

# Effects of Autumn Warming on Energy Consumption of Diapausing Fall Webworm (Lepidoptera: Arctiidae) Pupae

Lvquan Zhao<sup>1,\*</sup> and Wei Wang

Co-innovation Center for Sustainable Forestry in Southern China, College of Forestry, Nanjing Forestry University, Nanjing 210037, China and <sup>1</sup>Corresponding author, e-mail: [zhaolvquan@njfu.edu.cn](mailto:zhaolvquan@njfu.edu.cn)

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## Abstract

Since its invasion into China in 1979, the fall webworm, *Hyphantria cunea* Drury, has spread from Dandong city (about 40°N) in Liaoning Province to Nanjing city (about 32°N) in Jiangsu Province, and to other areas. Owing to geographic and latitudinal gradients in temperature, *H. cunea* will encounter temperature changes during the spreading process. In this study, we verified the hypothesis that autumn warming accelerates the energy consumption of *H. cunea* diapause pupae. We found that, after autumn warming, the body size and mass of diapause pupae decreased significantly and raised constant temperature accelerated carbohydrate and protein consumption in female pupae, while fluctuating temperature changes had a more pronounced effect on carbohydrate and protein consumption in male pupae. Contrary to expectations, the lipid content of diapause pupae did not decrease after autumn warming, and even increased significantly. We conclude that warming in autumn accelerates energy consumption by diapause pupae, and the autumn energy consumption of diapause pupae is dominated by carbohydrates, supplemented by protein when carbohydrates are overconsumed, while lipid use is dominated by anabolic metabolism during autumn.

**Key words:** energy storage, autumn warming, climate change, carbohydrate, lipid

Despite growing awareness of the ecological, economic, and social impacts of human-mediated insect spread, the rate of spread of invasive species is increasing significantly (Ward and Masters 2007, Seebens et al. 2017). The spread of exotic species is associated with changes in climatic conditions, in particular the warming of winter temperatures as insects spread from north to south in the northern hemisphere (Renault et al. 2018). As an intuitive outcome of warmer winter temperatures, cold-induced stress in insects is released, their survival rate is increased, and their distribution range is expanded (Crozier 2004, Jepsen et al. 2008). However, insects, being typical ectotherms, lack the ability to regulate their body temperature, and their metabolic rate varies with the external temperature. Warmer winter temperatures accelerate the metabolic rate of insects, increase their energy consumption during overwintering, and thus decrease energy reserves for individuals recovering from diapause status in spring, resulting in lower energy inputs for post-diapause life support and reproduction, and decreasing post-diapause population fitness (Irwin and Lee 2003, Hahn and Denlinger 2011, Williams et al. 2012). For example, autumn warming prior to winter significantly accelerated the consumption of carbohydrates during diapause in *Carposina sasakii* (Zhang et al. 2016). When *Eurosta solidaginis* larvae overwintered in the under-snow layer, they experienced relatively higher temperatures during overwintering and

therefore consumed significantly more lipids than did larvae that overwintered in upright stems. More importantly, the mortality of under-snow larvae was significantly higher during overwintering, and adult fecundity was reduced by ~18% after adult emergence (Irwin and Lee 2003).

To understand the geographic patterns of adaptation to temperature changes during spread of exotic species, it is important to recognize that there is a clear geographic latitudinal gradient between the mean temperature and the fluctuating temperature (Colinet et al. 2015, Renault et al. 2018). Exotic organisms not only experience mean temperature changes, but also experience fluctuating temperature changes during spread. Studies of the thermal adaptation of exotic species during spread may overlook the impact of geographic differences in fluctuating temperatures if we focus only on mean temperature changes. Not only does fluctuating temperature significantly affect insect biology, morphology, reproduction, and longevity (Colinet et al. 2015), it also has a significant impact on energy consumption (Williams et al. 2012). According to Jensen's inequality curve, the average metabolic rate of the accelerated portion of the curve increases with temperature; therefore, the warm portion of the daily thermal cycle will disproportionately increase the metabolic rate above the average expected temperature, and larger temperature fluctuations will further exacerbate the energy consumption

of insects (Ruel and Ayres 1999, Pazstor et al. 2000). For example, fluctuating temperature significantly increased energy consumption during overwintering in *Erynnis propertius* (Williams et al. 2012).

The fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae), a major invasive pest in China, is native to North America and is widely distributed in the northern United States, southern Canada, and Mexico (Ji et al. 2003). In the late 1940s, *H. cunea* spread from North America to Europe and was first found in Asia in Tokyo in 1945; in 1979, it was introduced to Dandong city (about 40°N) in Liaoning Province, China, and has now spread to Nanjing city (about 32°N) in Jiangsu Province and other areas (Ji et al. 2003, Liu et al. 2016).

*Hyphantria cunea* occurred in two generations per year when it was first introduced to China (Ji et al. 2003). In recent years, with climate change and its spread southward, *H. cunea* has been occurring in three generations per year in most parts of China (Wei et al. 2006). *Hyphantria cunea* overwinters as pupae, and the photoperiod sensed by young larvae determines whether the pupae enter diapause (Masaki 1968). Adult *H. cunea* is unable to feed because of mouthpart degeneration (Gomi 2000). The basic metabolism of *H. cunea* and the energy required for metamorphosis, eclosion, adult flight, and reproduction after overwintering come from the energy reserves in the larval stage. Therefore, the energy consumption during diapauses determines the survival rate during overwintering and the fitness of the population after overwintering (Irwin and Lee 2003).

*Hyphantria cunea* has spread from Dandong city (about 40°N) in Liaoning province to Nanjing city (about 32°N) in Jiangsu province following its introduction into China. Owing to geographic and latitudinal gradients of temperature, *H. cunea* will encounter temperature changes during the spread process. However, the effect of temperature changes during the spread process, especially the increase in autumn temperature before winter, on the energy consumption of *H. cunea* diapause pupae is not clear. To investigate the effect of increased autumn temperature on diapause pupae energy consumption during spread of *H. cunea*, we hypothesized that increased autumn temperature would increase the energy consumption of *H. cunea* diapause pupae and we predicted that both body mass and size and the lipid, carbohydrate, and protein content of diapause pupae would decrease with increasing autumn temperatures. We also predicted that temperature fluctuations would further exacerbate lipid, carbohydrate, and protein consumption by diapause pupae.

## Materials and Methods

### Source of Insects

Old larvae of *H. cunea* were collected in September 2018 from poplar trees (*Populus deltoids*) in Chuzhou District, Huai'an City (33.50°N, 119.14°E), and the collected larvae were transferred to the laboratory and placed in an incubator (RXM-358, Jiangnan) (L:D, 13:11) at 25°C. The larvae were fed with poplar leaves in a round container (10 cm in diameter, 8 cm high). The leaves were replaced every day, and the larvae were checked for pupation. After pupation, the pupae were moved to a new plastic container with a layer of absorbent cotton on the bottom of the container to maintain the humidity. The pupae were placed on a layer of filter paper, which was placed on the top of absorbent cotton. Given that the collected larvae were third-generation old larvae living under natural environmental conditions, these larvae were destined to enter the diapause state after pupation.

### Temperature Setting Scheme

Climatic data for the past 10 yr (2007–2017) at the specimen collection site were collected to calculate the average autumn temperature

in the area. The average autumn temperature at the collection site, Huai'an City, Chuzhou District, was 15°C. Under natural environmental conditions, daily temperatures are not constant, and to simulate the effect of autumn temperature changes on energy consumption in *H. cunea* diapause pupae, we set up two sets of experiments. In one set, three sets of constant temperatures of 15, 20, and 25°C were simulated, respectively; in the other set, two sets of fluctuating temperatures, 20 ± 5°C and 20 ± 10°C, were simulated, respectively (Fig. 1). We determined the sex of each pupa by the presence (female) or absence (male) of a line intersecting the first abdominal sternite after pupation (Loewy et al. 2013, Williams et al. 2015). The female and male pupae were randomly selected on the day of pupation and placed in the incubator at the five temperature temperatures described above, and the pupae were removed 30 d later.

### Body Size and Mass

To investigate the effect of autumn temperature changes on the body size and mass of diapause pupae, the length, width, and mass of pupae in the five temperature treatments were measured with a micrometer (0.1 cm) and electronic balance (AL104 Mettler-Toledo; Mettler-Toledo,  $d = 0.0001$  g), respectively, on the day of pupation and 30 d after. Forty female and 72 male pupae in control group; 10 female and 20 male pupae at 15°C, 10 female and 24 male pupae at 20°C, 10 female and 23 male pupae at 25°C in the constant temperature treatments; and 10 female and 20 male pupae at 20 ± 5°C, 10 female and 19 male pupae at 20 ± 10°C in the fluctuating temperature treatments, were used for body mass and body size analysis.

### Body Composition

Lipid, carbohydrates, and proteins were extracted from pupae on the day of pupation and after the five temperature treatments for 30 d according to the Lorenz's (2003) method. The sulphophosphovanillin method (adapted from Lorenz 2003) was used to quantify the lipid content. Samples were placed in sulfuric acid (1 ml) and boiled for 10 min at 100°C; after cooling, 5 ml of 0.2% phosphovanillin in 57% ortho-phosphoric acid was added to the mixture. The absorbance value of the sample was determined at 530 nm using cholesterol as the standard. We measured protein content by Coomassie blue staining: 25 µl of the sample was placed in 125 µl phosphate-buffered saline, and 2.85 µl Coomassie blue fast staining solution was added. The absorbance value was measured at 595 nm and the sample concentration was determined according to the bovine serum albumin standards. The anthrone method was used to quantify the carbohydrate content (Carroll et al. 1956). Following this, 100 µl of the sample

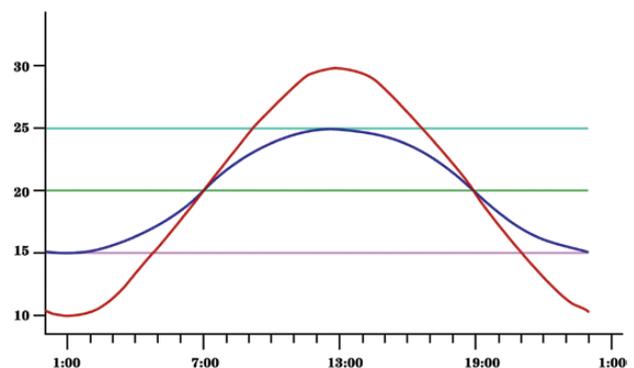


Fig. 1. Temperature setting scheme. Purple: 15°C; green: 20°C; cyan: 25°C; blue: 20 ± 5°C; red: 20 ± 10°C.

was placed in 900 µl of distilled water and 4 ml of 0.1% anthrone (1 g anthrone in 1,000 ml of 80% sulfuric acid) was added to the mixture. The mixture was heated to 100°C for 10 min, and the absorbance value was measured at 620 nm to determine the sample concentration using glucose as the standard. Twenty female and 30 male pupae on the day of pupation were used for body composition analysis. The number of samples used for body composition analysis in the constant and fluctuating temperature treatments group was the same as those for body mass and size analysis.

### Statistical Analysis

The experimental data were analyzed using the data processing system SPSS 22.0. We compared the body size and body mass of pupae on the day of pupation and 30 d after the five temperature treatments using analysis of variance with Tukey's multiple range test. First, we analyzed the normality and homogeneity of the data. The results showed that the data adhered to the assumptions of normality and homogeneity of error variances. A general linear model (GLM) was used to analyze the lipid, carbohydrate, and protein content on the day of pupation with the three constant temperature treatments and the constant 20°C treatment group with the two fluctuating temperature treatments with body mass as a covariate. The GLM showed that sex had a significant effect on the lipid, carbohydrate, and protein content, and there was no interaction between sex and temperature when the diapause pupae encountered constant temperature changes and fluctuating temperature changes so we compared the lipid, carbohydrate, and protein content of female and male pupae separately using analysis of covariance with body mass as a covariate and followed with Tukey's multiple range test.

## Results

### Body Mass and Size

Raised constant temperature significantly decreased the body mass and size of diapause pupae (body mass: female:  $F_{3,67} = 3.27$ ,  $P = 0.026$  and male:  $F_{3,142} = 4.57$ ,  $P = 0.0043$ ; body length: female:  $F_{3,67} = 3.13$ ,  $P = 0.031$  and male:  $F_{3,142} = 4.23$ ,  $P = 0.0062$ ; body width: female:  $F_{3,67} = 4.52$ ,  $P = 0.0060$  and male:  $F_{3,142} = 3.06$ ,  $P = 0.03$ ; Table 1). The body mass of female pupae after treatments at 15 or 20°C for 30 d was similar to their body mass on the day of pupation (15°C:  $P = 0.93$ ; 20°C:  $P = 0.89$ ), but the body mass of female pupae in the 25°C treatment group was significantly lower than their body mass on the day of pupation ( $P = 0.022$ ). At the same time, their body mass was also significantly lower than that of their counterparts in the 15 and 20°C treatment groups (15°C:  $P = 0.037$ ; 20°C:  $P = 0.031$ ). Similarly, body length and width of female pupae in the 15 and 20°C

treatment groups were similar to those on the day of pupation (body length: 15°C:  $P = 0.41$ , 20°C:  $P = 0.30$ ; body width: 15°C:  $P = 0.62$ , 20°C:  $P = 0.068$ ), while those in the 25°C treatment group were significantly lower than those on the day of pupation (body length:  $P = 0.010$ ; body width:  $P = 0.0011$ ) and also significantly lower than those in the 15 and 20°C treatment groups (body length: 15°C:  $P = 0.045$ , 20°C:  $P = 0.041$ ; body width: 15°C:  $P = 0.028$ , 20°C:  $P = 0.047$ ). Similar to female pupae, the body mass and size of male pupae after 30 d at 15 and 20°C were similar to those on the day of pupation (body mass: 15°C:  $P = 0.95$ , 20°C:  $P = 0.55$ ; body length: 15°C:  $P = 0.41$ , 20°C:  $P = 0.39$ ; body width: 15°C:  $P = 0.82$ , 20°C:  $P = 0.61$ ), whereas body mass and size of pupae in the 25°C treatment group were significantly lower than those on the day of pupation (body mass:  $P < 0.001$ ; body length:  $P = 0.014$ ; body width:  $P = 0.013$ ) and also significantly lower than those in the 15 and 20°C treatment groups (body mass: 15°C:  $P = 0.0020$ , 20°C:  $P = 0.019$ ; body length: 15°C:  $P = 0.023$ , 20°C:  $P = 0.037$ ; body width: 15°C:  $P = 0.026$ , 20°C:  $P = 0.042$ ).

The fluctuating temperature also significantly decreased the body mass and size of diapause pupae (body mass: female:  $F_{2,43} = 7.25$ ,  $P = 0.003$  and male:  $F_{2,64} = 13.9$ ,  $P < 0.0001$ ; body length: female:  $F_{2,43} = 6.48$ ,  $P = 0.005$  and male:  $F_{2,64} = 5.74$ ,  $P = 0.0051$ ; body width: female:  $F_{2,43} = 6.57$ ,  $P = 0.0047$  and male:  $F_{2,64} = 8.84$ ,  $P < 0.0001$ ; Table 2). The body mass of female pupae was  $151.4 \pm 30.5$  and  $149.2 \pm 39.3$  mg after 30 d at  $20 \pm 5^\circ\text{C}$  and  $20 \pm 10^\circ\text{C}$ , respectively, significantly lower than the body mass of female pupae at constant 20°C ( $20 \pm 5^\circ\text{C}$ :  $P = 0.025$ ;  $20 \pm 10^\circ\text{C}$ :  $P = 0.0097$ ). Similarly, the body length and width of female pupae in the  $20 \pm 5^\circ\text{C}$  and  $20 \pm 10^\circ\text{C}$  treatment groups were significantly lower than those in the constant 20°C treatment group (body length:  $20 \pm 5^\circ\text{C}$ :  $P = 0.028$ ,  $20 \pm 10^\circ\text{C}$ :  $P = 0.0039$ ; body width:  $20 \pm 5^\circ\text{C}$ :  $P = 0.033$ ,  $20 \pm 10^\circ\text{C}$ :  $P = 0.012$ ). Similar to female pupae, after 30 d at  $20 \pm 5^\circ\text{C}$  and  $20 \pm 10^\circ\text{C}$ , body mass and size of male pupae were significantly lower than those in the constant 20°C treatment group (body mass:  $20 \pm 5^\circ\text{C}$ :  $P = 0.042$ ,  $20 \pm 10^\circ\text{C}$ :  $P = 0.012$ ; body length:  $20 \pm 5^\circ\text{C}$ :  $P = 0.027$ ,  $20 \pm 10^\circ\text{C}$ :  $P = 0.0057$ ; body width:  $20 \pm 5^\circ\text{C}$ :  $P = 0.0063$ ,  $20 \pm 10^\circ\text{C}$ :  $P = 0.0023$ ).

### Effects of Constant Temperature on Body Composition

#### Lipid

There was no significant effect of different constant temperatures on the lipid content of female pupae ( $F_{3,46} = 0.34$ ,  $P = 0.82$ , Fig. 2A). However, the higher temperature significantly increased the lipid content in male pupae ( $F_{3,93} = 26.36$ ,  $P < 0.0001$ ; Fig. 2A). Thirty days after treatment at 15, 20, and 25°C, the lipid content of male pupae was significantly higher than that on the day of pupation ( $P < 0.001$ ).

**Table 1.** Effect of constant temperature on the body mass and size of diapausing fall webworm pupae

Temperature	Mass (mg)		Length (mm)		Width (mm)	
	Male	Female	Male	Female	Male	Female
Control	120.9 ± 20.5a	164.1 ± 25.5a	12.1 ± 0.7a	13.3 ± 0.9a	4.4 ± 0.3a	4.9 ± 0.3a
15°C	118.0 ± 29.1a	162.2 ± 17.7a	11.8 ± 0.9a	12.8 ± 0.8a	4.4 ± 0.4a	4.9 ± 0.4a
20°C	114.6 ± 16.4a	160.4 ± 32.8a	11.7 ± 0.8a	12.7 ± 0.9a	4.6 ± 0.2a	4.8 ± 0.4a
25°C	110.1 ± 19.7b	159.4 ± 22.4b	10.7 ± 0.8b	12.2 ± 0.9b	4.2 ± 0.3b	4.7 ± 0.2b

Data in the table are mean ± SD. The different letters following the data within a column indicate significant differences ( $P < 0.05$ ). Control: pupae on the day of pupation.

**Table 2.** Effect of fluctuating temperature on the body mass and size of diapausing fall webworm pupae

Temperature	Mass (mg)		Length (mm)		Width (mm)	
	Male	Female	Male	Female	Male	Female
20°C	114.6 ± 16.4a	160.4 ± 32.8a	11.7 ± 0.8a	12.7 ± 0.9a	4.6 ± 0.2a	4.8 ± 0.4a
20 ± 5°C	111.1 ± 16.5b	151.4 ± 30.5b	10.7 ± 0.7b	12.3 ± 0.8b	4.3 ± 0.2b	4.7 ± 0.4b
20 ± 10°C	108.9 ± 18.7b	149.2 ± 39.3b	10.2 ± 0.7b	12.2 ± 1.1b	4.2 ± 0.3b	4.7 ± 0.4b

The data for 20°C come from Table 1. Data in the table are mean ± SD. The different letters following the data within a column indicate significant differences ( $P < 0.05$ ).

for all temperatures), and the lipid content of male pupae at 20 and 25°C was significantly higher than that of the 15°C group (20°C:  $P = 0.0062$ ; 25°C:  $P = 0.0058$ ).

### Carbohydrates

The level of constant temperature significantly affected the carbohydrate content of diapause pupae (female:  $F_{3,46} = 31.64$ ,  $P < 0.0001$ ; male:  $F_{3,93} = 5.66$ ,  $P = 0.001$ ; Fig. 2B). Thirty days after treatment at 15, 20, and 25°C, the carbohydrate content of female pupae was significantly lower than that on the day of pupation ( $P < 0.001$  for all temperatures). Meanwhile, the carbohydrate content of the 25°C treatment group was significantly lower than those of the 15 and 20°C treatment groups (15°C:  $P = 0.0028$ ; 20°C:  $P = 0.0034$ ). Unlike the female pupae, which showed a significant decrease in carbohydrates with increasing temperature, the male pupae showed a tendency to increase and then decrease carbohydrates with increasing temperature; after 30 d at 15 and 20°C, the carbohydrate levels in the male pupae were significantly higher than those on the day of pupation (15°C:  $P = 0.0072$ , 20°C:  $P = 0.029$ ), but the carbohydrate levels in the male pupae at 25°C were significantly lower than those on the day of pupation ( $P = 0.042$ ).

### Protein

The protein content of diapause pupae was significantly affected by the value of constant temperature (female:  $F_{3,46} = 16.54$ ,  $P < 0.001$ ; male:  $F_{3,93} = 36.24$ ,  $P < 0.001$ ; Fig. 2C). Thirty days of treatment at 15°C produced no significant change in the protein content of female pupae compared with that on the day of pupation ( $P = 0.68$ ), but the protein content of female pupae at 20 and 25°C was significantly lower than that on the day of pupation (20°C:  $P = 0.026$ ; 25°C:  $P < 0.001$ ), and significantly lower than that of the 15°C group ( $P < 0.001$ ). In contrast to the significant decrease in the protein content of female pupae after 30 d at 15, 20, and 25°C, there was a tendency for the protein content of male pupae to increase and then decrease with temperature. The protein content of male pupae in the 15 and 20°C treatment groups was significantly higher than the protein content on the day of pupation ( $P < 0.001$ ), but the protein content of male pupae in the 25°C treatment group was significantly lower than the protein content in the 15 and 20°C treatment groups ( $P < 0.001$ ) and similar to the protein content on the day of pupation ( $P = 0.62$ ).

## Effect of Fluctuating Temperature on Body Composition

### Lipid

The lipid content of female pupae after 30 d of 20 ± 5°C and 20 ± 10°C treatment was not significantly different when compared with that of female pupae after 30 d of constant 20°C treatment (20 ± 5°C:  $P = 0.44$ ; 20 ± 10°C:  $P = 0.24$ ; Fig. 3A). Similar to female pupae, although the lipid content of male pupae in the 20 ± 10°C treatment group was higher than that in the 20 ± 5°C treatment

group ( $P = 0.035$ ), the lipid content of male pupae in the 20 ± 5°C and 20 ± 10°C treatment groups was not significantly different from that in the constant 20°C treatment group (20 ± 5°C:  $P = 0.45$ ; 20 ± 10°C:  $P = 0.41$ ).

### Carbohydrates

The fluctuating temperature did not have a significant effect on the carbohydrate content of female pupae ( $F_{2,49} = 2.24$ ,  $P = 0.26$ ; Fig. 3B) but significantly decreased the carbohydrate content of male pupae ( $F_{2,62} = 54.20$ ,  $P = 0.016$ ; Fig. 3B). The carbohydrate content of the 20 ± 5°C male pupae treatment group was not significantly different when compared with that of the constant 20°C male pupae treatment group ( $P = 0.36$ ), but the carbohydrate content of the 20 ± 10°C male pupae treatment group was significantly lower than that of the constant 20°C male pupae treatment group ( $P = 0.0090$ ).

### Protein

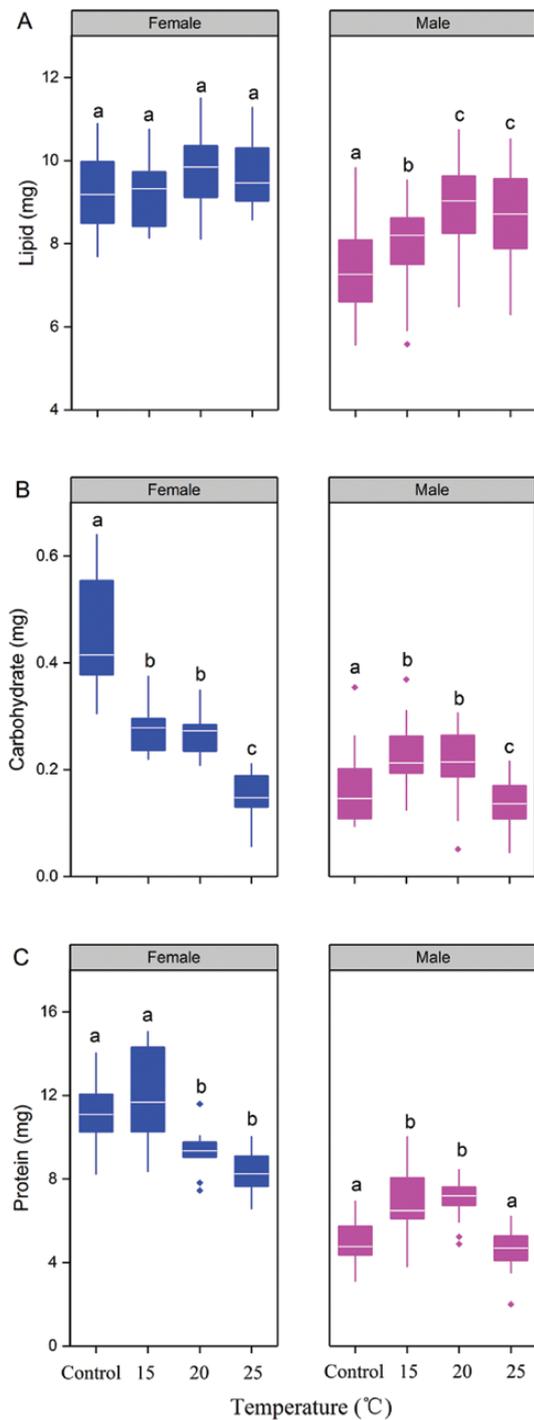
Similar to carbohydrates, fluctuating temperature had no significant effect on the protein content of female pupae ( $F_{2,49} = 0.82$ ,  $P = 1.04$ ; Fig. 3C), but significantly decreased the protein content of male pupae ( $F_{2,62} = 45.62$ ,  $P < 0.001$ ; Fig. 3C). Male pupae treated at 20 ± 5°C had similar protein content to those at constant 20°C ( $P = 0.84$ ; Fig. 3C), but male pupae treated at 20 ± 10°C had significantly lower protein content than those in the 20°C and 20 ± 5°C treatment groups (20°C and 20 ± 5°C:  $P < 0.001$ ; Fig. 3C).

## Discussion

Consistent with our expectations, autumn warming significantly reduced body mass and size in *H. cunea* diapause pupae. Meanwhile, raised constant temperature significantly increased carbohydrate and protein consumption by female pupae of *H. cunea*. The fluctuating temperature accelerated carbohydrate and protein consumption in diapause male pupae. Contrary to our expectations, the lipid content of diapause pupae did not decrease significantly after the autumn warming, and even increased significantly.

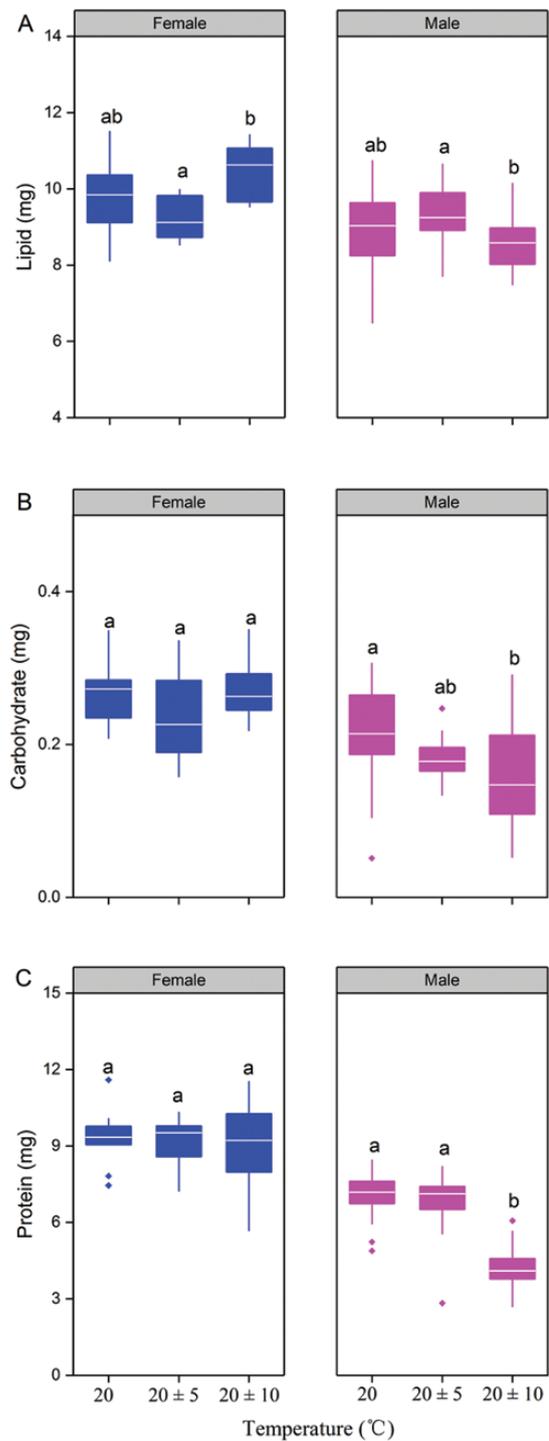
Larger individuals during diapauses can provide greater storage capacity for the nutritional reserves needed to survive in harsh environmental conditions (Hahn and Denlinger 2007, Shin et al. 2012). For example, the body size and mass of *H. cunea* diapause pupae were significantly greater than those of non-diapause pupae, and the lipid and carbohydrate reserves of diapause pupae were significantly higher than those of non-diapause pupae (Zhao et al., unpublished data). We found that autumn warming significantly decreased the body mass and size of diapause pupae, suggesting that autumn warming accelerated the energy consumption of *H. cunea* diapause pupae.

As lipids are the most abundant energy reserve of insects in diapause, it can meet their long-term energy requirements; therefore,



**Fig. 2.** Effect of constant temperature on energy consumption of *Hyphantria cunea* diapause pupae. (A) Lipids, (B) carbohydrates, and (C) protein. Blue: female pupae; purple: male pupae. Different letters within female or male pupae indicate significant differences between temperatures. Control: pupae on the day of pupation.

insects in diapauses tend to store more lipid than non-diapause insects, and most non-feeding insects consume mainly lipid during the diapause period (Sinclair 2015, Sinclair and Marshall 2018). However, the results of the present study did not support this conclusion. The warm autumn did not promote a decrease in lipid content in *H. cunea* diapause pupae, and there was even a significant increase in lipid content. This may be due to the fact that metabolism during insect



**Fig. 3.** Effect of fluctuating temperature on energy consumption of *Hyphantria cunea* diapause pupae. (A) Lipids, (B) carbohydrates, and (C) Protein. Blue: female pupae; purple: male pupae. Different letters within female or male pupae indicate significant differences between temperatures.

diapause is a dynamic process that can draw energy from other reserves at specific stages (Hahn and Denlinger 2007, Sinclair et al. 2011). For example, *Sarcophaga crassipalpis* mainly consumes lipid in the early stage of diapause, and then shifts its energy consumption from lipid to carbohydrates and other energy substances as diapause develops (Adedokun and Denlinger 1985). *Eurytoma Plotnikovi*, on the other hand, consumes mainly carbohydrates in the early stages

of diapause, and switches its energy consumption from carbohydrates to lipids as it develops (Mohammadzadeh et al. 2017). These results indicate that there are interspecies differences in the energy consumption patterns of insects in the early stages of diapause. The warm autumn did not reduce the lipid content of *H. cunea* diapause pupae, suggesting that carbohydrate, protein, or other energy consumption dominated the early stages of diapause in *H. cunea*.

In *Pieris napi*, lipid utilization by pupae mainly occurs during the post-diapause developmental and metamorphosis stages, suggesting that the insects need to use the lipid retained at the end of winter to provide energy for post-wintering activities (Lehmann et al. 2016). The eggs of many insects contain large amounts of lipids, which may be derived from their mother's energy stores during the larval stage; in addition, the post-diapause lipid content of some insects correlates with flight capacity (Arrese and Soulages 2010). The above results indicate that lipid protection during diapause is a key factor for successful overwintering of insects and for improving the fitness of populations after overwintering. Adult *H. cunea* lose their ability to feed due to the degeneration of their mouthparts (Gomi 2000); therefore, the energy consumption during diapause and the energy required for post-diapause metamorphosis, adult flight, and reproduction are derived from the energy stores at the larval stage. The energy consumption at the early stages of diapause is dominated by carbohydrates, protein, or other energy, which can reduce lipid consumption in the early stage of diapause and provide an energy source for post-diapause metamorphosis, adult flight, and reproduction, so as to improve the fitness of the population.

Carbohydrates and proteins are two other important energy reserves for insects during the diapause stage (Hahn and Denlinger 2007). Among them, glycogen, which can be rapidly converted to glucose to meet limited energy requirements and provide a source for the synthesis of cold tolerant polyols, is used to meet the energy needs of insects during the early diapause stage (Storey et al. 2004, 2012), while protein is used for catabolic respiratory metabolism during diapause and for functional recovery after diapause (Hahn and Denlinger 2011). In diapausing female pupae of *H. cunea*, carbohydrate consumption is accelerated by warmth in autumn, while protein consumption is not affected by relatively low temperature but is accelerated by relatively high temperature. The above phenomenon may be related to the autumnal energy consumption pattern in female pupae of *H. cunea*. Glycogen, the main source of autumnal energy consumption in female pupae of *H. cunea*, may be in short supply because autumn warming accelerates its rate of consumption. Lipid, which is required for the long winter and post-diapause spring metamorphosis and for adult reproduction, is in the storage mode rather than prioritized for usage at this point. On the other hand, protein, an important energy reserve for insects, is transported to meet the energy consumption needs of female pupae in diapause during the warm autumn.

Energy consumption by insects during diapause depends not only on the average temperature of the overwintering habitat but also on the variability of the habitat temperature (Williams et al. 2012). We found that the carbohydrate and protein consumption by male pupae of *H. cunea* is dependent on the magnitude of the temperature fluctuations. It is well-appreciated that an increase in temperature will result in an increase in the metabolic rate of an ectotherm. Jensen's inequality states that changes in the metabolic rate in response to fluctuating temperature are asymmetrical, with limited effects of decreasing temperatures and greater effects of increasing temperatures (Ruel and Ayres 1999). The warm part of daily thermal cycles will disproportionately increase metabolic rate beyond what would be predicted by mean temperatures, and the magnitude of this

effect will depend on the magnitude of the temperature fluctuations: larger amplitudes will have a greater impact (Pazstor et al. 2000). Thus, large amplitudes of fluctuating temperature accelerated carbohydrate and protein consumption in male pupae of *H. cunea*.

Increasing temperature during the diapause period accelerates insect energy consumption, leading to reduced energy reserves during overwintering and thus reducing insect survival and potential fecundity during overwintering (Irwin and Lee 2003). Moreover, excessive autumn energy consumption increases the temperature sensitivity of insects during overwintering and reduces their ability to adapt to cold environments. Thus, temperature-dependent energy consumption in autumn may adversely affect the viability of overwintering populations of diapause insects. Our results indicate that autumn warming accelerates energy consumption in *H. cunea* diapause pupae. *Hyphantria cunea* has spread from Dandong city (about 40°N) in Liaoning Province to Nanjing city (about 32°N) in Jiangsu Province after its invasion into China. Owing to geographic and latitudinal gradients in temperature, *H. cunea* will encounter autumn warming during the spreading process. *Hyphantria cunea* overwinter on the ground from October to May of the next year in Jiangsu Province, so they are vulnerable to temperature changes during the overwintering period. Therefore, we infer that high mean temperature and large amplitudes of fluctuating temperature in autumn in Nanjing city are bound to accelerate the energy consumption, and thus reduce the survival rate of overwintering pupa of *H. cunea*, resulting in lower post-diapause population fitness.

In conclusion, our results support the hypothesis that autumn warming promotes energy consumption in *H. cunea* diapause pupae. Consistent with our expectations, autumn warming significantly reduced body mass and body size in *H. cunea* diapause pupae. Raised constant temperature promoted carbohydrate and protein consumption by female pupae, while fluctuating temperature had a more pronounced effect on carbohydrate and protein consumption by male pupae. Surprisingly, the lipid content of diapause pupae did not decrease significantly after autumn warming, and even increased. Energy consumption in autumn in *H. cunea* diapause pupae is dominated by carbohydrates, supplemented by protein when carbohydrates are overconsumed. However, in autumn, the lipid is mainly synthetically stored to conserve energy for post-diapause development, metamorphosis, adult flight, and reproduction.

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## Authors' Contributions

L.Q.Z. conceived and designed the experimental protocol. W.W. conducted the experimental work. L.Q.Z. and W.W. analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

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