

1 **Winter fruit contribution to the performance of the invasive fruit fly *Drosophila suzukii***
2 **under different thermal regimes**

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14 **Running title:** *D. suzukii* performance in winter fruit

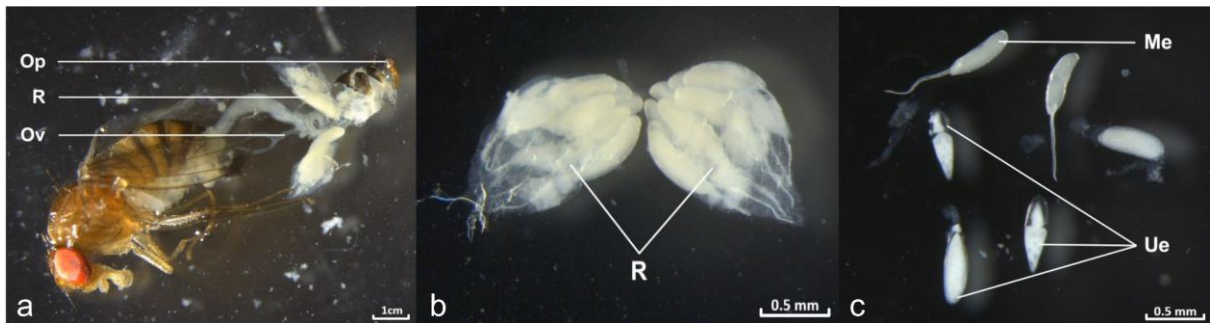
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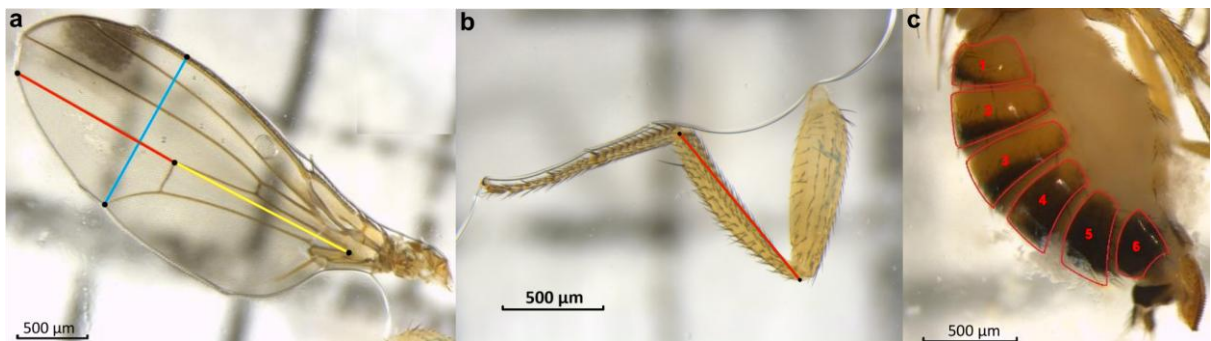
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28 **Supplementary Materials**

29 **Figure S1** Ovarian load of female *D. sukuzii*: (a) Dissected female with visible ripe ovaries; (b)
 30 ripe ovaries; (c) eggs. Legend: ripe ovaries (R), unripe (Ue) and mature eggs (Me), oviduct (Ov)
 31 and ovipositor (Op).
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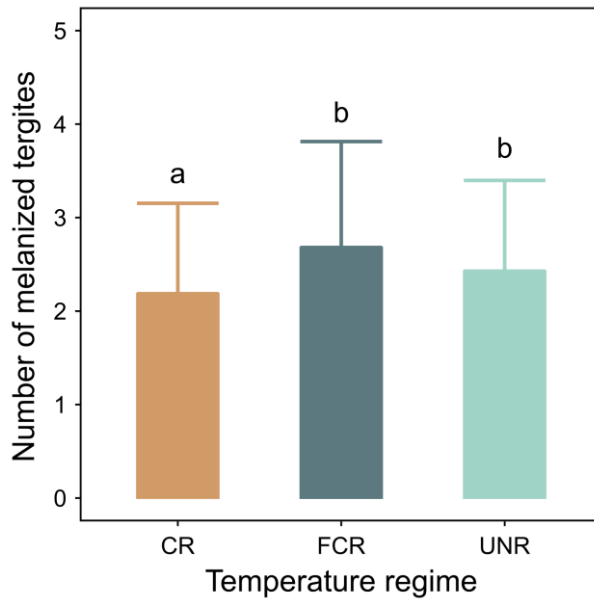
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34 **Figure S2** (a) Wing size measurement (red: wing length 1, yellow: wing length 2, blue: wing
 35 width); wing area index = (wing length 1 + wing length 2) × wing width (after Ulmer *et al.*,
 36 2024), (b) tibia size measurement and (c) count of the melanized abdominal tergites in *D.*
 37 *sukuzii*.
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42 **Figure S3** Number of melanized tergites (mean \pm sd) of *D. sukukii* flies that emerged under the
43 different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C
44 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023). Different
45 letters indicate significant differences at $p < 0.05$ (GLM with Poisson error distributions).

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47 **Table S1** Period of fruit collection.

Collection date	Fruits
25.01.2023- 27.01.2023	<i>Aucuba + Viscum</i>
01.02.2023- 03.02.2023	<i>Aucuba + Viscum</i>
08.02.2023- 10.02.2023	<i>Aucuba + Viscum</i>
15.02.2023- 17.02.2023	<i>Aucuba + Viscum</i>
24.03.2023	<i>Aucuba + Viscum + Elaeagnus</i>
29.03.2023- 31.03.2023	<i>Aucuba + Viscum + Elaeagnus</i>
05.04.2023- 07.04.2023	<i>Aucuba + Viscum + Elaeagnus</i>
12.04.2023	<i>Elaeagnus</i>
19.04.2023	<i>Elaeagnus</i>

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50 **Table S2** Total number of individuals available for each experiment (CR: 20°C; FCR:
 51 fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between
 52 24 March and 31 June 2023).

Temperature regime	Diet	Longevity	Starvation resistance	Ovarian load	Energy content	Morphometric measurement
CR	Artificial	49♂/40♀	64♂/58♀	30♀	20♀	65♂/94♀
	<i>Aucuba</i>	65♂/42♀	59♂/35♀	30♀	20♀	69♂/90♀
	<i>Elaeagnus</i>	65♂/38♀	44♂/28♀	33♀	20♀	57♂/80♀
	<i>Viscum</i>	126♂/60♀	149♂/106♀	32♀	20♀	62♂/92♀
FCR	Artificial	40♂/48♀	46♂/35♀	30♀	20♀	60♂/88♀
	<i>Aucuba</i>	37♂/26♀	33♂/23♀	20♀	20♀	58♂/66♀
	<i>Elaeagnus</i>	28♂/15♀	31♂/16♀	14♀	20♀	50♂/39♀
	<i>Viscum</i>	73♂/61♀	61♂/46♀	30♀	20♀	60♂/90♀
UNR	Artificial	35♂/35♀	33♂/34♀	33♀	20♀	60♂/90♀
	<i>Aucuba</i>	66♂/60♀	32♂/31♀	31♀	20♀	61♂/91♀
	<i>Elaeagnus</i>	27♂/30♀	0♂/0♀	0♀	16♀	22♂/23♀
	<i>Viscum</i>	41♂/56♀	40♂/36♀	32♀	20♀	57♂/80♀

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55 **Table S3** Differences in nutritional composition (proteins, glucose, P:C ratio) between fruits.

Proteins (square-root -transformed)					
Effect	Df	Sum of Squares	Mean Square	F	$P > \square^2 $
Fruit	2	15.64	7.822	80.95	$< 2e-16$
Residuals	187	18.07	0.097		
Glucose (log-transformed)					
Effect	Df	Sum of Squares	Mean Square	F	$P > \square^2 $
Fruit	2	156.22	78.11	600.6	$< 2e-16$
Residuals	186	24.19	0.13		
P:C (square-root-transformed)					
Effect	Df	Sum of Squares	Mean Square	F	$P > \square^2 $
Fruit	2	6.944	3.472	65.74	$< 2e-16$
Residuals	186	9.823	0.053		

Data were analyzed using ANOVA on square-root-transformed proteins and P:C ratio, and log-transformed glucose. Df: degrees of freedom; $P > |\square^2|$: p -value.

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57 **Table S4** Influence of diet, temperature and their interaction on *D. suzukii* emergence rate.

Emergence rate			
Effect	Df	LR Chisq	$P > \square^2 $
Diet	3	2974.64	$< 2.2e-16$
Temperature	2	457.25	$< 2.2e-16$
Diet \times Temperature	6	85.29	$2.9e-16$

Data were analyzed using generalized linear models (GLM) on binomial data with logit link function. Df: degrees of freedom; LR Chisq: likelihood ratio Chi-square; $P > |\square^2|$: p -value.

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59 **Table S5** Influence of diet, temperature and their interaction on *D. suzukii* sex ratio (M:F).

Sex ratio			
Temperature regime	Df	LR Chisq	$P > \chi^2 $
Diet	3	5.0542	0.1679
Temperature	2	14.3665	0.0008
Diet × Temperature	6	6.0839	0.4139

Data were analyzed using generalized linear models (GLM) on binomial data with logit link function. Df: degrees of freedom; LR Chisq: likelihood ratio Chi-square; $P > |\chi^2|$: p -value.

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61 **Table S6** Sex ratio (M:F) of emerging *D. suzukii* under different combinations of temperature
 62 regime (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled
 63 outdoor regime between 24 March and 31 June 2023) and diet. The sex ratio was calculated in
 64 each experimental tube to obtain the mean sex ratio ± sd.

Temperature regime	Diet	Ntot	Females	Males	Mean sex ratio ± sd (M:F)
CR	Artificial	393	200	193	0.49 ± 0.25
	<i>Aucuba</i>	292	151	141	0.50 ± 0.37
	<i>Elaeagnus</i>	321	164	157	0.48 ± 0.32
	<i>Viscum</i>	556	258	298	0.53 ± 0.23
FCR	Artificial	303	182	121	0.39 ± 0.28
	<i>Aucuba</i>	168	95	73	0.44 ± 0.42
	<i>Elaeagnus</i>	147	76	71	0.52 ± 0.35
	<i>Viscum</i>	543	303	240	0.45 ± 0.29
UNR	Artificial	334	176	158	0.49 ± 0.29
	<i>Aucuba</i>	410	220	190	0.48 ± 0.38
	<i>Elaeagnus</i>	77	48	29	0.38 ± 0.39
	<i>Viscum</i>	643	322	321	0.50 ± 0.30

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66 **Table S7** Influence of diet, temperature and their interaction on *D. suzukii* morphometric
 67 measurements (wing area index and tibia size), mass, energy content (J/mg of fly biomass) and
 68 development time.

Wing area index					
Effect	Df	Sum of Squares	Mean Square	F	<i>P</i> > F
Diet	3	90.06	30.021	223.548	< 2.2e-16
Temperature	2	52.78	26.391	196.522	< 2.2e-16
Diet × Temperature	6	4.58	0.764	5.689	8.09e-06
Residuals	910	132.19	0.134		
Tibia size					
Effect	Df	Sum of Squares	Mean Square	F	<i>P</i> > F
Diet	3	2.534	0.8446	205.037	< 2.2e-16
Temperature	2	0.406	0.2032	49.336	< 2.2e-16
Diet × Temperature	6	0.129	0.0215	5.228	2.64e-05
Residuals	910	3.749	0.0041		
Mass					
Effect	Df	Sum of Squares	Mean Square	F	<i>P</i> > F
Diet	3	31.279	10.426	131.987	< 2.2e-16
Temperature	2	0.523	0.262	3.312	0.0382
Diet × Temperature	6	2.111	0.352	4.455	0.0003
Residuals	224	17.695	0.079		
Energy content (log-transformed)					
Effect	Df	Sum of Squares	Mean Square	F	<i>P</i> > F
Diet	3	2.853	0.9511	11.357	6.02e-07
Temperature	2	1.575	0.7877	9.406	0.0001
Diet × Temperature	6	1.884	0.3140	3.749	0.0014
Residuals	216	18.089	0.0837		
Development time (log-transformed)					
Effect	Df	Sum of Squares	Mean Square	F	<i>P</i> > F

Diet	3	83.9	28.0	3634.5	< 2.2e-16
Temperature	2	854.9	427.5	55585.4	< 2.2e-16
Diet × Temperature	6	8.7	1.4	187.7	< 2.2e-16
Residuals	3822	29.4	0.0		

Data were analyzed using ANOVA; if a transformation was required to achieve normality, the transformation is indicated in parentheses. Df: degrees of freedom; $P > F$: p -value.

70 **Table S8** Morphometric measurement (mean \pm sd) of flies emerging from artificial diet, *Aucuba japonica*, *Elaeagnus* \times *submacrophylla* or *Viscum*
 71 *album* fruits under different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor
 72 regime between 24 March and 31 June 2023). Differences were analyzed using ANOVA followed by Tukey HSD's tests. Different letters indicate
 73 significant differences between diet \times temperature at $p < 0.05$.

	Temperature	Artificial		<i>Aucuba</i>		<i>Elaeagnus</i>		<i>Viscum</i>		Test	<i>p</i> -value
Mass (mg)	CR	1.91 \pm 0.23	a	0.73 \pm 0.21	d	1.22 \pm 0.32	bc	1.32 \pm 0.28	b	Anova	0.0003
	FCR	1.81 \pm 0.14	a	0.96 \pm 0.46	cd	1.19 \pm 0.38	bc	1.20 \pm 0.22	bc		
	UNR	2.04 \pm 0.22	a	1.14 \pm 0.21	bc	1.08 \pm 0.32	bc	1.28 \pm 0.24	b		
Tibia size (mm)	CR	0.83 \pm 0.05	bc	0.67 \pm 0.07	g	0.78 \pm 0.08	de	0.80 \pm 0.06	d	Anova	2.64e-05
	FCR	0.86 \pm 0.05	ab	0.73 \pm 0.07	f	0.80 \pm 0.08	cd	0.81 \pm 0.06	cd		
	UNR	0.88 \pm 0.04	a	0.76 \pm 0.07	ef	0.82 \pm 0.07	cd	0.82 \pm 0.07	cd		
Wing area index (mm ²)	CR	3.18 \pm 0.29	bc	2.18 \pm 0.43	f	2.74 \pm 0.41	e	2.90 \pm 0.29	de	Anova	8.09e-06
	FCR	3.63 \pm 0.29	a	3.03 \pm 0.47	cd	3.32 \pm 0.43	b	3.33 \pm 0.36	b		
	UNR	3.52 \pm 0.20	a	2.74 \pm 0.43	e	3.14 \pm 0.38	bcd	3.17 \pm 0.41	bc		

74 **Table S9** Influence of temperature regime on the number of melanized tergites of *D. sukuzii*.

Number of melanized tergites				
Effect	Df	Sum of Squares	F	<i>P</i> > F
Temperature	2	22.86	26.452	5.47e-12
Residuals	1310	566.07		

Data were analyzed using general linear models with Poisson error distributions. Df: degrees of freedom; *P* > F: *p*-value.

75 **Table S10** Energy content (mean \pm sd) of flies emerging from artificial diet, *Aucuba japonica*, *Elaeagnus* \times *submacrophylla* or *Viscum album* fruits under
 76 different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and
 77 31 June 2023). N = 20, except for Artificial and *Aucuba* in FCR (n = 18 and n = 17, respectively), Artificial, *Aucuba* and *Viscum* in UNR (n = 19), and
 78 *Elaeagnus* in UNR (n = 16). The reduced sample size is due to removal of aberrant values. Differences were analyzed using ANOVA on log-transformed
 79 (total energy content, fructose, glycogen, proteins) or square-root-transformed values (glucose, total carbohydrates, lipids) followed by Tukey HSD's tests.
 80 Different letters indicate significant differences between diet \times temperature at $p < 0.05$.

	Temperature	Artificial		<i>Aucuba</i>		<i>Elaeagnus</i>		<i>Viscum</i>		Test	<i>p</i> -value
Total energy content (J/mg fly biomass)	CR	4.50 \pm 1.21	abcd	4.13 \pm 1.24	cd	4.22 \pm 1.52	cd	4.37 \pm 1.19	bcd	Anova	0.0014
	FCR	5.96 \pm 1.55	a	4.70 \pm 1.60	abc	4.43 \pm 0.99	abcd	3.41 \pm 1.03	d		
	UNR	5.82 \pm 0.94	a	6.03 \pm 1.96	ab	4.85 \pm 1.34	abc	4.39 \pm 1.57	cd		
Glucose (mg/mg fly biomass)	CR	10.23 \pm 4.43	b	12.45 \pm 5.21	ab	9.05 \pm 3.27	b	8.47 \pm 3.95	b	Anova	0.0100
	FCR	9.12 \pm 1.42	b	12.52 \pm 9.57	ab	7.01 \pm 4.01	b	7.13 \pm 3.57	b		
	UNR	20.73 \pm 11.32	a	11.26 \pm 5.17	b	11.12 \pm 8.14	b	9.04 \pm 5.32	b		
Fructose (mg/mg fly biomass)	CR	1.10 \pm 2.12	bcd	1.78 \pm 2.24	abc	0.91 \pm 0.92	bcd	0.76 \pm 1.12	bcd	Anova	0.1434
	FCR	0.69 \pm 1.05	bcd	0.39 \pm 0.39	cd	0.44 \pm 0.85	cd	0.19 \pm 0.25	d		
	UNR	3.55 \pm 3.52	a	2.11 \pm 2.45	ab	1.83 \pm 2.14	abc	0.92 \pm 0.91	bcd		
Total carbohydrates (mg/mg fly biomass)	CR	11.33 \pm 6.41	bc	14.23 \pm 6.60	b	9.96 \pm 3.63	bc	9.23 \pm 4.21	bc	Anova	0.0077
	FCR	9.81 \pm 1.87	bc	12.92 \pm 9.85	bc	7.46 \pm 4.11	c	7.33 \pm 3.47	c		
	UNR	24.28 \pm 13.70	a	13.37 \pm 6.53	bc	12.95 \pm 9.32	bc	9.96 \pm 5.38	bc		

Glycogene (mg/mg fly biomass)	CR	8.09 ± 4.52	bc	14.85 ± 6.20	a	10.00 ± 4.35	abc	8.15 ± 2.45	bc	Anova	0.0293
	FCR	6.07 ± 2.36	c	11.79 ± 9.57	abc	9.16 ± 9.59	c	5.97 ± 1.24	c		
	UNR	15.73 ± 8.27	a	17.15 ± 16.08	ab	17.99 ± 13.37	a	8.64 ± 2.67	abc		
Proteins (mg/mg fly biomass)	CR	89.84 ± 26.35	ab	64.85 ± 27.91	b	101.37 ± 73.83	a	99.29 ± 51.43	a	Anova	0.0574
	FCR	88.58 ± 20.09	ab	72.67 ± 45.41	ab	80.35 ± 14.78	ab	66.62 ± 24.97	ab		
	UNR	84.15 ± 15.87	ab	75.38 ± 17.13	ab	84.63 ± 56.07	ab	84.32 ± 41.83	ab		
Lipids (mg/mg fly biomass)	CR	76.43 ± 27.28	c	76.01 ± 27.97	c	64.91 ± 22.45	c	70.04 ± 22.96	c	Anova	0.0047
	FCR	116.37 ± 37.90	a	88.54 ± 32.29	abc	80.23 ± 23.04	bc	59.26 ± 23.14	c		
	UNR	108.42 ± 25.33	ab	122.47 ± 47.84	a	87.26 ± 38.55	abc	76.66 ± 31.61	bc		

81 **Table S11** Influence of diet, temperature and their interaction on *D. sukuzii* female ovarian load.

Female ovarian load				
Effect	Df	Sum of Square	F	<i>P</i> > F
Diet	3	2449.15	110.16	< 2.2e-16
Temperature	2	220.11	14.85	7.03e-07
Diet × Temperature	5	159.77	4.31	0.0008
Residuals	304	2252.98		

Data were analyzed using general linear models with quasi-Poisson error distributions. Df: degrees of freedom; *P* > F: *p*-value.

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83 **Table S12** Influence of different diets under each temperature regime (CR: 20°C; FCR: fluctuating
84 controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31
85 June 2023) on *D. sukuzii* starvation resistance and longevity ('Survival with food for 15 days').

Starvation resistance	
Temperature regime	<i>p</i> -value
CR	2.37e-19
FCR	6.24e-12
UNR	2.13e-04
Longevity	
Temperature regime	<i>p</i> -value
CR	2.40e-05
FCR	0.17
UNR	2.49e-03

Data were analyzed using log-rank tests.

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88 **Table S13** Differences in oviposition choice between fruit types under each temperature regime (CR:
 89 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime
 90 between 24 March and 31 June 2023).

Oviposition choice				
Temperature regime	n	Df	Chisq	$P > \chi^2 $
CR	3	2	76.0	3.08e-17
FCR	3	2	51.9	5.49e-12
UNR	3	2	59.7	1.07e-13

Data were analyzed using Chi-squared tests with an expected proportion of 1/3 without any choice. Df: degrees of freedom; Chisq: chi-square; $P > |\chi^2|$: *p*-value.

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93 *Nutritional composition of collected fruit*

94 For each artificial infestation experiment, a randomly selected batch of 10 fruit was isolated
95 and stored at -80°C for further nutritional analysis of proteins and carbohydrates. The frozen fruit
96 were sliced on an ice-cold crystallizer. The material was weighed and then homogenized in tubes with
97 ice-cold PBS (Sigma P4417) and four tungsten beads. The tubes were placed in a bead-beater for 2
98 min at 25 Hz. Samples were then centrifuged for 5 min at $5000 \times g$ at 4°C . The clear supernatant was
99 removed and stored at -80°C before glucose and protein quantification. For glucose quantification,
100 samples were first deactivated by heating the tubes for 10 min at 70°C . Then, we used glucose
101 oxidase/peroxidase reagent (Sigma G3660) and o-dianisidine reagent (Sigma D2679). The procedure
102 involved the oxidation of glucose to gluconic acid and hydrogen peroxide by glucose oxidase. The
103 hydrogen peroxide then reacted with o-dianisidine in the presence of peroxidase to form a colored by-
104 product. The oxidized o-dianisidine then reacted with sulfuric acid to form a stable-colored product
105 whose intensity (pink coloration) was measured at 540 nm using a microplate reader (SAFAS Flx-
106 Xenius). A standard curve was generated using a glucose standard solution (Sigma G3285). For
107 protein quantification, we used the Bradford assay, a colorimetric protein assay based on an
108 absorbance shift of the dye Coomassie Brilliant Blue G-250 proportional to protein concentration. The
109 blue coloration was measured at 595 nm using a microplate reader (SAFAS Flx-Xenius). A standard
110 curve was prepared using a stock solution of BSA (bovine serum albumin).

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112 Analysis of *D. sukuzii* energy content

113 Individual females from each treatment combination were first solubilized in a phosphate-
114 lysis buffer. Then, total proteins were assayed using Bradford's reagent (B6916, Sigma-Aldrich) with
115 BSA as a standard. In a second step, a methanol:chloroform mixture and a sodium sulfate solution
116 were added to the homogenate to reach the proportions 2:5:10 v/v/v of water, chloroform and
117 methanol, respectively. After vortexing and centrifugation, glycogen was quantified from the pellet
118 while free-carbohydrates and total lipids were assessed from the supernatant. Carbohydrates (free
119 sugars and glycogen) were quantified using Anthrone reagent (319899, Sigma-Aldrich) with D-
120 glucose as a standard, and total lipid quantification was performed using Vanillin (V1104, Sigma-
121 Aldrich), with triolein as a standard. Optical density measurements were performed using a
122 spectrophotometer (NanoQuant, TECAN).

123