Supplementary data

Source of mealworms tested for depolymerization and degradation
 Source 1: the PetCo Pet Store Chain, Mountain View, California, the USA (supplied by
 Timberline Fisheries, Marion, Illinois, the USA)
 Source 2: the PetSmart Pet Store Chain, Sunnyvale, California (supplied by Bug Company,
 Ham Lake, Minnesota, the USA)
 Source 3: Timberline Fisheries, Marion, Illinois, the USA
 Source 4: Exotic Nutrition Pet Company, Newport News, Virginia, the USA
 Source 5: Rainbow Mealworms, Compton, California, the USA
 Source 6: A pet store in Belfast, North Ireland, UK
 Source 7: A pet store in Beljing, China
 Source 9: A mealworm farm in Tai'an County, Shandong Province, China
 Source 10: A pet store in Shanghai, China
 Source 11: A pet store in Shanghai, China

2. Procedures of molecular analysis

Before DNA from the larvae of *Tenebrio molitor* was extracted, the external surface of the larvae was disinfected with a 10% hypochlorite wash followed by a triple rinse in sterile water as previously described (Ridley et al., 2012). Two tissue types were investigated: midgut tissue, which was dissected with sterile instrumentation, and homogenized larvae (with alimentary canal and head removed). DNA extractions of pooled larval midguts (N = 3 per sample) and homogenized larvae were performed using the DNeasy Blood and Tissue kit (Qiagen) with addition of lysozyme (15 mg ml⁻¹, Invitrogen) during the lysis step. DNA was quantified using a Qubit dsDNS HS (High Sensitivity) Assay Kit (Invitrogen) and a Qubit 2.0 (Thermo Fisher). All DNA

preparations were stored at -20 °C until sequencing. Library construction and sequencing (2 x 250 bp paired-end reads, Illumina MiSeq) were performed by the Michigan State University Genomics Core Facility using a modified version of the protocol adapted for the Illumina MiSeq, described by Caporaso et al. (2011). Region-specific primers 515F/806R (5'-

GTGCCAGCMGCCGCGG-3', 5'-TACNVGGGTATCTAATCC-3') were used to amplify variable region 4 of the 16S rRNA gene according to previously described methods (Claesson et al., 2010; Caporaso et al., 2011). The raw reads were demultiplexed, assembled, and qualityfiltered in QIIME 2 (v 2017.12), using default settings (<u>https://qiime2.org</u>) (Kuczynski et al., 2011). DADA2 was used to filter chimeric reads and artifacts commonly present in Illumina amplicon data (Callahan et al., 2016). To classify filtered reads to taxanomic groups, a Naive Bayes classifier was trained using the 16S rRNA region (V4), the primer set and read length used (515F/806R, 250 bp), and the Greengenes 99% reference set (v 13.8,

http://greengenes.secondgenome.com) (DeSantis et al., 2006; McDonald et al., 2012; Werner et al., 2012). This trained feature classifier was then used to assign taxonomy to each read using the default settings in QIIME. Reads assigned to mitochondria or chloroplast were removed from each sample before analysis. For each taxanomic group at a certain level (e.g. phylum, class, genus, etc.), the relative abundance difference between groups was tested with Wilcoxon rank-sum tests (FDR correction) in R (v 2.4.4) (R Development Core Team R, 2017) and visualized using ggplot2 (v 2.2.1) (Wickham, 2016).

3. Supplementary Figures



Figure S1. Typical survival curves of mealworms fed with PS only or PS plus bran and unfed. (A) Mealworms from PetCo, USA (source 1) fed with protein plus PS, bran plus PS, PS only versus control (unfed). (B) Mealworms from Belfast, North Orland, UK (source 6) fed with bran plus PS, PS alone, and unfed. (C) Mealworms from Shanghai, China (source 11) fed with PS only and PS plus bran versus control (unfed). Single incubators with 410 mealworms each were used. Note: Figure S1A is adapted from Yang et al. (2018).



Figure S2. Comparison of survival rate (SR) curves of mealworms fed with bran, PS plus bran, PS only and without feed with mealworms from four USA sources. The tests were performed using mealworms from USA sources 2 (A), 3 (B), 4(C) and 5(D) at 25 °C. The initial number of mealworms in each incubator was 120 with duplicates. The data showed that the SR curves of mealworms fed with bran, bran plus PS and PS were similar during a 32 day period. The SRs of unfed mealworms of all sources were well below those fed with bran only, PS plus bran (P/B co-fed) and PS only during a 32 day period.



Figure S3. FTIR spectra of feed stock and frass samples of mealworms CP fed bran, PS and bran plus PS at three different temperatures (20, 25 and 30 °C). The results showed that the frass samples of mealworms fed with PS only and PS plus bran showed C-O bending near 800 but the samples fed with bran only did not. Strong C=O carbonyl group peak was observed in the samples fed with PS and PS plus bran. The mealworms used was from Source 1. Data from Yang et al. (2018).



Figure S4. FTIR graphs of mealworm frass fed with PS plus bran from various sources (1 -5, 7-11), which were similar to those fed with PS plus bran in Figure S3.

4. Supplementary Tables

Table S1. 32 day survival rate (%) of mealworms fed with bran only, PS plus bran, PS only and unfed from four USA sources

Feed	Bran only	PS+Bran co-fed	PS only	Unfed
Source 2	90.8 <u>±</u> 1.7	90.8 <u>+</u> 3.3	88.3 <u>+</u> 0.8	74.2 <u>+</u> 4.2
Source 3	87.5 <u>±</u> 1.7	88.3 <u>+</u> 2.5	87.5 <u>+</u> 1.7	70.8 <u>+</u> 5.0
Source 4	89.2 <u>±</u> 0.8	90.8 <u>+</u> 2.5	90.8 <u>+</u> 1.7	75.8 <u>+</u> 2.5
Source 5	85.0 <u>+</u> 2.5	84.2 <u>+</u> 1.7	82.5 <u>+</u> 1.7	69.2 <u>+</u> 3.3

Note: Source description in Section 1 SPI. The test condition is described in Fig. S2.

Table S2. Survival rate (%) and weigh changes of mealworms fed with PS plus bran, PS only and unfed from Shanghai (Source 11)

	Bran only		PS+Bran		PS only		Unfed	
Feed	SG,%	∆weight	SG,%	∆weight,%	SG,%	∆weight,%	SG,%	∆weight,%
Day 0	100	0	0	0	100	0	100	0
Day 14	100	3.5%	99.5	0.1%	97.8	-8.2%	78.8	-10.2%
Day 24	nd	13.0%	98.8	8.0%	95.6	-10.4%	73.9	-13.3%
Day 34	nd	25.0%	95.0	16.0%	92.0	-9.3%	68.3	-17.4%

Note: Source description in Section 1 SPI. The test condition is described in Fig. S2C. Test was performed with single incubator with 410 mealworms each. The initial average weight of mealworms was 96.0 \pm 0.6 mg/worm. Initially, 4.7 g of PS foam was added into each container. 8.5 g of bran was added at the beginning and then supplemented 8.5 every 5 days. A total of 8 times. The mealworms with PS only consumed 2.0 g PS while those fed with bran plus PS consumed 2.7 g PS.

Source of mealworms	Mn	$\mathbf{M}_{\mathbf{w}}$	M _n reduction compared with control PS	M _w reduction compared with control PS	
1	82000±2000	210000±1000	9.40±0.72	7.19±0.48	
2	78000±2000	200000±6000	13.58±3.53	10.78±2.57	
3	78000±3000	203000±8000	13.34±4.61	10.88±3.24	
4	76000±4000	206000±4000	15.64±4.73	9.66±2.14	
5	77000±2000	204000±6000	14.27±0.13	10.29±2.73	
6	84000±5000	182000 ± 9000	5.37±0.19	12.10±4.51	
7	78000±4000	215000±3000	12.92±2.51	5.55±1.12	
8	78000±2000	212000±1000	13.50±4.08	6.72±0.79	
9	82000±2000	213000±6000	9.05±0.18	6.50±2.06	
10	81000±4000	214000±3000	9.53±2.17	5.89±1.28	
11	77000±2000	216000±3000	14.08 ± 1.48	5.18±1.72	
12	83000±2000	231000±2000	11.19±1.48	9.02±1.09	

Table S3. Decrease in average molecular weights $(M_n \text{ and } M_w)$ of residual PS polymers in the frass of mealworms fed bran plus PS

Locations of numbered sources are provided in the main text. Molecular weights for PS from different sources: for sources 1-5, 7-11, M_n was 90000±2000, and M_w was 228000±12000; source 6, M_n was 89000±2000, and M_w was 207000±2000; for source 12, M_n was 93000±3000, and M_w was 254000±1000.

Table S4 T-test comparing Mn and Mw of PS feedstock to Mn and Mw values of residual p	polymer in the
frass of mealworms from the 12 sources when fed both bran and PS	

Feedstock PS source	Residual PS in frass from mealworm source	Value	<i>p-</i> value*	Significance (V/N)	Value	<i>p-</i> value*	Significance (V/N)
<u> </u>	1	M	0.0025	V	M	0.0017	V
A	1	IVIn	0.0025	I V	M	0.0017	I V
A	2	lVIn	0.0241	Y	\mathbf{W}_{W}	0.0184	Ŷ
А	3	M_n	0.0400	Y	M_w	0.0279	Y
А	4	Mn	0.0307	Y	M_{w}	0.0166	Y
А	5	Mn	0.0003	Y	$M_{\rm w}$	0.0231	Y
В	6	Mn	0.0450	Y	$M_{\rm w}$	0.0069	Y
А	7	Mn	0.0103	Y	$M_{\rm w}$	0.0132	Y
Α	8	Mn	0.0322	Y	$M_{\rm w}$	0.0049	Y
А	9	Mn	0.0005	Y	M_{w}	0.0311	Y
Α	10	Mn	0.0140	Y	$M_{\rm w}$	0.0158	Y
Α	11	Mn	0.0036	Y	M_{w}	0.0356	Y
С	12	Mn	0.0079	Y	$M_{\rm w}$	0.0051	Y

* The results indicated that the changes in molecular weights were significant. See text for feedstock sources and mealworm source locations.

Table S5 Number of total visible gut bacteria counted from the control mealworms and the antibiotic treated mealworms. Mealworms from the five USA sources were assayed after continuous feeding of gentamicin for 7 days

Sources of	Gut Bacteria Concentration (CFUs per gut)				
mealworms	Control tests	Antibiotic treatment tests			
USA source 1	$(4.79\pm0.31) \times 10^{6}$	$(1.91\pm8.56) imes 10^4$			
USA source 2	$(6.89\pm0.21) \times 10^5$	$(5.67\pm0.98) imes 10^3$			
USA source 3	$(3.46\pm0.18) imes 10^6$	$(1.64\pm0.13) imes 10^4$			
USA source 4	$(5.01\pm0.17) imes10^{6}$	$(1.27\pm0.16) \times 10^5$			
USA source 5	$(2.23\pm0.25) imes 10^{6}$	$(6.22 \pm 0.61) imes 10^4$			

Table S6 The molecular weights (M_n and M_w) of the PS and the frass samples from 5 USA sources after feeding with gentamicin for 15 days

Sample	$\mathbf{M}_{\mathbf{n}}$	M_w
PS feedstock	90000±2000	228000±1000
Frass from source 1	88000±2000	223000 ± 4000
Frass from source 2	87000±2000	223000 ± 3000
Frass from source 3	85000±3000	224000 ± 5000
Frass from source 4	85000±1000	225000 ± 3000
Frass from source 5	85000±2000	226000±4000

Value	Sample	Sample	n valua	Significance	Value	Sample	Sample	<i>p</i> -value	Significance
	1	2	<i>p</i> -value	(Y/N)	value	1	2		(Y/N)
Mn	PS	Frass 1	0.3502	Ν	$M_{\rm w}$	PS	Frass 1	0.2325	Ν
Mn	PS	Frass 2	0.2545	Ν	$M_{\rm w}$	PS	Frass 2	0.0629	Ν
Mn	PS	Frass 3	0.2622	Ν	$M_{\rm w}$	PS	Frass 3	0.1842	Ν
Mn	PS	Frass 4	0.0565	Ν	$M_{\rm w}$	PS	Frass 4	0.2730	Ν
M_n	PS	Frass 5	0.0769	Ν	$M_{\rm w}$	PS	Frass 5	0.7133	Ν
Mn	1	Frass 1	0.0378	Y	$M_{\rm w}$	F1-#1	Frass 1	0.0282	Y
Mn	2	Frass 2	0.0027	Y	$M_{\rm w}$	F1-#2	Frass 2	0.0140	Y
M_n	3	Frass 3	0.0287	Y	$M_{\rm w}$	F1-#3	Frass 3	0.0228	Y
Mn	4	Frass 4	0.0383	Y	$M_{\rm w}$	F1-#4	Frass 4	0.0182	Y
Mn	5	Frass 5	0.0300	Y	$M_{\rm w}$	F1-#5	Frass 5	0.0281	Y

Table S7 T-test statistical analysis of M_n and M_w of PS feedstock versus residual polymer extracted from frass samples of the mealworms previously fed gentamicin and receiving a diet of bran plus PS.

The results indicated no significant differences between PS feedstock versus residual PS polymers of the

frass from mealworms fed gentamicin. Significant differences were observed between the residual

polymers with and without gentamicin in the feed.

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Polystyrene foam consumption by yellow mealworms (*larvae of Tenebrio molitor*) obtained in Australia

Min Zheng, Ph.D

Advanced Water Management Centre, the University of Queensland, St. Lucia, QLD 4072, Australia

Source of mealworms: mealworms were purchased from a local Australian farm, located in Joyner, Brisbane. The mealworms were mature larvae with average weight of 115.1 mg per worm.

Test materials: Polystyrene (PS) foam plate was expanded PS packaging material product used in university laboratory. It has density of 0.0235 g/cm³.

Test set up: The test was set up with single incubators (high density polyethylene food containers) under two conditions. Each incubator contained 800 mealworms. In one incubator, 7.9895 g wheat bran and 7.936 g PS foam were initially added to test PS consumption in the presence of bran. Another incubator was supplemented with only 7.9891 g PS. Both incubators were maintained under dark condition at 22 °C. After start-up, the weight of plastic was recorded along the time.

Results. The results showed that, after two weeks (14 days), the PS foam were consumed in both incubators with the respective weight loss of 1.5390 g and 1.5766 g, indicating a total plastic reduction ratio around 20%, and about 200 m g-PS consumed per 100 mealworm or 13.4 mg PS per 100 mealworms per day. During incubation period, pupation was observed.

The results have verified that the mealworms obtained in Joyner, Brisbane, Australia have capability of consuming PS foam. Future test is need to verify if the ingested PS foam is depolymerized and then biodegraded or even mineralized to CO_2 .



Survey of Ingestion of Plastics by Yellow Mealworms

Contact: Dr. Wei-Min Wu, Department of Civil & Environmental Engineering, Stanford University, Stanford, CA 94305-4020, USA (Email: billwu@stanford.edu; phone: 1-650-723-5310)

The purpose of this survey is to understand the ubiquity of yellow mealworms (the larvae of Tenebrio molitor) eating plastics, especially polystyrene (PS in short) globally. The biodegradation of the plastics has to be further determined by analysis of frass egested by the mealworms. Your contribution will be greatly acknowledged.

1. School or organization (elementary, middle, high school, college, other)

Marshall School, Duluth MN

2. Address (city, state or province, country)

1215 Rice lake road, Duluth MN

3. Team group size

9 people, grades 4-8

4. Contact person and email (optional)

Noah Stevens: stev5848@marshallschool.org

5. Test time (yr., how many times)

Nov. 2015-Mar. 2016

6. Source of yellow mealworms (store or farming name, location, phone, email, website) Amazon.com

https://www.amazon.com/gp/product/B00CQMTP56?psc=1&redirect=true&ref_=oh_aui_detailpa ge_o01_s00

7. Plastic tested: Styrofoam (EPS), extruded PS foam (XPS), others (polyethylene foam, polypropylene foam, polyurethane foam, etc.)

Styrofoam cups, styrofoam plates, styrofoam packing material and styrofoam packing peanuts



8. Test temperature range (oC or oF) 69.5 F

9. Number of mealworms tested (optional)

500 with styrofoam and 500 with oat bran as a control.



10. Plastic mass used (optional)

45 grams

11. Eating activity: Not eating, slightly active, active and very active

Active in foam, more active and healthier in bran

12. Plastic removal efficiency (e.g. % in 30 days) (optional)

The mealworms ate 5 grams of styrofoam in the first 30 days. The mealworms ate approx. 4.5 grams of styrofoam in the 2nd 30 days.

The Styrofoam consumption rate for the first 30 days is about 33 mg per 100 worms per day

13. Photos of mealworms (optional)



Mealworms used



Bottom of container with mealworms and

styrofoam



Mealworms in container with bran (control group)





Mealworms in styrofoam



Mealworms in container with styrofoam



Size difference after 30 days. Bigger mealworm is

from control group with bran. Smaller mealworm is from the container with styrofoam.



Tapirs of the 8th Dimension Team members: First Row: Ben Austin, Usman Mallick, Noah Stevens, Ayaah Mallick, Xander Schroeder Second Row: Joe Perry-Spears, Cedar Melby, Micah Stevens, Ben Bergeron

14. Other description (Survival rates, plastic reduction) (optional)

The mealworms did better when eating the less dense packing peanuts and packaging foam, and ate less of the dense plates and cups.

Survival rate 465/500 x 100% =93% 15. Permission to cite your test results: yes or no Yes 16. Permission to use your photos for scientific conferences and academic publication if you attach photos: yes or no Yes

Signature if permission yes: yes (Karla Steven) Date: 07/02/17 Note: EPS = Styrofoam, XPS= extruded polystyrene foam. Figure source: Rani et al (2014), Chemosphere, 110, 111-119 za bg

- 1. School or organization (elementary, middle, high school, college, other) Dwight School
- 2. Address (city, state or province, country) 800 Columbus Ave, NY 10025, New York, USA
- 3. Team group size

1 person, 18 years old

- 4. Contact person and email (optional) So Eun Kim, soeunkim312@gmail.com
- 5. Test time (yr., how many times) November 2015 & January 2016, 2 times
- 6. Source of yellow mealworms (store or farming name, location, phone, email, website)

FM Feeders

https://www.amazon.com/250-Live-Mealworms-LARGE-Free/dp/B00EVUZV6S/ref=pd_y s_sabia_11?_encoding=UTF8&pd_rd_i=B00EVUZV6S&pd_rd_r=NQC06XR76HKE84K QTV2F&pd_rd_w=YDTGv&pd_rd_wg=ZQTXg&psc=1&refRID=NQC06XR76HKE84KQT V2F

- 7. Plastic tested: Styrofoam (EPS), extruded PS foam (XPS), others (polyethylene foam,
- polypropylene foam, polyurethane foam, etc.)

Styrofoam (EPS)

8. Test temperature range (^oC or ^oF)

 $21^{\circ}C \sim 26^{\circ}C$

- 9. Number of mealworms tested (optional) Five boxes, 100 mealworms each
- 10. Plastic mass used (optional)

10 g

- 11. Eating activity: Not eating, slightly active, active and very active Active
- 12. Plastic removal efficiency (e.g. % in 30 days) (optional) About 10% in 30 days
- 13. Photos of mealworms (optional)



First Experiment (100% wheat bran & 100% styrofoam)

14. Other description (Survival rates, plastic reduction) (optional)

- In the first experiment only four out of 100 mealworms alive after two days when it was fed solely on styrofoam. Most of the mealworms were alive when fed on wheat bran.
- The mealworms with styrofoam had a darker color.
- In the second experiment, almost half of the mealworms successfully became beetles although it was fed solely on styrofoam. More mealworms turned into beetles when styrofoam was mixed with wheat bran.
- The beetles which consumed bran lived longer than the beetles with styrofoam.
- When both wheat bran and styrofoam were present, mealworms only ate wheat bran. They started eating styrofoam when wheat bran was all consumed.

15. Permission to cite your test results: yes or no

Yes

16. Permission to use your photos for scientific conferences and academic publication if you attach photos: yes or no

Yes Signature if permission yes:

Eun Rim

Date: July 13, 2017

Survey of Ingestion of Plastics by Yellow Mealworms

Contact: Dr. Wei-Min Wu, Department of Civil & Environmental Engineering, Stanford University, Stanford, CA 94305-4020, USA (Email: <u>billwu@stanford.edu</u>; phone: 1-650-723-5310) The purpose of this survey is to understand the ubiquity of yellow mealworms (the larvae of *Tenebrio molitor*) eating plastics, especially polystyrene (PS in short) globally. The biodegradation of the plastics has to be further determined by analysis of frass egested by the mealworms. Your contribution will be greatly acknowledged.

1. School or organization (elementary, middle, high school, college, other) The Evergreen School (Preschool-8th grade)

Address (city, state or province, country)
 15201 Meridian Avenue North
 Shoreline, WA 98133

3. Team group size $5-5^{th}$ graders

Contact person and email (optional)
 Dr. Christopher Li
 cili@fredhutch.org

5. Test time (yr., how many times) Jan 2016-April 2016 – one time

6. Source of yellow mealworms (store or farming name, location, phone, email, website) Beta Hatch, Seattle, WA, USA

7. Plastic tested: Styrofoam (EPS), extruded PS foam (XPS), others (polyethylene foam, polypropylene foam, polyurethane foam, etc.) EPS board, XPS coffee cup, styrofoam food trays

8. Test temperature range (°C or °F) Room temperature only

 Number of mealworms tested (optional) ~500

10. Plastic mass used (optional) Didn't weigh

11. Eating activity: Not eating, slightly active, active and very active Active

12. Plastic removal efficiency (e.g. % in 30 days) (optional) We didn't measure this but ~50% in 30 days, we only tested with bran

13. Photos of mealworms (optional)

See attached video of the worms (most are with bran on top of a Styrofoam food tray) and photo of our setup (The works are in the top gray bin on the left side. We adapted a kitty litter box (see: https://www.amazon.com/Lift-Sift-Litter-Tray-System/dp/B01MT0NX45/ref=sr_1_77?s=pet-supplies&ie=UTF8&qid=1493404022&sr=1-77) for this purpose which allowed us to keep the worms in a dark place and we put a mesh on the bottom of the kitty litter sifter grid so only the frass would drop into the open tray right below it.)

14. Other description (Survival rates, plastic reduction) (optional)

The main challenge we had was with respect to the transition of the mealworms to bugs. In the end we had very few worms and a lot of bugs and we could not get the cycle going where they would hatch new worms. Our set up worked well though as we were able to separate the frass and use it as compost.

15. Permission to cite your test results: yes or no Yes

16. Permission to use your photos for scientific conferences and academic publication if you attach photos:

Yes

Signature if permission yes:

un (··

Date: 4/28/17