

## Effects of elevated CO<sub>2</sub> on the water hyacinth-biocontrol agent *Megamelus scutellaris* (Hemiptera: Delphacidae) and its yeast-like symbiotes

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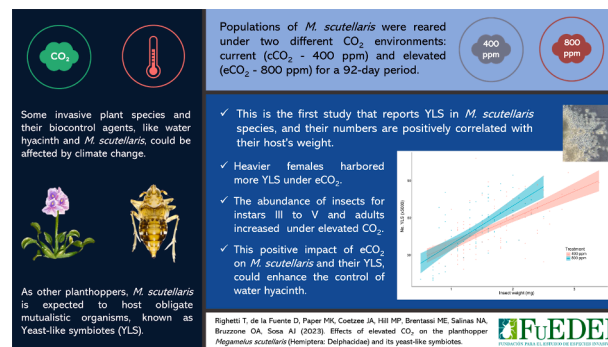
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### HIGHLIGHTS

- Some invasive plant species, like *Pontederia crassipes*, could be possibly affected by climate change.
- *Megamelus scutellaris* is one of the most commonly used biocontrol agents for *P. crassipes*.
- As other planthoppers, *M. scutellaris* is expected to host obligate mutualistic organisms, known as Yeast-like symbiotes (YLS).
- YLS are first reported in *M. scutellaris* species and their numbers are positively correlated with their host's weight.
- YLS numbers increased under elevated CO<sub>2</sub>, in heavier females.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Water hyacinth, *Pontederia crassipes*, is a highly invasive plant native to South America and one of the most invasive aquatic plants in the world. For its control, the planthopper *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), a phloem feeder also native to South America has been introduced to the USA and South Africa. Considering predicted climate change scenarios, understanding their impacts on biological control agents is crucial. An intriguing yet scarcely explored subject, is the effect of climatic changes on the obligate endosymbionts associated with sap-sucking feeders. Planthoppers establish an obligate relationship with yeast-like symbiotes (YLS), unicellular fungal microorganisms that play an important role in their development, providing missing nutrients in their diet. Considering that increased atmospheric CO<sub>2</sub> affects plant chemical composition, this might have a direct impact on their insect host and on their number of YLS. We evaluated the effect of two different CO<sub>2</sub> environments: current (cCO<sub>2</sub> – 400 ppm) and elevated (eCO<sub>2</sub> – 800 ppm) on the

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abundance of YLS (number of YLS cells/insect) of *M. scutellaris*, as well as the, age structure, sex, and weight of insects. Heavier females harbored more YLS under eCO<sub>2</sub> which underscores the importance of the interaction of CO<sub>2</sub> levels and insects' weight in shaping the abundance of YLS. Additionally, there was a significant increase in the total abundance of insects for instars III to V and adults under eCO<sub>2</sub> conditions. However, male number significantly exceeded that of females under both CO<sub>2</sub> conditions. Our results suggest a potential positive impact of eCO<sub>2</sub> on *M. scutellaris* populations, which could, in turn, enhance the control of *P. crassipes*.

## 1. Introduction

Two of the greatest threats to biodiversity are the increase in global greenhouse gas concentrations causing climate change, and the increase in biological invasions by exotic species (IPBES, 2023). Invasive plant species have spread across the planet as a result of the increase in global temperatures and atmospheric CO<sub>2</sub> levels, reaching higher latitudes in both northern and southern hemispheres (Capdevila-Argüelles et al., 2011), among other reasons. Therefore, the development of minimal environmental impact solutions in the management of invasive plants, that provide early warnings, climate change scenario analysis, and future response, is critical.

The average global temperature increase recorded over the past 50 years have more than doubled compared to any other time in human history (IPCC, 2023). The post-industrial increase in atmospheric CO<sub>2</sub> is unprecedented (Ehleringer and Cerling, 2002), with an increased rate of  $\pm 1$  parts per million (ppm)/year (Pachauri et al., 2014). The continuous increase in anthropogenic atmospheric CO<sub>2</sub> has led to irreversible changes in climate around the globe, such as reductions in the dry-season rainfalls or rises in the global average sea levels; these changes and their associated impacts would normally be expected over millennia instead of decades (Solomon et al., 2009).

Depending on the climate scenario used by the Intergovernmental Panel on Climate Change, atmospheric CO<sub>2</sub> levels are projected to rise above 800 ppm by the year 2100 if anthropogenic CO<sub>2</sub> continues to increase at the current rate (Meehl et al., 2007). Plant physiology is expected to be altered by predicted elevated CO<sub>2</sub> (eCO<sub>2</sub>) ratios, affecting photosynthesis, plant metabolism and phloem sap composition (Bowes et al., 1996; Sage et al., 1994; Ehleringer and Cerling, 2002; Ryan et al., 2015). eCO<sub>2</sub> is also expected to have impacts on a macroscopic level, by altering the structure and functions of ecosystems and potentially changing the ranges and interactions of communities inhabiting those ecosystems (Walther et al., 2002; Root et al., 2003; Hellmann et al., 2008). Some invasive plant species could take advantage of these environmental changes (Ziska, 2004; Hellmann et al., 2008; Ziska and Dukes, 2011), making biological invasions by alien plants more common in the future (Reeves, 2017).

Water hyacinth, *Pontederia* (= *Eichhornia*) *crassipes* Mart. (Pontederiaceae) is a highly invasive plant native to the del Plata Basin and Amazon Basin in South America and is considered one of the four most invasive and aggressive aquatic plants in the world (Alowe et al., 2004). This plant can alter invaded aquatic ecosystems by changing the structure and diversity of macrophyte communities, outcompeting and displacing native species. In many cases, invasions lead to increased fish mortality and degradation of wetlands and surrounding urban environments (Coetzee et al., 2014; Masifwa et al., 2001; Toft et al., 2003). This species was introduced in various countries as an ornamental plant and can be found invading water bodies in 55 countries in North and Central America, Europe, Africa, Asia, and Australia, reaching latitudes of 40° N (Portugal) and 40° S (New Zealand) (Julien, 2000) and potentially experiencing further range expansion due to the global increase of temperature (Hellmann et al., 2008; Hoveka et al., 2016). Due to the low effectiveness of mechanical and the environmental hazard that chemical control methods present, biological control is considered a viable alternative in economic and environmental terms (Julien, 2000), especially in water ecosystems.

Classical biological control using host-specific insects and pathogens

has been implemented to mitigate water hyacinth infestations around the world (Coetzee et al., 2017). The most recent agent released to control this species is the phloem-feeding planthopper, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) which was released in USA and South Africa in 2010 and 2013, respectively (Coetzee et al., 2021, Tipping et al., 2014). *M. scutellaris*, like other Delphacidae, exhibit wing dimorphism, with fully winged adults (macropterous) and others with reduced wings (brachypterous), which are unable to fly (Sosa et al., 2004). This species is a host-specific planthopper native to South America, with its whole life cycle tied to *P. crassipes* (Sosa et al., 2004, 2005, 2007). This sap-sucking insect feeds from phloem sap and reproduces on water hyacinth (Hernández et al., 2011). When the population density reaches about 100 insects per plant, it can cause severe leaf chlorosis and an overall reduction in plant vigour (Fitzgerald and Tipping, 2013; Miller et al., 2019). It is expected that under eCO<sub>2</sub> conditions, phloem-feeders such as *M. scutellaris*, substantially increase their feeding responses on their respective host plants (Paper et al., 2023).

Phloem presents a challenge to insects that feed on it, due to a high carbon-nitrogen ratio and low lipid levels available to the insect (Douglas, 2009; Hansen and Moran, 2014). Phloem sap-feeders thus depend on the interaction with microorganisms with which they establish a mutualistic association, playing a key role in their nutrition. *Megamelus scutellaris*, like other planthoppers, hosts obligate fungal mutualists in their fat body cells known as yeast-like symbiotes (YLS). This is the first time these are reported for this planthopper. YLS interact synergistically with their planthopper hosts, providing nutritional functions such as the synthesis of essential amino acids and steroids, and nitrogen recycling (Xue et al., 2014), and are vertically transmitted from females to their eggs by transovarial transmission and are present at all stages of host development (Cheng and Hou, 2001; Liljesthrom et al., 2017).

In order to assess how *M. scutellaris* and its YLS would react to eCO<sub>2</sub> conditions, we hypothesised that populations of *M. scutellaris*' YLS are affected as a direct consequence of developing under eCO<sub>2</sub> conditions.

Considering the influence of eCO<sub>2</sub> on the physiology of host plants, including changes in phloem sap composition, our hypothesis posits that the populations of *M. scutellaris* and their YLS may be affected under eCO<sub>2</sub> conditions. Consequently, this could potentially impact the performance of *M. scutellaris* as a biocontrol agent for water hyacinth.

The main aim of this study is to determine whether or to what extent eCO<sub>2</sub> affects the number of YLS, and different insect's parameters such as age structure, sex and weight under the two CO<sub>2</sub> conditions.

## 2. Materials and methods

### 2.1. Insect rearing conditions and experimental design

To estimate the effects of eCO<sub>2</sub> on *M. scutellaris* and its YLS, we carried out an experiment in 4 decagonal, 3 m in diameter x 2.8 m tall open-top chambers (OTCs) located in the Rhodes University Elevated CO<sub>2</sub> Facility, Eastern Cape, South Africa (33°18'41.0 "S 26°30'33.4 "E). These OTCs were surrounded with an F-Clean polyethylene film which permits 94 % of solar radiation in the visible and near-visible spectrum to penetrate. To ensure uniform distribution of air, a 34-centimeter-diameter diffuser, perforated with 300 1-centimeter holes and mounted 1 m above the floor, spanned the entire perimeter of each chamber, and was used to ventilate the chambers using a 3-phase fan. The velocity

of airflow in the diffuser was regulated by a fan speed that responded to changes in temperature and CO<sub>2</sub> concentration, controlled by Senlan SB150 variable frequency drives. Temperature and humidity were monitored using CS215 sensors by Campbell Scientific, while data were processed with the CR6 data logger and Logger-net software (Campbell Scientific) (Raubenhimer et al., 2022). In order to study the effect of eCO<sub>2</sub>, the atmospheric CO<sub>2</sub> concentrations inside two of the four OTCs were modified according to the Intergovernmental Panel on Climate Change's RCP 8.5 scenario for the year 2100 ("Business as usual" – 800 ppm) (Pachauri et al., 2014). The remaining two chambers were maintained at their regular ambient concentrations (400 ppm). Open-path CO<sub>2</sub> analysers (GMP343, Visala, Finland) controlled and measured atmospheric CO<sub>2</sub> in all four OTCs by modifying CO<sub>2</sub> injection into the chamber ventilation system. To ensure proper mixing, CO<sub>2</sub> was injected into the blower fan inlet and was regulated using a 2873 proportional valve (Burkert, Germany). Air and leaf temperature were almost constant and similar among CO<sub>2</sub> treatments during the whole experiment (Table 1, Supplementary material).

To study the impact of eCO<sub>2</sub> on *M. scutellaris* and its associated YLS, 96 mature *P. crassipes* plants were collected from the Centre for Biological Control Mass Rearing Facility in Makhanda, South Africa, on December 15, 2018, and grown in 20L cylindrical mesocosms in sets of four. Any dead material and daughter plants were removed from each mesocosm, leaving four mature plants of similar weight and size in each. Each mesocosm was then treated with 10 mgN/L Culterra Multisol® 6.1.3 foliage and 1.1mgFe/L 13 % Fe Chelate for 62 days to replicate the nutrient conditions of invaded sites in South Africa (Coetzee and Hill, 2012) and acclimate the plants to predicted eCO<sub>2</sub> conditions. 1440 first-instar *M. scutellaris* nymphs, also collected from the CBC Mass Rearing Facility's *P. crassipes* biological control programme, were randomly introduced in groups of 60 into each of the six mesocosms inside every OTC. The quantification of insect populations was determined through reference to estimations provided by Miller et al. (2019) and Sosa et al. (2007), both of which advocate around 60 insects for ensuring a successful establishment. 1:1 sex ratio was assumed due to the difficulty of determining it in first instar nymphs. Each mesocosm was carefully covered with fine mesh screens. This study was conducted over 92 days, from February to May 2019. After the experiment was completed, all insects were collected from each mesocosm and further analyses were performed to estimate the number of YLS, age structure and weight of the *M. scutellaris* populations. For this purpose, every insect was extracted from each mesocosm of the four OTCs and processed afterwards.

## 2.2. YLS quantification

The number of YLS is variable among stages and sexes. Female fat tissue contains a higher number of YLS, compared to males, as they transfer some of them to their eggs (Cheng and Hou, 2001; Liljeström et al., 2016). Also, males tend to decrease their YLS numbers with time (Noda, 1974; Liljeström et al., 2016). Because of this, to quantify the number of YLS per insect, 10 female adults of *M. scutellaris* were randomly selected from each container and weighted using an APX-200 scale (Denver Instrument). After being weighed, insects were

**Table 1**

Fixed-factors' estimated parameters (from YLS), deviations and *P* values from the generalized linear mixed model (GLMM), from the number of Yeast-like symbiotes (YLS) after the 92-day eCO<sub>2</sub> experiment. Significant differences are indicated with \*.

Fixed factor	Estimate	Std. Error	z value	<i>P</i>
Intercept	3.23	0.11	28.30	< 2e-16*
Weight	435.95	32.53	13.40	< 2e-16*
eCO <sub>2</sub>	-0.41	0.16	-2.61	0.0092*
Weight x eCO <sub>2</sub>	365.19	53.09	6.88	6.02e-12*

individually triturated with a steel micropestle in an Eppendorf® tube containing 0.5 ml of sterile water and homogenized with agitation. We counted each fungal yeast-like cell, characterized by a rod-shaped morphology (10–12 µm length) under an optical microscope (Leica DM750) using a hemocytometer (Neubauer chamber, BOECO, Germany) according to Noda (1974) and following the procedure described by Liljeström et al. (2016) for *Delphacodes kuscheli* Fennah (Hemiptera: Delphacidae).

## 2.3. Effect of CO<sub>2</sub>

The age structure of the population was determined by identifying each individual's instar (nymphs I to V and adults), recording length, number of teeth in the metatibial spur, and presence of the three-segmented tarsi on hind legs, wings, and genitalia. To determine the sex of the adults, morphological differences between males and females were carefully examined according to Sosa et al. (2004,2005) description. Individuals were examined under a Leica MZ12.5 stereoscopic microscope.

## 2.4. Statistical methods

To analyze the number of YLS in adult females we fitted Generalized Linear Mixed Models (GLMM) with interactions with binomial negative structure of errors. Thus, we modeled the number of YLS per insect as the response variable, with CO<sub>2</sub> treatments and insect weight as categorical and numerical fixed variables, respectively, and chamber (OTC) as random factors. To analyze the age structure of *M. scutellaris*, we fitted a GLMM with Poisson structure of errors (estimated using ML and Nelder-Mead optimizer) to predict the number of individuals of a particular stage with CO<sub>2</sub> treatments and stage as fixed factors in an interaction model. To analyze if the abundance of the adults depends on the treatment, sex, and the interaction of these two, we fitted GLMMs with interactions with the binomial negative structure of errors, modeling the number of adults as the response variable, with CO<sub>2</sub> treatments and sex as fixed categorical variables, and mesocosms as random factors.

## 2.5. Software

For all the analysis we used the *lme4* package (Bates et al., 2015). 95 % confidence Intervals (CIs) and *P*-values were computed using a Wald *z*-distribution approximation. Models were selected according to their performances in the *Performance* package (Lüdecke et al., 2021). All analyses were conducted with R 4.3.1 (R Core Team, 2022).

## 3. Results

### 3.1. Weight, CO<sub>2</sub>, and YLS

The GLMM analysis revealed significant interactions between weight and CO<sub>2</sub> treatment on the abundance of YLS in *M. scutellaris* (Table 1, GLMM, *p* < 0.05). The statistical model explained most of the variability of the data (conditional R<sup>2</sup> = 0.56), and the part related to the fixed effects alone (marginal R<sup>2</sup>) was 0.46. All the coefficients of the statistical model were significantly different from zero as shown in Table 1, which means that all the treatments caused a significant change in the mean of the YLS, alone and in the interaction with the other variable. According to the *P* value of each coefficient, the effect was more substantial for the weight, followed by the interaction, and the weakest one was the concentration of the CO<sub>2</sub>.

Our results indicated that the relationship between weight and number of YLS differed significantly between cCO<sub>2</sub> and eCO<sub>2</sub>. Heavier *M. scutellaris* adult females tended to harbor a higher number of YLS in both treatments. Additionally, the effect of the CO<sub>2</sub> treatment on the number of YLS changed according to the weight of the females. For individuals weighing 1 mg, no differences in the number of YLS between

treatments were found. In both cases, it was around 175,000 YLS per female. On the other hand, significant differences were found in heavier females. For individuals weighing 2 mg, the number of YLS was around 350,000 per female at eCO<sub>2</sub>, and around 275,000 at cCO<sub>2</sub> (Table 1, Fig. 1).

### 3.2. *Megamelus scutellaris* age structure

After a 92-day experiment, the total number of individuals obtained, considering all the populations of *M. scutellaris*, was 7613. Three of the 12 mesocosms under 800 ppm were discarded close to the end of the 92-day period, as populations experienced rapid declines, and it was suggested that insect escapes were likely a cause.

The age structure was similar in both treatments, but from instar 3 onwards, the number of individuals of each stage increased. Also, there were differences between CO<sub>2</sub> treatments, being eCO<sub>2</sub> higher (although not significant), and differences increasing with the stages, being much higher in the adult than in all the previous stages ( $P = 0.001$ , Fig. 2, Table 2). Hence, in the eCO<sub>2</sub> treatment, there were more adults than in the 400 ppm treatments. The statistical model explained almost all the data variability (conditional  $R^2 = 0.99$ ) and the part related to the fixed effects alone (marginal  $R^2$ ) was 0.79. The model's intercept corresponded to cCO<sub>2</sub> (Treatment = 400 ppm) and Stage = Adults. The estimated coefficients and their statistical significance are summarized in Table 3.

### 3.3. *Megamelus scutellaris* adult abundance related to sex

In both treatments, the GLMM analysis revealed more males than females (Table 4, Fig. 3, GLMM,  $p < 0.05$ ), and total adult numbers remained similar through CO<sub>2</sub> treatments. The model explained almost all of the variability (conditional  $R^2 = 0.94$ ), and the part related to the fixed effects alone (marginal  $R^2$ ) was 0.04. The model's intercept, corresponding to cCO<sub>2</sub> (Treatment = 400 ppm) and SEX = females, is at 3.40 (95 % CI [2.90, 3.90],  $p < 0.001$ ). The estimated coefficients and their statistical significance are summarized in Table 4.

## 4. Discussion

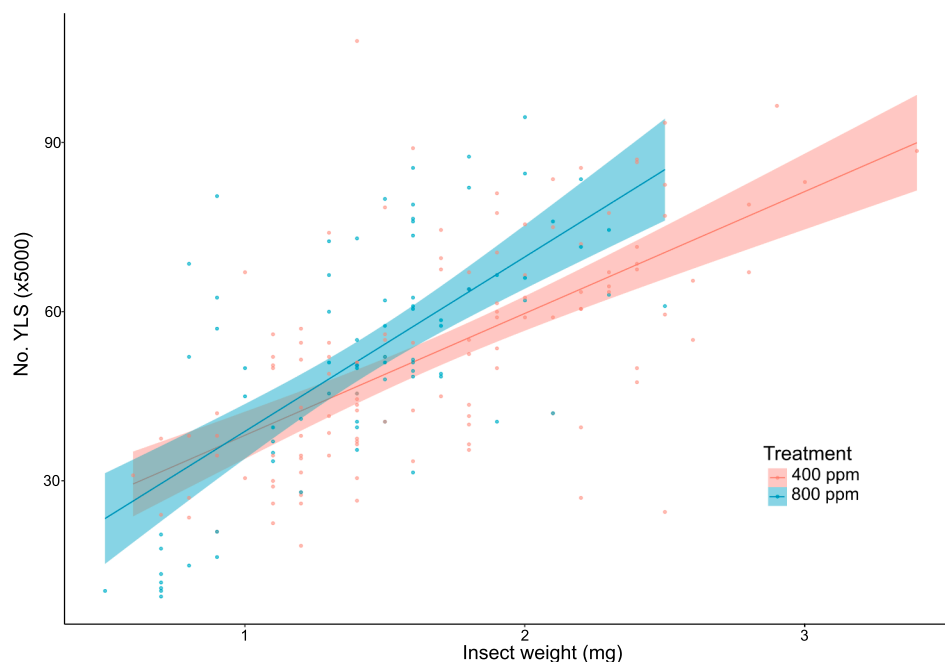
Due to the increasing rise of CO<sub>2</sub> in the atmosphere, numerous studies have been conducted to assess what its impact could be on plants and phytophagous insects (Whittaker, 1999; Stiling et al., 2002; Sun et al., 2016). Taking into account that obligate mutualistic relationships have an essential role in the life cycle of their insect hosts, it is relevant to study how symbiotic associations could be affected by atmospheric alterations in a climate change context. This study is the first report on the presence of yeast-like symbiotes in *Megamelus scutellaris*. Our analyses show some insights into the effects of elevated CO<sub>2</sub> on *M. scutellaris* and on their obligate mutualistic fungal associates.

Yeast-like symbiotes are responsible for performing certain nutritional functions for their hosts, such as providing amino acids, nitrogen recycling, and supplying lipids (Sasaki et al., 1996; Noda et al., 1979; Xue et al., 2014; Fan et al., 2015). At the same time, it has been reported that CO<sub>2</sub> directly influences the growth of some fungi, stimulating budding and the mechanisms that some fungi have to detect CO<sub>2</sub> (Bahn and Mühlshlegel, 2006; Volodyaev et al., 2013).

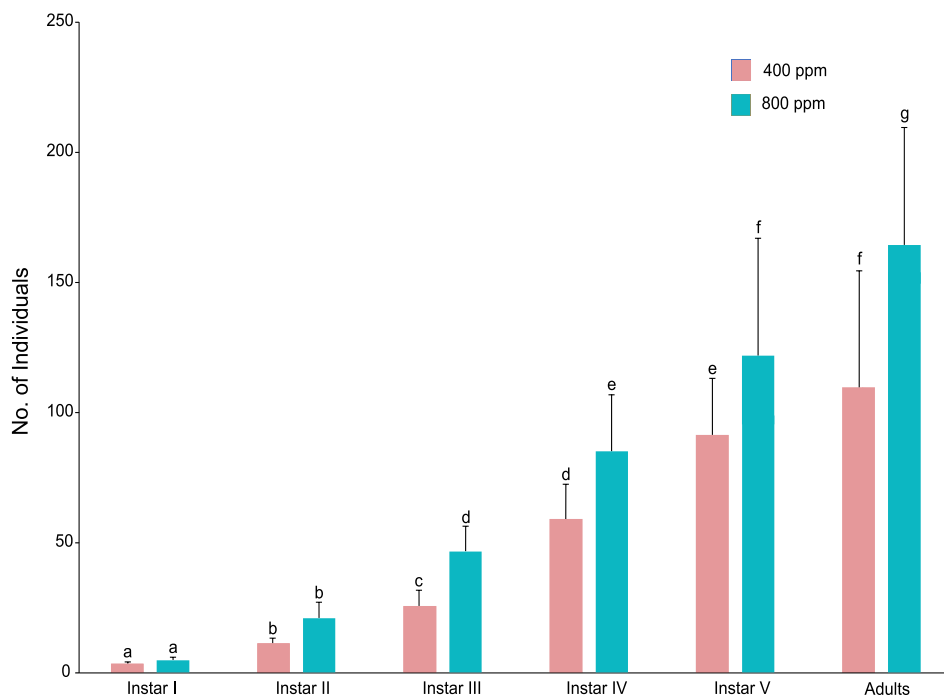
To date, the effect of elevated CO<sub>2</sub> on YLS has been only explored by Xiao-Na et al. (2011) in the planthopper pest of rice in Asia, *Nilaparvata lugens* (Stål.) (Hemiptera: Delphacidae). The authors concluded that elevated CO<sub>2</sub> can strongly affect the symbiotic association by decreasing the number of YLS in brachypterous females. In this study, we found that heavier *M. scutellaris* females tend to harbor more YLS under eCO<sub>2</sub> and our results underscore the importance of the interaction of both CO<sub>2</sub> levels with insect weight, in shaping the abundance of YLS.

The increase of YLS in heavier females under eCO<sub>2</sub> conditions is interesting, as previous studies on *N. lugens* showed that nymphs presented higher nutritional requirements (e.g amino acids) to respond to elevated concentrations of CO<sub>2</sub> (Zhao et al., 2020). Also, multiple studies have revealed that eCO<sub>2</sub> levels have an indirect impact on arthropod performance through alterations in plant chemical composition, particularly increasing carbon:nitrogen ratio (Taub et al., 2008; Carreras Navarro et al., 2020).

The implications of eCO<sub>2</sub> for arthropod herbivore performance were evidenced through a meta-analysis of different insect orders and feeding guilds performed by Robinson et al. (2012). Their results showed a



**Fig. 1.** Predicted model of YLS considering insect weight (mg) as explanatory variables and its interaction with the categorical variable CO<sub>2</sub> treatments, which are indicated in two colors. Curves and confidence bands (95%) came from generalized linear models.



**Fig. 2.** Age structure of *Megamelus scutellaris* populations after 92 days. Bars represent the total number of individuals of each stage (nymph instars and adults) for each treatment expressed as mean ± 1 Standard Error. Bars with different letters mean significant differences ( $p < 0.05$ , GLMM).

**Table 2**

Age structure of *Megamelus scutellaris* populations after 92 days. Mean (±Standard Error) Minimum and Maximum and Total number of YLS.

CO <sub>2</sub> treatment	Instar I	Instar II	Instar III	Instar IV	Instar V	Adult	Total
400 ppm	3.6 ± 0.9 (1–10) n = 12	11.4 ± 2.6 (1–30) n = 12	25.8 ± 6.0 (6–59) n = 12	59.2 ± 12.9 (15–139) n = 12	91.4 ± 21.6 (30–284) n = 12	109.8 ± 42.9 (24–569) n = 12	50.2 ± 9.3 n = 72
800 ppm	5.0 ± 1.0 (0–10) n = 9	21.1 ± 5.9 (2–53) n = 9	46.8 ± 9.7 (6–89) n = 9	85.2 ± 21.8 (21–228) n = 9	121.9 ± 22.9 (50–268) n = 9	164.4 ± 45.1 (28–371) n = 9	74.1 ± 11.8 n = 54
	43	137	309	710	1097	1317	3613
	45	190	421	767	1097	1480	4000

**Table 3**

Fixed-factors’ estimated parameters (Number of individuals), deviations and  $P$  values from the generalized linear mixed model (GLMM), from age structure of *Megamelus scutellaris* after the 92-day eCO<sub>2</sub> experiment. Significant differences are indicated with \*.

Fixed factor	Estimate	Std. Error	z value	P
Intercept	4.44	0.22	20.10	< 2e-16*
eCO <sub>2</sub>	0.52	0.34	1.54	0.12
Instar I	-3.24	0.25	-12.78	< 2e-16*
Instar II	-2.17	0.22	-9.96	< 2e-16*
Instar III	-1.41	0.21	-6.81	9.48e-12*
Instar IV	-0.58	0.20	-2.90	0.00379*
Instar V	-0.08	0.20	-0.43	0.67
CO <sub>2</sub> treatment x Instar I	-0.29	0.37	-0.77	0.44
CO <sub>2</sub> treatment x Instar II	0.010	0.32	0.02	0.99
CO <sub>2</sub> treatment x Instar III	0.10	0.31	0.32	0.75
CO <sub>2</sub> treatment x Instar IV	-0.16	0.31	-0.52	0.60
CO <sub>2</sub> treatment x Instar V	-0.17	0.30	-0.56	0.57

significant decrease in the relative growth rate under eCO<sub>2</sub>. However, while fecundity decreased for Coleoptera, Lepidoptera, and Orthoptera, it significantly increased in Hemiptera (alongside other biological parameters). Although there are usually positive responses from Hemiptera to eCO<sub>2</sub> conditions, these responses are frequently “species-specific” and it is hard to make a generalization. In aphids, for example, the response to eCO<sub>2</sub> in terms of fecundity, development, and population

**Table 4**

Fixed-factors’ estimated parameters (Number of adults), deviations and  $P$  values from the generalized linear mixed model (GLMM), from the *Megamelus scutellaris* adult abundance related to sex after the 92-day eCO<sub>2</sub> experiment. Significant differences are indicated with \*.

Fixed factor	Estimate	Std. Error	z value	P
Intercept	3.40	0.26	13.23	< 2e-16*
CO <sub>2</sub> treatment	0.11	0.39	0.29	0.77
Sex (0-females, 1-males)	0.33	0.09	3.67	0.000252*
CO <sub>2</sub> treatment x Sex (males)	0.031	0.14	0.23	0.82

growth, differed between different species, different hosts, or even different genotypes of the same host (Hughes and Bazzaz, 2001; Newman et al., 2003). Studies on planthoppers showed the positive effect of eCO<sub>2</sub> on their performance. For example, *N. lugens* increased their feeding rate and fecundity under eCO<sub>2</sub> conditions which resulted in a significant increase in its population (Guru-Pirasana-Pandi et al., 2018; Wen et al., 2019). Interestingly, when this species was exposed to three CO<sub>2</sub> levels (ambient, gradual increase, and abrupt increase) the fecundity of insects reared in the gradual CO<sub>2</sub> treatment was significantly higher than that in the other treatments (Liu et al., 2020). Furthermore, Wang et al. (2020) found that climatic treatment (ambient versus elevated temperature and CO<sub>2</sub>) generated variable effects on life-history



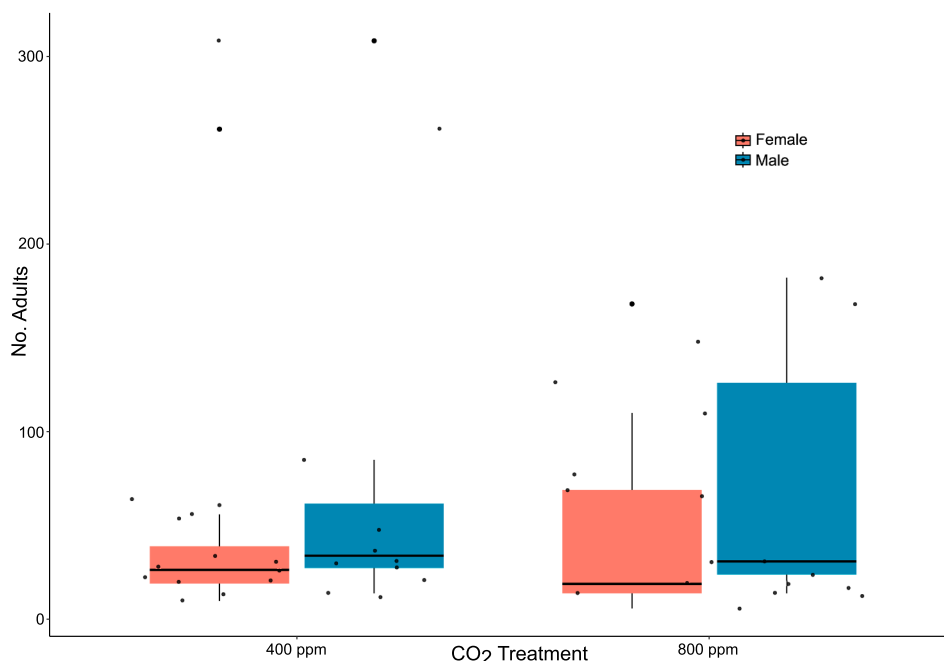


Fig. 3. Number of *Megamelus scutellaris* adults of each sex and CO<sub>2</sub> treatment after 93 days.

traits in one rice delphacid planthopper species but not on another under intraspecific and interspecific crowding.

In our study, we found a significant increase in the total abundance of insects for instars III to V and adults under eCO<sub>2</sub> conditions, after 92 days of experiment. This evidence suggests that eCO<sub>2</sub> conditions may exhibit a positive effect on *M. scutellaris* populations. However, no differences in the sex ratio between treatments were observed, as the male number was significantly higher than the number of females under both CO<sub>2</sub> conditions. This contrasts with the results obtained by [Guru-Pirassanna-Pandi \(2018\)](#) for another species of planthopper (*N. lugens*), where the authors found a significantly higher number of females under eCO<sub>2</sub>, than under cCO<sub>2</sub>.

Climate change has the potential to diminish the effectiveness of biocontrol methods and facilitate the invasion of plants, which could lead to significant economic and health implications ([Sun et al., 2022](#)). Elevated CO<sub>2</sub> levels (800 ppm) lead to increased plant biomass, higher leaf carbon-to-nitrogen ratios, and a reduction in feeding damage by biocontrol agents, making biological control of aquatic plants a challenge for future scenarios ([Baso et al., 2021](#)). However, our results showed that for *M. scutellaris* this must not be the case, providing valuable insights into the potential impact of eCO<sub>2</sub> on the number of YLS and the abundance of this biocontrol agent. Understanding the mechanisms driving insect-endosymbiotic associations has implications for the ecology and population dynamics of *M. scutellaris* and contributes to a broader understanding of insect-endosymbiont interactions under changing environmental conditions and improve the management of water hyacinth.

## 5. Conclusion

Our study highlights the complexity of species-specific responses to climatic changes and the need for a better understanding of the mechanisms driving such responses, especially on important biocontrol agents. Elevated CO<sub>2</sub> resulted in an increased adult population and heavier insects with higher numbers of YLS. It can thus be concluded that eCO<sub>2</sub> has a positive influence on *M. scutellaris*' YLS, increasing their populations and consequently enhancing *P. crassipes* control. However, we suggest further investigation to continue unveiling the mechanisms involved in the interactions between *M. scutellaris*, its YLS, and elevated

CO<sub>2</sub>, as well as other climatic changes.

## CRediT authorship contribution statement

**Righetti Tomás:** Conceptualization, Investigation, Writing – original draft, Visualization, Data curation. **de la Fuente Daniela:** Investigation, Writing – original draft. **K. Paper Matthew:** Investigation, Writing – review & editing. **E. Brentassi María:** Supervision, Writing – original draft. **P. Hill Martin:** Funding acquisition, Writing – review & editing. **A. Coetzee Julie:** Writing – review & editing. **A. Salinas Nicolás:** Investigation, Writing – review & editing. **A. Bruzzone Octavio:** Writing – review & editing. **J. Sosa Alejandro:** Conceptualization, Funding acquisition, Methodology, Formal analysis, Supervision, Writing – original draft.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT 3.5 / OpenAI in order to improve English. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2023.105433>.

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