body weight (mg) and fat body content (µg triglycerides/mg dry weight) of 10-d-old female and male *Coccinella septempunctata* and *Harmonia axyridis* adults

	Differences between T0 – T3 (within the species)			
	Female		Male	
	C. septempunctata	*H. axyridis*	*C. septempunctata*	*H. axyridis*
Consumption index (aphid days)	P = 0.0055*	P = 0.1357	p = 0.0060*	P = 0.0905
Body weight of 10-day-old adults (mg)	P = 0.0092*	P = 0.7350	P = 0.3997	P = 0.9803
Fat body content (µg triglyceride/mg dry weight)	P = 0.9583	P = 0.8837	P = 0.0495*	P = 0.9583

*: Significant differences (Simultaneous contrast with simulate test, p ≤ 0.05)

4.3.1.2 Experiment B

Mortality

The mortality rate from first instar to adult coccinellid ranged from 5.0% (T3) to 11.7% (T0) for *C. septempunctata* and from 7.7% (T0) to 10.4% (T3) for *H. axyridis*. Mortality decreased from first instars to adult in both species.

Developmental time

There were significant effects of treatment group (species x sex) on developmental time of *C. septempunctata* and *H. axyridis* ($\chi^2 = 224.314$; df = 7; $P < 0.0001$). *Coccinella septempunctata* and *H. axyridis* females and males differed significantly in terms of mean developmental time under T0 and T3 conditions (Table 8). It took *C. septempunctata* significantly longer to develop at normal temperatures (T0) than *H. axyridis*, but the opposite was true of coccinellids reared at elevated temperatures (T3) (Table 8).

Eclosion weight

There was no significant effect of treatment group (species x sex) on mean eclosion weight of coccinellids reared at T0 ($F = 0.07$; df = 134; $P = 0.7924$) and T3 temperatures ($F = 0.01$; df = 140; $P = 0.9370$). However, there was a significant effect of temperature regime on mean eclosion weight within the treatment groups (species x sex) ($F = 3.46$; df = 274; $P = 0.0168$). *C. septempunctata* females and males showed significant higher eclosion weights than *H. axyridis* females and males at temperature regime T3 (Figure 12).

Body weight

To determine the period of main temperature influence on body weight of 10-day-old coccinellids, we compared the body weights of coccinellids treated at the same temperatures during preimaginal period, but different temperatures during young adult period (T0-T0 versus T0-T3 and T3-T3 versus T3-T0) and those specimens treated at different temperatures during preimaginal period, but the same temperatures during young adult period (T0-T0 versus T3-T0 and T0-T3 versus T3-T3). Treatment group (species x sex) had a significant effect on the weight of 10-day-old adults ($F = 24.92$; df = 263; $P < 0.0001$). Within the species, the mean weights of treatment groups reared under same temperatures during preimaginal development but different temperatures during young adult period did not differ in all cases (Table 9 and 10). However, male *C. septempunctata* treated at different temperatures during preimaginal period but at the same T0 temperatures during the imaginal period (T0-T0 versus. T3-T0) significantly differed (P = 0.0043). *Harmonia axyridis* females treated at T0-T0 versus T3-T0 (P = 0.0151) and T0-T3 versus T3-T3 exhibited significant differences (P = 0.0039).

Fat body content

To determine the time of main temperature effect on fat body content of 10-day-old coccinellids, we compared the fat body content of young adult coccinellids treated at the same temperatures during preimaginal development but different temperatures during young adult period (T0-T0 versus T0-T3 and T3-T3 versus T3-T0) and those specimens treated at different temperatures during preimaginal period, but same temperatures during young adult period (T0-T0 versus T3-T0 and T0-T3 versus T3-T3). We observed significant effects of treatment group (species x sex) on fat body content (F = 17.52; df = 142; P < 0.0001). Within the species, the mean fat body content of treatment groups (species x sex) reared at the same temperatures during preimaginal development but different temperatures during young adult period did not differ in all cases (Figure 13, 14). In *C. septempunctata* males treated at different temperatures during preimaginal period, but at the same temperatures during the imaginal period, T0-T0 versus T3-T0 (P < 0.0001) and T0-T3 versus T3-T3 (P = 0.0012) significantly differed. Female *C. septempunctata* treated at different temperatures during preimaginal period, but at the same T3 temperatures during the imaginal period exhibited significant differences (T0-T3 versus T3-T3, P < 0.0001).

Table 8: Experiment B - Developmental time (in days) from first instar to adult for female and male *Coccinella septempunctata* and *Harmonia axyridis* at normal temperatures (T0) and elevated temperatures (T3)

Female									Male								
Coccinella septempunctata				*Harmonia axyridis*					*Coccinella septempunctata*				*Harmonia axyridis*				
n	Median	Q_1	Q_3	n	Median	Q_1	Q_3	P	n	Median	Q_1	Q_3	n	Median	Q_1	Q_3	P
T0																	
32	28.5	28.0	30.0	36	28.0	27.0	28.5	0.0139*	34	28.0	27.0	29.0	36	27.0	27.0	28.5	0.0409*
T3																	
31	20.0	20.0	21.0	32	21.0	20.5	22.0	0.0013*	44	20.0	20.0	21.0	37	21.0	21.0	21.0	< 0.0001*

*: Significant differences (Wilcoxon-test, $p \leq 0.05$)

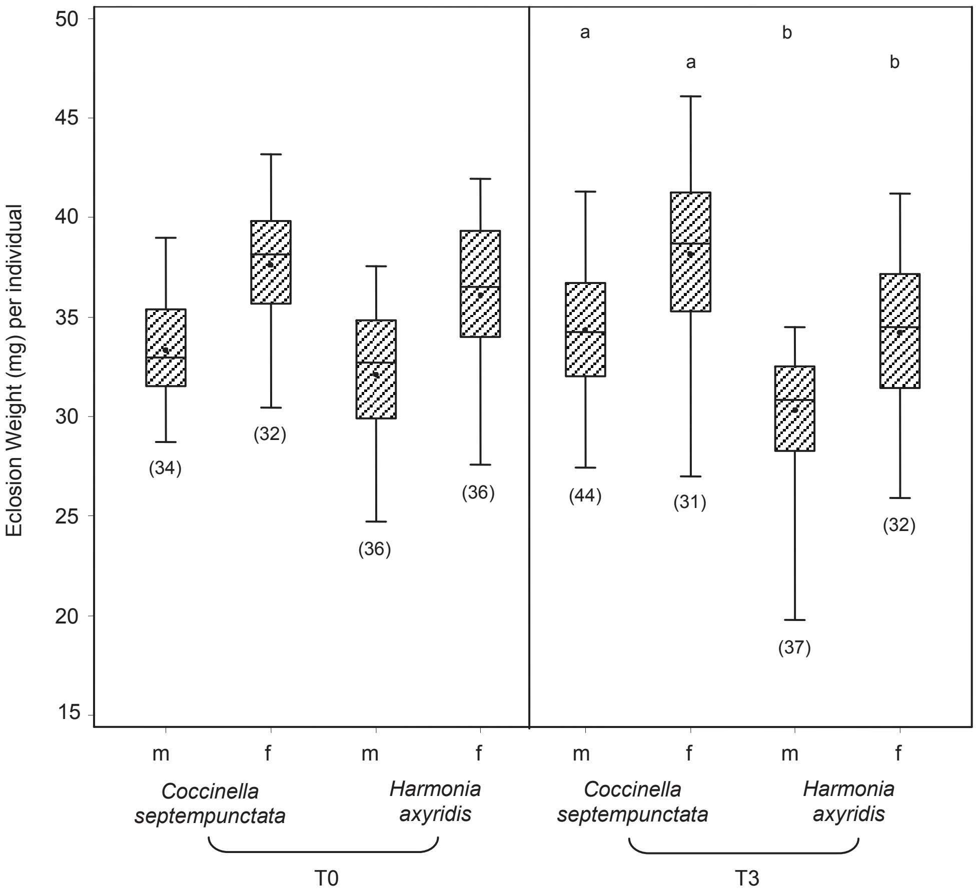

Figure 12: Experiment B - Eclosion weight (mg) of male (m) and female (f) *Coccinella septempunctata* and *Harmonia axyridis* under temperature regimes T0 and T3. Significant differences between species within sex and temperature are indicated by the letters "a" and "b". (Simultaneous contrast with simulate test, $p \leq 0.05$). Numbers in brackets indicate the number of replicates.

Table 9: Experiment B – Body weight (mg) of 10-d-old female and male *Coccinella septempunctata* adults under temperature regimes T0-T0, T0-T3, T3-T3 and T3-T0

	C. septempunctata									
	Female					Male				
	n	Mean (SEM)	n	Mean (SEM)	P	n	Mean (SEM)	n	Mean (SEM)	P
	T0 – T0		**T0 – T3**			**T0 - T0**		**T0 - T3**		
Body weight of 10-d-old adults (mg)	16	50.08 (1.65)	14	47.94 (1.76)	0.9004	18	39.52 (0.90)	16	38.84 (0.90)	1.0000
	T3 – T3		**T3 – T0**			**T3 – T3**		**T3 – T0**		
Body weight of 10-d-old adults (mg)	19	51.00 (1.60)	11	48.67 (1.72)	0.8740	18	41.92 (1.18)	26	44.99 (1.29)	0.3374

*: Significant differences (Simultaneous contrast with simulate test, P ≤ 0.05)

Table 10: Experiment B – Body weight (mg) of 10-d-old female and male *Harmonia axyridis* adults under temperature regimes T0-T0, T0-T3, T3-T3 and T3-T0

	H. axyridis									
	Female					Male				
	n	Mean (SEM)	n	Mean (SEM)	P	n	Mean (SEM)	n	Mean (SEM)	P
	T0 – T0		**T0 – T3**			**T0 - T0**		**T0 - T3**		
Body weight of 10-d-old adults (mg)	19	47.29 (1.49)	17	47.01 (1.05)	1.000	18	35.60 (0.73)	18	34.62 (0.90)	0.9988
	T3 – T3		**T3 – T0**			**T3 – T3**		**T3 – T0**		
Body weight of 10-d-old adults (mg)	15	40.64 (1.44)	17	41.95 (1.06)	0.9932	20	33.66 (0.41)	17	32.46 (0.85)	0.9940

*: Significant differences (Simultaneous contrast with simulate test, p ≤ 0.05)

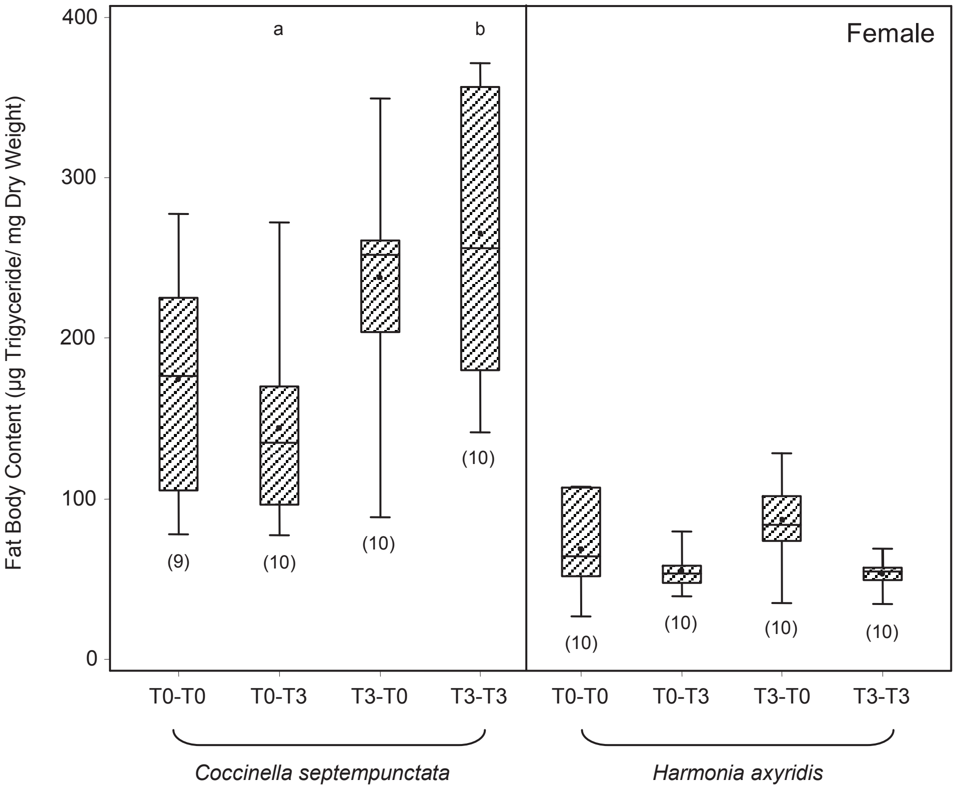

Figure 13: Experiment B - Fat body content (µg trigyceride/ mg dry weight) of 10-day-old female *Coccinella septempunctata* and *Harmonia axyridis* adults at temperature regime T0-T0, T0-T3, T3-T0 and T3-T3. Significant differences between temperatures within sex and species are indicated by the letters “a” and “b”. Simultaneous contrast with simulate test ($p \leq 0.05$). Numbers in brackets indicate the number of replicates.

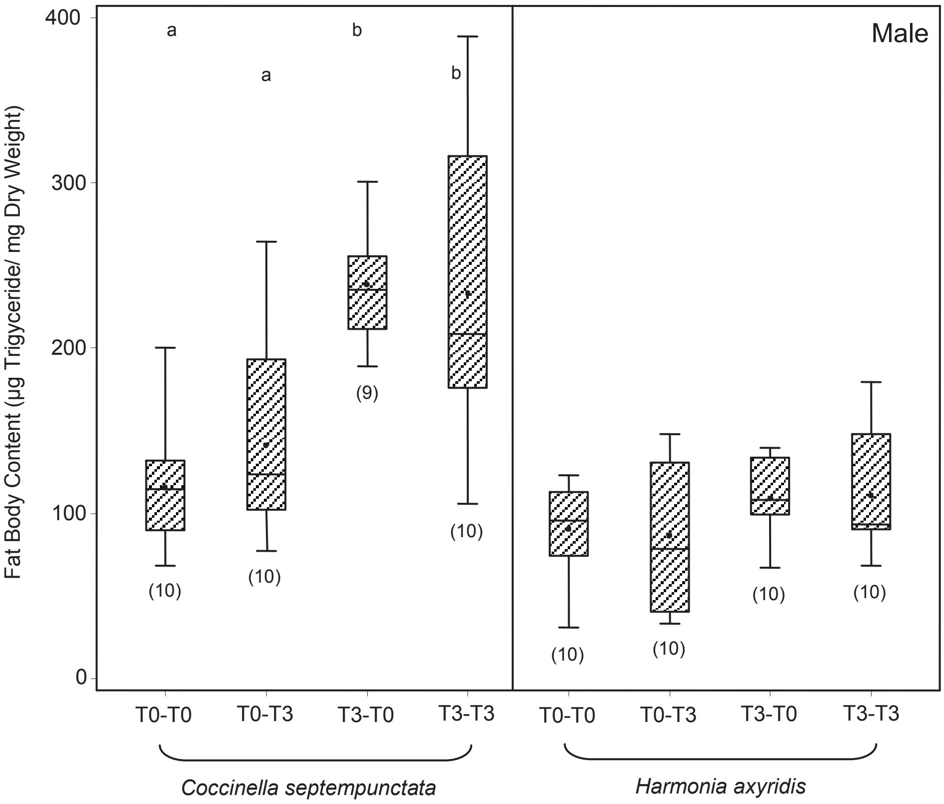

Figure 14: Experiment B - Fat body content (µg trigyceride/ mg dry weight) of 10-d-old male *Coccinella septempunctata* and *Harmonia axyridis* adults at temperature regimes T0-T0, T0-T3, T3-T0 and T3-T3. Significant differences between temperatures within sex and species are indicated by the letters “a” and “b”. Simultaneous contrast with simulate test ($p \leq 0.05$). Numbers in brackets indicate the number of replicates.

4.3.2 Study on effects of elevated temperatures on food conversion efficiencies

Eclosion weight

The two species had very similar eclosion weights under both temperature regimes. The only significant difference was that the eclosion weights of *H. axyridis* females were significantly higher than those of *C. septempunctata* females at T0 temperatures (Table 11). There was a slight but not significant tendency for higher eclosion weights of both sexes of *C. septempunctata* at elevated temperatures and for higher eclosion weights of both sexes of *H. axyridis* at normal temperatures (Table 12). In all cases, males had significantly lower eclosion weights than females (Table 13).

Aphid consumption

Coccinella septempunctata females and males consumed significantly more aphids during the first 10 d of adulthood than *H. axyridis* females (T0: df = 25.70, t-value = 3.58, P = 0.0014; T3: df = 33.58, t-value = 5.33, P < 0.0001) and males (T0: df = 28.8, t-value = 4.40, P = 0.0001 T3: df = 24.79, t-value = 8.50, P < 0.0001) at both temperatures (Figure 15). The two species showed a significant increase in aphid consumption at elevated temperatures (Csf: df = 33.79, t-value = -4.78, P < 0.0001; Csm: df = 26.30, t-value = -8.05, P < 0.0001; Haf: df = 44.45, t-value = -5.16, P < 0.0001 and Ham: df = 46.80, t-value = -4.39, P < 0.0001, Figure 15). *Coccinella septempunctata* females were significantly more voracious than males only at normal temperatures (df = 25.84, t-value = -4.32, P = 0.0002), whereas *H. axyridis* females consumed significantly more than males at both temperatures (T0: df = 30.60, t-value = -5.72, P < 0.0001; T3: df = 53.34, t-value = -6.86, P < 0.0001, Figure 15).

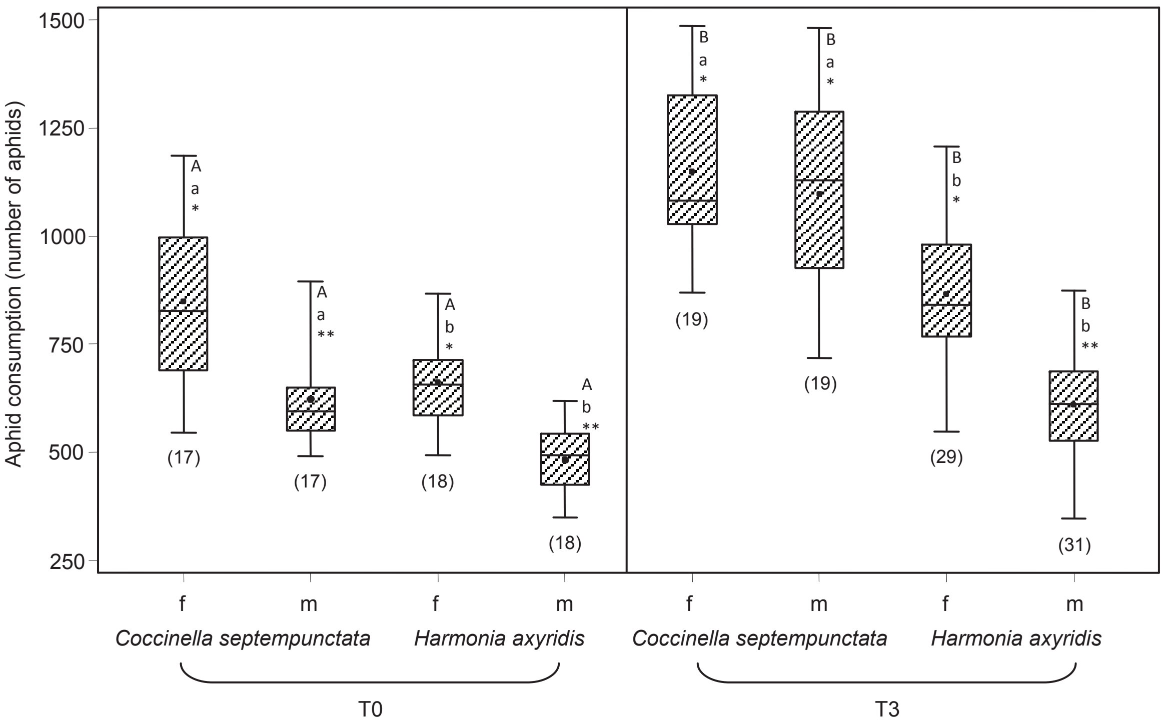

Figure 15: Aphid consumption (number of aphids per individual) during the first 10 d of adulthood of *Coccinella septempunctata* females (f) and males (m) and of *Harmonia axyridis* females (f) and males (m) at temperature profiles T0 (normal) and T3 (elevated) in climate chamber experiments A, C and D (Welch’s test for pairwise comparisons; p ≤ 0.05). Numbers in brackets represent replicates. “A” and “B” indicate significant differences between the two temperature regimes (T0 and T3) within the respective groups (Csf, Csm, Haf and Ham), “a and “b” denote significant differences between ladybirds of the same sex but different species under the same temperature regime (T0-Csf vs. T0-Haf; T0-Csm vs. T0-Ham, etc.) and “α” and “β” indicate significant gender differences within the same species and temperature groups.

Weight of 10-day-old adults

The weight of 10-day-old female *C. septempunctata* adults was significantly higher than that of female *H. axyridis* adults only at T3 temperatures, whereas the weight of 10-day-old male *C. septempunctata* adults was significantly higher than that of male *H. axyridis* adults at both T0 and T3 temperatures (Table 11). Significant increases in the weights of 10-day-old adults in response to elevated temperatures were observed only in *C. septempunctata* females and males; the weights of the 10-day-old *H. axyridis* adults remained approximately the same (Table 12). The 10-day-old adult females were significantly heavier than the 10-day-old adult males in all cases (Table 13).

Weight gain

Similar to the weight of 10-day-old adults, weight gain in *C. septempunctata* females during the first 10 d of adulthood was significantly higher than that in *H. axyridis* females only at T3 temperatures, whereas weight gain in male *C. septempunctata* adults was significantly higher than that in male *H. axyridis* adults at both temperatures (Table 11). Both female and male *C. septempunctata* adults but only male *H. axyridis* adults exhibited significantly higher weight gain at elevated temperatures than at normal temperatures (Table 12). In both species and temperature groups, females gained more weight during the first 10 d of adulthood than males (Table 13).

Fat body content

The fat body content of female and male 10-day-old *C. septempunctata* adults was significantly higher than that of 10-day-old female (T0: df = 54.40, t-value = 5.40, P < 0.0001, T3: df = 44.23, t-value = 9.31, P < 0.0001) and male *H. axyridis* adults (T0: df = 75.73, t-value = 2.02, P = 0.0469, T3: df = 73.48, t-value = 7.45, P < 0.0001) at T0 and T3 temperatures (Figure 16). Temperature elevation led to a significant increase in fat body content of female and male 10-day-old *C. septempunctata* adults (Csf: df = 73.64, t-value = -2.32, P = 0.0229; Csm: df = 71.29, t-value = -6.29, P < 0.0001) but to a significant decrease in that of female 10-day-old *H. axyridis* adults (df = 87.60, t-value = 5.20, P < 0.0001). The fat body content of 10-day-old male *H. axyridis* adults showed no significant response to elevated temperature, but rather stagnated (df = 83.50, t-value = -0.03, P = 0.9735, Figure 2). In

C. septempunctata, the fat body content of females was significantly higher than that of males at T0 temperatures (df= 64.61, t-value = -2.33, P = 0.0228) but was not different at T3 temperatures (df = 81.97, t-value = 0.85, P = 0.3955). In *H. axyridis*, on the other hand, the fat body content of females was significantly lower than that of males under both temperatures regimes (T0: df = 79.11, t-value = 2.38, P = 0.0195; T3: df = 71.30, t-value = 6.39, P < 0.0001, Figure 16).

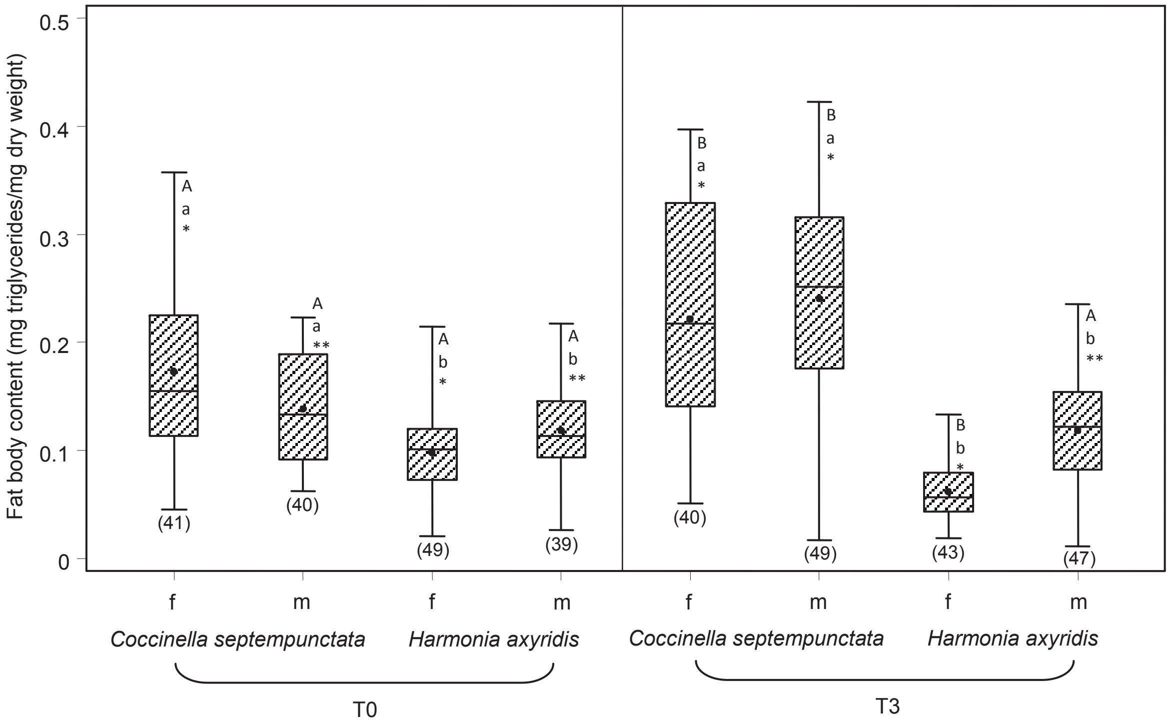

Figure 16: Fat body content (mg triglycerides/mg dry weight) of 10-day-old adult *Coccinella septempunctata* females (f) and males (m) and of 10-day-old adult *Harmonia axyridis* females (f) and males (m) at normal (T0) and elevated (T3) temperatures as determined in climate chamber experiments A, B, C and D (Welch's test for pairwise comparisons; p ≤ 0.05). Numbers in brackets represent replicates. "A" and "B" indicate significant differences between the two temperature regimes (T0 and T3) within the respective groups (Csf, Csm, Haf and Ham), "a and "b" denote significant differences between ladybirds of the same sex but different species under the same temperature regime (T0-Csf vs. T0-Haf; T0-Csm vs. T0-Ham, etc.) and "α" and "β" indicate significant gender differences within the same species and temperature groups.

Food conversion ratios

Overall, female *C. septempunctata* and *H. axyridis* required very similar amounts of aphid biomass (FCR_{WG}) to achieve 1 mg of weight gain. However, the *H. axyridis* females required less biomass at normal temperatures, whereas the *C. septempunctata* females required less at elevated temperatures. In particular, *H. axyridis* females exhibited the lowest food conversion ratios at elevated temperatures, whereas those of *C. septempunctata* remained rather constant (Table 14). Regarding males, *C. septempunctata* required considerably less biomass to accumulate body mass than *H. axyridis* at both T0 and T3 temperatures. Males of both species showed very constant food requirements at elevated temperatures. Overall, *H. axyridis* males had the lowest efficiency to convert aphid biomass into body mass under T0 and T3 conditions (Table 14). In terms of fat body content, female *C. septempunctata* required considerable less biomass than *H. axyridis* females to accumulate 1 mg triglyceride, the main type of fat body content. Females of both species exhibited the lowest food conversion efficiencies at elevated temperatures. This tendency was more distinct in *H. axyridis* females than in *C. septempunctata* females, who were the worst converters of consumed biomass into fat body content, particularly at T3 temperatures. Males only differed slightly and temperature had almost no effect on their efficiency to convert food into fat body content. In particular, *H. axyridis* exhibited gender differences between FCR_{FBC} (Table 14).

Table 11: Eclosion weight of adults (mg), body weight of 10-d-old adults (mg) and weight gain (mg) during the first 10 d of adulthood for *Coccinella septempunctata* and *Harmonia axyridis* females and males subjected to T0 and T3 temperatures in experiments A, B, C and D

	Female					Male				
	C. septempunctata		*H. axyridis*			*C. septempunctata*		*H. axyridis*		
	n	Mean (SEM)	n	Mean (SEM)	P	n	Mean (SEM)	n	Mean (SEM)	P
T0										
Eclosion weight of adults (mg per individual)	50	34.73 (0.51)	57	36.13 (0.47)	0.0474*	45	31,09 (0,46)	43	32.18 (0.65)	0.3988
Body weight of 10-day-old adults (mg per individual)	61	44.30 (0.87)	63	44.89 (0.74)	0.6051	55	37.48 (0.56)	53	33.84 (0.55)	<0.0001*
Weight gain within the first 10 d of adulthood (mg per individual)	50	9.91 (0.72)	55	8.46 (0.57)	0.1205	43	5.74 (0.38)	43	2.04 (0.28)	<0.0001*
T3										
Eclosion weight of adults (mg per individual)	46	36.24 (0.65)	42	35.49 (0.52)	0.3695	52	31.78 (0.44)	53	31.04 (0.40)	0.2155
Body weight of 10-day-old adults (mg per individual)	56	49.17 (0.85)	54	44.46 (0.75)	<0.0001*	60	40.85 (0.63)	57	33.88 (0.38)	<0.0001*
Weight gain within the first 10 d of adulthood (mg per individual)	45	12.92 (0.82)	42	9.06 (0.68)	0.0005*	51	9.06 (0.52)	53	2.81 (0.26)	<0.0001*

* Statistical analysis of differences between ladybirds of the same sex but different species treated under the same temperature conditions (Welch's test for pairwise comparison of samples with unequal variances, confidence interval p ≤ 0.05)

Table 12: Comparison of mean eclosion weight of adults (mg), body weight of 10-d-old adults (mg) and weight gain (mg) during the first 10 d of adulthood for *Coccinella septempunctata* and *Harmonia axyridis* females and males, respectively, to determine differences between T0 and T3

	Differences between T0 and T3 (within the species)			
	Females		Males	
	C. septempunctata	*H. axyridis*	*C. septempunctata*	*H. axyridis*
Eclosion weight of adults (mg per individual)	P = 0.0697	P = 0.3712	P = 0.6662	P = 0.1379
Body weight of 10-day old adults (mg per individual)	P = 0.0001*	P = 0.6860	P = 0.0001*	P = 0.9459
Weight gain during the first 10 d of adulthood (mg per individual)	P = 0.0073*	P = 0.5062	P < 0.0001*	P = 0.0457*

* Statistical analysis: Welch's test for pairwise comparison of samples with unequal variances; confidence interval p ≤ 0.05)

Table 13: Comparison of mean eclosion weight of adults (mg), body weight of 10-day-old adults (mg) and weight gain (mg) during the first 10 d of adulthood of *Coccinella septempunctata* and *Harmonia axyridis* in response to T0 and T3 temperature to determine differences between females and males

	Gender differences			
	T0		**T3**	
	C. septempunctata	*H. axyridis*	*C. septempunctata*	*H. axyridis*
Eclosion weight of adults (mg per individual)	P < 0.0001*	P < 0.0001*	P < 0.0001*	P < 0.0001*
Body weight of 10-day old adults (mg per individual)	P < 0.0001*	P < 0.0001*	P < 0.0001*	P < 0.0001*
Weight gain during the first 10 d of adulthood (mg per individual)	P < 0.0001*	P = 0.0002*	P < 0.0001*	P < 0.0001*

* Statistical analysis: Welch's test for pairwise comparison of samples with unequal variances; confidence interval $p \leq 0.05$)

Table 14: Food conversion ratio (FCR) related to weight gain (FCR_{WG} - mg aphids per mg WG) and to fat body content (FCR_{FBC} - mg aphids per mg triglyceride * mg dry weight^{-1}) for 10-d-old female and male *Coccinella septempunctata* and *Harmonia axyridis* adults subjected to T0 and T3 temperatures

		T0				**T3**			
		C. septempunctata		*H. axyridis*		*C. septempunctata*		*H. axyridis*	
		Female	Male	Female	Male	Female	Male	Female	Male
FCR_{WG}	Biomass (mg) per mg weight gain per individual	19.19	24.34	17.56	53.00	19.94	27.17	21.44	48.57
FCR_{FBC}	Biomass (mg) per mg triglyceride * mg dry weight^{-1} per individual	1099.10	1008.74	1516.24	912.75	1161.59	1022.62	3139.38	1152.35

4.3.3 Study on long-term effects of elevated temperatures on *Harmonia axyridis*

Development time from first instar to adult

The development time from first instar to adult eclosion did not differ significantly between *H. axyridis* males and females in theT0-T3 and T6-T3 groups at one and two years (Table 15). Significant differences between *H. axyridis* males and females in the T0-T3 and T6-T3 groups were only observed at one year (Table 16). In most cases, there were significant differences in development times between the two years, and only T6-T3 females showed no significant difference between the development times at one and two years (Table 16).

Eclosion weight of adults

H. axyridis males and females treated at T0-T3 and T6-T3 temperatures showed no significant differences in eclosion weights of adults one year or two years after initialization of the two strains (Table 17). Females generally had significantly higher eclosion weights than males at both one and two years (Table 18). T6-T3 adult females were the only group with differences between eclosion weights after one and two years of rearing, and they had significantly higher body weight after two years of rearing (Table 18).

Body weight of 10-day-old adults

Ten-day-old *H. axyridis* females were the only group where significant effects of T0-T3 and T6-T3 temperatures on body weights could be observed after one year of continuous rearing under these temperatures (Experiment A). T0-T3 females had significant higher body weights than T6-T3 females (Table 17). The 10-day-old females of both groups had higher body weights than their male counterparts in both years (Table 18). In terms of the body weight of 10-day-old adults, T0-T3 *H. axyridis* males were the only group with significantly higher body weights after two years of rearing compared to one year. All other treatments remained unaffected by the length of the rearing period (Table 18).

Weight gain within the first 10 days of adulthood

After two years of rearing (Experiment B), the T0-T3 and T6-T3 males differed significantly in terms of weight gain within the first 10 d of adulthood. The T6-T3 males gained approximately 2 mg more weight than the T0-T3 males. However, after one year of rearing for T0-T3 females showed significantly higher weight gain within the first 10 d of adulthood than T6-T3 females (Experiment A) (Table 17). Females gained significantly more weight than males in both temperature regimes and in both years (Table 18). The duration of rearing (one or two years) did not affect the weight gain of males or females within the first 10 d of adulthood under T0-T3 or T6-T3 conditions (Table 18).

Table 15: Development times (in days) of T0-T3 and T6-T3 female and male *Harmonia axyridis* at one year (Experiment A) and two years (Experiment B) of rearing

T0-T3									**T6-T3**								
Females				Males					Females				Males				
n	Median	Q_1	Q_3	n	Median	Q_1	Q_3	P	n	Median	Q_1	Q_3	n	Median	Q_1	Q_3	P
Experiment A (one year)																	
12	19.0	18.5	19.0	16	19.0	18.5	19.5	0.5694	10	20.0	19.00	21.0	15	19.0	19.0	21.0	0.7946
Experiment B (two years)																	
12	20.0	19.5	21.0	14	20.0	20.0	21.0	1.0000	15	21.0	20.0	21.0	13	21.0	20.0	21.0	0.8599

Comparison of females and males within the same groups in Experiments A and B. Asterisks (*) indicate significant differences, as determined by Wilcoxon two-sample test, $p \leq 0.05$

Table 16: Development times of female and male *Harmonia axyridis*: Differences between T0-T3 and T6-T3 and between Experiments A and B (one and two years of rearing)

Differences between T0-T3 and T6-T3

Experiment A		Experiment B	
Females	Males	Females	Males
P = 0.0204*	P = 0.0312*	P = 0.1628	P = 0.1322

Differences between Experiment A and B

T0-T3 Females	T0-T3 Males	T6-T3 Females	T6-T3 Males
P = 0.0008*	P = 0.0012*	P = 0.0528	P = 0.0122*

Asterisks indicate significant differences, as determined by Wilcoxon two-sample test, $p \leq 0.05$

Table 17: Eclosion weight (mg), body weight of 10-d-old adults (mg) and weight gain within the first 10 d of adulthood (mg) of female and male *Harmonia axyridis* adults treated under T0-T3 and T6-T3 conditions in Experiments A and B

	Females					Males				
	T0-T3		**T6-T3**			**T0-T3**		**T6-T3**		
	n	Mean (SEM)	n	Mean (SEM)	P	n	Mean (SEM)	n	Mean (SEM)	P
					Experiment A (one year)					
Eclosion weight of adults (mg per individual)	13	38.29 (0.85)	10	35.21 (1.25)	0.0580	16	32.38 (0.61)	15	31.02 (0.86)	0.2585
Body weight of 10-day-old adults (mg per individual)	12	48.81 (1.87)	10	42.34 (2.07)	0.0317*	16	32.90 (0.86)	15	33.67 (0.80)	0.5186
Weight gain within the first 10 d of adulthood (mg per individual)	12	10.67 (1.46)	10	7.12 (1.16)	0.0715	16	0.52 (0.82)	15	2.42 (0.62)	0.0482*
					Experiment B (two years)					
Eclosion weight of adults (mg per individual)	12	37.20 (0.79)	15	38.74 (0.70)	0.1591	14	33.74 (0.72)	13	32.24 (1.07)	0.3561
Body weight of 10-day-old adults (mg per individual)	12	45.94 (0.90)	54	44.46 (0.75)	0.5112	14	36.01 (0.91)	57	33.88 (0.38)	0.1784
Weight gain within the first 10 d of adulthood (mg per individual)	12	8.74 (0.84)	15	6.33 (0.81)	0.0496*	14	2.24 (0.60)	13	2.04 (0.47)	0.7715

Asterisks (*) indicate statistically significant differences, as determined by Welch’s test for comparison of two independent samples with unequal variances, $p \leq 0.05$

Table 18: Eclosion weight (mg), body weight of 10-day-old adults (mg) and weight gain within the first 10 d of adulthood (mg): Gender differences between female and male *Harmonia axyridis* adults treated under T0-T3 and T6-T3 conditions in Experiments A and B

	Differences between females and males					
	T0-T3			**T6-T3**		
	df	t value	P	df	t value	P
	Experiment A (one year)					
Eclosion weight of adults (mg per individual)	22.854	5.66	< 0.0001*	17.056	2.76	0.0134*
Body weight of 10-day-old adults (mg per individual)	15.620	7.73	< 0.0001*	11.732	3.90	0.0022*
Weight gain within the first 10 d of adulthood (mg per individual)	17.732	6.07	< 0.0001*	14.205	3.40	0.0042*
	Experiment B (two years)					
Eclosion weight of adults (mg per individual)	23.241	3.25	0.0035*	21.072	5.08	< 0.0001*
Body weight of 10-day-old adults (mg per individual)	23.857	7.77	< 0.0001*	25.934	8.38	< 0.0001*
Weight gain within the first 10 d of adulthood (mg per individual)	20.551	6.28	< 0.0001*	22.113	4.56	0.0002*

Asterisks (*) indicate statistically significant differences, as determined by Welch’s test for comparison of samples with unequal variances, $p \leq 0.05$

Fat body content of 10-day-old adults

In terms of the fat body content of 10-day-old male and female *H. axyridis* adults, no significant effects of either the T0-T3 nor the T6-T3 treatment were observed after one year (Experiment A: T0-T3-f vs. T6-T3-f: P = 0.6659; T0-T3-m vs. T6-T3-m: P = 0.8989) or two years of rearing (Experiment B: T0-T3-f vs. T6-T3-f: P = 0.0515; T0-T3-m vs. T6-T3-m: P = 0.3437) (Figure 17). Only T6-T3 males and females showed no significant gender differences (P = 0.0884) at one year (Experiment A). In all remaining treatment groups, females had significantly lower fat body contents than males (Figure 17). However, the differences in fat body content between the sexes were more prominent after two years (Experiment B) than after one (Experiment A). The rearing duration affected the accumulation of fat only in T6-T3 males (P = 0.0181), who had more fat body content after two years of rearing than after one. The other treatments were not affected by the duration of rearing (Figure 17).

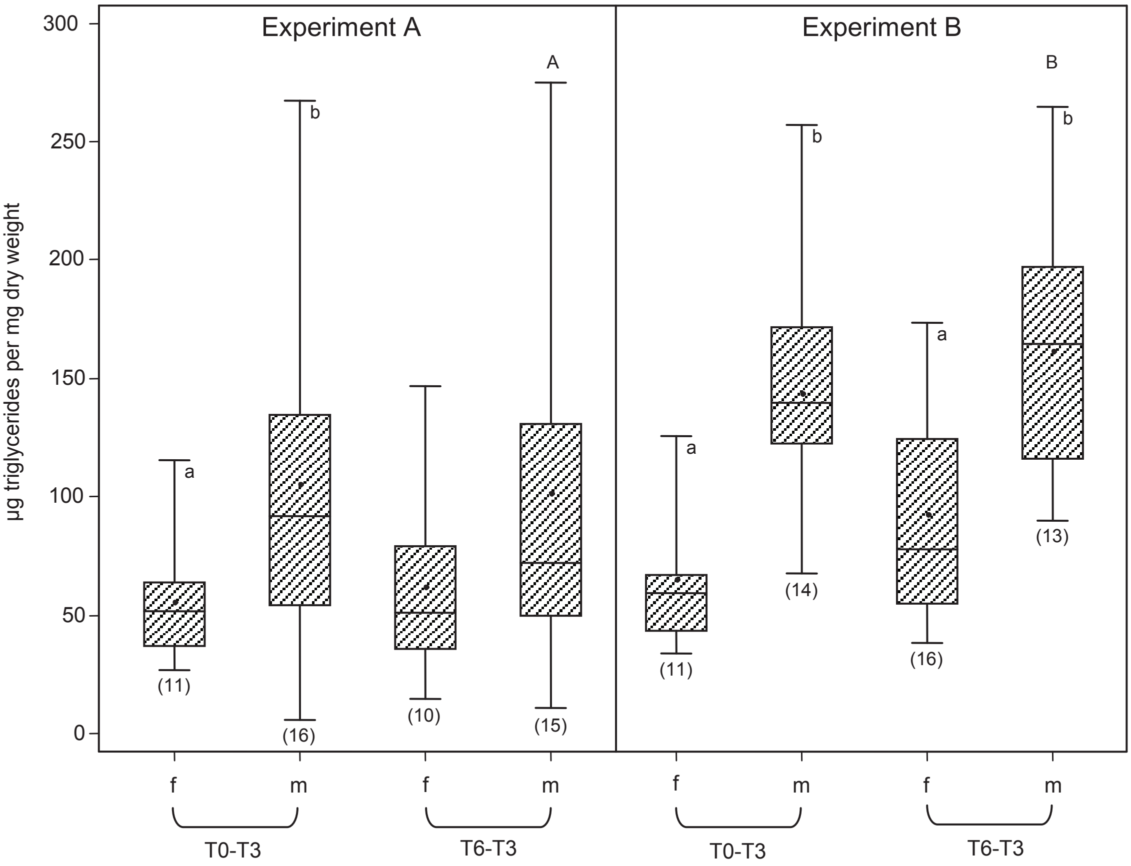

Figure 17: Fat body content (µg triglycerides/ mg dry weight) of female and male *Harmonia axyridis* adults treated at T0-T3 and T6-T3 temperatures in Experiments A and B. Differences between sexes within (a, b) and between the groups (A, B), as determined by Welch's test for comparison of two independent samples with unequal variances; $p \leq 0.05$

Antimicrobial activity

Measurement of the minimal inhibitory concentration (MIC) against *E. coli*, *B. subtilis* and the yeast *S. cerevisiae* in the hemolymph of 3rd, 6th and 10th generation of 10-day-old *H. axyridis* adults of the two strains reared at T0 and T6 temperatures yielded no gender differences in terms of antimicrobial activity. Therefore, the male and female MIC values were combined. The comparison of mean MIC values against *E. coli* and *B. subtilis* in the T0 and T6 groups, including males and females, revealed differences between the two temperature regimes in the 3rd and 6th generation (Figure 18, Table 19). The MIC values were lower, i.e., the antimicrobial activity was higher at the maximum temperature (T6). This effect disappeared in the 10th generation in both groups. Additionally, the generation number affected the MIC against *E. coli* and *B. subtilis.* The higher the generation number, the higher the MIC and the lower the antimicrobial activity of the two temperature regimes. In terms of *S. cerevisiae*, differences were only observed at the first analyzed generation 3 of the T0 and T6 strains (Table 19). Antimicrobial activity against this yeast was higher in the T6 strain. This effect was no longer detectable in the 6th and 10th generation.

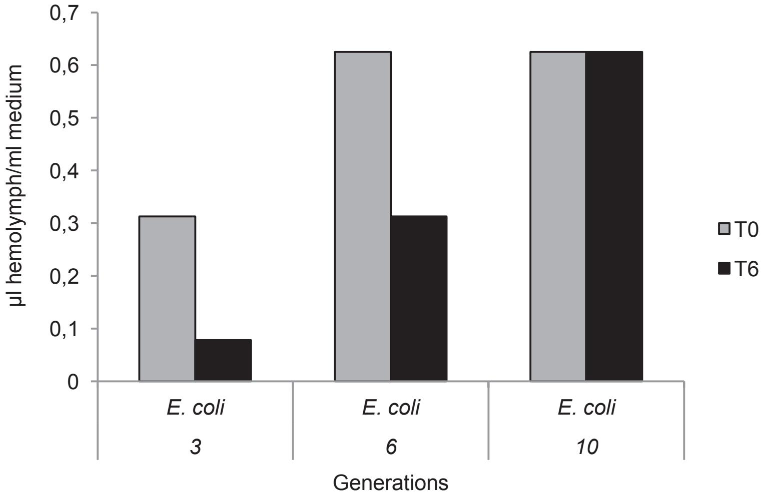

Figure 18: Minimal inhibitory concentration (µl hemolymph/ml medium) against (gram-negative) *Escherichia coli* measured in 3rd, 6th and 10th generation of *Harmonia axyridis* adults treated at T0 and T6 temperatures (at least n = 10 specimens per treatment)

Table 19: Minimal inhibitory concentration (µl hemolymph/ml medium) against *Bacillus subtilis* (gram positive) and *Saccharomyces cerevisiae* (yeast) in 3rd, 6th and 10th generation of at least n = 10 *Harmonia axyridis* adults treated at T0 and T6 temperatures (at least n = 10 specimens per treatment)

	Generation and temperature treatment					
	3th		**6th**		**10th**	
	T0	**T6**	**T0**	**T6**	**T0**	**T6**
B. subtilis	1.25	0.75	2.5	1.25	1.25	1.25
S. cerevisiae	0.157	0.077	0.157	0.157	0.157	0.157

4.4 Discussion

4.4.1 Study on effects of elevated temperatures on life table parameters

The seven-spot ladybird (*C. septempunctata*) is a successful native predator of aphids in arable crops worldwide. In central Europe, this species is accompanied by another coccinellid, *P. quatuordecimpunctata* (Poehling et al. 2007). We expect that the invasive multicolored Asian lady beetle (*H. axyridis*) will compete with these two native coccinellid species. The matter of how comparatively the two species respond to increasing temperatures in the context of global warming is of particular interest. Dunn (1952), Frazer and Gilbert (1976) and Triltsch et al. (1996) stated that elevated temperatures could lead to an increased predatory potential of coccinellids. We expect that both trophic levels – prey and predator – will benefit from increasing temperatures. However, there is evidence that aphid consumption by coccinellids increases more strongly than aphid reproduction rates, and this difference could enhance the predatory efficiency of coccinellids (Triltsch et al. 1996). Although many studies on the effects of temperature on ecological parameters of *C. septempunctata* and *H. axyridis* already exist, there are few comparative studies of these two species. In the few studies performed, the investigators used constant or alternating night and day temperature regimes. We did not find any reports of experiments carried out with realistic daily temperature curves. In view of these shortcomings, the present climate chamber study used daily temperature profiles to simulate real temperatures in the daily minimum to maximum range during the period of main predatory activity in the field (mid-June to the beginning of July). Hence, it provides useful and more realistic data on the effects of different temperature regimes (normal, elevated or both) on preimaginal mortality and developmental time, eclosion weight, and aphid consumption by adults as well as body weight and fat body content of adults of both species.

In terms of mortality, no clear tendencies were observed. Mortality rates were generally low (≤ 11.7%) and were not associated with any particular temperature conditions or species. However, one must consider that both experiments started with first instars and not with eggs, which are more sensitive. The highest mortality of coccinellids generally occurs in eggs due to cannibalism and intraguild predation (Sato et al. 2009, Kajita & Evans 2010). Survival rates increased during ongoing development, and later instars seemed to be more resistant than lower instars. Frazer (1988) reported much higher mortality (95.4%) from egg to young adult under environmental conditions. Srivastava and Omkar (2003) reported mortality rates of 52.1 and 31.1% for immature *C. septempunctata* at 20 °C and 30 °C, respectively. Srivastava and Omkar (2003) assumed that a thinner and softer cuticle of lower instars could be the cause of higher mortality rates in low immature stages of coccinellids. Our results, with lower mortality rates at later stages, support their assumption.

Corresponding to our previous investigations (Krengel & Freier 2010), the developmental times of both species were quite similar. There was only a small tendency of *H. axyridis* for faster development at normal temperatures (T0) and of *C. septempunctata* for faster development at elevated temperatures (T3); the difference was significant only in the second experiment. The current study as well as several others demonstrated that the developmental time for coccinellids is shorter at higher temperatures. Katsarou et al. (2005) reported 38.4 d and 18.7 d at a constant 17°C and 23°C, respectively, as the development time from first instar to adult eclosion of *C. septempunctata* (when feeding on *Myzus persicae* ssp. *Nicotinae* Blackman). In experiment A, *C. septempunctata* development times were 28.0 d for females and males at normal temperatures (mean: 17.8 °C) and 18.0 d (females) to 19.0 d (males) at elevated temperatures (mean: 20.8 °C). LaMana and Miller (1998) determined 34.9 d (at constant 18 °C) and 21.3 d (at constant 22 °C) as the development time from *H. axyridis* first instar to adult [feeding on *Acyrthosiphon pisum* (Harris)]. Stathas et al. (2011) estimated *H. axyridis* development time from first instar to adult to be 23.4 d at constant 20 °C when feeding on *Aphis fabae* Scopoli. In contrast, we determined *H. axyridis* development time to be 27.0 d (female) to 26.0 d (male) at normal temperatures (mean: 17.8 °C) and 19.0 d (females and males) at elevated temperatures (mean: 20.8 °C) in experiment A, for example.

Deviations within our data and from the literature data can have different causes. Different prey quality could be one reason. Aphid species are differently suitable as prey for both coccinellid species (Hukusima & Kamei 1970, Hauge et al. 1998, Stathas et al. 2011). In our own investigations, we used aphids and coccinellids produced from the same stocks. Because we conducted the investigations during different periods, we assume certain seasonal effects occurred, which could explain the deviations of developmental times in both experiments. The possibilities for comparison of our results with those of other authors are limited because the other authors only used constant temperature regimes. Nevertheless, temperature- and species-specific tendencies were similar in both of our experiments.

Our analysis of eclosion weight under the two temperature regimes revealed interesting differences between *C. septempunctata* and *H. axyridis*. Although the measured eclosion weights reflected the result of larval and pupal development, the weights of 10-d-old adults represented the results of lifetime development up to the 10th day of adulthood. In the majority of cases, the eclosion weights of *C. septempunctata* were higher than those of *H. axyridis*.

Experiment A investigated aphid consumption by adult coccinellids. Daily aphid consumption exhibited enormous variation between days, increasing within the first 10 d of adulthood. Thus, we used the aphid consumption index, which describes the area under the curve of daily feeding rates, for analysis of aphid consumption. The aphid consumption index did not differ significantly between species or temperature regimes, although aphid consumption rates for *H. axyridis* were lower than those for *C. septempunctata* in both temperature and sex groups. Conversely, Lucas et al. (2002) found that daily aphid consumption (*Aphis citricola* van der Goot) in *H. axyridis* was higher than that of *C. septempunctata* after 24 h of starvation at constant temperature (25 °C). Consistent with previous results from Freier and Triltsch (1996), Krengel and Freier (2010) and other authors (e.g. Hodek and Honek 1996), we observed that aphid consumption indices for both species were higher at elevated temperatures (T3) than at normal temperatures (T0). Elevated temperatures cause metabolic processes and therefore aphid consumption by coccinellids to increase (Freier & Triltsch 1996). Moreover, females of both species tended to consume more aphids than males. This tendency was more obvious at T0 than at T3. Oogenesis and oviposition are probable causes of higher consumption in females. Seagraves (2009) suggested that egg production by coccinellids requires an additional nutritional intake, and Beenakkers et al. (1985) reported that females of many insect species need additional reserves for egg production.

In our present and previous studies (Krengel 2009, Krengel & Freier 2010), the weights of the native species *C. septempunctata* were highest at elevated temperatures (T3) and those weights of the invasive species *H. axyridis* at normal temperatures (T0). Kawauchi (1979) also reported that the weights of adult *H. axyridis* reared at higher temperatures were lower. Regarding the strong increase in aphid consumption by both species at elevated temperatures, important differences between the species existed. Aphid consumption index values for *C. septempunctata* increased by 42 and 75%, and those for *H. axyridis* by 30 and 57% for females and males, respectively. *Coccinella septempunctata* consumed more aphids and weighed more. *Harmonia axyridis* weighed less despite higher consumption at T3 temperatures. In terms of weight, our data suggest that *C. septempunctata* seems to be better adapted to elevated temperature conditions than *H. axyridis*. However, Katsarou et al. (2005) and others did not observe any effect of temperature on adult weights of *C. septempunctata*. Zhao et al. (2010) also observed that the weights of adult *H. axyridis* are lower at higher temperatures (25 °C) than at lower temperatures (18 °C). The different responses in live weight of the two species may be indicative of a change in physiological demands created by the increased temperature. *Harmonia axyridis* is often described as particularly aggressive, mobile and active (Soares et al. 2005). It might be that higher activity of *H. axyridis* than *C. septempunctata* at elevated temperatures causes increased energy consumption and lower weights of *H. axyridis*. Even though we did not analyze gender differences, several investigators e.g. Anderson (1981) and Zhou et al. (1995) confirmed that the weights of male coccinellids were lower than those weights of females. In terms of body weight, the response of female coccinellids, particularly *H. axyridis,* to temperature elevation was much greater than that of males.

We also analyzed the fat body content of 10-d-old *C. septempunctata* and *H. axyridis* adults. The results of these experiments confirmed our previous findings showing considerable differences in fat body accumulation of these two species. We observed great differences not only in the measured content of lipids, but also in the response to elevation of temperature. A key finding of the present study was that *C. septempunctata* accumulated higher fat body content than *H. axyridis* under all conditions studied. In particular, females showed significant differences. The fat body content of *C. septempunctata* was higher at elevated temperatures (T3) than at normal temperatures (T0), whereas that of *H. axyridis* remained at a relatively low level. Thus, fat body accumulation per mg dry weight and its dependency on temperature seem to be strongly linked to body weight. Lower weights of *H. axyridis* at elevated temperatures corresponded with lower rates of fat body accumulation.

This phenomenon supports our hypothesis of increased energy consumption at higher activity levels because of increased temperatures.

The divergent fat body development, particularly among females, could be caused by species-specific differences in voltinism. In Central Europe, *C. septempunctata* usually produces only one generation per year (Hagen 1962, Majerus 1994, Klausnitzer & Klausnitzer 1997), whereas *H. axyridis,* a polyvoltine species, produces two or three generations per year (Koch 2003). Thus, *C. septempunctata* seems to be genetically programmed to prepare itself for hibernation early in the season, soon after emergence of the generation. Butler (1982) also assumed that *C. septempunctata* has a high tendency to univoltinism because of a lack of oviposition in newly hatched, fertilized females of that species. Conversely, the aim of *H. axyridis* seems to be to produce eggs to build up the next generation. Other studies of fat body content of diverse coccinellid species revealed higher fat body contents at the beginning of hibernation or diapause (El-Hariri 1966, Hodek & Landa 1971, Anderson 1981, Pfaender et al. 1981, Okuda & Hodek 1994, Zhou et al. 1995, Katsoyanno et al. 1997). We also found gender-specific differences in triglyceide accumulation by the fat body. Female *C. septempunctata* had higher fat body contents than males at normal temperature (T0), but comparable fat body contents at elevated temperatures (T3). In the case of *H. axyridis*, fat body content was higher in males than in females at T0 and T3 temperatures. This finding could be additional evidence that female *H. axyridis* sacrifice all their reserves for reproduction.

It was still unclear at which stage – larva and pupa or young adult – temperature elevation determines the body weight and fat body content of adult coccinellids. There were no significant differences in adult body weight or fat content between individuals reared under the same preimaginal but different post-eclosion temperatures (T0-T0 versus T0-T3 and T3-T3 versus T3-T0). Nevertheless, we observed several significant differences in those two life table parameters when coccinellids were treated at different temperatures during preimaginal development but the same temperatures during the imaginal period (T0-T0 versus T3-T0 and T3-T3 versus T0-T3). Thus, we assume that the temperatures experienced during preimaginal development determine the body weight and fat body content of both *C. septempunctata* and *H. axyridis* adults.

Regarding the validity of the findings of the current study, we emphasize that the data on developmental time, body weight and fat body content of *C. septempunctata* and *H. axyridis* exhibited exactly the same tendencies in both experiments, indicating that they are quite reliable. The investigated life table parameters (developmental time, live weight and fat body content) have been found to be valid markers of effects of increasing temperatures. The current study focused on the individual effect of different daily temperature profiles. We assume that additional factors such as solar radiation or humidity modify the assessed responses under natural settings. The present experiments studied only short-term effects of temperature within one generation. Additional investigations are needed to study the long-term effect of temperature over several generations. Processes such as metabolic programming (Langley-Evans 2009, Fernandez-Twinn & Ozanne 2010) are known to be relevant for invertebrate adaption to changing environmental conditions. Such processes may also affect the response of insects such as coccinellids to elevated temperature conditions.

4.4.2 Study on effects of elevated temperatures on food conversion efficiencies

Data on how native *C. septempunctata* and invasive *H. axyridis* convert consumed food into body mass can provide valuable information on their biology and ability to tolerate different environmental conditions (Omkar & Afaq 2011, Hodek & Honek 1996, Roy & Waijnberg 2008). Significant differences in food conversion efficiencies of these two species could indicate interesting findings on their competitive ability and predatory potential under elevated temperature conditions.

Contrary to many current investigations which use constant or alternating temperatures, we used daily temperature profiles to simulate real temperature fluctuations in the daily minimum to maximum range. Also Fischer et al. (2011) point to the fact that in nature temperature typically shows fluctuations over the course of the day. They result several effects of fluctuating compared to constant temperatures on development time, heat and cold stress resistance, heat-shock protein expression and immunocompetence of *Lycaena tityrus* (Poda) (Lepidoptera: Lycaenidae). Ellingsen (1969) and Mellors et al. (1984) investigated the effects of alternating versus constant temperatures on life table parameters of *Adalia bipunctata* L. and *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae) and also detected significant differences. Additionally, Mellors and Allegro (1984) concluded that the responses to alternating temperatures are not simply the sum of responses to constant temperatures.

The present analysis revealed that elevated temperatures led to significantly higher consumption rates (up to 75%) in both species, whereby *C. septempunctata* showed higher consumption rates than *H. axyridis*. Particularly striking differences of the two species were observed between males at T3 temperatures, where *C. septempunctata* consumed about 80% more aphids than *H. axyridis* males. Moreover, females of both species consumed up to 50% more aphids than the males. Only a few studies can be compared with the present experiments because different aphid species were used as prey. In contrast to our findings, Lucas et al. (2002) observed higher daily consumption rates in *H. axyridis* than in *C. septempunctata* feeding on *Aphis citricola* van der Goot at a constant temperature of 25 °C after 24 h of starvation. A reason for this different result could be that the aphid species are unequally suited as prey for the two coccinellid species investigated. However, our experiments since 2006 have consistently shown higher feeding rates for *C. septempunctata* feeding on *S. avenae* (Krengel et al. 2012). Regarding the effect of elevated temperatures, other authors including Freier and Triltsch (1996), Hodek and Honek (1996) and Katsarou (2005) have also observed higher consumption rates at elevated temperatures. Like Seagraves (2009) and Beenakkers et al. (1985), we also suggest that reproductive processes such as oogenesis and oviposition result in higher nutritional requirements for females than in males. Therefore, females need to consume more aphids than males to meet their nutritional requirements and build up reserves.

Eclosion weight is the result of all environmental conditions during development from egg to pupa. Overall, the eclosion weights of female and male *C. septempunctata* and *H. axyridis* adults differed only slightly in the four experiments. As expected, females of both species had significantly higher eclosion weights than males under T0 and T3 conditions. Phoofolo and Obrycki (1998), who used different prey (*Acyrthosiphon pisum* Harris), measured very similar weights of female (34.2 mg) and male (30.7 mg) *H. axyridis* adults directly after eclosion and observed almost identical gender-specific differences.

In terms of the weight and fat body content of 10-day-old adult *C. septempunctata* accumulated significantly higher values than 10-day-old *H. axyridis* adults. Despite higher consumption rates in both species, only female and male *C. septempunctata* adults strongly benefited from the elevation of temperature and had significant higher weights and fat body contents on day 10 of adulthood under T3 conditions. In contrast, Katsarou et al. (2005) did not observe any effect of temperature on adult weights of *C. septempunctata*. However, elevated temperatures did not influence the weights of 10-day-old *H. axyridis* females and males and led to decreased fat body content in females. Kawauchi (1979) and Zhao et al. (2010) even reported lower weights of *H. axyridis* adults reared at higher temperatures.

Contrary to the body weight of 10-day-old adults, most *H. axyridis* males accumulated higher, up to 2 times, fat body reserves than females. Studies on the behavior of *H. axyridis* have described this species as particular aggressive, mobile and active (Soares et al. 2005, Yasuda et al. 2001). Thus, we presume that the different weight responses of 10-day-old adults of the two species could be due to the higher activity rates of *H. axyridis* at elevated temperatures, possibly in connection with a higher energy turnover in *H. axyridis* than in *C. septempunctata*.

The present data on body weights reveal considerable weight gains ranging from 6% to 35% depending on the type of treatment. The following tendency was observed: the higher the consumption rate, the higher the weight gain, independent of species and sex. In terms of fat body content high fat body content was associated with a high consumption rate only in *C. septempunctata*, whereas *H. axyridis* exhibited the contrary tendency. These varying results can be disused in relation to the different biology of the two coccinellids. *Coccinella septempunctata* usually produces only one generation per year in Central Europe (Hagen1962, Majerus 1994, Klausnitzer & Klausnitzer 1997) and tries to prepare for hibernation early in the season. Butler (1982) also reported a high tendency of *C. septempunctata* to produce only one generation per year. *Harmonia axyridis*, on the other hand, tends to produce two or three generations per year, so females have to produce more eggs (Koch 2003).

Elevated temperatures had a relatively low effect on the ladybirds’ ability to convert aphid biomass into body weight. *Coccinella septempunctata* females and males required only 4% and 12% more aphids, respectively, at elevated (T3) than at normal (T0) temperatures. *Harmonia axyridis* females and males responded differently: the females consumed 22% more aphids whereas the males consumed 8% less at elevated temperatures. Surprisingly, the invasive ladybird *H. axyridis* clearly exhibited the lowest food conversion ratios. *Harmonia axyridis* males were the poorest converters of consumed biomass into body weight. In terms of the ability to transform aphid biomass into fat body content, *H. axyridis* females were the least efficient converters. Compared to all other groups, they required approximately 50% more aphids at T0 temperatures and even up to 3 times more aphids at T3 temperatures to gain 1 mg triglyceride per mg dry weight per individual.

Overall, the results regarding food conversion ratios reflect temperature-, species- and gender-specific differences in the ladybirds' ability to convert consumed aphid biomass into body weight and fat body content. *Harmonia axyridis* requires partially considerable higher consumption rates than *C. septempunctata* to produce body weight and fat body. Unfortunately, we found almost no comparable studies on the food conversion efficiencies of adults of these two species that were related or even unrelated to different temperatures. The studies identified focused on the larval stages of coccinellids. Işikber and Copland (2001), who studied food consumption and utilization by larvae of *S. levaillanti* and *C. sanguine*, observed size-dependent differences in conversion efficiencies and higher conversion efficiencies of *C. sanguine* larvae at elevated temperatures. They found that smaller *S. levaillanti* better converted consumed biomass. Işikber and Copland (2001) assume that the type of digestion is able to affect food conversion. Behmer (2009) also state that insects are able to regulate their nutrient intake using special pre- and post-ingestive mechanisms. Maurice and Kumar (2011) and Schuder et al. (2004) found that the amount of food supply additionally influences the food conversion efficiency.

In summary, the present study shows that the food conversion efficiency of the invasive species *H. axyridis* is remarkably lower than that of the native species *C. septempunctata*. This was observed either in females or in males. Elevated temperatures had a particularly strong effect on *H. axyridis* females. All these results provide evidence for substantial differences in biology regarding the ability of *H. axyridis* and *C. septempunctata* to adapt to changing environmental conditions.

4.4.3 Study on long-term effects of elevated temperatures on *Harmonia axyridis*

There is already a large amount of data available regarding the effects of different temperature conditions on life table parameters of predatory coccinellids. Nevertheless, all these studies have focused on short-term effects. As global warming will have a permanent effect on life table parameters of coccinellids, their prey and their interactions, it is of particular interest to obtain more information on how these predators respond to elevated temperature conditions over several parental generations. No scientific findings have been made so far towards this objective. In view of these shortcomings, the present study provides the first information on the long-term effects of temperature on diverse life table parameters and antimicrobial defense mechanisms of *H. axyridis*.

We observed significantly prolonged larval development time in both male and female progeny of *H. axyridis* reared at elevated temperature than in those reared at normal temperatures after one year of rearing, but not after two years. The values tended to be about one day longer for the development from first instar to adult at the corresponding experimental temperatures. These findings suggest that the differences in developmental time observed at one year disappeared after two years, which could be an indication of adaptation. Interestingly, all developmental times leveled out at similarly high values after two years. These results confirm previous studies finding similar development rates of males and females at the same temperatures (Krengel et al. 2012). As expected, long-term exposure of parental generations to high temperatures did not affect this gender similarity.

Even when continued for one or two years, treatment at elevated temperatures did not affect eclosion weight, which is the result of larval and pupal development of the offspring generation. The body weight of 10-day-old adults was the only parameter where we observed significantly lower values in female progeny and significantly higher weight gain in male progeny of the strain reared at elevated temperatures after one year. The tendency found in females was confirmed by the results of the second experiment (after two years), which indicated significantly lower weight gain. Thus, there are slight indications that female body weight seemed to be reduced while those of males seemed to increase in response to long-term rearing at elevated temperatures. While there are no comparable data on long-term rearing of coccinellids at elevated temperatures, numerous results do exist on the immediate effects of elevated temperatures on the weight of adult *H. axyridis*. Kawauchi (1979), Zhao et al. (2010) and Krengel et al. (2012) found that the weights of male and female *H. axyridis* adults decreased or stagnated at elevated temperatures.

We detected no significant differences between the fat body content of the progeny of the two *H. axyridis* strains after one and two years of rearing. That means that the fat body content also did not respond to long-term exposure to elevated temperatures. Only after two years of rearing did the offspring of the elevated-temperature strain show a slight but non-significant tendency towards higher fat body content in both genders. Furthermore, the experiment confirmed the previous finding that males accumulate more triglycerides in the fat body than females (Krengel et al. 2012). The difference between the genders even increased after two years of rearing, unaffected by the rearing temperature of the parental generations. As already assumed in previous sections, the reproductive behavior of *H. axyridis* could be a reason for these results (Krengel et al. 2012). In contrast to other coccinellid species, *H. axyridis* is polyvoltine, even in Central Europe (Koch 2003). It seems that female *H. axyridis* adults use their metabolic activities for reproductive purposes and not for accumulation of fat body reserves under stress conditions.

The evaluation of the antimicrobial activity in 3rd, 6th and 10th generation *H. axyridis* reared at T0 and T6 temperatures revealed no gender differences. Therefore, male and female minimal inhibitory concentrations against the three tested microorganisms (*E. coli, B. subtilis* and *S. Cerevisiae*) were combined to yield one common value per treatment. Regarding the MIC values against *E. coli* and *B. subtilis,* respectively, the values observed in *H. axyridis* ladybirds reared at highly elevated temperatures were equal to or lower than those of their counterparts reared at normal temperatures. That could be the first evidence of higher antimicrobial activity and of possible selection advantages at higher temperatures. However, this phenomenon disappeared in the 10th generation. Additionally, we observed an effect of generation number. The longer the beetles were kept under laboratory conditions, the higher the MIC. That implies that long-term rearing under laboratory conditions could have a negative effect on antimicrobial activity probably due to inbreeding effects (Gerloff et al. 2003). Another reason for decreasing antimicrobial activity could be the absence of microbial pathogen stimuli.

In summary, we conclude that exposure of several generations of *H. axyridis* to highly elevated temperatures over the course of two years has no significant long-term effect on life table parameters of their progeny. Only in terms of antimicrobial activity did the duration of rearing have a marked effect on the minimal inhibitory concentration against the target microorganisms. Maybe, the special isolated conditions in the laboratory minimized biological stress effects by pathogens. Nevertheless, there is evidence for an adaptation of diverse animal species to long-term exposure to specific environmental conditions (Lucas 1991, Bezemer et al. 1998, Bradshaw & Holzapfel 2001, Fernandez-Twinn & Ozanne 2010). However, considering the present results, *H. axyridis* seems to be enormously adaptable to new environmental conditions. This could be a selective advantage over other less adaptable species. It is difficult to estimate whether two years of *H. axyridis* rearing under laboratory conditions are enough to change behavioral or fitness patterns of the test populations. Bradshaw and Holzapfel (2001) observed genetic shifts in pitcher-plant mosquito populations (*Wyeomyia smithii)* over a period of 5 years, which they described as an adaptive evolutionary response to recent global warming. Bezemer et al. (1998) already detected adaptations to changed temperature conditions in My*zus persicae* and its parasitoid *Aphidius matricariae* after only 9 months of investigation. Although we could exclude inbreeding as a factor in *H. axyridis* rearing, the genetic pool remained relatively constant within the two years of study. Inbreeding can negatively affect various fitness components including immune system (Gerloff et al. 2003). But there are also other studies which report that *H. axyridis* is able to overcome inbreeding depressions (Facon et al. 2011). However, one has to consider that these investigations were performed using individuals of the 3rd generation of a laboratory stock based on field collected beetles. In contrast, our experiments were implemented with individuals up to the 17th generation over a time period of 2 years. Coccinellids may possibly have other ways to adapt to long-term environmental changes under field conditions while showing larger genetic diversity. The results of the present study did not provide any indicates in terms of epigenetic hypothesis that “sleeping genes” might be awaked in response to long-term exposure to elevated temperatures. Nevertheless, the investigation provided first findings on possible long-term adaptation of *H. axyridis* to highly elevated temperature conditions.

5. Conclusions

At the beginning of this doctoral research little comparative data was available on the responses of *C. septempunctata* and *H. axyridis* as natural enemies of cereal aphids to different temperature conditions, particularly elevated temperatures. Although an enormous amount of data exists on the different life table parameters of the two species the comparability of the different studies is strongly hampered by the use of different experimental conditions. The present doctoral research aimed to reduce this shortage of comparative data by conducting a set of several preliminary studies and three main studies. This raises the question of what data we were finally able to gather regarding this issue and what conclusions we were able to derive from this data.

In microcosm experiments, conducted within the scope of preliminary studies using alternating night and day temperatures, both coccinellids showed similar predation rates on *S. avenae* under laboratory conditions. Neither *C. septempunctata* nor *H. axyridis* clearly benefited from elevated temperatures in terms of predatory potential. If *H. axyridis*, the invasive Asian ladybird, becomes established in winter wheat this could be evidence for additional competition between the two species. We also conclude that the representativeness of the microcosm experiments was limited by factors such as high artificial aphid reproduction rates and the exclusion of inter- and intraguild competition. Nevertheless, these experiments were useful as they provided the first comparative data on the predatory potential of the two ladybird species.

In a mini field experiment including three cereal aphid species and different fractions of predators, we observed a strong dominance of coccinellids within this aphid predator community and a strongly increased number of predators after the three-week experimental period. The predators reproduction rates were seven times higher at elevated temperatures than at normal temperatures. Thus, a 3 K temperature increase seems to have an enormous potential to increase the number of progeny produced by the coccinellids. The optimal temperature for reproduction of *S. avenae* is reported to be 22 °C, which is lower when the optimal temperature range reported for the two coccinellids (Yuan et al. 1994, Obyrcki & Tauber 1981). This evidence suggests higher predatory efficiency at elevated temperatures as already assumed by Dunn (1952), Frazer and Gilbert (1976), and Triltsch et al. (1996). When placed in direct competition, *H. axyridis* showed decisive superiority over *C. septempunctata*.

Thus, it can be assumed that *H. axyridis* probably could have negative effects on *C. septempunctata* when existing in direct competition in the field. However, further studies must be conducted to draw clear conclusions regarding their interactions in the field and possible consequences of this in the context of other influencing factors.

There are some amazing similarities between the two ladybird species, such as their feeding range, size and developmental temperature requirements. Nonetheless, the laboratory experiments conducted using night and day temperatures as well as daily temperature profiles revealed some interesting differences in the responses of their life table responses to elevated temperatures. Based on the fact that both species responded similarly to elevated temperatures in terms of higher consumption rates but differently in terms of the conversion of consumed biomass and accumulation of weight gain and fat body content during adulthood, we assume that they could maybe benefit from elevated temperatures to different degrees. *Coccinella septempunctata* seems to be affected more positively by 3 K warming. In contrast to *H. axyridis*, it produces higher body weight and fat body content at elevated temperatures. However, the two species seem to have different strategies for this. After the eclosion of new adult beetles the single aim of *C. septempunctata* seems to be to prepare itself for hibernation. In contrast, *H. axyridis* aims to produce further progeny and to build up a new generation. It is very difficult to assess which of these two strategies is better. Building up reserves could increase a species´ ability to survive hibernation periods, and the production of further generations could enable a population to reach similar numbers after hibernation individuals due to the higher initial numbers of hibernating individuals. The investigation of long-term effects of elevated temperatures on *H. axyridis* revealed no clear adaptation processes, such as the awakening of sleeping genes. Instead, these experiments, conducted in *H. axyridis* alone, rather revealed a strong ability of the species to respond spontaneously to changed environmental conditions.

However, a lot of other factors, such as the increased probability of extreme weather events, projected within current climate change scenarios, are thought to influence the success of a species. Buttelmann et al. (2012) who investigated the effects of heat stress on *S. avenae* and their natural enemies *E. balteatus* and *Aphidius rhopalosiphi* (de Stefani-Perez) *(*Hymenoptera: Braconidae) assumed considerable influences*.* Further studies are much-needed to find more pieces of the puzzle of the effects of elevated temperatures on *C. septempunctata* and *H. axyridis* and their potential as natural enemies of cereal aphids.

6. References

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VI. Selbstständigkeitserklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe. Es wurden keine anderen als die in der Arbeit angegebenen Quellen und Hilfsmittel benutzt. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen sind als solche kenntlich gemacht.

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