Schildkröten im Fokus Online, Biebertal 2016 (1): 1–8

Which role may freshly molted (white) insect pupae play in the diet of omnivorous or carnivorous turtles or does self-medication occur in reptiles? Commented observations

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At a recently attended private meeting of herpetologists a report was presented about keeping and breeding the leaf turtle *Geoemyda spengleri*. The report was followed by a discussion why young hatchlings do not grow very well. Obviously many breeders experienced this problem. I mentioned that I have seen various species of carnivorous turtle hatchlings growing up and eating well when I maintained them at a constant temperature of 26–27 °C and that hatchlings which showed reduced agility and growth benefit a lot from providing them with freshly molted white pupae of mealworms. By feeding them that way it is possible that *Geoemyda spengleri* hatched in July grow up to 28 g of body weight before their first hibernation at the end of November (see also Schaefer 2005 who describes a body weight of 75 g at the age of 1 year including hibernation). But I also have to say that mealworm pupae or wax moth pupae should not be supplied as a regular food over longer periods, because it will cause abnormal growth patterns and malnutrition which may lead to malformations (for review see Bidmon 2010). Right away several of the participants commented that this type of diet should not be provided at all!

By that time I left it at that. However, I was a little surprised by such a categorical refusal of that dietary option, especially since some of the participants were affiliated with zoos or museums keeping reptile collections and I thought they were aware of the nutritional facts professional keepers should know about. I kept thinking about it and the question occurred to me whether the knowledge about the immune system of invertebrates and the special needs of holometabolic insects is really common knowledge among herpetologists

Self-sterilisation in holometabolic insects

Holometabolic insects form a group of invertebrates in which the larval stages (e. g. caterpillars, muggots) do not only look different than the adults (imagines), but in most cases also use different food resources. One of the best known examples are butterflies: e. g. the larvae of the cabbage white (*Pieris brassicae*) feeds on

a

cabbage leafs, whereas the adult butterfly collects nectar from flowers with its proboscis. The same is true for the flower beetle – even tough in this case the larvae uses the same diet (oatflakes, bread) as the imagines. And the same holds true for various other holometabolic insects which use various diets such as meat or fruits, for example that is also true for different species of flies such as Drosophila, houseflies or the blue bottle fly. These holometabolic insects have to rebuild their entire body when transforming from the larvae into the imagines. Hereby they use a special developmental stage, the so called pupal stage (or pupae), during which all organs and tissue including the digestive tract becomes transformed in order to create the tissue needed by the imagines. The caterpillar is transformed to the butterfly and the maggots turn into flies. The big challenge for all these insects is that the transformation of the tissue has to take place under more or less sterile conditions, because the entire digestive tract is restructured and has to be cleaned after feeding cessation of the last larval instar and prior to pupal formation. There is the need to eliminate the gut flora completely. What this requires is best explained by looking at the blowfly *Calliphora vicina* which deposits its eggs on dead meat or feces, which serve as the diet for its larval stages for a several days until pupation takes place. During that time the maggots already produce antibacterial substances in their salivary glands in order to savely use that diet (Chernysh & Gordia 2011). They then need to rebuild their whole digestive tract during the pupal

Fig. 1a–c
Freshly molted white flour beetle pupae (*Tenebrio molitor*) are eagerly taken by many carnivorous turtles even when they don't prefer the meal worms or adult beetles so much, as shown here for a 3 months old leaf turtle, *Geoemyda spengleri*. Especially sick or for other reasons weak young animals will start feeding on the white pupae. However, I like to mention that this food is not meant for continuous feeding! It should be a food source for special situations and emergency cases (see Bidmon 2010, see also video).

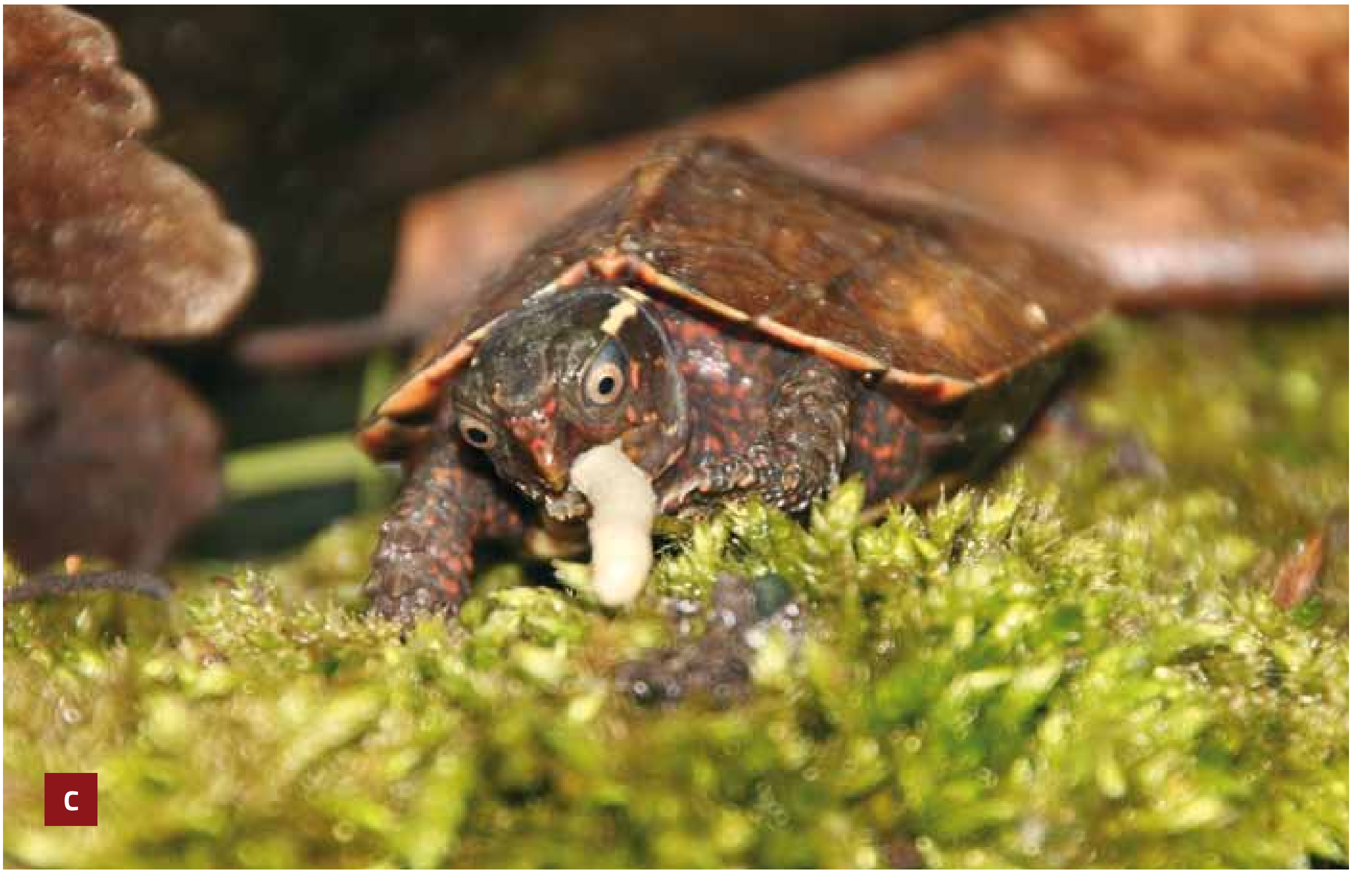

Fig. 2
Freshly molted white meal worms which have also the need to shed their protective, chitinous cuticle from parts of the feeding apparatus and the hindgut are protected during the molting cycle by defensines and antimicrobial proteins and may serve as "a special food source".

stage, because the imagines prefer more sweet diets e. g. sugared water. These fly species (as well as the goldfly *Lucilia sericata*) have been very well studied and one can even read about their medical use under the keyword "maggot-therapy" on Wikipedia (http:// de.wikipedia.org/wiki/Madentherapie). Last but not least, all holometabolic insects have the need for self-sterilisation of their digestive tract during formation of the pupa and the save restructuring of their internal and external tissues and organs.

The role defensines play in insects

For this „self-sterilization" insects use numerous highly efficient immunecompetent peptides and amino acid complexes. Most of these complexes have special names, but usually they are designated as AMP's (antimicrobial peptides) and one group is referred to as *defensines* (He et al. 2015, Méndez-Samperio 2013). These small substances cause less resistance compared to classical antibiotics and many of these AMP's are bactericidal and fungicidal and they also act against blood parasites (Chernysh et al. 2015, Hedegüs & Marx 2013, Pretzel et al. 2013). Such defensines are also known from vertebrates including man as well as birds and chelonians, e. g. for protection of the egg shell against molds and bacteria (van Hoek 2014). However, in molting holometabolic insects the level of this AMP's is high and some of them have been biochemically isolated from pupae (Yi et al. 2014). Also from the flower beetle *Tenebrio molitor* AMPs and defensines had been already isolated in the previous century (Lee et al. 1998).

Fig. 3
Meal worms can be fed with various food sources, so that their obvious nutritional deficiencies described earlier (Bidmon 2010) can be balanced to a vitamin enriched diet.

Fig. 4
Geoemyda spengleri taking a last larval instar of the wax moth. Last larval stadium of the wax moth, *Galleria mellonella* which empty their gut and prepare for the formation of the pupae represent also a rich source of defensines and AMP's and are eagerly taken. However it should be taken care that the larvae are taken directly from their cocoon. Nowadays many shops sell larvae which have been taken out and stored in a refrigerator for weeks which do not represent as the best food choice.

However, the knowledge about the presence of these substances within insects is still limited: There are approximately 700 publications listed on the „Web of Science" (from which I refer only to the most current ones) in comparison to knowledge and the huge number of publications referring to classical antibiotics. Nevertheless, the approximately 200 year old maggot-therapy of the pre-antibiotic period in human medicine has made use of the desinfecting and curing properties of these substances even without the exact knowledge of the therapeutic mechanisms (El Shazely et al. 2013, Nayduch et al. 2013, Ng et al. 2013, Gao & Zhu 2014, Zdybicka-Barabas et al. 2014). Currently AMPs and defensines are researched intensively for clinical application in humans (Wilmes & Sahl 2014, Pretzel et al. 2013, Chernysh et al. 2015). According to Professor Sahl from the University of Bonn the synthetic production of these molecules is currently very expensive. However, most recently they are used with patients infected with multiresistant bacteria, which do no longer respond to classical antibiotics therapy (Citation: http://www1.wdr.de/mediathek/audio/wdr5/audios-leonardo-102.html from Sept. 16., 2015).

Defensines act bactericidal as well as fungicidal

Defensines and turtle maintenance

To cut a long story short; I am a little bit surprised that this scientifically published knowledge has not yet been taken into account by zoo biology or for maintaining captive breeding collections. This is the only way that may explain why the audience came up with (or did not argue against) such a categorical refusal of feeding freshly molted pupae of holometabolic insects.

I am certainly not an expert for the maintenance of carnivorous turtles, but during the past I had the option to gain some experience by keeping and breeding *Geoemyda spengleri* and in addition I did collect some practical experience by cooperating with friends, who breed various turtle species (siehe Bidmon & Krug 2009, Krolak & Krolak 2011, 2015). As already mentioned in the introduction I observed that young *Geoemyda spengleri* or *Glyptemys inscupta* (wood turtles) do not prefer mealworms – and some even never touch them –, but that they do eagerly eat the freshly molted „snow white" mealworms as well as the freshly molted white pupae. Whether the above mentioned animals, which had been given to me and showed growth retardation, were affected by an infection or if they were just stressed due to prior maintenance conditions such as suboptimal temperatures is difficult to judge, because I did not do any specific diagnostic examinations when I got them. Only one of these turtles, a small *Geoemyda spengleri* with a body weight of 14 g which had stopped eating for two weeks, had been diagnosed

Fig. 5
Another leaf turtle with a wax moth larvae (details see fig. 4).

Fig. 6a–b
A small weak offspring of the wood turtle, Glyptemys insculpta eating a white meal worm pupae. It was given to me for a little nutritional enhancement in order to keep up with his siblings (see also video attachment online).

by a veterinarian to be infected with hexamites. But neither my friend Gerhard Jennemann nor I were able verify that diagnosis by examining the feces – although one sibling had supposedly died due to hexamite infection and therapeutic treatment. However, I would like to point out my observation that the growth retarded turtle hatchlings, which were kept on their own, in small enclosures, at a basic temperature of 26–27 °C in room with high relative humidity will start eating after a short time, especially when they were offered freshly molted "snow white" flower beetle pupae which were still moving (Fig. 1–9). Sure one has to select the right size of pupae, but usually there are different sizes of pupae in a flower beetle breeding stock. However, when the turtles get used to that kind of food, they will also take the bigger pupae. This diet results in continuing weight increase and higher activity. The major disadvantage however is that the turtles get quite used to that food, which makes it necessary to change the diet immediately after they have recovered to a more diversified one. This is not easy in some cases. Some keepers might be reluctant to change the diet again, because they may become afraid that the hatchlings lose weight again. But continuing this diet for longer periods of time will results in unnatural growth patterns

Defensines are also present in other insect pupae, but for example freshly molted fly pupae have the disadvantage of being immobile. The same – although less severe – applies to the pupae of the wax moth (*Galleria mellonella*) and others (Wang et al. 2013). However, these moth larvae can be used as food when the last larval instar starts to prepare for pupation by emptying the gut and spinning the cocoon as long as the larval-pupal transition is not completed. Also freshly molted pupae of the darkling beetle *Zoophoba moria* can serve as preferred diet for larger turtles.

Résumé / conclusion

Only laboratory analyses can provide clarity and all my observations and conclusions have to be considered as hypothesis. Whether the AMP's and

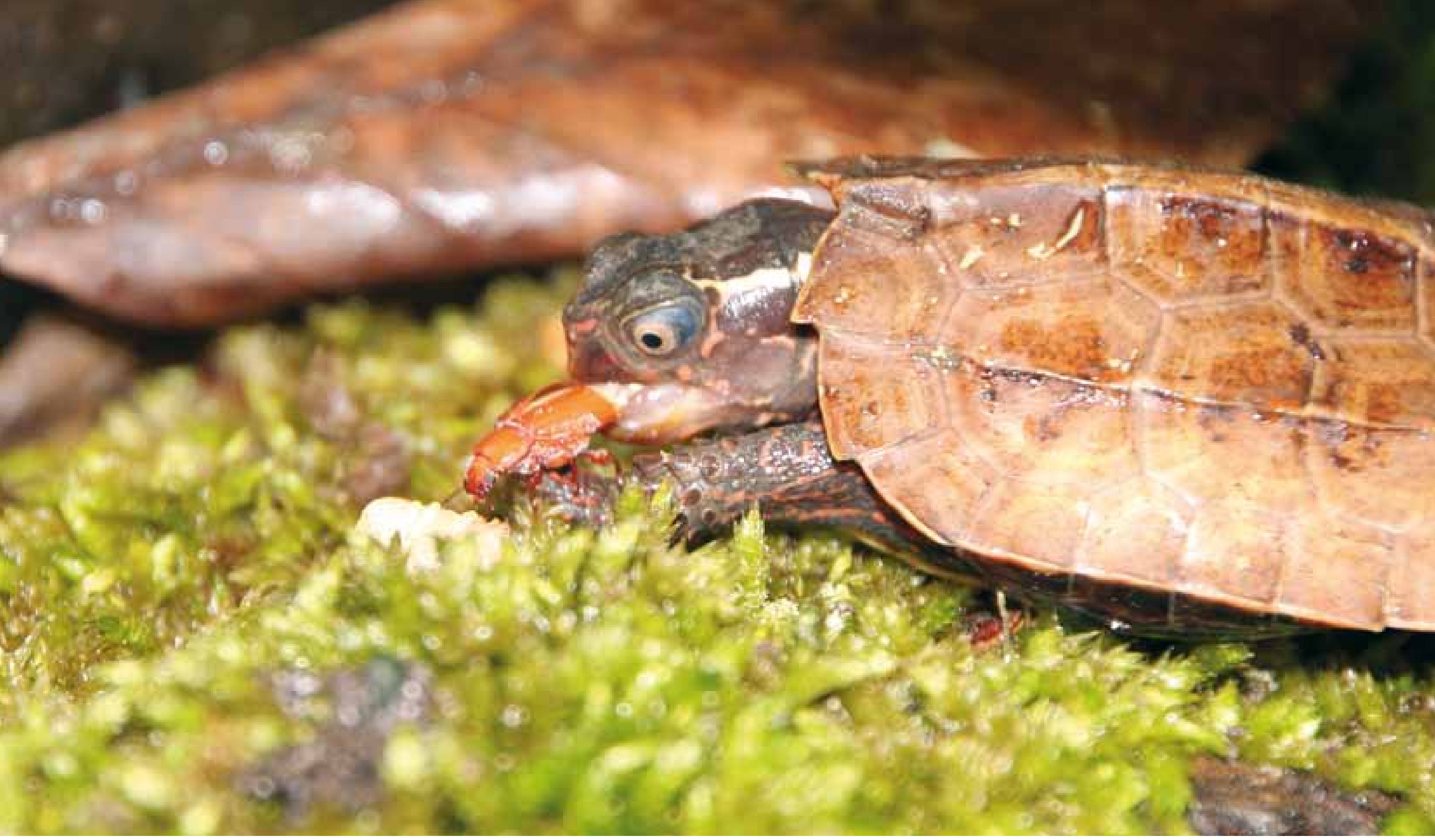

Fig. 7
Geoemyda spengleri will later also hunt for freshly molted flour beetles.

defensines present in that type of food are able to cure turtles and act against bacterial and fungal infections or gut parasites or whether they reduce the amount of „commensal microbes" (see Jennemann & Bidmon 2009) is still a matter of discussion. Also the constant temperature may have helped these animals (see Dubois et al. 2008). However, I would like to stress that my observations do not refer to a single case and that the benefits of this „white pupal diet" have been observed several times during the past years. Some of the growth retarded individuals were returned to their owners with a larger body weight compared than their siblings. In addition I would like to describe a recent case: A two year old *G. spengleri* which refused all types of presented food (earthworms, snails, woodlice) for several weeks after hibernation. This turtle was placed in the warm breeding room and freshly cocooned wax moth larvae were offered, which the turtle ate eagerly after the second trial. Whether the turtles are instinctively know which diet is helpful remains a matter of discussion, but all trials of offering earthworms or snails had failed. As a result of feeding cocooned wax moth larvae the turtle discharged smelling feces containing parts of non-digested larvae (white fat body and cuticula). However, after 11 days of feeding on wax moth larvae (every 2nd day 3–5 larvae) the feces appeared normal again and two days later the turtle ate a large earthworm without problems.

We should not negate the possibility of self-medication in animals – in that case carnivorous turtles – completely. Of course there is no well reported case and not a single scientific publication on self-medication in amphibians or reptiles. However, self-medi-

Abstract

Which role may freshly molted (white) insect pupae play in the diet of omnivorous or carnivorous turtles or does self-medication occur in turtles? Commented observations

Here I focus on the observation that hatchlings of various omnivorous and carnivorous turtles affected by growth retardation will easily feed on freshly molted white pupae and larvae of the mealworm *Tenebrio molitor* and/or waxmot *Galleria mellonella* if they are kept at an optimal temperature of 26–27 °C. This leads to a rapid onset of growth and agility. However great care must be taken to reestablish afterwards the feeding of a normal diversified diet in order to prevent malformations. These observations are discussed in the light of the role of defensins in holometabolous insects when it comes to establish a "sterile" internal pupal environment for the rebuilding and reorganization of their internal organs including the alimentary tract. In addition a possible self-medication behavior for turtles will be shortly discussed.

Keywords
Testudines, turtle, *Geoemyda, Glyptemys*, veterinary medical hypothesis, self-medication, holometabolous insects.

Fig. 8
A hatchling of a European pond turtle *Emys orbicularis* feeding on a white flour beetle pupae.

cation has been known for quite some time for animals. The best reports refer to primates (apes), which use a comparable strategy against gut parasites (Huffman 2003) as it has been proposed for antihelminthic treatment of tortoises by Jennemann (2009) using certain leafs. In addition, there are publications describing self-medication for ruminants (Amit et al. 2013), and surprisingly feeding behavior as a kind of self-medication has been studied more intensively for various insects (for example see Singer et al. 2009; Simone-Finstrom & Spivak 2012, Povey et al. 2014).

As a matter of fact so far nobody has studied self-medication in amphibians and reptiles. But this is something very different than the really negative finding that self-medication does not exist at all in these taxa. From an evolutionary point of view it is much more likely that amphibians and reptiles are able to make use of something which already exists in insects and probably in other invertebrates and that they have the possibility to do so. But I am saying

Fig. 9
Also hatchlings of loggerhead musk turtles, *Sternotherus m. minor* can be fed in special situations with white flour beetle pupae. As seen they usually prefer them more than the pieces of small earthworms.

this with caution. In the end, only the investigation under natural conditions and detailed field observations may as well as the use of specially designed feeding trails in captive management settings can experimentally test this hypothesis and provide sufficient evidence for self-medication in amphibians and reptiles.

SIF-LINK 160101

For this article several supplemental videos are available. Please type in the field SiF-Link under www.dauvi.de the number „160101" or use the following link www.dauvi.de/160101.

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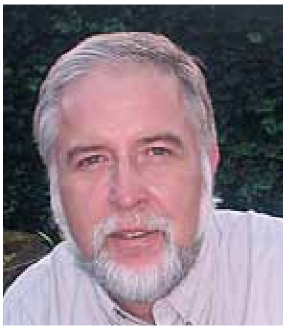

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