Studies on the influence of different diets and rearing conditions on the development and growth of the two-spotted cricket *Gryllus bimaculatus* de Geer

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Dedication

- I would like to dedicate this work to the spirits of my father, my brother Abdulaziz and my brother-in-law Dhafer.
- This work is for you my dear mother for that you have raised me, taught me and sacrificed the world for me. This is the fruit of your labour.
- I also dedicate this work to my respectable brother Ashour who are the source of enlightenment and through him I have known knowledge and the true importance of seeking knowledge.
- Last but not least I would like to dedicate this work to my lovely wife for her support and sacrifice throughout this long and difficult journey.
- To my lovely children; Robyan, Raghda and Mohamed.

Thank you all and may Allah bless you all. I could not have done this without you.

Abbreviations

AD	adult
AKH	adipokinetic hormone
BSA	bovine serum albumin
°C	degrees Celsius
CA	corpora allata
CC	corpora cardiaca
CEST	central European summer time
Ctrl	control
d	day
DAG	diacylglycerol
Fig.	figure
h	hour
L x W x H	length x width x height
g	gram
kJ	kilojoule
l	liter
DLM	dorso-longitudinal flight muscle
LL	last larvae
JH	juvenile hormone
L:D	light : dark
Mod.	modified
n.s.	not significant
PL	penultimate larvae
S.E.	standard error of the mean
TAG	triacylglycerol
w/	with
w/o	without

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1-INTRODUCTION

Although insects do not fascinate everyone, and indeed may repel some people, no one can afford to ignore the fact that we and most other species on this planet are dependent on them, directly or indirectly. Not only do they pollinate our crops; they also play a crucial role in a vast range of ecological processes, including the natural control of invertebrate species which can behave as pests, the cycling of organic matter and the fact that they are a food source for many vertebrate species **(Fry and Lonsdale, 1991)**.

Fruit, vegetable and ornamental plants are considered the most important crops, which are used as human and animal food in the entire world. As a result of the expansion of cultivated crops the problems of insect pests have been increasing during the last years. These crops or plants are subject to attack by a lot of harmful insect pests throughout the growing seasons or during storage. Among these insect pests, certain hemipteran insects such as Jassidae **(Hegab, 1981)**, white fly **(Rodrigues et al., 1997)**, scale insects **(Kosztarab and Kozar, 1988)** and aphids **(Blackman and Eastop, 1984; Komazaki, 1993; Darwish, 1998)** are of great economic importance. They cause immense damages either directly by sucking plant sap or indirectly as vectors of serious virus diseases.

On the other hand, many species are directly useful to us as a source of food, fuel, fiber, drugs, and many others have the potential to become useful in future **(Fry and Lonsdale, 1991)**. Beneficial insects such as *Apis mellifera* and *Bombyx mori* provide humans with certain useful materials such as honey, royal jelly and silk, respectively. Insects are considered a good model for the study of many biological and physiological aspects. This is due to their relatively short life cycle and small size, and the fact that they are easy to rear in the laboratory.

At present, no single control practice will solve all the arthropod pest problems. In the past, the excessive use of pesticides has often complicated the problem by creating a barren habitat void of natural enemies as well as resistant target insects **(Patterson, 1990)**. Predator and parasitoid insects play an important role in ecosystem.

The Orthoptera are a large group of “good jumpers” including crickets, locusts, grasshoppers, katydids and groundhoppers that can be found in most habitats. The two-spotted cricket *G. bimaculatus* de Geer (Orthoptera: Gryllidae) is one of the most abundant cricket species in tropical and subtropical areas where it is sometimes a major agriculture pest.

G. bimaculatus usually lives solitary in burrows or under stones **(Iba et al., 1995)**. The morphological characters of crickets are their jumping hind legs, three tarsal segments, and long tactile ceri bearing clumps of knobbed hairs, mandibulate mouthparts and a large

prothorax. The body length ranges from 1 mm to over 50 mm **(Alexander, 1968)**. Crickets have a sexual reproduction and male use songs to attract females that are typically silent. *G. bimaculatus* de Geer is a rather large and stocky cricket. The crickets of this species are insensible, slow moving and so robust that they can be reared at high density **(Nishioka and Matsuura, 1977)**. Generally, *G. bimaculatus* is one of many crickets species known as field cricket or black field cricket or as the African or Mediterranean field cricket or as the two-spotted cricket. It can be easily discriminated from other *Gryllus* species by the two dot-like marks on the base of its fore wings. *G. bimaculatus* has a good ability to fly. The locomotor activity of the nymphal (or larval) stage of the two-spotted cricket displays a diurnal rhythm whereas the adults show a nocturnal rhythm **(Tomioka et al., 1993)**. *G. bimaculatus* undergo incomplete metamorphosis (egg, larvae, adult). Usually females multiply mate, and have a long needlelike egg laying organ called an ovipositor. During the life cycle of *G. bimaculatus* and under optimal conditions a female lays between 2000-2500 eggs **(Espig and Hoffmann, 1985)**, and a maximum of 3000 eggs at different conditions of 30 or 34 °C **(Rivnay and Ziv, 1963)**. Larvae and adults usually occupy similar habitats and feed on similar foods. Larvae resemble the adults but are smaller and do not have wings; wing buds can be seen during the last two larval stages. They are widely distributed, easily collected, easily reared in the laboratory, easy to rear on a defined diet, have a simple life cycle, are large in size and large enough to dissect, easily sexed, produce many eggs, are easy to handle and have surfaces (pronotum), that permit easy marking for individual or group recognition. They do not sting, and most of them do not bite painfully **(Masaki and Walker, 1987; Wineriter and Walker, 1988)**. Since it does not enter diapause, it is available all year round. **Simmons (1986a, 1991)** stated that field crickets have been used as good model organisms for many important studies in the field and laboratory such as insect behavioural ecology, endocrinology **(Strambi et al., 1997)**, acoustic communication, reproductive biology **(Gäde et al., 2003)**, olfactory learning, pharmacology, electrophysiology **(Matsumoto and Mizunami, 2005)**, agonistic behaviour **(Killian and Allen, 2008)**, physiology, molecular mechanisms of development and regeneration **(Mito and Noji, 2008)**. On the other hand it is raised in captivity for use as a major food source for pets like snakes, lizards, spiders etc. *G. bimaculatus* are omnivores and scavengers likely to consume a variety of plant and animal matter during the course of their development. Some people in several countries also eat crickets. It is also used as an excellent diet for terrestrial anurans **(Nishioka and Matsuura, 1977)** and excellent source of proteins for many insectivores **(Ibler et al., 2009)**.

1.1 Isolated and crowded conditions

Not much information is available on the effects of isolated or crowded condition on life span of *G. bimaculatus.* The effect of population density on the survival, growth, and development of many species of animals varies greatly depending upon species and developmental stage **(Allee, 1934)**. **Chauvin (1958)** was the first to demonstrate that house crickets reared in groups grew more rapidly than those reared individually. Other effects of crowding are changes in body colour and wing development. Crowded hoppers of *G. bimaculatus* become lighter in colour than isolated ones **(Fuzeau-Braesch, 1960)**.

Generally environmental factors such as temperature, humidity, wind, light, abnormal nutrition, natural enemies, competitors and crowding have a profound influence on insect development e.g. number of instars, duration of larval development, final size, morphology and behaviour. **Bradley (1976)** determined that fecundity of the house cricket was decreased under crowded conditions. **Zutshi et al. (1980)** found insects reared under crowded conditions were more susceptible to insecticides than insects reared under isolated conditions.

The group effect in house crickets (*Acheta domesticus* L.) has been investigated by **McFarlane (1962)**, **McFarlane et al. (1984), McFarlane and Alli (1988)**, and **Clifford and Woodring (1990)**. **Gadot et al. (1989)** found that the ooctyes of females maintained in groups matured more quickly than did those of females kept alone. As reported in **Iba et al. (1995)** brain of crickets reared under crowded conditions contain significantly higher amounts of octopamine (also higher in corpora cardiaca), dopamine, and 5-hydroxytrytamine (5-HT; also higher in corpora allata) than that of isolated crickets; whereas, the level of N-acetyldopamine is highest in isolated crickets. These results unambiguously indicate that high population density affects aminergic systems which in turn probably modulate various biological events, such as development, growth and behaviour.

1.2. Fat body

The insect fat body is functionally analogous to mammalian liver and adipose tissue. It contains a single cell type which synthesizes, stores, and mobilizes lipid, protein, and glycogen. Fat body, unlike flight muscle, uses glycine and leucine as respiratory substrates, and it is suggested that the fat body acts like the vertebrate liver by trans deaminating amino acids and making them available for further metabolism by other tissues **(Clements, 1959; Beenakkers et al., 1985a; Canavoso et al., 2001**). In vitro incubation of young adult male *Leucophaea maderae* fat bodies with extracts of corpora cardiaca or intact corpora cardiaca results in stimulation of oxygen consumption but reduction in carbon dioxide evolved from

carbohydrate. The carbohydrate is preferentially used for trehalose synthesis, and the endogenous metabolism of the fat body appears to be supported by increased lipid utilization **(Wiens and Gilbert, 1965)**.

Haemolymph volume does not change significantly during 96 h of starvation. Haemolymph and fat body metabolites are depleted significantly in insects denied food for 96 h and the percentage of oocyte resorption is increased considerably by starvation. Differences in the utilization of lipid and protein under conditions of starvation between adult *Oxya japonica* and adult *Locusta migratoria* might be attributed to the migratory habit of the latter. The dry weight and metabolite content (lipid, carbohydrate and protein) of the fat body of insects starved for 96 h are significantly lower than in fed controls of the same age indicating that metabolites of the fat body are mobilized to supply the needs of the starving insects **(Lim and Lee, 1981)**. Although in many insects considerable somatic growth occurs during the adult stage prior to the onset of oogenesis, it is typical of insects that the larval stage is devoted to somatic growth and that the adult stage is devoted to ovarial growth. The fat body mass declines as yolk deposition progresses in *Melanoplus sanguinipes* and *Schistocerca gregaria* **(Hill et al., 1968; Gillot and Elliott, 1976)**. In orthopteroid insects, adult feeding provides the raw material from which the fat body synthesizes the specific precursors for oogenesis **(Hagedorn and Kunkel, 1979)**. The control of vitellogenin production and release from the fat body and the uptake of vitellogenin and lipids into the oocytes are primarily under the control of juvenile hormone (JH) **(Engelmann, 1983)**. In some insects mating is a stimulus for oogenesis or oviposition **(Loher and Edson, 1973)**.

The large amount of fat body present on day 0 in the adult female is produced and stored during the last larval stadium. Food consumption of mated, ovariectomized females and virgins during the first two days is high and the food is utilized to support somatic growth **(Woodring et al., 1979)**. Fat body mass is used up during oogenesis, but the mass of eggs ovulated is about 5 times the fat body mass lost. The combination of fat body reserves plus the food ingested permits the highest egg production rate to occur soon after the final moult. There are advantages in laying a large number of eggs quickly, even for a species that lays eggs continuously for the entire life span of the adult. Another advantage of large fat body mass in newly moulted females is that they can survive starvation much longer than females lacking the fat body **(Clifford and Woodring, 1986)**. Fat body sensitivity to AKH also varies in close synchrony with the lipid rhythm. Lipids are mobilized from the fat body as DAG, not free fatty acids as in vertebrate. Mobilization is induced by two types of hormones: adipokinetic hormone and octopamine.

1.3. Lipid

Lipids, especially the neutral lipids are important sources of energy for insects. The lipid content of the different stages of *Dysdercus koenigii* is about 10% of the fresh weight **(Agarwal and Rao, 1969)**. Lipids, carbohydrates and, in some insects, certain amino acids are used as respiratory fuels to supply the energy for flight. The amount and the composition of lipids in an insect vary considerably between developmental stages and tissues, and are influenced by several factors, including starvation, sex, hormones, and nutrition **(Beenakkers et al., 1985a)**. Lipids play key roles in insect biochemistry as sources of energy, structural components and as hormones **(Stanley-Samuelson et al., 1988)**. Triacylgcerols are the major lipid class in ovaries, fat body and newly laid eggs, whereas diacylgcerols and phospholipid predominate in the haemolymph **(Grapes et al., 1989)**. In insects using lipid as an energy source during flight or extended periods of rapid movement, diacylgcerols are the usual form in which fatty acids are transported in the haemolymph. Insects utilize lipids efficiently for development, reproduction and flight. Little information is available on the metabolic changes in the lipid content of tissues during the reproductive cycle, although insect vitellogenins are approximately 15% lipid and eggs contain appreciable amounts of lipid, up to 21% in *G. bimaculatus* eggs **(Lorenz, 2003)**. In *Manduca sexta* 40% of the dry weight of eggs is lipids **(Kawooya and Law, 1988)**. Oocytes of the yellow fever mosquito, *Aedes aegypti*, were found to contain 35% lipids **(Troy et al., 1975)**, although the percentage of lipid varies according to the size and nutritional status of the female **(Briegel, 1990)**. The two-spotted cricket belongs to the latter group, which starts adult life with significant amounts of lipid, glycogen, and protein in the fat body **(Lorenz and Anand, 2004)**. Due to intense feeding **(Woodring and Lorenz, 2007)** and a high rate of lipid synthesis **(Lorenz, 2001)**, the amount of lipid in the fat body is more than doubled within a short time and reaches a maximum on day 2 after adult emergence. In addition to lipids, the major component of the fat body, protein and glycogen also increase dramatically during these first 2 days of adult life. Furthermore, considerable amounts of energy-rich substrates are transferred to the developing flight muscles. Thereafter, the fat body stores are mobilised to fuel vitellogenic egg growth and about 2 days later, the flight muscles start to histolyse and probably provide additional substrates for oogenesis **(Lorenz and Anand, 2004; Lorenz, 2007)**. In nocturnal crickets, the concentration of lipid in the haemolymph is significantly higher during early scotophase than during early photophase. This increase also very likely depends on a higher concentration of AKH in the haemolymph and it has been shown that haemolymph titres of AKH in the cricket are indeed higher during early scotophase than during early photophase **(Lorenz and Gäde, 2009)**.

1.4. Carbohydrate

The quantity and quality of blood sugar can be influenced by diet. Both total carbohydrate and glucose concentrations are influenced by the amount of food and can be considerably lowered by starvation. The carbohydrate composition of the haemolymph is greatly influenced by the diet **(Hansen, 1964)**. Trehalose usually is the main sugar found in the haemolymph of many different insects **(Wyatt and Kalf, 1956; Wyatt, 1961)**. The principal blood sugar in the cricket haemolymph is trehalose **(Wang and Patton, 1969a)**. Carbohydrate is the predominant energy source for the first 20 to 30 min of flight in *Locusta migratoria* **(Jutsum and Goldsworthy, 1976)**.

In general, insects' carbohydrates are depleted during metamorphosis due to energy metabolism and metabolic interconversions **(Chippendale, 1978)**. Carbohydrates are stored in the fat body mainly in the form of glycogen, which can be rapidly hydrolyzed to release trehalose into the hemolymph **(Wyatt, 1967; Candy, 1985; Candy et al., 1997; Thompson, 2003)**. In adult crickets the blood carbohydrate titer displays two peaks in adult females, one towards the end of scotophase and another in the late photophase, but a single peak at the end of scoptophase is apparent in last instar larvae **(Das et al., 1993)**. Insects belonging to the orders Hymenoptera, Diptera and Blattoidea are examples of insects using carbohydrates as fuels for flight **(Anand, 2004)**.

1.5. Glycogen

In some insects the chief reserve substance is glycogen. Carbohydrate in insects is stored mainly in the form of glycogen, while the disaccharide trehalose may represent a more readily available source of energy. **Babers (1941)** suggested that glycogen may be of more importance in flight physiology. In the cockroach *Leucophaea maderae*, fat body glycogen serves as an energy source during the cycles of egg maturation in the adult female **(Wiens and Gilbert, 1967)**. Glycogen with lipid and protein form the three main storage materials in adult fat body of the black fly *Simulium vittatum* **(Liu and Davies, 1972)**. Glycogen, the major carbohydrate reserve, serves as an important energy source for *Hymenolepis diminuta* **(Read, 1972; Mied and Beuding, 1979)**. In insects, as in other organisms, glycogen serves as a glucose reserve for utilization at different points of the life cycle. Insects´ glycogen is most abundant in fat body, flight muscles and intestine, although there are deposits in other tissues, with the exception of haemolymph **(Brown and Nestler, 1985)**. The eggs of insects store various nutrients at different levels during oogenesis as resources for embryogenesis and the newly hatched larvae. The major resources are lipids and proteins which are mainly produced

by the extra ovarian organs and sequestered by the developing ovary. The third major component is glycogen which is synthesized in the ovary itself from haemolymph trehalose **(Yamashita and Hasegawa, 1985)**.

1.6. Protein

In several insects during the inter-moult, the fat body synthesizes proteins. In *Bombyx mori* the fat body synthesizes and releases proteins **(Shigematsu, 1958)**. Growth of the house cricket on diets containing 10 – 50% protein appears to be largely independent of protein level **(McFarlane, 1964a)**. In *Schistocerca* fat body protein synthesis is low before oocyte production, increases during yolk deposition and drops again at the end of oocyte development **(Hill, 1965)**. In *Locusta* during the last larval instar labelled haemolymph proteins are taken up by the fat body and other tissues **(Tobe and Loughton, 1969)**. During oocyte maturation the fat body synthesizes proteins which are released into the haemolymph and are taken up by the oocytes. It is now well know that in many insects at least one female specific protein is synthesized exclusively under the influence of JH **(Engelmann, 1970)**. The Australian sheep blowflies, *Lucilia cuprina*, become sexually receptive only after ingesting a protein meal **(Barton Browne et al., 1976)**. *G. bimaculatus* requires an average of 55 days and eight larval instars to reach adulthood on a high protein diet, while they stay 117 days across 10 larval instars on a low protein diet at an average temperature of 29 °C day, 11 °C night, and a 16: 8 LD photoperiod **(Merkel, 1977)**. Proteins and lipids in particular can accumulate in large quantities before metamorphosis **(Downer and Matthews, 1976; Levenbook, 1985)**. Nitrogen was often implicated as a limiting factor in insect reproduction **(McCaffery, 1975; Barton Browne et al., 1980; Hahn, 2005)**.

1.7. Flight muscle

Various degrees of changes occur in muscles during postembryonic development in insects **(Finlayson, 1975)**. The nervous system is another element controlling muscle growth and development **(Nüesch, 1985)**. Lipid is the major flight fuel in species of *Gryllus* **(Zera et al., 1999)**, as is the case for many other insects, especially Orthoptera **(Beenakkers et al., 1985b; Candy, 1989)**. In hemimetabola a complete set of adult muscles is present in the larval form. Most muscles are in use during nymphal stage. Flight muscles, however, remain small and functionless until the last larval instar and develop rapidly just before and after the imaginal moult **(Ready and Josephson, 1982; Novicki, 1989a, b)**. In crickets, flight muscle histolysis is common in LW morphs, and several develop their ovaries as rapidly as SW females **(Tanaka, 1976, 1986, 1994a, b; Zera and Denno, 1997)**. Adult *G. bimaculatus*

increase the mass of their flight muscles during the first 3 day after final ecdysis and decrease it thereafter through selective degeneration of the meta-thoracic muscles **(Shiga et al., 1991)**. Changes in the mass of flight muscles are likely to be caused by a change in the rates of synthesis and degeneration of the proteins comprising the tissues. However, no information is available about how these rates change during the course of the development and histolysis of flight muscles **(Gomi et al., 1995)**. Degeneration of flight muscles is known to occur in young adult insects of several orders **(Finlayson, 1975; Collatz and Wilps, 1986)**. It has been reported that in the cricket *Teleogryllus oceanicus* the dorsal longitudinal muscle atrophies and becomes white in appearance in late adult life **(Ready and Josephson, 1982; Ready and Najm, 1985)** and in the cricket *Acheta domesticus* the dorsal longitudinal muscle increases in size during the first 2 days after the imaginal moult, but begins to degenerate on the fourth day in the presence of juvenile hormones **(Chudakova and Bocharova-Messner, 1968; Srihari et al., 1975)**. In some species the muscles degenerate after the mating period and the adults lose the ability to fly. **Stegwee et al. (1963)** found that the flight muscles in the beetle *Leptinotarsa decemlineata* degenerate at the onset of adult diapause, due to a lack of juvenile hormone (JH). **Srihari et al. (1975)** studied morphogenesis and degeneration of the flight muscles in *Acheta domesticus* The dorso-longitudinal flight muscles (DLMs) degenerate during the fourth day after adult ecdysis and the dorso-ventral flight muscles (DVMs) on the fifteenth day. The flight muscles begin to degenerate soon after reaching a maximal size in all individuals, and the metathoracic DLMs display a higher degree of degeneration than in the mesothoracic DVMs. This difference may be related to the fact that the DLMs are exclusively used for moving the wings but the DVMs are also used in walking **(Shiga et al., 1991)**.

1.8. Haemolymph

The haemolymph of insects resembles the blood of vertebrates in that it contains cellular components, lipid, proteins, salt, free amino acids, carbohydrates, water, hormones, etc. The chemical composition of the haemolymph is nearly as diverse. Often there is variation in different stages of the same species and in individuals depending upon their nutritional background, environment, and physiological state. In locusts the amount of carbohydrate in the haemolymph is greater than that stored in other tissues **(Goldsworthy, 1969)**, but the opposite is true for fat reserves **(Jutsum et al., 1975)**. The haemolymph sometimes contains substances which, in the case of reflex bleeding, may protect the insect from attack **(Mellanby, 1939)**. In insect haemolymph, lipid transport is accomplished mainly by lipophorin **(Wang and Patton, 1969b; Chino, 1985; Shapiro *et al*., 1988)**. Food substances are transported and stored in the blood, which also carries hormones through the body. A

considerable variation of reducing power with age is typical of the haemolymph of *Schistocerca gregaria*. Diet has an effect on the values and a constant artificial diet gives more reliable figures than a natural diet of grass **(Howden and Kilby, 1960)**. Insects have a relatively high total blood lipid content ranging from 1.5% to 5.5%, which fluctuates under a variety of conditions such as developmental stages, locomotor activity, starvation, disease **(Mullins, 1985)**. In vivo and in vitro studies using several insect orders showed diacylglycerol (DAG) is the main lipid in the haemolymph after lipid digestion **(Canavoso and Wells, 2000)**.

1.9. Food consumption

Animals or insects raised on various diets do not grow at equal rates and differ in developmental periods. Feeding may affect the activity of the neuroendocrine system which may in turn affect egg development **(Highnam et al., 1966; Highnam and Mordue-Luntz, 1974)**. Food quality and temperature thus affect growth, mortality, development, and reproduction of insects **(Hoffmann, 1973, 1974; McCaffery, 1975)**. In general, growth rates and metabolic rates in house crickets as well as in other insects are directly related to food intake. The time of most rapid growth is always at the time of maximal feeding and the top metabolic rate **(Woodring et al., 1979)**. Many insects compensate for low food quality by increasing consumption **(Absigold and Simpson, 1987)**.

Many beetles, crickets, grasshoppers, moths, and other insects, but only two or three butterfly species, can be reared on artificial diets **(Singh, 1977)**. Larvae of *Acheta domesticus* reared on an artificial diet show the group effect not only in the first generation **(McFarlane et al., 1984)** but also in the second, third and fourth generations.

In crickets, it has been known that ovarain development and food consumption are influenced by various factors such as temperature, mating, and the presence or absence of oviposition substrate **(Loher and Edson, 1973; Merkel, 1977; Clifford and Woodring, 1986; Loher et al., 1987; Renucci et al., 1990)**. **Yanikoğlu (1999)** concluded that water has a significant effect on *G. bimaculatus* females.

Unfortunately, the studies of relationships among biotic potential factors of crickets, chemical composition of fat body, lipids, free carbohydrates, protein and glycogen have been greatly hampered by the lack of modern reference works. Consequently not much information is available. However, this great lack stimulated our attention to launch the present study.

Therefore, the scope of the present study was to contribute towards a better knowledge of the following:

1- Study the effect of isolated or crowded conditions from day 0 to day 5 of the adult female, *G. bimaculatus* on some biological aspects (increase in body mass, pronotum width, fat body mass, ovary mass, flight muscle mass, lipid, free carbohydrate, protein and glycogen content in the fat body and haemolymph lipid and carbohydrate concentrations and food consumption).
2- Study the effect of the presence or absence of adult males with adult female crickets from day 0 to day 5 under crowded conditions on the above-stated parameters.
3- Study the effect of feeding three different diets from day 0 to day 5 to adult female reared under isolated or crowded conditions on the above-stated parameters.
4- Study the effect of feeding three different diets throughout the penultimate and last larval stage until day 5 of the adult stage, reared under crowded conditions on the above-stated parameters.

2- MATERIALS AND METHODS

The present study was carried out in the laboratory of the Department of Animal Ecology I, University of Bayreuth, Germany.

2.1. Rearing of *Gryllus bimaculatus* de Geer

Colonies of the Mediterranean field crickets (two-spotted crickets), *Gryllus bimaculatus* de Geer (Orthoptera, Ensifera, Gryllidae) were reared under crowded conditions at a long day light cycle (16 h light, 8 h dark; lights on 06:00 CEST, lights off 22:00 CEST) on a mixture of four parts of breeding diet for rabbits (No. 2021), two parts of breeding diet for rats and mice (No. 1311) and one part of breeding/maintenance diet for cats (No. 5031), all from Altromin GmbH (Lage, Germany) and water ad libitum, at a constant temperature of 27 ± 1°C and 30 – 40% relative humidity. The colonies were maintained in white plastic boxes covered with netted lids and provided with eggs dividers (egg boxes) which served as shelter. The colony is regularly freshed up with crickets supplied by b.t.b.e. Futtertierzucht (Schnürpflingen, Germany).

All experiments were performed using female penultimate larvae or adult female crickets. The penultimate larvae or adult females were collected from breeding colonies and reared under the above-mentioned conditions. At the day of collection, the animals were 0-day-old. To test the effects of isolated or crowded conditions, of the presence or absence of adult males, of feeding three types of diets and of the time span the three different diets were provided, the following four experiments were conducted.

2.2. Experiment I

Effect of rearing adult females from day 0 to day 5 under isolated or crowded conditions.

Under isolated conditions, adult 0-day-old females were weighed and placed singly into plastic boxes (10 x 10 x 6 cm L x W x H) (192 replicates). A piece of egg divider was used as shelter and each animal was supplied with 1.5 g food mixture (standard diet 1:1 rabbit: rat/mouse) and a small glass vial plugged with cotton wool for water supply (Fig. 1). The remaining food was collected, dried and weighed as described under 2.12.

Under crowded conditions, 12 adult 0-day-old females were weighed and placed together in a white plastic box (16 x 11 x 6 cm L x W x H) (15 replicates). Egg dividers were

used as shelter and each 12 animals were supplied with 15 g food mixture (standard diet) and two bigger glass vials plugged with cotton wool for water supply (Fig. 2). The remaining food was collected, dried and weighed as described under 2.12.

The food mixture contained, 20% proteins, 4.5% lipids, 45.5% carbohydrates; (12.17 kJ/g).

2.3. Experiment II

Effect of rearing adult females from day 0 to day 5 with or without adult males under crowded conditions

2.3.1. Rearing under crowded conditions with adult males

About 20 – 25 newly ecdysed adult females were collected from the mass rearing colony, and their body weights were recorded. After five h, the insects were marked on the pronotum by using nail polish (Fig. 3) and placed in a white plastic box (60 x 40 x 30 cm, L x W x H), which contained about 200-300 crickets of both sexes and different stages (penultimate and last larve, adults, including adult males). They were provided with water and food ad libitum (Fig. 4).

2.3.2. Rearing under crowded conditions without adult males

The same method was repeated, however, this time all adult males were removed from the boxes every day. The diet used in Experiment II contained 22% proteins, 6% lipids and 42% carbohydrates for a total of 12.49 kJ/g (Mod. standard diet contains rabbit, rat/mouse and cat respectively in the following ratio 4:2:1).

2.4. Experiment III

Effect of feeding three different diets (normal starch and fat, high starch and low fat, high fat and low starch) under isolated and crowded conditions

The same method as described in part 2.2. was followed but this time three different diets were supplied (1.5 g per animal or 15 g per 12 animals, respectively). The three different diets are described in table 1.

Table 1: The diet formulations

Diet	Chemical compositions
A	Normal starch and fat (Diets were formulated by incorporating 30% protein, 5% fat, 38% N-free, 8% fiber); 12.75 kJ/g. 0.8288 g rat /rabbit (NO. 1311 and No. 2021). 0.1602 g CFM Whey (Cfm Instant Whey Isolat, Best Body Nutrition, Auerbach Germany). 0.0110 g Biskin (fat and oil from plant) (P. Köllon KGaA, Elmshorn Germany).
B	High starch, low fat (Diets were formulated by incorporating 30% protein, 2% fat, 50% N-free, 4% fiber); 13.55 kJ/g. 0.36644 g rat /rabbit (NO. 1311 and No. 2021). 0.2533 g CFM Whey (Cfm Instant Whey Isolat, Best Body Nutrition, Auerbach Germany). 0.3823 g food starch (cornstarch) (Feine Speisestärke, Fine food; A.C.L. Warenvertriebsgesellschaft mbH, Düsseldorf, Germany).
C	High fat, low starch (Diets were formulated by incorporating, 30% protein, 30% fat, 23% N-free, 5% fiber); 19.73 kJ/g. 0.5001 g rat /rabbit (NO. 1311 and No. 2021). 0.2247 g CFM Whey (Cfm Instant Whey Isolat, Best Body Nutrition, Auerbach Germany). 0.2752 g Biskin (fat and oil from plant) (P. Köllon KGaA, Elmshorn Germany).

Note: To examine energetic costs of development, we used energy conversion factors of 16.0 kJ g^{-1} for protein and carbohydrate and 37.5 kJ g^{-1} for lipid (**Adrian et al., 1988**).

2.5. Experiment IV

Effect of feeding three different diets to crowded females from day 0 of the penultimate larval stage to day 5 of the adult stage .

The same method as described in part 2.4. was followed, however this time only crowded conditions were applied and newly ecdysed female penultimate larvae were collected, weighed and placed in groups of 12 per fauna box (25 x 11 x 18 cm LxWxH) (Figs. 5 and 6) and supplied with 15 g per 12 animals (1.25 g per one cricket) of the three different diets. Newly ecdysed last instar larvae were weighed and transferred to new boxes containing fresh food. After the moult to the adult stage, the animals were weighed again and transferred to new boxes. For each stage the remaining food was collected, dried and weighed as described under 2.12.

As in the other experiment, the adult females were sacrificed on day 5.

2.6. Haemolymph samples

On the morning of the fifth day, immediately before the body weight of the crickets was determined, a haemolymph (HL) sample (2 µl) was taken from the base of a metathoracic leg. The 2 µl HL sample was blown into 200 µl of concentrated sulfuric acid and thoroughly mixed. The HL – sulfuric acid mixture was then divided into two equal aliquots for determination of lipid and carbohydrate titers.

2.7. Body weight

Immediately after haemolymph (HL) samples were taken, the body weight of the experimental animals was determined. The percent increase in body weight was calculated as (W2 – W1) / W1 x 100, where W1 is the body weight at the beginning of each instar and W2 is the body weight at the time specified for each experiment.

2.8. Determination of the pronotum width

Measurements of the pronotum (the tergum sclerite of the prothorax) width were made with a handheld digital calliper.

2.9. Dissection of fat bodies

Crickets were decapitated, opened on the ventral side by a longitudinal incision from the thorax to the anus, and fixed with insect needles on a styrofoam plate to expose the inner organs. Then, the complete gut and the ovaries were carefully removed.

Half fat bodies (i.e. the free abdominal fat body from one body side) were dissected and collected into pre-weighed 1.5 ml safe-lock Eppendorf tubes, containing 20 mg of Na_2SO_4 and 200 µl of 75% methanol in water to determine half fat body fresh weight and then kept at -20°C until they were analysed to estimate lipid, protein, glycogen, and free carbohydrate content according to a protocol described by **Lorenz (2003)**.

2.10. Ovarian growth

The ovaries from these females were dissected, carefully removed and collected into pre-weighed 1.5 ml non safe-lock Eppendorf tubes to determine ovary fresh weight on a microbalance.

2.11. Flight muscle weight

The dorsal longitudinal flight muscle of one body side was carefully removed and transferred into pre-weighed 1.5 ml non safe-lock Eppendorf tubes to determine their fresh weight on a microbalance.

2.12. Amount of food consumed

The faeces were removed and the remaining food was dried for 14 h at 100°C in an oven and subsequently weighed. The amount of food consumed was calculated as follows:

Calculated food consumption =

fresh mass of food supplied (mg) x 91.815* ÷100 - dry weight of remaining food.

* 100 mg of the crude fresh supplied food contains only 91.815 mg of dry matter; the remaining 8.185 mg refers to water content.

2.13. Determination of haemolymph lipid

Total lipid concentration in the haemolymph was measured using the sulphophosphovanillin method as described by **Zöllner and Kirsch (1962)** with some modifications. Samples (in 100 µl of concentrated sulfuric acid) were heated to 100°C for 10 min. After cooling 1 ml of 0.2% vanillin in 57% ortho-phosphoric acid was added, and the

tubes were put in the dark for 20-30 min. Samples were measured against cholesterol standards of 0 to 20 µg at 530 nm in a spectrophotometer **(Lorenz, 2003)**.

2.14. Determination of haemolymph carbohydrate

The concentration of total carbohydrate in the haemolymph was measured using the anthrone method described by **Mokrasch (1954)** with some modifications. 1 ml of anthrone reagent (0.13% anothrone in 67% sulfuric acid) was mixed with the samples, heated to 90 °C for 10 min and subsequently put in the dark for 20-30 min. After cooling the samples were measured against trehalose standards of 0 to 20 µg at 585 nm in a spectrophotometer **(Lorenz, 2003)**.

2.15. Extraction and separation of lipid, protein, glycogen and free carbohydrate from the fat body

The procedure for extraction and separation of lipid, protein, glycogen and free carbohydrate from half fat body was developed on the basis of methods by **Van Handel (1965)** and **Speck and Urich (1969)**. The method was optimised for a rapid and efficient separation in small volumes of solvents to allow the use of Eppendorf reaction tubes throughout **(Lorenz, 2003)** (Fig. 7).

2.15.1. Lipid estimation

Total body lipid was estimated by the sulphophosphovanillin method as described in 2.13.

2.15.2. Glycogen and free carbohydrate estimation

Total body glycogen and free carbohydrates were estimated by the anthrone method as described in 2.14.

2.15.3 Protein estimation

Protein was estimated by Roti-Quant universal solution based on a modified Bradford's protein assay. Protein samples in a final volume of 50 µl were mixed with 200 µl Roti-Quant universal solution on a 96-well micro-titer-plate (G080-F, G. Kisker GbR, Steinfurt, Germany) heated to 50°C for 20 min and measured at 515 nm against water. A standard curve

of 0 to 200 µg BSA was plotted against the absorption at 515 nm to determine protein concentration.

2.16. Data presentation and statistical analyses

All results are presented as mean values ± standard error (S.E.) of the number of individual measurements indicated in the legend of each figure. Mean values were compared by t- test, Mann-Whitney U-test or Kruskal-Wallis ANOVA. To isolate the group or groups that differ from the others a multiple comparison procedure was used. All pair-wise multiple comparison procedures were carried out using Dunn's method or Tukey Test. Stastistical significance is shown in the graphs (* $p<0.05$; ** $p<0.01$; *** $p<0.001$). All calculations were performed using Sigmaplot 11.0 software.

2.17. Instruments

Analytical balance A 7073 03; LC1201S; MC 210 P	Sartorius, Göttingen, Germany
Capillary 2µl Blaubrand ® intraEND	Brand GmbH, Wertheim, Germany
Centrifuge: Biofuge 13	Heraeus Sepatech GmbH, Osterode, Germany
Centrifuge: Sigma 3K12	Sigma Laborzentrifugen, Osterode, Germany
Digital calliper	T C M, Hamburg, Germany
ELx808 ™ Ultra Microplata Reader	Bio-Instruments Inc., Bad Friedrichshall Germany
Eppendorf tubes: (1.5 ml, 2.0 ml / safelock, non-safelock)	Eppendorf, Hamburg, Germany
Hand-held homogeniser, MHX / E	Xenox, Niersbach, Germany
Pipette Pipetman ® P	Gilson, Middleton, USA
Sonifier, Branson W- 250	Heinemann, Schwäbisch-Gmünd Germany
Spectrophotometer, Ultraspec III	Pharmacia LKB, Freiburg, Germany
Speed-Vac-Concentrator, Alpha RVC	Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany
Thermobloc TB1	Biometra, Göttingen, Germany
Grant Boekel BBA	Grant Instrument, Cambridge, UK
Ultrasonic bath, Transsonic 310	Elma, Singen / Htwl., Germany
Vortexer REAX 2000	Heidolph, Schwabach, Germany

2.18. Chemicals

Anthrone

BSA bovine serum albumin

Cholesterol

Roti®-quant

Vanillin

$CHCl_3$ Chloroform

EtOH Ethanol

C_6H_{14} Hexane

H_2O Water

H_2SO_4 Sulfuric acid

KOH Potassium hydroxide

MeOH Methanol

NaCl Sodium chloride

Na_2SO_4 Sodium sulfate

All chemicals were from Sigma, Steinheim, Carl Roth GmbH+Co, Karlsruhe, Fluka, Neu-Ulm or Merck, Darmstadt, in p.a grade.

2.19. Software

Microsoft® Office Excel 2003 Microsoft corporation

Microsoft® Office Word 2002 Microsoft corporation

Sigmaplot 11.0 (SYSTAT Software GmbH, Erkrath, Germany)

Figs. 1-6: Method for rearing the two-spotted cricket, Figs. 1, 2 and 5 show the used rearing box (a), food container with hole to enable the insect to get inside the container without scattering the food (b), glass vials plugged with cotton wool for water supply (c), egg divider for shelter (d). Fig. 3 shows remarkable red color on the pronotum of the adult. Fig. 4 shows original cages of mass culture contained about 200-300 crickets of both sexes and different stages and ages. Fig. 6 shows 0-day-old penultimate larvae (a), 0-day-old last larvae (b), 0-day-old adult female (c), and 5-day-old adult female (d).

Separation of lipid, glycogen, free carbohydrates and protein (mg-range)

- Put sample (fat body) into pre-weighed 1.5 ml Eppendorf tube (containing 20 mg Na_2SO_4 and 200 µl 75% MeOH in H_2O) and weigh. Difference in weight = fresh weight of the tissue sample.
- Homogenise (with electric homogeniser), rinse pestle directly into the Eppi with 600 µl $CHCl_3$: MeOH (1:1) vortex vigorously put into ultrasonic bath 5 min, vortex as before.
- Centrifuge (Sigma 3K12, 21 000g, 2 °C 10 min.
- Separate pellet and supernatant as follows:

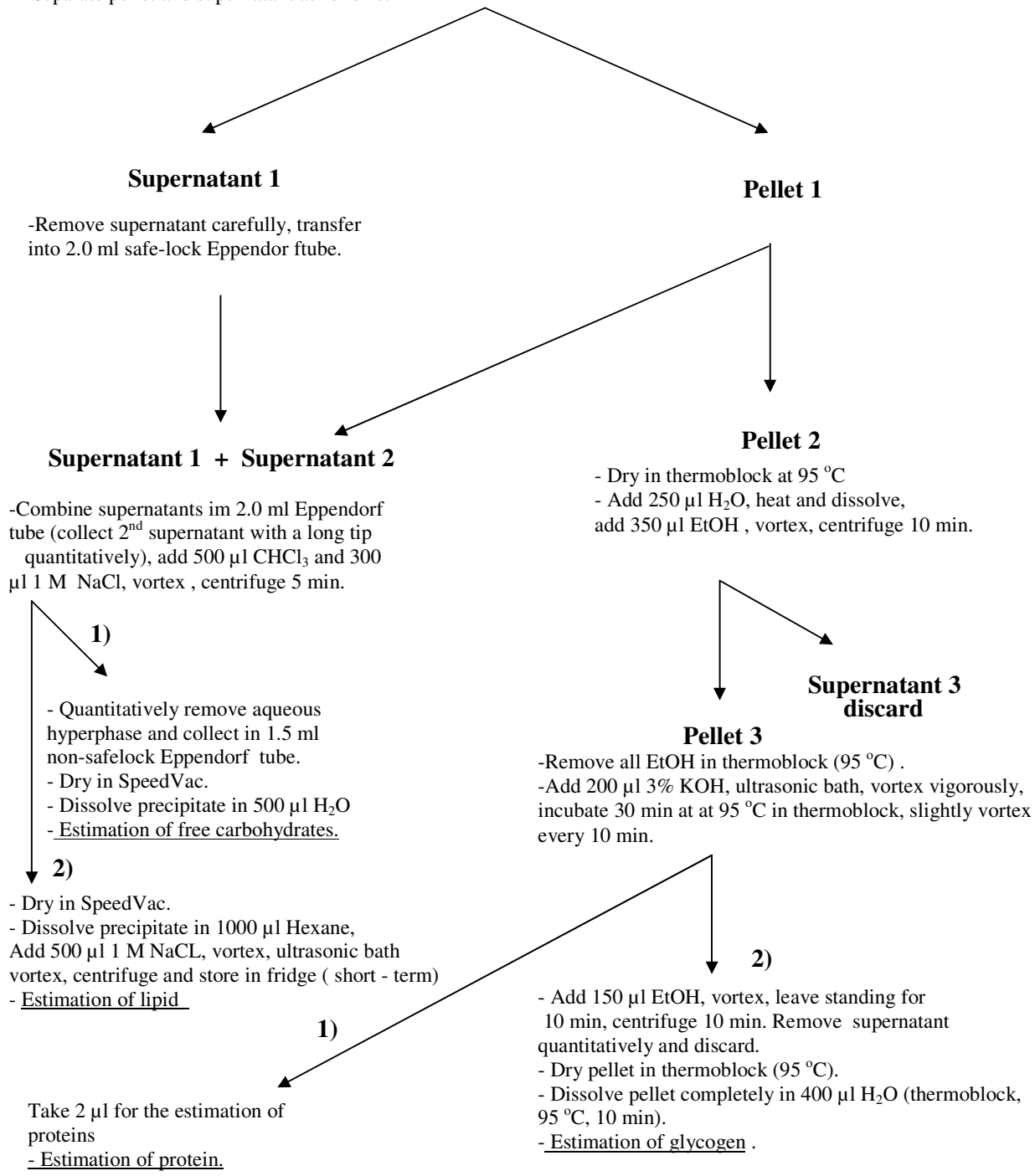

Fig. 7: Scheme showing the method used for the extraction and separation of organic substances from fat bodies was taken from **Lorenz (2003)**. All centrifugations steps were at 21 000g and 2 °C for either 5 or 10 min.

3- RESULTS

3.1. Experiment I

Effect of rearing adult females from day 0 to day 5 under isolated or crowded conditions

In addition to the Figures presented here, the results of the experiment can be found in table 2 (supplementary).

3.1.1. Pronotum width and percent increase in body mass

The data show that the pronotum width of females reared under isolated conditions was similar to that of females reared under crowded conditions (Fig. 8a).

The percent increase in body mass in females reared under isolated conditions was lower than that of females reared under crowded conditions (Fig. 8b).

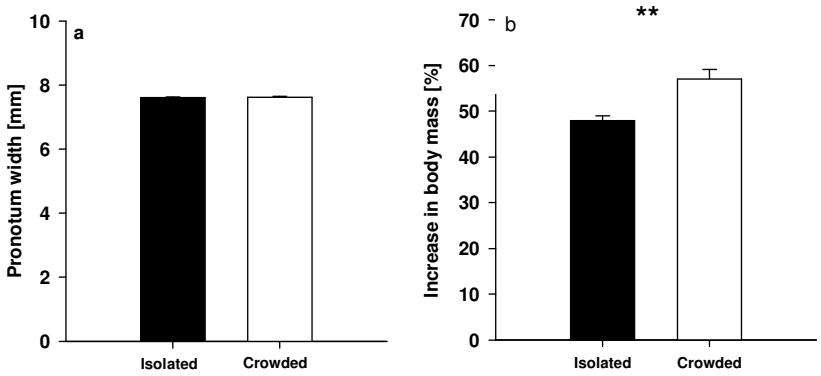

Fig. 8. Pronotum width (a) and percent increase in body mass (b) from d 0 to d 5 in adult female crickets reared under isolated or crowded conditions. Means ± S.E. of 192 (isolated) or 164 (crowded) determinations, **p<0.01(a: t-test and b: Mann-Whitney U-test).

3.1.2. Diet consumption

The amount of diet consumed by females reared under isolated conditions were slightly lower than that of females reared under crowded conditions, however, the differences were not significant (Fig. 9).

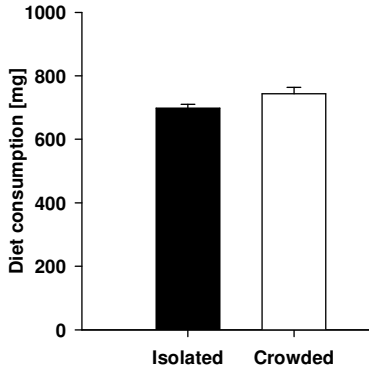

Fig. 9. Amount of diet consumed from d 0 to d 5 in adult female crickets reared under isolated or crowded conditions. Means ± S.E. of 192 (isolated) or 15 (crowded) determinations, p = 0.281, t-test.

3.1.3. Ovary fresh mass and whole fat body mass

Both ovary mass and fat body mass (Fig. 10) were slightly lower in females reared under isolated conditions, however, the differences were not significant.

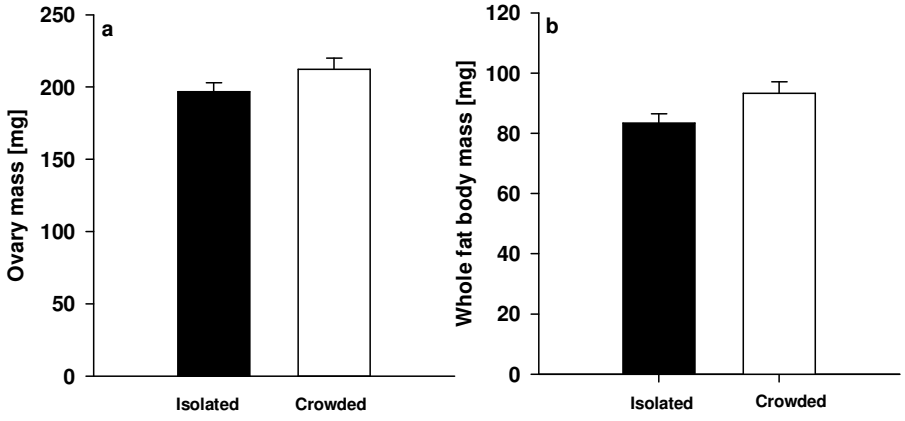

Fig. 10. Ovary fresh mass (a) and whole fat body mass (b) of 5-day-old adult female crickets reared under isolated or crowded conditions. Means ± S.E. of 192 (isolated) or 164 (crowded) determinations, p= 0.167 (ovary), p= 0.161 (fat body), Mann-Whitney U-test.

3.1.4. Lipid and protein content in mg per whole fat body and in µg per mg fat body fresh mass

The content of lipid per whole fat body (Fig. 11a) as well as that on a per mg basis (Fig. 11c) was higher in females reared under isolated condition. The total protein content and the protein content per mg fat body mass, did not differ between the two treatments (Fig. 11b, d).

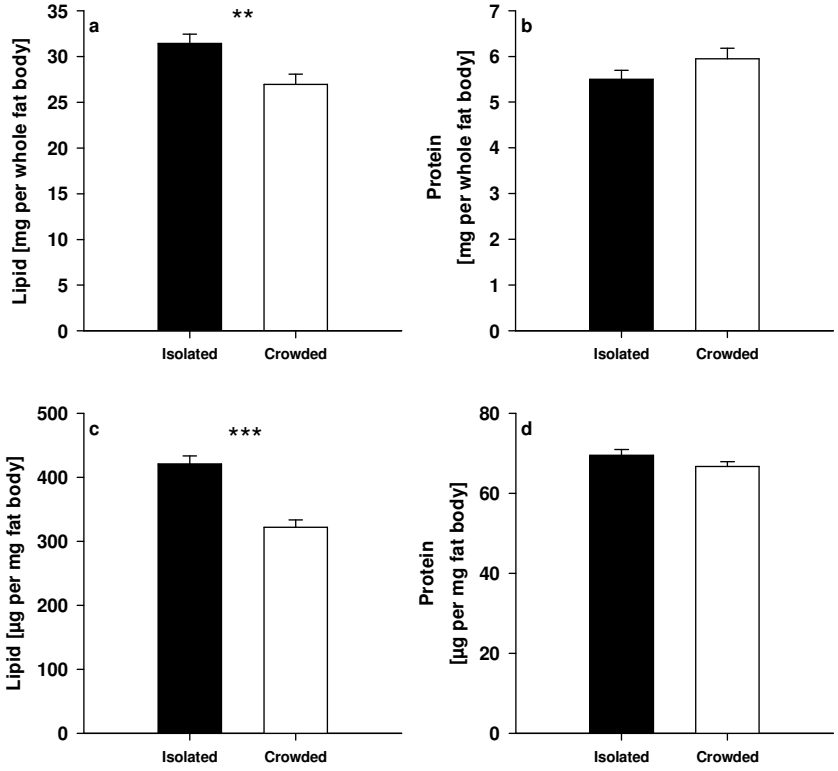

Fig. 11. Total lipid content (a) total protein content (b) lipid content per mg fat body fresh mass (c) and protein content per mg fat body fresh mass (d), of 5-day-old adult female crickets reared under isolated or crowded conditions. Means ± S.E. of 192 (isolated) or 164 (crowded) determinations, **p<0.01; ***p<0.001, Mann-Whitney U-test.

3.1.5. Glycogen and free carbohydrate content in mg per whole fat body and in µg per mg fat body fresh mass

The total glycogen content in mg per whole fat body and glycogen content in µg per mg fat body mass are given in Fig 12a and 12c respectively. This was seen to be lower for female individuals reared under isolated conditions, however, the differences were not significant.

Total free carbohydrate content per the whole fat body was approximately similar for both groups (Fig. 12b), however, per mg fat body mass, carbohydrate content was higher for females reared under isolated conditions (Fig 12d).

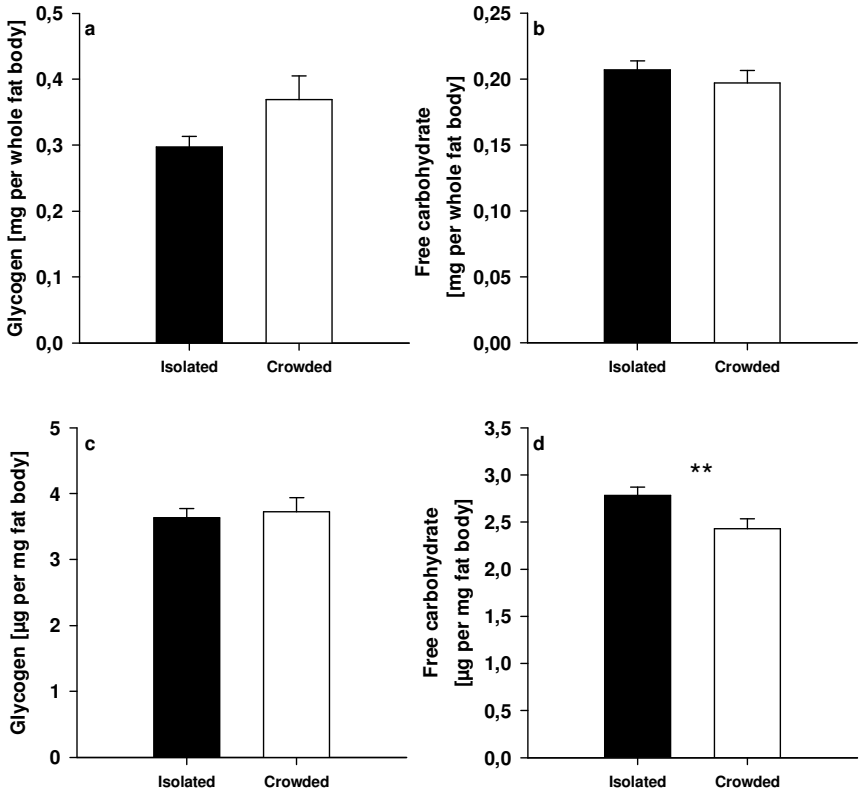

Fig. 12. Total glycogen content (a) total free carbohydrate content (b) glycogen content per mg fat body fresh mass (c) and free carbohydrate content per mg fat body fresh mass (d) of 5-day-old adult female crickets reared under isolated or crowded conditions. Means ± S.E. of 192 (isolated) or 164 (crowded) determinations, **$p<0.01$, Mann-Whitney U-test.

3.1.6. Haemolymph lipid and carbohydrate concentration

Results shown in Fig. 13a denote that the average value of haemolymph lipid in females reared under isolated conditions was higher than that of females reared under crowded conditions. On the other hand, haemolymph carbohydrate tended to be lower in females reared under isolated conditions (Fig. 13b).

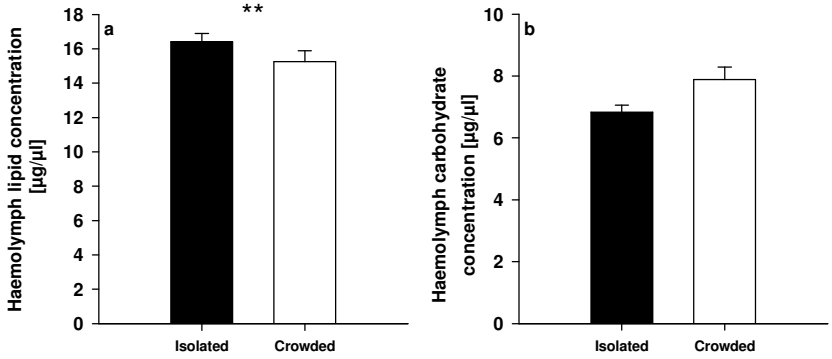

Fig. 13. Haemolymph lipid (a) and carbohydrate concentration (b) of 5-day-old adult female crickets reared under isolated or crowded conditions. Means ± S.E. of isolated (192) or crowded (164) determinations, **p<0.01, Mann-Whitney U-test.

3.2. Experiment II

Effect of rearing adult females from day 0 to day 5 with or without adult males under crowded conditions

In addition to the Figures presented here, the results of the experiment can be found in table

3 (supplementary).

3.2.1. Pronotum width and percent increase in body mass

Both pronotum width and percent increase in body mass (Fig. 14) were approximately similar for both groups.

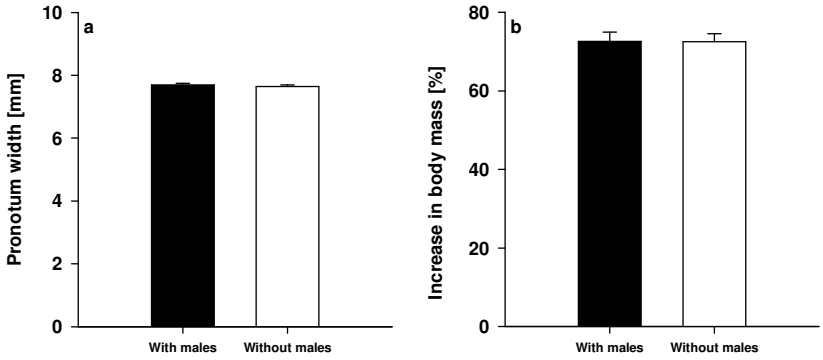

Fig. 14. Pronotum width (a) and percent increase in body mass (b) of 5-day-old adult female crickets reared with or without adult males. Means ± S.E. of 57 (with males) or 50 (without males) determinations, p= 0.430 (pronotum width), p= 0.998 (increase in body mass), (a: t-test and b: Mann-Whitney U-test).

3.2.2. Flight muscle, ovary and fat body mass

Both flight muscle and ovary mass (Fig. 15a, b) were approximately similar for both groups, whereas the fat body mass of females reared with males was lower than that of individuals reared without males (Fig. 15c).

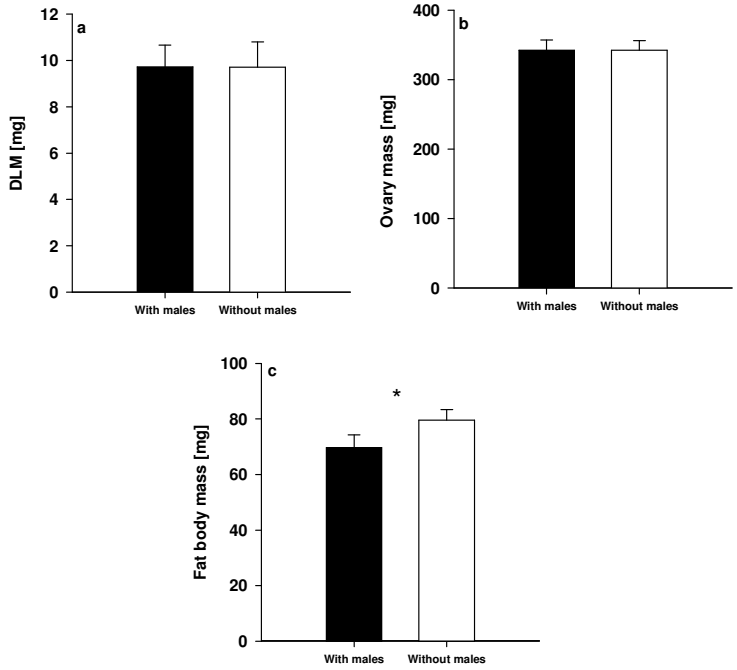

Fig. 15. Flight muscle mass (a), ovary mass (b) and fat body mass (c) of 5-day-old adult female crickets reared with or without adult males. Means ± S.E. of 57 (with males) or 50 (without males) determinations, *$p<0.05$, (a and c: Mann-Whitney U-test, b: t-test).

3.2.3. Total lipid and protein content in mg per whole fat body and lipid and protein content in µg per mg fat body fresh mass

The content of lipid in mg per whole fat body and lipid content in µg per mg fat body mass (Fig 16a, c) was higher in females reared under crowded conditions without males.

Total protein content per the whole fat body was approximately similar for both groups (Fig. 16b), however, per mg fat body mass, protein content was higher for females reared under crowded conditions with males (Fig. 16d).

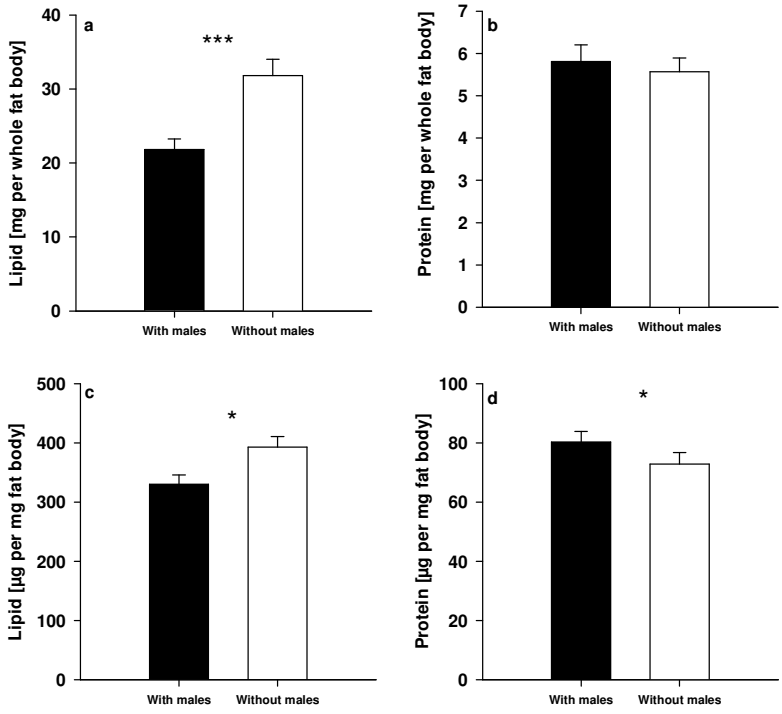

Fig. 16. Total lipid content (a), total protein content (b), lipid content per mg fat body fresh mass (c) and protein content per mg fat body fresh mass (d) of 5-day-old adult female crickets reared reared with or without adult males. Means ± S.E. of 57 (with males) or 50 (without males) determinations, *$p<0.05$; ***$p<0.001$ (a, b and d: Mann-Whitney U-test, c: t-test).

3.2.4. Total glycogen and free carbohydrate content in mg per whole fat body and glycogen and free carbohydrate content in µg per mg fat body fresh mass

Total glycogen and free carbohydrate content per whole fat body was higher for females reared under crowded conditions without males (Fig. 17a and 17b), however, per mg fat body mass, glycogen and free carbohydrate content was approximately similar for both groups (Fig. 17c and 17d).

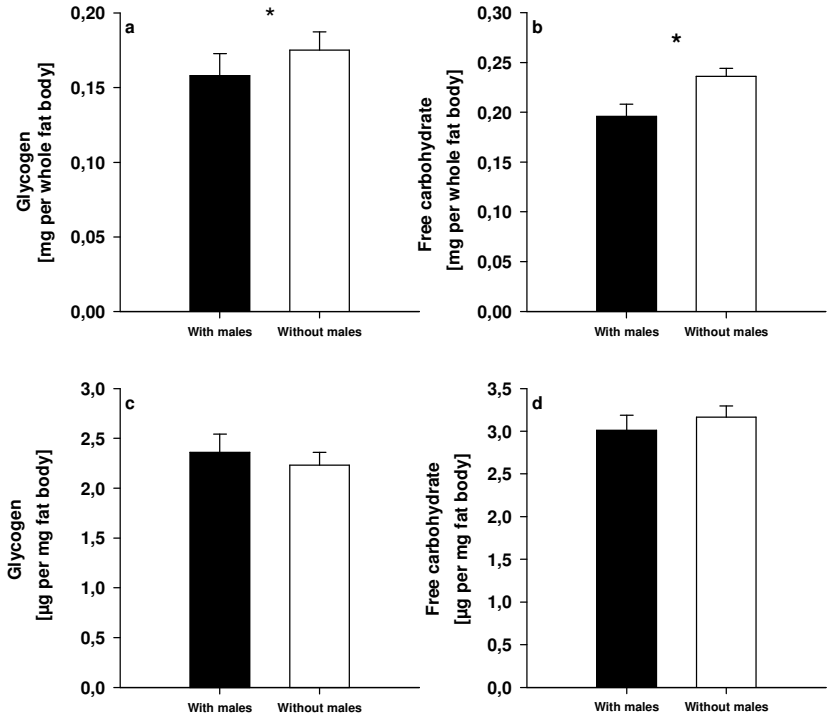

Fig. 17. Total glycogen content (a), total free carbohydrate content (b), glycogen content per mg fat body fresh mass (c) and free carbohydrate content per mg fat body fresh mass (d) of 5-day-old adult female crickets reared with or without adult males under crowded conditions. Means ± S.E. of 57 (with males) or 50 (without males) determinations, *$p<0.05$, Mann-Whitney U-test.

3.2.5. Haemolymph lipid and carbohydrate concentration

Both average values of haemolymph lipid (Fig. 18a). and carbohydrate concentration (Fig. 18b) were slightly lower in females reared under crowded conditions with males, however, the differences were not significant.

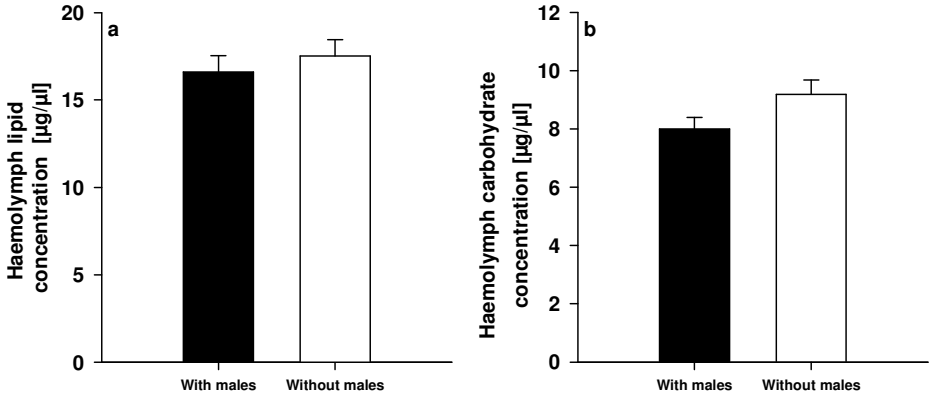

Fig. 18. Haemolymph lipid (a) and carbohydrate concentration (b) of 5-day-old adult female crickets reared with or without adult males. Means ± S.E. of 57 (with males) or 50 (without males) determinations, p=0.209 (haemolymph lipid), p=0.063 (haemolymph carbohydrate), (a: Mann-Whitney U-test, b: t-test).

3.3. Experiment III

Effect of feeding three different diets (normal starch and fat, A; high starch and low fat, B; high fat and low starch, C) under isolated and crowded conditions

In addition to the figures presented here, the results of experiment can be found in tables 4 and 5 (supplementary). Each group is exclusively fed only one of the three different diets (A, B and C).

3.3.1. Pronotum width and percent increase in body mass

Pronotum width was similar for females of both groups fed on the same diet (Fig. 19a).

Results given in Fig. 19b show a comparison between of the percent increase in body mass for female individuals under the different rearing conditions. Diet A gave the highest average increase in body mass followed by diet B and diet C which led to the least increase under isolated conditions. In females reared under crowded conditions diet A gave the highest average increase in body mass followed by diet C and diet B which showed the least. All these differences were not significant, though. Significant differences in body mass gain between isolated and crowded animals were found for females reared on diet A and C, with higher values in crowded animals. Crowded females fed on diet B only tended to grow heavier than isolated ones.

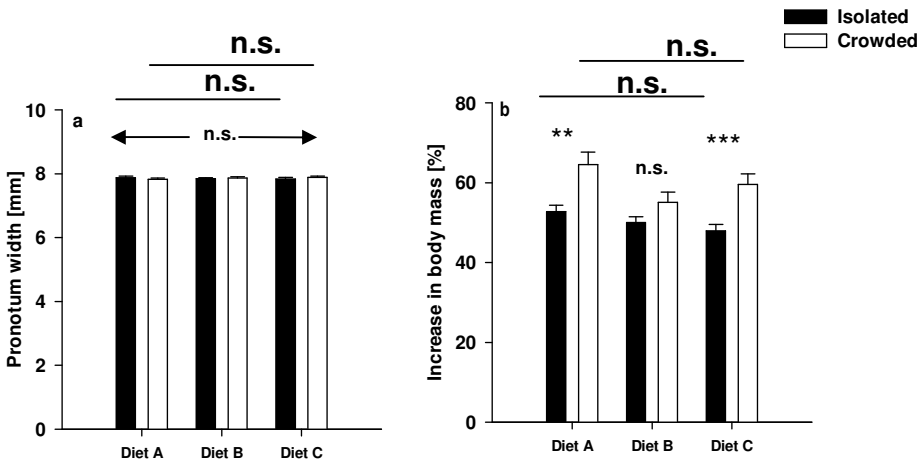

Fig. 19. Pronotum width (a) and percent increase in body mass(b) from d 0 to d 5 in adult female crickets reared under isolated or crowded conditions and fed on three different diets. Means ± S.E. of 73, 74 and 69 (isolated) or 73, 95 and 73 (crowded) determinations, **p<0.01; ***p<0.001. Difference between isolated and crowded females were tested by Mann-Whitney U-test, differences among the 3 different diets were tested by Kruskal-Wallis ANOVA.

3.3.2. Diet consumption

As shown in Fig. 20 the amount of diets consumed did not differ significantly under different conditions. Under both isolated and crowded conditions, diets A and B were consumed in equal amounts, whereas diet C was consumed significantly less.

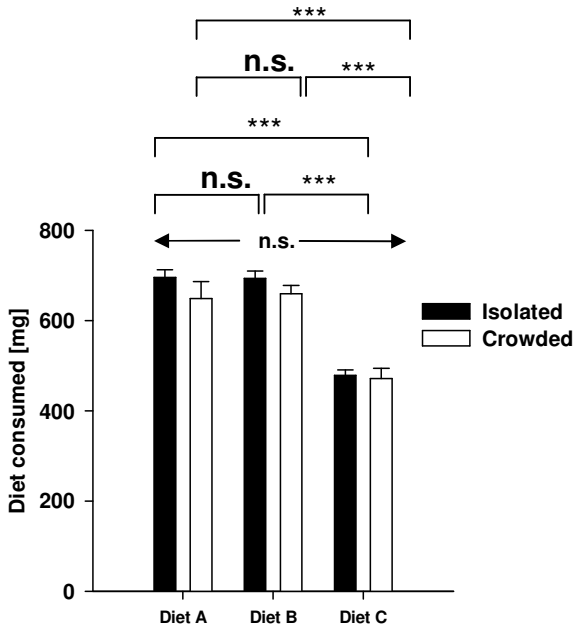

Fig. 20. Amount of diet consumed from d 0 to d 5 in adult female crickets reared under isolated or crowded conditions and fed on three different diets. Means ± S.E. of 73, 74 and 69 (isolated) or 7, 9 and 7 (crowded) determinations, ***p<0.001. Difference between isolated and crowded females were tested by Mann-Whitney U-test, differences among the 3 different diets were tested by Kruskal-Wallis ANOVA.

3.3.3. Flight muscle, ovary and whole fat body mass

The flight muscle mass did not differ significantly between animals fed on different diets (Fig. 21a), whereas the flight muscles of females reared under isolated conditions were significantly heavier than those of the crowded females, irrespective of the type of diet. In contrast, the ovaries of crowded females were larger than those of isolated females (Fig. 21b). There was a clear trend towards a diet- dependent ovary weight in isolated females with larger ovaries in females fed on diet A and smallest ovaries in females fed on diet C. The same trend (although not significant) was found in crowded females. An opposite trend was found for the fat body mass which was the largest in females fed on diet C (Fig. 21c). Except for the females fed on diet A, the fat body mass did not differ between isolated and crowded females.

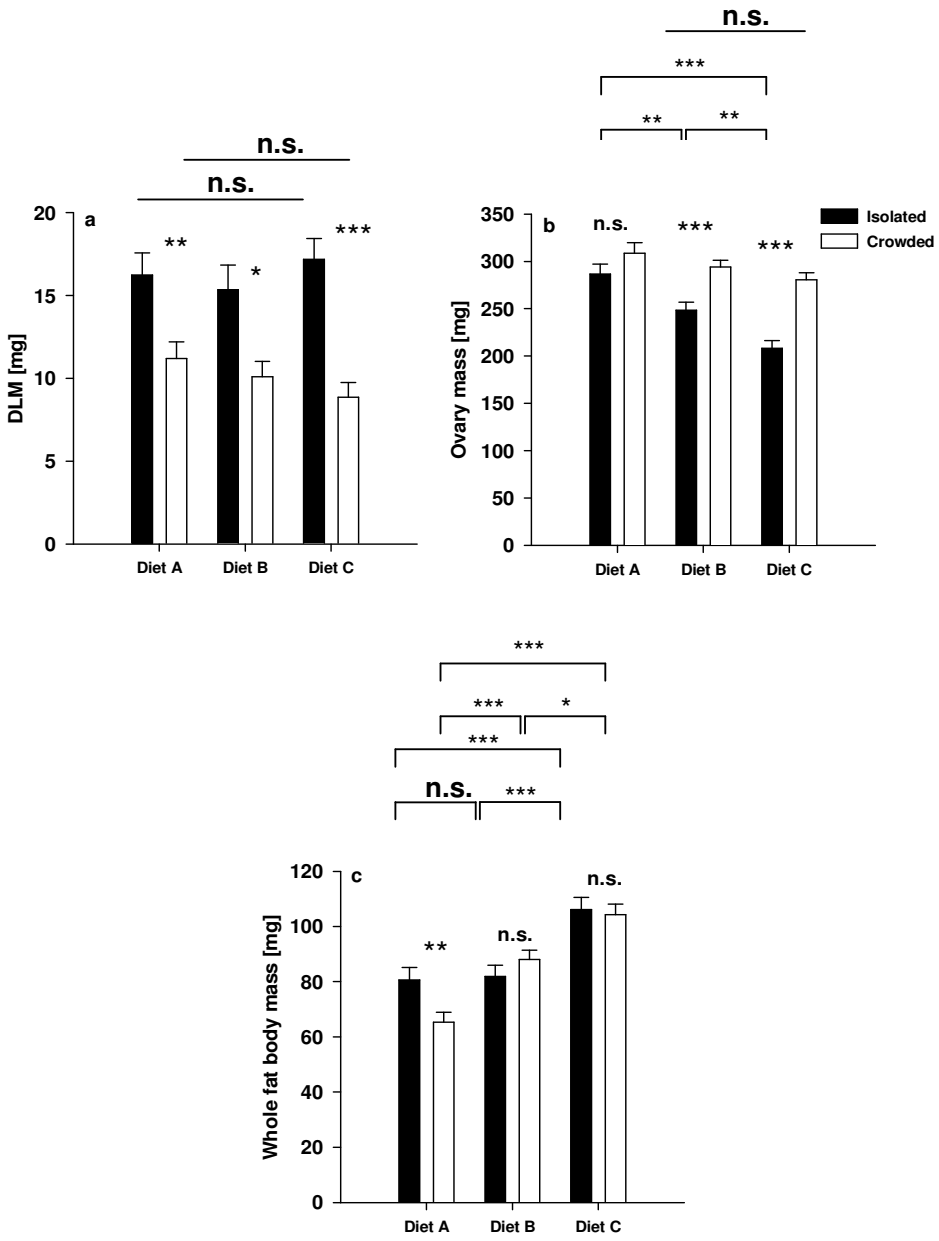

Fig. 21. Flight muscle (a), ovary (b) and fat body mass (c) of 5-day-old adult female crickets reared under isolated or crowded conditions and fed on three different diets. Means ± S.E. of 73, 74 and 69 (isolated) or 73, 95 and 73 (crowded) determinations, *p<0.05; **p<0.01; ***p<0.001. Differences between isolated and crowded females were tested by Mann-Whitney U-test, differences among the 3 different diets were tested by Kruskal-Wallis ANOVA.

3.3.4. Total lipid and protein content in mg per whole fat body and lipid and protein content in µg per mg fat body fresh mass

Both the total lipid (Fig. 22a) and the total protein content (Fig. 22b) in the fat body of isolated or crowded females was lowest in animals fed on diet A, intermediate in animals fed on diet B and highest in animals fed on diet C. Between isolated and crowded animals fed on the same diet no significant differences in lipid or protein content was found. On a per mg basis, no consistent trends for diet, or rearing, dependent lipid (Fig. 22c) or protein levels (Fig. 22d) were found.

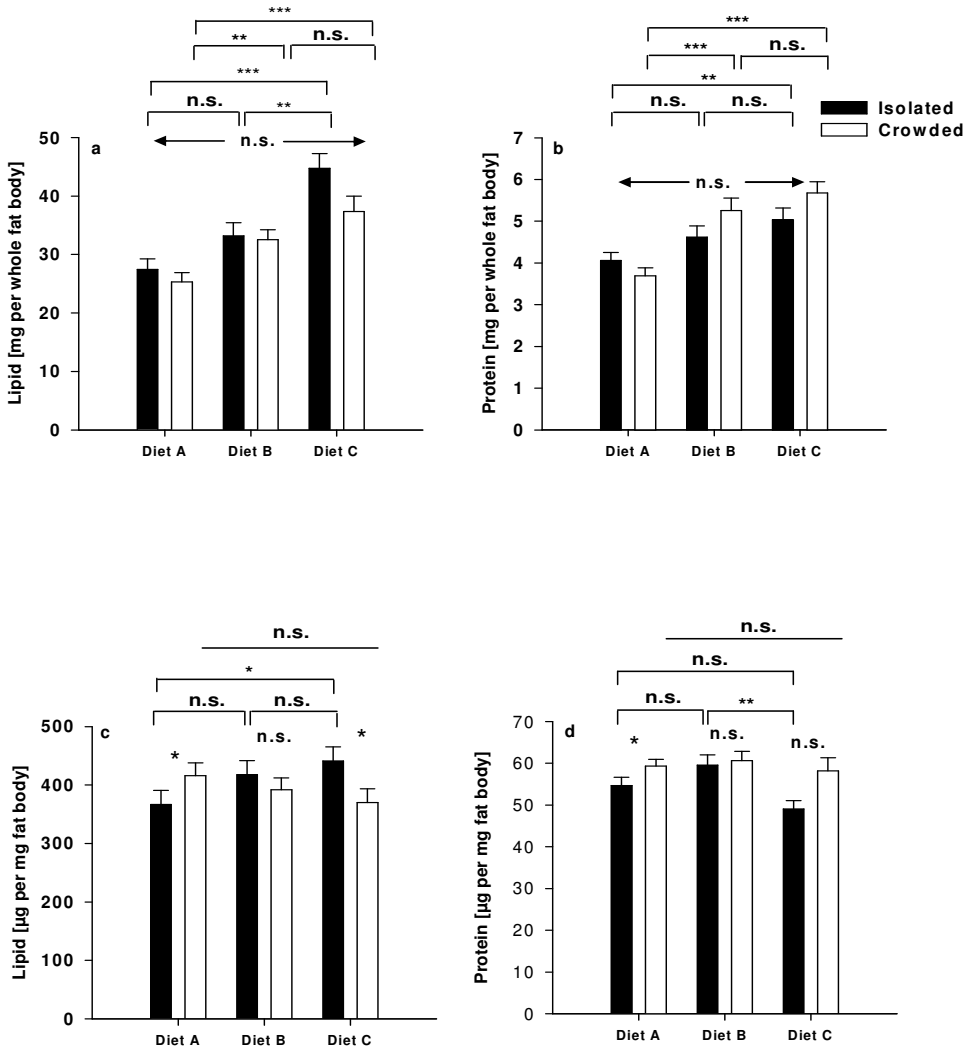

Fig. 22. Total lipid content (a), total protein content (b), lipid content per mg fat body fresh mass (c) and protein content per mg fat body fresh mass (d) of 5-day-old adult female crickets reared under isolated or crowded conditions and fed on three different diets. Means ± S.E. of 73, 74 and 69 (isolated) or 73,95 and 73 (crowded) determinations, *p<0.05; **p<0.01; ***p<0.001. Differences between isolated and crowded females were tested by Mann-Whitney U-test, differences among the 3 different diets were tested by Kruskal-Wallis ANOVA.

3.3.5. Total glycogen and free carbohydrate content in mg per whole fat body and glycogen and free carbohydrate content in µg per mg fat body fresh mass

According to the lipid and protein stores, the total glycogen content (Fig. 23a) in the fat body of isolated and crowded females increased depending on the diet in the order A < B < C.

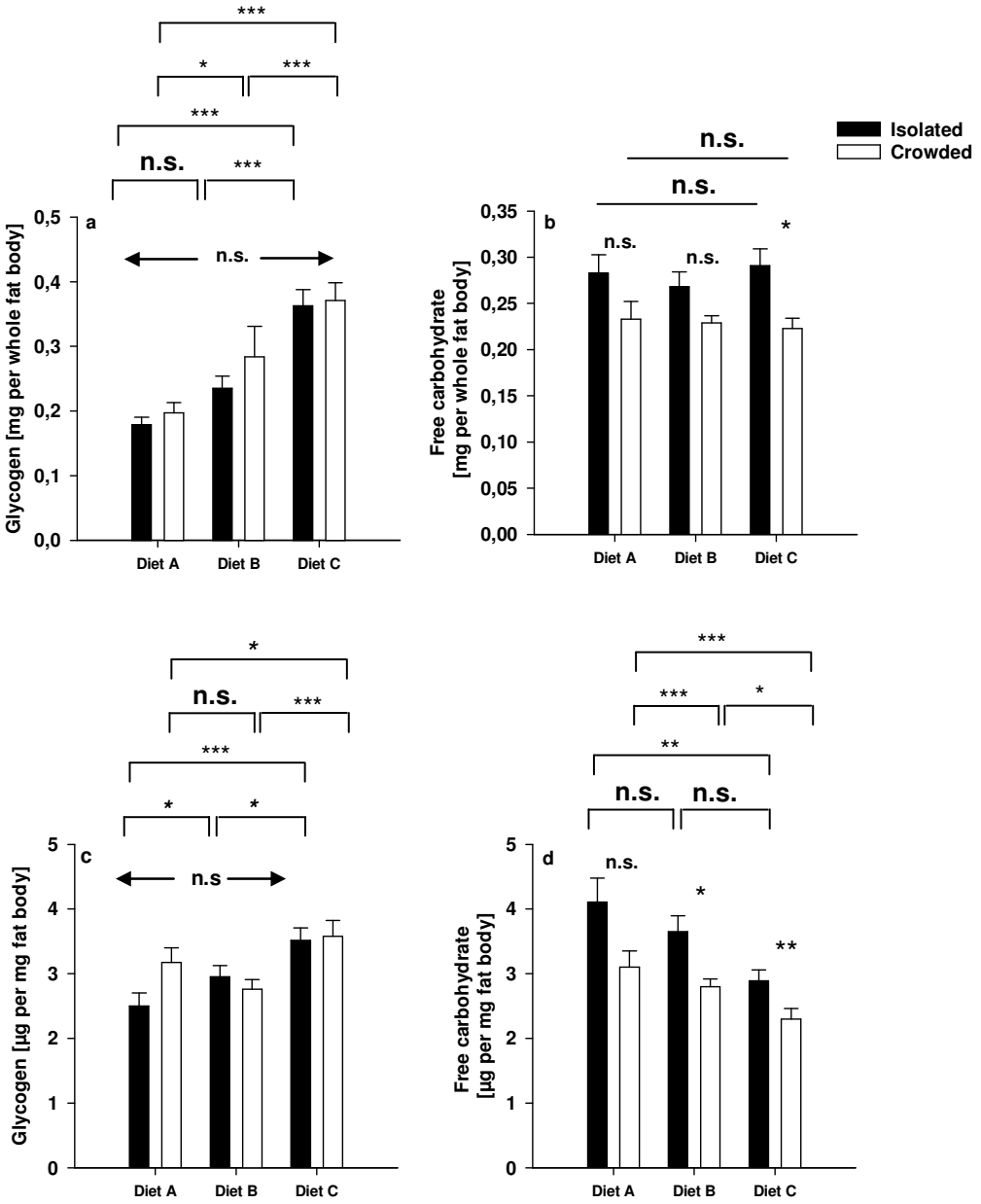

Fig. 23. Total glycogen content (a), total free carbohydrate content (b), glycogen content per mg fat body fresh mass (c) and free carbohydrate content per mg fat body fresh mass (d) of 5-day-old adult female crickets reared under isolated or crowded conditions and fed on three different diets. Means ± S.E. of 69-95 replicates except for diet A free carbohydrate determination in crowded where n =23 (b). Determinations, *p<0.05; **p<0.01; ***p<0.001. Differences between isolated and crowded females were tested by Mann-Whitney U-test, differences among the 3 different diets were
tested by Kruskal-Wallis ANOVA.

Isolation or crowding had no effects on the glycogen content. Total free carbohydrates (Fig. 23b) were generally lowest in the fat body of crowded females, although only in females fed on diet C was the difference significant. The kind of diet had no effect on the total free carbohydrate content. On a per mg fat body basis, glycogen did not differ between isolated and crowded females (Fig. 23c) but also differed depending on the diet consumed. Free carbohydrates per mg fat body were generally lower in crowded females (Fig. 23d) and also differed depending on the diet

3.3.6. Haemolymph lipid and carbohydrate concentration

The haemolymph lipid concentration did neither differ between isolated and crowded females (Fig. 24a) nor between females fed on diet A or B. Significantly higher lipid concentrations were found in isolated and crowded females fed on diet C. Depending on the diet, haemolymph carbohydrates differed significantly between isolated and crowded females (Fig. 24b). In addition, the kind of diet had some influence on carbohydrate titres in isolated females.

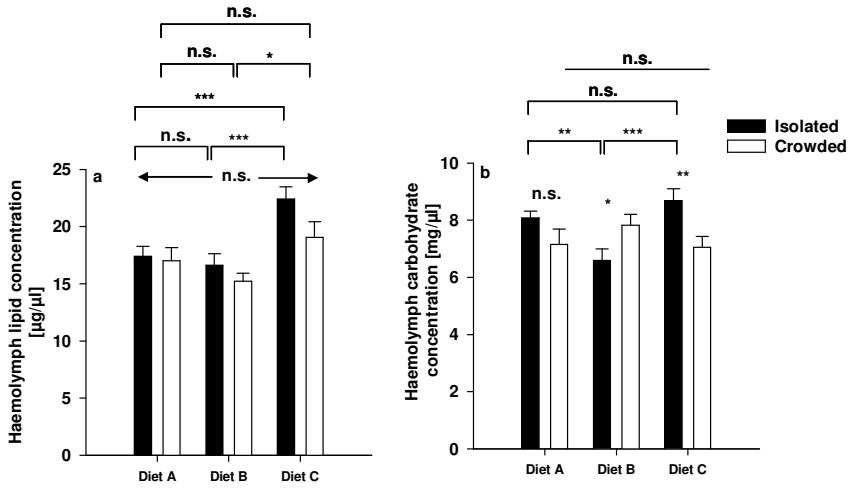

Fig. 24. Haemolymph lipid (a) and carbohydrate concentration (b) of 5-day-old adult female crickets reared under isolated or crowded conditions and fed on three different diets. Means ± S.E. lipid of 69, 55 and 66 (isolated) or 47, 87 and 53 (crowded) and carbohydrate of 73, 74 and 69 (isolated) or 66, 95 and 73 (crowded) determinations, *p=0.05; **p=0.01; ***p<0.001. Differences between isolated and crowded females were tested by Mann-Whitney U-test, differences among the 3 different diets were tested by Kruskal-Wallis ANOVA.

3.4. Experiment IV

Effect of feeding three different diets to crowded females from day 0 of the penultimate larval stage to day 5 adult stage

In addition to the Figures presented here, the results of the experiments can be found in tables 6 and 7 (supplementary). Each group was exclusively fed only one of the three different diets (A, B and C)

3.4.1. Percent increase in body mass from penultimate larvae 0 day to adult 5 day

The highest increase in body mass was found in animals fed on diet A throughout (Fig. 25). Whereas during the PL stage diet C enabled a similar increase, the increase was somewhat lower during the LL stage and significantly lower during the adult stage, Irrespective of the developmental stage, diet B enabled the lowest increase in body mass.

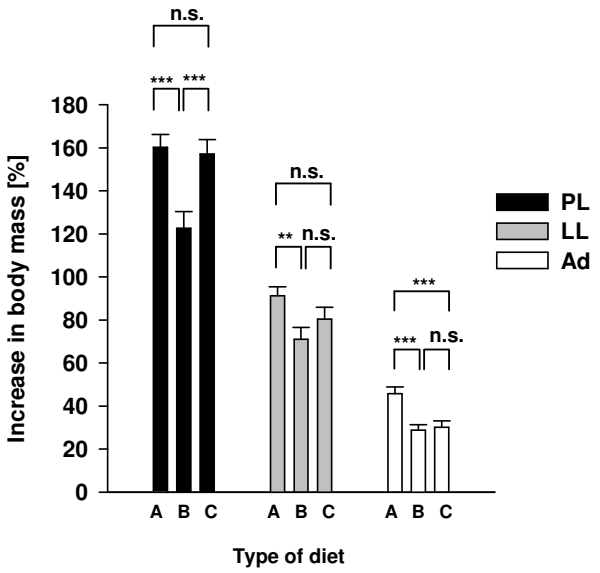

Fig. 25. Percent increase in body mass from day 0 PL to day 0 LL, day 0 LL to day 0 Ad and day 0 Ad to day 5 Ad. Female crickets reared under crowded conditions and fed on three different diet. Means ± S.E. of PL (70, 68 and 61), LL (69, 57 and 59) and Ad (66, 55 and 58) determinations, **p<0.01; ***p<0.001. Kruskal-Wallis ANOVA.

3.4.2. Diet consumption from penultimate larvae 0 day to adult 5 day

In general, the amount of diets consumed increases from PL to the adult stage (Fig. 26). No differences in consumption of the three different diets was found in the PL stage; however, in the LL and the adult stage diet B and C were consumed to a lesser extent than diet A.

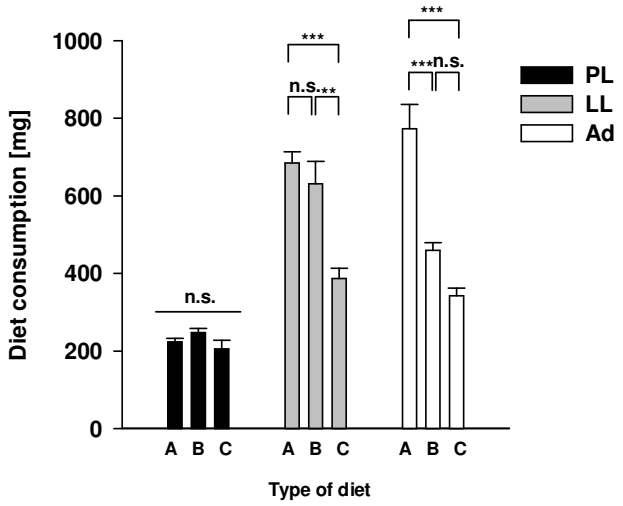

Fig. 26. Amount of diet consumed from day 0 PL to day 0 LL, day 0 LL to day 0 Ad and day 0 Ad to day 5 Ad female crickets reared under crowded conditions and fed on three different diet. Means ± S.E. of 6 determinations, **p<0.01; ***p<0.001, Kruskal-Wallis ANOVA.

3.4.3. Pronotum width

Diet A gave the highest average pronotum width of 5-day-old adult females, followed by diet C and diet B.

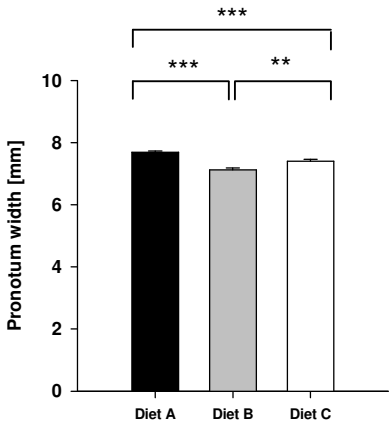

Fig. 27. Pronotum width of 5-day-old adult female crickets reared under crowded conditions and fed on three different diets. Means ± S.E, of 66, 55 and 58 determinations, **p<0.01; ***p<0.001, Kruskal-Wallis ANOVA.

3.4.4. Flight muscle, ovary and fat body mass

Diet B significantly affected the flight muscle mass (Fig. 28a), whereas the ovaries were significantly smaller in females fed on diet B and C (Fig. 28b). The highest fat body mass was reached in animals fed on diet C (Fig. 28c).

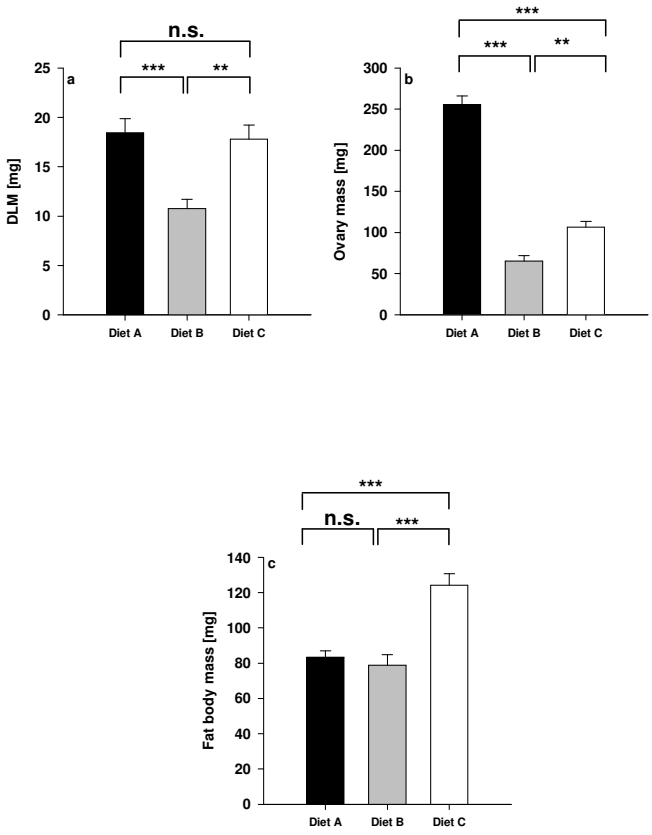

Fig. 28. Flight muscle (a), ovary (b) and fat body mass (c) of 5-day-old adult female crickets reared under crowded conditions and fed on three different diets. Means ± S.E. of 66, 55 and 58 determinations, **p<0.01; ***p<0.001, Kruskal-Wallis ANOVA.

3.4.5. Total lipid and protein content in mg per whole fat body and lipid and protein content in µg per mg fat body fresh mass

Both total lipid (Fig. 29a) and total protein content (Fig. 29b) were lowest in animals fed on diets A and B, whereas they were significantly higher in animals fed on diet C. On a per mg fat body basis, lipid (Fig. 29c) and protein (Fig. 29d) were lowest in animals fed on diet A.

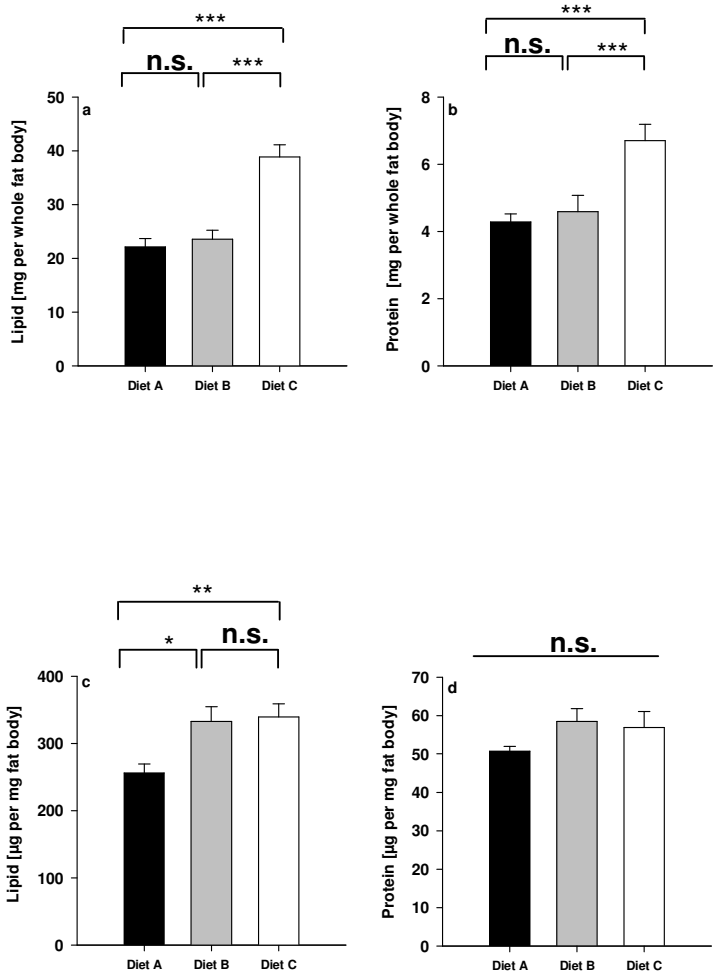

Fig. 29. Total lipid content (a), total protein content (b), lipid content per mg fat body fresh mass (c) and protein content per mg fat body fresh mass (d) of 5-day-old adult female crickets reared under crowded conditions and fed on three different diets. Means ± S.E. of 66, 55 and 58 (a and c) 66, 50 and 58 (b and d) determinations, *p<0.05; **p<0.01; ***p<0.001, Kruskal-Wallis ANOVA.

3.4.6. Total glycogen and free carbohydrate content in mg per whole fat body and glycogen and free carbohydrate content in µg per mg fat body fresh mas

The highest glycogen content in the fat body was found in animals fed on diet C (Fig. 30a). Total free carbohydrates did not differ significantly between the diets although there was a trend for higher levels in animals fed on diet A and lower levels in animals fed on diet C (Fig. 30b). On a per mg fat body basis, both glycogen and free carbohydrates were highest in animals fed on diet B (Fig. 30c, d).

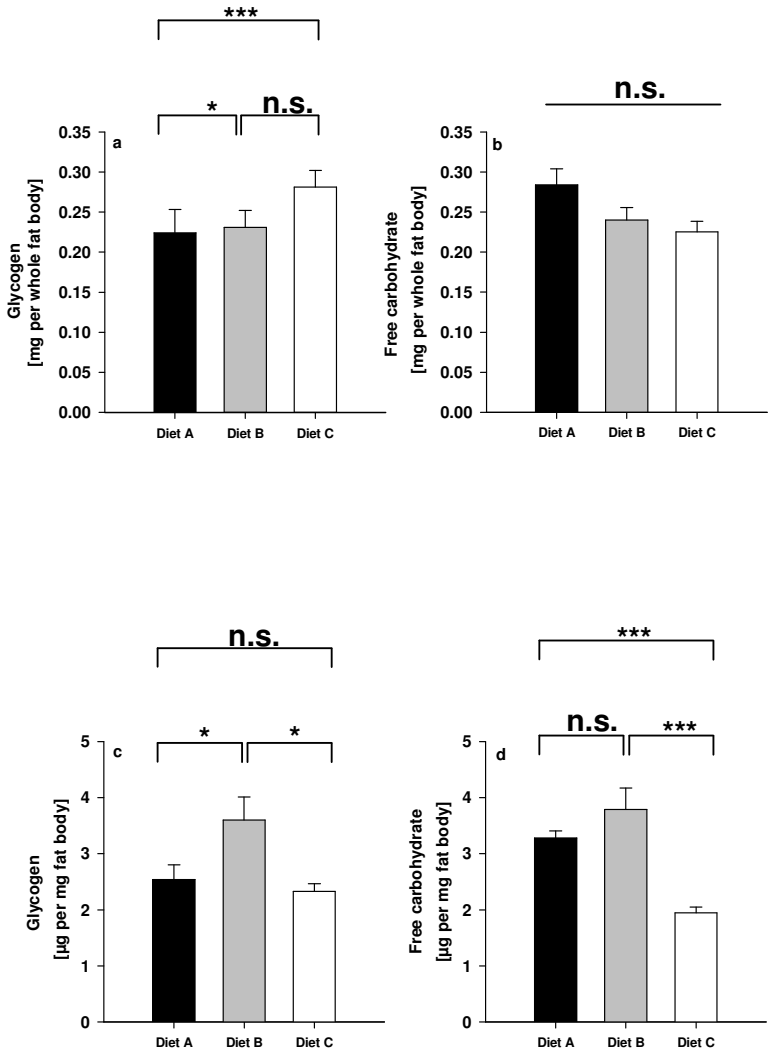

Fig. 30. Total glycogen content (a), total free carbohydrate content (b), glycogen content per mg fat body fresh mass (c) and free carbohydrate content per mg fat body fresh mass (d) of 5-day-old adult female crickets reared under crowded conditions and fed on three different diets. Means ± S.E. of 66-50, determinations, *p<0.05; ***p<0.001, Kruskal-Wallis ANOVA.

3.4.7. Haemolymph lipid and carbohydrate concentration

The haemolymph lipid concentration (Fig. 31a) increase in the order A < B < C, whereas the highest carbohydrate titre was found in animals fed on diet B (Fig. 31b).

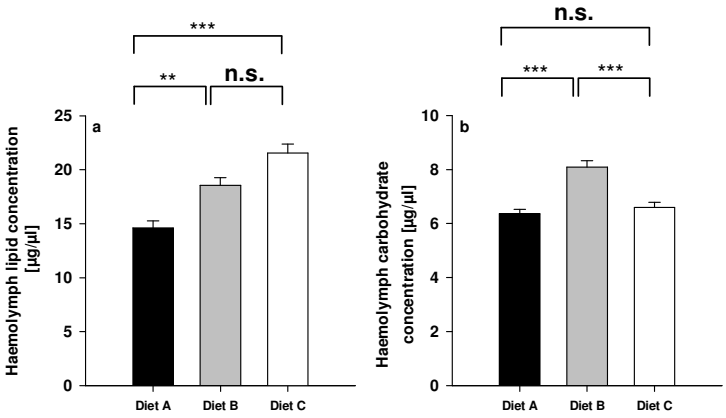

Fig. 31. Haemolymph lipid (a) and carbohydrate concentration (b) of 5-day-old adult female crickets reared under crowded conditions and fed on three different diets. Means ± S.E. of 66, 55 and 58 determinations, **p<0.01; ***p<0.001, Kruskal-Wallis ANOVA.

4- DISCUSSION

There is an increased need to understand basic biotic factors such as nutritive and protective potential (flight ability, adaptation, development of behaviour, structure and size of body), interactions that occur among pests, trophic and edaphic factors and natural enemies of various crop production systems.

Therefore, the objective of the present work was to investigate the ecology and biology of *G. bimaculatus* under laboratory conditions. In this work, the author inspected effects of population density by rearing the two-spotted crickets under both isolated and crowded conditions. Also, to evaluate the efficiency of aggregation and types of diets on rate increase of body mass, food consumption, flight muscles, fat body and ovary masses as well as the biochemical composition of the fat body were investigated.

Since the two-spotted cricket usually lives solitary in the field, the author considered that the results yielded with isolated insects reflect normal biological features in this species. It is worth mentioning that both penultimate and last larval (or nymphal) instars of the two-spotted cricket possess wing buds on meso- and metathorax, which makes them easily recognizable, as it is easy to distinguish between female and male. In the following discussion, the data obtained in Experiment I on 5-day-old adult females reared under isolated and crowded conditions fed on standard diet were considered as a control treatment.

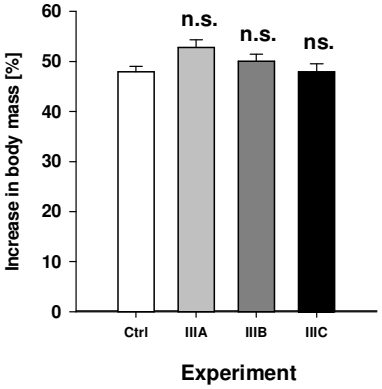

Fig. 32. Comparison among increase in body mass of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 194 (Ctrl l), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations. Differences among the increase in body mass were tested by Kruskal-Wallis ANOVA.

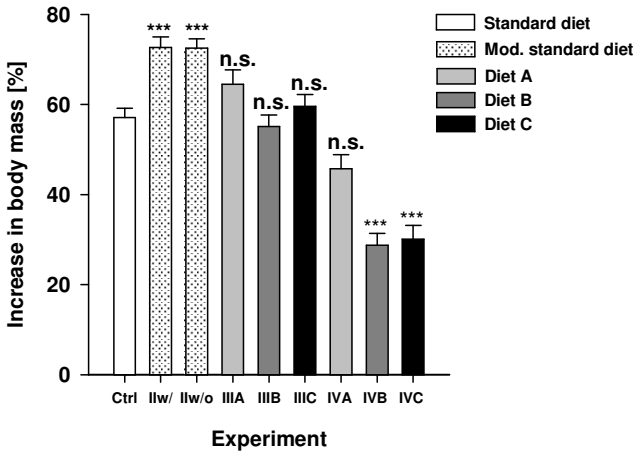

Fig. 33. Comparison among increase in body mass of 5-day-old adult female crickets reared under crowded conditions (Experiment I through IV) Means ± S.E. of 164 (Ctrl), 57 (IIw/), 50 (IIw/o), 73 (IIIA), 95 (IIIB), 73 (IIIC), 66 (IVA), 55 (IVB), 58 (IVC) determinations, ***p<0.001. Differences among the increase in body mass were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

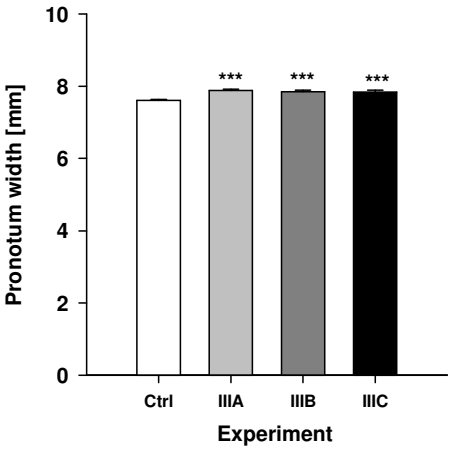

Fig. 34. Comparison among pronotum width of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 192 (Ctrl), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations, ***p<0.001. Differences among the pronotum width were tested by Kruskal-Wallis ANOVA. Treatment were tested against Ctrl by Dunn's post hoc test.

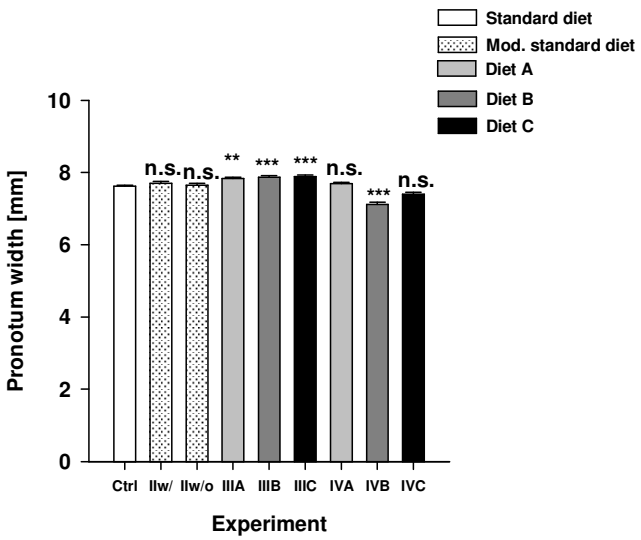

Fig. 35. Comparison among pronotum width of 5-day-old adult female crickets reared under crowded conditions (Experiment I through IV) Means ± S.E. of 164 (Ctrl), 57 (IIw/), 50 (IIw/o), 73 (IIIA), 95 (IIIB), 73 (IIIC), 66 (IVA), 55 (IVB), 58 (IVC) determinations, **p<0.01; ***p<0.001. Differences among the pronotum width were tested by Kruskal-Wallis ANOVA. . Treatment were tested against Ctrl by Dunn's post hoc test.

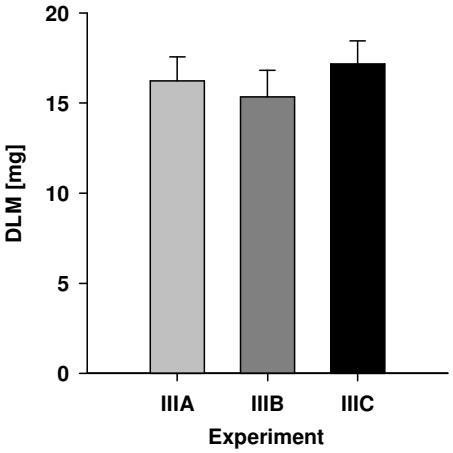

Fig. 36. Comparison among flight muscle mass of 5-day-old adult female crickets reared under isolated conditions (Experiment III) Means ± S.E. of 192 (control), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations (see Fig. 21)

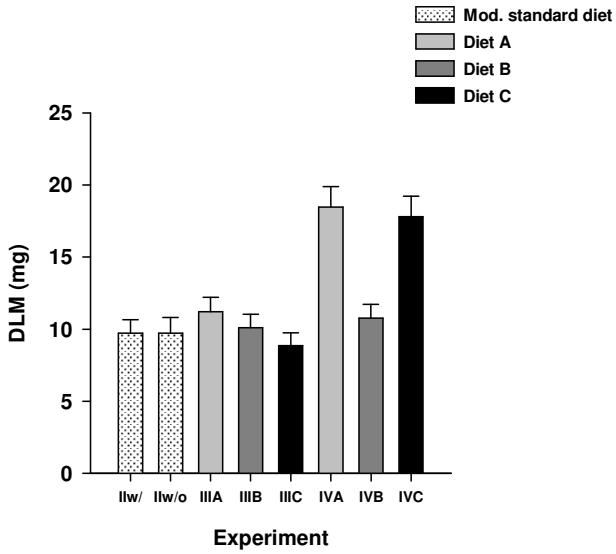

Fig. 37. Comparison among flight muscle mass of 5-day-old adult female crickets reared under crowded conditions (Experiment II through IV) Means ± S.E. of 57 (IIw/), 50 (IIw/o), 73 (IIIA), 95 (IIIB), 73 (IIIC), 66 (IVA), 55 (IVB), 58 (IVC) determinations (see Figs. 15, 21 and 27)

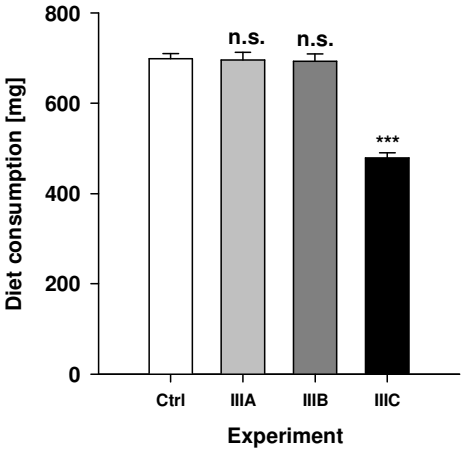

Fig. 38. Comparison among amount of diet consumed of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 192 (Ctrl), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations, ***p<0.001. Differences among the amount of diet consumed were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

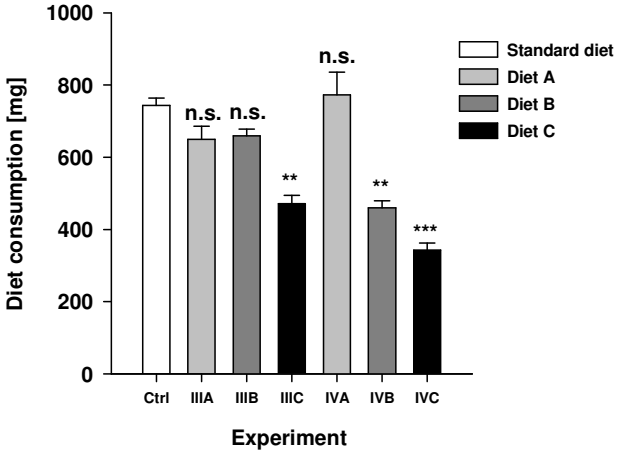

Fig. 39. Comparison among amount of diet consumed of 5-day-old adult female crickets reared under crowded conditions (Experiment I through IV) Means ± S.E. of 15 (Ctrl), 7 (IIIA), 9 (IIIB), 7 (IIIC), 6 (IVA), 6 (IVB), 6 (IVC) determinations, **p<0.01; ***p<0.001. Differences among the amount of diet consumed were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

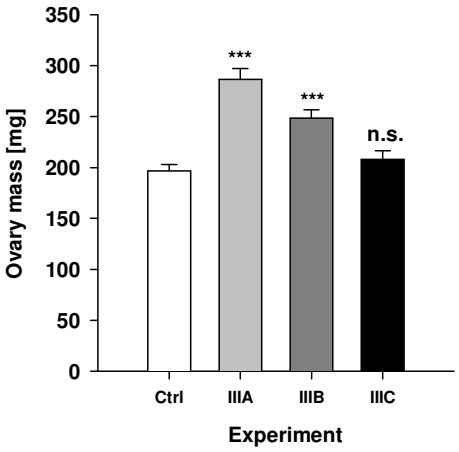

Fig. 40. Comparison among ovary mass of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 192 (Ctrl), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations, ***p<0.001. Differences among the ovary mass were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

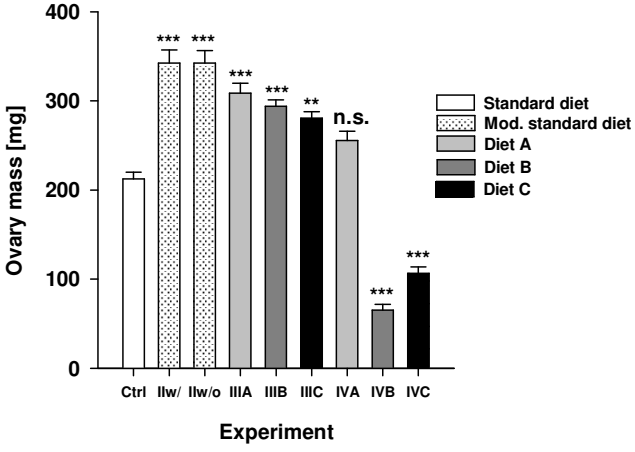

Fig. 41. Comparison among ovary mass of 5-day-old adult female crickets reared under crowded conditions (Experiment I through IV) Means ± S.E. of 164 (Ctrl), 57 (IIw/), 50 (IIw/o), 73 (IIIA), 95 (IIIB), 73 (IIIC), 66 (IVA), 55 (IVBl), 58 (IVC) determinations, **p<0.01; ***p<0.001. Differences among the ovary mass were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

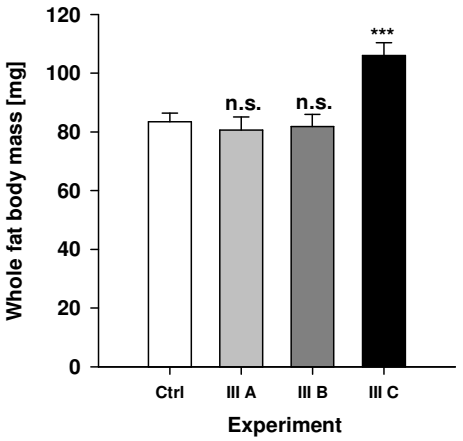

Fig. 42. Comparison among fat body mass of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 192 (Ctrl), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations, ***p<0.001. Differences among the fat body mass were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

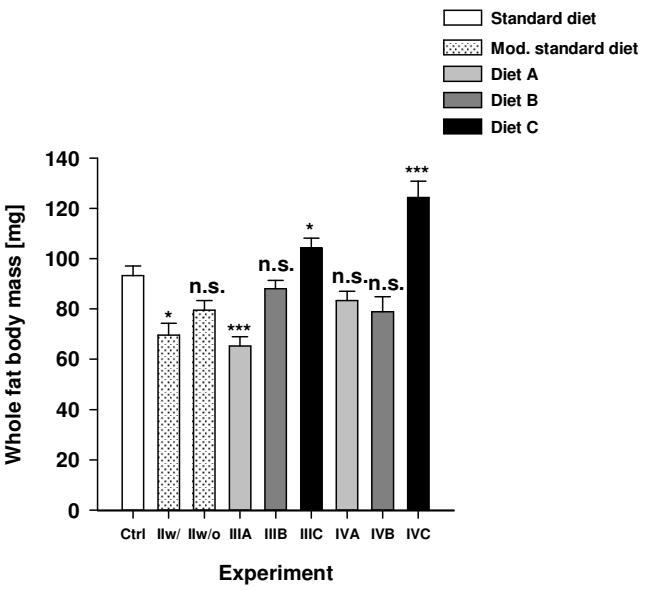

Fig. 43. Comparison among fat body mass of 5-day-old adult female crickets reared under crowded conditions (Experiment I through IV) Means ± S.E. of 164 (Ctrl), 57 (IIw/), 50 (IIw/o), 73 (IIIA), 95 (IIIB), 73 (IIIC), 65 (IVA), 55 (IVB), 58 (IVC) determinations, *p<0.05; ***p<0.001. Differences among the fat body mass were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

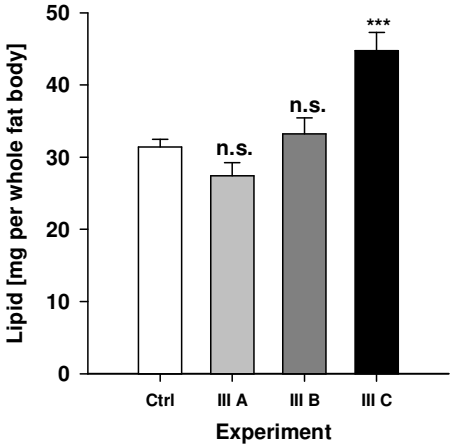

Fig. 44. Comparison among total lipid content per mg fat body fresh mass of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 192 (Ctrl), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations, ***p<0.001. Differences among the total lipid were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

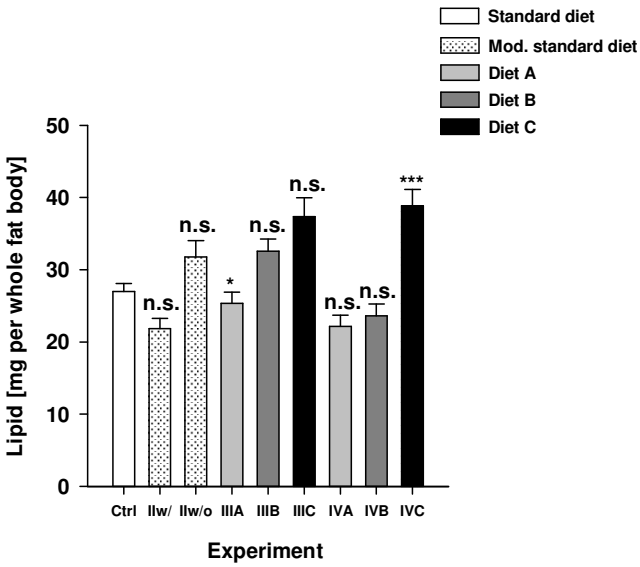

Fig. 45. Comparison among total lipid content per mg fat body fresh mass of 5-day-old adult female crickets reared under crowded conditions (Experiment I through IV) Means ± S.E. of 164 (Ctrl), 57 (IIw/), 50 (IIw/o), 73 (IIIA), 95 (IIIB), 73 (IIIC), 66 (IVA), 55 (IVBl), 58 (IVC) determinations, *p<0.05; ***p<0.001. Differences among the total lipid were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

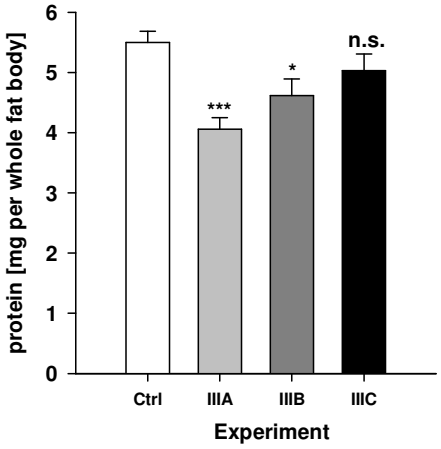

Fig. 46. Comparison among total protein content per mg fat body fresh mass of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 192 (Ctrl), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations, *p<0.05; ***p<0.001. Differences among the total protein were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

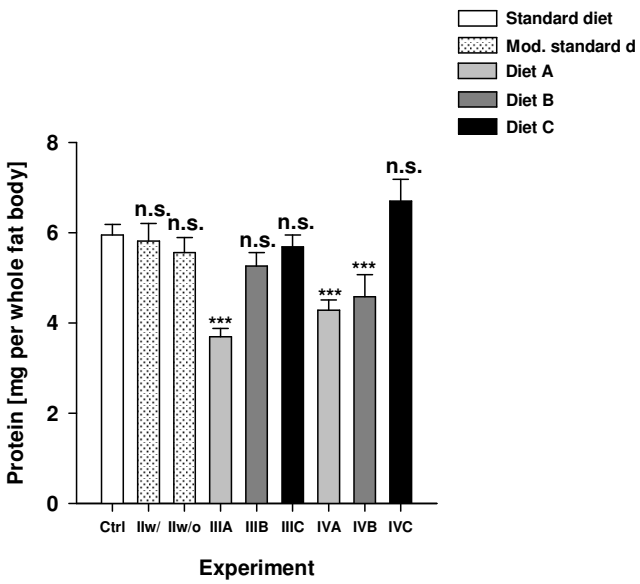

Fig. 47. Comparison among total protein content per mg fat body fresh mass of 5-day-old adult female crickets reared under crowded conditions (Experiment I through IV) Means ± S.E. of 164 (Ctrl), 57 (IIw/), 50 (IIw/o), 73 (IIIA), 95 (IIIB), 73 (IIIC), 66 (IVA), 55 (IVB), 58 (IVC) determinations, ***p<0.001. Differences among the total protein were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

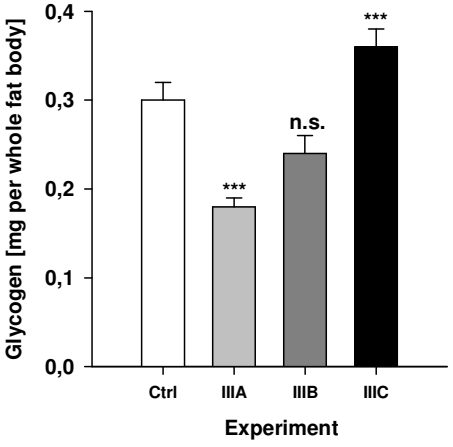

Fig. 48. Comparison among total glycogen content per mg fat body fresh mass of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 192 (Ctrl), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations, ***p<0.001. Differences among the total glycogen were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

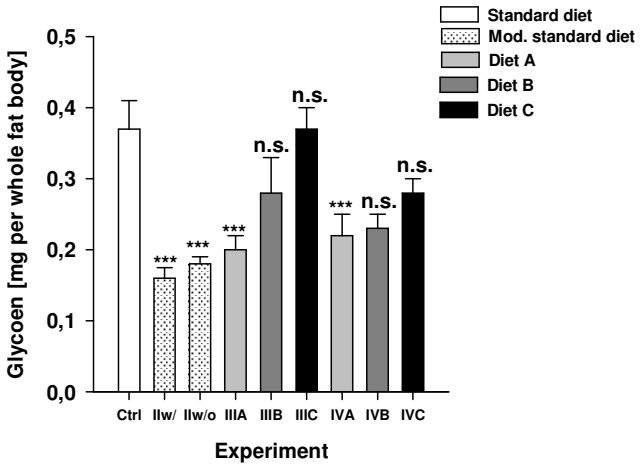

Fig. 49. Comparison among total glycogen content per mg fat body fresh mass of 5-day-old adult female crickets reared under crowded conditions (Experiment I and III) Means ± S.E. of 164 (Ctrl), 57 (IIw/), 50 (IIw/o), 73 (IIIA), 95 (IIIB), 73 (IIIC), 66 (IVA), 55 (IVBl), 58 (IVC) determinations, ***p<0.001. Differences among the total glycogen were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

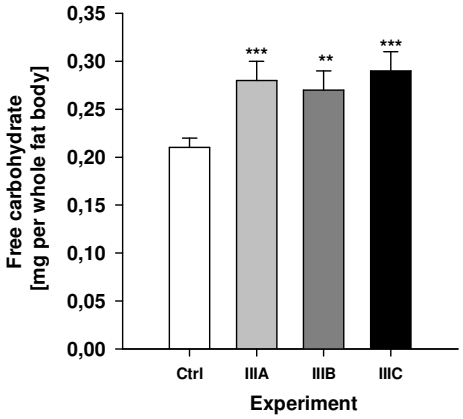

Fig. 50. Comparison among total free carbohydrate content per mg fat body fresh mass of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 192 (Ctrl), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations, **p<0.01; ***p<0.001. Differences among the total free carbohydrate were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

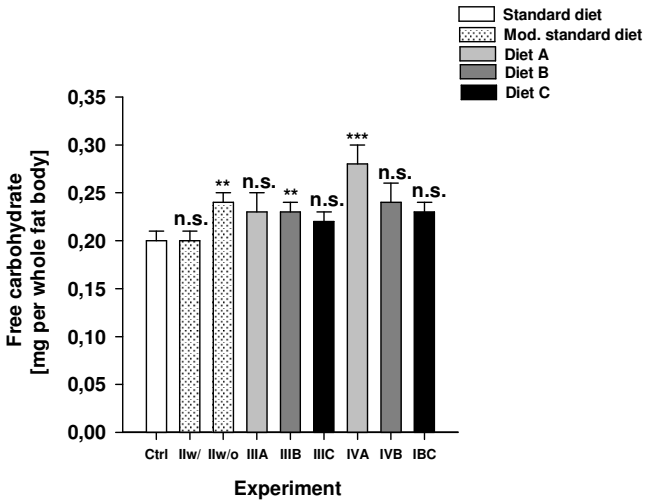

Fig. 51. Comparison among total free carbohydrate content per mg fat body fresh mass of 5-day-old adult female crickets reared under crowded conditions (Experiment I through IV) Means ± S.E. of 164 (Ctrl), 57 (IIw/), 50 (IIw/o), 23 (IIIA), 84 (IIIB), 64 (IIIC), 66 (IVA), 55 (IVBl), 58 (IVC) determinations, **p<0.01; ***p<0.001. Differences among the total free carbohydrate were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

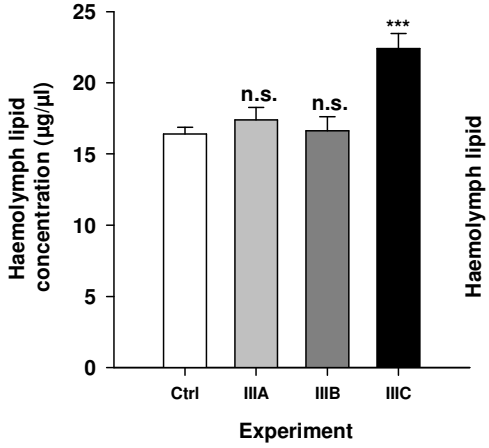

Fig. 52. Comparison among haemolymp lipid concentration of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 192 (Ctrl), 69 (IIIA), 55 (IIIB), 66 (IIIC) determinations, ***p<0.001. Differences among the total haemolymph lipid were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

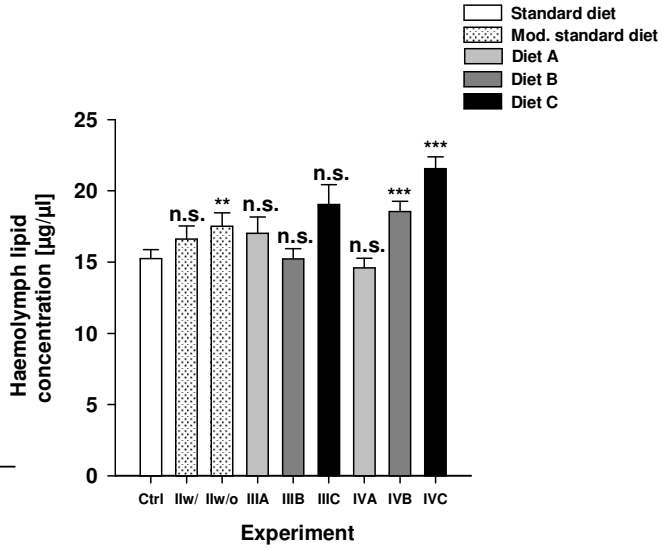

Fig. 53. Comparison among haemolymp lipid concentration of 5-day-old adult female crickets reared under crowded conditions (Experiment I through IV) Means ± S.E. of 164 (Ctrl), 57 (IIw/), 50 (IIw/o), 47 (IIIA), 87 (IIIB), 53 (IIIC), 66 (IVA), 55 (IVBl), 58 (IVC) determinations,**p<0.01; ***p<0.001. Differences among the total haemolymph lipid were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

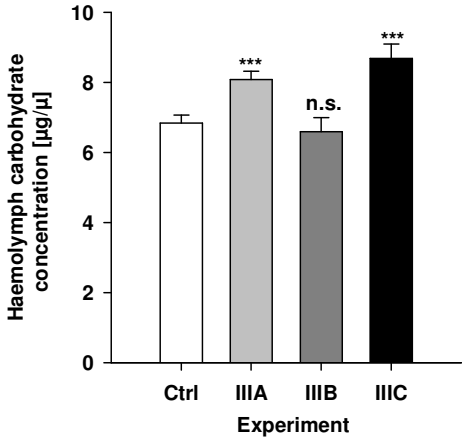

Fig. 54. Comparison among haemolymp carbohydrate concentration of 5-day-old adult female crickets reared under isolated conditions (Experiment I and III) Means ± S.E. of 192 (control), 73 (IIIA), 74 (IIIB), 69 (IIIC) determinations, ***p<0.001. Differences among the total haemolymph carbohydrate were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

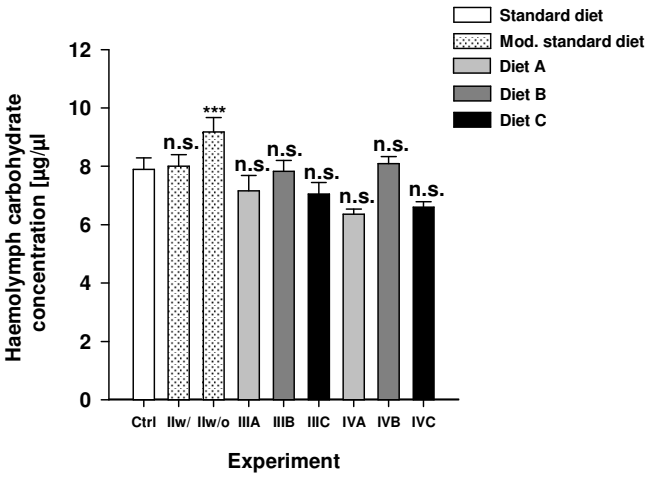

Fig. 55. Comparison among haemolymp carbohydrate concentration of 5-day-old adult female crickets reared under crowded conditions (Experiment I through IV) Means ± S.E. of 164 (Ctrl), 57 (IIw/), 50 (IIw/o), 66 (IIIA), 95 (IIIB), 73 (IIIC), 66 (IVA), 55 (IVBl), 58 (IVC) determinations, ***p<0.001. Differences among the total haemolymph carbohydrate were tested by Kruskal-Wallis ANOVA. Treatments were tested against Ctrl by Dunn's post hoc test.

4.1. Experiment I

Based on our results from this study, the average percent increase in body mass of individuals which were reared under isolated conditions was lower (47.93 ± 1.08 %) than that of females reared under crowded conditions (57.06 ± 2.05 %) (Fig. 8b). Crowded conditions lead to increased physical activity in adult female crickets and increased excitability inevitably leads to higher locomotor activity due to interaction between the animals **(Lorenz and Gäde, 2009)**. Moreover, it has to be underlined that under crowded conditions there is a widely presence of chemical and tactile stimuli and touch sensing may be more efficient: stimulation by fresh excreta and the tactile sense by antenna may play important part in the perception of other individuals. The previous results are in line with those of **McFarlane (1962)** who noted that rearing *A. domesticus* in groups of 10 individuals accelerated growth compared to single rearing.

The finding of **Iba *et al.* (1995)** that in crowded *G. bimaculatus* the rate of increase in body weight was slow, and also the day of imaginal moult was late, when compared to the isolated ones is in contrast to the results of the present study. Development and growth were thus suppressed in the crowded group. Also, it was noticed that isolated females were more

aggressive than crowded ones. The differences between the results of the present study and that of **Iba *et al.* (1995)** are probably caused by the variation in population density and nutrient contents.

Chauvin (1958) noticed that the group effect is mediated by the nervous system, with particular involvement of the antenna and cerci. But when the antenna and cerci were removed, grouped crickets grew similarly to isolated crickets.

In the house cricket *A. domesticus* larvae reared in groups grew faster than those reared in isolation **(Chauvin, 1958)**. **Watler (1982)** showed that crowded nymphs of *A. domesticus*, which mature faster than isolated ones, consumed more food per mg of body weight and converted it to body tissue more efficiently than did isolated nymphs during the fifth and sixth instar. Survival was also increased in crowded over isolated crickets **(McFarlane et al., 1984)**. For isolated conditions (Fig. 32), there were two Experiments I and III, in which the adult female crickets were reared under isolated conditions. As mentioned above, the results from Exp. I are considered as control treatments. In this experiment, the crickets displayed the lowest average increase in body mass, while the individuals which were reared under isolated conditions in Exp. III and fed on artificial diet A gave the highest average increase in body mass (diet A > diet B > diet C > Ctrl), although the differences were not statistically significant.

The results of the present study illustrate that the effect of isolated or crowded conditions had no effect on pronotum width (isolated: 7.61 ± 0.02 mm; crowded: 7.61 ± 0.03 mm) (Fig. 8a). In addition, this study found weak or no correlations between the body weight and pronotum width. Therefore, the pronotum width varied from one moult to another and increased in width. **Simmons (1986b)** denoted that the pronotum width of *G. bimaculatus* was highly correlated with other measures of body size and mass, but was not prone to the changes that can occur in body mass over time within the adult stage. However, I also observed similar results under both rearing conditions, where no significant differences were noted in food consumption, ovary mass and whole fat body mass, but all were slightly lower in females reared under isolated conditions (Fig. 9 and 10a, b). On the other hand, we found that females fed on Ctrl diet (Exp. I) under isolated conditions had significantly smaller ovaries compared with females fed on diets A and B in Exp. III (Fig. 40). **Clifford and Woodring (1986)** suggest that the combination of fat body reserves plus the food eaten permits the highest egg production rate that occurs soon after the final moult. Our experimental results revealed that solitary individuals contain a high amount of lipid (Fig. 11a, c), probably due to the fact that they produce less eggs and are less active than

individuals that lived under crowded conditions. **Lorenz (2003)** reported that eggs contain approximately 21% of lipid, 15% of proteins and 2% of carbohydrate (glycogen plus free carbohydrate) in *G. bimaculatus*.

The results of this study show that females reared under isolated or crowded conditions, had no significant differences in fat body protein content (Fig. 11b, d). From data illustrated in Fig. 46 it could be indicated that protein content in the fat body of 5-day-old adult female crickets reared under isolated condition was significantly higher in individuals fed on Ctrl diet in Exp. I than in individuals fed on diets A and B in Exp. III. In the present study the protein content of Ctrl diet was 20%; as reported in **Woodring et al. (1979)** the content of 20% is more than enough for the complete somatic and ovarial growth, besides some proteins were catabolised for energy. **McFarlane (1964b)** reported that the optimal level for both growth and wing development of cricket *Gryllodes sigillatus* was 20% dietary protein. **Merkel (1977**) found that *G. bimaculatus* required an average of 55 days and eight larval instars to reach adulthood on a high protein diet, while they stayed 117 days across 10 larval instars on a low protein diet at average temperatures of 29 °C day, 11 °C night, 16: 8 LD photoperiod.

Our study illustrates that the glycogen content in fat body was not significantly different in both groups (Fig. 12a, c), but, in comparison with other experiments (Fig. 49) it can be seen that glycogen content in the fat body of 5-day-old adult female crickets was relatively high in individuals fed on Ctrl diet (Exp. I) and individuals fed on diet C in Exp. III under crowded conditions. The present results show that the content of glycogen in the fat body mass of 5-day-old crickets was lower than the levels of lipids and proteins. The decrease in glycogen concentration in fat body is due to glycogen mobilization to supply energy for the synthesis and transfer of materials to the oocytes. Similar to the present study, **Anand (2004)** found that glycogen forms a minor part of the fat body in the penultimate larval instar crickets, while lipid and protein form the main part. However, in adults the glycogen content of fat body was about 1%, in the last larval instar about 3% and in the penultimate larval instar about 9%.

The present observations show that the lowest value of free carbohydrate in the fat body was found in groups reared under crowded conditions (Fig. 12b, d). From data illustrated in Fig. 50 and 51 the lowest value was found in individuals fed on Ctrl diet (this study) under isolated conditions and individuals fed on Ctrl diet (this study) and modified standard diet (Exp. II) with males under crowded conditions.

Higher concentrations of carbohydrates were present in larger individuals than smaller ones, while lipid concentrations tended to decrease with size **(Wheeler and Buck, 1992)**. Fat

body carbohydrate reserves were so limited in larval and adult crickets, that starvation quickly led to the reduction of fat body lipids and proteins **Woodring et al. (1979)**.

As reported under isolated conditions, the haemolymph lipid concentration of 5-day-old adult females had the highest values (Fig. 13a). It is know that when crickets are reared in groups, a higher locomotory activity is observed and haemolymph lipids are also higher than in the isolated crickets **(Faβold et al., 2010)**. In our experiment, to the contrary isolated crickets had higher haemolymph lipid titres than crowded ones and this may be due to the fact that insect females often produce huge number of eggs rich in proteins, lipids and carbohydrates that are transported in the haemolymph. Also, in the present study, we took haemolymph samples in the morning (2 h after light on), when the activity of crickets is low and the differences between isolated and crowded crickets may be less obvious than during scotophase (**Lorenz and Gäde, 2009; Faβold et al., 2010). Faβold et al. (2010)** suggested that an increased release of adipokinetic hormone during the scotophase is responsible for the higher locomotory activity and higher haemolymph lipid titers in active crickets.

The present study shows that the concentration of carbohydrate in the haemolymph of 5-day-old adult females reared under both isolated and crowded conditions, was lower than that of lipid. Our data are in agreement with that of **Woodring et al. (2002)** who showed that haemolymph sugar titers are generally lower than the lipid level, and sugars in larval and adult animals seem to participate less in generating energy reserves than lipids. In addition, haemolymph carbohydrates in *G. bimaculatus* under crowded conditions when compared with isolated conditions, we found not significantly different, but tended to be lower in females reared under isolated conditions (Fig. 13b). **Woodring et al. (2002)** reported that total carbohydrate and lipid concentrations in the haemolymph of female and male last instar larvae of *G. bimaculatus* were high at the beginning and the end of the larval stage but low approximately 24 h after moult. Except during the first few hours of the larval stage, lipids exceeded the sugar content in all ages in both sexes. Carbohydrate and lipid concentrations in the haemolymph of adult females and males changed slightly during the first 5 days of adult life, but both decreased with increasing age. **Jutsum et al. (1975)** found that the decrease of carbohydrate goes with a three to four fold increase of total lipid concentration in haemolymph of *Locusta migratoria migratorioides.*

4.2. Experiment II

The diet adopted here was slightly different compared with the diet used in first experiment. Here, the food mixture contained 22% proteins, 6% lipids and 42% carbohydrates for a total of 13.02 kJ/g (Mod. standard diet contains rabbit, rat/mouse and cat respectively in

the following ratio 4:2:1), while in Exp. I, the diet used contained, 20% protein, 4.5% lipids, 45.5% carbohydrates for a total of 12.71 kJ/g (standard diet 1:1 rabbit: rat/mouse). Moreover in Exp. II a completely different kind of rearing was adopted: the females were reared only under crowded conditions with or without adult males in the original cages of mass culture (containing about 200-300 crickets of both sexes with different stages and ages). Whereas in Exp. I animals under crowded conditions were reared in a white box (16 L x11 W x 6 H cm, 12 insects / box), in Exp. II, the cages were (60 L x 40 W x 30 H cm with 200-300 insects / cage). To have an idea about significant differences in population density we established the number of insects per square metre (m^2) that was around 682/m^2 in Exp. I, and about 883-1250/m^2 in Exp. II. Not only the population density plays an important role in morphological, physiological and behavioural changes within individuals, also, the number of animals acts as an important factor affecting the overall activity of the insects. However, under the experimental conditions of this study the diet is plentiful and optimal in both quantity and quality. It is known that individuals reared at high population density are much more active (this study) and respond to tactile cues, visual, olfactory stimuli, courtship and offspring than those reared under isolation; on the other hand, effects of rearing density and presence or absence of males were each due to change in different suites of movement behaviour such as walking, jumping, running etc. **Peters and Barbosa (1977)** clarified that the influence of population density on an insect population is a complex topic. Similar to the present study, **Simpson et al. (1999)** found in the desert locust *Schistocerca gregaria* that there is a simultaneous increase of activity and attraction among individuals that have been reared at high population density. The population density affected females more than males in *Drosophila melanogaster* **(Chu, 2009)**. The increase in metabolic rate is attributable to higher activity of individuals under crowded conditions. **Woodring et al. (1979)** demonstrated that the time of most rapid growth is always at the time of maximal feeding and the highest metabolic rate. As reported in **Iba et al. (1995)** brains of crickets reared under crowded conditions contain significantly higher amounts of octopamine (also higher in corpora cardiaca), dopamine, and 5-hydroxytrytamine (5-HT; also higher in corpora allata) than that of isolated crickets whereas the level of N-acetyldopamine is highest in isolated crickets. These results unambiguously indicate that high population density affects aminergic systems which in turn probably modulate various biological events, such as development, growth and behaviour.

In *G. bimaculatus*, the pronotum width (Fig. 14a) of 5-day-old adult female crickets reared under crowded conditions with or without males, did not differ significantly; this may

be explained by a positive correlation between adult size and the food utilization ability during the larvae stage.

Our results (Fig. 14b, 15a, b) show that increase in body mass, flight muscle mass (dorso-longitudinal flight muscle, DLM) and ovary mass with or without males, were approximately similar.

As illustrated in Fig. 33 and 41, I found a higher increase in body mass and ovary mass of the females reared in presence or absence of adult males as compared with other experiments. Based on these observations, I conclude that: (i) the fact that these females were reared in the original cages of mass culture determines a situation that may stimulate crickets to eat higher amounts and, thus, influence development and body mass; (ii) interactions between the individuals play an important role in regulating colony activities; (iii) the animals are affected by different factors such as diets, diet availability, diet quality and quantity, food intake and rearing conditions; (iv) in general, in some insects presence or absence of males is not essential for ovarian growth, egg maturation and ovary mass; (v) tactile stimulation leads to a number of behavioural responses in *G. bimaculatus*; (vi) presence of males in the same culture does not have any effect on growth or egg production in *G. bimaculatus*; and (vii) effects of rearing density and presence or absence of males were each due to a change in different suites of movement behaviour such as walking, speed etc. As reported, **Woodring et al. (1979)** showed that the presence of males was not required for ovarian growth or maturation of eggs in house crickets, but males were required to initiate and maintain oviposition.

As noted by **Norris (1962)**, the rapid maturation of the ovaries is probably the result of both mutual activity stimulation of individuals living in a group and the presence of a chemical stimulant.

In *G. bimacultus*, generally, under crowded conditions the increase in body mass and ovary mass of 5-day-old adult females was higher than under isolated conditions. Moreover, our study reports significant positive correlation between the increase in body mass and ovary mass and accompanied with the production of eggs, high food consumption and fat storage.

In this study the presence or absence of adult and sexually mature males does not make any difference or effect on pronotum width, increase in body mass, DLM mass and ovary mass (Fig. 14a, b, 15a, b).

Our data from all experiments underlines that the flight muscle mass of adult female crickets reared under isolated conditions (Fig. 36) was higher than in females reared under crowded conditions (Exp. II and III, Fig. 37). In addition, the data shown in Figs. 36, 37, 40 and 41 underline that there is a close relationship between flight muscle mass (DLM) and

ovary mass. Note that there is no control in this part because no measurement of the flight muscle mass was taken in the first experiment, which was considered as control treatment in all experiments. Our data are in agreement with that of **Lorenz (2007)** who reported that after the final moult of *G. bimaculatus* the flight muscle mass increases significantly to a maximum at days 2 and 3. The highest flight activity was also observed on day 2. Between days 2 and 3 the ovary weight starts to increase rapidly due to vitellogenic egg growth, which continues at a high rate until day 10. With the onset of ovarian growth, flight performance decreases and the flight muscles starts to histolyse. A high correlation between flight muscle mass and the content of protein, lipid, glycogen and free carbohydrate in the flight muscle indicates that energy-rich substrates from the degrading flight muscles are used to fuel oogenesis.

Food consumption could not be measured in this experiment, however, it was most likely higher than the quantities of food consumed by adult females in other experiments. More body growth and egg production was achieved. In crickets, it has been known that ovarian development and food consumption are influenced by various factors such as temperature, mating, and the presence or absence of oviposition substrate **(Loher and Edson, 1973; Merkel, 1977; Clifford and Woodring, 1986; Loher et al., 1987; Renucci et al., 1990)**. **Woodring et al. (1979)** showed that a higher percentage of consumed food is used as a fuel in adults than in larvae, suggesting that this might be due to the greater activity of adults.

The present result for *G. bimaculatus* demonstrate that fat body mass (Fig. 15c) of females reared with adult males was lower than that of individuals reared without adult males (69.64 ± 4.57 mg/whole fat body, 79.52 ± 3.85 mg/whole fat body, respectively). One possibility is that the difference might be caused by different activity levels in the presence or the absence of males, where males play an important role in regulation colony activities. Due to the increased activity or stress of females *G. bimaculatus* caused by the males, females significantly lost fat body lipids. This is corroborated by the observation that the total amount of lipid in the fat body was higher in females reared without males. There is a positive correlation between fat body mass in 5-day-old adult female crickets reared without males and the higher lipid content in the fat body (Fig. 15c, 16a, c).

The present data show that the highest level of protein in the fat body was found in grouped females reared with males (Fig. 16b, d). I suggest that the presence of adult males enhanced females to store higher amounts of protein in the fat body after the imaginal moult to be ready for oviposition. In the laboratory, a source of protein usually suffices and is critical for egg maturation, oviposition etc. The previously presented results are supported by

Telfer (1965) who stated that in some insects the proteins deposited as yolk appear to be synthesized not in the ovary, but in the fat body. **Boggs (1990)** summarized that male insects of many species donate nutrients to their females at mating and the females can use these nutrients for somatic maintenance and egg production. **Gwynne (1988)** found that increasing male nutrient donations in *Requena verticalis* produced an increase in egg number and size when females were maintained on either a low or a high protein diet. He suggested that males donate some special protein not available to females from any other source. In *G. bimaculatus* the spermatophore is small and probably of no nutritive value for the females. **Osborne et al. (1968)** noticed that in adult *S. gregaria* females protein synthesis was low soon after the moult, but rapidly increased to a peak 1 or 2 days later.

In insects during the life cycle many changes in physiological and behavioural characteristics occur that can involve food consumption, body weight, fat body, haemolymph, ovarian proteins, chemical stimuli released etc. The present study underlines that the significantly reduced fat body weight glycogen and free carbohydrate content in the fat body of females reared with adult males could indicate a higher investment into reproduction (Fig. 17a, b). In *G. bimaculatus* from day 0 to day 4 about 50 % of the wet weight of fat body is formed of lipid compared to less than 1 % of glycogen and free carbohydrate **(Lorenz and Anand, 2004)**. Our experimental crickets are kept under crowded conditions at relatively high population densities and the presence of males in laboratory insect cultures increases e.g. walking activity, running, jumping and increasing stress brought about by the constantly courting males. Besides locomotion, events such as larval development, moulting and reproduction require huge amounts of energetic substrates that have to be mobilised from the fat body stores **(Lorenz and Anand, 2004; Anand and Lorenz, 2008)**.

The haemolymph lipid (Fig. 18a) and carbohydrate concentration (Fig. 18b) were slightly, but not significantly, lower in females reared with males. Haemolymph lipid content and composition vary with the physiological state of the animals. The present results suggest that it may be due to the high stress level (locomotor activity, excitability, effect of the light or darkness, sexual behaviour, etc). Insect haemolymph composition is known to change in response to age, feeding, temperature and other factors **(Woodring et al., 1977, 1978)**. Compared with the other experiments, the groups kept with many additional animals with or without males on Mod. Standard diet (high carbohydrate, 42%) had the highest average carbohydrate levels in the haemolymph (Fig. 55). Our results suggest a significant trend in responses to carbohydrate in the diet and rearing conditions. **Clifford and Woodring (1986)** showed that adult male and female crickets display circadian rhythms of feeding, drinking,

locomotor activity, and oxygen consumption that typically peak during the first half of the scotophase. In *Locusta migratoria* the amount of carbohydrate in the haemolymph is greater than that stored in other tissues **(Goldsworthy, 1969)**.

However, to understand the significance of crowded conditions and group effects in *G. bimaculatus* in relation to the presence or absence of adult males, more laboratory and field investigations are required.

4.3. Experiment III

This experiment is quite similar to Exp. I (same rearing conditions, isolated vs. crowded, start of the experiment at day 0 adult); the only difference is the type of diet used (see table 1, diet formulations). Our results demonstrate for 5-day-old female crickets which were reared on the same diet, isolated or crowded conditions had no effect on pronotum width (Fig. 19a). Comparison among pronotum widths (Exp. I, II, III and IV) of 5-day-old adult female crickets reared under isolated or crowded conditions is illustrated in Figs. 34 and 35. From the data obtained it can be deduced that pronotum widths display approximately similar averages within experiment III and significant differences between Ctrls of Exp. I and Exp. III under isolated conditions. Similar results were observed under crowded conditions, with higher values in animals fed on diet A, B and C (Exp. III). Unfortunately, little is known about the influences of rearing conditions and diets on pronotum width of *G. bimaculatus* and further experiments should focus on these potential effects.

In the present study (Fig. 19b) significant differences in body mass gain between isolated and crowded animals were found, with higher values for females reared under crowded conditions. Similarly, the ovaries of crowded females were larger than those of isolated females (Fig. 21b). These data show that group effects played an important role in increase in body and ovarian mass.

Similar to the present study **Lorenz (2007)** found that the body mass increase in females was due mainly to the fast ovarian development.

The food intake (Fig. 20) tended to be very slightly lower in crowded animals. On the other hand, there was a significantly lower mass of flight muscles (Fig. 21a) that was accompanied by an increase in ovary mass (Fig. 21b) and a decrease in fat body mass (Fig. 21c) in animals reared under crowded conditions. Animals raised under different rearing conditions and diets do not grow at equal rates and differ in development periods.

Considering the diet A this was the most similar to Ctrl diet (higher protein but quite similar starch and lipid content). Although the diet A may be less tasty for the crickets, we did

not find any adverse effect due to this possibility. In this experiment the amount of food intake differed according to the diet. Diet C reduced the amount of food consumed in both groups (Fig. 20), likely because it contained higher caloric equivalents (19.73 kJ/g), due to the higher lipid content than the others diets (diet A and B contain 12.75 kJ/g and 13.55 kJ/g, respectively).

Animals generally require a dietary supply of various nutrients, because their biosynthetic capabilities are limited. The amount of caloric equivalents consumed over a period of 5 days under isolated and crowded conditions, was 8.87 and 8.27 kJ for diet A, 9.39 and 8.93 kJ for diet B and 9.44 and 9.30 kJ for diet C. This is probably due to the fact that females compensate the high dietary lipid level reducing the amount of food consumed, thereby keeping intake of caloric equivalents relatively constant.

Actually, digestion of high amounts of fats is quite complex for the crickets. It is known that the approximate digestibility depends on the diet (**Hoekstra and Beenakkers 1976**). The digestion of fat in insects still remains poorly understood **(Turunen and Chippendale, 1989; Arrese et al., 2001)**. In *G. bimaculatus* a low lipase level under starved and fed conditions in all parts of the gut was found (**Woodring et al., 2007**). The lipase and amylase ratio can indicate the adaption of an insect to its diet. Lipases cause the breakdown of lipid (triacylgycerol) and therefore, lead to increased titers of lipids (diacylgycerol) in the haemolymph (**Lorenz and Gäde, 2009**). *G. bimaculatus* appears to secrete digestive enzymes continuously, and a considerable loss of enzymes may occur at certain times through egestion (**Woodring et al., 2007**). **Thomas and Nation (1984)** found that the enzyme activity measurement and absorption indicate that the hindgut in gryllids and in mole crickets is a major site for digestion and absorption of digestion products. Also, **Thomas and Nation (1984)** showed that the midgut is the major source of lipase, amylase and protease in *Gryllus* and *Scapteriscus*.

Our results show that the total amount of protein consumed over the feeding period (0-5 days after adult emergence) under isolated and crowded conditions was higher for animals fed on diet A (3.34 kJ, 3.11 kJ) and B (3.32 kJ, 3.16 kJ), while lower amounts were consumed by animals fed on diet C (2.29 kJ, 2.26 kJ), even though all diets had the same protein content (30%). This reduced amount of consumed protein should have some consequences (e.g. lower flight muscle mass, lower egg production). It is known that egg production is dependent on the availability of proteins and lipids contained in the diet.

Diet C was consumed significantly less in both isolated and crowded females and, therefore, these animals consumed less protein. Interestingly, we did not find smaller DLM in those crickets fed on diet C. This is an important point because it demonstrates that DLM

mass (Fig. 21a) is not significantly affected by the diets A, B and C. In *Acheta*, almost all of somatic and ovarian growth occurs during larval and adult stage respectively **(Woodring et al., 1979)**. The DLM in some individuals degenerates shortly after adult emergence **(Gomi et al., 1995)**. In another study some individuals retained the muscles without undergoing degeneration for up two weeks **(Shiga et al., 1991)**. **Gomi et al. (1995)** argue this difference between the two studies may be due to differences in cricket strains or rearing conditions. Physiological mechanisms regulating flight muscle histolysis in crickets does not require synthesis of any new protein in the muscle **(Gomi et al., 1995)**.

Under crowded conditions the ovary mass was significantly higher than under isolated ones (Fig. 21b). The data obtained provide a strong negative relationship between flight muscle mass (Fig. 21a) and ovary mass: females that have large ovarian mass have a low flight muscle mass whereas females that have small ovarian mass have the highest value in flight muscle mass. It is known that the survival, growth, and egg production are often responsive to variation in the quality of the diet (**Joern and Behmer, 1997**). Our data are in line with those of **Abdel Rahman (2001)** who found that the influence of different diets on egg production might be attributed to the different nutritional efficiencies of these diets.

Insects preferentially preserve lipids as their energy reserve in the fat body. This source of energy is mobilized to fuel vitellogenesis (**Lorenz, 2003; Lorenz and Anand, 2004**). Lipid reserves are the most important source of energy used by insects during metamorphosis **(Downer, 1985)**.

Under isolated as well as under crowded conditions crickets which fed on diet C (recognized as a diet which has a high fat, low starch, and contained a higher caloric value than the other diets), had the highest average fat body mass (Fig. 42, 43).

The total quantity of lipid, protein, glycogen and free carbohydrate in the fat body is shown in Fig. 22 and 23. It is clear that there was no consistent effect of the three different diets under both rearing conditions. **Woodring and Lorenz (2007)** stated that lipids, the major component of the fat body, protein and glycogen also increase dramatically during the first 2 days of adult life. **Anand (2004)** found that during the initial phase of the larval instar, lipogenic activity is very high, which is followed by the peak of lipid and protein content of the fat body. As illustrated in Fig. 44-48 under both isolated and crowded conditions, not only the lipid but also the glycogen content in the fat body of 5-day-old adult female crickets was relatively high in individuals fed on diet C in Exp. III.

Lipid concentration in deposits into the haemolymph showed similar pattern between the isolated and crowded females (Fig. 24a). In addition, under isolated conditions, data illustrated in Fig. 52 show that haemolymph lipid concentration of 5-day-old adult female

crickets reared under isolated condition were in descending order: individuals fed on diet C (Exp. III) had the highest values, while the isolated individuals fed on standard diet had the lowest average. **Lorenz et al. (2004)** found that the titre of lipid in the haemolymph was relatively low 2 h after lights-on, increased significantly 2 h after lights-off, and decreased to the basal level during the next photophase.

On the other hand, influence of diets upon haemolymph carbohydrate concentration was significantly different for females reared under isolated and crowded conditions (Fig. 24b). The haemolymph carbohydrate concentrations of 5-day-old adult female crickets reared under isolated conditions (Fig. 54) were in descending order: individuals which were fed on diet C (Exp. III) had the highest average, followed by individuals which were fed on diet A, then individuals fed on Ctrl diet in (Exp. I), while the isolated individuals fed on diet B had the lowest value. There were significant differences in Exp. III.

Generally, these data agree well with those of **Hansen (1964)** who found that the carbohydrate composition of the haemolymph is greatly influenced by the diet. As reported in **Wang and Patton (1969a)** the quantity and quality of blood sugar can be as well influenced by the diet. Our results suggest some interesting interactions between diet composition, feeding, reproduction and some other biological aspects in the cricket *G. bimaculatus*.

4.4. Experiment IV

This experiment is different from the other three previous experiments, since three different diets were dispend (long term) from d 0 of the penultimate larval stage onwards; animals were reared under crowded conditions only. The population density was about 440 crickets/m^2.

This study demonstrated that the highest increase in body mass takes place during the PL stage with respect to the increase in body mass during PL and LL stage (Fig. 25). There were no obvious differences between diet A and diet C, but during the adult stage, animals fed on diet B and C gained significantly less weight than animals reared on diet A. Irrespective of the developmental stage, diet B enabled the lowest increase in body mass. On the other hand, it has been observed that the poor effect of diet B on body mass gain is not only observed in PL stage, but also in LL stage and Ad stage. Considering the developmental period and the percentage reaching adult stage, diets A and C are the most suitable diet to rear larvae. This result suggest that larvae play an important role in providing nutritional feedback to adults. However, as indicated in Fig. 33, the crickets that were reared under crowded conditions and fed on three different types of diets from the penultimate larval to adult stage (Exp. IV)

showed a lower increase in body mass compared with those reared under crowded or even isolated conditions during adult stage only. One explanation for this could be that individuals in Exp. IV reared in small groups of 12 on various artificial diets for a long period of time starting from 0-day-old penultimate larvae until they reached adulthood 5-day-old (produce small adults). In *Acheta*, almost all of somatic and ovarian growth occurs during larval and adult stage**,** respectively **(Woodring et al., 1979)**. Larvae reared on an artificial diet showed the group effect not only in the first generation **(McFarlane et al., 1984)** but also in the second, third and fourth generations.

Our finding in Fig. 26 shows that the different diet consumptions can thus be arranged in the following descending order of preference, PL stage (diet B > diet A > diet C), LL stage (diet A > diet B > diet C), and Ad stage (diet A > diet B > diet C). During the PL stage the results presented here suggest that larvae can easily benefit and better masticate diet B than other diets because it is more soft. On the other hand, mouthparts appear weakly sclerotised during larval stage. Our result from previous experiments (Exp. I, Exp. III) and this Experiment show little or no correlations between increase in body mass and different diets consumption on *G. bimaculatus*. This may be affected by behavioural, reproductive, rearing and physiological conditions.

As indicated in Fig. 27 there was a significant difference among pronotum width of 5-day-old adult female crickets. As a matter of fact, the adult feeding or rearing method has effects on the insect body as well as the pronotum width. However, in this experiment where the crickets were fed on different diets from the penultimate larval stage onwards, diets A, B and C affected the growth of the animals, including pronotum width. Therefore, the pronotum width varied from one moult to another and increased in width. These results are in general harmony with those of **Ibler et al. (2009)** who noticed that body length, length of the antenna and pronotum width of *G. bimaculatus* varies gradually from moult to moult. The contradicting finding of **Zajitschek al. (2009)** shows that in the cricket *Teleogryllus commodus* no significant effect of larval diet on developmental time and pronotum width for either sex was observed. The differences between the results of the present study and that of **Zajitschek al. (2009)** are probably caused by the variation in population density, environmental factors and diet composition.

Diet B led to a significantly lower increase in body mass, pronotum width, DLM mass, ovary mass and fat body mass (see Fig. 27, 28). This may be explained by the fact that diet B contains more complex carbohydrates (50%) and less lipids (2%) than the other diets,

determining (i) the negative effect upon the growth and development of crickets (ii) the suppression of physiological and metabolic functions (iii) the inability of digestive enzymes to cope with a high degree of complex carbohydrate and (iv) the obvious lack of lipids in diet B. Carbohydrates are no essential nutrients because insects can convert lipids and amino acids to carbohydrates via gluconeogenesis **(Behmer, 2006)**.

The amount of calories consumed by PL, LL and Ad under various food regimes were diet A (2.85 kJ, 8.72 kJ, 9.85 kJ), diet B (3.35 kJ, 8.55 kJ, 6.22 kJ) and diet C (4.04 kJ, 7.63 kJ, 6.76 kJ) respectively. The total amount of carbohydrates consumed over the course of the feeding period (PL, LL and Ad) were diet A (1.35 kJ, 4.16 kJ, 4.69 kJ), diet B (1.98 kJ, 5.04 kJ, 3.67 kJ) and diet C (0.75 kJ, 1.42 kJ, 1.26 kJ), respectively. It was highest in individuals fed on diet B in PL, LL and in those fed on diet A during the adult stage. The amount of food consumed from 0 to 5-day-old adult female crickets reared under isolated or crowded conditions and fed on different diets is illustrated in Figs. 38 and 39. Diet C (Exp. III and IV) which was formulated by incorporating 30% protein, 30% fat, 23% carbohydrate (a "high fat and low starch diet") reduced the amount of food consumed in both groups, because it contained more caloric equivalents than the others diets. During this experiment, the treatments were started from the penultimate larval instar. This and the following larval instar tended to build up huge energy stores, which are then used during adult stage; so any nutrient factor affects its growth.

The present results are supportive of the findings of **Merkel (1977)**, who clearly demonstrated that cricket larvae when reared under optimal conditions, store huge amounts of lipids and proteins, but low amount of polysaccharides in their fat body. The present results are also in line with those of **Hahn (2005)**, who stated that proteins, lipids and carbohydrates are carried over from larval feeding into adulthood.

Generally, carbohydrates are depleted during metamorphosis due to energy required for metabolism and metabolic interconversions in insects **(Tate and Wimer, 1974, Chippendale, 1978)**. **Woodring et al. (1979)** found that some carbohydrate was used for lipid synthesis and most was used for energy production but very little was used for growth. The same authors found that when 5% dietary lipid is fed to both larvae and adults, the animals had to synthesize additional lipid from carbohydrates to supplement absorbed lipids for growth demand. **Simpson et al. (2002)** showed that lipid deposition is positively correlated with the amount of lipid or carbohydrate in the diet. **Özalp and Emre (2001)** and **Yanikoğlu (1982)** found that starch and glycogen caused a significant decrease in the life span of *Pimpla*

turionellae adult females. Although the insect utilized these polysaccharides in the larval stage, the effect observed in the mature stage shows that some changes occur in the metabolic functions that are dependent upon the nutritional regime. Diets containing 70% of carbohydrate assure optimal growth in the flour beetle *Tenebrio*. When carbohydrate concentration is less than 40% *Tenebrio* fails to develop **(Chapman, 1971).** A study on *Schistocerca gregaria* demonstrates that when diet is very poor in digestible carbohydrates the insects grow very slowly and fail to complete their development. On the other hand, insect growth and development is normal and has a good rate when the carbohydrate content is 13%-26%. However, normal development and slow growth are observed when carbohydrate content is 39% of the diet (**Dadd, 1960a, b and c, 1961**).

In the present study, diets B and C caused a significantly reduced gain in body and ovary mass in adult 5-day-old females when compared with animals fed on the same diets used in Exp. III. This observation suggests that: (i) while adults in Exp. III reared on diet B and C for five days only did not show a significantly reduced gain in body and ovary mass compared with the Ctrl diet. The larvae and adults in Exp. IV fed on the same diets for a long period (18 - 20 days), obviously had problems to cope with diet B and C which have a drastically altered lipid and carbohydrate content (table 1); (ii) varying the composition of the diet could also affect its texture, or stickiness, leading to differences in dietary intake; (iii) due to feeding on suboptimal artificial diet for long periods, the animals appear to slow down the growth rate compared with natural diets and (iv) body weight and ovary weight was significantly affected by the protein, lipid and carbohydrate content in the diet, diet availability and diet quality and quantity. The quality of diet eaten by larval insects will affect traits such as gamete production, fat reserves, muscle bulk and body size in the adult (**Ryne et al., 2004**).

Additionally, flight muscle and fat body mass (Fig. 37, 43) were significantly higher in adult 5-day-old females fed on diets A, B and C in Exp. IV than in females fed on the same diet in Exp. III, except diet B in Exp. IV, which caused a significantly lower value in fat body mass because diet B contains less fats and, therefore, has a negative impact on lipid stores. Considering that the more food larvae ate the more weight they gained, the quality and quantity of food uptake can influence flight muscle mass. However, a correlation between an increase in flight muscle and fat body mass and a decrease in ovary mass was obvious. These results were expected and provide strong support for the inverse relationship between flight muscle (DLM) and ovary mass. The weight of flight muscle in day-5-old is relatively very

low compared to day 1 or 2; this decrease is due to flight muscle histolysis (**Shiga et al., 1991; Lorenz, 2007**).

As found in this study there are some interesting interactions between diet composition and flight muscle mass (A > C > B), ovary mass (A > C > B), and fat body mass (C > A > B) (see Fig. 28). Our result suggest significant trends in response to lipid, protein and carbohydrate in the diet. Diet B may provide much less favourable substrates for development of *G. bimaculatus* than diet A and C. This might be due to the negative physiological effects of diet B. On the other hand, the present result for *G. bimaculatus* 5-day-old adult females fed on diet B (Exp. IV) demonstrate that increase in body mass (28.78 ± 2.59 %) and ovary mass (65.30 ± 4.49 mg) was much lower than in individuals which were fed on Ctrl diet (Exp. I), individuals fed on diet A, B and C in Exp. III and individuals fed diet A and C in Exp. IV (Fig. 33, 41).

The present study found that larvae and adults of *G. bimaculatus* responded to an increase of lipid content in the diet with a raised lipid concentration in the fat body. Dietary lipid may improve growth by improving the palatability of the diet and may provide convenient lipid stores that must otherwise be synthesized.

In the present study, under isolated (Exp. III) and crowded conditions (Experiment III and IV) crickets which were fed on diet C had the highest average fat body mass. This might be due to the positive physiological effects of a high fat diet and possibility that diet C provided more favourable substrates for the development of *G. bimaculatus* than the other diets (Fig. 42, 43). In general, these data agree well with those of **Beenakkers et al. (1985a)** who found that the amount and the composition of lipid in an insect vary considerably according to developmental stage and considered tissues. Moreover, the lipid content is influenced by several factors: nutrition, starvation, sex and hormones. **Anand and Lorenz (2008)** mentioned that crickets feed more voraciously during the first half of the last larval stage. With the onset of feeding, fat body lipid synthesis increases, leading to increasing lipid stores in the fat body reaching the maximum on day 5. Generally, in insects lipid content increases from early to late larval instar **(Slansky and Scriber, 1985)**.

The levels of lipid and protein in the fat body tended to be higher in animals fed on diets B and C, whereas no clear trend could be observed for the glycogen and free carbohydrate fat body content (Fig. 29, 30). Under crowded conditions, as shown in Fig. 45, the average lipid content in the fat body of 5-day-old adult females was highest in animals fed on diet C. Isolated crickets fed on diet C (Fig. 44) contained the maximal amount of lipid compared with

females fed on the same diet under crowded conditions and on other diets under both rearing conditions. The present results suggest that variation in the fat body lipid content depends on the availability of fat in the diet as well as on the rearing conditions. **Downer and Matthews (1976)** stated that during insect evolution lipids have assumed a significant role in facilitating several important morphogenetic and physiological strategies. The study conducted by **Hahn (2005)** suggested that larval derived lipid stores are more important to adult fitness than those of carbohydrates or proteins.

The results show that females fed on Ctrl diet under isolated conditions (Fig. 46) and on diet C (Exp. IV) under crowded conditions (Fig. 47) had the highest amount of protein in the fat body, whereas the same samples had significantly smaller ovaries (Fig. 40, 41). With regard to animals fed on diet A and reared under isolated or crowded conditions an opposite trend could be verified: the protein content of the fat body was lower whereas the ovary mass was higher (Fig. 40, 41). Our results suggest an inverse relationship between fat body protein content and ovary mass probably due to transfer of materials during oocyte development, and other data suggest these differences in the development and reproduction are influenced by several factors including diet. **Chinzei et al. (1981)** found that lipid, protein and carbohydrate are transported from fat body to eggs during oogenesis. **Lorenz and Anand (2004)** and **Anand (2004)** found that a large amount of lipid is synthesized and stored by the cricket during the development: the highest lipid content of the PL fat body is about 10 mg and then increases to about 50 mg in the adult stage. A similar trend can be observed in the protein content which increases about three fold (from about 2 mg to about 6 mg). In contrast, the highest glycogen content of the larval fat body is about 2 mg and decreases to about half a milligram in the adult stage. The free carbohydrate content of the fat body is minute during both the stages (less than half a milligram) and hence does not seem to have significant role in the energy storage. This indicates that there is a major shift in the physiology, where carbohydrates seem to have a more important role during the larval stage than in the adult stage.

The concentration of total lipid in the haemolymph is high in 5-day-old adult crickets fed on diet C as compared with diets A and B (Fig. 31a). Therefore, it seems that variation in the haemolymph lipid level is a response of the insect to the lipid content in diet. The haemolymph lipid concentration of 5-day-old adult female crickets reared under isolated and crowded conditions is compared in Fig. 52 and 53. Isolated and crowded crickets fed on diet C had the highest level of haemolymph lipids. There is a strong correlation between the diet C (high fat content) and the highest haemolymph lipid levels.

Crowded conditions lead to increased physical activity in adult females. This is accompanied by higher titres of lipids in the haemolumph **(Lorenz and Gäde, 2009)**. Our findings show a significant increase in total haemolymph carbohydrate (Fig. 31b) in animals fed on diet B that could be explained by the higher level of carbohydrate in diet B (50%). The stored lipid is subsequently utilized during adult life together with the lipid and carbohydrate ingested from the diet **(Walker et al., 1970)**.

4.5. Outlook

There are only few reports on the dietary needs of the two-spotted cricket *G. bimaculatus*. Well-documented and systematic studies were only conducted in other insects such as locusts. Based on this fact, the current study is of great significance for further researches associated with physiological ecology such as nutritional effects on *G. bimaculatus*. The scope of the present study was to understand the effect of different diets on larvae and adults under different laboratory conditions. Intensive measurements and comparisons were performed in order to shed some light on the impact of diet and rearing conditions on developmental and reproductive rate. In our study important observations extracted include: *G. bimaculatus* can serve as a valuable animal model in physiological experiments (e.g. diet composition, diet consumption, group effect and fat body reserves). Data obtained from this study can serve as basis offering a great support for many of the relevant future studies in this area. Therefore future research on two-spotted cricket should focus more intensively on effects of rearing conditions, presence or absence of males, diet and other biological aspects in this species and other species **(Lorenz and Anand, 2004; Lorenz and Gäde, 2009)**.

5. SUMMARY

- The effect of rearing conditions and different diets on larvae and adults of the two-spotted cricket, *Gryllus bimaculatus* de Geer (Orthoptera: Gryllidae), was studied.
- The percent increase in body and ovary mass of adult females reared under crowded conditions was higher than that of those reared under isolated conditions.
- Higher population density increased reproductive investment in adult females, regardless of the presence or absence of adult males.
- The presence or absence of adult males appeared to have no significant effect on the pronotum width, increase in body mass, DLM mass and ovary mass of adult females.
- Not only the population density plays an important role in morphological, physiological and behavioural changes, but also the absolute number of animals in a population may influence the above-mentioned parameters.
- A strong positive correlation was found between increase in body and ovary mass and decrease in flight muscle and fat body mass; the loss of flight muscle and fat body mass is due to a high egg production.
- Five different diets were used: standard diet (ca. 20 % protein, 4.5 % lipid, 45.5 % carbohydrate), modified standard diet (ca. 22 % protein, 6 % lipid, 42 % carbohydrate), and three semi-artificial diets, all containing 30 % protein but differing amounts of lipid (A: normal, B: low, C: high).
- The amount of diet consumed highly depends on the type of diet given.
- Weak relationships were obtained between increase in body mass and different amounts of diets consumed.
- The amount of the different storage products in the fat body of 5-day-old adult females can be arranged in the following order: lipid > protein > glycogen > free carbohydrate.
- The total lipid concentration in the haemolymph was generally much higher than carbohydrate concentration.
- When fed on a high calory diet, the amount of food consumed was reduced.

- When crickets were fed on artificial diets from penultimate larval stage, they displayed a lower increase in body mass and some reduction in flight muscle and ovary mass compared with crickets fed on artificial diet from the day of the adult moult only.
- With respect to body and ovary mass, when fed to adult females modified standard diet was heartier, followed by diet A > B > C > standard diet.
- When fed to penultimate larvae onwards, however, diets B and C resulted in a significantly reduced body and ovary mass. This indicates that when diets too low or too high in lipid content are fed for a longer period, cricket cannot compensate for it.
- The development and reproductive investment of *Gryllus bimaculatus* shows a high degree of dependence on diet availability, diet quality and quantity, and food intake. Therefore, crickets raised on the different diets did not grow at equal rates.
- The results unambiguously show that population density dramatically alters cricket behavioural, morphological and physiological characteristics; as a matter of fact there was a strong correlation between crowded conditions and an increase in metabolic rate, which in turn had an influence on the effects of different diets.

6. Zusammenfassung

- Die Auswirkungen von Haltungsbedingungen und verschiedenen Diäten auf Larven und Adulte der Mittelmeerfeldgrille, *Gryllus bimaculatus* de Geer (Ensifera, Gryllidae), wurden untersucht.
- Die prozentuale Zunahme des Körper- und Ovargewichts adulter Weibchen war höher in Tieren, die in Gruppen gehalten wurden, als in Tieren in Einzelhaltung.
- Eine höhere Populationsdichte erhöhte die reproduktive Investition adulter Weibchen, unabhängig von der Anwesenheit bzw. Abwesenheit adulter Männchen.
- Das Vorhandensein oder Fehlen adulter Männchen hatte keine Auswirkung auf die Pronotumbreite und das Körper-, Flugmuskel- und Ovargewicht der adulten Weibchen.
- Nicht nur die Populationsdichte sondern auch die absolute Anzahl an Tieren beeinflusste die Veränderung von morphologischen, physiologischen und ethologischen Parametern.
- Zwischen der Zunahme der Körper- und Ovarmasse und der Abnahme der Flugmuskel- und Fettkörpermasse bestand eine deutliche Korrelation; die Abnahme der Flugmuskel- und Fettkörpermasse ist auf die erhöhte Eiproduktion zurückzuführen.
- Fünf verschiedene Diäten wurden verfüttert: Standarddiät (20 % Protein, 4.5 % Lipid, 45.5 % Kohlenhydrat) modifizierte Standarddiät (22 % Protein, 6 % Lipid, 42 % Kohlenhydrat) sowie drei semiartifizielle Diäten mit einem Proteingehalt von jeweils 30 % und unterschiedlichen Lipidgehalten (A: Normal, B: Niedrig, C: Hoch).
- Die Höhe des Nahrungskonsums hing stark von der Art der verfütterten Diät ab.
- Zwischen der Körpergewichtszunahme und der Art der verfütterten Diät bestand nur eine schwache Korrelation.
- Die Menge der verschiedenen Speicherstoffe im Fettkörper 5 Tage alter adulter Weibchen betrug Lipid > Protein > Glykogen > freie Kohlenhydrate.
- Die Lipidkonzentration in der Hämolymphe war generell höher, als die Kohlenhydratkonzentration.
- Bei Fütterung einer Diät mit hohem Kaloriengehalt reduzierten die Tiere ihre Nahrungsaufnahme.

- Wenn künstliche Diäten ab dem vorletzten Larvenstadium verfüttert wurden, war die Körpergewichtszunahme deutlich reduziert und das Flugmuskel- und Ovargewicht etwas niedriger als bei Tieren, die künstliche Diäten erst ab der Adulthäutung bekamen.
- Bezüglich der Körper- und Ovarmasse wurden mit der modifizierten Standarddiät die besten Resultate erzielt, gefolgt von Diät A > B > C > Standard diät.
- Die Fütterung der Diäten B und C ab dem vorletzten Larvenstadium führte jedoch zu einer signifikant reduzierten Körper- und Ovarmasse der resultierenden adulten Weibchen. Dies zeigt, dass Grillen einen zu niedrigen oder zu hohen Lipidgehalt der Nahrung nicht kompensieren können.
- Die Larvalentwicklung und die reproduktive Investition von *Gryllus bimaculatus* hängt in hohem Maß von der Verfügbarkeit, Art und Menge der Diät sowie von der Nahrungsaufnahme ab, weswegen die Grillen nicht auf allen Diäten im gleichen Maße wuchsen.
- Die Ergebnisse zeigen eindeutig, dass die Populationsdichte das Verhalten sowie morphologische und physiologische Parameter deutlich beeinflusst; es bestand eine deutliche Korrelation zwischen erhöhter Populationsdichte und einem Anstieg des Metabolismus, der wiederum die Auswirkungen verschiedener Diäten beeinflusste.

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8- Supplementary

Table 2: Means ± S.E. of some biological aspects of *G. bimaculatus* 5-day-old adult females reared under isolated or crowded conditions. Mean values were compared by t- test and Mann-Whitney U-test.

Biological aspects	Isolated conditions			Crowded conditions			Tests of Statistical Analyses and Probability			
	N	Mean	S.E.	N	Mean	S.E.	T T	P	MWRST	P
% increase in body mass from d 0 – d 5	192	47.93	1.08	164	57.06	2.06	-	-	**	P < 0.01
Pronotum width	192	7.607	0.03	164	7.618	0.03	n.s.	P = 0.764	-	-
Diet consumption mg from d 0 – d 5	192	698.4	11.56	15	743.5	20.03	n.s.	P = 0.281	-	-
Ovary fresh mass [mg]	192	196.72	6.35	164	212.28	7.79	-	-	n.s.	P = 0.167
Fat body mass [mg]	192	83.43	3.03	164	93.28	3.86	-	-	n.s.	P = 0.161
Total Lipid (A) mg / whole fat body	192	31.43	1.03	164	26.98	1.10	-	-	**	P < 0.01
Lipid (B)[µg / mg fat body]	192	421.09	12.46	164	321.84	11.62	-	-	***	P < 0.001
Total Protein (A) mg / whole fat body	192	5.50	0.19	164	5.95	0.23	-	-	n.s.	P = 0.193
Protein (B) [µg / mg fat body]	192	69.48	1.48	164	66.70	1.27	-	-	n.s.	P = 0.246
Total Glycogen (A) mg/whole fat body	192	0.30	0.02	164	0.37	0.04	-	-	n.s.	P = 0.420
Glycogen (B) [µg / mg fat body]	192	3.63	0.14	164	3.73	0.21	-	-	n.s.	P = 0.503
Total Free carbohydrate (A) mg/whole fat body	192	0.21	0.01	164	0.20	0.01	-	-	n.s.	P = 0.054
Free carbohydrate [µg / mg fat body]	192	2.79	0.09	164	2.43	0.11	-	-	**	P = 0.002
Haemolymph lipid concentration [µg / µl]	192	16.40	0.49	164	15.25	0.63	-	-	**	P <0. 01
Haemolymph carbohydrate concentration [µg / µl]	192	6.84	0.23	164	7.89	0.40	-	-	n.s.	P = 0.296

N, number of determinations; S.E., Standard Error; TT, t-Test; P, Probability; n.s., not significant; **, p < 0.01; ***, p < 0.001; MWRST, Mann-Whitney U- Test. -, not determined.

Table 3: Means ± S.E of some biological aspects of *G. bimaculatus* 5-day-old adult females reared under crowded conditions with or without adult males. Mean values were compared by t- test and Mann-Whitney U-test.

Biological aspects	Crowded female with males			Crowded female without males			Tests of Statistical Analyses and Probability			
	N	Mean	S.E.	N	Mean	S.E.	T T	P	MWRST	P
% increase in body mass from d 0 – d 5	57	72.64	2.32	50	72.54	2.01	-	-	n.s.	P = 0.998
Pronotum width	57	7.70	0.05	50	7.65	0.05	n.s.	P = 0.430	-	-
Flight muscle (DLM) mass [mg]	57	9.71	0.94	50	9.71	1.10	-	-	n.s.	P = 0.620
Ovary fresh mass [mg]	57	342.38	14.80	50	342.29	13.85	n.s.	P = 0.996	-	-
Fat body mass [mg]	57	69.64	4.58	50	79.53	3.86	-	-	*	P = 0.021
Total Lipid (A) mg / whole fat body	57	21.84	1.41	50	31.79	2.22	-	-	***	P < 0.001
Lipid (B)[µg / mg fat body]	57	330.34	15.96	50	392.82	17.78	*	P = 0.010	-	-
Total Protein (A) mg / whole fat body	57	5.81	0.39	50	5.56	0.33	-	-	n.s.	P = 0.757
Protein (B) [µg / mg fat body]	54	80.32	3.56	50	72.89	3.90	-	-	*	P = 0.048
Total Glycogen (A) mg/whole fat body	57	0.16	0.015	50	0.18	0.01	-	-	*	P = 0.036
Glycogen (B) [µg / mg fat body]	57	2.36	0.18	50	2.23	0.13	-	-	n.s.	P = 0.396
Total Free carbohydrate (A) mg/whole fat body	57	0.20	0.01	50	0.24	0.01	-	-	*	P = 0.018
Free carbohydrate [µg / mg fat body]	57	3.01	0.18	50	3.17	0.13	-	-	n.s.	P = 0.172
Haemolymph lipid concentration [µg / µl]	57	16.61	0.93	50	17.52	0.93	-	-	n.s.	P = 0.209
Haemolymph carbohydrate concentration [µg / µl]	57	8.00	0.40	50	9.18	0.49	n.s.	P = 0.063	-	-

N, number of determinations; S.E., Standard Error; TT, t-Test; P, Probability; n.s., not significant; *, p < 0.05; ***, p < 0.001; MWRST, Mann-Whitney U- Test. -, not determined.

Table 4: Means ± S.E. and analysis of variances of some biological aspects for 5-day-old adult females reared under isolated or crowded conditions and fed on three different diets.

Biological aspects	Type of diet	Means ± S.E. of some biological aspects of *G. bimaculatus* fed on three different diets under.							
		Isolated conditions				Crowded conditions			
		N	Mean ± S.E.	Probability		N	Mean ± S.E.	Probability	
% increase in body mass from d 0 – d 5	A	73	52.79 ± 1.57	n.s.	P = 0.086	73	64.51 ± 3.14	n.s.	P = 0.052
	B	74	50.02 ± 1.44			95	55.10 ± 2.52		
	C	69	47.96 ± 1.58			73	59.55 ± 2.65		
Pronotum width	A	73	7.88 ± 0.04	n.s.	-	73	7.83 ± 0.04	n.s.	-
	B	74	7.85 ± 0.04			95	7.87 ± 0.04		
	C	69	7.84 ± 0.05			73	7.89 ± 0.04		
Flight muscle (DLM) mass [mg]	A	73	16.23 ± 1.34	n.s.	-	73	11.20 ± 1.01	n.s.	-
	B	74	15.35 ± 1.48			95	10.09 ± 0.94		
	C	69	17.18 ± 1.27			73	8.86 ± 0.89		
Amount of diet consumed from d 0 – d 5	A	73	696.0 ± 16.76	***	P < 0.001	7	649.2 ± 36.70	***	P < 0.001
	B	74	693.3 ± 16.17			9	659.5 ± 18.04		
	C	69	478.6 ± 11.94			7	471.8 ± 22.33		
Ovary fresh mass [mg]	A	73	286.6 ± 10.57	***	P < 0.001	73	308.7 ± 11.13	n.s.	P = 0.087
	B	74	248.5 ± 8.24			95	293.9 ± 7.12		
	C	69	208.0 ± 8.42			73	280.6 ± 7.40		
Fat body mass [mg]	A	73	80.58 ± 4.56	***	P < 0.001	73	65.30 ± 3.63	***	P < 0.001
	B	74	81.87 ± 4.14			95	88.07 ± 3.31		
	C	69	106.1 ± 4.39			73	104.2 ± 3.93		
Total lipid (A) mg / whole fat body	A	73	27.42 ± 1.83	***	P < 0.001	73	25.33 ± 1.56	***	P < 0.001
	B	74	33.21 ± 2.23			95	32.55 ± 1.69		
	C	69	44.74 ± 2.51			73	37.33 ± 2.62		
Lipid (B) [µg / mg fat body]	A	73	366.7 ± 23.84	*	P = 0.017	73	416.2 ± 21.90	n.s.	P = 0.064
	B	74	417.6 ± 24.33			95	392.2 ± 19.83		
	C	69	441.3 ± 24.13			73	370.2 ± 23.69		
Total protein (A) mg / whole fat body	A	73	4.06 ± 0.19	**	P = 0.009	73	3.69 ± 0.19	***	P < 0.001
	B	74	4.62 ± 0.27			95	5.26 ± 0.30		
	C	69	5.03 ± 0.28			73	5.68 ± 0.27		
Protein (B) [µg / mg fat body]	A	73	54.64 ± 2.02	**	P = 0.003	73	59.32 ± 1.62	n.s.	P = 0.116
	B	74	59.57 ± 2.46			95	60.65 ± 2.20		
	C	69	49.03 ± 2.00			73	58.22 ± 3.09		
Total glycogen (A) mg / whole fat body	A	73	0.18 ± 0.01	***	P < 0.001	73	0.20 ± 0.02	***	P < 0.001
	B	74	0.24 ± 0.02			95	0.28 ± 0.05		
	C	69	0.36 ± 0.02			73	0.37 ± 0.03		
Glycogen (B) [µg / mg fat body]	A	73	2.50 ± 0.20	***	P < 0.001	73	3.17 ± 0.22	***	P < 0.001
	B	74	2.95 ± 0.18			95	2.76 ± 0.15		
	C	69	3.51 ± 0.20			73	3.58 ± 0.25		
Total free carbohydrate (A) mg / whole fat body	A	73	0.28 ± 0.02	n.s.	-	23	0.23 ± 0.02	n.s.	-
	B	74	0.27 ± 0.02			84	0.23 ± 0.01		
	C	69	0.29 ± 0.02			64	0.22 ± 0.01		
Free carbohydrate (B) [µg / mg fat body]	A	73	4.11 ± 0.37	**	P = 0.006	73	3.10 ± 0.25	***	P < 0.001
	B	74	3.65 ± 0.25			95	2.80 ± 0.12		
	C	69	2.89 ± 0.17			73	2.30 ± 0.16		
Haemolymph lipid concentration [µg / µl]	A	69	17.41 ± 0.85	***	P < 0.001	47	17.02 ± 1.15	**	P < 0.025
	B	55	16.63 ± 0.99			87	15.23 ± 0.71		
	C	66	22.40 ± 1.07			53	19.05 ± 1.38		
Haemolymph carbohydrate concentration [µg / µl]	A	73	8.08 ± 0.24	***	P < 0.001	66	7.16 ± 0.53	n.s.	P = 0.346
	B	74	6.59 ± 0.40			95	7.83 ± 0.37		
	C	69	8.69 ± 0.41			73	7.05 ± 0.39		

N, number of determinations; S.E., Standard Error; *, p < 0.05; **, p < 0.01; ***, p < 0.001; n.s., not significant; -, not determined.

Table 5: Means ± S.E. of some biological aspects of 5-day-old adult females reared under isolated or crowded conditions and fed on three different diets.

Biological aspects	Comparison between means ± S.E. of isolated and crowded fed on the three different diets.							
	Type of diet	Isolated conditions Vs. Crowded conditions				Test of statistical analysis		
		N	Mean ± S.E.	N	Mean ± S.E.	EVT	MWRST	Probability
% increase in body mass from d 0 – d 5	A	73	52.79 ± 1.57	73	64.51 ± 3.14	**	-	P < 0. 01
	B	74	50.02 ± 1.44	95	55.10 ± 2.52	n.s.	-	P = 0.106
	C	69	47.96 ± 1.58	73	59.55 ± 2.65	***	-	P < 0.001
Flight muscle (DLM) mass [mg]	A	73	16.23 ± 1.34	73	11.20 ± 1.01	-	**	P = 0.003
	B	74	15.35 ± 1.48	95	10.09 ± 0.94	-	*	P = 0.013
	C	69	17.18 ± 1.27	73	8.86 ± 0.89	-	***	P < 0.001
Ovary fresh mass [mg]	A	73	286.6 ± 10.57	73	308.7 ± 10.57	n.s.	-	P = 0.152
	B	74	248.5 ± 8.24	95	293.9 ± 7.12	***	-	P < 0.001
	C	69	208.0 ± 8.42	73	280.6 ± 7.40	***	-	P < 0.001
Fat body mass [mg]	A	73	80.58 ± 4.56	73	65.30 ± 3.63	-	**	P = 0.007
	B	74	81.87 ± 4.14	95	88.07 ± 3.31	-	n.s.	P = 0.196
	C	69	106.1 ± 4.39	73	104.2 ± 3.93	-	n.s.	P = 0.778
Lipid (B) [µg / mg fat body]	A	73	366.7 ± 23.84	73	416.2 ± 21.90	-	*	P = 0.018
	B	74	417.6 ± 24.33	95	392.2 ± 19.83	-	n.s.	P = 0.274
	C	69	441.3 ± 24.13	73	370.2 ± 23.69	-	*	P = 0.010
Protein (B) [µg / mg fat body]	A	73	54.64 ± 2.02	73	59.32 ± 1.62	-	*	P = 0.011
	B	74	59.57 ± 2.46	95	60.65 ± 2.20	-	n.s.	P = 0.644
	C	69	49.03 ± 2.00	73	58.22 ± 3.09	-	n.s.	P = 0.074
Total free carbohydrate (A) mg / whole fat body	A	73	0.28 ± 0.02	23	0.23 ± 0.02	-	n.s.	P = 0.178
	B	74	0.27 ± 0.02	84	0.23 ± 0.01	-	n.s.	P = 0.391
	C	69	0.29 ± 0.02	64	0.22 ± 0.01	-	*	P = 0.010
Free carbohydrate (B) [µg / mg fat body]	A	73	4.11 ± 0.37	23	3.10 ± 0.25	-	n.s.	P = 0.225
	B	74	3.65 ± 0.25	84	2.80 ± 0.13	-	*	P = 0.014
	C	69	2.89 ± 0.17	64	2.30 ± 0.16	-	**	P = 0.004
Haemolymph carbohydrate concentration [µg / µl]	A	73	8.08 ± 0.24	66	7.16 ± 0.53	n.s.	-	P = 0.102
	B	74	6.59 ± 0.40	95	7.83 ± 0.37	*	-	P = 0.027
	C	69	8.69 ± 0.41	73	7.05 ± 0.39	**	-	P = 0.004

N, number of determinations; EVT, Equal Variance Test; MWRST, Mann-Whitney U- Test; *, p < 0.05; **, p < 0.01; ***, p < 0.001; n.s., not significant; -, not determined.

Table 6: Means ± S.E. and analysis of variance of food consumption and increase in body weight from d 0 PL to d 0 LL to d 0 adult females reared under crowded conditions and fed on three different diets from d 0 PL onwards.

Biological aspects	Type of Diet	Means ± S.E. of some biological aspects of *G. bimaculatus* adult female fed on three different diets					
		Crowded conditions		Test of statistical analysis and probability			
		N	Mean ± S.E.	NT	EVT	KWA	P
% increase in body mass from 0 d PL – 0 d LL.	A	70	160.33 ± 5.87	Failed	-	***	P < 0.001
	B	68	122.68 ± 7.66				
	C	61	157.16 ± 6.75				
% increase in body mass from 0 d LL – 0 d Ad.	A	69	91.29 ± 4.06	Failed	-	**	P = 0.005
	B	57	70.95 ± 5.61				
	C	59	80.45 ± 5.42				
Amount of diet consumed by 0 d PL – 0 d LL.	A	6	223.58 ± 8.89	Failed	-	n.s.	P = 0.075
	B	6	247.50 ±10.83				
	C	6	205.00 ± 22.57				
Amount of diet consumed by 0 d LL – 0 d Ad.	A	6	684.44 ± 28.71	Passed	***	-	P < 0.001
	B	6	631.00 ± 57.51				
	C	6	387.00 ± 25.93				

N, number of determinations; NT, Normality Test; PL, Penultimate larvae; LL, Last larvae; EVT, Equal Variance Test; KWA, Kruskal-Wallis –ANOVA; - No analysis; **, p < 0.01; ***, p < 0.001; n.s., not significant.

Table 7: Means ± S.E. and analysis of variance of some biological aspects for 5-day-old adult females reared under crowded conditions and fed on three different diets from d 0 PL onwards.

Biological aspects	Type of diet	Means ± S.E. of some biological aspects of *G. bimaculatus* adult female fed on three different diets.					
		Crowded conditions		Test of statistical analysis and probability			
		N	Mean ± S.E.	NT	EVT	KWA	P
% increase in body mass from d 0 – d 5	A	66	45.75 ± 3.08	Failed	-	***	P < 0.001
	B	55	28.78 ± 2.59				
	C	58	30.10 ± 3.05				
Pronotum width	A	66	7.69 ± 0.04	Failed	-	***	P < 0.001
	B	55	7.12 ± 0.06				
	C	58	7.40 ± 0.05				
Flight muscle (DLM) mass [mg]	A	66	18.46 ± 1.42	Failed	-	***	P < 0.001
	B	55	10.76 ± 0.95				
	C	58	17.80 ± 1.43				
Amount of diet consumed from from d 0 – d 5	A	6	772.90 ± 62.59	Failed	-	***	P < 0.001
	B	6	459.75 ± 19.21				
	C	6	342.91 ± 19.38				
Ovary fresh mass [mg]	A	66	255.55 ± 10.42	Failed	-	***	P < 0.001
	B	55	65.30 ± 6.50				
	C	58	106.55 ± 7.09				
Fat body mass [mg]	A	65	83.33 ± 3.70	Failed	-	***	P < 0.001
	B	55	78.90 ± 6.00				
	C	58	124.27 ± 6.56				
Total lipid (A) mg / whole fat body	A	66	22.14 ± 1.57	Failed	-	***	P < 0.001
	B	55	23.60 ± 1.64				
	C	58	38.83 ± 2.29				
Lipid (B) [µg / mg fat body]	A	64	256.01 ± 13.12	Failed	-	**	P = 0.004
	B	54	332.83 ± 21.57				
	C	58	339.09 ± 19.60				
Total protein (A) mg / whole fat body	A	66	4.28 ± 0.23	Failed	-	***	P < 0.001
	B	50	4.58 ± 0.49				
	C	58	6.70 ± 0.48				
Protein (B) [µg / mg fat body]	A	65	50.69 ± 1.27	Failed	-	n.s.	P = 0.400
	B	50	58.46 ± 3.30				
	C	58	56.89 ± 4.15				
Total glycogen (A) mg / whole fat body	A	66	0.22 ± 0.03	Failed	-	***	P < 0.001
	B	55	0.23 ± 0.02				
	C	58	0.28 ± 0.02				
Glycogen (B) [µg / mg fat body]	A	65	2.54 ± 0.26	Failed	-	***	P < 0.001
	B	54	3.60 ± 0.41				
	C	58	2.33 ± 0.14				
Total free carbohydrate (A) mg / whole fat body	A	66	0.28 ± 0.02	Failed	-	n.s.	P = 0.114
	B	55	0.24 ± 0.02				
	C	58	0.23 ± 0.01				
Free carbohydrate (B) [µg / mg fat body]	A	65	3.28 ± 0.12	Failed	-	***	P < 0.001
	B	54	3.79 ± 0.38				
	C	58	1.95 ± 0.10				
Haemolymph lipid concentration [µg / µl]	A	66	14.60 ± 0.67	Failed	-	***	P < 0.001
	B	55	18.55 ± 0.72				
	C	58	21.56 ± 0.82				
Haemolymph carbohydrate concentration [µg / µl]	A	66	6.36 ± 0.17	Passed	***		P < 0.001
	B	55	8.09 ± 0.24				
	C	58	6.60 ± 0.19				

N, number of determinations; NT, Normality Test; EVT, Equal Variance Test; KWA, Kruskal-Wallis –ANOVA; - No analysis; **, p < 0.01; ***, p < 0.001; n.s., not significant.

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Hiermit erkläre ich, dass ich die Arbeit selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilftsmittel benutzt habe.

Ferner erkläre ich, dass ich nicht anderweitig mit oder ohne Erfolg versucht habe, eine Disseration einzureichen oder mich der Doktorprüfung zu unterziehen.

Hassan I. H. EL-Damanhouri

Bayreuth, den 16 February 2011