Załącznik 3

Self-Report

1. First and last name: Oskar Wasielewski

2. Degrees in science / art – including city and year of receiving, and the title of PhD thesis

1. MSc in biology, 2001, thesis title: Miotropic and hemolytic activity of peptides from the frogs skin secretion of *Rana esculenta* complex.
2. PhD in biology, specialization: animal physiology, Faculty of Biology, Adam Mickiewicz University, 2005; PhD thesis title: Mechanism of neurohormonal regulation of gonadotropic cycle in *Tenebrio molitor* (Coleoptera).

3. Appointments

- 2006 - Present: Institute of Zoology, Faculty of Animal Breeding and Biology, Poznań University of Life Sciences, Assistant Professor

4. Indication of the achievement in accordance with the article 16, act 2 about scientific degrees and scientific title and about degrees and title in art, March 14 2003 (Dz. U. nr 65, poz. 595 ze zm.):

a. title of achievement

"Physiology and regulation of imaginal diapause in red mason bee females, *Osmia bicornis* L."

b. (author / authors, title/ titles of article, year of edition, publication name)

[1] **Wasielewski O.,** Szczepankiewicz D., Giejdasz K., Wojciechowicz T., Bednarova A., Krishnan N. (2014) The potential role of adiponectin- and resistin-like peptides in the regulation of lipid levels in the hemolymph of over-wintering adult females of *Osmia bicornis*. Apidologie DOI: 10.1007/s13592-013-0264-z [IF 2012: 2,155]

[2] **Wasielewski O.**, Wojciechowicz T., Giejdasz K., Krishnan N. (2013) Overwintering strategies in the red mason solitary bee - Physiological correlates of midgut metabolic activity and turnover of nutrient reserves in females of *Osmia bicornis*. Apidologie 44: 642–656 [IF 2012: 2,155]

[3] **Wasielewski O.**, Wojciechowicz T., Giejdasz K., Krishnan N. (2011) Influence of methoprene and temperature on diapause termination in adult females of the over-wintering solitary bee, *Osmia rufa* L. Journal of Insect Physiology 57: 1682–1688 [IF 2012: 2,379]

[4] **Wasielewski O**., Giejdasz K., Wojciechowicz T., Skrzypski M. (2011) Ovary growth and protein levels in ovary and fat body during adult-wintering period in the red mason bee, *Osmia rufa*. Apidologie 42: 749-758 [IF 2012: 2,266]

c. scientific goals and results of studies presented above, including potential application of obtained results

For several years can observe an increase interest in wild bees living in many countries of the world. This interest comes not only from a cognitive but also for practical reasons. They are used for pollination of a number of crops in both field crops and greenhouses, where in many respects exceed the honeybees. Therefore, the aim is to get to know bionomy and ethology of that species and to develop rearing methods and their practical use. The most popular are solitary bees because of their biodiversity. In Poland, as reported Banaszak (1993) there are about 450 species of solitary bees belonging to 6 families. Noteworthy are species of bees belonging to the family Megachilidae, Anthophoridae and Melittidae. Studies on the some of solitary bees initiated in Poland back in the sixties (Wojtowski, 1964), aiming to determine their effectiveness of crops pollination and the development of rearing methods. Solitary bees select for breeding, reproduction and utilization must present satisfy certain conditions: characterized by a prevalence in large groups, nesting in an artificially prepared sites, exhibit high population growth with an appropriate sex ratio and their trophic requirements should be address of many plant species (Wilkaniec, 1991). The above criteria are complied by solitary bee, *Osmia bicornis* (rufa) L. belonging to the Megachilidae family. Polish name “red mason bee” was named this species by Wojtowski (1979), thus emphasizing the ecological characteristics of the bee pollinate almost fruit trees and shrubs, as well as some decorative plants found in the gardens and orchards. Studies on this promising group of bees in order to use them for pollination are conducted in many countries around the world. In Poland, the most attention among the bees of the Osmia genus focused on species *Osmia bicornis*. Red mason bee is a univoltine species, and its development from egg to imago occurs during spring and summer period. The female builds a nest and lay an eggs from April till June. Development of a new generation of bees runs through the summer and adult appear in cocoons in the end of September (Giejdasz and Wilkaniec, 2002). After imaginal moulting

bees start the overwintering period, including diapause time between November and January. This period is necessary to obtain physiological maturity in adults (Giejdasz and Wilkaniec, 1998). A very important element in facilitating increase the efficiency of rearing *O. bicornis* is to understand the physiological processes occurring during the obligatory diapause. It is one of the adaptations allowing for survival of unfavorable environmental conditions.

All organisms have mechanisms to tolerance or avoidance of changes in environmental conditions. When the impact of local environmental factors exceed the capacity of organisms to adapt and migrate to a more favorable environment is not possible, the only mechanism that will ensure that they can survive is to produce a quiescent forms resistant to periodic impact of unfavorable conditions. Insects have developed through evolution specific mechanisms that allow them to survive very different environmental conditions. One of these adaptations is diapause - temporary inhibition of development - allowing insects to survive unfavorable environmental conditions. Insects during diapause stage are characterized by a very low metabolism and special biochemical adaptations (Denlinger, 2002). Before the diapause will entered insects usually accumulate in their body large reserves of fats, proteins and carbohydrates (Robich and Denlinger, 2005). Insect diapause can be induced by many different environmental factors such as temperature, quality and availability of food, humidity and day length (photoperiod) (Numata and Denlinger, 2005). There are several types of diapause, including imaginal diapause, that present in red mason bee, *O. bicornis*. This type of diapause is characterized primarily by inhibition of reproduction. Diapausing insects exhibit many characteristic changes in behavior and metabolic processes. Nutrition, physical activity, reaction to light and mechanical stimuli are changed. The level of oxygen consumption in comparison with non-diapausing insects is also lower. Diapausing insects accumulate also in their bodies reserves of fat and glycogen. This is particularly important because diapausing insects do not intake food (Denlinger, 2002). Besides the environmental factors a very important role in the regulation of diapause plays an endocrine system and associated hormones: juvenile hormone (JH) and diapause hormones (Munyiri and Ishikawa, 2004). In short photoperiod conditions the JH production and release is inhibited. It is connected with inhibitory effect of brain on *corpora allata* (CA), glands where the JH are produced and released. This changes in endocrine system initiates imaginal diapause. During the next days and months of imaginal diapause activity of CA is gradually increased, which results in JH level raising and initiate the development of the gonads (Eizaguirre et al, 2005). Until now there was no a difference in the ecdysteroids level, suggesting that these hormones are not involved directly in the regulation of imaginal diapause (Denlinger and Lee, 1997). So far managed to identify only two diapause hormones regulate embryonic diapause in the

silkworm, *Bombyx mori*. These are respectively Bom-DH-(19Cys) (Imai et al, 1991) and Bom-DH-(19Trp) (Sato et al, 1992) differ by a single amino acid at position 19. In this species the diapause hormone is located in neurosecretory cells of suboesophageal ganglion and is responsible for the induction of embryonic diapause. Synthesis and release of diapause hormone is under control of factors synthesized in the brain cells (Nässel, 2002).

In Poland, despite many papers and research trends focused mainly on rearing and plants pollination by *O. bicornis*, still must to be done in the field of broadly defined bionomy this species and its controlled rearing methods. Until our team has started some experiments connected with an explanation of the physiological and biochemical aspects of imaginal diapause regulation, there was no precise data on the physiology and biology of this species from the diapause period. Well understanding of this process, particularly the aspects related with the role of particular hormones, will conduct controlled rearing of this species and the development of effective methods of reproduction.

Scientific achievement is a series of four articles on the physiological characteristics and various aspects of the regulation of imaginal diapause mandatory occurring in solitary bee *Osmia bicornis* L. The most important achievements of the research on diapause of this species can include:

1. The precise definition of time periods during the development cycle of red mason bee from the appearance of the imago in the pre-wintering time until the imago emerged early spring. For the first time to determine the diapause duration the indicators such as ovary development and ovary and fat body proteins level were used.
2. Determining the role of juvenile hormone in the regulation of diapause and its influence on its termination.
3. A comprehensive assessment of the management of metabolic reserves during overwintering and the role of metabolic axis: gut – fat body – hemolymph.
4. Indication for the first time on the presence of peptides with structure similar to mammals adiponectin and resistin and their role in regulating lipid levels, the main energetic reserves used during overwintering.

(1) Wasielewski O., Giejdasz K., Wojciechowicz T., Skrzypski M. (2011) Ovary growth and protein levels in ovary and fat body during adult-wintering period in the red mason bee, *Osmia rufa*. Apidologie 42: 749-758

One of the most common types of diapause in insects is an adult diapause, which involves the arrest of reproductive development. The shortening of the day-length in autumn, or the change from long to short-day photoperiod coupled with the lowering of temperature is sufficient to induce adult diapause. The normal adult diapause is characterized by the ovaries ceasing to develop and by hypertrophy of the fat body. A different situation exists in *Osmia* genus. In the following months of wintering, the development of oocytes is progressively continued in the vitellarium region which implies that the ovaries are not completely inactive during wintering, including diapause period. A similar situation occurs in the case of fat body as a source of vitellogenin to the developing ovary. The information about the time of diapause initiation, maintenance and termination in *Osmia* genus are very scanty and refer to fragmentary knowledge obtained during research on *Osmia lignaria*, a spring-flying solitary bee from North America.

Pre-wintering period in *O. bicornis* females started at the beginning of September after the immatures reach adulthood. During first two months of wintering, when the outdoor temperature is relatively high, we noted that the size of terminal oocytes increased. The protein content in fat body tissue decreased from September to October. Contrary, in ovary the protein content rose at the same time. In the following months (Nov-Jan), the protein concentration in both tissues fluctuated but the changes were not significant. The next increase of the terminal oocyte in November was recorded and was the last before the start of winter months. Based on our data October was the last month of pre-wintering period. In late November, when the temperature declined, the ovary growth reached plateau. During December and January, the ovary growth has been halted but not fully arrested. Our observations indicate that *O. bicornis* entered the diapause in November and finished in January, after the temperature rose. In our study, oocyte size increased in February and March as the temperature did. This time in our opinion corresponds to a post-diapause quiescence, during which low metabolic rates are maintained exogenously while temperatures are still too cold for morphogenesis (or emergence). Until now, it has been really difficult to construct a model of diapause in *O. bicornis*. In this species the diapause is obligate and it occurs in complete darkness. Thus, using the fact that oocyte size could provide a good indicator of morphogenesis resumption in this species, it seems that the most probable model is late diapause termination followed by short period of post diapause quiescence. Furthermore, we

recorded that besides growth, the oocytes progressively increased in number in particular months of over-wintering. We noted that the increase of oocyte number was faster in pre-wintering months (Sep-Oct), then in diapause months (Nov-Jan) has slightly fluctuated and during post diapause quiescence increased again. During post diapause quiescence ovary protein concentration rapidly increased. As opposed to ovary proteins at the same time the protein content in fat body tissue significantly decreased. In our opinion the protein concentration can be another diapause indicator in this species. Using ecophysiological terminology the different phases of diapause can be identified in *O. bicornis*. Generally the whole time of overwintering can be divide into three periods of time. A period September – October we recognize as a pre-diapause (also called pre-wintering). Second from November to January we consider for the diapause. After results analysis, we assume that the initiation of diapause occurs in November, maintenance in December – January and termination after mid-January. After diapause termination the next period, February - March is the time of post-diapause quiescence (also called the post-wintering).

Our results demonstrated, for the first time, that changes in ovary morphology and proteins concentration in ovary and fat body in female *O. bicornis* are accompanied throughout the pre-wintering and wintering period. The contribution to knowledge of over-wintering time, including the diapause period in *O. bicornis* will improve the management of this bee for crop pollination. Necessity of synchronous adult emergence of pollinator with blooming period requires termination of diapause or continuation over-wintering period under laboratory conditions.

(2) Wasielewski O., Wojciechowicz T., Giejdasz K., Krishnan N. (2011) Influence of methoprene and temperature on diapause termination in adult females of the over-wintering solitary bee, *Osmia rufa* L. Journal of Insect Physiology 57: 1682–1688

In many insects, the duration of diapause is controlled by more than one factor. Beyond the inducing factors, photoperiod and temperature, diapause is regulated by neuroendocrine processes. Endocrine regulation is important especially in reproductive diapause (i.e. a resting state with reduced metabolic activity) where juvenile hormone (JH) titers appear to play a crucial role. A decrease in JH production in the *corpus allatum* (CA) induces cessation of reproduction, specifically, the arrest of vitellogenesis and regression of the ovaries. The appearance of the favorable environmental factors e.g. day length or temperature raises result in acting many physiological processes, with an increase CA activity and thereby increase of

JH level. The higher level of JH acts on both ovary and fat body and stimulates them to complete the maturation of the ovaries. This is usually placed during post-diapause time, when an unfavorable environmental conditions do not allow yet to emerge.

Based on our previous results respect to initiation, maintenance and termination of diapause the experiments focused on attempt to stop the obligatory diapause occurring in O. *bicornis* were planned. According to these data, experiments were performed on the beginning of January, during deep diapause stage, when the physiological processes was at the lowest level. To early terminate diapause juvenile hormone analogue – methoprene was applied in a suitable dose for five consecutive days in the beginning of January. To enhance the effect induced by the hormone we added also another factor, temperature. To the evaluate the impact of both factors the reproductive resumption indicators are used such as the size and number of oocytes and the concentration of protein in ovarian and fat body tissue. After the application of methoprene, independently of the temperature used, after five days, in the all proposed indicators the clearly changes were observed. Methoprene significantly increase the growth of terminal oocytes but the effect was also accelerate by temperature. The fastest effect of oocyte growth observed after the first application of hormone, was obtained in female groups kept at 20 ^{0}C. In these group of females a progressive increase in the number of oocytes was also observed. Termination of diapause and acceleration of ovary growth occurred even at the lowest temperature (4 ^{0}C), which shows an important role of JH in the termination of diapause. Other proposed indicators, protein concentration of protein in the ovary and fat body tissue also has changed. As in the case of the ovary growth, the rate of changes of proteins content depended on the temperature at which the different groups of females were kept. At lower temperatures the change the protein concentration (increase in ovarian tissue, decrease in fat body tissue) was delayed compared to the highest temperature used in the experiment. It should also be noted that both parameters (ovary and fat body proteins concentration) were negatively correlated with respect to each other. During the same period of the experiment the proteins level in ovary was increased but in fat body tissue was decreased. This correlation is due to specific interactions and role of both organs to the resumption of reproductive activity, where fat body is a source of proteins (vitellogenins) and ovary a depositions site. In red mason bee diapause finishes in the end of January after the temperature raises. It appears that the temperature can play a very important role in the diapause termination, but in our opinion it is not a crucial factor. Our results indicate that it plays a secondary role compared to the juvenile hormone and accelerates ovary growth and maturation. After analyzing the rate of ovary development in females exposed only to the different temperature we recorded the differences between females kept in different

temperatures. The largest differences were observed between females with extreme experimental groups e.g. 4 ^{0}C and 20 ^{0}C which indicated the role of temperature in diapause. However, a marked changes in the ovarian development was observed only after methoprene application. Our interpretation concerning the role of JH as a main factor in diapause regulating confirm our previous research on the role of temperature as a factor in the bees overwintering termination. Gradually increasing the temperature favored emerging from the coocons, but freshly eclosed females were not physiologically ready for reproduction. Females only activated by higher temperature have an immature ovaries and are not able to lay eggs. This shows that in the process of ovary maturation must be involved another factor than temperature, in our opinion the juvenile hormone.

In this study, we have demonstrated for the first time the presence of a strong relationship between JH and temperature as regulating factors of diapause in *O. bicornis*. We believe that the development of hormonal methods for the control of diapause (reduce or eliminate diapause) may be used in the future to obtain adult insects beyond the period of their natural life cycle. Control of diapause will open up the possibility for additional generations of pollinators and enable the use of this bee species as a managed pollinator in greenhouses and field plots outside its natural flight period. Finally, our results may also be used to devise methods to control diapause in *Osmia bicornis*, which will enable the activation of adult bees at various times.

(3) Wasielewski O., Wojciechowicz T., Giejdasz K., Krishnan N. (2013) Overwintering strategies in the red mason solitary bee - Physiological correlates of midgut metabolic activity and turnover of nutrient reserves in females of *Osmia bicornis*. Apidologie 44: 642–656

In temperate and cold climates, insect life cycles are correlated closely with seasonal changes. This ensures that insect development and reproduction are restricted to periods of the year when climatic conditions are favourable and there are suitable sources of food. Diapause is a strategy to survive seasons with environmental conditions that are inadequate for sustaining continuous development or maintenance of the organism. Both diapausing and direct-developing insects store metabolic reserves of the same three macronutrients groups: lipids, carbohydrates, and amino acids, as well as essential micronutrients such as vitamins and minerals. In insects that diapause as adults, nutrient reserves accumulated in fat body prior to diapause are critical for restoring post-diapause functions, including the rebuilding of tissues

atrophied as part of the diapause program and for providing energy for post-diapause activities, such as dispersal and reproduction. The gut may also act as a novel nutrient storage reserve in solitary bees, in addition to the fat body. Managing metabolic resources is critical for insects during diapause when food is limited or unavailable and adequate nutrient budgeting and use enables survival of overwintering period and ensures post-diapause development.

In our work, we have focused on the changes in the concentration of the three groups of energetic substrates: lipids, carbohydrates and proteins. In the case of carbohydrates we estimated the changes in the concentration of free hemolymph carbohydrates and glycogen. For the determination of each substrate we collected the tissues directly involved in the management of metabolic reserves, e.g. fat body, midgut, hemolymph, and crop. Furthermore, we also determined the activity of the two classes of enzymes that play a major role in the hydrolysis of the energetic compounds: proteases and amylases. Tissues, in which the nutrient content and enzymes activity were determined we collected during whole period of overwintering, from September until March. Changes in the concentration of different metabolic reserves and enzymes activity were associated with the different periods of red mason bee development, e.g. pre-wintering, wintering (including the diapause) and the post-wintering. During whole time of overwintering, the lipid concentration is gradually increased, especially in fat body homogenates and hemolymph. Metabolization of this group of nutrients dominated during the first period of overwintering (from the imago appearance till the diapause was entered). A slightly different situation was observed for protein concentration. Rapid increase of concentration of this substrates in the hemolymph and the gut tissue was noted at the beginning of overwintering (between September and October). Later, despite the gradually increasing concentration of protein in the hemolymph there was no specific absorptive metabolic activity of the midgut towards the proteins. Only during post-wintering period the proteins content significantly increased. A third group of nutrients, carbohydrates, was metabolized in two periods of the cocooned imago development, during pre-wintering and post-wintering time. During the diapause we did not detect any significant changes in the carbohydrates concentration. Despite the constantly decreasing of these substrates concentration in the hemolymph, in other tissues, midgut and crop, the carbohydrates concentration elevated in the month before the imago emerged from the cocoon. A similar pattern of utilization we observed in the case of glycogen accumulated in the fat body, which the lowest level was found in the winter months. The above changes in the concentrations of various nutrients have been correlated with a different profile of enzymes involved in the metabolization of these substrates. The proteases has shown a relatively low activity during

the most time of overwintering (pre-wintering and wintering). The significant increase of activity was detected after diapause termination and was connected with an increase of metabolic activity in the towards proteins. A quite different was the amylase activity profile. Similarly to carbohydrates concentration, in the case of amylase activity two characteristic peaks were observed. The first activity peak was noted during pre-wintering months, second after diapause termination. These changes of enzymes activity profile were correlated with concentration of carbohydrates in midut tissue.

Osmia species overwinter on a fixed energetic budget. That is, pre-wintering Osmia adults are not able to take advantage of benign conditions during pre-wintering to feed and accumulate additional energy reserves. In these species metabolic reserves for wintering are entirely dependent on the food allocated by the nesting female to each offspring within its nest cell. This implies, that the economic utilization of reserves during overwintering is necessary. An early consumption of reserves would disrupt emergence from the cocoon, flying to the nearest flowers to sip nectar or even nesting. In *O. bicornis* cocooned adults metabolize various energy substrates depending on the overwintering period and physiological condition. Our observations indicate that the main energy substrates used in the early phases of development (pre-wintering time) are lipids. In the following months of overwintering lipids level gradually decreased and reached the lowest value just before emergence. It is understandable to use the lipids during pre-wintering time because of their high energy value, which is essential for very active, but closed in a cocoon, imago. In the next months the activity of imago slowly decrease which is associated with decreasing outdoor temperature. In contrast to the lipids concentration, during the two developmental periods the proteins content stays at the same level. These results demonstrate that probably these substrates may be used for purposes other than energy. The increase of proteins level after diapause termination may be connected with two processes: tissue remodeling and ovary growth (vitellogenins). Similarly to lipids, carbohydrates are also extensively metabolized energy substrates, in both trehalose and glycogen. Both sugars exhibit a very characteristic fluctuation during the overwintering period of imago development. We can observe two characteristic peaks of concentration: at the beginning and the end of overwintering. In *O. bicornis* at the beginning of pre-wintering, we noted the highest level of hemolymph carbohydrates. During pre-wintering, despite the closure of the cocoon, the imago inside is still very metabolically active. Therefore, in our opinion, a high concentration of carbohydrates, which are metabolised during pre-wintering, are a pre-requisite, till the bees enter diapause. The gradual and significant decline of trehalose levels in hemolymph indicates that it likely may not have any specific role as a cryoprotectant. The increase of concentration during post-wintering time is probably related

to the fact that the bees as a specific group of insects which exclusively utilize carbohydrates to power flight. Therefore it is very important an increase in amylase activity, especially in the March before imago emergence and active flight. In red mason bee, as in many others wintering insects species, the glycogen level depends on environmental conditions. In winter the low content of glycogen in the fat body cells is associated with the mobilization and transformation into cryoprotectant molecules that protect insect before freezing. However, after the diapause termination, when the outside temperature raises, the concentration of glycogen is increased as a result of re-conversion of cryoprotectants.

(4) Wasielewski O., Szczepankiewicz D., Giejdasz K., Wojciechowicz T., Bednarova A., Krishnan N. (2014) The potential role of adiponectin- and resistin-like peptides in the regulation of lipid levels in the hemolymph of over-wintering adult females of *Osmia bicornis*. Apidologie DOI: 10.1007/s13592-013-0264-z

Triacylglyceride fat stores are the most common energy reserve in most diapausing insects, often accounting for as much as 80–95% of the total lipid content. They are important because of their high caloric content, low hydration state, and perhaps relatively high yield of metabolic water. The fat body is the primary site of fatty acid synthesis, triacylglyceride production, and triacylglyceride storage in insects, although all cells can store some triacylglycerides and substantial stores can occur in tissues such as the large, metabolically active flight muscles. Managing metabolic resources such as lipids but also carbohydrates and amino acids is critical for insect during diapause when food is limited or unavailable. An adequate nutrient utilization promotes tiding over the diapause period and enables post-diapause development. The range of information about hormonal regulation in these processes is limited. Currently, the only group of hormones expected to be a regulators of metabolism during diapause are peptide hormones from adipokinetic family (AKH). AKHs comprise a family of peptide hormones that are synthesized, stored and released predominantly by neurosecretory cells of the *corpora cardiaca* (CC), neuroendocrine glands connected to the brain. The most widely recognized action of the AKH family of peptides is their role for mobilizing carbohydrates, lipids and the amino acid proline from reserves to support the extreme energetic demands of insect flight. However, evidence of the involvement of AKH in diapause metabolism is currently limited to one study. At the functional level, AKHs resembles the vertebrate peptide hormone, glucagon. Other vertebrate candidates whose function can be compared with AKHs are adiponectin and resistin, peptide hormones

discovered in the last decade from vertebrate adipose tissue, which modulate a number of metabolic processes, including glucose regulation and fatty acid catabolism. The primary structure of adiponectin and resistin differs substantially from that of members of the AKH family. Moreover, there is no information available on adiponectin-like and resistin-like peptides or their activity in insects. Therefore, the aim of our study focused on identification the factors other than AKH involved in lipid metabolism during diapause.

Based on a specific antibody assays (RIA, ELISA) against adiponectin and resistin the changes of peptides profiles with structure similar to peptide hormones were checked. After analysis of fat body tissue extracts, we found that the concentration of these peptides was different for the varied periods of development of the cocooned imago. At the beginning, during pre-wintering time the concentration of peptides defined as adiponectin- and resistin-like peptides was very high. Especially high concentration for adiponectin-like peptides we noted in first two months (September – October) of overwintering. In following periods of development, the level of both peptides declined and reached its lowest value during post-dipause time (March). The presence of adiponectin- and resistin-like peptides in fat body cells was confirmed by performing a western-blot analysis, which highlighted the differences in the concentration of both peptides in different development times in cocooned imago and indicated the pre-wintering time as a period with the highest peptide concentration. The obtained results show that the presence in fat body cells both of peptides are correlated with the lipids content hemolymph and their utilization as main energy reserves. To present the physiological role of adiponectin- and resistin-like peptides in the regulation of lipid metabolism, we made physiological assays with direct injection of various doses of synthetic adiponectin and resistin and various doses of the extract from the fat body tissue. Injections were performed in three different periods: pre-winter, winter (diapause) and post-winter. The biological assays showed a mobilizing effect of both synthetic peptide hormones (adiponectin and resistin) and fat body extracts on lipid concentration in the hemolymph. Additionally, the physiological effect of using factors (hormones and extracts) depends on the development period of cocooned imago. The results from physiological assays confirmed the highest impact of tested factors at the beginning of overwintering (in pre-wintering time).

Until now in the literature we do not notice any information relating to the presence of the insect peptide hormones with a structure similar to mammalian adiponectin and resistin (adipocytokines). The specific and sensitive tests performed in our laboratory clearly show that in the fat body cells of insect diapausing as imago, may be synthesized peptides structurally related to mammalian adipocytokines involved in the regulation of the level of free lipids in the hemolymph.

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5. Other scientific – research achievements

In 1996 I started my study at the Faculty of Biology, Adam Mickiewicz University in Poznań. Already at the second year of study I started my scientific work in Biology Students Scientific Society. Together with the colleagues we have created a section of Experimental Biology, which in the Department of Animal Physiology had conducted research, under the direction of supervisor Dr. Lesław Pilc, on the effects of various environmental factors on development of the mealworm beetle, *Tenebrio molitor*. Working in the Students Scientific Society resulted laboratory experience and numerous scientific communications presented at Student's Scientific conferences. Involvement in the scientific and academic results have been awarded numerous scholarships and awards, including the Ministry of Education scholarship. Since 2001, I started a PhD study at the Faculty of Biology (UAM). The theme of my PhD thesis was the hormonal regulation of the reproductive cycle of mealworm beetle, *Tenebrio molitor*. During my study I tested the role of different hormones in the process of eggs maturation (eg, Neb-TMOF or Neb-colloostatin) or their effects on oviducts contractility (eg. CCAP or Lem-MS). After performing of initial experiments for continuation our research we obtained a grant of which I was member of a team (2003-2005 *Neurohormonal regulators of oocyte develpment and ovarian contractility in Tenebrio molitor L. females*). During my PhD study I started collaborating with the Department of Organic Chemistry (University of Wroclaw). In this Department the oostatic hormones was synthesized and modified. The result of our collaboration were numerous communications at national and international conferences (6 communications) and 3 original scientific papers published in peer-reviewed journal Pesticides. At the turn of 2004 and 2005, after receiving a MENiS scholarship I had a 6-month research internship in the Department of Animal Physiology (Institute of Entomology, Czech Academy of Sciences). During my stay in the Institute of Entomology, I was responsible for a series of experiments focused at the identification sites of synthesis and metabolic role of neurohormones family: allatostatin existed in firebug, *Pyrrhocoris apterus*. Involvement in this type of research has given me the opportunity to learn a lot of new detection techniques such as HPLC, ELISA, RIA. Cooperation with Prof. Kodrik also allowed me to perform part of my PhD thesis in his laboratory (identification of AST-like peptide from the brain of *T. molitor*). The result of our cooperation was the communicate at an international conference and publication (*Archives of Insect Biochemistry and Physiolology* 2009 71: 223-235). My results for the first time showed the presence of AST-like peptides in the brain of *T. molitor* and their role in the regulation of gonadotropic cycle. The result of scientific internship in the Prof. Kodrik laboratory was my participation in 2008 in an

international scientific project headed by Prof. Kodrik (2008-2009 "*Insect neuropeptides – can they be used as biorationale insecticides?*").

After finished my PhD study, in June 2005 year I was employed as an assistant professor in the Department of Zoology at the Agricultural Academy in Poznan. In the new workplace I continued scientific cooperation with the Faculty of Biology (UAM). The result was an intercollegiate grant AR-UAM, of which I was the manager (2006-2007 *„Diversity of hemocytes class in different populations of invertebrate fauna as an indicator the environment degradation"*). It was a continuation of my research conducted during the MSc study on the influence of abiotic factors on model organisms (2006-2007 *„Diversity of hemocytes class in different populations of invertebrate fauna as an indicator the environment degradation"*). For the purposes of the research project I had a few days internship at the Department of Evolutionary Immunology (Jagiellonian University; Prof. B. Płytycz). During this short internship I learned to collected and indicate different classes of hemocytes, which variability was supposed to be an indicator of environmental pollution. In that time I also published in two scientific papers works based on my results obtained during PhD study. First manuscript was a summary of a research on the role of oostatic hormone in the regulation of the reproductive cycle in *T. molitor* females (*Archives of Insect Biochemistry and Physiology* 2007 64: 131-141), the second for the first time showed a pleiotropic activity of two myotropic peptides, CCAP and Lem-MS (*Journal of Comparative Physiology* B 2008 178: 877-885). Changing workplace was also associated with a change of my research topics. I started to cooperate with two other departments of my university: Department of Insect Breeding and Department of Animal Physiology. As a result of our cooperation, we created a team working on some aspects of the regulation of diapause in solitary bee, *Osmia bicornis*. Our scientific goal was supported by the grant (2008-2011 *„Hormonal regulation of diapause in red mason bee, Osmia rufa L. (Apoidea: Megachilidae)"* in which I was the manager. Scientific effect of the grant was to publish the results in the form of 6 scientific publications in prestigious journals indexed in the JCR database (Apidologie, Journal of Insect Physiology, Environmental Entomology, Insect Science). Moreover, since 2011, I am cooperating with Dr. N. Krishnan, who is the head of Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology (Mississippi State University, USA). Till this time as a result of this collaboration we published together several manuscripts in high-impacted Entomology journals. In order to the continuation of research on the regulation of diapause I was able get two grants for young researchers founded by University of Life Sciences (2011-2012 *„Mobilization of nutrient storage in overwintering red mason bee females, Osmia rufa"*; 2012-2013 *„Impact of climate change on development red mason bee, Osmia rufa L."*) ". An

extension of scientific interests focused mainly on solitary bees was to highlight to other social insects, ants. For the project, whose main aim was to clarify the mechanism of social evolution of ants we obtained the grant funding by the National Science Centre (2011-2013 *„Mechanisms of social evolution of ants: impact of potential fertility analysis on behavior and division of labor”*) in which I was a manager. The ants project was finished in November last year and the result so far is oral and poster presentation at an international conference on social insects. After completion the part of basic research on diapause control in red mason bee our team entered to the competition announced by National Centre for Research and Development. The main purpose created by the NCBiR Applied Research Programme was to finance projects only about application pattern, and obtained after the project completion product for commercialization. In 2012 to better understand the specifics of PBS programme I had a three-month internship in a company Novazym Poland organized by the Marshal's Office in Poznań ("Support for cooperation between science and business"; internship goal: Preparation of highly specific ELISA assay for determination of juvenile hormone level in insects). I had also a two-week training in projects commercialization organized by the INNO-GENE S.A. Our greatest success as a team was to obtain in 2013 a research project funded by the NCBiR within the Programme of Applied Research, in which I have the honor to be a manager (2013 to 2016 *„Modification of life cycle of red mason bee Osmia rufa to obtain the bees for crop pollination under greenhouses during winter time”*). Although the our project started in October 2013 we presented first results on the national beekeeping conference in March 2014. During the time I started my work at University of Life Sciences to this moment I published 13 articles, including 11 manuscripts in journals indexed in the JCR database. In most of them I am the first author and corresponding. This was also the period of time I managed to get funding for my research in the form of 8-financed projects by various institutions (including one international project). My involvement and academic achievements have been appreciated in the form of Scientific Rector Award (2008; 2013). My research activity focused not only on the manuscript publication and grants realization. During my research I made also two reviews of research projects (Poznań Science and Technology Park, National Science Centre) and two manuscripts (Pest Management Science, Journal of Experimental Biology). In addition, since 2011, I go regularly several times a year for a period of 7 to 14 days to scientific internships for Institute for Advanced Study, Technische Universität München, Germany (Prof. T. Sparks).

6. Teaching and organizational activities

Since 2005 I conduct at the University of Life Sciences in Poznań, lectures and classes for students of the Faculty of Animal Breeding and Biology (Animal Breeding: Zoology and ecology; Biology: General Zoology; Systematic Zoology) and Faculty of Food Sciences and Nutrition (Parasitology) . For General Zoology and Parasitology I am a lecture leader and author of syllabuses. Before the start of Parasitology classes, to improve my teaching skills , I had an internship at the Laboratory of Biology and Ecology of Parasites (ZUT Szczecin , Prof. K. Kavetska) and a course in raising and breeding of trematodes larvae (Laboratory of Biology and Ecology of Parasites, ZUT Szczecin). I was also a supervisor of bachelor (2 person) and master (3 person) thesis. It is worth emphasizing that the results of one bachelor's study have been published in the journal indexed in the JCR database (Turkish Journal of Zoology 2012 36: 1-5). I act organizationally for the Faculty of Animal Breeding and Biology participating in the work of the various committees (Faculty Recruitment Committee, Faculty Science Commission, Faculty Education Quality Commission. Since the bachelor's exams were introduced, I am also a member of the Examination Committee. Moreover, since 2012 I am a member of the Council of the Faculty of Animal Breeding and Biology.

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Piotr Wasielewski