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Effects of polystyrene diet on *Tenebrio molitor* larval growth, development and survival: Dynamic Energy Budget (DEB) model analysis☆

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ABSTRACT

The presence of polystyrene (PS) waste increases constantly. Styrofoam, the most popular form of PS, is one of the major plastic pollutants in the environment. An efficient and environmentally friendly method of PS recycling is still needed. The biodegradation of PS by insects has been presented by researchers as a promising alternative to chemical, mechanical and thermal methods. The main aim of this study was to assess the survival, growth, and development of yellow mealworms (the larvae of *Tenebrio molitor*) fed with PS to determine if the insects are able to use PS as a source of mass and energy. The Dynamic Energy Budget (DEB) model was used to analyze the effects of food type on the growth trajectory and metabolism of tested organisms. We investigated five possible modes of influence of PS diet on DEB model parameters including a decrease of food availability, an increase in somatic maintenance power, an increase in costs for structure, allocation of energy, and a decrease in somatic maintenance power. Our results show that changes in the development of larvae fed with PS are mainly caused by a decrease in reserves density and reaction of the organism to the insufficient food supply. The inability or difficulty in completing the life cycle of *T. molitor* larvae fed with PS raises doubts about the use of mealworms as an effective technology for utilizing polystyrene.

1. Introduction

Styrofoam, the most popular form of polystyrene (PS), is widely used for the production of foamed insulation boards and packaging including disposable food packaging. The production of styrofoam reaches nearly 2 million tons per year in Europe (Source: PlasticsEurope (PEMRG)/Consultic/ECEBD). This high demand is strictly related to the enormous PS waste production. Styrofoam is light and therefore easily spread by wind and floats on water, polluting shores and waterways (Kwon et al., 2014). Moreover, PS is hardly degradable and is resistant to photo-oxidation (Bandyopadhyay and Basak, 2007). There are different methods of PS recycling including mechanical, chemical, and thermal methods (Maharana et al., 2007). Involving living organisms in polystyrene degradation seems to be an environmentally friendly and economically justified idea.

Biodegradation of PS with the participation of insects was described among others by Yang et al., in 2015 (Yang et al., 2015a, 2015b). The authors focused on the larvae of *Tenebrio molitor* Linnaeus, a species of darkling beetle belonging to the Tenebrionidae family, which ate Styrofoam. These insects are classified as holometabolic; they undergo four life stages: egg, larva, pupa, and imago. Adult individuals lay eggs in soft ground and after few weeks usually die. The optimum temperature for completing their life cycle is around 25 °C (Yang et al., 2018b). In nature, mealworms occur in the decaying bark of deciduous trees. They also live in warehouses for cereal products, poultry farms, and dovecotes

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where they feed on cereal products or dead animals. In the experiment by Yang et al. (2015a) plastic was efficiently degraded. Mealworms fed with Styrofoam survived and behaved normally during 1 month of the experiment. The depolymerization process of long-chain PS molecules in the larval gut was observed. Yang et al. (2015a) estimated that 47.7% of the ingested Styrofoam carbon was converted into CO_2 and the residue (49.2%) was excreted in faeces (frass), with a very limited fraction incorporated into biomass (ca. 0.5%). In the following experiments, Yang et al. (2015b) proved the unique role of gut bacteria in the biodegradation process. They isolated a PS-degrading bacterial strain, *Exiguobacterium* sp. strain YT2, which was able to form a biofilm on PS and create pits and holes in the plastic (Yang et al., 2015b). What is more, Tang et al. (2017) claimed that they isolated two bacterial strains, TM1 and ZM1 (cocci-like and short rod-shaped Gram-negative bacteria) from mealworm gut, which grown in medium with PS as sole carbon source. Furthermore, it was shown that antibiotics can suppress gut microbiota and inhibit PS biodegradation indicating that PS degradation is gut-microbe dependent (Peng et al., 2019; Yang et al., 2018b, 2018a; Yang et al., 2015b).

Therefore, mealworms, and their gut bacteria, seem to be a promising 'tool' for the degradation of PS waste. However, a great number of factors including temperature, humidity, photoperiod, oxygen concentration, population density, parental age, food quality, and toxic substances influence directly the development of larvae. We decided to investigate whether the larvae can be used as an effective technology for recycling PS. Therefore, the main aim of this study was to assess the survival, growth, and reproduction of larvae fed with PS.

2. Dynamic Energy Budget model description

The rationale for the observed effect of PS on mealworms can be provided by mathematical models. They are used to deduce the reasons for observed phenomena; this, in turn, can lead to solutions to some emerging problems. One of the most powerful models used to describe life cycles of different organisms is the Dynamic Energy Budget (DEB) model. DEB theory describes the energy and mass fluxes in organisms and is used to assess changes in physiological processes (maintenance, growth, development, and reproduction). The DEB model provides a framework to predict individual-level responses to environmental stress. It has already and successfully been used to predict the toxicity of different compounds, the influence of changes in physicochemical properties of the environment, reaction to lack of food and many other factors (Jager et al., 2016, 2014, 2013; Kooijman, 2010). There are several versions of DEB models in the literature. Among them, special attention should be paid the standard model DEB, which in most cases was used as a starting point to derive new equations in new model versions (Jager et al., 2013; Jager and Zimmer, 2012; Kooijman, 2010; Llandres et al., 2015). The scheme of energy fluxes in a standard DEB model is presented in Fig. 1. The energy is assimilated from the food and accumulated as a reserve. Energy from the reserves is mobilized and used for maintenance, growth, maturation or reproduction (Kooijman, 2010).

However, its application to describe the life cycle of holometabolic organisms is a relatively new issue. Only a few papers have been published concerning this type of organism in the DEB context. DEB model for holometabolic insects can be treated as an extension of the standard DEB model with some specific assumptions and equations added (Llandres et al., 2015). Another version of the DEB model which allows for significant simplifications of standard DEB model equations is the simplified DEB model (Jager and Zimmer, 2012). It is dedicated to assessing the impact of toxic substances on the energy budgets of organisms which is also a premise for using this model to assess the impact of PS on mealworms. In this study, the extended simplified DEB model was used to analyze the effects of food type on the growth trajectories and metabolism of *T. molitor* at the larval stage (not the complete life cycle). The model is derived on the basis of standard DEB, simplified and extended by assumptions for the holometabolic insects DEB model. Therefore, assumptions for the standard DEB model (Kooijman, 2010), the model for holometabolic insects (Llandres et al., 2015) and simplified DEB model (Jager and Zimmer, 2012) should be satisfied and consistent. The most important model assumptions, directly related to the growth and development of *T. molitor* larvae and relevant to the derivation of equations, are listed and commented below:

Assumptions *for standard DEB model (all 10 assumptions for the standard DEB model can be found in* (Kooijman, 2010)*; non-listed assumptions are met for the present case)*

a) Structures and reserves have a constant composition (strong homeostasis) – it holds for the control sample; however, a change in food composition, for instance, the addition of PS, can affect the reserve composition and reserve dynamics;
b) The ratio between structure and reserve amounts is constant when the food availability is constant (weak homeostasis) – it holds for the control sample; when the food availability will change the ratio between amounts of reserve and structure will

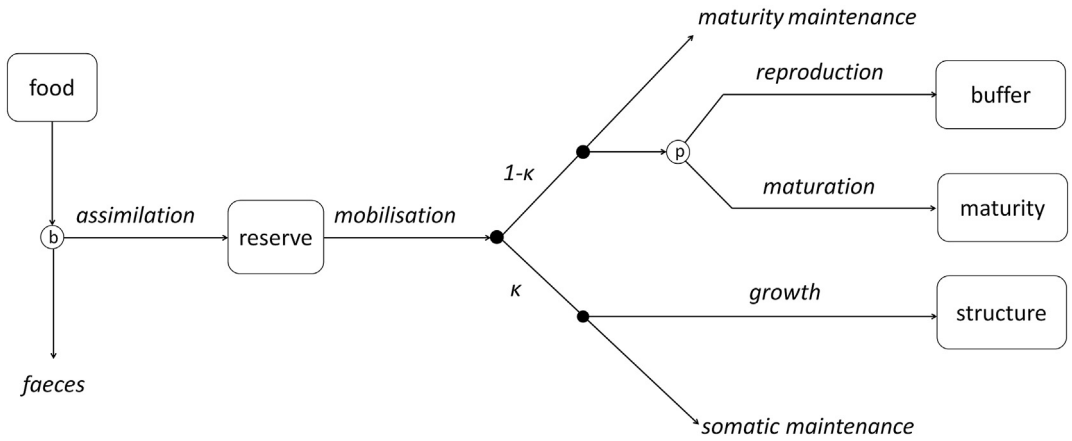

Fig. 1. Energy flows in a standard DEB model. Symbol *b* indicates a switch to feeding after birth; symbol *p* indicates a switch to reproductive investment after puberty. The *kappa* rule is applied for mobilization flux (Kooijman, 2010).

also change. The transition dynamics between steady states at different food availability levels can be used to determine the DEB model parameters. For instance, if the loss of reserves during starvation can be observed as a loss of mass or volume of organisms, the energy conductance can be determined;

c) Individuals do not change shape during growth (isomorphism) - for T. molitor the change of shape can be noted between different life stages; however, we deal only with larvae (adult in DEB context) which can be assumed to be isomorphs, as they do not change shape during growth (note that V0 and V1 - morphy can be applied to different species or life stages);

Assumptions *for holometabolous insects* (Llandres et al., 2015)

d) The reproduction buffer is accumulated in the larval phase; when it exceeds a critical value, pupation occurs;
e) Maturity of larvae does not change, and their cells do not differentiate; therefore, there is no need to consider maturation flux. The larvae are adults in the DEB context; they feed and they use reserves to feel reproduction buffer. Maturity maintenance is constant; it does not change during the time of larval growth;

Assumptions *for simplified DEB model* (Jager and Zimmer, 2012)

f) For juveniles and embryos, there is always a constant ratio between structure and maturity – this assumption is not applicable as we treat larvae as adults in the DEB context;
g) The cost of making an egg is constant – in the case of larvae when the reproduction buffer reaches threshold value pupation occurs;
h) The reproduction buffer can be scaled.

These assumptions are compatible with each other and met for the description of *T. molitor* larval development. Therefore, a simplified DEB model (with further extensions) can be used to analyze the growth of the larvae and reserve dynamics. A comprehensive description of the simplified DEB model with an application example can be found in the paper of Jager and Zimmer (2012). The model can be expressed by three main equations:

$$\frac{de}{dt} = (f - e)\frac{\dot{v}}{L} \tag{1}$$

$$\frac{dL}{dt} = \frac{\dot{k}_M g}{3(e+g)}\left(e\frac{\dot{v}}{\dot{k}_M g} - L\right) \tag{2}$$

$$\dot{R} = \frac{\dot{R}_m}{L_m^3 - L_p^3}\left(\left(\frac{\dot{v}}{\dot{k}_M}L^2 + L^3\right)\frac{e}{e+g} - L_p^3\right) \tag{3}$$

Equation (1) describes reserves dynamics, equation (2) describes changes in the structural length of organisms, and equation (3) describes the reproduction rate. The model variables and parameters are introduced in Table 1.

Equation (2) can be expressed with only two parameters:

$$\frac{dL}{dt} = \dot{r}_B(L_m - L) \tag{4}$$

where L_m is the maximum body length and $\dot{r}_B$ is the Von Bertalanffy growth rate (both at given constant scaled reserve density e). These parameters are related to other DEB model parameters:

$$\dot{r}_B = \frac{\dot{k}_M g}{3(e+g)} \tag{5}$$

$$L_m = e\frac{\dot{v}}{\dot{k}_M g} \tag{6}$$

Equation (4) can be analytically solved when $f = const.$ and $e = const$. The solution takes the form given by equation (7):

$$L(t) = L_{m0}\left(1 - e^{-\dot{r}_B t}\right) + L_0 \tag{7}$$

where L_0 is the structural length at the beginning of the experiment; L_{m0} is the maximum structural length above L_0 ($L_0 + L_{m0} = L_m$). When $\dot{r}_B$ and L_m are estimated, there is a possibility of calculating $\dot{k}_{M0}$ and g_0 in the control sample, assuming that $f = 1$ and therefore $e = 1$, and the value of $\dot{v}$ was estimated in a different experiment. According to DEB theory, parameters $\dot{k}_M$ and g can be expressed using primary DEB model parameters:

$$\dot{k}_M = \frac{[\dot{p}_M]}{[E_G]} \tag{8}$$

$$g = \frac{[E_G]}{\kappa[E_m]} \tag{9}$$

These relationships can be useful for the interpretation of larval growth patterns in different feeding conditions. Additional assumptions and extensions of the simplified DEB model specific for *T. molitor* larvae are introduced in *Materials and Methods* section.

3. Materials and methods

3.1. The experiment

The experiments were carried out on *T. molitor* larvae (from 'Cricket Farm' in Lublin, Poland). Larvae were divided and allocated into seven groups with different feeding conditions, with 50 specimens in each. The mass of each specimen in each group at the beginning of incubation was in the range 0.02–0.05 g, except one group where the mealworms were lager (see *PS large* group). The insects were reared for 3 months (91 days, up to three times longer than in previous studies (Yang et al., 2018a; Yang et al., 2015a)) at 25 °C and 80% humidity in the laboratory and supplied with water. The groups of mealworms were as follows:

- *Control* – larvae fed with oatmeal *ad libitum*;
- *Starvation 1* – larvae with no source of food; each individual was kept in seclusion without the possibility of eating droppings and moult residues (in contrast to all other groups);
- *Starvation 2* – larvae with no source of food, kept together with the possibility of eating droppings and moult residues;
- *PS* – larvae fed with PS (Styrofoam chips for ensuring safe transport package, commercially available);
- *PS large* – older and larger larvae compared to the *PS* group; with a mass in the range 0.06–0.1 g each, fed with PS;
- *PS + oat.* – larvae fed with PS and crushed oatmeal; oatmeal was served every 10 days, left for 24 h and then removed;
- *PS + oat. + vit.* – larvae fed with PS; vitamins and minerals (see Supporting Information for full composition) were mixed with crushed oatmeal and added every 10 days, left for 24 h and then removed.

Every second or third day of the experiment, the number of

Table 1
DEB model main variables and parameters. The units are expressed by: l − length of the organism; e − energy; t − time.

Symbol	Description	Units
Variables		
L	Structural body length	l
L_V	Volumetric body length	l
L_w	Physical body length	l
L_E	Volumetric length of reserve	l
$\dot{R}$, $\dot{R}_S$	Reproduction rate/scaled reproduction rate	t^{-1}
R, R_S	Integrated reproduction/scaled integrated reproduction	[-]
e	Scaled reserve density	[-]
E	Amount of energy in reserve	e
$[E]$	Reserve density	el^{-3}
E_R	Amount of energy in reproduction buffer	e
$[E_R]$	Density of energy in reproduction buffer	el^{-3}
Parameters		
f	Scaled functional response	[-]
$\dot{v}$	Energy conductance	lt^{-1}
$\dot{k}_M$	Somatic maintenance rate coefficient, in control $\dot{k}_{m0}$	t^{-1}
g	Energy investment ratio, in control g_0	[-]
$\dot{R}_m$, $\dot{R}_{Sm}$	Maximum reproduction rate (at $f = 1$, $L = L_m$), in control $\dot{R}_{m0}$/Scaled $\dot{R}_m$, in control $\dot{R}_{Sm0}$	t^{-1}
L_m (e), $L_{Vm}(e)$	Maximum structural/volumetric body length − for given constant e used as a parameter	l
L_0, L_{V0}	Structural/volumetric length at the beginning of the experiment	l
L_{m0}, L_{Vm0}	Maximum structural/volumetric length above L_0 and L_{V0}	l
L_p, L_{Vp}	Structural/volumetric body length at puberty	l
L_{Em}	Maximum volumetric length of reserve for given L and e	l
L_{S0}	Structural body length at the beginning of starvation	l
$L_{survive}$	Critical structural length	l
$[\dot{p}_M]$	Volume − specific somatic maintenance power	$et^{-1}l^{-3}$
$[E_G]$	Volume − specific costs for structure	el^{-3}
$[E_m]$	Maximum reserve density	el^{-3}
E_0, E_T	Energy content of a single egg/total number of eggs	e
$[E_0]$, $[E_T]$	Energy density threshold for a single egg/ total number of eggs	el^{-3}
$[E_R^j]$	Energy density threshold for pupation	el^{-3}
κ	Fraction of mobilized reserves allocated to the soma	[-]
κ_R	Fraction of allocated reserves fixed in eggs	[-]
$\dot{r}_B$	Von Bertalanffy growth rate − for given constant e used as a parameter	t^{-1}
u_V	Proportionality coefficient equal to constant ratio $\frac{L_V}{L}$	[-]
u_R	Proportionality coefficient equal to constant ratio $\frac{L_E}{L}$	[-]

surviving larvae and the number of pupae were counted and a picture of them was taken (Canon EOS 400D with Tokina macro lenses ATX Pro). The physical length L_w of larvae (the perimeter of the orthographic projection of larvae; see Supporting Information for detailed description) was measured from the pictures. The length in pixels was converted into units [mm] using a marker (5.1 mm long). The physical length (L_w) was then converted into volumetric length L using a shape coefficient. The density of larvae and the shape coefficient were determined earlier. A full description of the estimation of these parameters can be found in the Supporting Information.

3.2. Changes in larvae volume

It has to be pointed out that in this work we assume that the volume of larvae L_V^3 is the sum of the volume occupied by structures L^3 and reserves L_E^3:

$$L_V^3 = L^3 + L_E^3 \tag{10}$$

Therefore, the change in the volume of larvae is given by:

$$\frac{dL_V^3}{dt} = \frac{dL^3}{dt} + \frac{dL_E^3}{dt} \tag{11}$$

It should be noted that according to standard DEB model (DEB for holometabolic insects and simplified DEB) the energy accumulated in the reproduction buffer cannot be returned to reserves and used to sustain life and growth. We assumed that the changes in the volume of reproduction buffer do not affect equations (10) and (11).

Furthermore, we have extended the assumptions of strong and weak homeostasis and assume that the amount of reserves is proportional to the volume of reserves, and therefore for constant feeding condition the ratio between the volume of reserves L_E^3 and structure volume L^3 is constant during larval growth. According to these assumptions, it can be shown that:

$$\frac{dL_V}{dt} = \frac{\dot{k}_M g}{3(e+g)}\left(u_V e \frac{\dot{v}}{\dot{k}_M g} - L_V\right) \tag{12}$$

where u_V is a proportionality coefficient equal to constant ratio $\frac{L_V}{L}$ and therefore according to equations (4), (6), (7) and (12):

$$L_{Vm} = u_V L_m \tag{13}$$

$$\frac{dL_V}{dt} = \dot{r}_B(L_{Vm} - L_V) \tag{14}$$

$$L_V(t) = L_{Vm0}\left(1 - e^{-\dot{r}_B t}\right) + L_{V0} \tag{15}$$

The full derivation can be found in Supporting Information. Equation (15) was used to estimate parameters in the control sample.

The reserves are preferred to be used to maintain structures. Therefore we assumed that during starvation, volumetric length L_V can decrease because of the decrease of scaled reserves density; the structures are preserved. Furthermore, we assumed that growth, the increase in structure volume given by equation (4), does not occur during starvation ($\frac{dL_S^3}{dt} = 0$) and the scaled reserve density can be expressed as:

$$e(t) = \frac{L_E^3(t)}{L_{Em}^3} \tag{16}$$

where L_{Em}^3 is the maximum volume of reserves for $f = 1$ and $e = 1$, and depends on L. Therefore, the change in volumetric length caused by a decrease in reserves density can be derived from equations (1), (11) and (14) and is expressed as:

$$\frac{dL_V}{dt} = \frac{1}{3}\left(\frac{fL_{Em}^3 + L_{S0}^3}{L_V^2} - L_V\right)\frac{\dot{v}}{L_{S0}} \tag{17}$$

where, in the case of a lack of structures growth L_{Em},L_{S0} are parameters along with $f$and $\dot{v}$. This equation considers reserves dynamics and cannot be derived from equation (4) which describes changes in structures. The full derivation can be found in Supporting Information.

3.3. PS mass loss

The mass loss of PS was determined after 20, 30, 40, and 50 days of the experiment. Styrofoam polystyrene chips were removed from rearing boxes with larvae, gently cleaned of faeces and food, weighed, and returned into boxes.

3.4. Survival in DEB context

According to the DEB model, an organism can die if the somatic maintenance costs can no longer be paid (Kooijman, 2010; Muller and Nisbet, 2000). The criterion for larvae to survive can be expressed as:

$$L(t) \le L_{survive} = \frac{e(t)\dot{v}}{\kappa \dot{k}_M g} \tag{18}$$

The right-hand side of this inequality should have an equal or higher value than the certain structural length of the organism. In a situation when after a change of feeding conditions, the right side of inequality (18) would reach a value lower than the structural length obtained before this change ($L_{survive}$), the organism will not survive.

3.5. Time of pupation

According to the paper by Llandres et al. (2015) the pupation of larvae occurs when the density of the reproduction buffer achieves a certain threshold. We are not able to obtain data for the density of the reproduction buffer; however, we can estimate the time when the threshold is reached.

In order to do it, we have to look at equation (3) which, according to definition (Jager and Zimmer, 2012) and proportionality coefficient u_V, can be rewritten as a:

$$\dot{R} = \frac{\kappa_R \frac{dE_R}{dt}}{E_0} = \frac{\dot{R}_m}{L_{Vm}^3 - L_{Vp}^3}\left(\left(\frac{\dot{v}}{\dot{k}_M}L_V^2 + L_V^3\right)\frac{e}{e+g} - L_{Vp}^3\right) \tag{19}$$

and its integrated form:

$$R = \int \dot{R}dt = \frac{\kappa_R E_R(t)}{E_0} \tag{20}$$

equation (20) describes the amount of energy invested into eggs per energy content of a single egg. We can also rewrite this equation for energy densities – the amount of energy per structural volume of the organism:

$$R = \frac{\kappa_R E_R(t)}{E_0} = \frac{\kappa_R \frac{E_R(t)}{L^3}}{\frac{E_0}{L^3}} = \frac{\kappa_R [E_R](t)}{[E_0]} \tag{21}$$

Here it should be pointed out that equation (19) describes the energy flux from the reserve into reproduction buffer, and further, multiplied by the consonant κ_R, the fraction of energy which flows from reproduction buffer directly into the eggs (the remainder is lost as overheads). The pupation does not use energy accumulated in the reproduction buffer, pupal reserves are supplied from larva structures and reserve (Llandres et al., 2015). The reproduction buffer would be used for egg production in later development stages (eg imago). Therefore, in the case of larvae, we can simplify the equation by removing κ_R - the parameter which is useless during the larval stage when the eggs are not produced. Furthermore, we can replace E_0 by the total energy content of all eggs which can be produced in the future during the imago stage E_T. The substitution is consistent with the derivation of equation (19) (Jager and Zimmer, 2012):

$$R_S = \frac{E_R(t)}{E_T} = \frac{[E_R](t)}{\left[E_R^j\right]} \tag{22}$$

In this case $[E_T]$ is a reproduction buffer (energy) density threshold for the total number of eggs, which is analogous to the reproduction buffer (energy) density threshold for pupation $[E_R^j]$ described by Llandres et al. (2015). Therefore, R_S is an integrated reproduction R scaled by the total number of eggs possible to be produced during the imago stage $\frac{E_T}{E_0}$. Note, that the value of E_0 can be used to calculate the number of eggs, however, it does not have any influence on the amount of energy stored in the reproduction buffer.

According to equation (22), when the reproduction buffer (energy) density threshold is reached ($E_R(t) = E_T$) the integrated scaled reproduction R_S is equal to 1 and pupation can begin. However, to use this value we have to know when it would be reached. The time needed to obtain critical value $[E_R^j]$ can be estimated from the pupation dataset. The threshold for pupation is reached most likely when most of the larvae pupate (if we started an experiment with the larvae approximately at the same age and size). If we assume that this threshold is normally distributed, we have to determine the time when the highest value of the density curve, which indicates the highest probability of pupation, occurs. Before and after this time, pupation can also occur but less frequently. The accumulated number of pupae, scaled by the number of specimens, occurring in a sample can be then described by a sigmoidal distribution curve (log-logistic model) (Ritz, 2010):

$$P(t) = 1 - \frac{1}{1 + \left(\frac{t}{\tau}\right)^{b}} \quad (23)$$

where P is the probability of achieving threshold after a given time t (values between 0 and 1); t is the time [days]; n describes the slope of the distribution curve, and; τ is the time at which pupation occurs most likely (inflection point of distribution curve) [days] – so this is exactly the value we are looking for. Therefore, the integrated version of equation (19) scaled by $\frac{E_T}{E_0}$ and additional data point $R_S(\tau) = 1$ can be used in model parameters estimation.

3.6. Analysis of growth patterns and parameter estimation

A lot of information can be obtained from the analysis of growth curves. Therefore, model parameter estimation was divided into five steps, which are described in Supporting Information.

3.7. Modes of action

The influence of PS diet on *T. molitor* larvae can be caused by different possibilities which include the influence of insufficient food supply and the toxic effects of PS metabolites and impurities present in Styrofoam (Brandon et al., 2020). Using a simplified DEB model, we can consider:

- a simple decrease in food availability in the environment, decrease in f;
- toxicity of PS which can be considered in a few ways depending on the mode of toxic action (Jager and Zimmer, 2012). The possible modes of affecting DEB parameters are (for a constant value of $\dot{v}$ – assumption in simplified DEB model): increase in somatic and maturity maintenance $[\dot{p}_M]$; increase in costs for structure and maintenance $[E_G]$; increase of overheads costs of making an egg; hazard during oogenesis. We cannot measure the number of new eggs produced – when the reproduction buffer is filled, we observe pupation, which happens only once during the life of an organism. Furthermore, we do not deal with oogenesis. Therefore, we do not consider the last two modes of toxic action.
- the reaction of larvae to starvation which can be quantified by the change of different primary DEB model parameters (Kooijman, 2010): allocation of energy – an increase of the kappa κ parameter; a decrease in somatic maintenance costs $[\dot{p}_M]$ (with constant kappa κ); use of structures as reserves (shrinking); faster production of eggs (seeds in plants). We assume that the observed decrease in length is caused by the decrease in reserves, as they are preferred to maintain life (Kooijman, 2010). We also did not observe faster pupation as an analogue to faster egg production which is a typical reaction to starvation in plants. Therefore, we focused on the first two mechanisms.

The chosen possible mechanisms are summarized in Table 2. We showed which DEB parameters are affected in each case and how other parameters can be affected.

4. Results and discussion

The density of larvae in the *Control* sample did not change during their growth and had a value of $\boldsymbol{\rho} = 0.89$ g/mL (see Table S2 and Figs. S3 and S4 in Supporting Information). A lack of changes in density can support the assumption that maturation does not occur during the larval stage of *T. molitor* (Llandres et al., 2015). The shape coefficient for mealworms has a value $\sigma = 0.1027$ (see Table S2 and Fig. S2). This value did not change during the growth of larvae, which confirms the assumption of isomorphic growth.

4.1. Mass loss of PS

To be sure that larvae eat PS, we measured the mass loss of PS chips during the experiment. Results of mass loss in each sample variant are presented in Fig. 2A and in Table S5.

The differences in the accumulated PS mass loss among the four groups (*PS*, *PS large*, *PS + oat*, and *PS + oat. +vit.*) were probably due to the differences in larval survival rates (see section *Survival*). This could be shown when comparing data after, e.g., 50 days of the experiment (Table S5). The highest number of larvae, 46 specimens, was alive in the *PS + oat.* group, and the highest mass loss was noted – 51.4%. In the *PS + oat. + vit.* group, containing 45 specimens, after 50 days we observed lower mass loss – 39%. The lowest mass loss was noted for *PS large* – 32.6% for 39 specimens, and for *PS* – 16.7% for 15 specimens. The mass loss presented in Fig. 2A was comparable to the results obtained by Yang et al. (2015a) - 31% after 30 days for 500 specimens at the beginning of the test and by Yang et al. (2018a) - approximately 46% after 32 days for 120 specimens at the beginning. The differences between the results obtained in the mentioned studies could be caused also by the differences in the PS material used, especially its density which can have a large effect on its biodegradability (Yang et al., 2018a). The addition of diet supplements seems to extend the time of high eating rate (the slope of the curves in Fig. 2A) by an improvement of the larvae's conditions, providing nutrients necessary for their survival and proper functioning, needed for the synthesis of biomass and new enzymes, etc. Our results confirm the findings of Yang et al. (2018a) who observed 67.6% and 76.8% consumption of PS by larvae additionally fed with bran and soy protein respectively, compared to 39.1% when larvae were fed only with PS. It should be pointed out

Table 2
Possible mechanisms of reaction of *T. molitor* larvae to PS diet.

Possible mechanism	Parameter changes	Possible change in $\dot{r}_B$, L_m and $\dot{R}$
(a) – food availability		
1. Decrease of food availability in environment	$f \downarrow$ and $e \downarrow$	$\dot{r}_B \uparrow$ $L_m \downarrow$ $\dot{R} \downarrow$
(b) – PS toxic action		
2. Increase in somatic maintenance power $[\dot{p}_M]$	$\dot{k}_M \uparrow$ and $\dot{R}_m \uparrow$	$\dot{r}_B \uparrow$ $L_m \downarrow$ $\dot{R} \uparrow$
3. Increase in costs for structure $[E_G]$	$\dot{k}_M \downarrow$ and $g \uparrow$ [a]	$\dot{r}_B \downarrow$ $L_m = const.$ [a] $\dot{R} \uparrow\downarrow$
(c) – reaction to starvation		
4. Allocation of energy – increase of kappa κ parameter	$g \downarrow$	$\dot{r}_B \downarrow$ $L_m \uparrow$ $\dot{R} \downarrow$
5. Decrease in somatic maintenance power $[\dot{p}_M]$	$\dot{k}_M \downarrow$	$\dot{r}_B \downarrow$ $L_m \uparrow$ $\dot{R} \downarrow$

[a] We assume the same stress factor value for both parameters (the change is in $[E_G]$) (Jager and Zimmer, 2012).

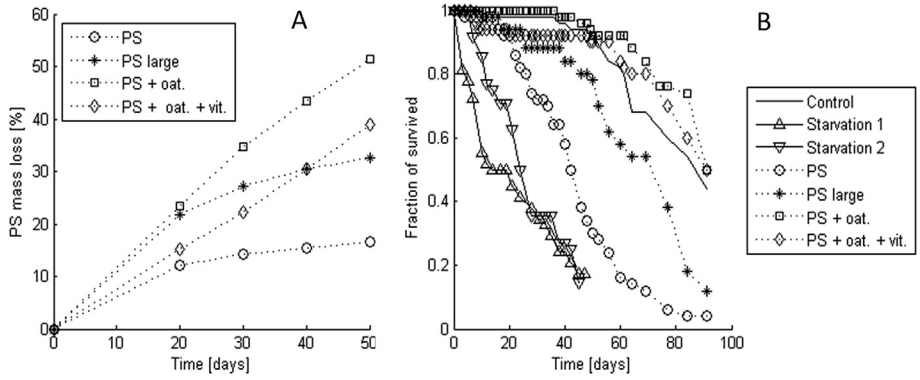

Fig. 2. A - Accumulated mass loss of PS chips during the experiment. **B -** Fraction of surviving larvae in different feeding conditions.

that we noticed that larvae ate PS; however, we still do not know if it is decomposed and biodegraded in their gut so they can use it as a mass and energy source for somatic maintenance, growth, and reproduction.

4.2. Survival

The survival curves can give us some information about the energetic costs paid for somatic maintenance. Fractions of surviving larvae in different feeding conditions are shown in Fig. 2B.

The results obtained by Yang et al. (2015a) (approx. 86%, 30 days) and Yang et al. (2018a) (86,7%, 32 days) showed no significant differences between PS-fed samples and controls. After 30 days, we observed a similar survival probability in the *PS large* group (88%) and one a bit lower in the *PS* group (72%). The survival rate in the *Starvation* group was lower than in the *PS* group and reached a value of 34–35% after 30 days. This confirms the finding of Yang et al. (2018a) who also noted a lower survival rate in the unfed larva population (54.2%, 32 days) compared to a population fed only with PS.

These results could suggest that larvae are able to survive when they eat PS. However, which is of great importance, the substantial differences in survival between groups with different feeding conditions were noted for longer than 30 days incubation times, up to 3 months. Some examples of survival rates after 98 days can be found in Yang et al. (2018b). In our study, the highest mortality was observed in the *Starvation 1*, *Starvation 2*, *PS* and *PS large* groups. After 42 days of the experiment, 25% of larvae in the *Starvation 1* and 20% in *Starvation 2* were alive. After 91 days, only 4% were alive in *PS* and 12% in the *PS large* group. In comparison, in the *Control*, *PS + oat.* and *PS + oat. + vit.* groups, we observed that after 42 days more than 92% of specimens were still alive, and after 91 days the fraction surviving was 0.44, 0.5 and 0.5 respectively in each group. This indicates that the addition of diet supplements increases the survival probability (Fig. 2B), which was also observed by Yang et al. (2018a). In the case of *Starvation* and *PS* groups, the criterion given by inequality (18) has not been met. The most probable explanation for these observations is that reserves were not supplied sufficiently and therefore the scaled reserve density $e(t)$ decreased to a level which was not sufficient for somatic maintenance. The larvae fed with PS and supplemented with oatmeal had a similar survival rate compared to controls. The supplementation was sufficient to pass the criterion given by inequality (18).

4.3. Time of pupation

The number of pupae observed in *Control* group between days 42 and 84 was sufficient to estimate the average time of pupation which was equal to $\tau = 81$ days from the beginning of the experiment (Table S3 in Supporting Information). We did not note any pupae in other groups besides one pupa in *PS + oat. + vit.* This indicates a strong influence of diet on the life cycle of *T. molitor* and confirms the findings obtained by Yang et al. (2018a) where only larvae fed with PS and supplemented with bran were able to complete their life cycle. It can be also supported by the results obtained by Morales-Ramos et al. (2010) who observed that the number of instars varied from 10 to 16 depending on the type of food available. They found out that the addition of a diet supplement which made up only 20% of the total diet affected the development time. The authors concluded that some nutrients like starch, protein, and vitamins are beneficial and significantly influence the growth and development of larvae. A change in feeding conditions can affect the scaled reserve density *e*. According to equation (3), a decrease in *e* causes a decrease in reproduction rate $\dot{R}$ and can extend the time needed to reach the reproduction buffer threshold and form pupae. Therefore, bad feeding conditions can lead to a situation where the threshold will not be reached before the death of specimens. However, it is possible that when starvation occurs when the reproduction buffer is already partially filled, the reserves accumulated will be sufficient for specimens to form a pupa. Connat et al. (1991) observed that large larvae of *T. molitor* (>50 mg at the beginning of incubation) have instars of longer duration and are able to complete larval development and moult to pupae during starvation. This supports the idea that the reproduction buffer is filled from reserves.

4.4. Growth

The results of the DEB model growth curve fitting (*Steps 1–5*) are shown in Table 3 and Fig. 3. The low values of the sum of squared errors (SSE) and root mean square error (RMSE) indicate a good model fit. Low values of R^2 indicate that the function is almost parallel to the X axis.

In the control sample, we observed an increase of volumetric length (Steps 1 and 3; Fig. 3A and B1). The increase of biomass in the control previously noted in research carried out by Yang et al. (2015a) was equal to 33.6% over 16 days and by Yang et al. (2018a, b) 32% during 32 days.

Both studies also indicated a decrease in the biomass of unfed larvae – 24.9% after 16 days (Yang et al., 2015a) and 2.6% after 32 days (Yang et al., 2018a). Our results showed a decrease of volumetric length of larvae in the *Starvation 1* and *Starvation 2* groups as shown in Fig. 3C and D. We did not observe any increase in volumetric length in these groups even at the beginning of the

Table 3
The results of DEB model growth curve fitting (*Step 1–5*).

	Parameters									Goodness of fit		
	$\dot{r}_B$	L_{Vm0}	L_{Vm}	L_{S0}	$\dot{v}$	f	$\dot{k}_M$	g	$\dot{R}_{Sm}$	R^2	SSE	RMSE
Step 1 control	0.10	1.4	5.1			1[a]				0.37	275.3	0.47
Step 2 Starvation 1				3.58	0.54	0[a]				0.74	0.19	0.10
Step 3 control					0.54[a]	1[a]	0.82	0.58	0.014	0.85	0.61	0.10
Step 4 PS + oat.	0.04	0.51	4.0							0.09	180.7	0.32
PS + oat. + vit.	0.02	0.47	4.1							0.01	153.3	0.30
*Step 5*** Starvation 2	≈0[a]			3.40	0.54[a]	0[a]				0.05	0.14	0.09
PS	≈0[a]			3.45	0.54[a]	0[a]				0.52	0.51	0.13
PS large	≈0[a]			4.31	0.54[a]	0[a]				0.47	0.32	0.11

[a] Not estimated, fixed parameters; additional calculated constants can be found in Supporting Information (Table S4).

starvation period and therefore we assumed that there was no increase in larval structures (basically, it can be interpreted as $\dot{r}_B = 0$ or $L = L_m$). We assumed that the decrease in volumetric length is caused by a decrease in reserve density e and that the food availability f is equal to zero. This assumption allowed us to estimate the energy conductance $\dot{v}$ (Step 2, Table 2) in *Starvation 1*. The energy conductance was assumed to be constant and we used it as a fixed parameter in further steps. The *Starvation 2* group was used to get information about the energy available for larvae from droppings and moult residues.

Really interesting information can be obtained from groups *PS + oat.* and *PS + oat. + vit.* in which the survival rate was almost the same as in *Control* but the growth and reproduction were partially inhibited. In both groups, we observed a decrease in DEB model parameters: $\dot{r}_B\downarrow$, $L_m\downarrow$, and in $\dot{R}\downarrow$ (Table 3). This pattern can be compared with the possible modes of action listed in Table 2 and concerning food availability, toxicity, and physiological response to starvation.

Supplementation of the diet in these groups could be still insufficient to fill the reserves, and therefore reserve density e could decrease (mode 1) as was observed in the *Starvation 1* group. This mode of action could explain the decrease in L_m and $\dot{R}$ as pupation was not observed or occurred less often than in the control sample. However, it cannot explain the decrease in $\dot{r}_B$; values were equal to 0.04 and 0.02 in *PS + oat.* and *PS + oat. + vit.* respectively (Step 4, Table 3). Therefore, probably one of the remaining modes of action 2–5 influenced the parameter values. An increase in somatic maintenance $[\dot{p}_M]$ (mode 2) can be eliminated and not taken into account because it would increase $\dot{r}_B$ and increase $\dot{R}$ which was not observed. Mode 3 can be eliminated because of changes in $[E_G]$ are very unlikely as the composition of structures and reserves did not change significantly in larvae fed with PS (only 0.5% of eaten PS could be found in biomass (Yang et al., 2015a), which supports the assumption of strong homeostasis; moreover according to the method used to determine carbon mass balance (Yang et al., 2015a) there is also a possibility that this very small amount of PS is in the gut of the organism, and is not incorporated into the biomass). Elimination of modes 2 and 3 led us to the conclusion that PS and PS remainings and metabolites (e.g., aliphatic hydrocarbons, phenyl derivatives and a fragment of small molecules, which can occur in faeces (Yang et al., 2015b)) are not toxic to mealworms. Modes 4 and 5, concerning reaction to starvation, could decrease $\dot{r}_B$ and $\dot{R}$. Furthermore, the increase of L_m caused by modes 4 and 5 could be lower than the decrease caused by mode 1. Therefore, we conclude that *T. molitor* larvae use one of these modes as a strategy to stay alive during insufficient feeding. However, we need more results and proofs or assumptions to choose between modes 4 and 5. To summarize, changes of development of larvae fed with PS (*PS + oat.* and *PS + oat. + vit.*) are mainly caused by a decrease in reserve density (mode 1) and by the physiological reaction of the organism to insufficient food supply (modes 4 or 5). These results support the earlier assumption that the value of parameter $\dot{r}_B$ is equal or close to zero and/or $L = L_m$ in the *Starvation 1* group, and the growth of structures in this group does not occur.

We did not observe any increase of volumetric length in *Starvation 2*, *PS* or *PS large* groups. However, our results show a pattern of changes of volumetric length (Fig. 3D, G and H) in these groups similar to those noted in the *Starvation 1* group (Fig. 3C). Taking into account the earlier conclusions, probably a lack of available energy in the environment led to starvation which forced the larvae to the physiological response manifested in a decrease in DEB model parameters $\dot{k}_M$ and g (modes 4 or 5). A large decrease in the values of these parameters can completely inhibit the growth of structures. Therefore, specimens in *Starvation 2*, *PS* and *PS large* groups probably used their reserves only to maintain life. The decrease in reserve density was observed as a decrease in volumetric length (Fig. 3D, G and H). This led to the conclusion that PS and the droppings and moult residues are an insufficient diet to maintain the life of larvae. The reserves are probably not filled or filled at a much lower rate than used by the organism. In other words, assimilation of energy is lower than mobilization or assimilation does not occur. The lower the value of f, the lower the availability of food and its assimilation. For modeling, purposes we assumed $f = 0$ in *Starvation 1, Starvation 2*, *PS* and *PS large* groups. The volumetric length in each group decreases quickly until the reserves are used almost completely and the volume of structures (built before starvation period) L_{S0} is reached (values of L_{S0} are given in Table 2; L_{V0}, L_{Em} and u_V in Table S4 in Supporting Information). It can be concluded that there is no difference between food availability in these four groups of larvae. The small differences in u_V values for each group indicate differences between accumulated reserves before the starvation period. The addition of PS into the larva diet does not increase food availability f above the value obtained in the group where the only possible source of food was droppings and moult residues (*Starvation 2*). It should be noted that PS contains only C and H atoms, so the lack of macro- and microelements can be a limiting factor for maintaining life (Yang et al., 2018b). Therefore, PS is an insufficient or null source of mass and energy for *T. molitor* larvae. This conclusion can be supported by survival data (Fig. 3): a large decrease in the survival of larvae in *PS* and *PS large* groups was noted, indicating a lack of energy for somatic maintenance. When

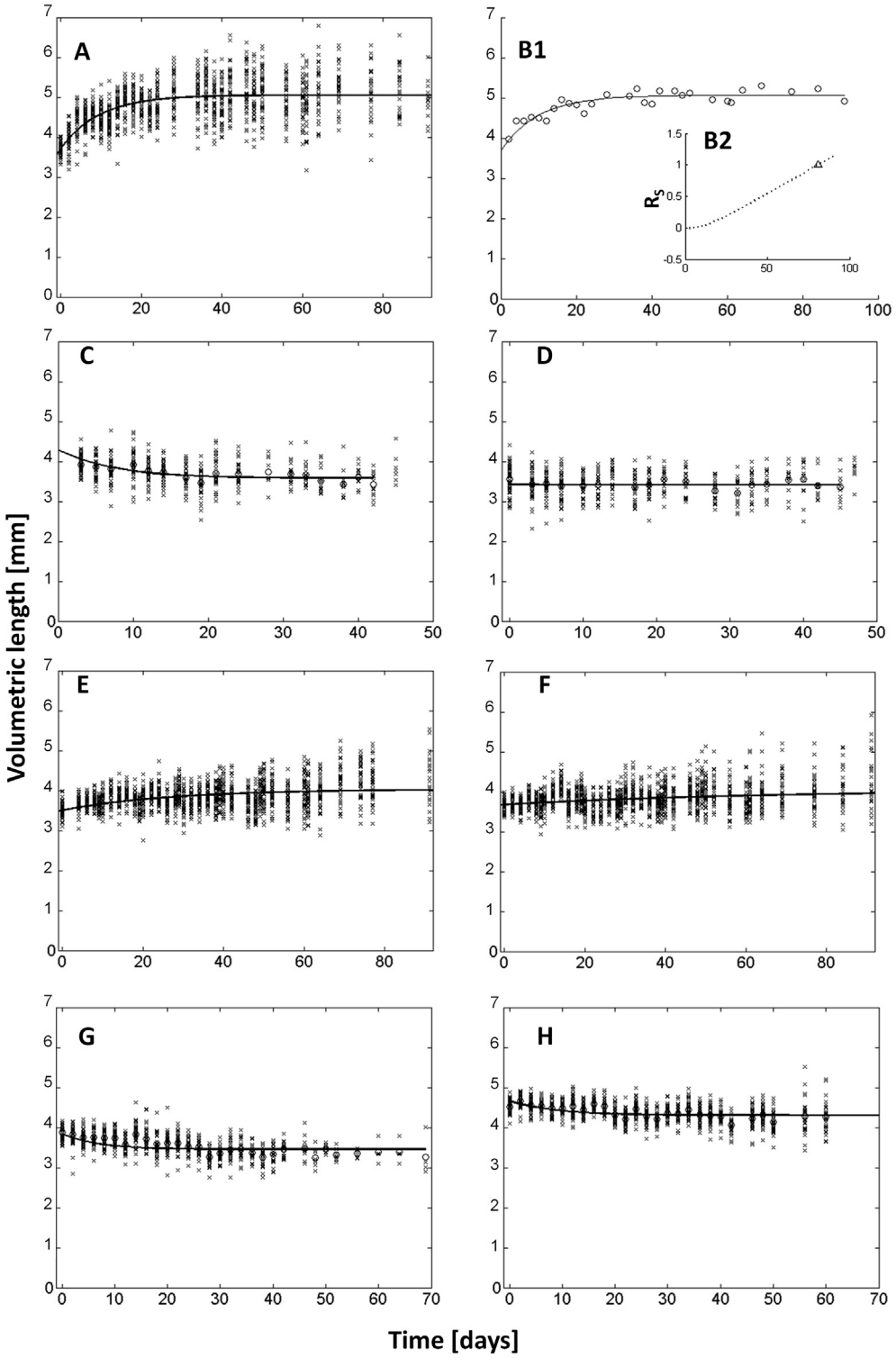

Fig. 3. Growth patterns of *T. molitor* larvae under different feeding conditions and changes in reproduction buffer in control; A – growth in *Control* (Step 1); B1 – growth in *Control*, mean values of length were used (Step 3); B2 – changes in integrated scaled reproduction buffer in *Control* (Step 3); C – decrease in volumetric length in *Starvation 1* group (Step 2); D – decrease in volumetric length in *Starvation 2* group (Step 5); E – growth in *PS + oat.* group (Step 4); F – growth in *PS + oat. + vit.* group (Step 4); G – decrease in volumetric length in *PS* group (Step 5); H – decrease in volumetric length in *PS large* group (Step 5).

the reserves cannot be refilled, and when their density decreases to a critical value, death occurs. Moreover, concerning the survival criterion $\frac{e(t)\dot{v}}{\dot{k}\dot{k}_M g}$, values of parameters $\dot{k}_M$ and g could decrease (see modes 4 and 5 in Table 2) to extend the life of the larvae.

In comparison, Yang et al. (2015a) and Yang et al. (2018a) did not observe changes in biomass after 16 and 32 days respectively for larvae fed only with PS. These results led them to the conclusion that PS, 47.7% of which they found to be converted into CO_2 in larva gut and only 5% built into biomass (Yang et al., 2015a) is a sufficient

source of energy to maintain life but insufficient to continue growth. However, the decrease in biomass and volumetric length probably was difficult to notice in these studies because different statistical methods were used (Yang et al., 2018a; Yang et al., 2015a) compared to the mechanistic model used by us. Moreover, the experiments were conducted for 1 month which is not enough time to observe differences in the survival probability of larvae in different feeding conditions.

Our results showed that the viability of larvae in starvation conditions, which occurs also when they are fed only with PS, is strongly related to the amount of reserves accumulated and the rate at which they are used to maintain life. A physiological reaction of larvae to starvation conditions probably occurs and its mechanism is based on changes in energy distribution in the organism. In some cases, there is a hypothetical possibility that even when starvation occurs, the amount of accumulated reserve would be sufficient to fill the reproduction buffer and form a pupa. This possibility could lead to some misinterpretation in the assessment of the growth, survival, and development of larvae for which the source of energy was assigned to PS, and the reserves were omitted. The importance of reserves has been described in a few studies which support our findings. The metabolic response to food deprivation has been studied in *Alphitobius diaperinus* Panzer which belongs to the same family (Tenebrionidae) as *T. molitor*. The most important changes were recorded in the levels of triglycerides, which is in line with the fact that oxidation of fatty acids and glycerol (stored as triglycerides) is the basic source of energy for insects. Fat is accumulated in the insect fat body (Renault et al., 2002). What is more, the level of triglycerides in the body has an exact impact on the survival of the larva during a food shortage. Interestingly, the level of glycogen was not affected until the fuel reserves (lipids) were present. Carbohydrates are critically important as they are present in the haemolymph of insects and play a key role in sustaining the nervous system and other organs. The protein content is also very important for body functioning. No special forms of protein are accumulated for energetic purposes but if they are obtained endogenously, they are utilized during starvation. In a long period of starvation, proteins are usually hydrolysed and used as a source of energy (Renault et al., 2002).

5. Conclusions

The results of the DEB model analysis show that changes in the development of *T. molitor* larvae strongly depends on reserve dynamics. The model cannot be used to confirm or deny that the PS is biodegraded by microbes in mealworms guts. However, whether or not degradation occurs, the PS and/or possible products of its degradation are none or insufficient source of mass and energy for larvae. The inability or difficulty in completing the life cycle of *T. molitor* larvae fed with PS raises doubts about the use of mealworms as an effective technology for utilizing PS.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

CRediT authorship contribution statement

Konrad Matyja: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization, Supervision, Project administration. **Justyna Rybak:** Validation, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Beata Hanus-Lorenz:** Validation, Investigation, Resources, Writing - review & editing. **Magdalena Wróbel:** Investigation. **Radosław Rutkowski:** Investigation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114740.

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