UNIVERSITÄT BAYREUTH

Endocrine control of fat body composition and effects of the insect growth regulators methoprene and pyriproxyfen on the development and reproduction of the Argentinian cockroach, *Blaptica dubia* Serville (Blattaria: Blaberidae)

Dissertation
zur Erlangung des Doktorgrades
der Fakultät für Biologie, Chemie und Geowissenschaften
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vorgelegt von

Ahmad Hussein Alamer

Aus Deir Azzor (Syria)

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To, my dear mother and spirit of my father

This study was performed between summer 2008 and winter 2011 in the Department of Animal Ecology I, University of Bayreuth, Germany, under supervision of Prof. Dr. Klaus Hubert Hoffmann.

Vollständiger Abdruck der von der Fakultät Biologie, Chemie und Geowissenschaften der Universität Bayreuth zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften genehmigten Dissertation.

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Abbreviations

20-HE	20-hydroxyecdysone
AKH	adipokinetic hormone
BSA	bovine serum albumin
°C	degree Celsius
CA	corpora allata
CC	corpora cardiaca
d	day
E	ecdysone
Ec	ecdysteroids
Fig.	figure
HL	haemolymph
HPLC	high performance liquid chromatography
H x W x L	height x width x length
IGR	Insect growth regulator
IPM	Integrated pest management
LC-MS	liquid chromatography-mass spectrometry
GPAH	glycogen phosphorylase activating hormone
JH	juvenile hormone
JHA	juvenile hormone analogue
JHBPs	JH binding proteins
L : D	light : dark
min	minute/s
n.s.	not significant
PTTH	prothoracicotropic hormone
S.E.	standard error of the mean
TAG	triacylglycerol
v/v	volume/volume
Vg	vitellogenin

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1 Introduction

“Insects are the most numerous and successful life form on the planet. They are helpful models that can facilitate our general understanding of biology. These creatures exist everywhere on earth and humans cannot avoid them. In extreme habitats such as the sea, some insects live and swim, even in the cold depth water of Antarctic and Arctic. Insects could also be found alive in pools of crude oil. In one word, insects play a very important part in any ecosystems” (McGavin, 1997).

Cockroaches (Blattodea) are among the oldest insect orders (Cruden and Markovetz, 1987). There are almost 4000 known species of cockroaches. Only a small number of them are worldwide indoor pests, but these species can cause severe health problems (cockroaches are one of the most important public health pest), such as the American cockroach *Periplaneta americana*, which can contaminate human food with bacteria (including the species of *Salmonella* and *Shigella*) that cause food poisoning. German cockroaches, *Blattella germanica,* may cause hepatitis, typhoid fever, dysentery and gastrointestinal disorders by transmitting bacteria, viruses and other microorganisms, because they deposit their saliva and feces in our food. Asthma and allergies are common diseases associated with cockroach infestation of houses in many parts of the world (Arruda et al., 2001; Eggleston and Arruda, 2001).
Cockroaches are nocturnal and generally live in warm and moist tropical and subtropical forests. Roaches like to hide in dark, warm areas, tend to congregate in corners, and to stay in cracks where they are well protected.
Most adult female cockroaches produce an egg capsule, called an ootheca or egg case.

The Argentinian cockroach, *Blaptica dubia* (Serville 1839) (Blattaria, Blaberidae), an ovoviviparous cockroach, carries the ootheca (in each ootheca about 20-35 eggs) during a gestation period of more than one month until eggs are ready to hatch. Under stress conditions, the females may drop their ootheca earlier. *B. dubia* cockroaches have the great advantage of being extremely easy to sex; they are sexually dimorphic, males and females look different in body shape (only males are

winged) and size. They have become very popular feeder for reptiles and tarantula. *B. dubia* have an only low pest potential but may act allergic.

Immature cockroaches (nymphs) resemble adults and have similar feeding habits, but are smaller, do not have wings and are not reproductively active. After molting, a newly molted cockroach is white in color, but usually within hours their cuticle darkens as it hardens. Adults are dark brown to black with somewhat lighter orange spot/stripe patterning sometimes visible only in bright light, while nymphs are grey/brown. *B. dubia* roaches are a non-flying species of roach. Although the males have fully developed wings, they do not fly whereas the adult females possess only tiny wing buds. Males reach a body length of just over 4 cm including their wings, while females can exceed 4.5 cm. They are rather slow movers, don't jump and cannot climb up smooth surfaces (glass or plastic), which makes it easier to care for them and to breed them. They are quiet and do not make any noise unlike crickets.

The Dubia cockroaches are tropical roaches found in Central and South America (French Guyana and Brazil to Argentina). The Argentinian cockroach is also known as orange spotted roach or Guyana spotted cockroach.

These cockroaches prefer higher moisture (above 60% relative humidity) than many other species and consequently breed better at those conditions. They need to be kept warm at a temperature between 25 and 30°C. If temperatures are lower, their growth and reproduction will be much slower.

Dubia roaches are a better feeder species than most other insects for pets. They are popular for feeding reptiles and amphibians because they contain a high amount of protein (a high quality herp food source). They are soft-bodied and have more meat for example than crickets making them an excellent food item for example for arachnids and small lizards.

Dubia cockroaches are prolific and give birth to live young. This together with the long life cycle (adults live up to 2 years, where the females seem to be more long-lived than the males) makes the species highly suitable for experiments on the endocrine control of development and reproduction.

The insect fat body

The fat body of insects is a large multifunctional organ, analogous in function to the liver of vertebrates and the adipose tissue. It is also a center of intermediary metabolism for the synthesis of haemolymph components, and it acts during the metamorphosis of holometabolous insects as storage for nutrient reserves. Fat body is distributed throughout the insect body, occupies the space among the insect organs, preferentially underneath the integument and surrounding the gut and reproductive organs (Kilby, 1963; Wyatt, 1975; Dean et al., 1985).

The fat body of insects plays an important role in energy storage and energy utilization. Fat body cells synthesize most of the haemolymph proteins and other circulating metabolites, and they control the synthesis and utilization of energy reserves. The fat body synthesizes and stores lipids, glycogen, free carbohydrates and proteins, in addition to other metabolites.

The free abdominal fat body is a very important source of energy for flight and reproduction in insects, such as shown for the cricket *Gryllus bimaculatus* (Lorenz, 2003). It is the major source for vitellogenins, which means that the females need considerable amounts of lipids, proteins, glycogen and free carbohydrates during vitellogenic growth of the oocytes (Keeley, 1985; Law and Wells, 1989; Hoffmann, 1995; Lorenz, 2003; Lorenz and Anand, 2004). The vitellogenins are then transported to the growing oocytes and incorporated into the maturing eggs. Lipid and carbohydrate metabolism, protein synthesis, and amino acid and nitrogen metabolism in general takes place in the fat body.

The basic cells of the fat body are the adipocytes and the urocytes, which have been described in the fat bodies of cockroaches and locusts (Dean et al., 1985; Willott et al., 1988).

Fat body lipids

Lipids are the main constituents of the fat body fresh mass and an important source for energy in insects. Lipids are mainly stored in the insect as energy reserve during larval development, and are subsequently utilized during adult life, for example during flight and vitellogenesis (Walker et al., 1970). Main storage forms of lipids (up to 90%) are the (acyl) triglycerides (Bailey et al., 1975; Canavoso et al., 2001).

In *Manduca sexta*, *Locusta migratoria*, and *G. bimaculatus*, it was found that these insects mainly use lipids as a flight fuel to supply energy for flight (Oudejans et al., 1993; Oda et al., 2000).

Juvenile hormones and ecdysteroids act on the formation and possibly also on the release of fat body lipids (Zhao and Zera, 2002).

Lipids are synthesized by the ovaries only in very small quantities, if at all (Ziegler, 1997). Therefore, most of the lipids in the oocytes are derived from fat body lipid stores (Arrese et al., 2001; Jouni et al., 2003).

Fat body carbohydrates

Carbohydrates are other major sources of energy during the life of an insect, especially important during processes such as molting, adult gonadal and reproductive growth, vitellogenesis in females and muscular activity. In addition, high levels of blood sugar are required as energy during flight and fight situations.

Fat body carbohydrates are an important source for trehalose, the blood-sugar in insects. Carbohydrates are stored in the fat body mostly in the form of glycogen. Glycogen is hydrolyzed to free carbohydrates like glucose for synthesis of the disaccharide trehalose, which is then released into the haemolymph (Wyatt, 1967; Keeley, 1978; Goldsworthy and Gäde, 1983; Candy, 1985; Candy et al., 1997; Thompson, 2003).

In some orders of insects, such as the Hymenoptera, Diptera and Blattoidea, free carbohydrates are directly used as fuel for flight (Anand, 2004).

Adipokinetic Hormone (AKH), secreted from the corpora cardiaca (CC), controls carbohydrate (and also lipid) metabolism in most insect species (Siegert and Ziegler, 1983; Van der Horst and Rodenburg, 2010).

Fat body glycogen

Glycogen, the major fat body carbohydrate reserve, is mobilized for use by other tissues, mainly in the form of trehalose, the blood sugar of insects (Thompson, 2003). Glycogen can be synthesized also in the ovary itself, from haemolymph trehalose as major source (Yamashita and Hasegawa, 1985).

In females of the cockroach *Leucophaea maderae*, glycogen from the fat body was shown to serve as major energy source during the first gonadotropic cycle (Wiens

and Gilbert, 1967). In adults of *M. sexta*, glycogen phosphorylase is activated during flight and during starvation. Glycogen phosphorylase activating hormone (GPAH) from the CC controls the activation of fat body glycogen phosphorylase in larvae of *M. sexta* during starvation (Siegert and Ziegler, 1983; Ziegler et al., 1985; Ziegler and Schulz, 1986).

In most insects, an intimate relation exists between concentrations of glycogen and trehalose in insect tissues and the physiological events of moulting, reproduction, flight, etc.

Subsequent to injection of corpus cardiacum extracts (AKH) in *P. americana* and *Blaberus discoidalis*, the concentration of haemolymph trehalose increased while the glycogen content of the fat body decreased (Steele, 1961; Bowers and Friedman, 1963; Ralph and McCarthy, 1964).

Fat body proteins

The insect fat body synthesizes and secretes large amounts of proteins such as storage proteins, which are used as an amino acid reservoir for morphogenesis, lipophorins, which are responsible for the transport of lipids in the haemolymph, or vitellogenins, which are used during egg maturation in females (Keeley, 1985).

The major egg protein, vitellin, is synthesized as vitellogenin by the fat body in huge amounts, then secreted into the haemolymph and selectively taken up by the developing oocytes through receptor-mediated endocytosis (Wyatt and Pan, 1978; Kunkel and Nordin, 1985; Hoffmann, 1995).

Ecdysteriods (especially 20-hydroxyecdysone, 20-HE) activate and stimulate the uptake of storage and transport proteins by the fat body in lepidopteran and dipteran species (Tojo et al., 1982; Ueno et al., 1983; Ismail and Dutta-Gupta, 1990; Burmester and Scheller, 1995). At least one ovarian protein of females is synthesized in the fat body exclusively under the impact of juvenile hormone (JH) in most insects, the vitellogenin (Engelmann, 1970). In some dipterans, however, vitellogenin synthesis is under the control of ecdysteroids (Bownes, 1982; Pondeville et al., 2008).

In locust larvae a correlation between changes in protein synthetic activity and changes in ecdysone titer of the haemolymph were observed (Tobe and Loughton 1969; Bouthier et al., 1975).

Insect developmental hormones

Insect metamorphosis, behaviour, caste determination, reproduction, and polymorphism are generally under hormonal control, especially under the control of juvenile hormones (JH) and 20-hydroxyecdysone (20-HE) (Hoffmann, 1995; Emlen and Nijhout, 1999; Gilbert et al., 2000).

Juvenile hormones are major developmental and reproductive hormones in insects, which are synthesized and secreted by the corpora allata (CA). The function of the CA, and that means the synthesis of JH, is regulated by activating (allatotropin) or inhibiting (allatostatin) neuropeptides from the brain (Stay et al., 1983). There are several insect JH homolgs (i.e. JH I to JH III, JH 0, and iso-JH 0, several JH bisepoxides). Lepidopteran species produce JH I, II, and III, whereas most non-lepidopteran species biosynthesize only JH III (Schooley et al., 1984; Baker 1990). Recently, JH bisepoxides have been found in the Diptera (Richard et al., 1989; Yin et al., 1995) and Heteroptera (Kaihara et al., 2012).

JH exerts many effects on physiological processes in insects by regulating gene expression in a variety of tissues (Nijhout 1994). A distinct JH receptor, however, is not yet known.

Juvenile hormones play important roles during insect life-cycle especially in larval development (morphogenetic role) and in adult reproduction (gonadotropic role) (Koeppe et al., 1985; Gäde et al., 1997). JH has also an effect on female sexual behaviour, flight and migration. During larval development, immature larvae need relatively high concentration of JH to grow and pass through larval molts induced by molting hormone secretion. JH concentration in the haemolymph decreases very sharp shortly before pupation and must be almost zero in the pupa to guarantee metamorphosis development to the imago (Retnakaran et al., 1985; Krishna Kumaran, 1990; Westerlund and Hoffman 2004).

In the viviparous cockroach *Leucophaea maderae*, it was shown that JH stimulates sundry processes which are associated with ovarian maturation (Koeppe and Ofengand, 1976). JH induces vitellogenesis in many insect species and suppresses pupation in holometabolous insects (Eto, 1990).

The transport of JH in the haemolymph of insects is performed by JH binding proteins (JHBPs) (Goodman and Chang, 1985).

As mentioned above, in many insects, presence of JH is necessary for vitellogenin synthesis in the adult female fat body (Hagedorn and Kunkel, 1979; Engelmann, 1979, 1980; Koeppe et al., 1985). Fat body lipid and glycogen levels were reduced when JH was topically applied to newly emerged queens of *Bombus terrestris* (Röseler and Röseler, 1988).

Juvenile hormones (JHs) regulate many physiological events (ecdysis, molting, metamorphosis, diapause, reproduction, migration) throughout the insect life cycle together with ecdysteroids (Ec) (20-hydroxyecdysone, 20-HE, is the active ecdysteroid) (Truman, 1985; Gelman et al., 2006). For example, vitellogenesis and maturation of the ovary in adult crickets are controlled by JH and ecdysteroids (Gäde et al., 1997; Hoffmann and Lorenz, 1997; De Loof et al., 2001).

The two major ecdysteriods are ecdysone and 20-hydroxyecdysone. 20-HE is the molting hormone of most insects (Chang, 1993, Hua et al., 1994; Gäde et al., 1997). Ecdysone or some other “prohormones” (e.g. 3-dehydroecdysone; Kiriishi et al., 1990) are secreted from the prothoracic gland (ring glands or ventral glands in some orders) under control of the brain neuropeptide PTTH (prothoracicotropic hormone). 20-HE initiates the molting process, whereas JH modulates the ecdysteroid activity (Nijhout, 1994).
In some dipteran species such as the mosquito *Aedes aegypti*, ecdysteroids regulate female reproduction (yolk protein synthesis and deposition during the vitlllogenic stage) (Bai et al., 2010).
In cockroaches that transport an ootheca, such as *Blattella germanica*, the production of JH is very low during the period of ootheca transport, which decreases oocyte growth and prevents the premature deposition of the ootheca (Osorio et al., 1997). In *B. dubia*, courtship behaviour, mating and vitellogenesis seem to be controlled entirely by JH (Hintze-Podufal and Vetter, 1996).

Insect growth regulators

The application of insect growth regulators (IGR) is one of the new biological control methods, and widely used to control pest insects (Kubo et al., 1983). IGR act as hormone analogues (e.g., ecdysteroid mimetica such as RH-5992, tebufenozide) or antihormones (e.g., the anti-juvenile hormone precocene). They induce a variety of

reproductive, developmental and morphogenetic effects on insects, because of their hormonal activity. IGR usually induce a potent suppression of embryogenesis, metamorphosis and formation of adult insects (Meola et al., 2001; Liu, 2003). JH analogues like methoprene, hydroprene, kinoprene, fenoxycarb or pyriproxyfen disrupt the larval and adult developmental processes in insect pests by mimiking the action of JH (Sehnal, 1983). Another group of IGR act as chitin synthesis inhibitors (cuticle formation) (diflubenzuron, buprofezin, lufenuron) (Tunaz and Uygun, 2004).

IGR disrupt specific physiological processes in target insects, and topically perturb enzymatically and hormonally regulated processes that are relatively specific in insect growth and development (Retnakaran et al., 1985; Dhadialla et al., 1998).

IGR, such as analogues of JH, are not or only slightly toxic to vertebrates and harmless to many natural enemies of the insects. They can be rapidly degraded in the natural environment and are, therefore, used as insecticides in integrated pest management control programs (IPM). IGR are one of the most promising alternatives to conventional insecticides for insect pest control (Staal, 1975; Ishaaya, 1990; Degheele, 1990; Dhadialla et al., 1998).

IGR interfere with development, growth and/or reproduction of insect pests; therefore, they are in wide use to control a variety of insect pests (Yu, 2008).
Ecdysone agonists such as tebufenozide, and juvenile hormone mimics such as methoprene or pyriproxyfen interfere with the hormonal control of molting, metamorphosis and vitellogenesis/ovarian development in many insect orders (Dhadialla et al., 1998). Some IGR affect spermatogenesis of the insects (King and Bennett, 1989).
IGR need not necessarily be toxic, but can have devastating effects on the target insects, by leading to an abnormality which impairs the survival of the insect (Siddall, 1976). Others inhibit vitellogenesis and egg production, thus keeping population growth rate of the pest species low.

Methoprene, one of the commercially most important juvenile hormone analogues, is a terpenoid compound used primarily against household and community pests

because of its low activity against agricultural pests and low residual on plants under field conditions (Smith, 1995).

The approved uses of methoprene appear to be environmentally harmless, and it is effective against a wide range of insects, including the orders Diptera, Lepidoptera and Coleoptera (Miura and Takahashi, 1973; Glare and O´Callaghan 1999).

Methoprene (Altosid®) was formerly used as a mosquito larvicide (selective larvicide), but it is now widely used in other pest control and horticultural programs (Sehnal, 1983).

Methoprene prevents the metamorphosis of mosquito larvae into adults; therefore, one of the main uses of methoprene was in the control of mosquitoes (Retnakaran et al., 1985; Dhadialla et al., 1998). It appears to prevent the target insect from becoming mature when it is constantly present. Moreover, it interferes with the insects general life cycle and disrupts metamorphosis and normal insect development (Breeden et al. 1981).

Treatment of *L. migratoria* larval females with methoprene caused the precocious appearance of female-specific protein band in the blood, and also resulted in premature sexual differentiation, because methoprene treatment causes precocious development of the fat body into its adult form (Cotton and Anstee, 1990).

Methoprene has also been shown to possess great potential for use as a specific control agent against the pest beetle *Rhyzopertha dominica* (Samson et al., 1990).

Pyriproxyfen has a high JH mimic activity against insects. It is a JH analogue that was used to control public health pests such as houseflies, mosquitoes and cockroaches, and was first registered in Japan in 1991 for controlling mosquitoes. Pyriproxyfen suppresses powerfully embryogenesis and formation of adults. It is available for use against household and horticultural/agricultural pests such as control of scale, whitefly, aphids and fire ants. It has been used for controlling a variety of insect pest with relatively low toxicity to mammals. It acts on the endocrine system by inhibiting molting (insects treated with pyriproxyfen are unable to molt successfully to adult stage) and subsequently inhibiting reproduction of adults (Yokoyama and Miller 1991; Langley et al., 1993; Miyamoto et al., 1993; Dhadialla et al., 1998; Sullivan and Goh, 2008).

Under laboratory conditions, pyriproxyfen application caused reduction of longevity, inhibited progeny production of *Thrips tabaci*, and it could cause high mortality (Liu, 2003).

Application of pyriproxyfen to *Spodoptera litura* caused reduction in oviposition (Hatakoshi, 1992). On the contrary, butterfly females of *Bicyclus anynana* that had been treated with pyriproxyfen exhibited an increase in egg-laying rates and fecundity started early in adult life, but pyriproxyfen caused a reduction of longevity compared with controls (Steigenga et al., 2006).

According to the ovicidal, larvicidal and reproductive effects of pyriproxyfen against the Asian citrus psyllid *Diaphorina citri*, pyriproxyfen is suitable for integration into an IPM program for *D. citri*. It caused reduction of female fecundity and egg viability in addition to morphological abnormalities in adults (Boina et al., 2010).

The use of pyriproxyfen as cockroach control agent has also been reported. Pyriproxyfen caused sterility in the German cockroach (*B. germanica*) due to incomplete development of the internal reproductive organs in addition to deformation and degeneration in the ovaries and accessory glands. Sterile roaches also showed various morphological abnormalities (Fathpour et al., 2007).

Topical application or contact of cuticle with pyriproxyfen caused nymphal death in *B. germanica*, and population growth was suppressed by the inhibition of reproduction (Kawada et al., 1989).

Aim of the study

Actually, not much information is available on the endocrine regulation of ovarian development and reproduction in females of the cockroach *Blaptica dubia*. Therefore, the present study focuses on several physiological and endocrine aspects of *B. dubia* female life.

In the first part of this work, growth and gain in weight of females and males were analyzed (fresh weight, ovary weight, fat body weight) during the first 100 (160) days of adult life, and also was analyzed the chemical composition of the fat body of the animals in terms of the levels of total lipids, proteins and carbohydrates.

Since hormones control vitellogenesis, ovarian development and oviposition in insects, the titers of JH III and the two major ecdysteroids (ecdysone and 20-

hydroxyecdysone) were determined in the haemolymph of *B. dubia* females during the first 100 days of adult life.

In the second part of the work, we determined effects of the insect growth regulators methoprene and pyriproxyfen on development and reproduction of *B. dubia* females.

2 Methods and materials

The present study was carried out in the laboratory of Animal Ecology I, University of Bayreuth, Germany during July, 2008 and December, 2011.

2.1 Rearing of *Blaptica dubia* Serville

A colony of the Argentinian cockroach (the Dubia cockroach), also known as orange-spotted cockroach or Guyana spotted cockroach, *Blaptica dubia* (Dictyoptera, Blattaria, Blaberidae), was maintained in our laboratory under crowded conditions in a climate chamber until use.

Colonies of the Dubia cockroach were reared at a constant temperature of 28ºC (need to be kept warm because Dubia roaches are tropical roaches) with a relative humidity of about 70% and a long day light cycle (16 h light, 8 h dark; lights from 06:00 to 22:00 CEST).

The colonies were maintained in white plastic boxes (35 x 45 x 60 cm, h x w x l), covered with netted lids to prevent roaches from getting out and other animals from getting in, and provided with egg dividers (egg boxes) or cardboard egg flats (stacked horizontally), which served as shelter and gave the roaches a place to hide and breed. Breeding boxes were kept clean with attention to sweep or scoop the bottom of the enclosure from their waste and frass as required, usually every month.

The Dubia roaches were supplied with a mixture of one part of breeding diet for rats and mice (No. 1311) and one part of breeding diet for rabbits (No. 2021), all provided by Altromin Spezialfutter GmbH & Co. KG (Lage, Germany).

Dubia roaches preferred and consumed breeding diet for rabbits more than for rats and mice. The food was placed in the corners directly on the container floor.

Rats and mice food (No. 1311) ingredients:

22.5% protein; 5.0% fat; 4.5% fibres; 6.5% ash; 0.9% calcium; 0.7% phosphor; 0.2% sodium.

Rabbits (No. 2021) ingredients:

17.5% protein; 4.0% fat; 14.5% fibres; 8.0% ash; 0.9% calcium; 0.7% phosphor; 0.2% sodium.

The mixture of breeding diet provided a high proteinous food at all times. It was important to keep the diet dry, because wetness will promote mold and will offer breeding grounds for bacteria, mites and beetles that are a threat to roach colony.

The animals were fed also with fresh fruit or vegetables at least once a week. Any fruit or vegetables not eaten within a few hours should be removed to prevent mold.

Drinking water was supplied ad libitum in watering containers (Heuser, Haan, Germany) which were changed every two days.

All experiments (except body weight measurements) were performed using adult females of the Argentine cockroach *B. dubia*. Newly-emerged 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. The newly emerged adults were isolated from the stock culture within one hour after the final moult and placed in a fauna transparent plastic box (20 x 20 x 35 cm, h x w x l), covered with a transparent plastic lid, under similar conditions as described above.

2.2 Experiment I

Lifecycle, chemical composition of the fat body and juvenile hormone (JH) and ecdysteroid (Ec) titers in the haemolymph of *B. dubia* females from day 0 to 80/100, and from day 130 to 160 after ecdysis

2.2.1 Culture of *B. dubia* in the laboratory and life cycle

Newly emerged adults (10 adult 0 day old females and 4 males) were collected from the mass rearing colony each 5 days from day 0 to 100 and 130 to 160, respectively, weighed and placed together in fauna plastic boxes (20 x 20 x 35 cm, h x w x l) with a transparent plastic lid, under similar conditions as described above.

Fresh weight changes of adult females and males, fat body and ovary weight changes in females, formation of the ootheca, ootheca deposition and larval hatching were determined during the first 100 days of adult life.

Under above mentioned conditions, the first mating occurred on day 5 after adult emergence. First occurrence of the ootheca including undeveloped eggs took place around day 19 ± 4 after adult emergence. The ootheca deposition and hatching of the larvae/nymphs started on day 70 ± 5 after adult ecdysis.

From each ootheca, 15-30 larvae hatched. Larval development took about 3-4 months. Under our rearing conditions the number of larval stages was six.

2.2.2 Haemolymph sampling and JH III and free ecdysteroid titer measurements

Up to ten females were collected from each age group every 5 days between days 0 and 100, and 130 and 160, respectively. After the body weight of the cockroaches had been determined, a haemolymph (HL) sample (20 µl) was taken.

For the determination of JH III, ecdysone (E) and 20-hydroxyecdysone (20-HE) titers in the haemolymph of *B. dubia* liquid chromatography (HPLC)-mass spectrometry (HPLC-MS; Westerlund and Hoffmann, 2004) was used.

The haemolymph was obtained by fixing the insect on its back with rubber on a white board and puncturing the cuticle of the thorax under the hind legs with a preparation needle, then slightly pressing the female so that the haemolymph appears. The haemolymph was taken off by a (20 µl) disposable micropipette with ring mark (ringcaps® Duran®, Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt, Germany) fixed in front of a syringe. After haemolymph sample (20 µl) had been collected, it was blown into sample Fisher Scientific glass vials that were filled with 200 µl methanol/isooctane - mixture (MIM) (ratio methanol: isooctane = 1:1, v/v; ratio haemolymph: MIM = 1:10, v/v). After vortexing the Fisher vials for 20 sec, the samples stayed for 20 min at room temperature, were vortexed again, and then put into Eppendorf tubes (1.5 ml) with cut caps and centrifuged at 10.000 *g* for 20 min. The upper isooctane phase and the lower methanol phase were each removed with a syringe and the samples were transferred into new sample Fisher Scientific glass vials. All sample vials were sealed with aluminium foil and parafilm and stored at -80°C until use. JH III, E, and 20-HE titers were measured by LC-MS as described by Westerlund and Hoffmann (2004) with slight modifications.

Hormone stock solution concentrations

The concentrations of standard stock solution (ng/µl) were as follows:

JH I: 6.48 – 6.64 JH II: 5.77 – 6.66 JH III: 5.09 – 6.12

E: 3.76 – 11.81 20-HE: 11.94 – 20.62

Table 1: The dilutions of the hormone standard stock solutions for calibration curve

Sample name	**Volume of standard stock solution in µl**	**Volume of haemolymph in µl**	**Total volume in µl**
HL-matrix	0	50	50
Con. 1	2	48	50
Con. 2	5	45	50
Con. 3	10	40	50
Con. 4	15	35	50
Con. 5	20	30	50
Con. 6	25	25	50
Con. 7	40	10	50
Con. 8	50	0	50

2.2.3 Body weight

Immediately before haemolymph (HL) samples were taken, the body weight of the experimental animals was determined.

2.2.4 Extraction and separation of lipids, proteins, glycogen and free carbohydrates from the fat body

The fat body was analysed by estimating lipid, protein, glycogen, and free carbohydrate content according to a protocol described by Lorenz (2003).

Females were decapitated and fixed with insect needles on a styrofoam plate to expose the inner organs. They were opened on the ventral side by a longitudinal incision from the thorax to the anus.

Then, the complete gut and the ovaries were carefully removed (ovaries were weighed) and the free abdominal fat body from one body side was dissected. The half fat body was divided into quarters (Lorenz, 2001). 1/8 of each whole fat body was put 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 the fat body fresh weight and then kept at - 20°C until use.

The other half fat bodies were collected into 1.5 ml safe-lock Eppendorf tubes to determine whole fat body fresh weight (weight of the fat body of females from day 0 to 100 after emergence).

On the basis of methods by Van Handel (1965) and Speck and Urich (1969) a procedure was developed to allow the use of Eppendorf reaction tubes for a separation of organic compounds in small volumes of solvents (Lorenz, 2003) (Fig. 1).

Lipid estimation

The sulphophosphovanillin method was used as described by Zöllner and Kirsch (1962) with some modifications to measure total lipid concentration in the fat body. After adding 100 µl of concentrated sulfuric acid, samples were heated to 100°C for 10 min. After cooling, 1 ml of vanillin reagent (0.2% vanillin in 57% ortho-phosphoric acid) was added, and the tubes were put in the dark for 25 min, and then vortexed for a short time. Samples were measured against cholesterol standards (cholesterol in chloroform 1 mg/ml) of 0 to 20 µg in a spectrophotometer at 530 nm (Lorenz, 2003).

Carbohydrate estimation

Free carbohydrates in the fat body were measured by the anthrone method as described by Mokrash (1954) with some modifications. Samples were mixed with 1 ml of anthrone reagent (0.13% anthrone in 67% sulfuric acid), heated for 10 min to 90°C and put in the dark for 25 min. After cooling, samples were vortexed for a short time and absorbance was measured at 585 nm against glucose standards (1mg/ml) of 0 to 20 µg in a spectrophotometer (Lorenz, 2003).

Glycogen estimation

The concentration of total glycogen in the fat body was estimated by the anthrone method as described for the free carbohydrates (Lorenz, 2003).

The samples were measured against glycogen standards of 0 to 20 µg at 585 nm in a spectrophotometer.

Protein estimation

Roti-Quant universal solution based on a modified Bradford's protein assay was used to estimate proteins in the fat body. Protein samples in a final volume of 50 µl (samples of 5 µl diluted with water) were mixed with 200 µl Roti-Quant universal solution on a 96-well plate, heated to 50°C for 20 min and measured at 515 nm

against water. A protein standard curve of 0 to 200 µg BSA (bovine serum albumin) was used, to determine protein concentrations.

2.2.5 Ovarian and ootheca weight

The ovaries and the ootheca were carefully removed from the females before fat body dissection and collected into pre-weighed 1.5 ml non safe-lock Eppendorf tubes to determine ovary and ootheca fresh weight on a microbalance.

2.3 Experiment II

Repeated determination of lipids, proteins, glycogen, and free carbohydrates in the fat body of *B. dubia* females, and measurement of JH III and ecdysteroid titers in the haemolymph from day 0 to 100 after emergence

About 10 newly ecdysed adult females and about 4 males were collected every 5 days from the mass rearing colony, and placed in a fauna transparent plastic box (20 x 20 x 35 cm, h x w x l). They were provided with water and food ad libitum as described.

Under the rearing conditions described in chapter 2.1, the first mating occurred about 5-7 days after adult emergence. First ootheca deposition took place around day 19 ± 4 after adult emergence. The hatching of larvae started around day 70 ± 5 after adult ecdysis. From each ootheca, 15-30 larvae hatched.

Body, ovary, ootheca, and fat body weight were determind as described. Estimation of lipids, proteins, glycogen, and free carbohydrates in the fat body, as well as titers of JH III, E and 20-HE in the haemolymph were measured as described.

2.4 Experiment III

Effects of the insect growth regulator (IGR) methoprene, a JH analogue, on reproduction and hormone titers of *B. dubia* females

2.4.1 Insects

From the mass rearing colony 40 newly ecdysed adult females were collected and their body weights were recorded. Each 10 females were placed in a fauna transparent plastic box (20 x 20 x 35 cm, h x w x l), which contained 4-5 males, and animals were provided with water and food ad libitum. At the day of collection, the animals were 0 day old. Under the conditions as described in chapter 2.1 the first mating started on day 4 after adult emergence.

First oviposition of unfertilized eggs in the ootheca took place around day 19 ± 4 after adult emergence. The oviposition (from which larvae hatched later) started around day 69 ± 5 after adult ecdysis.

2.4.2 Treatment of experimental insects

Collected females were divided into two groups:

The first group contained 10 insects as control and 10 as hormone treated animals. Treatment occurred from day 2 to day 20 after emergence.

Application of methoprene and acetone (control) was as follows:

On days 2, 5, 10, 15, and 20 after emergence application of methoprene (100 µg in 5 µl acetone).

On days 2, 5, 10, 15, and 20 after emergence application of acetone (5 µl).

Topical applications were performed onto the dorsal surface of the thorax and abdomen, respectively, always during daytime. Haemolymph (HL) samples (20 µl) were taken shortly before treatment with methoprene or acetone at days 5, 10, 15, 20 (and also at days 0 and 25). Body weights of females were recorded (control and treated) every 5 days from day 0 to day 25.

At day 25 females were dissected and the ovary and ootheca weights were recorded.

The second group of *B. dubia* females again consisted of 10 insects as control and 10 as hormone treated animals, but treatment occurred from day 30 to day 70 after emergence.

Application of methoprene and acetone (control) was as follows:

On days 30, 35, 40, 50, 60, and 70 after emergence application of methoprene (100 µg in 5 µl acetone).
On days 30, 35, 40, 50, 60, and 70 after female emergence application of acetone (5 µl).
Haemolymph samples (20 µl) were taken shortly before treatment with methoprene and acetone at days 30, 35, 40, 45, 50, 55, 60, 65, and 70 (and also at day 75). Body weights of females were recorded (control and treated) every 5 days from day 0 to day 75.
At day 75, the females were dissected, and weights of the ovaries and the ootheca were determined.

2.4.3 Determination of JH III, E, and 20-HE titers in the haemolymph of methoprene treated *B. dubia*

To evaluate the effects of methoprene on reproductive events during the adult life, the titers of JH III, E, and 20-HE in the haemolymph of *B. dubia* females (control and treated) were measured using the LC-MS method according to Westerlund and Hoffmann (2004) as described in chapter 2.2.2.

2.5 Experiment IV

Effects of pyriproxyfen, a juvenile hormone mimic, on female adult development, egg hatching, and hormone titers

2.5.1 Insects

From the mass rearing colony 40 newly ecdysed adult females were collected, and their body weights were recorded. Each 10 females were placed in a fauna transparent plastic box (20 x 20 x 35 cm, h x w x l), covered with a transparent plastic lid, which contained 4-5 males, and animals were provided with water and food ad libitum. At the day of collection, the animals were 0 day old. Under the conditions as described in chapter 2.1, the mating occurred on day 6 ± 1 after adult emergence, and first occurrence of the ootheca containing undeveloped eggs took place around day 20 ± 5 after adult emergence.The birth (hatching of larvae) started around day 70 ± 5 after adult ecdysis.

2.5.2 Topical application of pyriproxyfen

Collected females were divided to two groups:

The first group consisted of 10 insects as control and 10 as treated animals.

Application of pyriproxyfen and acetone (control) was on days 2, 5, 10, 15, and 20 after emergence. Pyriproxyfen was dissolved in acetone and topically administered at 100 µg in 5 µl acetone. Control females were treated with 5 µl of acetone. At day 25, the females (control and treated) were dissected and the ovary and ootheca weights were recorded.

The second group of *B. dubia* females also consisted of 10 insects as control and 10 as treated animals.

Application of pyriproxyfen and acetone (control) was as in the first group, but on days 30, 35, 40, 50, 60, and 70 after emergence.

At day 75 after emergence the females were dissected (control and treated), and weights of the ovaries and ootheca were determined.

2.5.3 Determination of JH III, E, and 20-HE titers in the haemolymph of pyriproxyfen treated *B. dubia*

Hormone titers were determined as described in chapter 2.2.2.

2.6 Chemicals

Acetone GR for analysis; E. Merck KgaA, Darmstadt, Germany

Anthrone 97%, A.C.S. Reagent; Sigma-Aldrich Chemie GmbH, Steinheim, Germany

BSA Albumin FraktionV, Protease-frei; Carl Roth GmbH & Co. KG, Karlsruhe, Germany

Cholesterol from lanolin; Sigma-Aldrich Chemie GmbH, Steinheim, Germany; Fluka Chemie AG, Buchs, Switzerland

$CHCl_3$ Chloroform, Rotipuran® ≥ 99%, p.a.; Carl Roth GmbH & Co. KG, Karlsruhe, Germany

$C_6H_{12}O_6$ D(+)-Glucose anhydrous for biochemistry; E.Merck KgaA, Darmstadt, Germany

C_2H_6O Ethanol, Rotipuran® ≥ 99,8%, p.a.; Carl Roth GmbH & Co. KG, Karlsruhe, Germany

Glycogen Type II from oyster; Sigma-Aldrich Chemie GmbH, Steinheim, Germany

C_6H_{14} n-Hexane, Rotipuran® ≥ 99%, ACS; Carl Roth GmbH & Co. KG, Karlsruhe, Germany

$C_{27}H_{44}O_6$ Ecdysone and $C_{27}H_{44}O_7$ 20-hydroxyecdysone; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany

H_2O Water Eau, Rotipuran® p.a., ACS; Carl Roth GmbH & Co. KG, Karlsruhe, Germany

H_2O Water, Lichrosolv® for chromatography; E. Merck KgaA, Darmstadt, Germany

Isooctane for analysis, Emsure® ACS, Reag. Ph. Eur.; E. Merck KgaA, Darmstadt, Germany

$C_{18}H_{30}O_3$ JH I, $C_{17}H_{28}O_3$ JH II; SciTech, Prague, Czech Republic

$C_{16}H_{26}O_3$ JH III; Fluka Chemie AG, Taufkirchen, Germany

KOH Potassium hydroxide pellets, puriss. p.a., Reag. Ph. Eur.; Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany

MeOH Methanol, Rotipuran® ≥ 99,9%, p.a., ACS, ISO; Carl Roth GmbH & Co. KG, Karlsruhe, Germany

MeOH Methanol for liquid chromatography, Lichrosolv®; E. Merck KgaA, Darmstadt, Germany

MeOH Methanol hypergrade for LC-MS, Lichrosolv®; E. Merck KgaA, Darmstadt, Germany

$C_{19}H_{34}O_3$ Methoprene Pestanal®; Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany

NaCl Sodium chloride puriss. p.a, Reag. ACS, Reag. ISO, Reag. Ph. Eur.; Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany

Na_2SO_4 Sodium sulfate anhydrous GR for analysis; E. Merck KgaA, Darmstadt, Germany

$C_{20}OH_{19}NO_3$ Pyriproxyfen Vetranal; Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany

2-Propanol Lichrosolv® gradient grade for liquid chromatography; E. Merck KgaA, Darmstadt, Germany

Roti®-Quant Universal Colorimetric protein concentration analysis:

Roti®-Quant Universal Reagenz 1: 500 ml (0120.1)/200 ml (0120.2)

Roti®-Quant Universal Reagenz 2: 40 ml (0120.1)/16 ml (0120.2); Carl Roth GmbH & Co. KG, Karlsruhe, Germany

$C_8H_8O_3$ Vanillin; Sigma-Aldrich Chemie GmbH, Steinheim, Germany

2.7 Instruments and materials

Eppendorf tubes (1.5 ml, 2.0 ml, safelock, non-safelock); Eppendorf AG, Hamburg, Germany

Hand-held homogeniser, MHX / E; Xenox, Niersbach, Germany

Grant Boekel BBA4; Grant Instrument (Cambridge) Ltd., UK

Grant QBT4; Grant Instrument (Cambridge) Ltd., UK

Pipette Pipetman®; Gilson, France

Pipette microman; Gilson®, France

Pre-assembled pipette capillaries and pistons; Gilson S.A for Microman®, Villiers-Le-Bel, France

Vortexer Heidolph REAX 2000; Heidolph, Schwabach, Germany

Mini Vortexer VWR; MFG. By Henry Troemner LLC, USA

Centrifuge, Biofuge 13; Heraeus Instruments GmbH, Osterode, Germany

Centrifuge, Sigma 3K12 B. Braun Biotech International; Sigma Laborzentrifugen, Osterode am Harz, Germany

Analytical balance A 7073 03; LC1201S; MC 210 P; Sartorius AG, Göttingen, Germany

Ultrasonic bath, Transsonic 310 Elma®; Singen / Htw., Germany

EL 808 ™ Ultra Microplate Reader; Bio-Tek Instruments Inc., Bad Friedrichshall Germany

Spectrophotometer, Pharmacia LKB. Ultraspec III®; Freiburg, Germany

Disposable cuvettes, semi-micro PMMA, Plastibrand®; Brand GmbH & Co. KG, Wertheim, Germany

Speed-Vac-concentrator, Alpha RVC; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany

Heating-magnetic stirrer, RCT B; Janke & Kunkel GmbH & Co. KG, IKA Labortechnik Staufen, Germany

LCMS-2010 Liquid chromatography mass spectrometer; Shimadzu Corporation, Kyoto, Japan

Sonifier, Branson W- 250; G. Heinemann, Schwäbisch Gmünd, Germany

Parafilm, Bemis® Laboratory Film; Neenah, WI, USA

Hamilton Microliter™ syringes; Hamilton Bonaduz AG, Bonaduz, Switzerland

Brown glass vials, Rotilabo®- 2 ml amber screw neck vial; Carl Roth GmbH & Co. KG, Karlsruhe, Germany

Fisher Scientific tubes, Test tubes heavy-walled; Assistant, Germany

Septum Silikon/Teflon; Carl Roth GmbH & Co. KG, Karlsruhe, Germany

Screw Cap with hole, Schraubkappen mit Loch; Carl Roth GmbH & Co. KG, Karlsruhe, Germany

Autosampler vials with silicon-teflon septum, Rotilabo®; Carl Roth GmbH & Co. KG, Karlsruhe, Germany

Reaction tubes 0.5 ml; Eppendorf, Hamburg, Germany

Disposable micro pipettes with ring mark, ringcaps® Duran®; Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt, Germany

Oven, Heraeus; Hanau, Germany

Micro-titer-plate G080-F; G. Kisker GbR, Steinfurt, Germany

2.8 Statistical analysis

Results are presented as the mean ± standard error (S.E.). The age and number of animals tested in each experiment are given in the results. Mean values were compared by t-test, Mann-Whitney U-test or ANOVA. Pairwise comparisons were made using the Dunn's method or Tukey test.

2.9 Software

Computer programs: LCMS solution; SigmaPlot 11.0

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

-Add sample (fat body) into pre-weighed 1.5 ml Eppendorf tube (containing 20 mg Na_2So_4 with 200 µl 75% MeOH in H_2O) and weigh. Difference in weight of the tissue sample.
-Homogenisa (with electric homogeniser), rinse pestle directly into the Eppendorf tube. With 600 µl $CHCl_3$:MeOH (1:1) vortex vigorously put into ultrasonic bath 5 min, vortex as before.
-Centrifuge (Sigma 3K12, 21 000 *g*, 2˚C 10 min, progr. 8).
-Separate pellet and supernatant as follows:

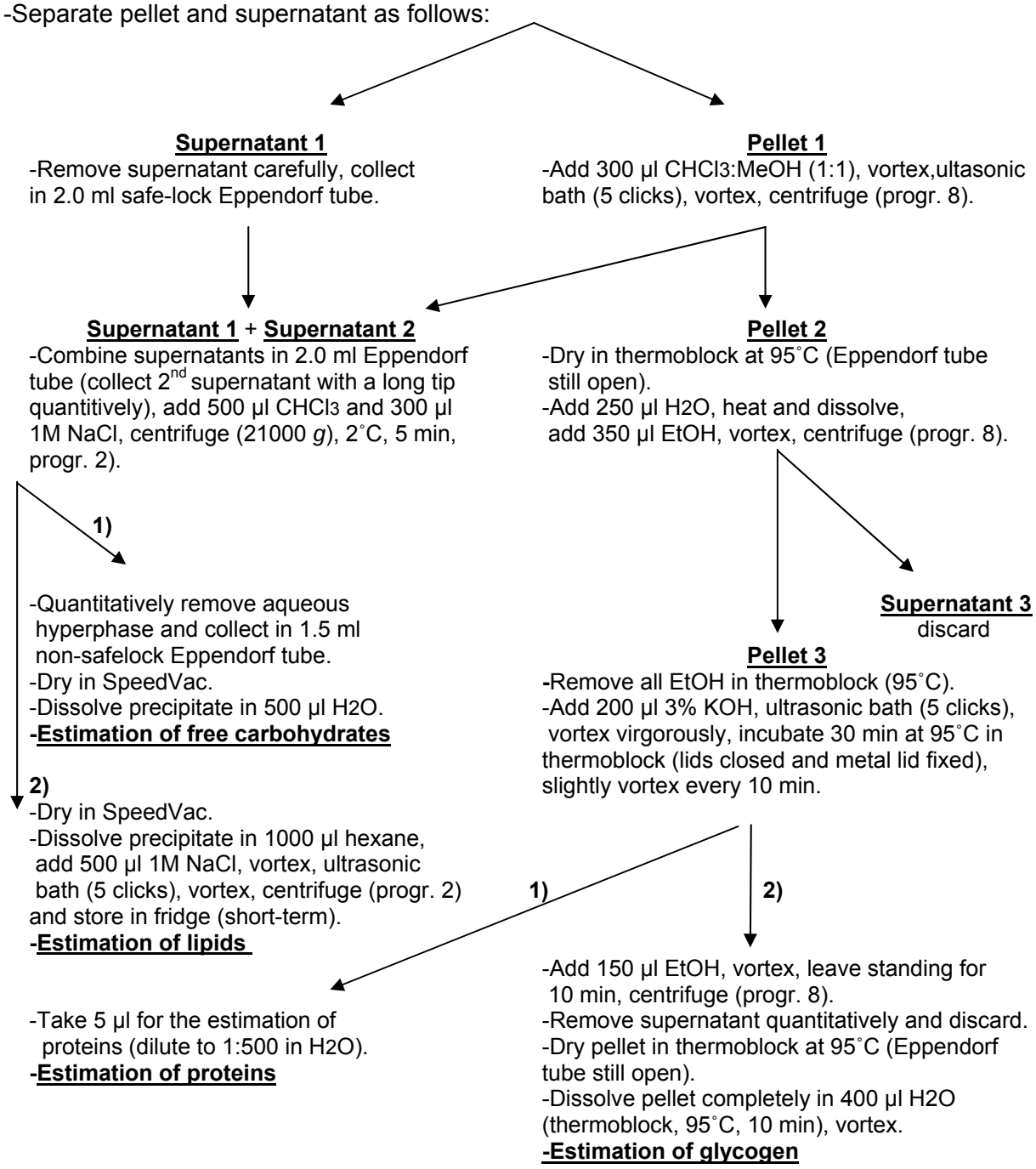

Fig. 1: Scheme showing the method used for the extraction and separation of organic substances from fat bodies (Lorenz, 2003).

3 Results

3.1 Experiments I, II

Determination of lipids, proteins, glycogen, and free carbohydrates in the fat body of *B. dubia* females, and measurement of JH III and ecdysteroid titers in the haemolymph from day 0 to 80/100, and from day 130 to 160 after ecdysis

3.1.1 Fresh weight of adult males and females

The weight of males slightly increased after adult ecdysis till first mating with females on day 5, and then decreased to day 30 after emergence. A significant increase in the body weight of males was noticed between day 30 and 40 of adult life (Fig. 2). Older males stayed fairly constant in weight.

The weight of females began to increase sharply during the first 10 days after emergence and reached a plateau value around day 35. A decrease in body weight of the females was observed after day 70, the time of ootheca deposition and hatching of the first nymphs (Fig. 3).

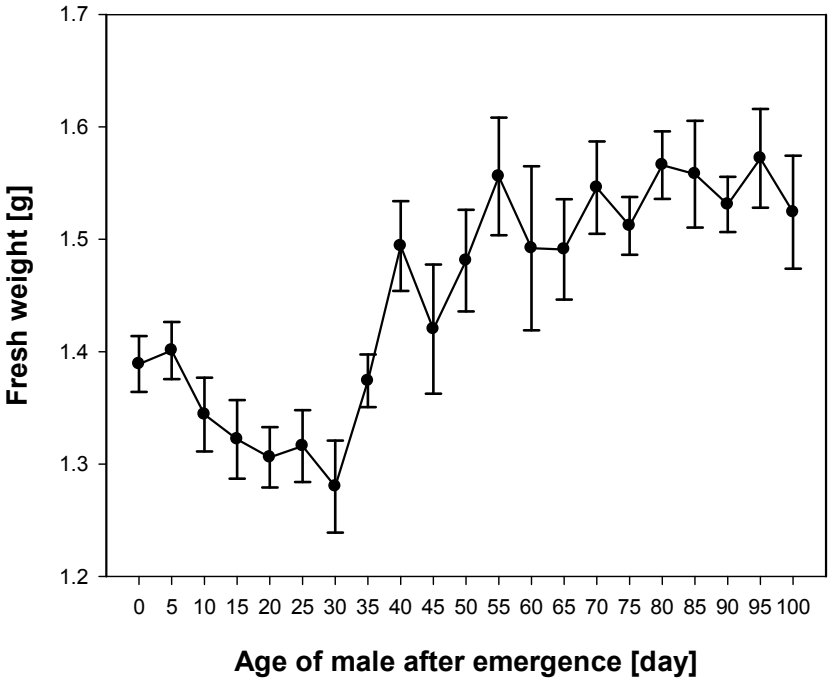

Fig. 2. Fresh weight of cockroach *B. dubia* males. Means ± S.E. For significant differences in fresh weight at different ages (One Way ANOVA with posthoc-test: Holm-Sidak) see Table 2; n = 20. Results from experiment I and II were pooled.

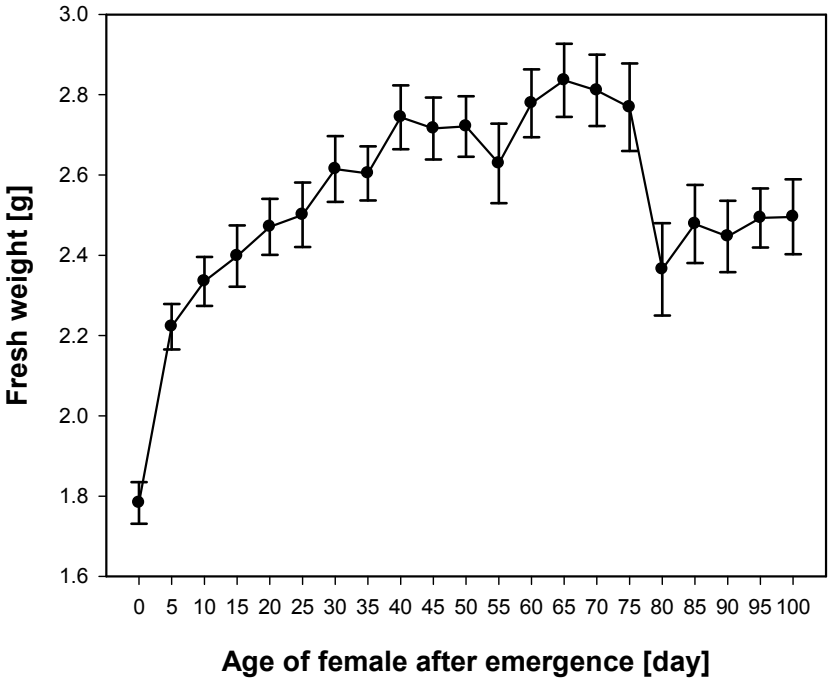

Fig. 3. Fresh weight of cockroach *B. dubia* females. Means ± S.E. For significant differences in fresh weight at different ages (One Way ANOVA on Ranks with posthoc-test: Tukey) see Table 3; n = 20. Results from experiment I and II were pooled.

Shortly after adult emergence, femeles had an about 400 mg higher fresh weight than males and this difference was even greater in older animals (maximum of 2.8 g in females versus 1.55 g in males).

3.1.2 Ovary weight, ootheca fresh mass and fat body mass

There was a clear trend towards increasing ovary weights during the first 15-20 days after emergence of the females when ovaries grew up and the ootheca was formed. The significant increase in ootheca mass from day 25 to days 50 to 65 of female adult life (gestation period) went in line with a significant decrease in the ovary weight. Thus, ovaries were the smallest when the ootheca contained developing eggs. No differences were observed in ovary and ootheca development between experiment I and experiment II (Fig. 4a, b). Another increase in the ovary weight after day 80 of adult life indicates the beginning of the second gonadotropic cycle (Fig. 4b).

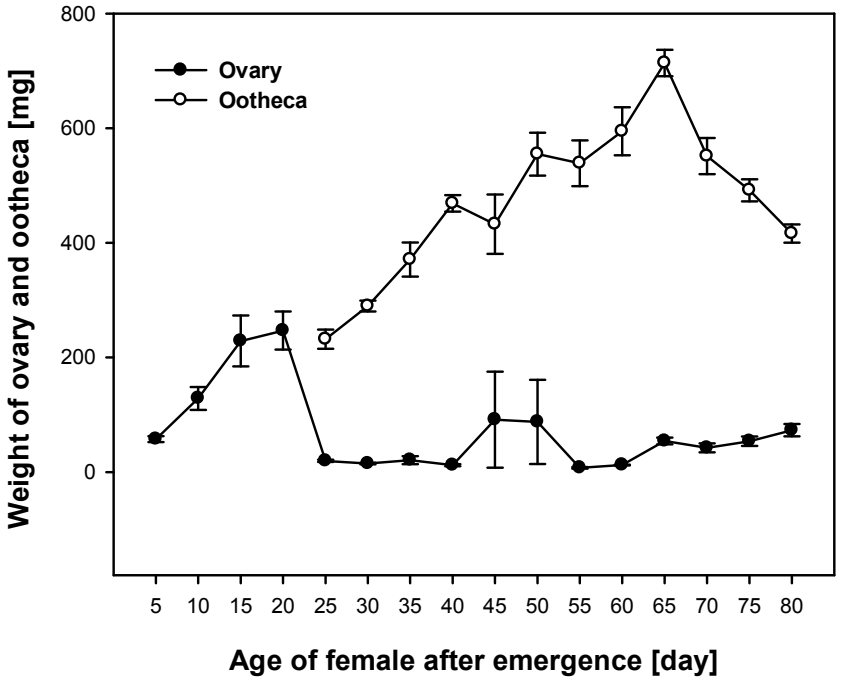

Fig. 4a. The weight of ovary and ootheca of *B. dubia* females (experiment I). Means ± S.E. For significant differences in ovary and ootheca weight at different ages (One Way ANOVA on Ranks with posthoc-test: Dunn´s) see Tables 4 a, b; n = 10.

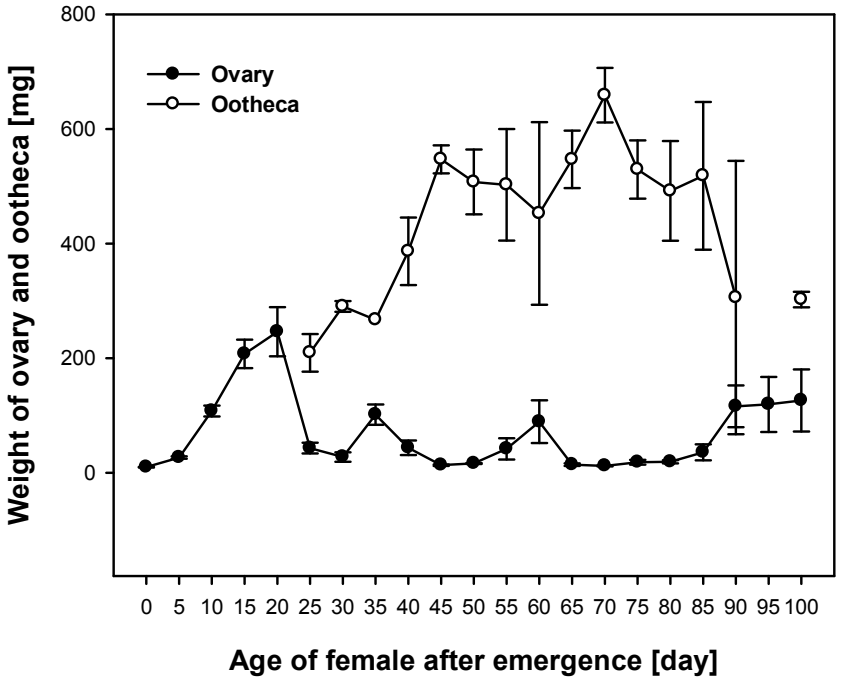

Fig. 4b. The weight of ovary and ootheca of *B. dubia* females (experiment II). Means ± S.E. For significant differences in ovary and ootheca weight at different ages (One Way ANOVA on Ranks with posthoc-test: Dunn´s) see Tables 5 a, b; n = 10.

The weight of the female fat body decreased from about 400 mg at adult emergence to 300 mg at days 15 to 25 after moulting, when the first eggs were found in the ootheca (Fig. 5). A significant increase in the fat body weight up to about 500 mg occurred between day 25 and 40 to 55 of adult life, at the time of ootheca growth and egg development during gestation. A slight drop in fat body fresh weight was observed at the end of the gestation period (day 70 ± 5 after ecdysis). Another increase in fat body weight after day 80 of female adult life indicates the beginning of the second gonadotropic cycle.

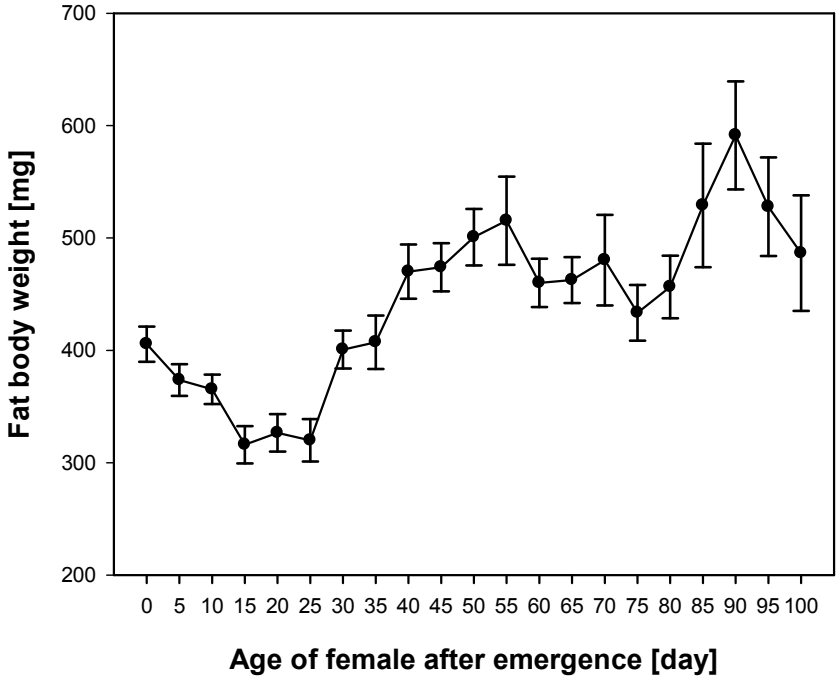

Fig. 5. The whole fat body weight of *B. dubia* females. Means ± S.E. For significant differences in fat body weight at different ages (One Way ANOVA on Ranks with posthoc-test: Dunn´s) see Table 6; n = 20. Results from experiment I and II were pooled.

3.1.3 Lipid content in mg per fat body and in µg per mg fat body fresh mass

Absolute amounts and changes in fat body lipid content per whole fat body were approximately similar in both experiments I and II during the first 80 days of female adult life (Figs. 6a, b). The lipid content of the fat body dropped from 130-160 mg per

fat body at early days after emergence to a minimum of 80-100 mg per fat body around days 15 to 20. The following increase in the fat body lipid content ended in another drop of lipids at days 55 to 60 of adult life. However, because of high variance in individual fat body lipid values, the differences were not statistically significant. A significant increase in fat body lipid content was observed beween day 20 and 85 of adult life in experiment II.

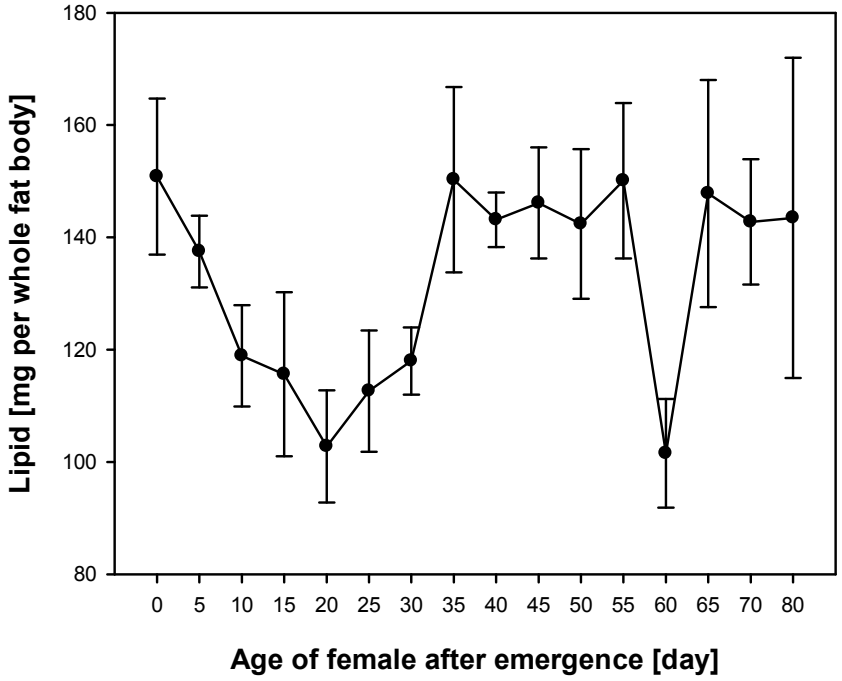

Fig. 6a. Total lipid content per fat body [mg] of female *B. dubia* (experiment I). Means ± S.E. No significant differences in lipid content at different ages was observed (One Way ANOVA on Ranks with posthoc-test: Dunn´s); n = 10.

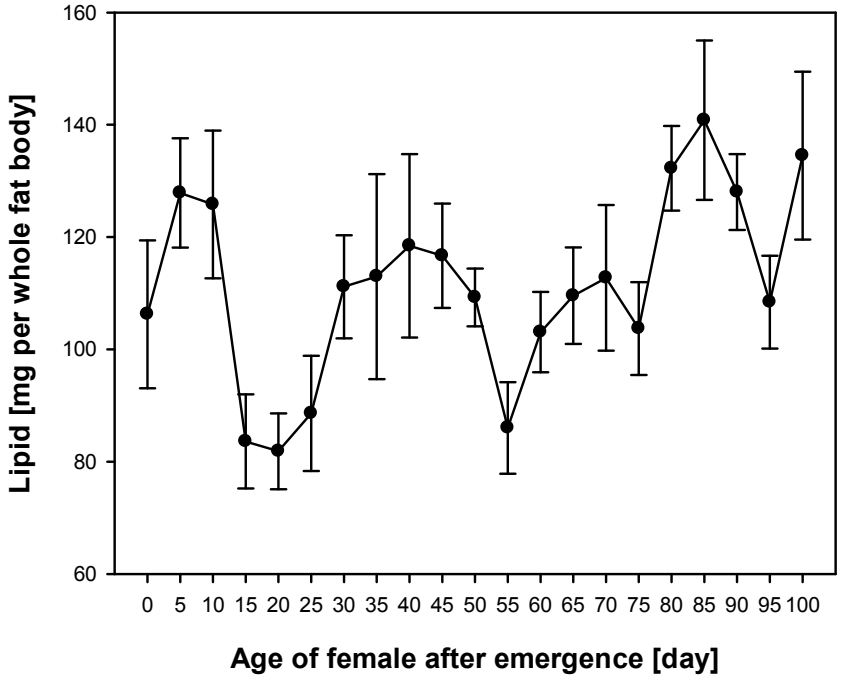

Fig. 6b. Total lipid content per fat body [mg] of female *B. dubia* (experiment II). Means ± S.E. Significant differences where oberved between day 20 and day 85 (One Way ANOVA with posthoc-test: Holm-Sidak); n = 10.

When the lipid contents of the fat body calculated per mg fat body mass (Figs. 7a, b), a significant decrease in fat body lipids was observed from adult emergence to days 50 to 55 of adult life in both experiments I and II. In experiment II, where lipid contents were followed until day 100 of adult life, another significant increase in lipids occurred from day 55 to 80.

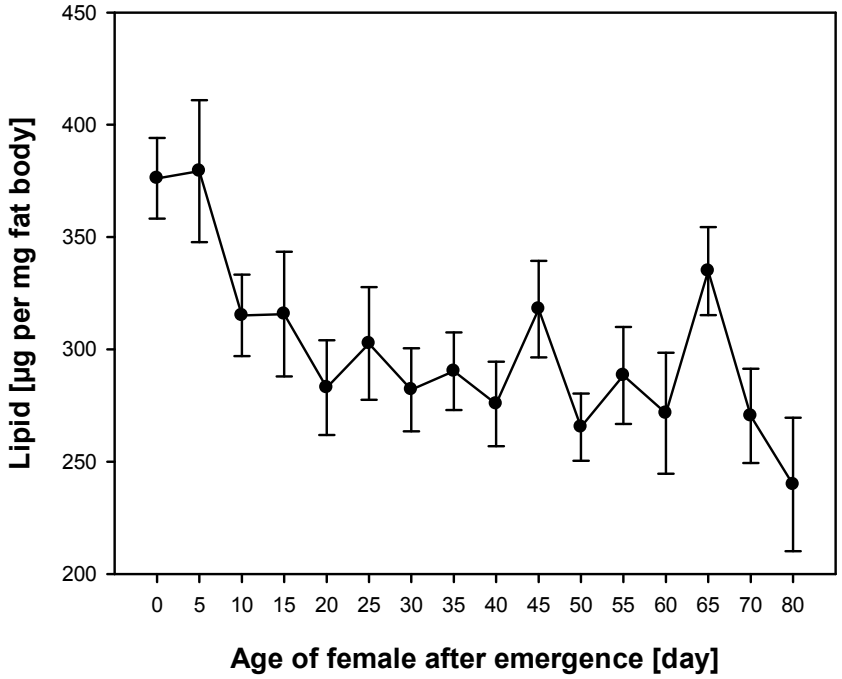

Fig. 7a. Lipid content per mg fat body fresh mass of *B. dubia* females (experiment I). Means ± S.E. There is significant difference ($p<0.05$) between day 5 and 50, and day 5 and 80, respectively (One Way ANOVA with posthoc-test: Holm-Sidak); n = 10.

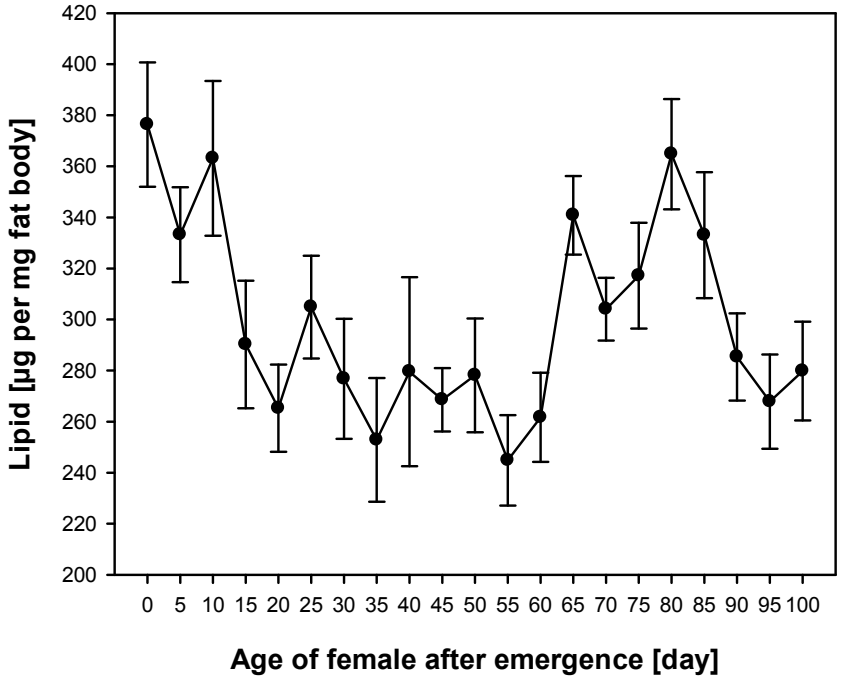

Fig. 7b. Lipid content per mg fat body fresh mass of *B. dubia* females (experiment II). Means ± S.E. For significant differences in lipid content at different ages (One Way ANOVA with posthoc-test: Holm-Sidak) see Table 7; n = 10.

3.1.4 Protein content in mg per fat body and in µg per mg fat body fresh mass

Both the total protein content and the protein content per mg fat body mass of females was low in animals after ecdysis, and high in animals during the egg case carrying period until day 70 (Figs. 8a, b and Figs. 9a, b). This increase in protein content of the fat body was statistically significant in both experiments I and II, but protein contents were generally higher in the second than in the first experiment. A distinct but statistically not significant increase in the protein content of the fat body was also observed during the ovarian growth period of the first gonadotropic cycle (day 15 in experiment I).

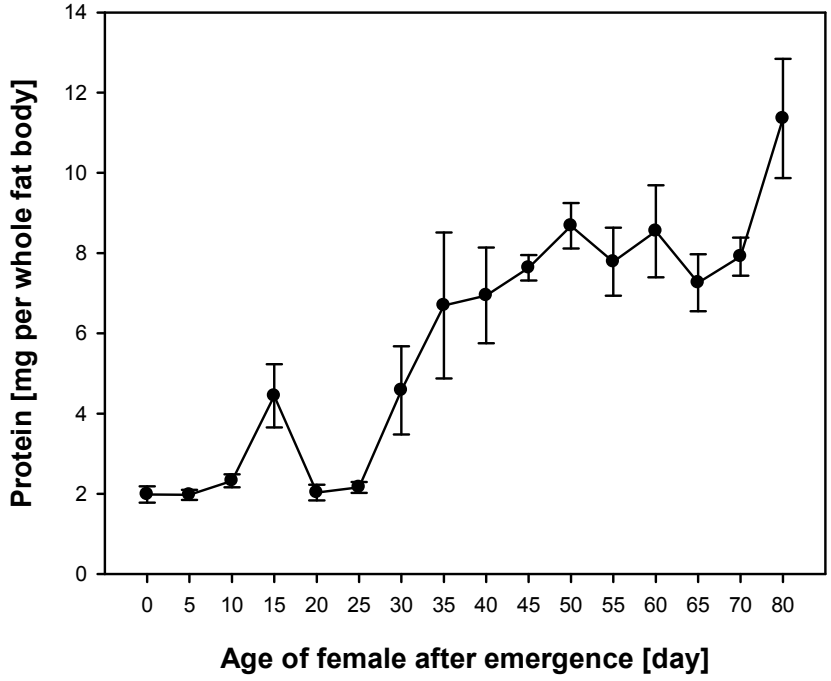

Fig. 8a. Total protein content per fat body [mg] of *B. dubia* females (experiment I). Means ± S.E. For significant differences in protein content at different ages (One Way ANOVA on Ranks with posthoc-test: Dunn´s) see Table 8a; n = 10.

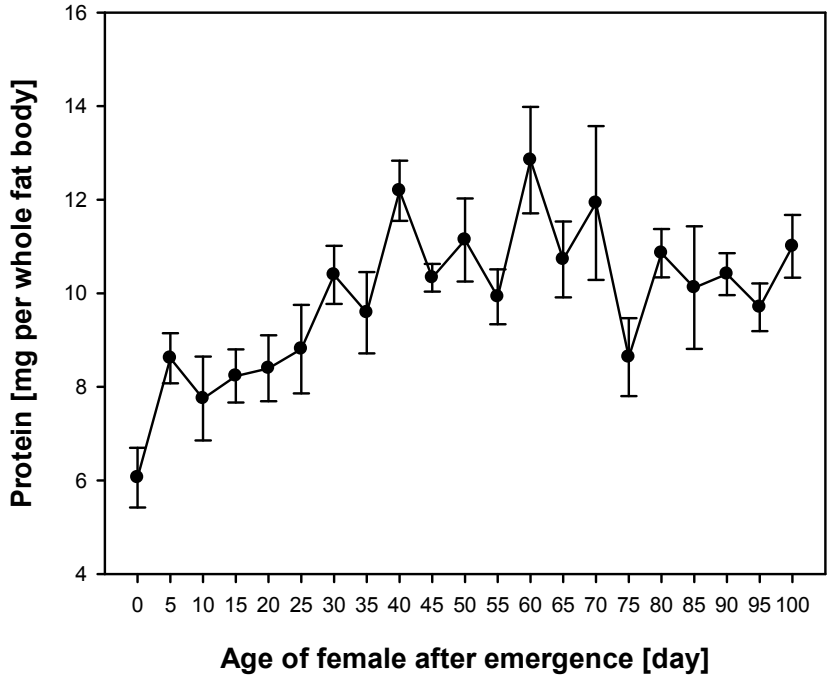

Fig. 8b. Total protein content per fat body of *B. dubia* females (experiment II). Means ± S.E. For significant differences in protein content at different ages (One Way ANOVA on Ranks with posthoc-test: Tukey) see Table 9; n = 10.

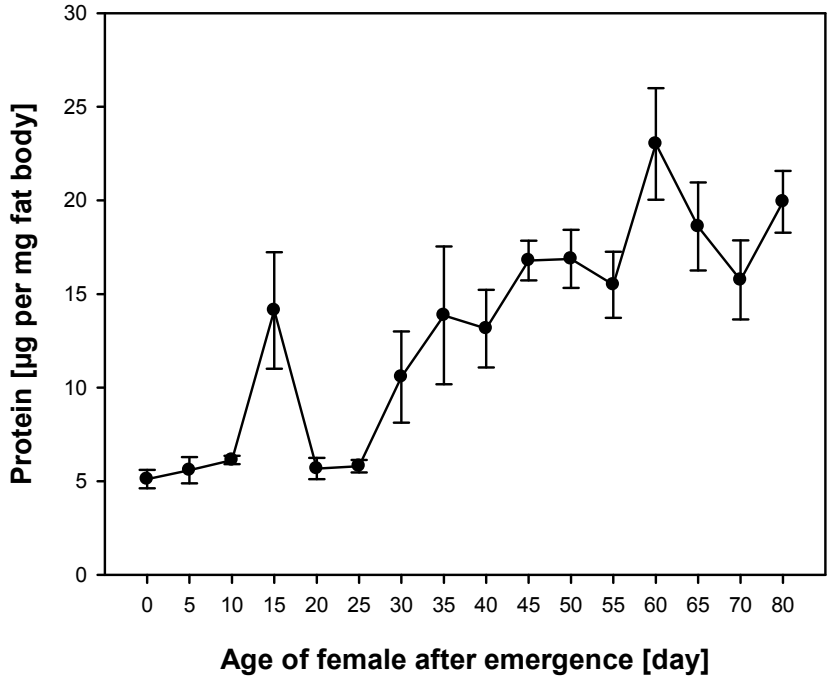

Fig. 9a. Protein content per mg fat body fresh mass of *B. dubia* females (experiment I). Means ± S.E. For significant differences in protein content at different ages (One Way ANOVA on Ranks with posthoc-test: Dunn´s) see Table 8b; n = 10.

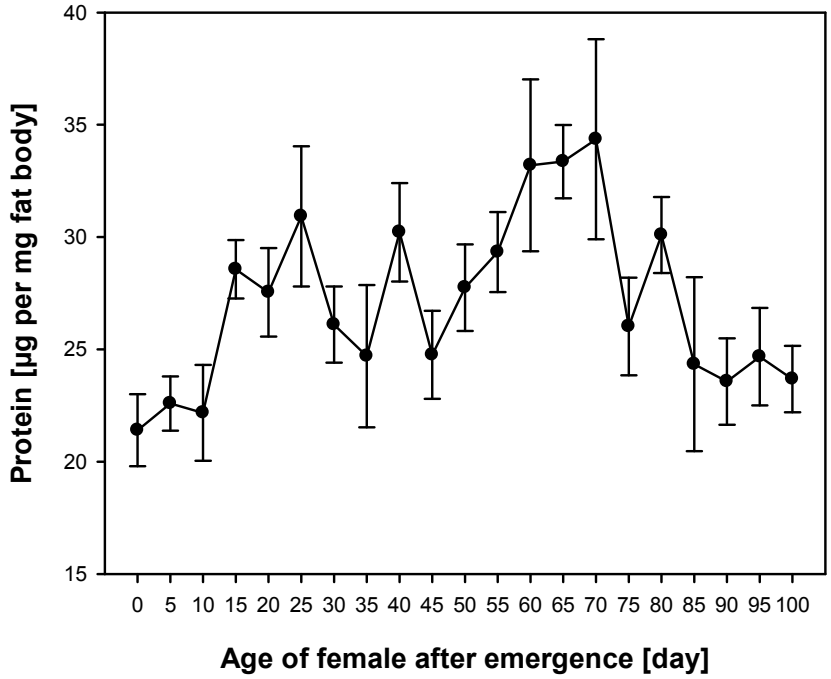

Fig. 9b. Protein content per mg fat body fresh mass of *B. dubia* females (experiment II). Means ± S.E. A significant difference ($p<0.05$) in the protein content between day 0 and day 65 was observed (One Way ANOVA on Ranks with posthoc-test: Tukey); n = 10.

3.1.5 Free carbohydrate content in mg per fat body and in µg per mg fat body fresh mass

In both experiments free carbohydrate content per whole fat body was highest in the midst of the gestation period (days 40 to 45) and dropped towards hatching of the nymphs. Similar, but statistically not significant changes of free carbohydrates per fat body were observed during the ovarian growth period around day 15 (Figs. 10a, b). When free carbohydrate contents were calculated per mg fat body fresh mass, results were very similar to those shown above (Figs. 11a, b). In the second experiment some higher carbohydrate values were measured at all ages compared to the first experiment.

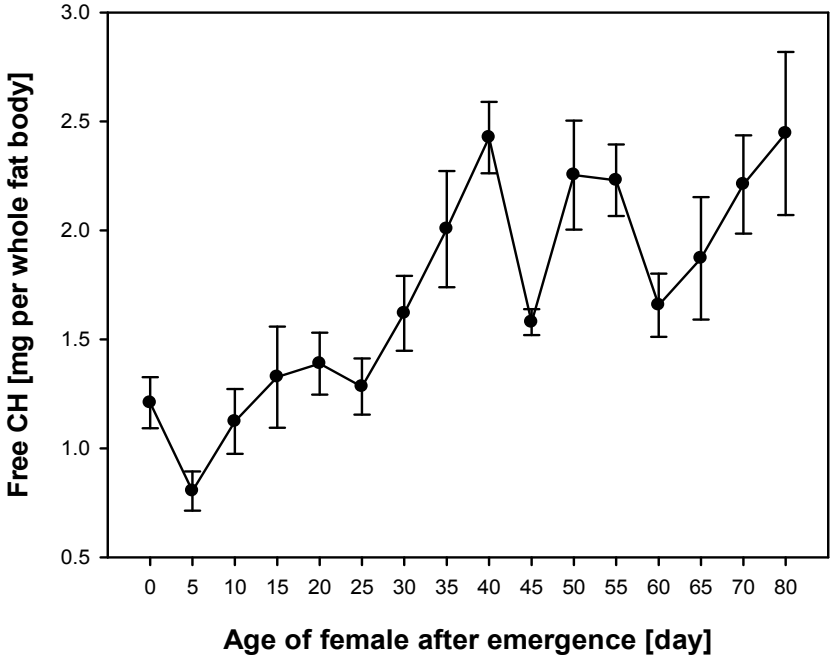

Fig. 10a. Free carbohydrate content per fat body of *B. dubia* females (experiment I). Means ± S.E. For significant differences in free carbohydrate content at different ages (One Way ANOVA on Ranks with posthoc-test: Dunn´s) see Table 10a; n = 10.

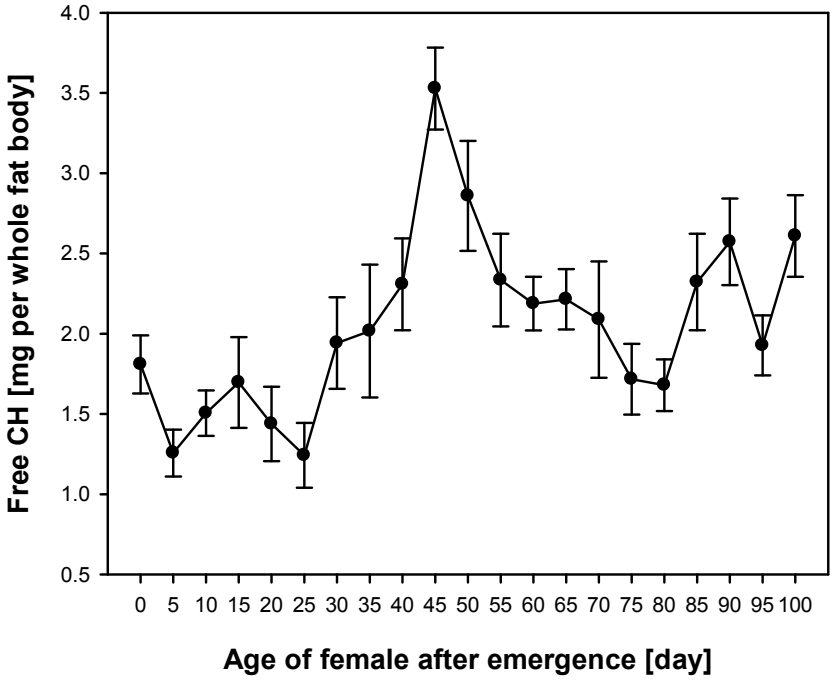

Fig. 10b. Free carbohydrate content per fat body of *B. dubia* females (experiment II). Means ± S.E. For significant differences in free carbohydrate content at different ages (One Way ANOVA on Ranks with posthoc-test: Tukey) see Table 11a; n = 10.

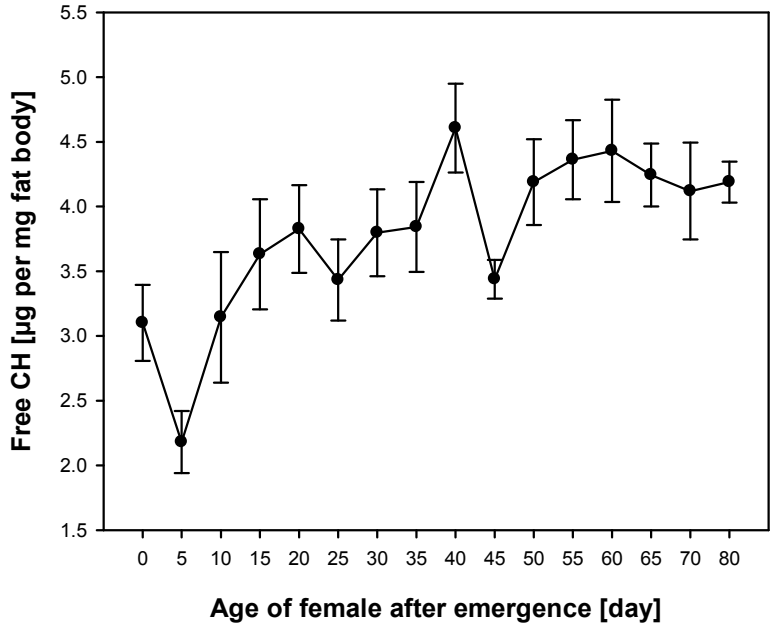

Fig. 11a. Free carbohydrate content per mg fat body fresh mass of *B. dubia* females (experiment I). Means ± S.E. For significant differences in free carbohydrate content at different ages (One Way ANOVA with posthoc-test: Holm-Sidak) see Table 10b; n = 10.

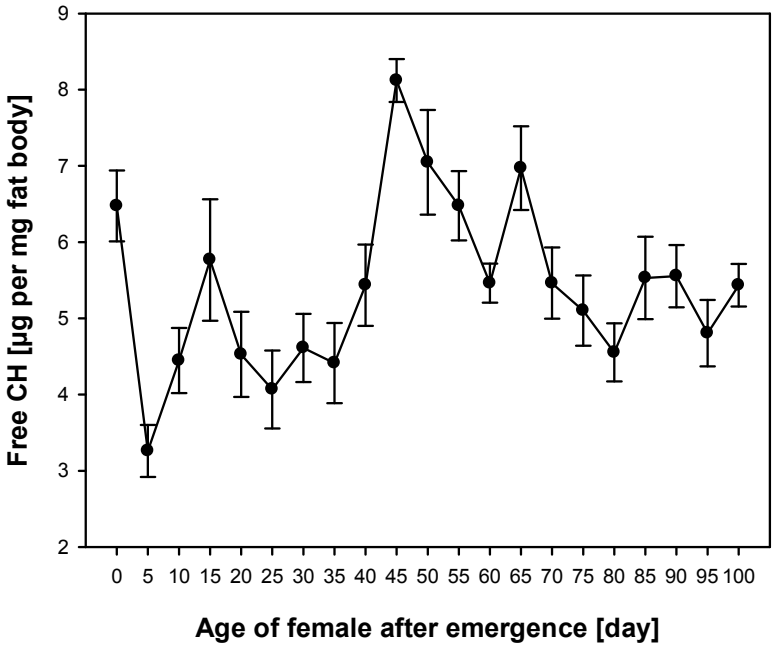

Fig. 11b. Free carbohydrate content per mg fat body fresh mass of *B. dubia* females (experiment II). Means ± S.E. For significant differences in free carbohydrate content at different ages (One Way ANOVA with posthoc-test: Holm-Sidak) see Table 11b; n = 10.

3.1.6 Glycogen content in mg per fat body and in µg per mg fat body fresh mass

The glycogen content of the fat body roughly paralleled that for the free carbohydrates, with high values in the midst of ovarian growth period and during the gestation period and low values at the times of ootheca formation and hatching of the nymphs (around days 20 and 70, respectively). Changes during the ovarian growth period, however, were much lower than during the gestation period. In the long-term experiment II, another significant increase in glycogen content towards a second gonadotropic cycle is indicated (day 90) (Fig. 12b). Results for the glycogen content expressed in µg per mg fat body mass do not differ much from those per fat body (Figs. 13a, b).

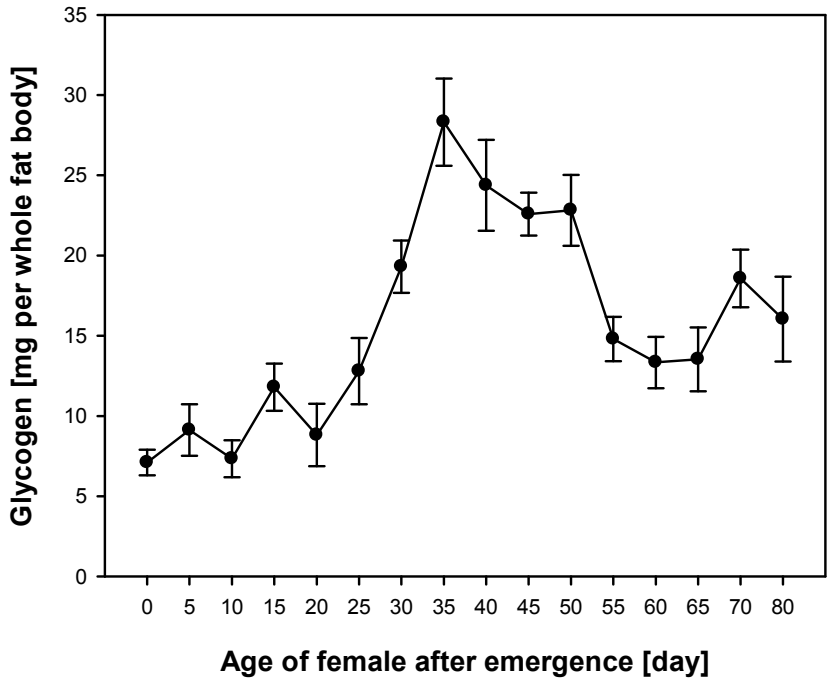

Fig. 12a. Total glycogen content per fat body of *B. dubia* females (experiment I). Means ± S.E. For significant differences in glycogen content at different ages (One Way ANOVA with posthoc-test: Holm-Sidak) see Table 12a; n = 10.

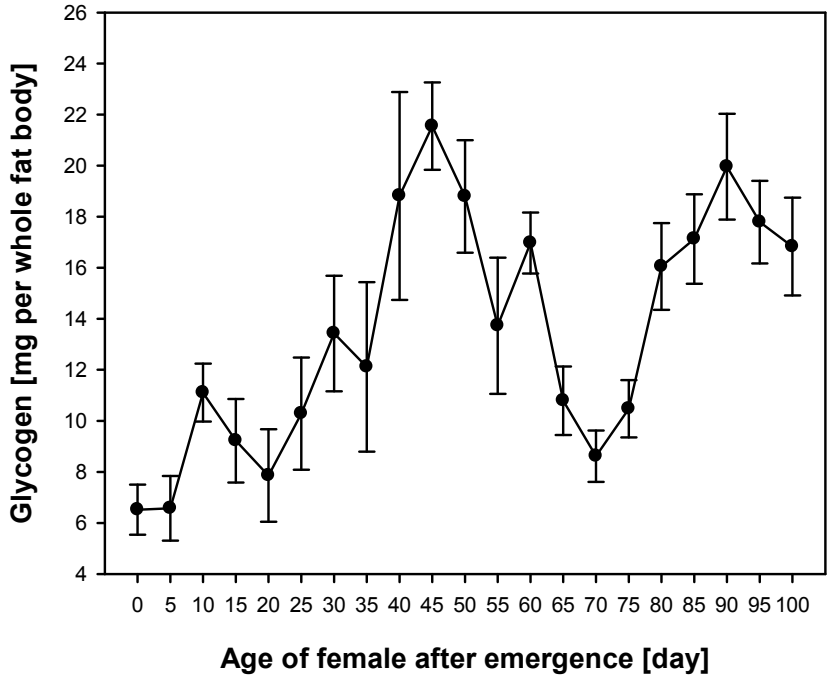

Fig. 12b. Total glycogen content per fat body of *B. dubia* females (experiment II). Means ± S.E. For significant differences in glycogen content at different ages (One Way ANOVA on Ranks with posthoc-test: Tukey) see Table 13a; n = 10.

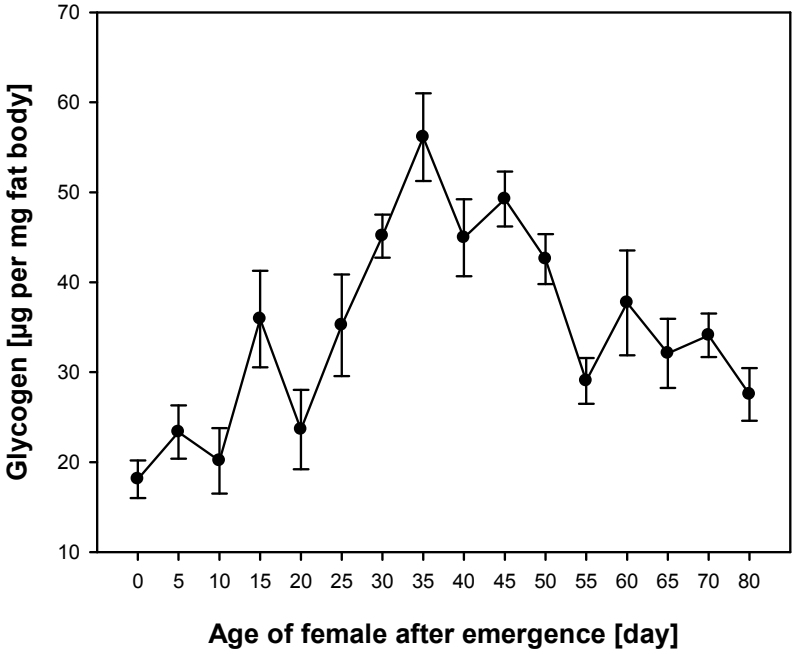

Fig. 13a. Glycogen content per mg fat body fresh mass of *B. dubia* females (experiment I). Means ± S.E. For significant differences in glycogen content at different ages (One Way ANOVA on Ranks with posthoc-test: Dunn´s) see Table 12b; n = 10.

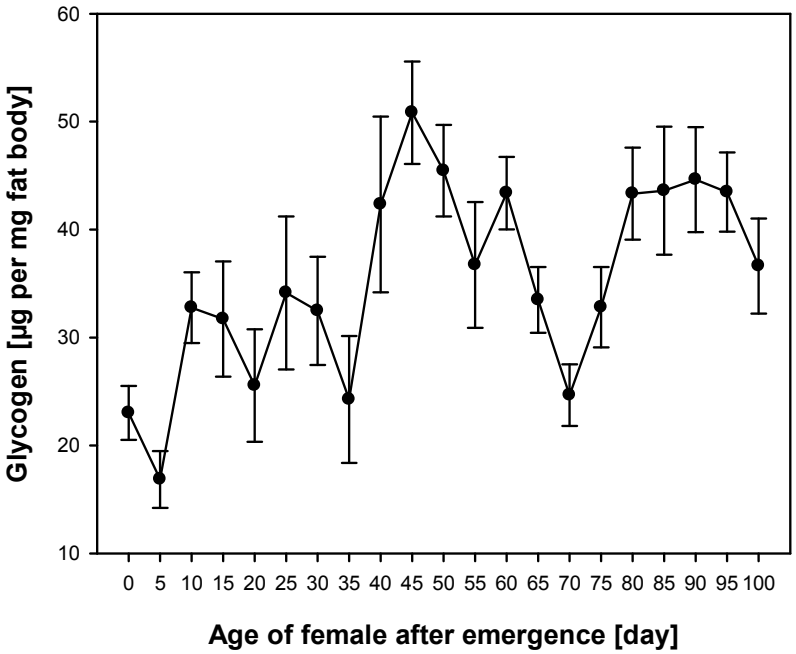

Fig. 13b. Glycogen content per mg fat body fresh mass of *B. dubia* females (experiment II). Means ± S.E. For significant differences in glycogen content at different ages (One Way ANOVA on Ranks with posthoc-test: Tukey) see Table 13b; n = 10.

3.1.7 Juvenile hormone (JH) and ecdysteroid (Ec) titers in the haemolymph of *B. dubia* females from day 0 to 100, and from day 130 to 160 after ecdysis

Since it is well known that juvenile hormones and ecdysteroids are involved in the regulation of vitellogenesis, ovarian growth and oviposition of most insects, we measured the titers of JH III, ecdysone and 20-hydroxyecdysone in the haemolymph of adult female cockroaches during the ovarian growth period and the gestation time by HPLC-mass spectrometry. JH III was the only JH homologue found in the haemolymph of the animals. Results from both experiments I and II (Figs. 14a, b) show low concentrations of JH III at the beginning of the ovarian growth and of the gestation period (day 0 and day 30, respectively), but significant peaks towards the end of each period (maxima at days 10 to 15 and 60 to 70, respectively). Another significant increase in the JH III titer around day 85 indicates the vitellogenesis during the second gonadotropic cycle in the females. In older females (130-160 days after emergence) (Fig. 14c), however, only slight changes in the JH III titer of the haemolymph were observed. The drop in haemolymph JH III concentration at day 155 may indicate ootheca deposition of the second gonadotropic cycle.

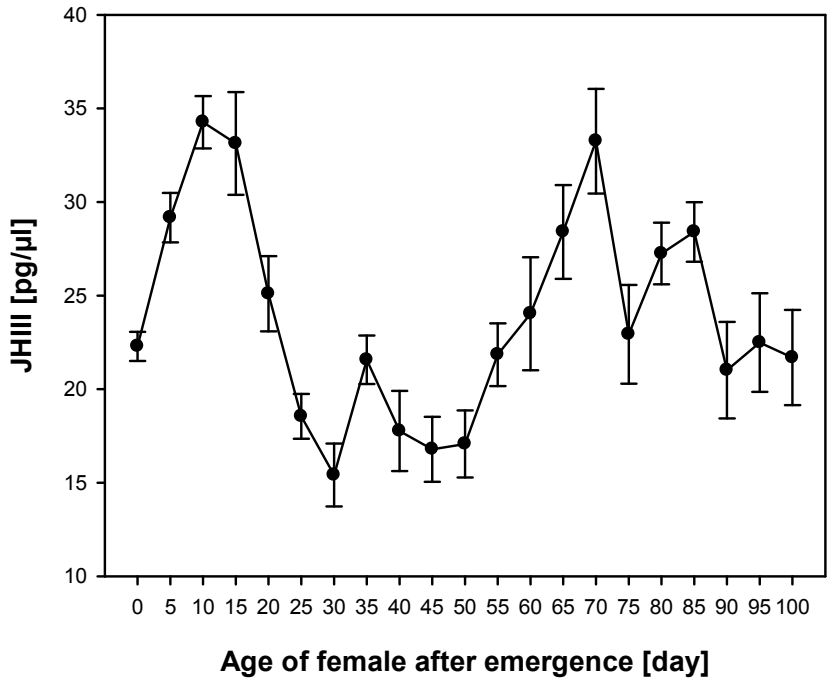

Fig. 14a. JH III titer in the haemolymph of *B. dubia* females (experiment I). Means ± S.E. For significant differences in JH III titer at different ages (One Way ANOVA with posthoc-test: Holm-Sidak) see Table 14a; n = 10.

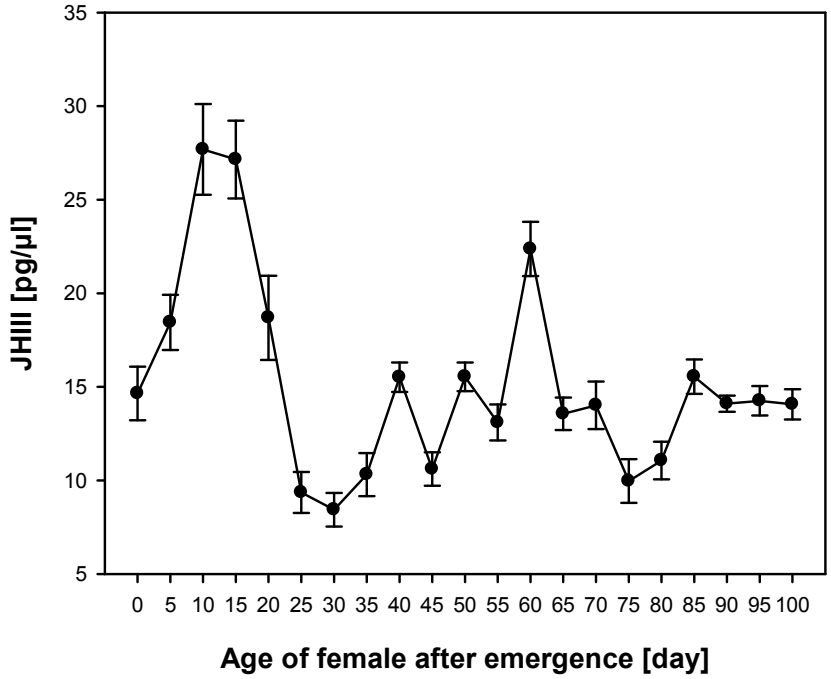

Fig. 14b. JH III titer in the haemolymph of *B. dubia* females (experiment II). Means ± S.E. For significant differences in JH III titer at different ages (One Way ANOVA on Ranks with posthoc-test: Tukey) see Table 14b; n = 10.

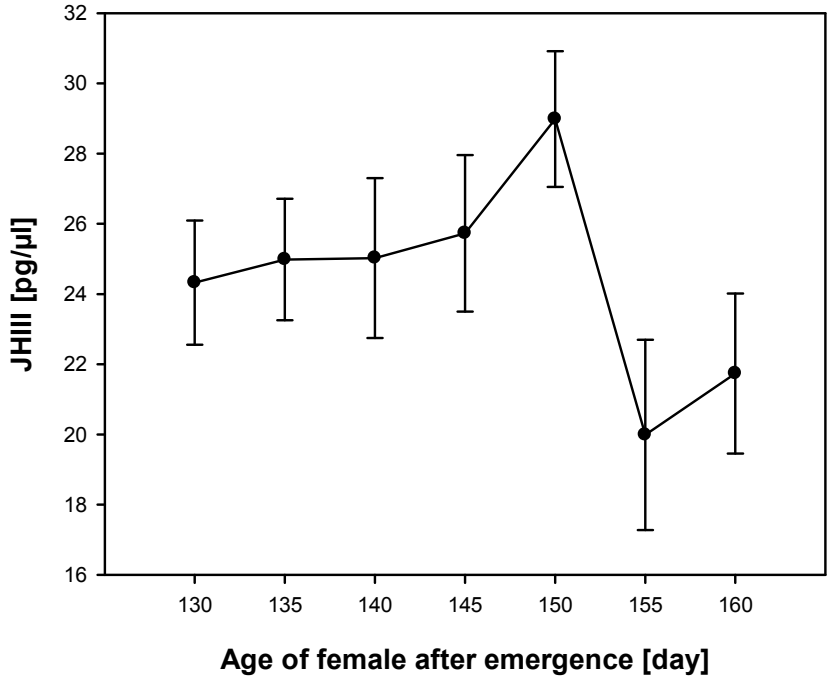

Fig. 14c. JH III titer in the haemolymph of older *B. dubia* females. Means ± S.E. There are no significant differences in the JH III titer at different ages (One Way ANOVA on Ranks); n = 10.

Results for the concentration of ecdysone (Fig. 15a) and 20-hydroxyecdysone (Fig. 16a) in the haemolymph of adult females from experiment I show some age-and development-dependent changes, which however are not statistically different. Much clearer changes in haemolymph ecdysteroid concentrations were found in the second experiment (Fig. 15b). Haemolymph ecdysone concentration was high during early ovarian development and in the earlier gestation period, but dropped significantly to a minimum a few days before ootheca formation and shortly before ootheca deposition and hatching of the nymphs. Another peak at day 75 indicates the start of a second gonadotropic cycle, but ecdysone titer remained constant in older females (130-160 days) (Fig. 15c). The slight drop in haemolymph ecdysone concentration at day 155 again may indicate the deposition of the ootheca in the second gonadotropic cycle. Changes in the titer of 20-hydroxyecdysone in the haemolymph were less clear than for ecdysone (Fig. 16a-c).

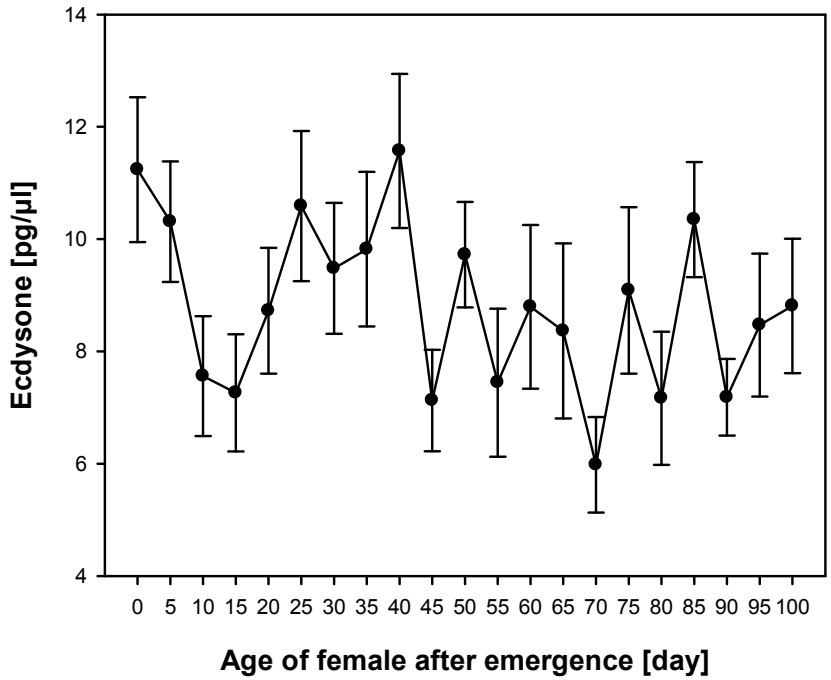

Fig. 15a. Ecdysone titer in the haemolymph of *B. dubia* females (experiment I). Means ± S.E. No significant differences (One Way ANOVA on Ranks) were observed; n = 10.

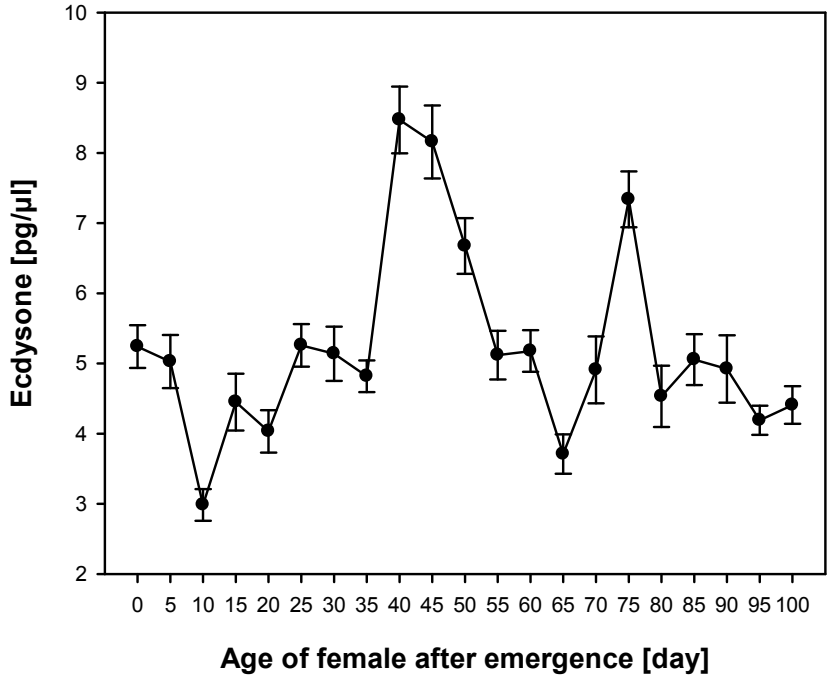

Fig. 15b. Ecdysone titer in the haemolymph of *B. dubia* females (experiment II). Means ± S.E. For significant differences in ecdysone titer at different ages (One Way ANOVA with posthoc-test: Holm-Sidak) see Table 15; n = 10.

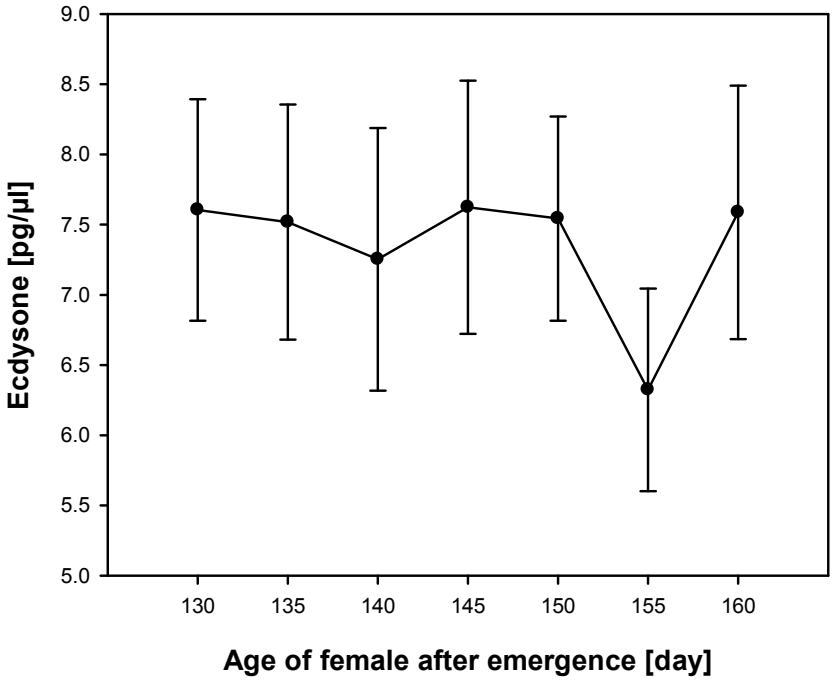

Fig. 15c. Ecdysone titer in the haemolymph of older *B. dubia* females. Means ± S.E. No significant differences (One Way ANOVA) were observed; n = 10.

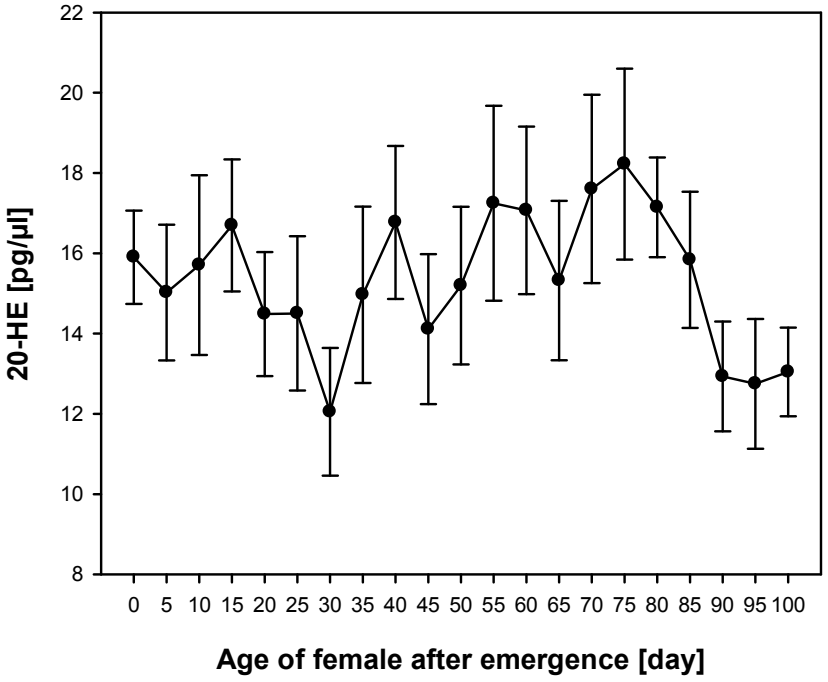

Fig. 16a. 20-Hydroxyecdysone titer in the haemolymph of *B. dubia* females (experiment I). Means ± S.E. No significant differences (One Way ANOVA on Ranks) were observed; n = 10.

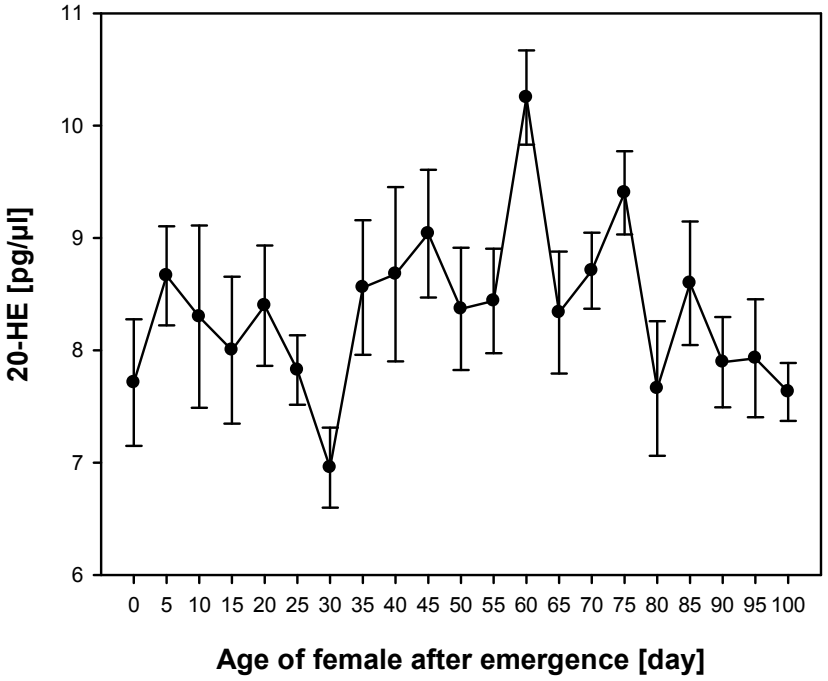

Fig. 16b. 20-Hydroxyecdysone titer in haemolymph of *B. dubia* females (experiment II). Means ± S.E. There is a significant difference ($p<0.05$) in 20-HE titer between day 30 and day 60 (One Way ANOVA on Ranks with posthoc-test: Tukey); n = 10.

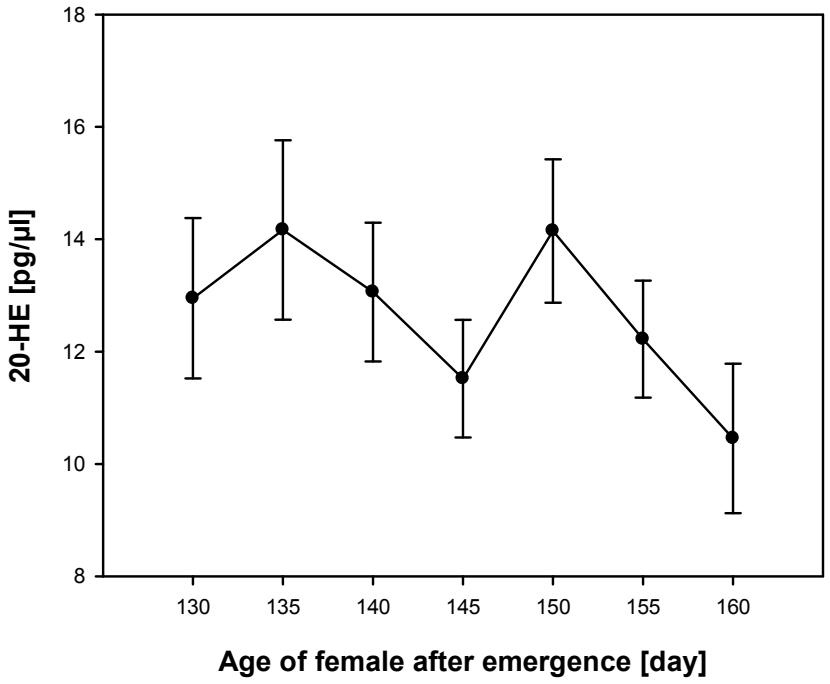

Fig. 16c. 20-Hydroxyecdysone titer in the haemolymph of older *B. dubia* females. Means ± S.E. No significant differences (One Way ANOVA) were observed; n = 10.

In general, changes in the concentration of JH III in the haemolymph of adult females are closely related to ovarian growth, ootheca formation and oviposition and are more pronounced than changes in the concentration of the ecdysteroids. In the following experiments on the effects of IGR on cockroach reproduction, therefore, we used modulators of JH titers, namely the JH analogue methoprene in experiment III and another JH mimic, pyriproxyfen in experiment IV.

3.2 Experiment III

Effects of the insect growth regulator (IGR) methoprene, a JH analogue, on reproduction and hormone titers of *B. dubia* females during the periods of ovarian growth and gestation in the first gonadotropic cycle

3.2.1 Fresh weight of adult females, ovaries and ootheca fresh mass (control and treated with methoprene) during the period of ovarian growth

Untreated cockroach females increase in fresh weight from about 1.8 g at the time of adult emergence to 2.5 g at the time of ootheca formation (day 19) (see Fig. 3). In

acetone as well as in methoprene treated animals, first oviposition occurred at day 19 ± 5, such as in untreated females, but the fresh weight of the animals did not reach more than 2.25 g in the case of the acetone treated controls and 2.08 g for the methoprene treated females (Fig. 17). This maximal body weight was reached already on day 10 after emergence. The lower weight of the methoprene treated females at that time was significantly different from the body weight of the acetone treated controls.

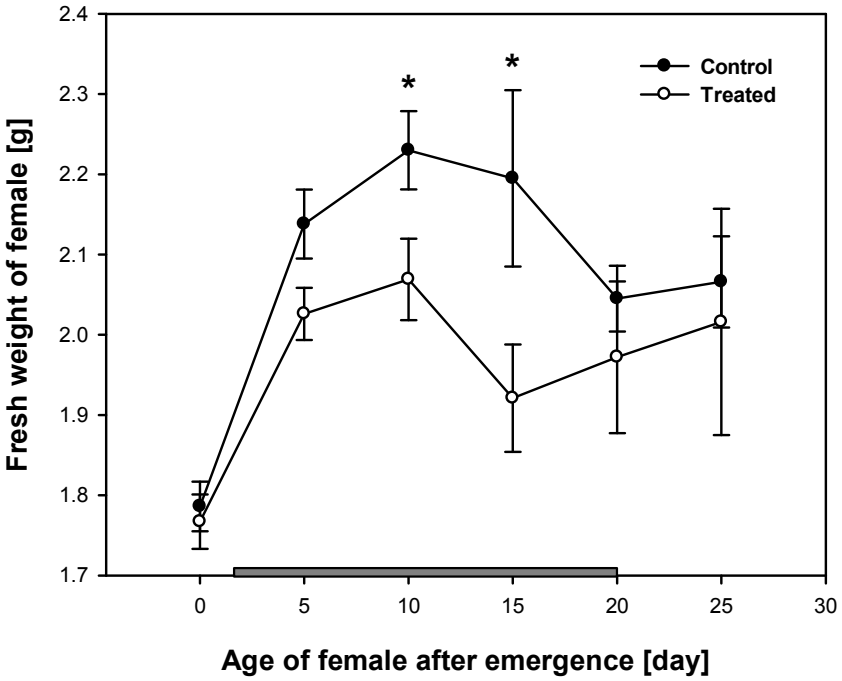

Fig. 17. Changes in the fresh weight of *B. dubia* females after treatment with methoprene during the period of ovarian growth. Control animals were treated with the solvent acetone. Data are expressed as means ± S.E. (* $p<0.05$) indicates significant differences between control and methoprene treated females (t-test d 0-15; Mann-Whitney Rank Sum Test d 20, 25). Grey horizontal bar refers to treatment time; 100 µg methoprene per treatment; n = 10.

Untreated females (see Figs. 4a, b) as well as acetone treated control animals (Fig. 18) had a very low ovarian weight at day 25 after emergence (less than 20 mg) because they had produced an ootheca at that time with a weight of about 250 mg. Methoprene treatment abolished the formation of an ootheca and the ovary weight remained high (about 150 mg) up to day 25 after emergence.

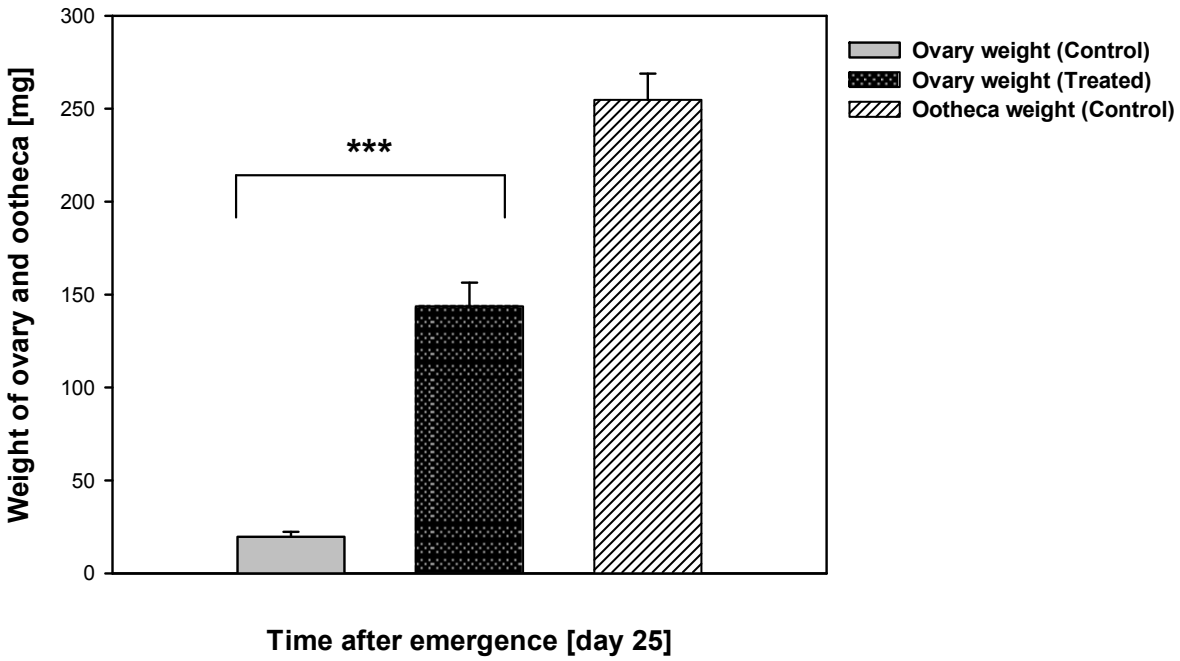

Fig. 18. The weight of ovary and ootheca from *B. dubia* females on day 25 after emergence for acetone treated controls and the ovary weight of methoprene treated females. Means ± S.E. Treatment (100 µg/female) occurred during the ovarian growth period (days 2 to 20). Methoprene treated females did not produce an ootheca. (*** $p < 0.001$) indicates a significant difference between control and methoprene treated ovary. (Mann-Whitney Rank Sum Test); n = 10.

3.2.2 Juvenile hormone (JH) and ecdysteroid (Ec) titers in the haemolymph of *B. dubia* females treated with methoprene during the period of ovarian growth

Results in Fig. 19 show a highly significant decrease in the haemolymph JH III titer of *B. dubia* females after treatment with methoprene during the entire period of the ovary growth. In acetone treated controls, JH variations in time as well as absolute amounts of JH III in the haemolymph were similar to those in untreated animals (see Fig. 14a).

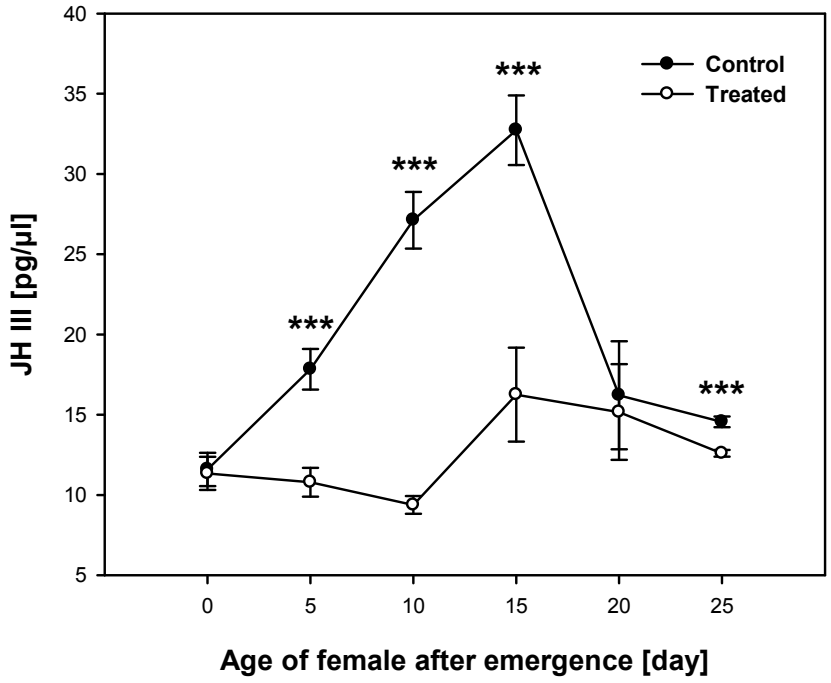

Fig. 19. JH III titer in the haemolymph of *B. dubia* females, treated with methoprene (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) during the period of ovarian growth. Data are expressed as means ± S.E. (*** p< 0.001) indicate significant differences between control and methoprene treated females (t-test d 0, 5, 15, 25; Mann-Whitney Rank Sum Test d 10, 20); n = 10.

Concentrations of ecdysone and 20-hydroxyecdysone in the haemolymph of the females were slightly higher in the methoprene treated animals compared to the acetone treated controls (Figs. 20 and 21). In the case of 20-HE, the pattern of concentration changes during ovarian growth roughly followed that for untreated females (see Fig. 16b).

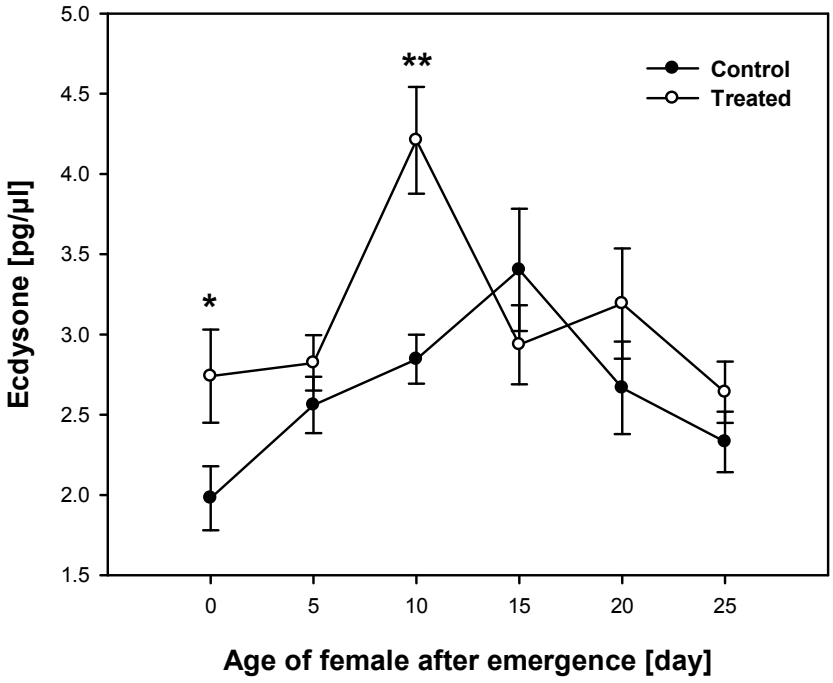

Fig. 20. Ecdysone titer in the haemolymph of *B. dubia* females treated with methoprene (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) during the period of ovarian growth. Data are expressed as means ± S.E. (* $p < 0.05$; ** $p<0.01$) indicate significant differences between control and methoprene treated females (t-test d 0, 5, 15, 25; Mann-Whitney Rank Sum Test d 10, 20); n = 10.

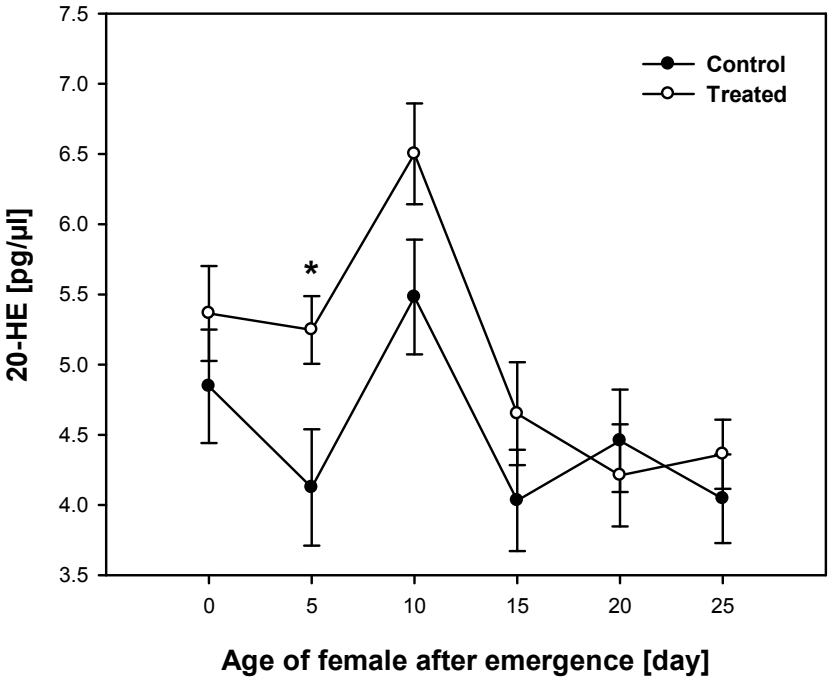

Fig. 21. 20-Hydroxyecdysone titer in the haemolymph of *B. dubia* females treated with methoprene (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) during the period of ovarian growth. Data are expressed as means ± S.E. (* $p < 0.05$) indicates a significant difference between control and methoprene treated females (t-test); n = 10.

3.2.3 Fresh weight of adult females, ovaries and ootheca fresh mass (control and treated with methoprene) during the period of gestation

The data in Fig. 22 show that following methoprene treatment from day 30 after emergence onwards, the fresh weight of the females significantly decreased compared to the acetone treated control. The final weight gain of the acetone treated controls (+ 0.8 g) was slightly lower than that of the untreated females (+ 1.0 g; see Fig. 3).

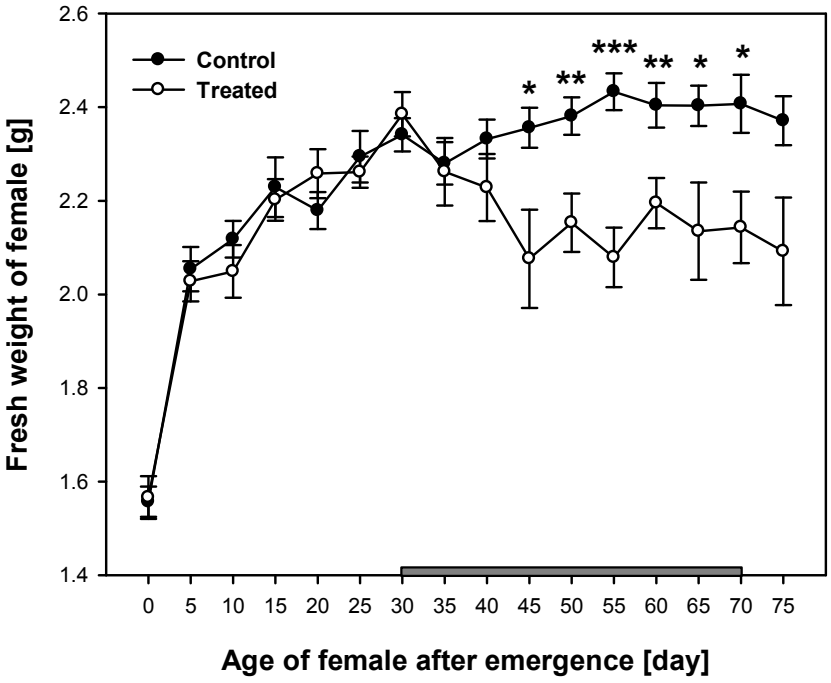

Fig. 22. Changes in the fresh weight of *B. dubia* females after treatment with methoprene during the period of gestation. Control animals were treated with the solvent acetone. Data are expressed as means ± S.E. (* $p<0.05$; ** $p<0.01$; *** $p< 0.001$) indicate significant differences between control and methoprene treated females (t-test; Mann-Whitney Rank Sum Test d 45, 65, 70, 75). Grey horizontal bar refers to treatment time; 100 µg methoprene per treatment; n = 10.

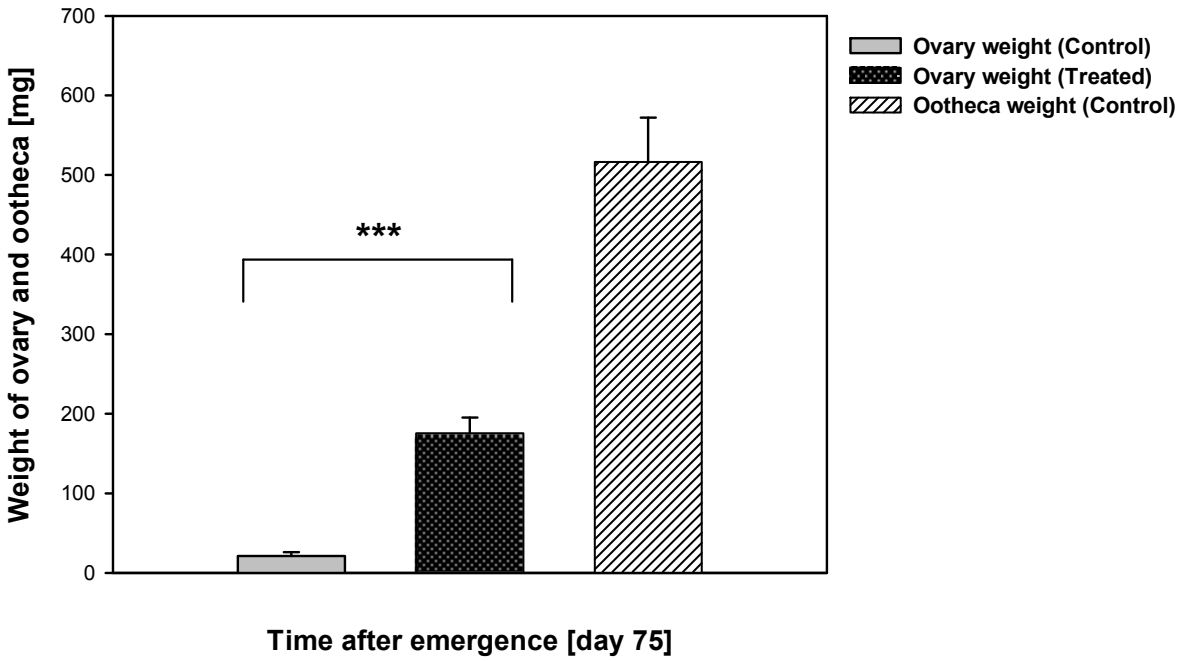

Fig. 23. The weight of ovary and ootheca from *B. dubia* females on day 75 after emergence for acetone treated controls and the ovary weight of methoprene treated females. Treatment (100 µg/female) occurred during the period of gestation (days 30 to 70). Methoprene treated females did not have an ootheca on day 75. (*** $p < 0.001$) indicates a significant difference between control and methoprene treated ovary. (Mann-Whitney Rank Sum Test); n = 10.

Untreated females (see Figs. 4a, b) as well as acetone-treated control animals (Fig. 23) had a very low ovarian weight at day 75 after emergence (less than 50 mg) because they had produced an ootheca, which had reached a mass of 550-700 mg at that time and oviposition/hatching had started. Methoprene treatment during the period of gestation led to a resorption of the ootheca and to a significant increase in the ovary weight at day 75 after emergence (Fig. 23).

3.2.4 Juvenile hormone (JH) and ecdysteroid (Ec) titers in the haemolymph of *B. dubia* females treated with methoprene during the period of gestation

Results in Fig. 24 show a distinct increase in haemolymph JH III titer of animals from both experimental groups in the midst of the egg case carrying period, similar to that in untreated females (see Fig. 14a). In methoprene treated females, however, haemolymph JH III titer significantly dropped towards days 65/70, when the ootheca was reabsorbed.

Titers of ecdysone (Fig. 25) and 20-hydroxyecdysone (Fig. 26) decreased more or less continuously from day 30 to days 50 to 75 with only slight differences between the acetone treated controls and the methoprene treated females.

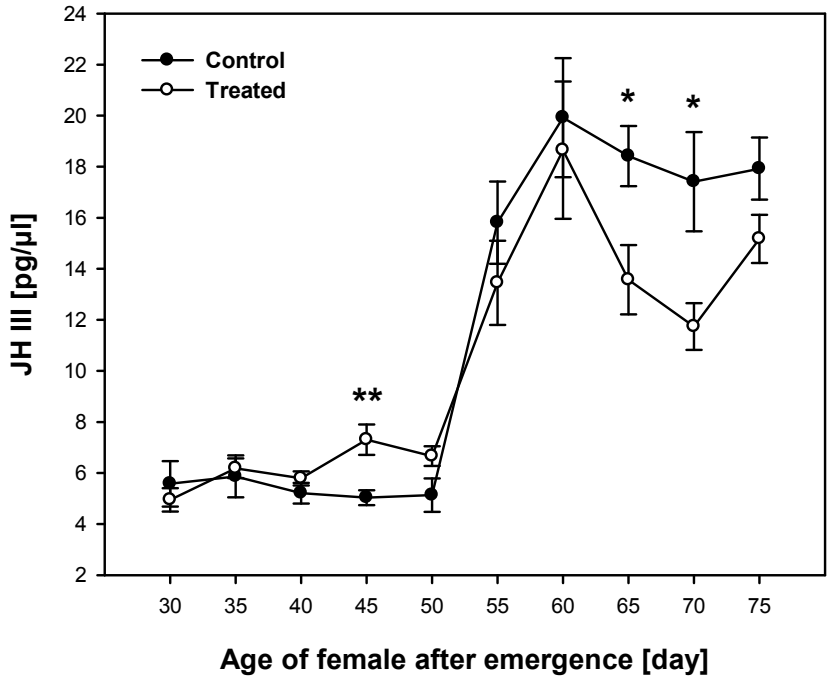

Fig. 24. JH III titer in the haemolymph of *B. dubia* females, treated with methoprene (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) during period of gestation (days 30 to 70). Data are expressed as means ± S.E. (* $p < 0.05$; ** $p < 0.01$) indicate significant differences between control and methoprene treated females (t-test; Mann-Whitney Rank Sum Test d 30, 35, 45); n = 10.

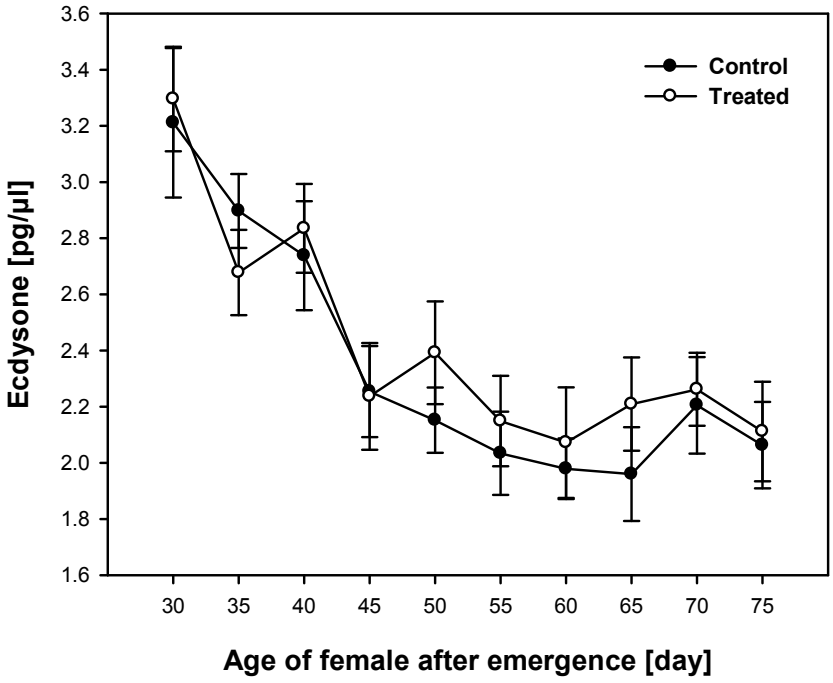

Fig. 25. Ecdysone titer in the haemolymph of *B. dubia* females treated with methoprene (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) from day 30 to 70 of adult life. Data are expressed as means ± S.E. No significant differences between control and methoprene treated animals were observed (t-test; Mann-Whitney Rank Sum Test d 45); n = 10.

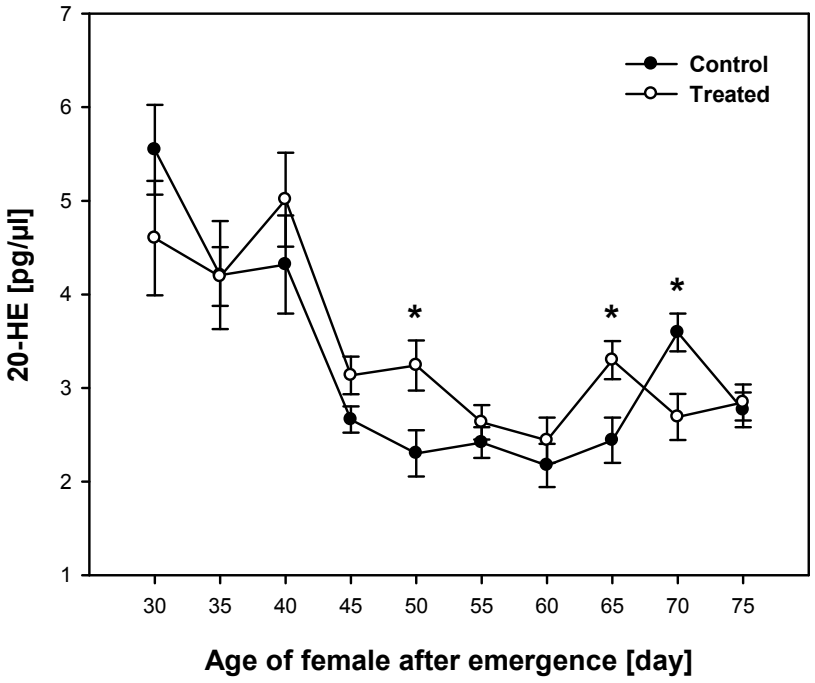

Fig. 26. 20-Hydroxyecdysone titer in the haemolymph of *B. dubia* females treated with methoprene (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) from day 30 to 70 of adult life. Data are expressed as means ± S.E. (* $p < 0.05$) indicates significant differences between control and methoprene treated animals (t-test; Mann-Whitney Rank Sum Test d 45); n = 10.

3.3 Experiment IV

Effects of pyriproxyfen, a juvenile hormone mimic, on reproduction and hormone titers of *B. dubia* females during the periods of ovarian growth and gestation in the first gonadotropic cycle

3.3.1 Fresh weight of adult females, ovaries and ootheca fresh mass (control and treated with pyriproxyfen) during the period of ovarian growth

In females from both experimental groups, an increase in fresh weight during the period of ovary growth was observed, but this increase was significantly lower in pyriproxyfen treated animals than in the acetone treated controls (Fig. 27).

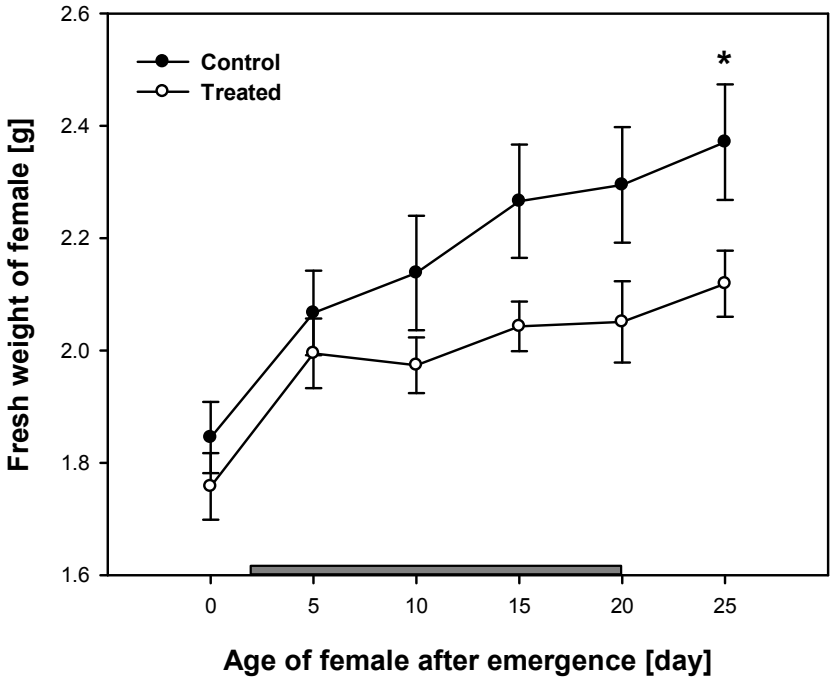

Fig. 27. Changes in the fresh weight of *B. dubia* females after treatment with pyriproxyfen during the period of ovarian growth. Control animals were treated with the solvent acetone. Data are expressed as means ± S.E. (* $p < 0.05$) indicates a significant difference between control and pyriproxyfen treated females (t-test; Mann-Whitney Rank Sum Test d 15). Grey horizontal bar refers to treatment time; 100 µg pyriproxyfen per treatment; n = 10.

Acetone treated females had a very low ovarian weight at day 25 after emergence (less than 25 mg) because they had produced an ootheca with a weight mass of about 250 mg (Fig. 28). Treatment with pyriproxyfen prevented the formation of an ootheca and the ovary weight remained high (ca. 170 mg).

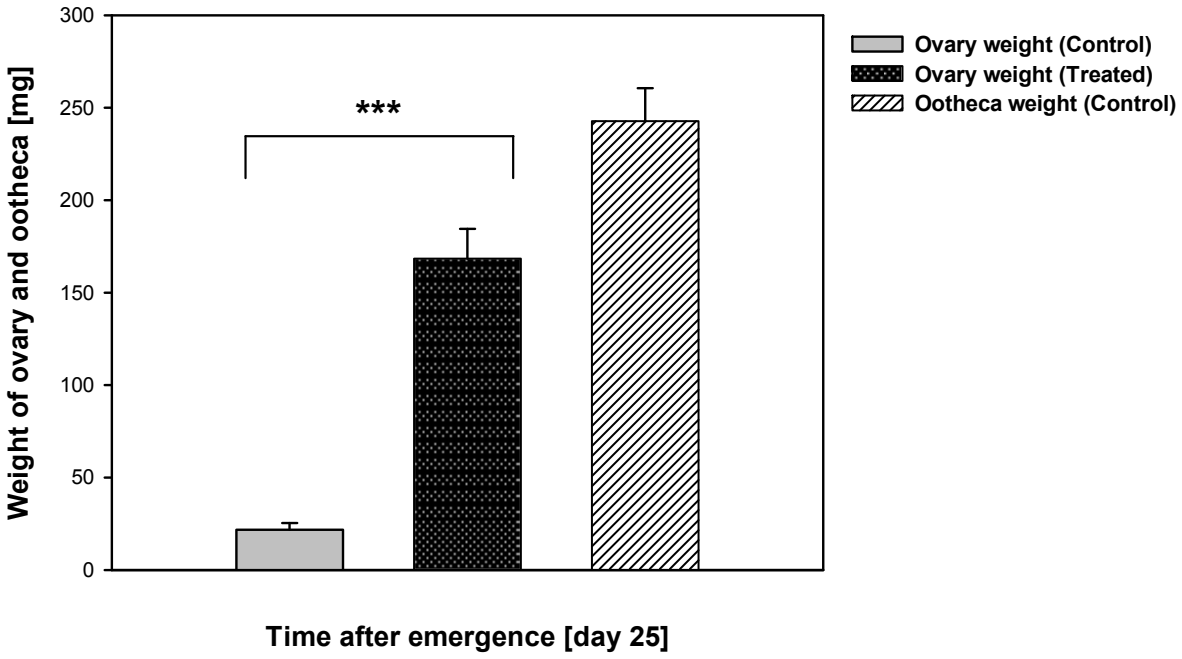

Fig. 28. The weight of ovary and ootheca from *B. dubia* females on day 25 after emergence for acetone treated controls and the ovary weight of pyriproxyfen treated females. Means ± S.E. Treatment (100 µg/female) occurred during the period of ovary growth (days 2 to 20). Pyriproxyfen treated females did not produce an ootheca. (*** $p < 0.001$) indicates a significant difference between control and pyriproxyfen treated ovary. (Mann-Whitney Rank Sum Test); n = 10.

3.3.2 Juvenile hormone (JH) and ecdysteroid (Ec) titers in the haemolymph of *B. dubia* females treated with pyriproxyfen during the period of ovarian growth

Results in Fig. 29 show a highly significant decrease in the haemolymph JH III titer of *B. dubia* females after treatment with pyriproxyfen during the entire period of the ovary growth. In acetone treated controls, JH variations in time as well as absolute amounts of JH III in the haemolymph were similar to those in untreated animals (see Fig. 14a).

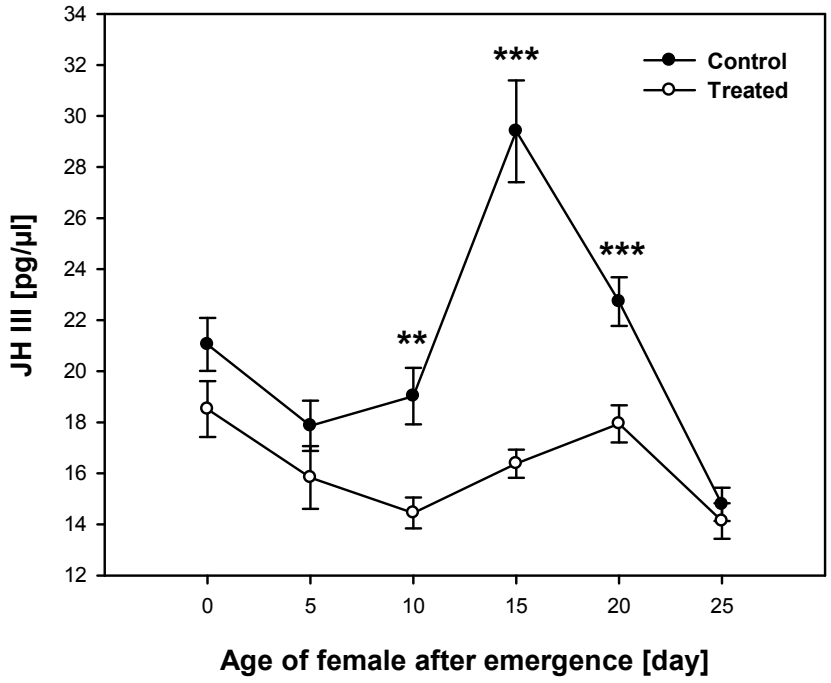

Fig. 29. JH III titer in the haemolymph of *B. dubia* females, treated with pyriproxyfen (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) during the period of ovarian growth. Data are expressed as means ± S.E. (** p< 0.01; *** p< 0.001) indicate significant differences between control and pyriproxyfen treated females (t-test; Mann-Whitney Rank Sum Test d 0, 15); n = 10.

Concentrations of ecdysone and 20-hydroxyecdysone in the haemolymph were similar to that for the treatment with methoprene (Figs. 30, 31).

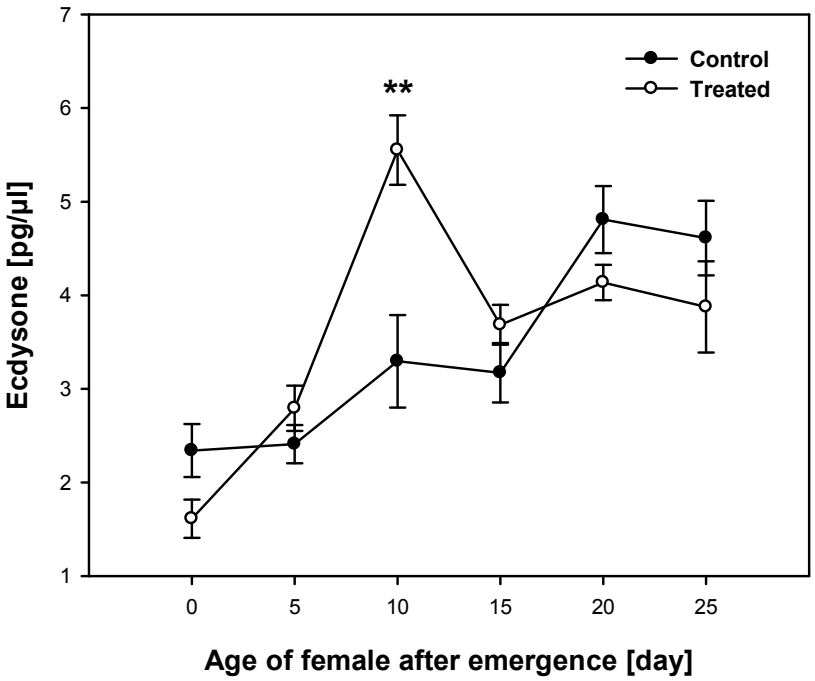

Fig. 30. Ecdysone titer in the haemolymph of *B. dubia* females treated with pyriproxyfen (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) during the period of ovarian growth. Data are expressed as means ± S.E. (** $p<0.01$) indicates a significant difference between control and pyriproxyfen treated females (t-test); n = 10.

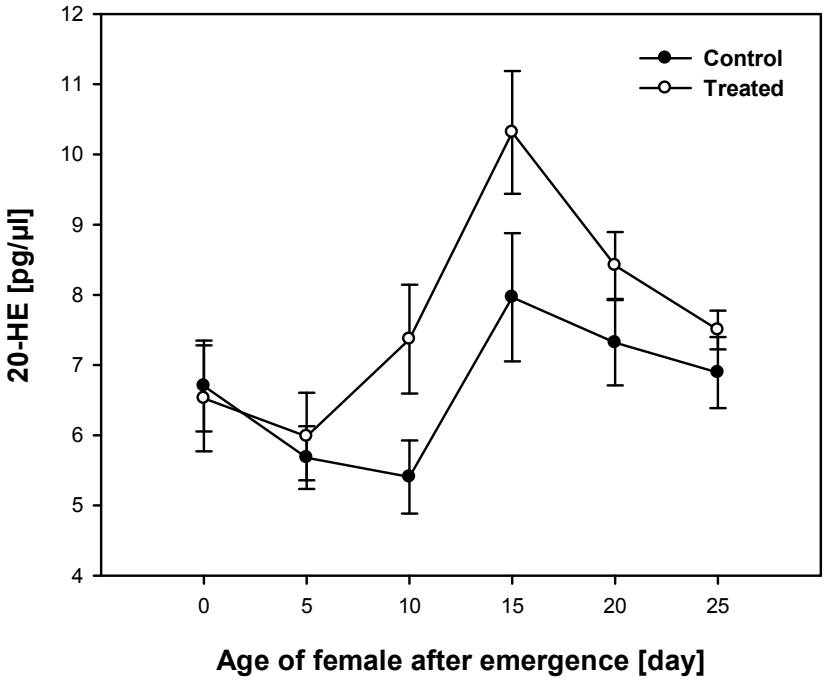

Fig. 31. 20-Hydroxyecdysone titer in the haemolymph of *B. dubia* females, treated with pyriproxyfen (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) during the period of ovarian growth. Data are expressed as means ± S.E.
No significant differences between control and pyriproxyfen treated females were observed (t-test; Mann-Whitney Rank Sum Test d 0, 10); n =10.

3.3.3 Fresh weight of adult females, ovaries and ootheca fresh mass (control and treated with pyriproxyfen) during the period of gestation

Following treatment with pyriproxyfen, the body weight of adult females decreased significantly compared to the acetone treated control animals (Fig. 32). The weight difference was similar to that after treatment with methoprene (see Fig. 22).

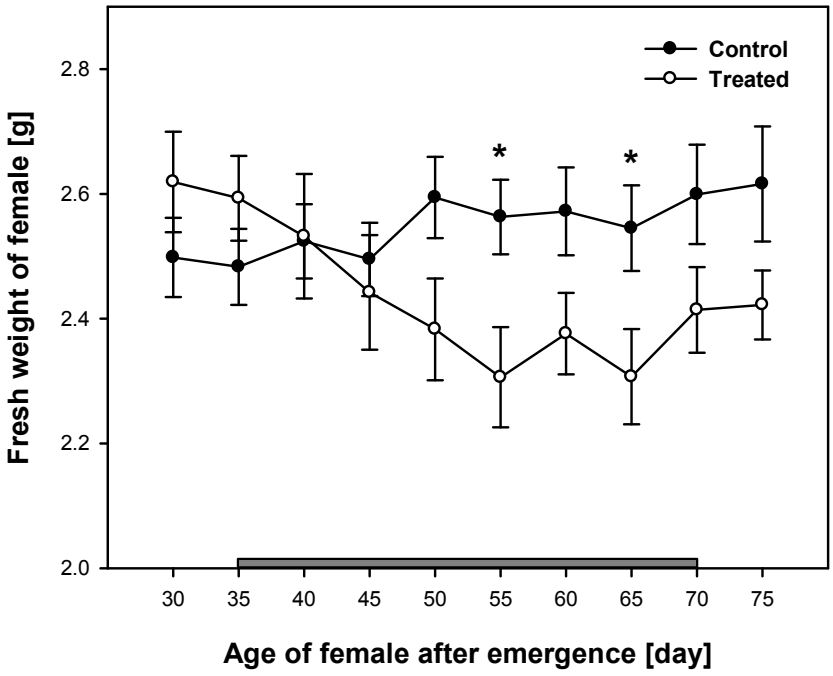

Fig. 32. Changes in the fresh weight of *B. dubia* females after treatment with pyriproxyfen during the period of gestation. Control animals were treated with the solvent acetone. Data are expressed as means ± S.E. (* $p < 0.05$) indicates significant differences between control and pyriproxyfen treated females (t-test; Mann-Whitney Rank Sum Test d 40). Grey horizontal bar refers to treatment time; n = 10.

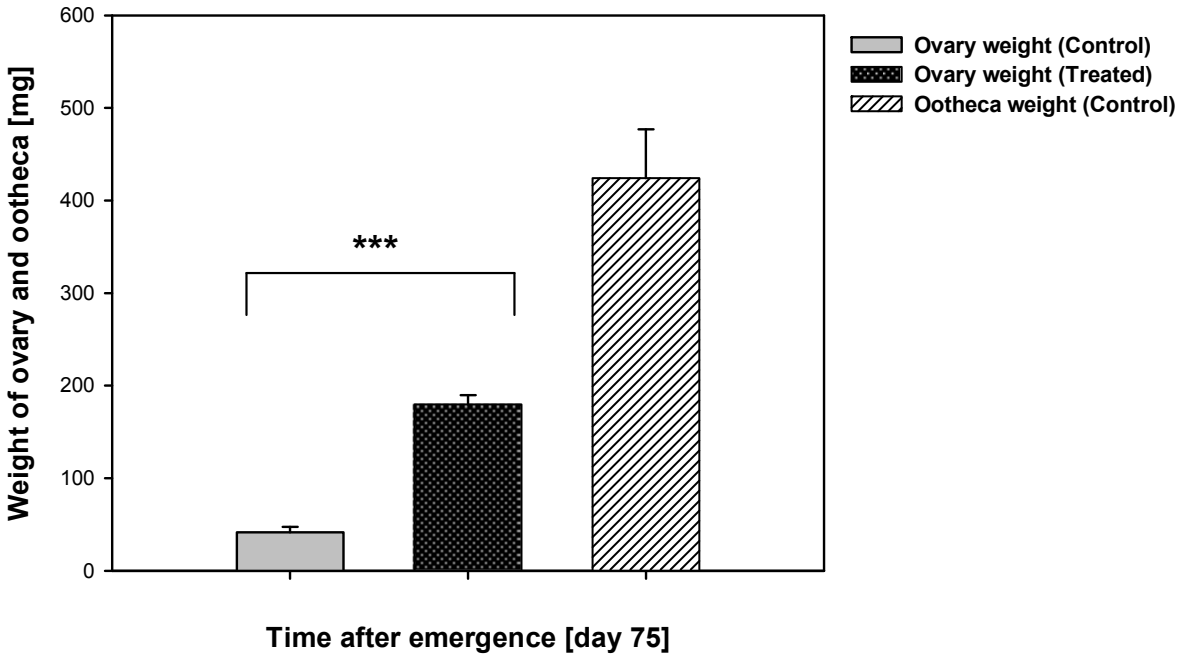

Fig. 33. The weight of ovary and ootheca from *B. dubia* females on day (75) after emergence for acetone treated controls and the ovary weight of pyriproxyfen treated females. Means ± S.E. Treatment (100 µg/female) occurred during the period of gestation (days 30 to 70). Pyriproxyfen treated females did not have an ootheca on day 75 after emergence. (*** $p < 0.001$; Mann-Whitney Rank Sum Test); n = 10.

3.3.4 Juvenile hormone (JH) and ecdysteroid (Ec) titers in the haemolymph of *B. dubia* females treated with pyriproxyfen during the gestation period

As in the case of methoprene treatment, the JH III titer in the haemolymph of pyriproxyfen treated females was significantly lowered at the end of the gestation period, when the ootheca was reabsorbed (Fig. 34).

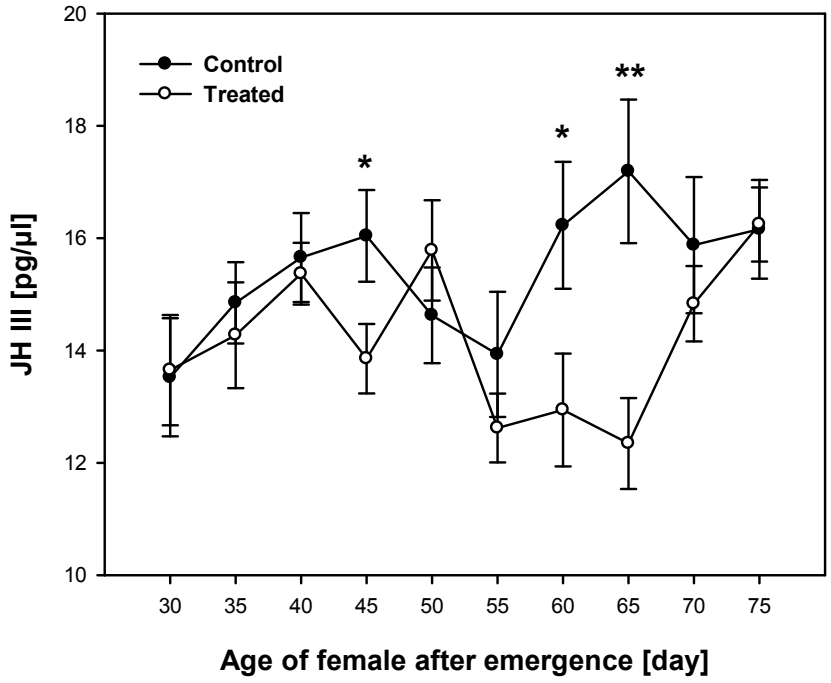

Fig. 34. JH III titer in the haemolymph of *B. dubia* females treated with pyriproxyfen (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) during the period of gestation (days 30 to 70). Data are expressed as means ± SE. (* $p < 0.05$; ** $p < 0.01$) indicate signifigant differences between control and pyriproxyfen treated females (t-test; Mann-Whitney Rank Sum Test d 55); n = 10.

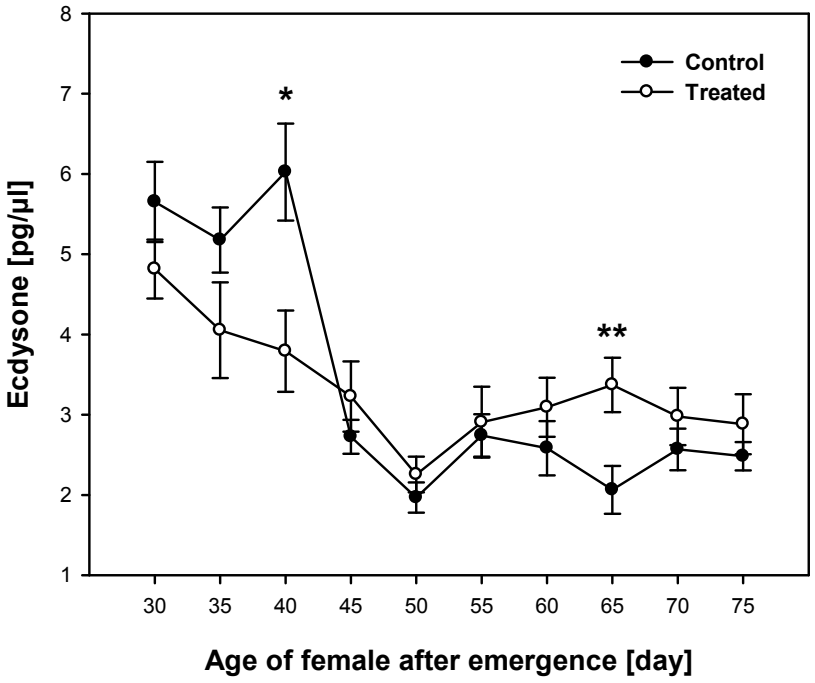

Fig. 35. Ecdysone titer in the haemolymph of *B. dubia* females treated with pyriproxyfen (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) from day 30 to day 70 of adult life. Data are expressed as means ± S.E.
(* p< 0.05; ** p< 0.01) indicate significant differences between control and pyriproxyfen treated females (t-test); n = 10.

Concentrations of ecdysone and 20-hydroxyecdysone in the haemolymph were similar to that for the treatment with methoprene (Figs. 35, 36).

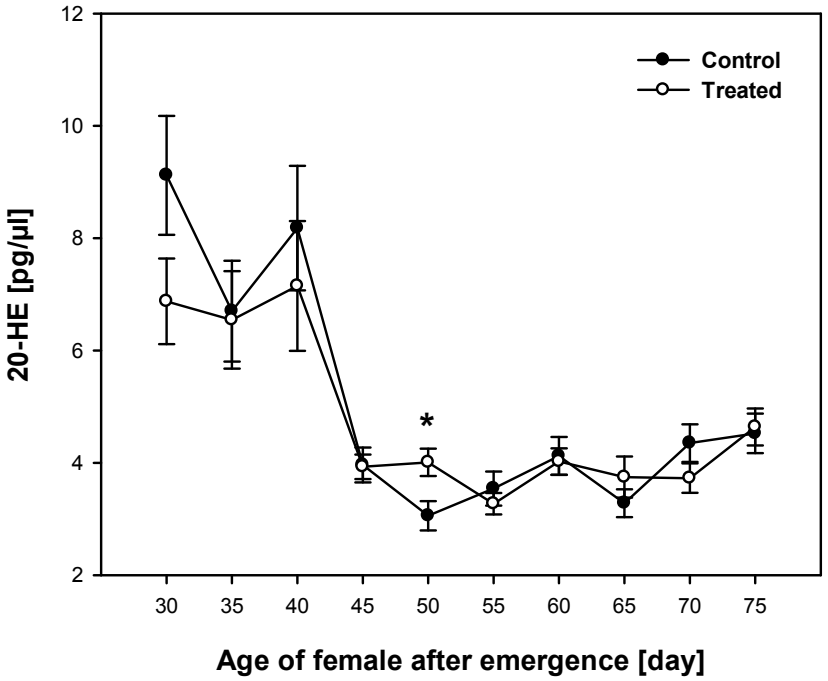

Fig. 36. 20-Hydroxyecdysone titer in the haemolymph of *B. dubia* females treated with pyriproxyfen (100 µg/female) (open circles) or with 5 µl acetone (control, closed circles) from days 30 to 70 of adult life. Data are expressed as means ± S.E.
(* $p < 0.05$) indicates a signifigant difference between control and pyriproxyfen treated females (t-test; Mann-Whitney Rank Sum Test d 40); n = 10.

4 Discussion

Only few physiological studies exist on the ovoviviparous Argentinean cockroach *B. dubia* and no information is available on the developmental hormone titers of that species. Therefore, the present study focused on the endocrine regulation of reproduction in adult females of *B. dubia*. The haemolymph titers of juvenile hormone (JH III) and free ecdysteroids (Ec, ecdysone and 20-hydroxyecdysone) were measured during ovarian maturation and gestation to see whether correlations exist between the hormone titers and the reproductive processes such as oogenesis, formation of the ootheca, gestation and oviposition/birth of the hatchings. Subsequent experiments were performed to determine whether the hormonally regulated developmental processes are reflected in changes of the chemical composition of fat body and ovarian tissues.

Another major goal of the present study was to identify and evaluate the effects of two insect growth regulators (IGR, methoprene and pyriproxyfen) on adult development, ovary growth and oviposition, hormone titers, and ovary mass of *B. dubia* females.

The term insect hormone mimics or insect growth regulators (IGR) is used to describe “third generation pesticides”, agents that disrupt the normal development of insects. The IGR include various chemical classes with different modes of action, such as the mimics of juvenile hormone [juvenile hormone analogues (JHA)] hydroprene, methoprene and phenoxycarb, anti juvenile hormones, such as the precocenes, chitin synthesis inhibitors, and ecdysteroid agonists and antagonists. IGR with juvenile hormone and anti-JH activity interrupt the life cycle of insects through inhibiting their growth and metamorphosis, but also the development of reproductive tissues, oogenesis and egg laying. JH-analogues and anti-juvenile hormones are widely used to control harmful insect species in a variety of practical applications (for example the control of stored product insects) (Dhadialla et al. 1998). Many IGR interfere with the molting process of harmful insects such as cockroaches or fleas, preventing them from reaching maturity, thus reducing the reproductive activity and disrupting the life cycle of these insects. It is not necessary that IGR show toxicity to the target species, instead they may cause different

abnormalities in development and reproduction, which may lead to a significant reduction in population density, and later to the death of the pests (Siddall, 1976; Oberlander et al., 1997; Tunaz and Uygun, 2004).

Because of their rapid biodegradation, IGR do not persist for longer time in the environment, and their high selectivity together with the low toxicity to non arthropods, such as mammals and humans, make them particularly advantageous in pest control and more environmentally safe than conventional insecticides (Dhadialla et al., 1998; 2005; Zibaee and Bandani, 2009).
IGR are designed to disrupt arthropod-specific physiological processes and typically perturb enzymatically and hormonally regulated reactions (Retnakaran et al., 1985).

Different types of hormones, ecdysteroids, juvenile hormones, and various peptides (neuropeptides) have been shown to control all aspects of insect reproduction (Gäde and Hoffmann, 2005; Stay and Tobe, 2007). For example, not only the brain (brain neuropeptides, allatostatins and allatotropins) may regulate the activity (JH biosynthesis) of the corpora allata (CA), but also the ovary is an important regulator of CA activity in a stimulatory or inhibitory manner. In the cockroach *Diploptera punctata,* the ovary acts on the brain to stimulate JH biosynthesis in the CA by inhibiting allatostatin release (Stay and Rankin 1986; Loher et al., 1987; Chiang et al., 1991; Ferenz and Aden, 1993; Stay et al., 1994).
Juvenile hormones play an important role in the reproduction of females of the Hemimetabola (e.g., in cockroaches, Dictyoptera). Juvenile hormones act vitellogenic by activating vitellogenin (Vg) gene expression in the fat body and in ovarian follicular epithelium. Moreover, JH stimulates the consequent control of Vg uptake into the oocytes; briefly JH is the main hormone regulating Vg synthesis and uptake in many insect orders (Engelmann, 1983, 2003; Wyatt and Davey, 1996; Belles, 2004).
In adult females of the German cockroach, *Blattella germanica*, the production of JH stimulates oogenesis and vitellogenesis in the early adult phase, but is very low during most of the period of ootheca transport, this reducing oocyte growth, and subsequently avoiding the premature deposition of the ootheca. JH is the major gonadotropic hormone also in other cockroaches, and stimulates the production of accessory gland proteins that form the ootheca (egg case), controls sexual

receptivity, and coordinates sexual behavior of the females (Burns et al., 1991; Schal and Chiang, 1995; Osorio et al., 1997).

The JH III titer in the haemolymph of adult females of *B. germanica* reached maximal concentrations during the vitellogenic cycle (Treiblmayr et al., 2006). Uzsák and Schal (2012) found that social conditions may influence the reproductive rate of *B. germanica* females, but only when their CA become active or competent to produce and release JH.

On the other hand, the effects of ecdysteroids (e.g., the physiologically active insect steroid hormone 20-HE) on molting cycle and metamorphosis, but also on oogenesis and vitellogenesis, demonstrate the importance of these hormones in regulation of particular events during female development and reproduction, and especially their influence on ovarian growth.

In several insect groups with holometabolous development, like mosquitos and flies, the ovaries produce ecdysteroids, which induce vitellogenin production in the fat body (Hagedorn et al., 1975; Bownes et al., 1984; Hagedorn, 1985).

In contrast, in the cockroach *D. punctata* ovarian ecdysteroids appear to inhibit JH synthesis, and thus vitellogenesis (Stay et al., 1980). In another cockroach, *Leucophaea maderae*, Engelmann (2002) has shown that there are two independent mechanisms how ecdysone inhibits vitellogenesis (a yolky egg): first by direct inhibition of the CA which results in inhibition of JH production and second by direct inhibition of Vg synthesis in the fat body.

In generally, almost all insect species need both hormones – juvenile hormones and ecdysteroids – for complete adult development and maturation of eggs that means for oogenesis and vitellogenesis, and during the oviposition period (Lorenz et al., 1999).

In addition to the main role of the fat body as major center for intermediary energy metabolism and nutrient storage (it synthesizes, stores, and mobilizes lipids, proteins, and carbohydrates; Beenakkers et al., 1985b), the fat body has an important vital role in Vg production during vitellogenesis, and also for general egg growth and development, for example by the transport of lipids from the fat body to the ovaries and the uptake of lipids into the oocytes. Thus, the fat body is very essential for reproductive processes in insects (Hoffmann, 1995; Anand, 2004; Anand and Lorenz, 2008).

The haemolymph of the insects as transport medium contains similar chemical components as the fat body, that means high total blood lipid content, a great amount of carbohydrates and proteins, and other components such as salts, free amino acids, water, and hormones which are also carried in the blood through the body (Goldsworthy, 1969; Mullins, 1985).
Several metabolic hormones control the insect fat body development and function, such as the insect neuropeptide adipokinetic hormone (AKH), which affects the fat body during flight or oogenesis, when energy has to be mobilized and stored products have to be transported from the fat body to the flight muscles or to the ovaries for oocyte growth (Anand and Lorenz, 2008; Liu et al., 2009; Lorenz and Gäde, 2009).

4.1. Experiments I, II

Subsequent experiments were carried out on adult females of *B. dubia* during the first and second reproductive cycle and whilst the period of ootheca transport (gestation) and oviposition/hatching of the first instar nymphs.
Our results show that no differences were observed in female body weight changes, male body weight changes, and in fat body weight between Experiment I and Experminent II. Therefore, results from the two experiments were pooled.
The body weight changes of females are closely related with reproductive processes. The females' body fresh weight increased from day 0 after emergence to reach a highest value around day 35, the time when embryogenesis is underway. In some females one could see an ootheca on the back of the animals. The body weight then sharply decreased at the time of ootheca deposition and hatching of the first nymphs (after day 70) (see Fig. 3).
The body weight of males at the time of emergence was generally lower than that of the females and began to decrease even more after the time of first mating with females until day 30 after adult emergence. A high increase in body mass of adult males was observed between day 30 and 40 of adult life, reaching a plateau value of about 1.6 g which is still > 1 g less than the maximal body weight of the females (see Fig. 2). In general, the body weight of the females changes much more during adult

life than that of the males, thus reflecting the reproductive events of ovary weight gain, ootheca formation, and egg deposition.

Female cockroaches not only grew faster than males, but consumed more food per mg of body weight (personal observation) and converted it to body tissue more efficiently than males. The increase in ovary weight during the first 20 days after emergence of females together with the increase in ootheca mass from day 25 to days 50 to 65 of female adult life well reflect the changes in total body weight of the females (females reached the heighest body weight on day 65 in experiment I and on day 70 in Exp. II, respectively, when the ootheca contained eggs with almost fully developed embryos) (see Fig. 4a, b).

The body weight of the females decreased sharply after day 70, the time of ootheca deposition and hatching of the first nymphs. However, almost at the same time (day 80) another increase in the ovary weight indicates the beginning of another gonadotropic cycle.

The biochemical composition of the fat body was investigated to see whether some correlations exist between JH titer in the haemolypmph, ecdysteroid titer in the haemolymph, changes in fat body storage compounds (lipids, carbohydrates, glycogen, and proteins), and the reproductive processes in *B. dubia* females.

The role of the insect fat body as center of intermediary (catabolism) and synthetic metabolism (anabolism) (Clements, 1959; Gilmour, 1961; Kilby, 1963; Chefurka, 1965; Gilby, 1965) has been mentioned above. Therefore, the present results are discussed concerning the fat body as storage for lipids, carbohydrates including glycogen, and proteins, and the use of the energy stores in reproductive processes under control of the developmental hormones JH and ecdysteroids.

The weight of the female fat body decreased from about 400 mg at day 0 to 300 mg at days 15 to 25 after adult moulting, when the first mature eggs were found in the ootheca, which means that a large amount of fat body mass (especially lipids, see Fig. 6a, b) was required for growing of ovaries and eggs, respectively. The weight of the ovaries increased dramatically during that time. The weight of fat body increased again after day 25 when embryogenesis starts.

During gestation, the time of ootheca growth and embryonic development within the eggs, a significant increase in the fat body weight between days 25 and 40 to 55 of adult life occurred. This increase correlates well with an increase in the ootheca

weight, but also with rather high concentrations of proteins, free carbohydrates and glycogen in the fat body (see Figs. 8 to 13). A slight drop in fat body fresh weight was observed at the end of the gestation period after the ootheca had been deposited.
Our data emphasize that fat body compositions play a major role in the development and growth of ovary and ootheca formation. The relationship between ovary development and fat body content can be clearly noticed. Another increase in the fat body weight after day 80 of female adult life indicates the beginning of the second gonadotropic cycle. A significant increase in the JH III titer around day 60 seems to induce vitellogenesis in the second gonadotropic cycle. This happens at least 10 days before the ootheca of the first gonadotropic cycle will be deposited and the first instar larvae start to hatch.

Lipids are the most important source of energy for many insects. In insects, the majority of the lipids are stored in the fat body (more than 90%) as triacylglycerol (TAG), the major lipid class not only in the fat body, but also in ovaries and newly laid eggs (Beenakkers et al., 1985; Candy, 1985; Downer, 1985; Grapes et al., 1989; Arrese and Wells, 1997; Canavoso et al., 1998).
Lipids are transported during the reproductive cycle together with vitellogenins from the fat body into the eggs, which contain appreciable amount of lipids (Lorenz, 2003).
The lipid content per whole fat body dropped from 130-160 mg per fat body at early days after emergence to a minimum of 80-100 mg per fat body around days 15 to 20, when the first eggs were found in the ootheca. A significant increase in the fat body lipid content up to about 150 and 120 mg (in experiments I and II, respectively) occurred between days 20 and 50 to 55 of adult life, at the time of ootheca growth during gestation (Fig. 6a, b). However, because the fat body weight also increased during that period of gestation, a significant decrease in lipid content per mg fat body from day 0 to days 50 to 55 was observed in both experiments. This decrease in lipid content per mg fat body reflects the use of lipids during oocyte maturation as well as during gestation. Lipids can represent significant constituents of the egg´s dry weight (almost 30-40%) (Troy et al., 1975; Kawooya and Law, 1988; Briegel, 1990) and are an important energy source during embryogenesis.
The present results show that the content of lipids in the fat body of *B. dubia* females is much higher than the levels of glycogen, proteins, and carbohydrates.

Such “lipid-driven” insects not only contain high amounts of lipids in the fat body, but also in the haemolymph (Hoffmann, 1995).

From data illustrated in Figs. 8a, b and 9a, b it can be seen that the total fat body protein and the protein content per fat body as well as per mg fat body mass is high during the period of ootheca carrying. This may indicate that proteins are also used as nutrient for the developing embryos during gestation. The protein content per mg fat body drops after ootheca deposition. Similar changes than for proteins were found for carbohydrates during gestation.

Free carbohydrates are major source of energy during many physiological events, such as moulting, gonadal and reproductive growth, vitellogenesis and muscular activity. In most insects carbohydrate reserves are present as glycogen and trehalose is released into the haemolymph, which can be readily converted to glucose.

As appear from Figs. 10a, b and 11a, b, free carbohydrate content per whole fat body and per mg fat body fresh mass reached the heighest levels on day 40 to 45 during gestation period in both experiments, but gradually dropped at the end of the egg case carrying period.

Moreover, free carbohydrates per fat body increased during the ovarian growth period between day 5 and 25, and decreased when the growth of the ootheca is low within the second phase of gestation.

It should be noticed that the content of carbohydrates in the fat body was higher in the second experiment compared to the first one.

Changes in the glycogen content of the fat body of adult *B. dubia* females were similar to those for free carbohydrates. This supports the idea that free carbohydrates have to be released from fat body glycogen stores during oogenesis as well as during the period of gestation (see Figs. 12b, 13b). Another peak in the fat body glycogen content on day 90 indicates the second gonadotropic cycle. It is well known from other adult female cockroaches, *Leucophaea maderae*, that fat body glycogen serves as important energy source during reproduction (Wiens and Gilbert, 1967).

In summary, the results of the present study illustrate that the main constituents of the fat body stores of reproducing *B. dubia* females are lipids, followed by glycogen,

proteins and free carbohydrates. Changes in fat body chemical constituents correlate well with ovarian growth, ootheca formation, gestation and deposition of the ootheca. In addition, good correlations were found between fat body weight changes and ovary growth, ootheca formation and oviposition.

In most insect species, high juvenile hormone titers in the haemoylmph of females are associated with vitellogenesis and oocyte maturation (Belles 2004; Raikhel et al. 2005).

In many insects (such as in the milkweed bug *Oncopeltus fasciatus*), oviposition starts only when JH titers reach their peak, and relatively higher JH titers are associated with completion of ovarian development (Rankin and Riddiford, 1978; Rankin, 1991).

Our results show that there is firm association between egg growth and JH III titer in the haemolymph of *B. dubia* females. As expected, JH III was the only JH homologue found in *B. dubia*. The JH III titer was positively correlated with ovarian mass and ootheca formation, with significant peaks during vitellogenesis and ovarian growth, and during the period of later gestation at days 10 to 15 and 60 to 70 of adult life, respectively (Fig. 14a, b). This second peak in JH III may induce vitellogenesis of the second gonadotropic cycle, even when the ootheca of the first gonadotropic cycle had not yet been deposited. In addition, results from both experiments show a significant increase in the JH III concentration at the beginning of oocyte growth in the second gonadotropic cycle around day 85.

Our results also reveal that JH III concentrations become low when ovarian growth is finished and eggs are packed into the ootheca as well as when the ootheca is deposited (the egg-case is dropped), and first nymphs are hatching. Thus our results clearly implicate JH III as a regulator of oocyte growth, egg formation and oviposition in *B. dubia*.

Rates of juvenile hormone synthesis and JH titers have been determined in several cockroach species with different reproductive strategies. Mated females of the American cockroach, *Periplaneta americana*, produce about 10 oothecae per vitellogenic cycle, which are placed on or in the soil a few hours after dropping. Nymphs emerge from egg cases in 6 to 8 weeks (oviparous). Total generation time of *P. americana* is about six months at optimal rearing conditions (30°C), but more than

500 days at 22°C. The first gonadotropic cycle takes about 4 days and is characterized by a peak in JH biosynthesis of the corpora allata (CA) (data on JH titer in the haemolymph of reproducing *P. americana* females are lacking) on day 2 after emergence, but a sharp decline of JH synthesis towards completion of the rapid oocyte growth (Weaver et al., 1995).

The only viviparous cockroach species in which the developing embryos derive nourishment, the Pacific beetle cockroach *Diploptera punctata*, mates immediately after emergence and ovulation or oviposition starts at day 8. Parturition or birth of nymphs following a period of pregnancy or gestation in the uterus occurs around day 60 after emergence (Roth and Stay, 1961). Rates of JH release from the CA as well as haemolymph JH titers of adult females of *D. punctata*, increase from the day of emergence and reach a maximum at day 4/5 of adult life, at the time of maximal vitellogenesis. Both parameters then drop sharply towards oviposition and remain low during the entire gestation period (Tobe et al., 1985).

The German cockroach, *Blattella germanica*, shows similar development pattern as that of *B. dubia,* but generation time is shorter. Females mate shortly after emergence and produce the first ootheca within one week, which is carried by the female for about 17 days during which embryogenesis takes place. Each of the 4 to 8 oothecae produced during adult life contains 20 to 50 eggs. Increasing JH levels in the haemolymph of 3 to 5 day old females correlate with the processes of vitellogenesis and oocyte maturation. During the period of ootheca transport haemolymph JH remains low but increases dramatically one day before the ootheca is deposited (Treiblmayr et al., 2006). Almost undetectable haemolymph JH titers during the period of ootheca transport maintain the arrest of gonadotropic activities and allow the retention of the ootheca in the genital atrium. During that time, JH biosynthesis in the CA is inhibited by allatostatic neuropeptides from the brain (type-A allatostatins). Animals reduce feeding and show low locomotoric activity (Vilaplana et al., 1999).

In the cricket *Gryllus firmus*, a positive correlation between JH titer and ovarian mass or terminal oocyte length was found, but not between JH titer and the number of post-vitellogenic eggs (Cisper et al., 2000). Hoffmann et al. (1996) reported that JH is essential for the expression of sexual behavior, production of yolk protein, ovary development and the development of various accessory reproductive glands.

In the first experiment of this study, we did not find any significant changes in the concentrations of ecdysone and 20-hydroxyecdysone in the haemolymph of adult female *B. dubia*. The results of the second experiment, however, illustrate that the concentrations of E (see Fig. 15b) and 20-HE (see Fig. 16b) in the haemolymph of adult females reached two peak values, one during early ovarian development and another one during the gestation period at around day 40 to 60 of adult life. These changes in the concentrations of free ecdysteroids may indicate that JH III and free ecdysteroids together will induce ovarian growth and gestation. Another peak in free ecdysteroids at day 75 indicates the beginning of the second gonadotropic cycle. The significant drops in free ecdysteroids to minimum values a few days before ootheca formation at day 10 and shortly before ootheca deposition and hatching of the nymphs around day 65 (especially pronounced for ecdysone) are found at times, when JH III concentrations were high.

Hatle et al. (2003) found that haemolymph ecdysteroids in adult females of the lubber grasshopper, *Romalea microptera*, neither regulated vitellogenesis nor acted as a source of ecdysteroids for the ovary. In the fall armyworm, *Spodoptera frugiperda*, however, JH III and 20-hydroxyecdysone induced vitellogenesis (Sorge et al., 2000). 20-Hydoxyecdysone is supposed to induce vitellogenesis in the female noctuid, whereas JH seems to be essential for a continuous uptake of Vg into the developing oocytes and may trigger 20-HE biosynthesis in the ovary. In the two-spotted field cricket, *Gryllus bimaculatus*, JH III also does not have an all-embracing control on vitellogenesis, though it does exert a marked quantitative effect on vitellogenesis and the rate of egg production (Hoffmann and Sorge, 1996).

In conclusion, these data demonstrate that changes in the concentrations of JH III and free ecdysteroids in the haemolymph of *B. dubia* adult females are strongly related to reproductive processes of ovarian growth, ootheca formation and egg/ootheca deposition, which on their part are closely related to qualitative and quantitative changes in the chemical composition of the fat body.

4.2. Experiment III

Methoprene is a chemical compound that mimics the action of juvenile hormones (JHs) in insects and is used as a safe and commercially available insecticide against pest species (Staal, 1975; Retnakaran et al., 1985). Methoprene exhibits a juvenile hormone analogue mode of action. It interferes with the maturation stages through which an insect goes and makes it impossible to reach the adult stage. Methoprene, therefore, prevents insects from reproducing. Methoprene is one of the most widely used and successful insect growth regulators (Retnakaran et al., 1985). It is used in the production of a number of food, but also in aquatic areas to control mosquitoes, and in the control of ants, flies, lice, moth, beetles and fleas. It is available in liquid, solid and aerosol formulations. Nino et al. (2009) have recently shown that progeny survival of the Scarabaeid beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae) is significantly reduced on manure treated with methoprene. Arthur and Fontenot (2012) demonstrated that the emergence of flour beetle adults, *Tribolium castaneum* and *T. confusum*, was reduced with more malformed adults appearing when larvae were topically treated with methoprene.

When female cockroaches, *B. dubia,* were topically treated with 100 µg methoprene dissolved in acetone every day during the ovarian growth period from day 2 to 20 after emergence, the fresh weight of the animals increased until day 10/15, but decreased thereafter. Similar changes in fresh weight were also observed in acetone treated controls (see Fig. 17). Untreated females increased in body mass until day 40 after emergence (see Fig. 3). Body fresh weight of the females on day 19, when the ootheca can be seen at first in untreated animals, was 2.4 g in the untreated females, but only about 2 g in the acetone and methoprene treated animals, respectively. Maximum body mass of treated cockroaches on day 10 to 15 of adult life was significantly lower following methoprene treatment compared to the acetone treated controls. The reason for the lower body mass of females following methoprene treatment is that such treated animals did not produce an ootheca until day 25 after emergence (see Fig. 18). On day 25 of adult life, methoprene treated females still contained a considerable amount of unmature eggs in their ovary. Topical application of methoprene, therefore, seems to delay ovarian development and egg maturation in *B. dubia* females. Acetone treatment slightly affected the fitness of the animals, as

can be seen from the lower body mass increase, but did not prevent egg maturation and formation of the ootheca.
Inhibition of ootheca formation following methoprene treatment went in line with significantly reduced titers of JH III and free ecdysteroids in the haemolymph of such treated females.
Treatment with methoprene at high concentrations is supposed to either inhibt JH biosynthesis in the corpora allata by a negative feedback mechanism (Tobe and Stay, 1979; Park and Raina, 2004) or to stimulate JH degradation by activating JH esterase activity (Slade and Wilkinson, 1973; Edwards et al., 1987; Kamita et al., 2011). The decrease in JH titer may then result in the observed arrest of ovarian development following methoprene treatment.

Methoprene treatment of females, which had developed normally during the first 30 days of adult life and had produced an ootheca, from day 30 to 70 of adult life (gestation period) resulted in complete resorption of the ootheca, whilst the ovaries again contained unmature eggs (see Fig. 23). Resorption of the ootheca went in line with a significant decrease in the body mass of these animals (see Fig. 22). The JH III titer in the haemolymph of such treated animals was also reduced, whereas ecdysteroid titers were hardly affected (see Figs. 24 to 26).

In conclusion, our results show that besides its well known ovicidal, embryocidal and larvicidal activity, methoprene may interfere with ovarian development, gestation and egg deposition/hatching of nymphs, and is thus suitable for control of even adult cockroaches.
Literature data on methoprene effects in adult insects, however, are still controversial. Daglish and Pulvarenti (1997) reported on reduced fecundity of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) following exposure of adults to methoprene. Treatment of sexually mature females of *B. germanica* cockroaches with methoprene also caused a significant reduction in the number of oocytes, but increased the ovarian protein content, which resulted in an increase in the size of the basal oocytes (Maiza et al., 2004). In contrast, Tanaka (1994) showed that topical application of methoprene induced flight muscle histolysis in both intact and neck ligated long-winged adults of a wing dimorphic cricket, *Modiogryllus confirmatus*, but a stimulation of ovarian development and an increase in egg production. Recently,

Sun et al. (2012) demonstrated the influence of methoprene treatments on the reproduction of the rice leaf roller, *Cnaphalocrocis medinalis,* on day 1 post emergence (JH-sensitive stage), and found that methoprene treatments significantly shortened the preoviposition period, but did not affect total fecundity, oviposition period, or adult longevity. Methoprene treatments also led to reduction in whole-body levels of triacylglyceride content in *C. medinalis* females on day 3 post emergence. In the African malaria mosquito, *Anopheles gambiae*, topical application of methoprene at 6 hours post blood meal delayed ovarian development and egg maturation by suppressing the expression of ecdysone-regulated genes in the females (Bai et al., 2010).

4.3. Experiment IV

Whereas methoprene strongly resembles in its basic terpenoid structure to juvenile hormone and may act, at least in part, as a true JH agonist, more recently several highly active compounds have been synthesized with less apparent similarity in structure to juvenile hormones. One of those is pyriproxyfen. Pyriproxyfen is known to be active against a number of mosquitoes, flies, whiteflies, aphids, scale insects, psyllids, and thrips (Dhadialla et al., 1998; Liu et al. 2003). Hatakoshi et al. (1986) found that pyriproxyfen was much more potent in inducing supernumerary larvae than methoprene and JH I when injected into last instar larvae of the tobacco cutworm *Spodoptera litura.* Application of pyriproxyfen to day 0 pupae of *S. litura* inhibited oviposition but not development of eggs in adult females. In the predatory bug, *Podisus maculivents,* pyriproxyfen strongly prevented embryogenesis, and disrupted metamorphosis and adult formation (De Clercq et al., 1995). Treatment of mole crickets, *Scapteriscus abbreviatus,* with pyriproxyfen caused significant reduction in progeny (egg and nymphs) and reduced survival of adult animals (Parkman and Frank, 1998). Kawada et al. (1989) found that exposure of *B. germanica* nymphs to pyriproxyfen suppressed adult emergence and caused morphological abnormalities in emerged adults. In addition pyriproxyfen treatment caused reduction in reproductive capacity of the German cockroach. Recently, Boina et al. (2010) demonstrated that pyriproxyfen reduces female fecundity and inhibits egg hatching in both younger and older eggs of the Asian citrus psyllid, *Diaphorina citri*. JH mimics such as pyriproxyfen seem to be especially effective against insect

pests of stored products. Abo-Elghar et al. (2004) demonstrated that pyriproxyfen treatment caused complete suppression of adult emergence in the cowpea weevil, *Callosobruchus maculatus.* A comparative analysis on the biological activity of two juvenile hormone agonists, methoprene and pyriproxyfen, and two ecdysone agonists, RH-5849 and tebufenozide, against three stored product insects, *Tribolium castaneum, Rhizopertha dominica*, and *Sitophilus oryzae*, showed that pyriproxyfen was the most effective compound among the four insect growth regulators. A concentration of 0.1 ppm could completely inhibit the F1 adult occurrence of both S- and R-strains of *T. castaneum* and its LC90s for controlling *R. dominica* and *S. oryzae* were 0.1 and 1.2 ppm, respectively (Kostyukovsky et al., 2000).

Köhler and Patterson (1991) already speculated that pyriproxyfen affects the hormonal balance (JH) in insects, which results in strong suppression of embryogenesis, metamorphosis, and adult formation. Zibaee and Bandani (2009) demonstrated that pyriproxyfen induces hormonal changes associated with the earliest development stages of larvae shedding in the Sunn pest, *Eurygaster integriceps.* Following pyriproxyfen exposure, larvae cannot shed their old cuticle and when they move to the following developmental stage, the survivals have abnormality in shape and die later.

Our present studies on topical application of pyriproxyfen on adult females of *B. dubia* gave results very similar to those for the methoprene treatment. Both pyriproxyfen as well as methoprene treatment caused a reduction of JH III in the haemolymph, going in line with delayed ovarian growth and failure of ootheca formation (see Fig. 28). Animals which had produced an ootheca earlier and where then treated with pyriproxyfen absorbed there ootheca and did not produce progeny (see Fig. 33). The decrease in JH III concentration in the haemolymph following pyriproxyfen treatment went in line with some other symptoms like a darker color of the integument than in the controls. Such treated animals also became slower in movement (personal observations).

The obtained results clearly show that pyriproxyfen as well as methoprene can be used most successfully as biocontrol agents against female adult *B. dubia* cockroaches when used in a dose of 100 µg per treatment. Besides their embryonic

and larvicidal activity, both JH analogues show a clear ovicidal activity resulting in sterilization of the females.
When adults and oothecae of the German cockroach, *Blattella germanica*, were treated topically with another juvenoid, fenoxycarb, reproduction was also suppressed (King and Bennett, 1990). Production of offspring even was reduced when males treated with fenoxycarb were mated with untreated females. The sterility seems to have been transferred from males to females, which suggests effects on sperm. However, no reproductive or ovicidal effects were caused by 1 to 10 µg JH analogue per treatment. *In P. americana,* the *in vitro* biosynthesis of JH III by isolated CA was strongly inhibited in the presence of a high concentration of fenoxycarb, but topical treatment of the adult females with fenoxycarb did not reduce the subsequent rate of JH III biosynthesis in the CA *in vitro* (Edwards et al., 1987).

When the use of compounds with insect hormone activity was proposed as “third generation insecticides”, insects were believed to be unable to develop resistance to molecules that mimic their own hormones (Dhadialla et al. 1998). This assumption, however, has not proved true. Several instances of JH analogue resistance have been documented. For example, resistance to methoprene has been shown to take place at the receptor level in methoprene resistant mutants of *Drosophila melanogaster* (Wilson and Fabian, 1986), and resistance to pyriproxyfen has been observed in the whitefly, *Bamesia tabaci* (Horowitz and Ishaaya, 1994).

In conclusion, the present study shows that ovarian growth and ootheca formation in the ovoviviparous Argentinian cockroach *Blaptica dubia* are controlled by the two insect developmental hormones, juvenile hormone III and free ecdysteroids. Ovarian development and the formation of the ootheca go in line with significant changes in the chemical composition of the fat body. Topical treatment of adult females with the two JH analogues methoprene and pyriproxyfen resulted in prevention of ootheca formation or in ootheca resorption, depending on the time of treatment. This demonstrates a clear ovicidal activity of the JH analogues besides their well known larvicidal activity.

5 Summary

- The present study analysis physiological and endocrine aspects of female adult life of the ovoviviparous Argentinian (Dubia) cockroach, *Blaptica dubia*. Experiments were done during the first (days 5 to 25 after emergence) and second gonadotropic cycle (days 80 to 100 after emergence), and whilst the period of oothecal transport (gestation) and hatching of the nymphs (around day 70 of adult life for the first gonadotropic cycle).
- Body weight changes of adult females are closely related with the reproductive processes of ovarian growth, ootheca formation, ootheca deposition, and hatching of the nymphs.
- The biochemical composition of the fat body was analyzed and revealed the storage lipids as main constituents, followed by glycogen, proteins and free carbohydrates. Changes in fat body chemical constituents as well as in fat body fresh weight correlate well with the reproductive processes.
- Concentrations of juvenile hormone (JH) III and free ecdysteroids (ecdysone, 20-hydroxyecdysone) were measured in the haemolymph of adult females by the use of HPLC-mass spectrometry. The JH III titer shows significant peaks during vitellogenesis and ovarian growth, and towards the end of the period of gestation. Changes in the concentration of free ecdysteroids in the haemolymph were less clear.
- Treatment of adult females with the juvenile hormone analogue methoprene during the first 20 days of adult life (topical application of 100 µg methoprene in acetone per day) retarded ovarian development and blocked the formation of the ootheca. The inhibition of the ootheca formation went in line with reduced titers of JH III and free ecdysteroids in the haemolymph of such treated females.
- Methoprene treatment of females, which had normally developed during the first gonadotropic cycle, from day 30 to 70 of adult life resulted in a complete reabsorption of the ootheca. The JH III titer of such treated females was also reduced, whereas free ecdysteroids were hardly affected.
- Treatment of adult females with another JH mimic, pyriproxyfen (100 µg per treatment) gave similar results than those for methoprene usage.

- The results show that both JH analogues have a clear ovicidal activity resulting in “sterilization” of the females and can be used as “third generation pesticides” in adult cockroach biocontrol.

6 Zusammenfassung

- In der vorliegenden Studie werden physiologische und hormonelle Aspekte im Adultleben der eilebendgebärenden Argentinischen Waldschabe, *Blaptica dubia*, analysiert. Die Untersuchungen wurden während des ersten (Tag 5 bis 25 nach der Adulthäutung) und zweiten gonadotropen Zyklus (Tag 80 bis 100 des Adultlebens) der Weibchen sowie währed der Periode des Oothekentransports (Trächtigkeit) und des Schlüpfens der Erstnymphen (um Tag 70 des Adultlebens für den ersten gonadotropen Zyklus) durchgeführt.
- Veränderungen im Körpergewicht der adulten Weibchen stehen im Zusammenhang mit dem Wachstum der Ovarien, der Bildung der Oothek, der Ablage der Oothek und dem Schlüpfen der Nymphen.
- Die chemische Zusammensetzung des Fettkörpers wurde analysiert. Speicherfette bilden den Hauptbestandteil des Fettkörpers, gefolgt von Glykogen, Proteinen und freien Kohlenhydraten. Veränderungen in der chemischen Zusammensetzung des Fettkörpers und im Fettkörperfrischgewicht korrelieren mit den Prozessen der Fortpflanzung.
- Die Konzentrationen an Juvenilhormon (JH) III und freien Ecdysteroiden (Ecdyson und 20-Hydroxyecdyson) in der Hämolymphe adulter Weibchen wurden mittels HPLC-Massenspektrometrie bestimmt. Der JH Titer zeigt einen signifikanten Peak während der Vitellogenese und des Ovarienwachstums und einen weiteren am Ende der Trächtigkeit. Veränderungen im Häutungshormontiter der Weibchen sind weniger klar zu erkennen.
- Behandlung der adulten Weibchen mit dem Juvenilhormon-Analogon Methopren während der ersten 20 Tage des Adultlebens (Applikation von 100 µg Methopren in Aceton pro Tag) verzögert die Ovarienentwicklung und verhindert die Bildung einer Oothek. Das Ausbleiben der Oothekbildung geht mit reduzierten Titern an JH III und freien Ecdysteroiden in der Hämolymphe einher.
- Behandlung von Weibchen, die sich normal entwickeln konnten, mit Methopren von Tag 30 bis 70 nach der Adulthäutung, führte zu einer vollständigen Resorption der Oothek. Bei diesen Tieren war auch der JH III

Titer in der Hämolymphe stark reduziert, währen bei den freien Ecdysteroiden keine signifikanten Veränderungen zu beobachten waren.

- Behandlung der adulten Weibchen mit einem anderen JH-Analogon, Pyriproxyfen (100 µg Pyriproxyfen in Acteon pro Tag) führte zu ähnlichen Ergebnissen wie die Methopren-Behandlung
- Die Ergebnisse zeigen, dass beide JH-Analoga eine deutliche ovicide Aktivität aufweisen, was die Bildung von Nachkommen verhindert. Die beiden JH-Anaolga sind somit für einen Einsatz als „Insektizide der dritten Generation“ bei der Bekämpfung adulter Tiere der Argentinischen Waldschabe geeignet.

7 References

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8 Supplementary material

Table 2: Significances in fresh weight of cockroach *B. dubia* males from day 0 to day 100 of adult life. Mean values were compared by one way ANOVA with posthoc-test: Holm-sidak.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-
5			-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-
10				-	-	-	-	-	-	-	-	+	-	-	+	-	+	+	-	+	-
15					-	-	-	-	-	-	-	+	-	-	+	-	+	+	+	+	+
20						-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+
25							-	-	-	-	-	+	-	-	+	-	+	+	+	+	+
30								-	+	-	-	+	+	+	+	+	+	+	+	+	+
35									-	-	-	-	-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 3: Significances in fresh weight of cockroach *B. dubia* females from day 0 to day 100. Mean values were compared by one way ANOVA on Ranks with posthoc-test: Tukey.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
5			-	-	-	-	-	-	+	+	+	-	+	+	+	+	-	-	-	-	-
10				-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
15					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 4a: Significances in ovary weight of *B. dubia* females from day 0 to day 80 (experiment I). Mean values were compared by one way ANOVA on Ranks with posthoc-test: Dunn´s.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5			-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
10				-	-	-	-	-	+	-	-	+	+	-	-	-	-
15					-	-	-	+	+	-	-	+	+	-	-	-	-
20						-	-	+	+	-	-	+	+	-	-	-	-
25							-	-	-	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-
50												-	-	-	-	-	-
55													-	+	-	-	+
60														-	-	-	-
65															-	-	-
70																-	-
75																	-
80																	

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 4b: Significances in ootheca weight of *B. dubia* females from day 0 to day 80 (experiment I). Mean values were compared by one way ANOVA on Ranks with posthoc-test: Dunn´s.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10				-	-	-	-	-	-	-	-	-	-	-	-	-	-
15					-	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	-	+	+	+	+	+	-	-
30								-	-	-	+	+	+	+	+	-	-
35									-	-	-	-	-	+	-	-	-
40										-	-	-	-	+	-	-	-
45											-	-	-	+	-	-	-
50												-	-	-	-	-	-
55													-	-	-	-	-
60														-	-	-	-
65															-	-	+
70																-	-
75																	-
80																	

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 5a: Significances in ovary weight of *B. dubia* females from day 0 to day 100 (experiment II). Mean values were compared by one way ANOVA on Ranks with posthoc-test: Dunn´s.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+
5			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10				-	-	-	-	-	-	+		-	-	+	+	+			-	-	-
15					-	-	-	-	-	+	+	-	-	+	+	+	+	+	-	-	-
20						-	-	-	-	+	+	-	-	+	+	+	+	+	-	-	-
25							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	+	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 5b: Significances in ootheca weight of *B. dubia* females from day 0 to day 100 (experiment II). Mean values were compared by one way ANOVA on Ranks with posthoc-test: Dunn´s.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-	-	-	-	-
40											-	-	-	-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 6: Significances in whole fat body weight of *B. dubia* females from day 0 to day 100. Mean values were compared by one way ANOVA on Ranks with posthoc-test: Dunn´s.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-
5			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
10				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
15					-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	-
20						-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	-
25							-	-	+	+	+	+	-	-	-	-	-	+	+	+	-
30								-	-	-	-	-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 7: Significances in lipid content per mg fat body fresh mass of *B. dubia* females from day 0 to day 100. Mean values were compared by one way ANOVA on Ranks with posthoc-test: Holm-Sidak.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-
5			-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-
10				-	-	-	-	-	-	-	-	+		-	-	-	-	-	-	-	-
15						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	+	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 8a: Significances in total protein content per whole fat body of *B. dubia* females from day 0 to day 80. One way ANOVA on Ranks with posthoc-test: Dunn´s.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
0		-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	+
5			-	-	-	-	-	-	-	+	+	+	+	+	+	-	+
10				-	-	-	-	-	-	-	+	-	-	-	-	-	+
15					-	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	+	+	+	+	-	+	-	+
25							-	-	-	-	+	+	+	-	+	-	+
30								-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-
50												-	-	-	-	-	-
55													-	-	-	-	-
60														-	-	-	-
65															-	-	-
70																-	-
75																	-
80																	

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 8b: Significances in protein content per mg fat body fresh mass of *B. dubia* females from day 0 to day 80. One way ANOVA on Ranks with posthoc-test: Dunn´s.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
0		-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	+
5			-	-	-	-	-	-	-	+	+	-	+	+	-	-	+
10				-	-	-	-	-	-	-	-	-	+	-	-	-	-
15					-	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	-	-	-	+	-	-	-	-
25							-	-	-	-	-	-	+	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-
50												-	-	-	-	-	-
55													-	-	-	-	-
60														-	-	-	-
65															-	-	-
70																-	-
75																	-
80																	

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 9: Significances in protein content per whole fat body of *B. dubia* females from day 0 to day 100. One way ANOVA on Ranks with posthoc-test: Tukey.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	-	-	-	-	-	-	+	-	+	-	+	+	+	-	+	-	+	-	+
5			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10				-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
15					-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																			-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 10a: Significances in free carbohydrate content per whole fat body of *B. dubia* females from day 0 to day 80. One way ANOVA on Ranks with posthoc-test: Dunn´s.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
0		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
5			-	-	-	-	-	+	+	-	+	+	-	-	+	-	+
10				-	-	-	-	-	+	-	-	-	-	-	-	-	-
15					-	-	-	-	+	-	-	-	-	-	-	-	-
20						-	-	-	-	-	-	-	-	-	-	-	-
25							-	-	+	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-
50												-	-	-	-	-	-
55													-	-	-	-	-
60														-	-	-	-
65															-	-	-
70																-	-
75																	-
80																	

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 10b: Significances in free carbohydrate content per mg fat body fresh mass of *B. dubia* females from day 0 to day 80. One way ANOVA with posthoc-test: Holm-Sidak.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5			-	-	-	-	-	-	+	-	+	+	+	+	+	-	-
10				-	-	-	-	-	-	-	-	-	-	-	-	-	-
15					-	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-
40											-	-	-	-	-	-	-
45											-	-	-	-	-	-	-
50												-	-	-	-	-	-
55													-	-	-	-	-
60														-	-	-	-
65															-	-	-
70																-	-
75																	-
80																	

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 11a: Significances in free carbohydrate content per whole fat body of *B. dubia* females from day 0 to day 100. One way ANOVA on Ranks with posthoc-test: Tukey.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5			-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+
10				-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
15					-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-	-	-	-	-
45											-	-	-	-	-	+	+	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 11b: Significances in free carbohydrate content per mg fat body fresh mass of *B. dubia* females from day 0 to day 100. One way ANOVA with posthoc-test: Holm-Sidak.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5			-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-
10				-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
15					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	+	+	-	-	+	-	-	-	-	-	-	-
30								-	-	+	-	-	-	-	-	-	-	-	-	-	-
35									-	+	+	-	-	+	-	-	-	-	-	-	-
40										+	-	-	-	-	-	-	-	-	-	-	-
45											-	-	+	-	+	+	+	+	+	+	+
50													-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 12a: Significances of glycogen content per whole fat body of *B. dubia* females from day 0 to day 80. One way ANOVA with posthoc-test: Holm-Sidak.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
0		-	-	-	-	-	+	+	+	+	+	-	-	-	+	-	-
5			-	-	-	-	+	+	+	+	+	-	-	-	+	-	-
10				-	-	-	+	+	+	+	+	-	-	-	+	-	-
15					-	-		+	+	+	+	-	-	-	-	-	-
20						-	+	+	+	+	+	-	-	-	+	-	-
25							-	+	+	+	+	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-
35									-	-	-	+	+	+	+	-	+
40										-	-	+	+	+	-	-	-
45											-	-	+	-	-	-	-
50												-	+	+	-	-	-
55													-	-	-	-	-
60														-	-	-	-
65															-	-	-
70																-	-
75																	-
80																	

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 12b: Significances in glycogen content per mg fat body fresh mass of *B. dubia* females from day 0 to day 80. One way ANOVA on Ranks with posthoc-test: Dunn´s.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
0		-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-
5			-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
10				-	-	-	+	+	+	+	-	-	-	-	-	-	-
15					-	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	+	-	+	-	-	-	-	-	-	-
25							-	-	-	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-
50												-	-	-	-	-	-
55													-	-	-	-	-
60														-	-	-	-
65															-	-	-
70																-	-
75																	-
80																	

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 13a: Significances in glycogen content per whole fat body of *B. dubia* females from day 0 to day 100. One way ANOVA on Ranks with posthoc-test: Tukey.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-
5			-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-
10				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15					-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
25							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-	-	-	-	-
45											-	-	-	-	+	-	-	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 13b: Significances in glycogen content per mg fat body fresh mass of *B. dubia* females from day 0 to day 100. One way ANOVA on Ranks with posthoc-test: Tukey.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
5			-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	+	+	-
10				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30								-	-	-	-	-	-	-	-	-	-	-	-	-	-
35									-	-	-	-	-	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-	-	-	-	-
45											-	-	-	-	-	-	-	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 14a: Significances in JH III titer of the haemolymph of *B. dubia* females from day 0 to day 100. One way ANOVA with posthoc-test: Holm-Sidak.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
5			-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-
10				-	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	+
15					-	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	+
20						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
30								-	-	-	-	-	-	+	+	-	+	+	-	-	-
35									-	-	-	-	-	-	+	-	-	-	-	-	-
40										-	-	-	-	-	+	-	-	-	-	-	-
45											-	-	-	+	+	-	-	+	-	-	-
50												-	-	+	+	-	-	+	-	-	-
55													-	-	+	-	-	-	-	-	-
60														-	-	-	-	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	+	-	+
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 14b: Significances in JH III titer of the haemolymph of *B. dubia* females from day 0 to day 100. One way ANOVA on Ranks with posthoc-test: Tukey.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5			-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10				-	-	+	+	+	-	+	-	+	-	-	-	+	+	-	-	-	-
15					-	+	+	+	-	+	-	+	-	+	-	+	+	-	-	-	-
20						-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25							-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
30								-	-	-	-	-	+	-	-	-	-	+	-	-	-
35									-	-	-	-	+	-	-	-	-	-	-	-	-
40										-	-	-	-	-	-	-	-	-	-	-	-
45											-	-	+	-	-	-	-	-	-	-	-
50												-	-	-	-	-	-	-	-	-	-
55													-	-	-	-	-	-	-	-	-
60														-	-	+	+	-	-	-	-
65															-	-	-	-	-	-	-
70																-	-	-	-	-	-
75																	-	-	-	-	-
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

Table 15: Significances in ecdysone titer of the haemolymph of *B. dubia* females from day 0 to day 100. One way ANOVA with posthoc-test: Holm-Sidak.

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0		-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-
5			+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-
10				-	-	+	+		+	+	+	+	+		+	+	-	+	+	-	-
15					-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	-
20						-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	-
25							-	-	+	+	-	-	-	-	-	+	-	-	-	-	-
30								-	+	+	-	-	-	-	-	+	-	-	-	-	-
35									+	+	-	-	-	-	-	+	-	-	-	-	-
40											-	+	+	+	+	-	+	+	+	+	+
45											-	+	+	+	+	-	+	+	+	+	+
50												-	-	+	-	-	+	-	-	+	+
55													-	-	-	+	-	-	-	-	-
60														-	-	+	-	-	-	-	-
65															-	+	-	-	-	-	-
70																+	-	-	-	-	-
75																	+	+	+	+	+
80																		-	-	-	-
85																			-	-	-
90																				-	-
95																					-
100																					

+, refer to p< 0.05, significant; -, refer to p≥ 0.05, not significant.

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Erklärung

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

Ferner erkläre ich, dass ich anderweitig mit oder ohne Erfolg nicht versucht habe, eine Dissertation einzureichen. Ich habe keine gleichartige Doktorprüfung an einer anderen Hochschule endgültig nicht bestanden.

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Bayreuth, den 10. Januar 2013