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## How variation in temperature and diet affect adult lifespan of the yellow mealworm beetle, *Tenebrio molitor* L (Coleoptera: Tenebrionidae)

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### Abstract

As Earth warms and human populations soar, the yellow mealworm beetle, *Tenebrio molitor*, may prove an invaluable alternative to mainstream food sources. The current study evaluates feasibility of maintaining *T. molitor* adults on a simplified, reduced water-footprint diet and at two ambient temperatures. Pupae were isolated (i.e., one pupa/vial) and assigned to one of four temperature and diet treatments. Adult lifespan generally declined at the higher temperature (30 °C) and with simplified diet. Unlike higher temperature, however, simplified diet did not consistently reduce lifespan, as female longevity on standard oats-and-carrot diet did not differ from that of females limited to only oats at 30 °C. While others have noted importance of temperature and (separately) diet, this study is the first to quantify relative effects of two major environmental variables—ambient temperature together with diet—affecting mealworm cultivation. The current findings are discussed in light of large-scale insect farming and global climate change.

**Keywords:** sustainability, longevity, desiccation resistance, water footprint

### Introduction

Entomophagy, or insect-eating, is longstanding practice in human societies. Since Vincent Holt's <sup>[1]</sup> reference to insect-eating in classical Greek and biblical times, Bodenheimer <sup>[2]</sup> documents reliance by past civilizations on “locust, cicada, and larvae of the cossus beetle (*Cerambyx cerdo*)” and of widespread insectivory in contemporary Australia and Asia (p. 50) <sup>[3]</sup>. DeFoliart <sup>[4]</sup> cites the Mideast origins of European agriculture to explain Western aversion to insects as food. In fact, while many around the world still subsist on insects, human consumption of beef, pork, chicken, and fish trends upward <sup>[5]</sup>.

Human population growth necessitates more intensive food production <sup>[6]</sup>. Fernández-Armesto <sup>[7]</sup> posits that snail cultivation presaged selective breeding of ruminants, suggesting that after humans learned to drive migratory game into enclosures hunting came to reliably supplement traditional gathering activities. Ritualized feasts from successful hunts then elevated social status of hunters over those gathering less adventurous game, e.g., snails and insects. With increased tribal population numbers, taming and breeding ruminants proved more economical than hunting increasingly scarce game <sup>[8, 9]</sup>. Fernández-Armesto <sup>[7]</sup> further notes that contemporary aquaculture practices e.g., <http://www.fao.org/fishery/culturedspecies/search/en> continue to evolve likewise away from hunting to systematic management to farming—that is, from tracking migratory wildlife to containment and selective breeding of food commodities, e.g., salmon (<http://www.marineharvest.ca>) and tuna (<http://www.cleanseas.com.au/>).

By land or sea, intensive food production greatly impacts freshwater resources. Of all the Earth's water, only 0.1% is freshwater <sup>[10]</sup>. Consumptive use of freshwater—termed “virtual water” or “water footprint”—in agricultural production reached a staggering 92% in 2010 <sup>[11, 12]</sup>. In terms of measurable volume, cattle and swine require about 40 m<sup>3</sup> H<sub>2</sub>O per kg of edible protein, and broilers half as much <sup>[13]</sup>. Current aquaculture practices improve on the relatively low water footprint of meat birds <sup>[14]</sup>, but vary widely: For example, Indonesian trout-tilapia polyculture consumed 0.877 – 1.121 m<sup>3</sup> H<sub>2</sub>O per kg of marketable product <sup>[15]</sup>, while Armenian trout farms ranged 42 – 1,262 m<sup>3</sup> H<sub>2</sub>O per kg product <sup>[16]</sup>.

The yellow mealworm beetle, *Tenebrio molitor*, seems ideal for studying how best to minimize water footprint in contemporary food production. Though its exact origin remains unknown, as a stored grains pest, *T. molitor* probably has as global a geographic range as

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humans [17, 18]. With its low space requirements and generalist diet [19, 20], *T. molitor* displays superior feed-to-protein conversion, i.e., amount of edible protein produced per amount of livestock feed consumed [5]. Often grown commercially as “pet feed,” *T. molitor* appears capable of reproducing well at industrial scale [21]. Thus, any water-saving practices during small-scale cultivation of *T. molitor* can translate to significantly lower water footprints at larger scale.

The current study explores the feasibility of cultivating yellow mealworm beetle, *T. molitor*, on a simplified dietary regimen. It is hypothesized that *T. molitor* could survive long enough for later breeding purposes when switched as adults to a reduced water footprint, oats-only diet since these animals presumably are adapted to the generally xeric conditions of stored grain environments [20]. Moreover, while Rho and Lee [19], Ribeiro *et al.* [20], Cortes Ortiz *et al.* [21], and others document the physical and physiological effects of temperature and (separately) dietary variation on *T. molitor*, no study has yet quantified the extent to which ambient temperature together with dietary factors can affect farming of this insect.

## Materials & Methods

Stocks originated from ~200 adults (unknown sex ratio) acquired locally (W.V. Hix, Ila, Georgia, U.S.A.). These ‘wildtype’ founders were housed in a 32-cm × 18-cm × 22-cm plastic bin (Tom Aquarium & Reptile Products) containing a shallow (~5 mm) layer of oats (*Avena sativa*, Bob’s Red Mill Extra-Thick Rolled Oats Whole Grain, Milwaukee, Oregon, U.S.A.) as a food base, carrot sticks for water and supplementary vitamins, and high-protein feline kibble (Nestlé Purina, St. Louis, Missouri, U.S.A.) at 24 °C, 12-h daily light, and >40% RH. Adults were removed four weeks after colony founding to minimize cannibalism.

Test subjects were sexed [22] and isolated as pupae into 8-dram vials (BioQuip part #8808P)-one pupa per vial. These space-saving containers held ~1 gram of whole grain rolled oat flakes and were kept loosely capped. As individual isolation prevents cannibalism, emergent adults were free to melanize fully at stock temperature and photoperiod before treatment assignment.

Four treatment groups experienced the same photoperiod (12L: 12D) but different ambient temperatures and diets. A positive-control (or “24 STANDARD”) group was maintained at standard stock temperature (24 °C) and diet, with carrot replacement on alternating days. An oats-only diet group (or “24 SIMPLIFIED”) ran concurrently with the positive controls but without carrot supplementation. A “30

STANDARD” group mirrored the positive controls but at 30 °C ambient temperature that optimizes larval growth rate [23]. The last group-“30 SIMPLIFIED” treatment-was set up like the “30 Standard” group but without carrot supplementation.

Adult survival was assessed daily. Beetles remaining completely motionless after gentle manipulation with forceps and through two consecutive days were considered deceased. Beetles surviving through the last recorded observation were refrigerated (4 °C) overnight and then, as with earlier deceased adults, suspended in 90% isopropanol.

As in prior studies [24, 25], raw observations of adult lifespan were transformed to satisfy normality assumptions of parametric analyses. Per van der Waerden normal scores testing [26], variates were ranked without ties before transformation to standard-normal scale ( $N, \mu=0, \sigma=1$ ). Thus, the present factorial design facilitated application of the following ANOVA model:

$$Y_{ijkn} = \mu_{...} + TEMP_i + DIET_j + SEX_k + (TEMP \times DIET)_{ij} + (TEMP \times SEX)_{ik} + (DIET \times SEX)_{jk} + (TEMP \times DIET \times SEX)_{ijk} + \varepsilon_{n(ijk)}$$

Where  $Y_{ijkn}$  is normalized-rank adult lifespan,  $\mu_{...}$  and  $\varepsilon_{n(ijk)}$  are population constants,  $TEMP_i$  is fixed-effect ambient temperature (24 °C versus 30 °C),  $DIET_j$  is fixed-effect diet (standard versus simplified), and  $SEX_k$  is random-effect sex of *T. molitor* individual. Any interaction term involving  $SEX_k$  was considered random.

Statistical description and hypothesis-testing were accomplished with a few software programs. ANOVA results were generated via *proc glm* in SAS 9.4 (SAS Institute Inc., Cary, North Carolina, U.S.A.), with final P values obtained online [27], effect size estimates from Eq. 13 in Lakens [28], and *post-hoc* statistical power ( $1-\beta$ ) from G\*Power [29]. The *proc varcomp* (featuring the ReML option) algorithm in SAS was then used to estimate relative contribution of each ANOVA factor to total variation. Finally, all pairwise comparisons of treatment means were performed via Student t-test [30], followed by *post hoc* Bonferroni adjustment of P values to maintain  $\leq 5\%$  experiment-wide Type I error rate.

## Results

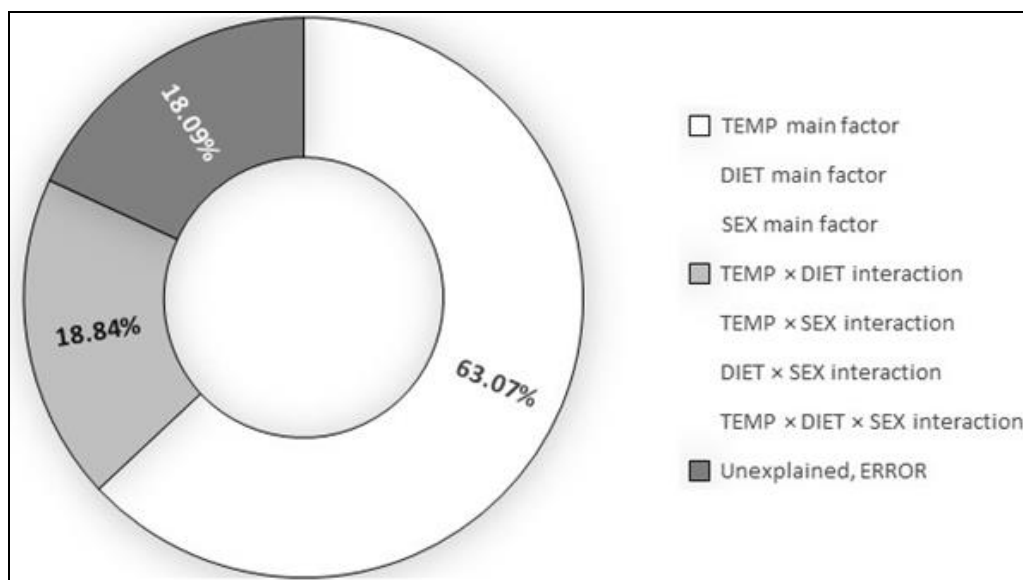
Ambient temperature greatly affected lifespan of virgin *T. molitor* adults. Of the three main ANOVA factors, only temperature contributed significantly (Table 1,  $F_{1,1}=1545.109$ ,  $P<0.5$ , effect size  $\eta_p^2>0.999$ , statistical power  $1-\beta>0.99$ ), explaining greater than 63% of total variance in virgin adult lifespan (Fig. 1).

**Table 1:** Three-way analysis of variance of virgin adult lifespan in the beetle, *Tenebrio molitor*. Please see Materials & Methods for description of variance factors.

Source of Variation	df	Type III MS	Observed F	P	$\eta_p^2$	1- $\beta$
Ambient temperature, TEMP	1	25.088	1545.109	0.016	0.999	>0.99
Diet (standard v. simplified), DIET	1	2.517	4.506	0.280	0.818	>0.99
Sex of beetle, SEX	1	0.015	0.592	0.583	0.372	0.68
TEMP×DIET interaction	1	4.758	182.185	0.047	0.995	>0.99
TEMP×SEX interaction	1	0.016	0.055	0.808	0.002	0.05
DIET×SEX interaction	1	0.558	1.906	0.175	0.049	0.06
TEMP×DIET×SEX interaction	1	0.026	0.089	0.766	0.002	0.05
Unexplained, ERROR	37	0.293				

Of the four ANOVA interactions, only that between temperature and diet appeared statistically significant (Table 1,  $F_{1,1}=182.185$ ,  $P<0.05$ , effect size  $\eta_p^2=0.995$ , statistical

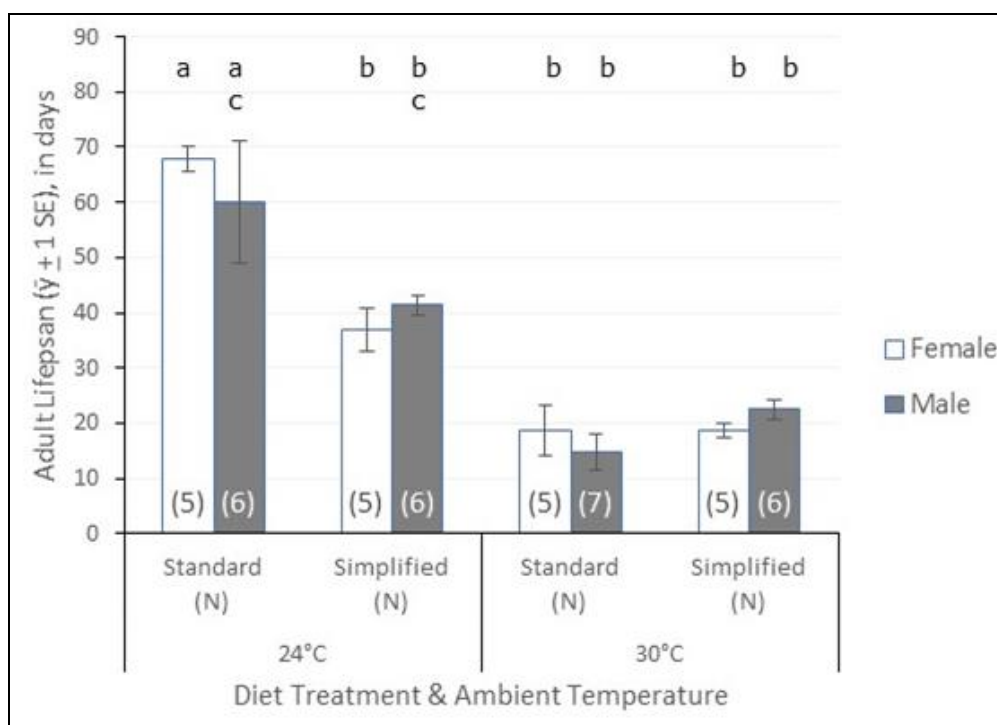
power  $1-\beta>0.99$ ), and was the second largest variance source (18.84%; Fig. 1).



**Fig 1:** Variance-components analysis on virgin adult lifespan in the beetle, *Tenebrio molitor*. Please see Materials & Methods for description of variance factors.

In general, the decline in adult lifespan from 24 °C to 30 °C appeared steeper for beetles maintained on the standard *versus* simplified diet (Fig. 2). And while tending to be numerically

lower on the simplified diet at 24 °C, there appeared to be no difference in adult lifespan at the hotter (30 °C) ambient temperature.



**Fig 2:** Environmentally mediated adult lifespan of virgin *Tenebrio molitor*. Shared lower-case letter above each column indicates no difference at 5% experiment-wide Type I error rate after Bonferroni adjustment for *post hoc* multiple comparisons.

**Discussion**

The current results appear to support the view that current standards for sustaining *T. molitor* colonies could be simplified to reduce maintenance costs. Given the range of ambient temperatures that *T. molitor* larvae can grow [23], it may be preferable to keep *T. molitor* colonies at lower, less energetically expensive temperatures in order to optimize adult lifespan-on a simplified (i.e., oats only) diet, *T. molitor* survived 2-3 weeks post-eclosion at 30 °C, and approximately twice as long at 24 °C, though difference in adult lifespans between 24 °C *versus* 30 °C oats-only groups was not

statistically significant (Fig. 2). In all, ambient temperature alone contributed the most to adult lifespan variation-here, greater than 63%-with diet moderating its effect as the only other significant variance contributor (18.84%).

The current results also allow the first estimation of water footprint reduction when *T. molitor* adults are switched from a standard oats-and-carrot diet to a simplified, oats-only diet. At 24 °C, *T. molitor* adult life span without weekly carrot supplement was roughly 38 d (~5.43 weeks) compared to about 68 d (~9.71 weeks) on the standard oats-and-carrot diet (Fig. 2). About 0.80 g of carrot per week per adult beetle

(female or male) in the standard diet treatment was used, which translates to (5.43 weeks  $\times$  0.80 g carrots/week  $\times$  2 beetles/carrot)  $\sim$ 8.688 g, or 0.00869 kg, of carrots saved. Parajuli *et al.* [31] estimate that 4.4 L tap water is used just to clean 1 kg of harvested carrot: Assuming the same amount of water for growing, packaging, and transporting carrots, the water savings in the current operation would be (0.00869 kg  $\times$  4.4 L water/kg  $\times$  4)  $\sim$ 0.153 L H<sub>2</sub>O savings per beetle pair. If each adult beetle pair on simplified, oats-only diet were to produce (very conservatively) 100 harvestable larvae, with each larva weighing  $\sim$ 0.15 g [20], then the amount of fresh mealworm product per beetle pair would be (100 larvae  $\times$  0.15g/larva)  $\sim$ 15 g, or 0.015 kg. Thus, the estimated reduction in water footprint for the current operation is (0.153 L  $\div$  0.015 kg)  $\sim$ 10.2 L H<sub>2</sub>O, or 0.0102 m<sup>3</sup>, per kg of mealworms produced. Ooninx and de Boer [32] provides a more comprehensive life cycle assessment that includes global warming potential, energy consumption, and land use (but not water footprint) for an industrial mealworm production operation, indicating consumption of 260,000 kg of carrots per 83,200 kg of mealworms, or 3.125 kg carrots $\cdot$ kg<sup>-1</sup> product—that is, a crop harvest (or its water equivalent) that, instead, could have been used more directly to sustain people. Of course, temperature- and diet-related reductions in adult lifespan of *T. molitor* prompt careful reflection on stock management. While surviving long enough for selective breeding, virgin *T. molitor* on the simplified diet might possibly cease reproduction altogether. As reproductive diapause seems absent in *T. molitor* [18, 20], nonetheless, tenebrionids maintained on an oats-only diet may sacrifice reproduction for somatic maintenance, a stress-related response observed in other organisms [33-36]. Comprehensive yet accessible reviews that explain specifically how environmental factors might significantly alter *T. molitor* life history traits are most welcomed.

The unexpected higher cost of maintaining *T. molitor* adults at 30 °C might also reflect the challenges farmers and food distributors could face on an increasingly warming planet. As the current results suggest, at higher ambient temperatures, higher stock mortality may outpace any gains in protein production originating from increased metabolism of ectotherms. Also, unlike other studies that focus on maximizing productivity of insect culturing at industrial scale, the present study is the first to consider the global water crisis [11] that industrialized insect farming could inadvertently exacerbate. Furthermore, since CO<sub>2</sub> and methane are major drivers of climate change [37], greater attention to keeping water consumption and greenhouse gas emission minimal in *T. molitor* farming activities, e.g., life cycle assessment of the kind performed by Ooninx and de Boer [32], is very much needed.

## Conclusion

Yellow mealworm beetle, *Tenebrio molitor*, survive a substantially long time when switched from an enriched, carrot-supplemented diet as larvae to an oats-only diet as adults. Animal breeders might also best prolong lifespan of *T. molitor* adults at 24 °C than at 31 °C. Such reductions of material and energy inputs not only save on maintenance costs for insect farming, but also help keep *T. molitor* positioned as a more sustainable high-protein alternative to vertebrate animal meat for the growing global human population.

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