JOURNAL OF
EMERGING INVESTIGATORS

Article

# Two wrongs could make a right: food waste compost accelerated polystyrene consumption of *Tenebrio molitor*

**Kwun Him Fu*, Cheuk Hin Yeung*, Sze Chit Hung*, Andrew Ching-Yuet To**
Po Leung Kuk Laws Foundation College, Tseung Kwan O, Hong Kong
*These authors contributed equally to the research

## SUMMARY

**Expanded polystyrene (EPS) is a type of plastic that poses serious impact to the environment. The ingestion of EPS can kill animals and cause harm to the entire food chain. It takes one thousand years for the complete degradation of EPS in nature. Previous work showed that mealworms, the larvae of *Tenebrio molitor*, are capable of digesting EPS. However, the use of mealworms for EPS degradation is not popular because of the slow rate. We herein report that food waste compost (FWC) is an effective additive to accelerate EPS consumption in mealworms. In our experiment, we investigated the effect of FWC and oats on the EPS consumption rate and lifecycle of mealworms. The effect of FWC on the EPS consumption rate of superworm (*Zophobas morio*) and greater waxworm (*Galleria mellonella*) was also studied. EPS consumption rate of mealworm gained a 78% increase when FWC was added. Oats, a common feed for mealworms, increased the EPS consumption rate, but the effect was significantly lower than that by FWC. FWC and oats promoted weight gain and survival of mealworms. A stronger positive correlation between weight gain and EPS consumption was observed in mealworms that were co-fed with FWC when compared with that with oats. In addition, the larval period of mealworms fed with FWC was significantly longer than that fed with oats. Finally, we found that FWC also accelerated EPS consumption in superworm and greater waxworm, but FWC-accelerated EPS consumption by mealworms showed the highest efficiency in terms of mass-to-mass ratio. These findings shed light on a potential industrial application for degrading EPS. Not only could the method degrade EPS in a more efficient manner, it could also provide a proper use of food waste.**

## INTRODUCTION

Expanded polystyrene (EPS) is a type of plastic that is commonly used for making food containers and packing material. According to the United States Environmental Protection Agency, more than 14 million tonnes of EPS are produced globally each year (1). EPS is the second most abundant marine litter worldwide (2). EPS pollution poses a serious threat to the environment. The ingestion of non-biodegradable EPS can kill animals and causes harm to the entire food chain, which ultimately impacts humans as well (3). EPS is extremely stable; it is estimated that one thousand years are required for the complete degradation of EPS in a landfill (4). There are only a limited number of ways to reduce the amount of EPS. For instance, incineration of EPS reduces its size, but produces harmful chemicals such as polycyclic aromatic hydrocarbons (PAHs) (5). The rate of EPS recycling is low due to technical difficulties; only 19% of EPS can eventually be recycled (6). Therefore, an effective method for EPS degradation is desperately required.

Since 2016, several publications have shown that larvae of *Tenebrio molitor*, commonly known as mealworms, are able to degrade EPS (7-9). In 2018, Yang et al. found that mealworm gut microorganisms digest EPS and respire carbon dioxide (7). Moreover, research found that superworm (*Zophobas morio*) and greater waxworm (*Galleria mellonella*) can also digest plastics (10, 11). In 2019, study found that harmful chemicals would not accumulate in mealworms after digesting EPS, suggesting that the process is environmentally safe (12). Nonetheless, biodegradation of EPS using worms could not be popularized because of its ineffectiveness. It is estimated that it takes about 30 days for 4,720 mealworms to completely consume a 17g foam container (13). Therefore, an effective way for accelerating the EPS consumption by worms is needed.

Addition of nutrient-rich supplements could be a way to accelerate the EPS consumption by worms, because high nutrient content might promote appetite and aid digestion. In this research, we proposed that oats, food waste (FW) and food waste compost (FWC) might contain high nutrient content to accelerate the EPS consumption by worms. Oats is a common feed for mealworms and mealworms could feed on oats solely to grow. FW is defined as the uneaten food produced by human. FW is the biggest waste problem in some developed countries. According to the Natural Resources Defense Council, 40% of edible food is thrown away by Americans (14). Disposing of FW in landfills does not meet the principle of sustainable development. FW is nutrient-rich and should not be wasted. FW can be recycled to produce FWC for agricultural needs. Complex compound in FW could be broken down into simple compound to give FWC. FW and FWC contain different types of nutrients. They might affect the appetite and digestion of worms differently.

In this research, we investigated the effect of oats, FW and FWC on the EPS consumption rate, survival rate, weight and pupation rate in mealworms. We also compared the

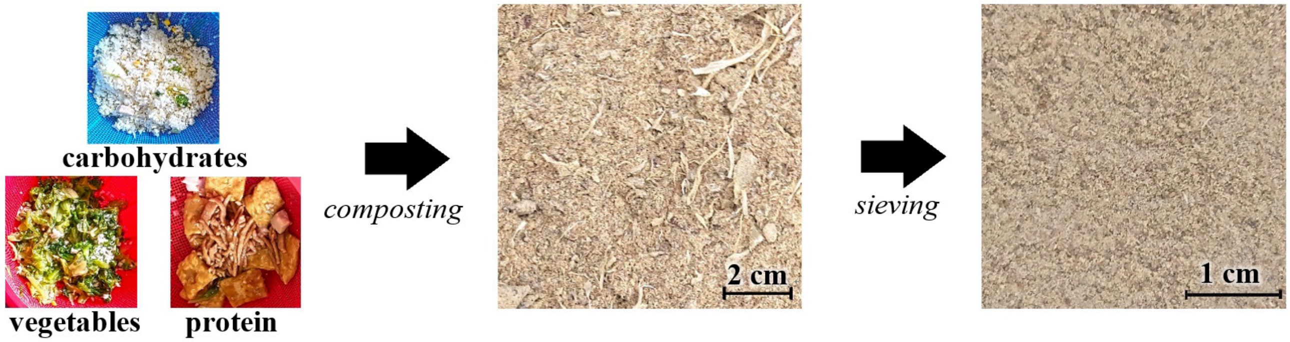


**Figure 1: Production of food waste compost.** Food waste consisting of carbohydrates, vegetables, and protein are composted and sieved to produce fine compost powder.

effect of FWC on the EPS consumption rate of mealworms, superworms and greater waxworms. In our experiment, we found that the rate of EPS consumption by mealworms remained unchanged when FW was added, while it was nearly doubled with FWC. Oats also increased the EPS consumption rate but it was significantly lower than that of FWC. Both FWC and oats promoted weight gain and survival of mealworms. A stronger positive correlation between weight gain and EPS consumption was observed in mealworms that were co-fed with FWC when compared with those with oats. In addition, the pupation rate of mealworms fed with FWC was significantly lower than those fed with same weight of oats, meaning that mealworms could gain a longer larval period for the consumption of EPS when FWC was added. We suggest that FWC is a more effective addictive for accelerating EPS consumption in mealworms when compared with FW and oats. Finally, although we found that FWC also accelerated EPS consumption in superworms and greater waxworm, FWC-accelerated EPS consumption by mealworms showed the highest efficiency in terms of mass-to-mass ratio. All these findings suggest that FWC-accelerated EPS degradation by mealworms could be a potential method to biologically degrade EPS in an industrial scale. Not only could the method degrade EPS in a more efficient manner, it could also provide a proper use of FW.

## RESULTS

### Production and Characterization of Food Waste Compost

We first collected FW from a secondary school in Hong Kong to produce FWC. After the composting process, FW turned into a brownish compost. We removed indigestible vegetable fiber from the compost and produce fine FWC powder (**Figure 1**). As revealed by a colorimetric NPK soil test kit combined with a spectrophotometer, the concentration of nitrate, phosphate, and potassium increased significantly after composting (**Table 1**). The moisture content lowered from 72% to 13% after composting. The FW became more acidic after the composting process, having its pH value dropped from pH 7.2 to pH 4.4. The number of viable microorganisms per gram increased around 200-fold after composting. These parameters indicate that we successfully composted FW. Microorganisms have thrived to decompose organic FW into simple inorganic nutrients.

### Effect of Food Waste, Food Waste Compost, and Oats on the Consumption Rate of EPS in Mealworms

To assess the EPS consumption rate of mealworms in the presence of oats, FW, and FWC, we randomly selected 40 mealworms and added them to each of the 8 different set-ups (**Table 2**). FWC and oats, rather than FW, significantly accelerated the EPS consumption rate of mealworm (**Figure 2A**). The Basal EPS consumption rate of the mealworms was 0.483 mg/day. The addition of uncomposted FW did not affect the EPS consumption rate. Interestingly, the EPS consumption rate increased to 0.639 mg/day when co-fed with oats, and to 0.859 mg/day with FWC. The average rate of EPS consumption increased by approximately 32% when co-fed with oats, and approximately 78% when co-fed with FWC; these differences were statistically significant (*p-value* $< 0.05$). This change in EPS consumption rate indicates that the addition of FWC is more effective than that of oats in accelerating EPS consumption in mealworms. The EPS consumption of mealworms with the addition of oats or FWC added was then continuously monitored throughout the larval stage of the mealworms (**Figure 2B**). We observed increased EPS consumption rate in oats and FWC throughout the 21-day period. The increase of EPS consumption rate is the highest in the first three days.

### Effect of EPS, Compost, and Oats on the Life Cycle of Mealworms

Since we found that FWC and oats could effectively accelerate EPS consumption of mealworms, we further analyzed their effect on the life cycle of mealworms. We first

| | Before Composting | After Composting |
|---|---|---|
| Moisture Content (%) | 72% | 13.23% |
| pH Value | 7.2 | 4.4 |
| Nitrate Content (relative unit) | 0.097 | 0.629 |
| Phosphate Content (relative unit) | 0.302 | 0.784 |
| Potassium Content (relative unit) | 0.064 | 0.102 |
| Microbial Count (CFU/g) | 210 | 4070 |

**Table 1:** Table summarizing the moisture content, pH value, nitrate content, phosphate content, potassium content, and microbial content in FW before and after the composting process.

| | EPS (g) | FW (g) | FWC (g) | Oats (g) |
|---|---|---|---|---|
| (1) EPS only | 0.5 | / | / | / |
| (2) FW only | / | 0.5 | / | / |
| (3) FWC only | / | | 0.5 | / |
| (4) Oats only | / | / | / | 0.5 |
| (5) EPS + FW | 0.5 | 0.5 | / | / |
| (6) EPS + FWC | 0.5 | / | 0.5 | / |
| (7) EPS + Oats | 0.5 | / | / | 0.5 |
| (8) Starvation control | / | / | / | / |

**Table 2:** Table summarizing the amount of food component added in each of the 8 set-ups. Each set-up contains 40 mealworms. The "/" symbolizes that a food component is absent.

investigated the survival rate of mealworms under the addition of oats and FWC (**Figure 3A**), because a longer survival time of mealworms could allow a longer time for EPS consumption. The survival rate of the starvation control was approximately 55%. EPS significantly increased the survival rate during the 21-day period, which suggests that mealworms could rely on the EPS to survive. The survival rates of mealworms in set-ups other than starvation control were approximately 85 to 95% at day 21, indicating that EPS, FWC, and oats could support the life of mealworms. We then investigated the percentage change in weight of mealworms to determine the conversion of EPS into biomass (**Figure 3B**). The weight loss of mealworms fed with EPS only was very similar to that of the starvation control, which may be explained by the lack of nutrients. Mealworms in other conditions showed increases in weight. At day 21, the weight gain of mealworms fed with oats alone was the highest, about 80%, while that with FWC alone was approximately 46%. Under the addition of EPS, the weight gain of mealworms co-fed with oats and that with FWC was approximately 56%. Later, we plotted the percentage change in weight of mealworms against the cumulative weight of EPS consumed to determine the correlation of the two variables (**Figure 4**). In the set-up with EPS alone, the percentage change in weight of the worms is independent of the intake of EPS, as concluded by a low $R^2$-value (0.09693, $p$-value = 0.1695). Positive correlation was observed when EPS was co-fed with oats or FWC. The correlation between weight gain and EPS intake in the set-up with FWC was much higher than that with oats, since the set-up with FWC has a $R^2$ value of 0.8147 ($p$-value < 0.0001) while the $R^2$ value of that with oats is 0.1908 ($p$-value < 0.05). The positive correlation might suggest the conversion of EPS into biomass. Interestingly, feeding mealworms with FWC resulted in a slower pupation rate when compared with feeding with same weight of oats in the presence or absence of EPS (**Figure 5**). The difference in pupation rate between EPS-eating mealworms fed with FWC and those with oats at day 21 was statistically significant ($p$-value < 0.05). This suggests that feeding with FWC could allow a longer larval period for the consumption of EPS.

### Effect of food waste compost on EPS consumption rate of three different EPS-digesting species

Ideally, to apply organismal EPS digestion on an industrial scale, we must maximize the mass of EPS to mass of organism ratio in order to digest the maximum amount of EPS in the smallest infrastructure. We also tested the effect of FWC on other EPS-digesting worms, including superworm and greater waxworm. We found that FWC accelerated EPS consumption 2-fold in all of the species (**Figure 6**). Among the tests, mealworms showed the highest EPS-to-worms mass-to-mass ratio in consuming the EPS. One gram of mealworms could consume around 150mg of EPS in the presence of FWC, indicating that this combination is the most effective way to consume EPS.

## DISCUSSION

Our research found that FWC, rather than FW, accelerated EPS-consumption in mealworms. We propose that the reason

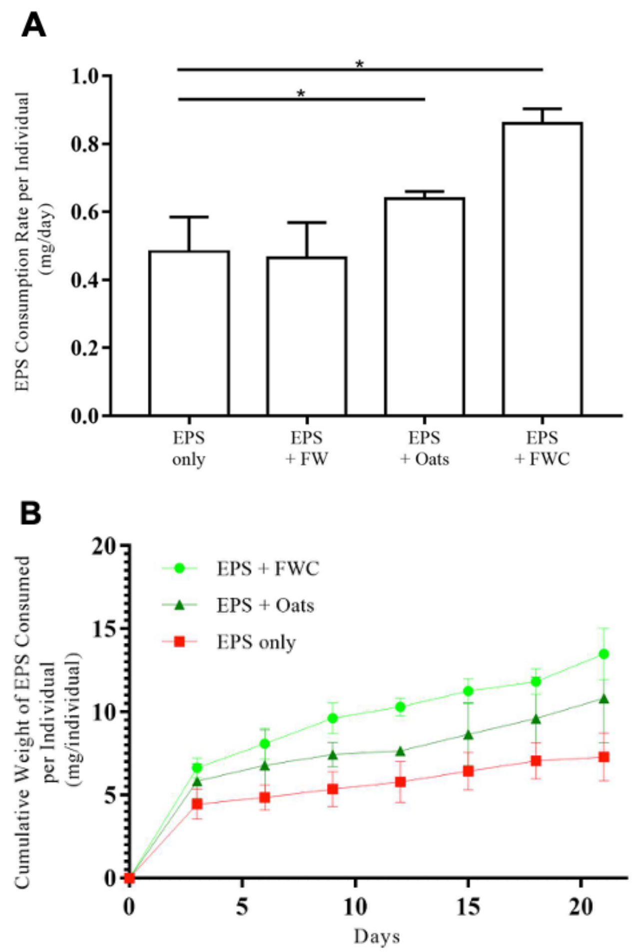


**Figure 2: Effect of food waste, food waste compost, and oats on the consumption rate of EPS in mealworms**. (**A**) Average individual EPS consumption rate under the addition of food waste (FW), food waste compost (FWC), and oats measured at the first 12 days. (Data represent the mean; Error bars represent standard deviation; n=3; unpaired t-test; *, $p$-value < 0.05)
(**B**) Cumulative weight of EPS consumed per individual throughout the larval stage in 21 days. (Data represent the mean; Error bars represent standard deviation; n=3)

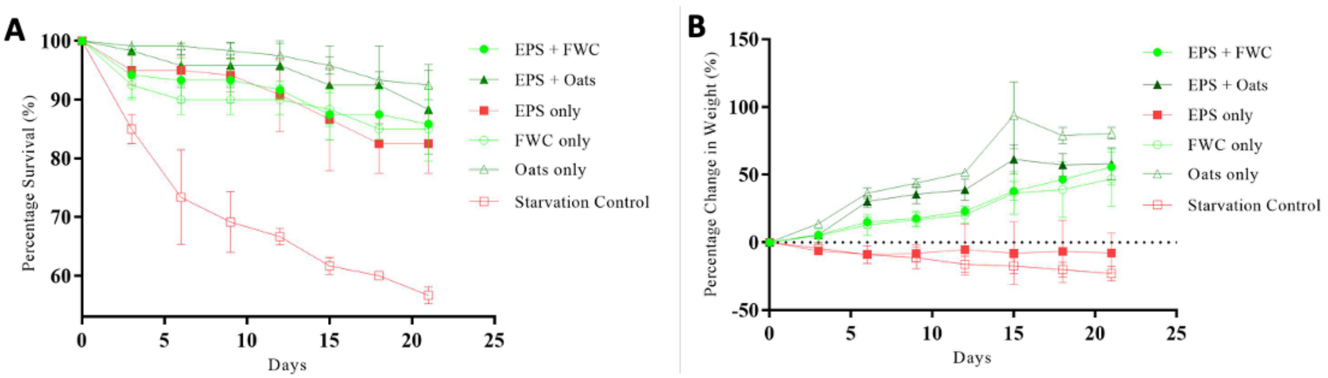


**Figure 3: Effect of EPS, compost, and oats on the life cycle of mealworms.** (**A**) Percentage survival of mealworms throughout 21 days. (Data represent the mean; Error bars represent standard deviation; n=3). (**B**) Percentage change in weight of mealworms throughout 21 days. (Data represent the mean; Error bars represent standard deviation; n=3)

may lie in mealworms' gut. According to previous research, the biodegradation of EPS is done by microorganisms in mealworms' gut (8). FWC contains a large number of probiotic microorganisms that may boost the mealworms' gut microbiota and aid the digestion of EPS. Research has shown that the increase in microbiota diversity in insects' gut can enhance their appetite and digestion (15). In addition, composting FW can break down large organic chemicals into smaller digestible substances. Many organic and inorganic nutrients may also be found in FWC. These factors could have facilitated the digestion of EPS in mealworms' gut when co-fed with FWC.

Apart from FWC, oats also accelerated EPS consumption in mealworms. Although oats seemed to have a stronger effect on promoting the survival rate and weight gain of mealworms, the effect on accelerating EPS consumption in mealworms was lower than that of FWC. FWC might contain fewer growth-related nutrients, but it should possess more chemicals or microorganisms that promote the decomposition and assimilation of EPS. The correlation between weight gain and EPS intake in the condition with FWC was much higher than that with oats. The higher correlation might suggest that more EPS could have been converted to biomass when FWC is added. In addition, the intake of FWC could result in a longer larval period for the consumption of EPS. Together with the fact that using FWC is more economic and environmentally friendly than using oats, we propose FWC is a better additive than oats for effective EPS consumption. We also showed that FWC accelerated EPS consumption in superworms and greater waxworms. However, FWC-accelerated EPS consumption by mealworms showed the greatest effectiveness in terms of mass-to-mass ratio.

We believe that our findings could facilitate the application of using mealworms to degrade EPS in an industrial setting. In an industrial setting, mealworms would be placed in a large-scale bioreactor, rather than a petri dish. Assuming that each mealworm weighs 50mg and the density of EPS is approximately 20kg/m$^3$, according to our experiment, 3150 m$^3$ (63 tonnes) of EPS would be digested by 220 tonnes (the weight of 12 double-decker buses) of mealworms in a month. But if FWC is added to facilitate the process, an additional 2550 m$^3$ (size of an Olympic-size swimming pool, weighs 50 tonnes) of EPS could also be processed per month. On the contrary, it takes one thousand years for EPS to decompose naturally without mealworms (4).

Previous research has shown that the EPS consumption of mealworms depends on different factors, including temperature, humidity, and the density and chemistry of EPS. The basal EPS consumption rate that we measured is 0.483 mg/day/mealworm, which is approximately double previously published data that measured 0.235 mg/day/mealworm (7). The difference may be explained by the difference in density of EPS. The density of EPS tested in the previous research is 0.02g/cm$^3$, which is a double that in our study. A lower density

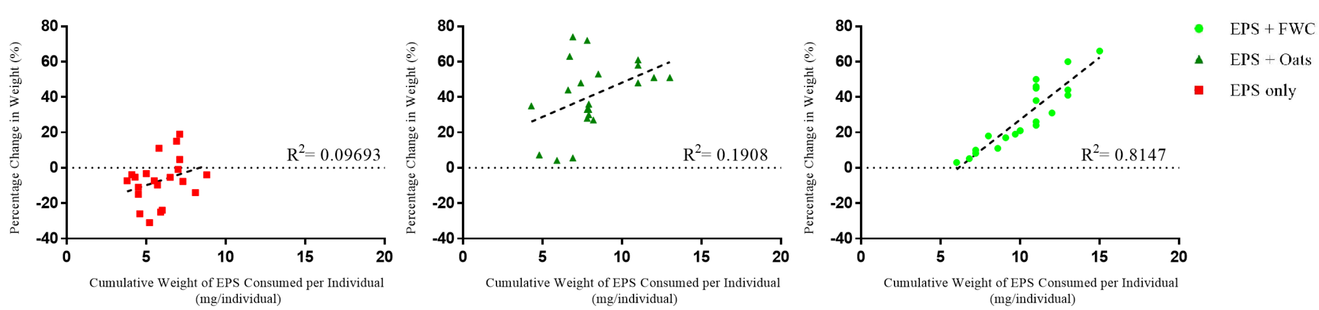


**Figure 4.Correlation between percentage change in weight and EPS consumed per mealworm.** $R^2$ represent the coefficient of determination. Dashed line represent the best-fit line.

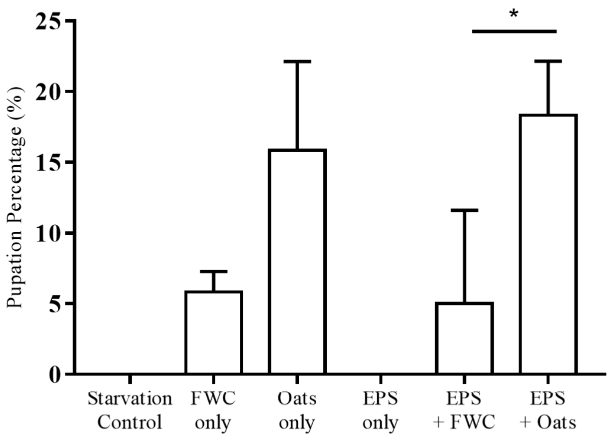


**Figure 5: Effect of EPS, compost, and oats on the pupation of mealworms.** Percentage of mealworms that had undergone pupation at day 21. (Data represent the mean; Error bars represent standard deviation; n=3; unpaired t-test; *, $p$-value < 0.05)

of EPS could result in a faster rate of EPS consumption (13). Limitations of our study also include the unknown molecular weight of the EPS that we used. In addition, the FW that we used was from an Asian diet, which is heavy in carbohydrates. Future work could explore the effect of FWC and EPS consumption with different EPS molecular weight and density. The effect of FWC generated from different styles of diet should also be tested.

In summary, we believe that FWC-accelerated EPS consumption by mealworms could be a promising method to biologically degrade EPS in an industrial setting. Current landfill disposal of FW and EPS does not meet the principle of sustainable development. Not only does it waste valuable resources, it also poses tremendous impact to the environment. By using FWC to accelerate the consumption of EPS in mealworms, FW and EPS could be turned into organic matter in a fast manner. The organic matter produced has the potential to become fertilizer. Future research could optimize other factors that affect the rate of EPS consumption in mealworms and explore ways for this finding to be implemented in an industrial setting.

## METHODS

### Composting of Food Waste

FW from a secondary school (Po Leung Kuk Laws Foundation College, Hong Kong) was collected at lunchtime on eight separate days during one month. A total of 30kg of FW was collected. The FW was classified into carbohydrates (mainly rice), protein (mainly meat), and vegetables during collection. The total carbohydrate, protein, and vegetable weights collected were 10.3kg, 6.2kg, and 12.9kg, respectively, meaning a weight-to-weight ratio of around 2:1:2. FW was composted in a composting machine (Oklin, GG-CMO-02). Composting microbe (Oklin, Acidulo) was added to the machine following the manufacturer's protocol. The compost was collected after one month. The compost was sieved to obtain a fine powder of compost (**Figure 1**).

### Characterization of Food Waste and Food Waste Compost

The moisture content and pH value were measured using the standard procedure documented by the International Soil Reference and Information Centre (16). To measure the moisture content, 5g of the FW or FWC was dried in an oven at 105°C for 1 hour. The difference in weight before and after heating indicated the mass of water. To measure the pH, 20g of the FW or FWC was shaken with 50mL of distilled water at a frequency of 250rpm for 2 hours. pH was measured by a pH meter (Milwaukee, ph600) after sedimentation. The NPK content of the compost was measured by a commercial colorimetric NPK soil test kit (LaMotte, 3-5880). Relative amounts of the nitrate, phosphate, and potassium content were measured by a spectrophotometer at 700nm, 460nm, and 600nm respectively. To measure the colony-forming unit (CFU) of FW and compost, 10g of FW or FWC was mixed with 10mL of distilled water and shaken at 250rpm for 30 minutes. 100μL of the resulting solution was then spread on Plate Count Agar (PCA) and incubated at 37°C overnight. CFU was obtained by counting the number of colonies on the plate. The CFU/gram was calculated by multiplying the CFU on agar plate with the dilution factor. All of the procedures were repeated three times and the average was reported.

### Mealworms and Other Materials

Approximately 3,000 mealworms, 300 superworms (*Zophobas morio*), and 300 greater waxworms (*Galleria mellonella*) were purchased from a local breeder in Mong Kok, Hong Kong. The identity of the species was confirmed by their appearance. Contamination of *Alphitobius diaperinus*

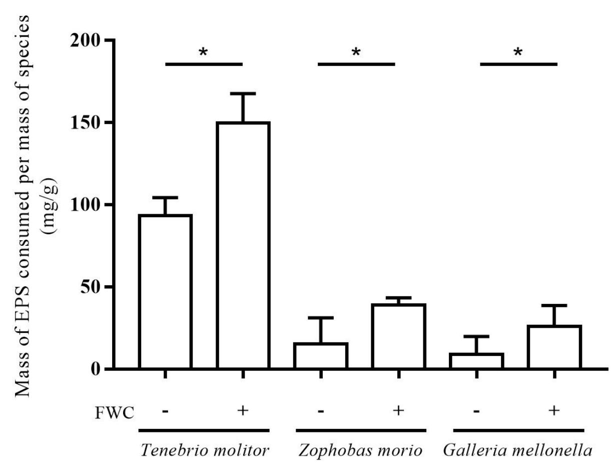


**Figure 6: Effect of food waste compost on EPS consumption rate of three different species.** *Tenebrio molitor* (mealworm) showed the highest efficiency in consuming EPS under the presence of FWC when compared with *Zophobas morio* (superworm) and *Galleria mellonella* (greater waxworm). (Data represent the mean; Error bars represent standard deviation; n=3; unpaired t-test; *, $p$-value < 0.05)

was found among mealworms and removed. Organisms were subject to a 48-hour starvation period before initiating the feeding test. Oats was purchased from a supermarket. EPS was purchased from a local stationary store in Hong Kong. The density of EPS was measured to be 0.01g/$cm^3$, which can be classified as a type of low-density EPS. The density is comparable to that of common EPS products such as cups and packaging material.

### Expanded Polystyrene feeding test

To assesses the EPS consumption rate, survival rate, pupation rate, and weight change of mealworms, 40 randomly selected mealworms were added to each of the 8 different set-ups (**Figure 2**), including: 1) 0.5g EPS only, 2) 0.5g FW only, 3) 0.5g FWC only, 4) 0.5g oats only, 5) 0.5g EPS mixed with 0.5g FW, 6) 0.5g EPS mixed with 0.5g FWC, 7) 0.5g EPS mixed with 0.5g oats, and 8) starvation control. The mealworms were incubated in petri dishes in an incubator (Digisystem, DSI-D) at approximately 25°C, 70% humidity, and in complete darkness for 25 days. The number and weight of the mealworms and the mass of plastic were measured every 3-4 days. A brush was used to clean the worms and plastic before each measurement. Dead mealworms, feces, and debris were removed after each measurement. New compost, oats, FW, or FWC were replaced in the corresponding set-up after each measurement. To assess the EPS consumption rate of superworms and greater waxworms, the same procedure was used except the worm sample size was reduced to 10 of each worm type. All tests were performed in triplicate.

### Statistical analysis and graph plotting

Raw data was input into Microsoft Excel (2016). The cumulative weight of EPS consumed per individual (mg/individual) was calculated by dividing the decreased weight of EPS by the number of survived larvae on the day of measurement. The individual EPS consumption rate (mg/day) was calculated by dividing the cumulative weight of EPS consumed per individual by the total number of days. Percentage change in the average weight of individual mealworm was reported as percentage change of weight (%) of mealworms. Percentage of survived mealworms was reported as percentage survival (%). Percentage of pupa among the living larva was reported as pupation percentage (%). The processed data obtained was input into GraphPad Prism version 7.00 (17). An unpaired t-test function was used to report the *p*-value of test and test for a significant difference. A linear regression function was used to report the R-squared ($R^2$) for the correlation analysis.

## ACKNOWLEDGEMENTS

We would like to thank Ms. Lin Mei Yu for providing guidance regarding composting of food waste and all the support given by our school Po Leung Kuk Laws Foundation College.

**Received:** February 28, 2020
**Accepted:** June 8, 2020
**Published:** July 13, 2020

## REFERENCES

1. Geyer, Roland *et al*. "Production, Use, And Fate Of All Plastics Ever Made". *Science Advances*, vol 3, no. 7, 2017, p. e1700782.
2. Moore, S.L *et al*. "Composition And Distribution Of Beach Debris In Orange County, California". *Marine Pollution Bulletin*, vol 42, no. 3, 2001, pp. 241-245.
3. Cannon, Seon M.E. *et al*. "Plastic Ingestion By Fish In The Southern Hemisphere: A Baseline Study And Review Of Methods". *Marine Pollution Bulletin*, vol 107, no. 1, 2016, pp. 286-291.
4. Kaplan, D L *et al*. "Biodegradation Of Polystyrene, Poly(Metnyl Methacrylate), And Phenol Formaldehyde.". *Applied and Environmental Microbiology*, vol 38, no. 3, 1979, pp. 551-553.
5. Elomaa, Matti, and Erkki Saharinen. "Polycyclic Aromatic Hydrocarbons (Pahs) In Soot Produced By Combustion Of Polystyrene, Polypropylene, And Wood". *Journal Of Applied Polymer Science*, vol 42, no. 10, 1991, pp. 2819-2824.
6. Epsindustry.Org, 2020, http://epsindustry.org/sites/default/files/2018%20RRR.pdf.
7. Yang, Shan-Shan *et al*. "Biodegradation Of Polystyrene Wastes In Yellow Mealworms (Larvae Of Tenebrio Molitor Linnaeus): Factors Affecting Biodegradation Rates And The Ability Of Polystyrene-Fed Larvae To Complete Their Life Cycle". *Chemosphere*, vol 191, 2018, pp. 979-989.
8. Brandon, Anja Malawi *et al*. "Biodegradation Of Polyethylene And Plastic Mixtures In Mealworms (Larvae Of Tenebrio Molitor) And Effects On The Gut Microbiome". *Environmental Science & Technology*, vol 52, no. 11, 2018, pp. 6526-6533.
9. Peng, Bo-Yu *et al*. "Biodegradation Of Polystyrene By Dark (Tenebrio Obscurus) And Yellow (Tenebrio Molitor) Mealworms (Coleoptera: Tenebrionidae)". *Environmental Science & Technology*, vol 53, no. 9, 2019, pp. 5256-5265.
10. Bombelli, Paolo *et al.* "Polyethylene Bio-Degradation By Caterpillars Of The Wax Moth Galleria Mellonella". *Current Biology*, vol 27, no. 8, 2017, pp. R292-R293.
11. Yang, Yu *et al*. "Biodegradation And Mineralization Of Polystyrene By Plastic-Eating Superworms Zophobas Atratus". *Science Of The Total Environment*, vol 708, 2020, p. 135233.
12. Brandon, Anja Malawi *et al*. "Fate Of Hexabromocyclododecane (HBCD), A Common Flame Retardant, In Polystyrene-Degrading Mealworms: Elevated HBCD Levels In Egested Polymer But No Bioaccumulation". *Environmental Science & Technology,* 2020, vol. 54, pp. 364-371.
13. Green, Sierra, and Katherine Green. "The Effects of Post-Consumer Waste Polystyrene on the Rate of Mealworm

Consumption." *Journal of Emerging Investigators*. 2018.
14. "Wasted: How America Is Losing Up To 40 Percent Of Its Food From Farm To Fork To Landfill". NRDC, 2020, https://www.nrdc.org/resources/wasted-how-america-losing-40-percent-its-food-farm-fork-landfill.
15. Leitão-Gonçalves, Ricardo *et al*. "Commensal Bacteria And Essential Amino Acids Control Food Choice Behavior And Reproduction". *PLOS Biology*, vol 15, no. 4, 2017, p. e2000862.
16. Reeuwijk, L. P. van. Procedures For Soil Analysis. ISRIC, 1995.
17. "Graphpad Software". Graphpad.Com, 2020, https://www.graphpad.com/quickcalcs/.