


**Please cite the Published Version**

Michaels, Christopher J, Ferguson, Amanda, Newton-Youens, Jade , Harland, Robert and Hickles, Ross (2022) Effects of Sex and Whole Life Cycle UVB Irradiation on Performance and Mineral and Vitamin D3 Contents in Feeder Crickets (*Gryllus bimaculatus*). Journal of Zoological and Botanical Gardens, 3 (3). pp. 488-498.

**DOI:** <https://doi.org/10.3390/jzbg3030036>

**Publisher:** MDPI AG

**Version:** Published Version

**Downloaded from:** <https://e-space.mmu.ac.uk/630484/>

**Usage rights:**  [Creative Commons: Attribution 4.0](https://creativecommons.org/licenses/by/4.0/)

**Additional Information:** This is an open access article published in Journal of Zoological and Botanical Gardens by MDPI.

**Data Access Statement:** Data are available for download at <https://github.com/CJMichaels/Cricket-UVB>

**Enquiries:**

If you have questions about this document, contact [openresearch@mmu.ac.uk](mailto:openresearch@mmu.ac.uk). Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from <https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines>)

Article

# Effects of Sex and Whole Life Cycle UVB Irradiation on Performance and Mineral and Vitamin D<sub>3</sub> Contents in Feeder Crickets (*Gryllus bimaculatus*)

Christopher J. Michaels <sup>1,\*</sup>, Amanda Ferguson <sup>1</sup>, Jade Newton-Youens <sup>2</sup>, Robert Harland <sup>1</sup> and Ross Hickles <sup>1</sup>

<sup>1</sup> Zoological Society of London, London NW1 4RY, UK

<sup>2</sup> Department of Natural Sciences, Manchester Metropolitan University, Manchester M1 5GD, UK

\* Correspondence: christopher.michaels@zsl.org

**Abstract:** Captive insectivore nutrition is challenging due to the differing nutritional profiles of wild and captive diets and an incomplete understanding of both. Ultraviolet B (UVB)-irradiation has recently been explored as a means of improving prey-insect vitamin D<sub>3</sub> and Ca content. Although short-term irradiation has been successful in some species, it has been unsuccessful in black field crickets (*Gryllus bimaculatus*)—a commonly cultured feeder insect. We exposed crickets to UVB irradiation from hatchling to adult stages and measured the vitamin D<sub>3</sub> and mineral contents of crickets by sex. We did not detect vitamin D<sub>3</sub> (detection limit 0.5 iU/g) or an effect of UVB irradiation on mineral content under either UV+ or UV− conditions. We identified large differences between sexes in Ca, K, Mg and P (females higher) and Cu, Fe, S and Zn (males higher), likely linked to reproductive investment. The differences do not straddle the minimum recommended concentrations of minerals for vertebrate growth and thus may be most relevant to animal nutrition in contexts of particular sensitivity or need. We demonstrate a UV-linked trade-off in cricket performance between individual cricket size and the numbers of crickets produced and characterise the energy costs associated with UVB provision. Our results do not support the use of UVB lighting for *G. bimaculatus* to improve nutrition but demonstrate previously unreported differences in the nutritional profiles between sexes in this species.

**Keywords:** livefood; nutrition; insectivore; calcium



**Citation:** Michaels, C.J.; Ferguson, A.; Newton-Youens, J.; Harland, R.; Hickles, R. Effects of Sex and Whole Life Cycle UVB Irradiation on Performance and Mineral and Vitamin D<sub>3</sub> Contents in Feeder Crickets (*Gryllus bimaculatus*). *J. Zool. Bot. Gard.* **2022**, *3*, 488–498. <https://doi.org/10.3390/jzbg3030036>

Academic Editor: Steven Monfort

Received: 11 August 2022

Accepted: 14 September 2022

Published: 19 September 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Captive insectivores are prone to numerous nutritional deficiencies, especially of vitamins and minerals, e.g., [1–7]. These may cause chronic or acute, mild or severe pathologies that affect welfare, e.g., [8], and can compromise conservation initiatives [9]. In all cases, this is at least partly driven by the narrow diversity of invertebrates bred commercially as viable staple diets for insectivores and the inherent associated nutritional imbalances [10]. Such commercially raised insects are especially deficient in minerals such as calcium, vitamins including A, B<sub>1</sub>, D and E and the essential fatty acid alpha-linolenic acid (18:3 (*n*-3)), while tending to contain excess bioavailable phosphorus, in comparison with both wild invertebrates and the nutritional requirements of vertebrates [7]. Nutritional profiles now exist for a large proportion of available feeder invertebrate species [7], which offer a starting point for improving the nutritional composition of captive insectivores by dietary manipulation, but more data are required to underpin exactly how diets are improved. Required data include more information on demographic nutritional variation within species [11] and how environmental conditions may be manipulated within invertebrate cultures to adjust nutritional content. While some nutrients, especially calcium and phosphorus, may be manipulated in feeder invertebrates according to well-researched gut-loading and dusting protocols [12], similarly established and effective protocols do not exist for others.

Moreover, additional evidence to better understand the nutritional profiles of many feeder invertebrate species in the context of biological categories such as age and sex is needed.

Vitamin D<sub>3</sub> and its derivatives are physiologically important in vertebrate animals, especially for calcium homeostasis [13] but also for other, less understood functions [14]. Animals derive vitamin D<sub>3</sub> from a combination of diet and photobiosynthesis [15], but the relative importance of these two routes varies by species and is largely dependent on the ecology of taxa [13]. Captive insectivores, especially those requiring live prey to stimulate feeding and those that are less capable of endogenous vitamin D synthesis through UVB irradiation, may develop hypovitaminosis D with acute or chronic detrimental effects, especially through the perturbation of calcium metabolism [15]. Recent work has demonstrated that the UVB irradiation of adult feeder insects of some species for around thirty days increases vitamin D<sub>3</sub> content, although the physiological mechanisms controlling the metabolism of this compound in invertebrates remains poorly understood [16]. In another work [17], the short-term irradiation (48 h)—aligned with typical timescales for gut-loading feeder invertebrates [18]—of another common feeder insect (black cricket, *Gryllus bimaculatus*) showed that this did not result in vitamin D<sub>3</sub> accumulation, at least above the threshold of detection in that study (0.5 iU/g), and thus was not suitable to meet the recommended estimated vertebrate vitamin D intake of 1111 iU/kg [8]. There was also no effect of the irradiation on the accumulation of calcium in the insects. The longer-term exposure of insects throughout their life cycle to UVB irradiation may result in the integration of synthesised vitamin D<sub>3</sub> into tissues better than short-term irradiation and therefore potentially on calcium accumulation by influencing calcium metabolism (although the underpinning physiology in insects is unknown). Conversely, UVB irradiation may have other effects on the performance of crickets and therefore on the efficiency of cricket production, as well as substantial resource costs through the procurement of equipment and energy use. In order to extend existing research in this area beyond short-term irradiation, we investigated this by exposing black crickets to UVB radiation from hatching until final instar in the context of a working large-scale live-food production facility in a zoo setting.

In addition to the impacts of UVB exposure on vitamin D<sub>3</sub> content, we designed our experiment to enable us to characterise the mineral profiles in this species of cricket and explore the differences in the mineral content between male and female crickets. Cerreta et al. [11] qualitatively showed that male and female cockroaches (*Blaberus giganteus*, *Blaptica dubia*, *Blatta lateralis* and *Gromphadorhina portentosa*) appear to differ in terms of several nutritional components, but few other studies have explored the potential for sex differences in nutritional content in feeder insects. Imagoes of male and female crickets have substantially different body shapes, sizes and gross appearances due to sex-linked adaptations including wing morphology and investment in eggs. Due to these morphologies, male or female crickets may be deliberately chosen to feed specific individual insectivores (pers. obs. CM), and there is thus potential for nutritional differences between insect sexes to influence the nutritional state of predators feeding on them.

## 2. Materials and Methods

### 2.1. Ethical Approval

Although crickets are not covered by the Animals (Scientific Procedures) Act 1986 and as such are not subject to ethical protection under law, ethical approval for all methods in this study was nonetheless gained from the Zoological Society of London Ethics Committee (23 February 2022).

### 2.2. Experimental Design

Hatchling black crickets (*Gryllus bimaculatus*) bred at ZSL London Zoo under non-irradiated conditions were housed from the hatching stage in each of four large plastic boxes (145ltr Really Useful boxes, Really Useful Products, Castleford, UK), the lids of which were replaced with mesh panels. A total of 10 g of hatchling crickets were added to each box. The boxes were filled with stacks of cardboard eggs crates. The boxes were

housed in a temperature-controlled cricket breeding facility at a temperature range (mean) of UV−: 21.6–40 (27.8) and UV+: 20.1–38 (28.06) °C. Each box was illuminated using a hydroponic lighting unit (Growth Technology, Taunton, UK) containing four HO T5 fluorescent lamps with reflectors, two of which were standard domestic lamps (24W Cool white HO T5, Philips, Amsterdam, Netherlands) and two of which were specialist UVB-emitting lamps produced for the reptile keeping industry (24W 12% D3 T5 HO lamps, Arcadia Reptile (part of Monkfield Nutrition), Ely, UK). Two of the lighting units were covered with a UVB-absorbing film known to absorb all wavelengths of UVB from fluorescent lamps but transmit other wavelengths, including UVA and Infrared (CLS200 XSR Clear Ultraviolet Filter, Westgate Group, Stafford, UK; [12]). This resulted in two boxes under a UVB+ treatment and two boxes under a UVB− treatment, for which all other lighting variables were identical. Lighting was turned on for 7 h per day. Temperature and relative humidity were both measured twice daily (morning and afternoon) using an Eidyer Digital Thermometer Hygrometer. UVB was measured at the start of the experiment and once weekly thereafter as Ultra-Violet Index (UVI), a unitless measure of UVB irradiance weighted for the biological relevance of wavelengths (see Baines et al., 2016), using a Solarmeter 6.5 (Solartech/Solar Light Company, Glenside, USA). UVI measurements were taken on the top of the stack of cardboard egg cartons used for the crickets to live on, approximately 20 cm beneath the T5 lamps, as this reflected the maximal irradiation available to crickets. Crickets could choose exposure to a UVI of 0 by sheltering underneath the egg carton. Crickets were fed daily, ad libitum, on a mixture of chopped vegetables (carrot, swede, potato, sweet potato, beetroot and parsnip), chick crumb (Dodson and Horrell, Nantwich, UK) and soaked Mazuri Zoo Diet A (Mazuri, St. Louis, MO, USA), lightly sprayed with tap water to provide moisture. The vegetables used varied arbitrarily on a daily basis, but all crickets were offered the same food on a given day. Food was weighed in and out each day, and the total quantity consumed was calculated across the entire experiment, incorporating a desiccation factor.

The crickets were maintained in these conditions for 35 days until they had moulted into their final instar (winged adults, L5).

### 2.3. Sample Processing

After 35 days, when the crickets had moulted into winged adults, the animals were euthanised by veterinary nurses by exposure to isoflurane gas, followed by freezing once all movement had ceased (following the British and Irish Association of Zoos and Aquariums guidelines; [19]). The total mass of crickets produced by each box was weighed prior to euthanasia, as was the total mass of waste, comprising frass and exuvia, left in the culture containers. Four cricket samples, each weighing 50 g  $\pm$  2 g wet weight and consisting of equal numbers of male and female crickets, were immediately collected from each box for vitamin D<sub>3</sub> analysis. Simultaneously, five samples, each consisting of two male and two female crickets, were collected for mineral analyses. These crickets were also weighed individually immediately after death.

### 2.4. Nutritional Analyses

All samples were immediately frozen at −20 °C and sent for nutritional analyses following the methods presented by Bah Nelson et al. [17]. Frozen crickets were sent to Sciantec (Stockbridge Technology Centre, Cawood, North Yorkshire YO8 3SD, UK) for vitamin D<sub>3</sub> analysis (minimum detection threshold 0.50 IU/g) via high performance liquid chromatography (HPLC), SOP Number: S1181.

Frozen cricket samples were sent to the Department of Natural Science, Manchester Metropolitan University (Manchester M1 5GD, UK) for ICP-AES mineral analysis. For mineral analysis, the frozen samples were transferred into 25 mL falcon tubes. Males and females from each sample were separated to give a total of 40 samples. The samples were then moved to a drying oven and dried for 72 h at 70 °C. Due to restrictions of the capacity of the oven, the samples were split into two groups and analysed separately. The samples

were split evenly across treatment groups and males/females. After drying, the samples were transferred to microwave digestion vessels and weighed to yield the dry weight. To each vessel, 10 mL nitric acid (>68% PrimerPlus—Trace analysis grade) was added and left for 6 h to digest. An additional four blanks were included. The vessels were then transferred to the microwave (CEM Mars Xpress 5) and run on the following cycle: 1. 5 min ramp and 5 min hold at 90 °C; 2. 10 min ramp and 10 min hold at 170 °C. The cycle was repeated to ensure complete digestion. After cooling, 5 mL of grade 1 deionised water was added. Each sample was then filtered (Whatman, 40, Ashless; Sigma, St. Louis, USA) into 100 mL volumetric flasks, which had been acid washed for 24 h prior to use. During filtering, the digestion vessels were repeatedly rinsed with grade 1 water to ensure all samples were filtered. The flasks were then made up to 100 mL and mixed. A total of 20 mL was then transferred to the falcon tubes to be used in the ICP analysis. Mineral analysis was conducted using a Thermo scientific iCAP6300 Duo. Ten metals were analysed: Mg, Ca, Na, Mn, Fe, K, Cu, Zn, Al, P and S. Three repeats were run for each sample, which were then averaged to give the final reading for each metal. The readings were then adjusted to account for any traces of target minerals in reagents by averaging the values in the blanks, which were typically very low, and subtracting from each reading. The values were then converted from mg/L to mg/kg and corrected for mass and dilution. To do so, the readings were multiplied by 1/dry mass of the sample and multiplied by the dilution factor (100).

### 2.5. Statistical Analysis

Statistical analyses were performed in the stats package in R version 4.1.1 [20] using RStudio 1.4.1717 for Windows 10. The data were analysed using ANOVAs via the aov function. Two-way nested ANOVA testing the effects of UV treatment and cricket sex on response variables, controlling for the culture box nested within the treatment, was used. The balanced design of the experiment with no missing values makes ANOVA a robust analytical approach in this context. The effect size, using partial eta-squared values, was estimated using the effectsize package [21]. Conformation to the assumptions of the homogeneity of variance and normal distribution was confirmed through a Levene's test and a Shapiro–Wilk test.

## 3. Results

### 3.1. Environmental Parameters

The UVi was consistently 0 throughout the study in UV– boxes and ranged between 8.5–9 in UV+ boxes. Temperature and relative humidity were very similar between treatments (Table 1).

**Table 1.** Environmental parameters and cricket performance data from UV– and UV+ crickets.

Treatment	UVi Min-max (Mean) across Ex- periment	Temperature Range (Mean) across Experiment (°C)	Humidity Range (Mean) across Experiment (%RH)	Mass Crickets Produced (g)	Total Mass Food Consumed (g)	Total Mass Waste Produced (g)	Sex	Mean Mass (g)	Standard Deviation of Mass
UV–	0–0	21.6–40 (27.8)	29–75 (48.8)	884	12,293	2607	M	0.78	0.14
							F	1.24	0.12
UV+	8.5–9	20.1–38 (28.06)	30–78 (50.2)	1790	12,908	2644	M	0.71	0.10
							F	1.00	0.23

### 3.2. Vitamin D<sub>3</sub>

All analyses returned an undetectable amount of vitamin D<sub>3</sub> in cricket samples from both treatments, that is, <0.5 iU/g; no statistical analyses were therefore performed.

### 3.3. Mineral Content, Dry and Wet Mass

Statistical outcomes and summary values from the ANOVA analyses are given in Table 2 and Figure 1. The box (treatment) never had a significant effect on the response variables. There were significant effects of cricket sex on Wet and Dry Mass, calcium, phosphorus, sulphur, zinc, copper, potassium, iron and magnesium content (see Table 2 for effect size and direction). These effects were strong, with partial Eta squared values of 0.17–0.93 (Table 2), and all significant values remained significant after correction for False Discovery Rate ([22]; original *p* values shown in Table 2 for simplicity). There was an effect of UV treatment on both Wet and Dry Mass; the effect sizes were medium to strong (partial eta squared = 0.09–0.21; Table 2). The mineral concentrations are compared with the results from other studies on the same cricket species in Figure 2.

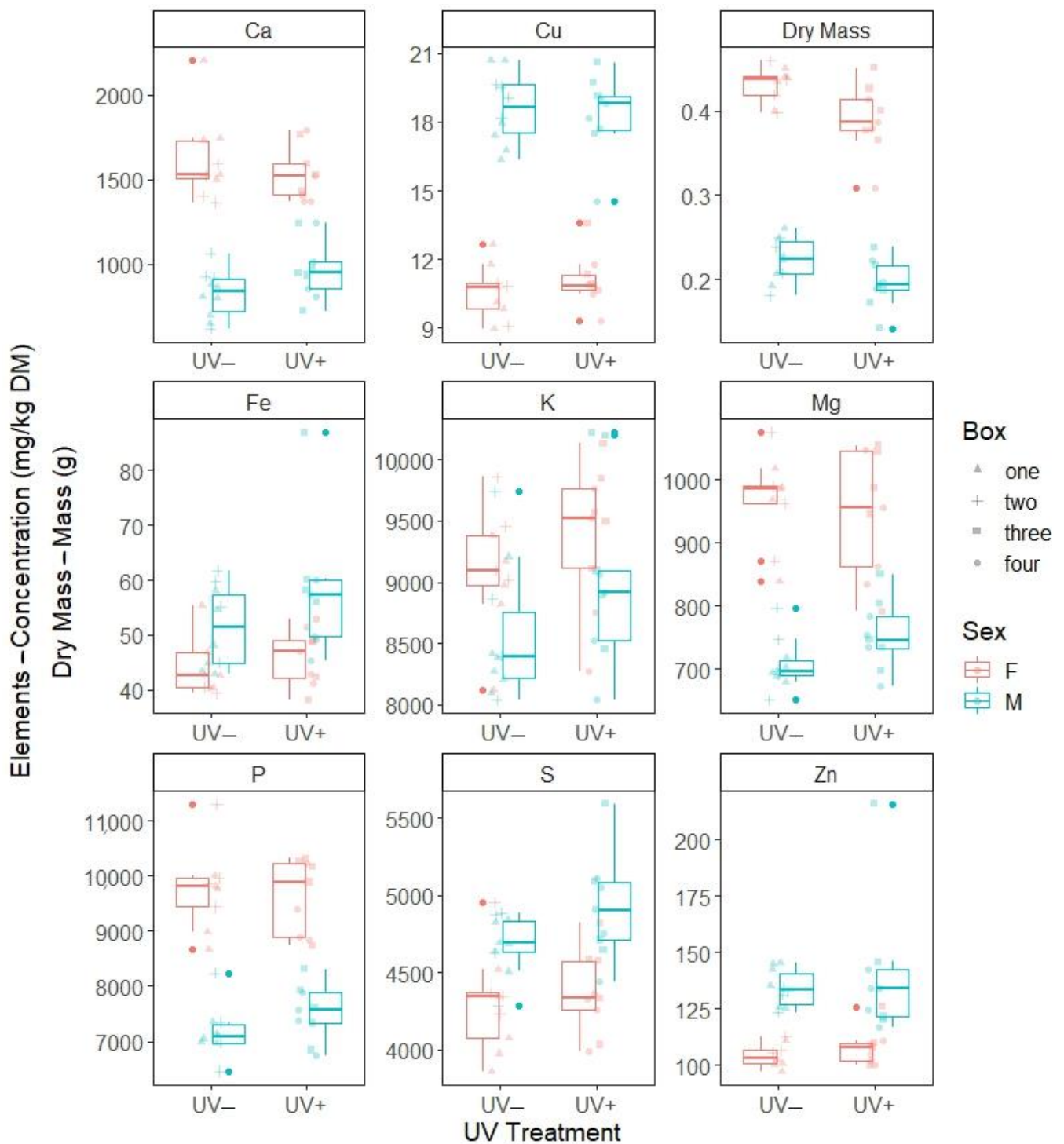
**Table 2.** Statistical outcomes of ANOVAs testing for the effects of UV treatment, sex and box (treatment) on the dry mass, wet mass, mineral content and Ca:P ratios of crickets. Significant *p* values are in bold; all remained significant after correcting for False Discovery Rate (adjusted *p* values not shown).

Response	Variable	Test Statistic	<i>p</i>	Direction of Effect	Effect Size (Partial Eta Squared)	Group Means (SDs)
Wet mass	Sex	$F_{1,75} = 185.10$	<b>&lt;0.001</b>	Males < Females	0.71	Male: 0.76 (0.18) g Female: 1.18 (0.19) g
	Treatment	$F_{1,75} = 7.43$	<b>0.01</b>	UV− < UV+	0.09	UV−: 1.00 (0.28) g UV+: 0.90 (0.28) g
	Box (Treatment)	$F_{2,75} = 2.04$	0.14	-	N/A	-
Dry mass	Sex	$F_{2,32} = 421.10$	<b>&lt;0.001</b>	Males < Females	0.93	Male: 0.21 (0.03) g Female: 0.41 (0.04) g
	Treatment	$F_{2,32} = 8.81$	<b>0.01</b>	UV− < UV+	0.21	UV−: 0.64 (0.22) g UV+: 0.59 (0.21) g
	Box (Treatment)	$F_{2,32} = 1.060$	0.36	-	N/A	-
Calcium	Sex	$F_{2,32} = 114.60$	<b>&lt;0.001</b>	Males < Females	0.77	Male: 891.6 (172.8) mg/kg Female: 1575.6 (210.8) mg/kg
	Treatment	$F_{2,32} = 0.62$	0.44	-	N/A	-
	Box (Treatment)	$F_{2,32} = 1.05$	0.36	-	N/A	-
Phosphorus	Sex	$F_{2,32} = 145.87$	<b>&lt;0.001</b>	Males < Females	0.82	Male: 7332.6 (498.6) mg/kg Female: 9691.5 (688.6) mg/kg
	Treatment	$F_{2,32} = 0.89$	0.35	-	N/A	-
	Box (Treatment)	$F_{2,32} = 1.64$	0.21	-	N/A	-
Ca:P	Sex	$F_{1,32} = 145.87$	<b>&lt;0.001</b>	Males < Females	0.49	Male: 0.12 (0.02) Female: 0.16 (0.02)
	Treatment	$F_{1,32} = 0.38$	0.54	-	N/A	-
	Box (Treatment)	$F_{2,32} = 1.54$	0.23	-	N/A	-
Aluminium	Sex	$F_{1,32} = 3.18$	0.08	-	N/A	Overall mean: 12.98 mg/kg
	Treatment	$F_{1,32} = 0.10$	0.75	-	N/A	
	Box (Treatment)	$F_{2,32} = 0.39$	0.68	-	N/A	

Table 2. Cont.

Response	Variable	Test Statistic	<i>p</i>	Direction of Effect	Effect Size (Partial Eta Squared)	Group Means (SDs)
Sulphur	Sex	$F_{1,32} = 26.90$	<b>&lt;0.001</b>	Males > Females	0.46	Male: 4795.9 (287.2) mg/kg Females: 4330.1 (288.5) mg/kg
	Treatment	$F_{1,32} = 2.43$	0.13	-	N/A	-
	Box (Treatment)	$F_{2,32} = 1.70$	0.20	-	N/A	-
Zinc	Sex	$F_{1,32} = 35.53$	<b>&lt;0.001</b>	Males > Females	0.52	Male: 136.7 (21.2) mg/kg Females: 106.2 (16.7) mg/kg
	Treatment	$F_{1,32} = 0.47$	0.5	-	N/A	-
	Box (Treatment)	$F_{2,32} = 1.31$	0.28	-	N/A	-
Copper	Sex	$F_{1,32} = 274.62$	<b>&lt;0.001</b>	Males > Females	0.90	Male: 18.5 (1.6) mg/kg Females: 10.8 (1.2) mg/kg
	Treatment	$F_{1,32} = 0.33$	0.89	-	N/A	-
	Box (Treatment)	$F_{2,32} = 1.37$	0.27	-	N/A	-
Potassium	Sex	$F_{1,32} = 6.37$	<b>0.02</b>	Females > Males	0.17	Male: 8789.2 (673.6) mg/kg Females: 9242.7 (536.8) mg/kg
	Treatment	$F_{1,32} = 3.17$	0.07	-	N/A	-
	Box (Treatment)	$F_{2,32} = 3.19$	0.06	-	N/A	-
Iron	Sex	$F_{1,32} = 14.03$	<b>&lt;0.001</b>	Males > Females	0.30	Male: 54.57 (10.12) mg/kg Females: 45.01 (5.03) mg/kg
	Treatment	$F_{1,32} = 2.56$	0.12	-	N/A	-
	Box (Treatment)	$F_{2,32} = 0.99$	0.38	-	N/A	-
Manganese	Sex	$F_{1,32} = 0.02$	0.882	-	N/A	Overall mean: 21.01 mg/kg
	Treatment	$F_{1,32} = 2.00$	0.17	-	N/A	
	Box (Treatment)	$F_{2,32} = 0.58$	0.57	-	N/A	
Sodium	Sex	$F_{1,32} = 0.49$	0.49	-	N/A	Overall mean: 3342.96 mg/kg
	Treatment	$F_{1,32} = 0.88$	0.36	-	N/A	
	Box (Treatment)	$F_{2,32} = 3.12$	0.06	-	N/A	
Magnesium	Sex	$F_{1,32} = 96.89$	<b>&lt;0.001</b>	Females > Males	0.75	Male: 728.54 (51.47) mg/kg Females: 956.36 (83.83) mg/kg
	Treatment	$F_{1,32} = 0.76$	0.39	-	N/A	-
	Box (Treatment)	$F_{2,32} = 0.76$	0.48	-	N/A	-





**Figure 1.** Mineral concentrations and dry mass of male and female crickets under UV+ and UV- treatments, split by box (treatment). Only response variables where significant effects of treatment and/or sex were detected are shown; see Table 2 for full statistical outcomes. Wet mass is not shown, as it presented identical patterns to dry mass.

### 3.4. Cricket Performance

UV- boxes (boxes 1 and 2, Figure 1) yielded a total of 424 g and 460 g of crickets, while UV+ boxes (boxes 3 and 4, Figure 1) yielded a total of 758 g and 1032 g of crickets. By dividing the mean production mass by the mean cricket mass for each treatment (UV- = 1.01 g, UV+ = 0.86 g), and assuming a 1:1 sex ratio, UV- produced 516 crickets per



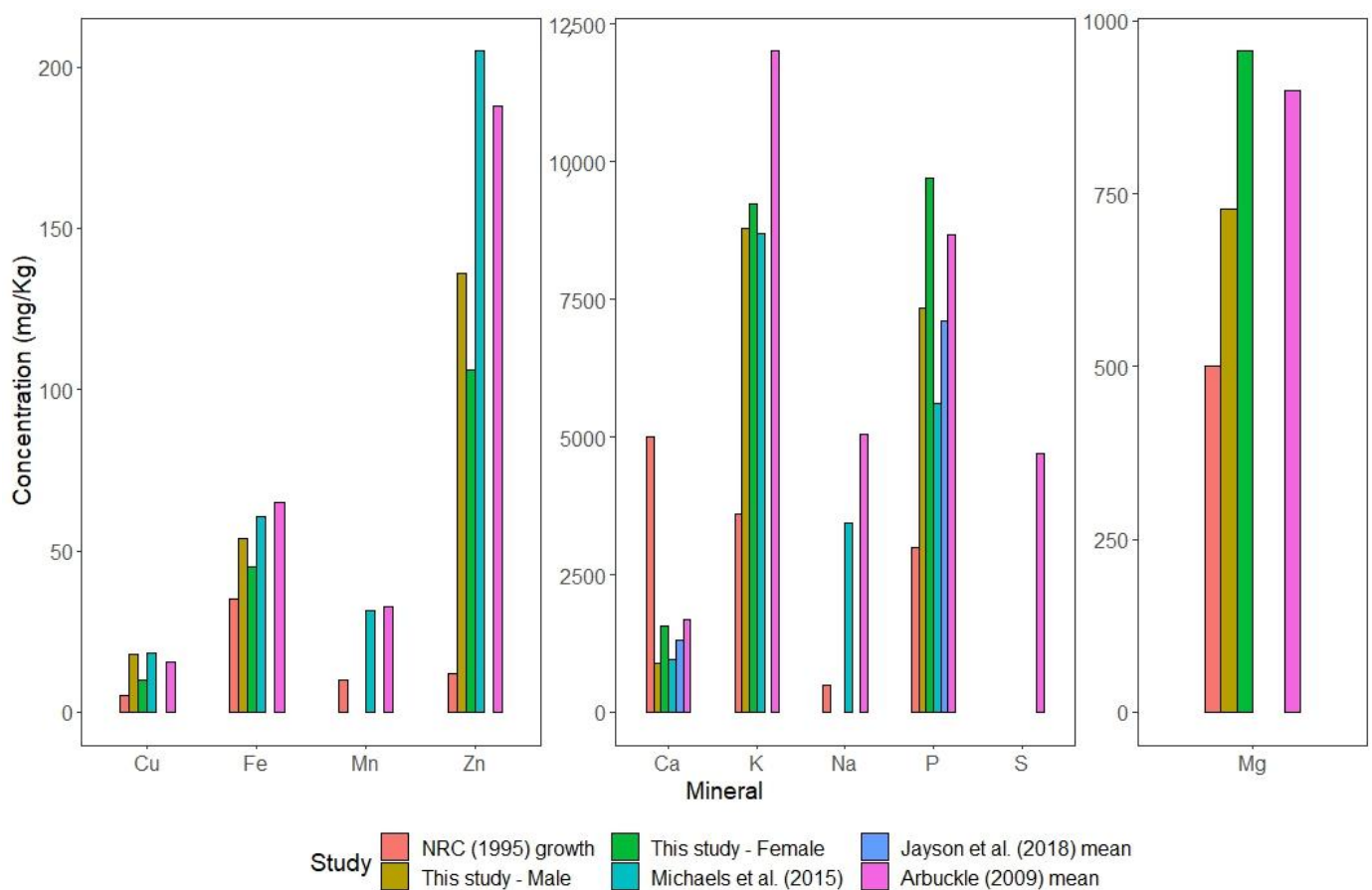
box, while UV+ produced 888 crickets per box. Similar quantities of food were consumed (UV− 12,293 g, UV+ 12,908 g), and similar quantities of waste were produced (UV− 2607 g, UV+ 2644 g; Table 1). The feed conversion ratios were therefore 13.9:1 and 7.21:1 for UV− and UV+, respectively.

#### 4. Discussion

Previously, Bah-Nelson et al. [17] showed that the short-term irradiation of black field crickets did not increase the vitamin D<sub>3</sub> content of the insects within the detection limits of the available analyses. Despite the fact that crickets were typically seen basking under the T5 lamps, as well as being fed underneath them, our data show that the long-term irradiation of crickets across their entire post-ovum life cycle is also unsuccessful in increasing the vitamin D<sub>3</sub> content of this species of feeder cricket. As with Bah-Nelson et al.'s [17] study, the lowest detection limit of the assays was 0.5 IU/g, and we cannot rule out effects on vitamin D<sub>3</sub> content below this threshold, as was detected in other insect species by Oonincx et al. [16]. However, vitamin D<sub>3</sub> contents below this value are relatively low compared with the minimum estimated vertebrate requirements (1.11 iU/g; [8]) and offer a less useful route of provision for this nutrient compared with other potential strategies, including dusting and gut-loading [18]. Equally, there was no effect of treatment on the calcium content of crickets; this is consistent with there being no effect on vitamin D<sub>3</sub> content, which might be hypothesised to drive any differences in calcium uptake based on the current understanding of at least vertebrate calcium metabolism [13]. Further research into the physiology of vitamin D<sub>3</sub> synthesis in insects is needed to better understand any potential of UVB irradiation to increase concentrations of this nutrient.

The mineral profiles we detected in our crickets are broadly similar to those found by other studies in the same species (Figure 2; [12,23,24]) and showed mineral concentrations above the minima required for vertebrate growth [25], with the exception of calcium, and particularly high concentrations of phosphorus, potassium and zinc compared with the minimum requirements. There is, however, substantial variation between studies, potentially due to insect diets, rearing conditions or analysis methodology; although our data are no more distinct from other studies than those are from one another, these comparisons highlight the relevance of context in determining the nutritional content of feeder insects. Importantly, the nutritional profiles of other cricket species (e.g., *Acheta domesticus*) may be very different [10], and species identity constrains the extrapolation of data.

Our findings of sex differences in multiple mineral concentrations in crickets, independent of UVB exposure, are perhaps more surprising and important. The effects of sex were not only significant but often very strong with large estimates of effect size, indicating important real-world differences. This was especially the case for Ca, Mg, P and Cu, where females contain approximately double the concentration of these minerals than males. These differences are potentially linked to investment in reproduction, as females generate large numbers of ova, each of which requires the investment of nutrients to enable the development and survival of nymphs, but may also be the result of behavioural factors including dietary selectivity, or structural factors including differences in exoskeletal form (e.g., males developing larger, more robust wing cases). We were unable to extend analyses to include nutrients such as protein, fat and vitamins other than D<sub>3</sub> due to resource constraints, but it is likely that a similar asymmetry in concentrations between sexes might also be present, and further investigation is required. Although the effect sizes are substantial, a comparison with the requirements of vertebrates for growth ([25]; Figure 2) shows that male and female means do not straddle minimum nutritional thresholds, and as such, practical implications may be limited in general. In cases where predators may be more sensitive to mineral concentrations (e.g., debilitated individuals), the provision of specifically male or female crickets may be beneficial.



**Figure 2.** Mean mineral concentrations (mg/kg Dry Mass) of *Gryllus bimaculatus* crickets from this study and three others, compared with the recommended minimum concentration required for the growth of rodents [25]; no data are available for Sulphur. Data from Jayson et al. [23] represent crickets without supplementary dusting averaged across the two institutions reported. Data from Arbuckle [2] represent the mean of four gut-loaded treatments reported. Data from Michaels et al. [12] represent starved adult crickets; data for minerals other than Ca and P are the previously unpublished results of this study.

Ref. [11] showed that male and female cockroaches have differing nutritional profiles, but otherwise, there is very little extant literature in this area, and almost all existing research on nutritional content and manipulation in feeder invertebrates does not consider sex as a factor. Although in most cases one can assume equal proportions of male and female invertebrates (where two distinct sexes exist) in treatment groups, our study opens up the possibility of differing requirements for nutritional manipulation between sexes and of interactive effects between manipulation attempts and invertebrate sex. We strongly encourage the inclusion of sex as a factor in future research in this field, as well as the investigation of a broader range of invertebrates in order to better understand the impact of sex on nutrition in feeder invertebrates. Our results also have implications for some special contexts where insectivores may not have access to an equal proportion of male and female insect prey. In some conservation translocations, invertebrates of only one sex are supplied as food to prevent the establishment of feeders as alien invasives [26], while insectivore species with sexually dimorphic body sizes may also feed dichotomously on smaller male or larger female crickets. For some cultured invertebrates, such as parasitoid wasps, single prey items are used as hosts for larvae; the sex of the prey insect used in these circumstances may influence reproductive success and the quality and/or sex of emerging offspring. This, too, requires further research in light of our results.

The production of livefoods must be economically efficient and meet the nutritional requirements of insectivores. Our data suggest a trade-off between growth rates and mortality under the different UV treatments; UV – crickets grew larger (UV+ crickets were 85% of the mass of UV – crickets, on average), but fewer adult crickets were eventually produced (by both total mass and calculated numbers of crickets; see above and Table 1). The total food consumption and waste production were similar between treatments. We suggest that this trade-off was the result of density-dependent growth, but due to our experimental design, we are unable to fully explore this statistically or determine whether this was driven by increased mortality leading to increased individual sizes under UV – conditions or whether UV+ conditions drove greater growth rates, leading to greater mortality by competition. Temperature and humidity were very similar between treatments and are thus unlikely to explain differences in performance. Additionally, the provision of UV lighting increases the cost base of live food production, with, in our study, approximately 0.8 kwh of additional energy use per culture box required. Further research might investigate the links between lighting, culture density and culture productivity.

In conclusion, we have demonstrated that UVB irradiation across the life cycle of *G. bimaculatus* is, as with the short-term exposure of adults, insufficient to increase vitamin D<sub>3</sub> above the limit of detection or to justify the additional costs of necessary equipment. Based on this work, it may be advisable to focus on other means of increasing concentrations of this nutrient in feeder invertebrates—for example, through gut-loading and supplementary dusting (although these areas are also poorly evaluated and in need of further research), as well as through the appropriate irradiation of insectivores themselves. Arguably more strikingly, we demonstrate meaningful differences in nutritional composition between the sexes of crickets, with implications for future research and for the captive husbandry of insectivores.

**Author Contributions:** Conceptualisation, C.J.M. and A.F.; methodology, C.J.M. and A.F.; formal analysis, C.J.M.; investigation, C.J.M., J.N.-Y., R.H. (Ross Hickles) and R.H. (Robert Harland); resources, R.H. (Robert Harland) and J.N.-Y.; data curation, C.J.M. and R.H.; writing—original draft preparation, C.J.M.; writing—review and editing, C.J.M., A.F., J.N.-Y., R.H. (Ross Hickles) and R.H. (Robert Harland); visualisation, C.J.M.; supervision, C.J.M.; project administration, C.J.M. and R.H. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Ethical review and approval were waived for this study, as insects are not included in the Animals (Scientific Procedures) Act 1986 in the UK. However, all methods were informally reviewed and approved by the ZSL Ethics Committee and followed best practice (see text).

**Data Availability Statement:** Data are available for download at <https://github.com/CJMichaels/Cricket-UVB> (accessed 16 September 2022).

**Acknowledgments:** The authors would like to thank the members of the animal husbandry teams who helped to maintain the cricket cultures used in this research.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Ferguson, G.W.; Jones, J.R.; Gehrmann, W.H.; Hammack, S.H.; Talent, L.G.; Hudson, R.D.; Dierenfeld, E.S.; Fitzpatrick, M.P.; Frye, F.L.; Holick, M.F.; et al. Indoor husbandry of the panther chameleon *Chamaeleo Furcifer pardalis*: Effects of dietary vitamins A and D and ultraviolet irradiation on pathology and life-history traits. *Zoo Biol.* **1996**, *15*, 279–299. [\[CrossRef\]](#)
2. Miller, E.A.; Green, S.L.; Otto, G.M.; Bouley, D.M. Suspected hypovitaminosis A in a colony of captive green anoles (*Anolis carolinensis*). *J. Am. Assoc. Lab. Anim. Sci.* **2001**, *40*, 18–20.
3. Pessier, A. Suspected hypovitaminosis A in captive toads (*Bufo* spp.). In *Proceedings of the American Association of Zoo Veterinarians/American Association Wildlife Veterinarians/American Zoo and Aquarium Association Nutrition Advisory Group Joint Conference*; Charlotte, K.B., Ed.; American Association of Zoo Veterinarians: Nassau County, FL, USA, 2005; p. 57.
4. Crawshaw, G.; Pienkowski, M.; Lentini, A.; Dutton, C.; Delnatte, P.; Russell, D.; Berkvens, C.; Barker, I.; Smith, D. Brown skin disease: A syndrome of dysecdysis in puerto rican crested toads (*Peltophryne lemur*). *Zoo Biol.* **2014**, *33*, 558–564. [\[CrossRef\]](#)

5. Hoby, S.; Wenker, C.; Robert, N.; Jermann, T.; Hartnack, S.; Segner, H.; Aebischer, C.P.; Liesegang, A. Nutritional metabolic bone disease in juvenile veiled chameleons (*Chamaeleo calyptrotatus*) and its prevention. *J. Nutr.* **2010**, *140*, 1923–1931. [\[CrossRef\]](#)
6. Feldman, S.H.; Formica, M.; Brodie, E.D. Opisthotonus, torticollis and mortality in a breeding colony of *Anolis* sp. lizards. *Lab Anim.* **2011**, *40*, 107. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Oonincx, D.G.A.B.; Finke, M.D. Nutritional value of insects and ways to manipulate their composition. *J. Insects Food Feed* **2021**, *7*, 639–659. [\[CrossRef\]](#)
8. Ferrie, G.M.; Alford, V.C.; Atkinson, J.; Baitchman, E.; Barber, D.; Blaner, W.S.; Crawshaw, G.; Daneault, A.; Dierenfeld, E.; Finke, M.; et al. Nutrition and health in amphibian husbandry. *Zoo Biol.* **2014**, *33*, 485–501. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Pessier, A.P.; Baitchman, E.J.; Crump, P.; Wilson, B.; Griffith, E.; Ross, H. Causes of mortality in anuran amphibians from an ex situ survival assurance colony in Panama. *Zoo Biol.* **2014**, *33*, 516–526. [\[CrossRef\]](#)
10. Finke, M.D. Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biol.* **2015**, *34*, 554–564. [\[CrossRef\]](#)
11. Cerreta, A.J.; Smith, D.C.; Ange-Van Heugten, K.; Minter, L. Comparative nutrient analysis of four species of cockroaches used as food for insectivores by life stage, species, and sex. *Zoo Biol.* **2022**, *41*, 26–33. [\[CrossRef\]](#)
12. Michaels, C.; Antwis, R.E.; Preziosi, R.F. Manipulation of the calcium content of insectivore diets through supplementary dusting. *JZAR* **2014**, *2*, 77–81.
13. Uhl, E.W. The pathology of vitamin D deficiency in domesticated animals: An evolutionary and comparative overview. *Int. J. Paleopathol.* **2018**, *23*, 100–109. [\[CrossRef\]](#)
14. Holick, F.M. Vitamin D: Evolutionary, physiological and health perspectives. *Curr. Drug Targets* **2011**, *12*, 4–18. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Antwis, R.E.; Browne, R.K. Ultraviolet radiation and Vitamin D<sub>3</sub> in amphibian health, behaviour, diet and conservation. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2009**, *154*, 184–190. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Oonincx, D.G.A.B.; Van Keulen, P.; Finke, M.D.; Baines, F.M.; Vermeulen, M.; Bosch, G. Evidence of vitamin D synthesis in insects exposed to UVB light. *Sci. Rep.* **2018**, *8*, 10807. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Bah-Nelson, I.; Newton-Youens, J.; Ferguson, A.; Michaels, C.J. Calcium accumulation and loss and vitamin D<sub>3</sub> content of feeder black field crickets (*Gryllus bimaculatus*) fed on a high calcium diet with and without UVB irradiation. *J. Zool. Bot. Gard.* **2021**, *2*, 382–387. [\[CrossRef\]](#)
18. Finke, M.D. Gut loading to enhance the nutrient content of insects as food for reptiles: A mathematical approach. *Zoo Biol.* **2003**, *22*, 147–162. [\[CrossRef\]](#)
19. Pellett, S.; Pizzi, R.; Trim, S.; Bushell, M.; Clarke, D.; Wood, J. *BIAZA Recommendations for Ethical Euthanasia of Invertebrates* (1.2 ed.); British and Irish Association of Zoos and Aquariums: London, UK, 2017.
20. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2022; Available online: <https://www.R-project.org/> (accessed on 16 September 2022).
21. Ben-Shachar, M.S.; Lüdtke, D.; Makowski, D. Effectsize: Estimation of Effect Size Indices and Standardized Parameters. *J. Open Source Softw.* **2020**, *5*, 2815. [\[CrossRef\]](#)
22. Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. B Stat. Methodol.* **1995**, *57*, 289–300. [\[CrossRef\]](#)
23. Jayson, S.; Ferguson, A.; Goetz, M.; Routh, A.; Tapley, B.; Harding, L.; Michaels, C.J.; Dawson, J. Comparison of the nutritional content of the captive and wild diets of the critically endangered mountain chicken frog (*Leptodactylus fallax*) to improve its captive husbandry. *Zoo Biol.* **2018**, *37*, 332–346. [\[CrossRef\]](#)
24. Arbuckle, K. *Influence of Diet on Mineral Composition of Crickets Used as Prey for Captive Amphibians, Specifically Hylidae*; University of Glasgow: Glasgow, UK, 2009.
25. NRC. *Nutrient Requirements of Laboratory Animals*; National Academy Press: Washington, DC, USA, 1995.
26. Nicolson, D.J.; Tapley, B.; Jayson, S.; Dale, J.; Harding, L.; Spencer, J.; Sulton, M.; Durand, S.; Cunningham, A.A. Development of in-country live food production for amphibian conservation: The Mountain Chicken Frog (*Leptodactylus fallax*) on Dominica, West Indies. *Amphib. Reptile Conserv.* **2017**, *11*, 59–68.