Feeding and reproduction of a tropical coastal copepod across warming and copper gradients

Running title: Thermal modulation of copper effects

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Abstract

Tropical coastal ecosystems in Southeast Asia are facing rapid warming and increasing pollution. Shallow coastal waters now frequently exceed 34 °C during marine heatwaves, potentially pushing tropical ectotherms beyond their thermal optimum while they are simultaneously exposed to copper (Cu) contamination, especially from aquaculture and shipping activities. However, how warming alters Cu toxicity in dominant tropical zooplankton remains poorly understood. We examined the effects of Cu (0 - 40 µg L^{-1}) across a realistic temperature gradient (26 - 35 °C) on the calanoid copepod *Pseudodiaptomus annandalei*, a dominant grazer of coastal plankton communities. Adult survival, cumulative faecal pellet production (as a proxy for energy intake), and cumulative nauplii production were quantified over seven days. No significant effects of temperature or Cu on adult survival were detected, likely reflecting age-dependent variability among wild-collected individuals. In contrast, temperature strongly structured feeding and reproductive performance, which peaked at 29 - 32 °C and declined at 35 °C. Cu exposure alone had no significant effects at 26 - 32 °C due to high variability in responses. At 35 °C, however, cumulative nauplii production decreased significantly at 30 µg Cu L^{-1} but increased at 20 µg Cu L^{-1}, while faecal pellet production was reduced in Cu-exposed copepods. These findings indicate that warming can modify contaminant effects in tropical zooplankton and highlight the importance of incorporating realistic thermal regimes and natural population variability into ecological risk assessments under climate change.

Keywords: Energy allocation trade-offs, multiple stressors, reproduction, tropical coastal ecosystems, temperature-dependent toxicity

1. Introduction

Tropical coastal ecosystems are increasingly exposed to multiple, overlapping anthropogenic stressors (Halpern et al., 2025, Barlow et al., 2018, Dinh, 2019). Rapid expansion of aquaculture, urbanization, and industrialization in Southeast Asia has resulted in substantial pollutant inputs to estuarine and nearshore environments (Crain et al., 2008, Halpern et al., 2025, Dinh, 2019). Among these pollutants, copper (Cu) is of particular concern due to its widespread use in antifouling paints, algaecides, and aquaculture practices (U.S. Environmental Protection Agency, 2009, Ebenezer et al., 2014). Although Cu rapidly adsorbs to particles and sediments, recurrent inputs from aquaculture and shipping activities may result in chronic exposure for planktonic organisms.

Tropical marine ecosystems are increasingly affected by climate warming and extreme temperature events (Lee et al., 2023, Smale et al., 2019, Gruber et al., 2021, Yao and Wang, 2021). Marine heatwaves (MHWs) have become more frequent, longer-lasting, and more intense over the past decades (Frölicher et al., 2018, Smale et al., 2019). In shallow coastal waters of Southeast Asia, temperatures of 34 °C or higher are frequently recorded during warm seasons (Doan et al., 2018). Tropical marine ectotherms typically live close to their upper thermal limits (Tewksbury et al., 2008, Deutsch et al., 2008). Consequently, even small increases above their thermal optimum may result in disproportionate declines in survival, growth, and reproduction (Nguyen et al., 2011, Doan et al., 2019).

Importantly, warming and pollutants can interact in non-additive manners, resulting in an intensifying or alleviating combined effects (Crain et al., 2008, Dinh et al., 2022). Understanding how warming interacts with contaminants has become a major challenge in aquatic ecology and ecotoxicology in the past two decades (Sokolova and Lannig, 2008, Noyes et al., 2009, Jackson et al., 2016, Dinh et al., 2022). Most studies have focused on temperate species, and are limited to two stressor levels (e.g., the absence *vs.* presence), which do not allow for testing for the non-linear ecological responses (Dinh et al., 2022, Orr et al., 2024), even ecological surprises (e.g., mass mortality Darling and Côté, 2008, Garrabou et al., 2009), especially at sub- and or supra-optimal thermal conditions (Dinh et al., 2022, Orr et al., 2024). Verheyen and Stoks (, 2023) showed that pesticide toxicity was minimal near optimal temperatures but increased at both lower and higher thermal extremes. In addition, most laboratory experiments rely on highly standardized cohorts of organisms, which may not fully capture the demographic variability typical of natural populations (Rohr et al., 2016). Differences in age, reproductive stage, and physiological condition can influence organismal responses to environmental

stressors, potentially altering the magnitude or detectability of contaminant effects under realistic ecological conditions (Muyssen and Janssen, 2007, Dinh et al., 2022, Schmid et al., 2023, Rohr et al., 2016). This may complicate extrapolation from laboratory assays to natural populations (Rohr et al., 2016, Dinh et al., 2022).

Despite growing recognition of temperature-dependent toxicity, few studies have evaluated contaminant effects across a realistic thermal gradient (but see e.g., Verheyen and Stoks, 2023). This gap is particularly relevant for short-lived planktonic organisms such as copepods, which form the foundation of coastal food webs (Chew et al., 2012). Calanoid copepods of the genus *Pseudodiaptomus* are dominant components of tropical plankton communities and play a critical role in transferring primary production to higher trophic levels (Truong et al., 2022, Truong et al., 2014, Chew et al., 2012). Because of their short generation time (5–12 days depending on temperature; Doan et al., 2019, Nguyen et al., 2020), changes in survival or reproduction can rapidly alter population dynamics and ecosystem functioning.

In this study, we investigated the sensitivity of *Pseudodiaptomus annandalei* to a gradient of temperatures (26 - 35 °C) and copper concentrations (0 - 40 µg L^{-1}) over seven days. By quantifying adult survival, cumulative faecal pellet production (as a proxy for food intake and energy assimilation, Isla et al., 2008), and cumulative nauplii production, we aimed to evaluate how warming and Cu exposure affect energy allocation on survival and reproduction, two of the most critical parameters for population growth. Specifically, we addressed three hypotheses (H1-H3): **H1)** Increasing temperature beyond the optimal range reduces feeding activity and reproductive output of *P. annandalei*, with stronger effects on reproduction than on survival. **H2)** Exposure to elevated copper concentrations reduces survival, feeding, and reproductive performance of *P. annandalei*, particularly at higher Cu levels. **H3)** Combined exposure to elevated temperature and copper results in stronger negative effects than either stressor alone, reflecting increased physiological costs under heat stress. By linking feeding activity and reproduction within an energy allocation framework, this study provides insights into how warming and pollution may interact in tropical coastal ecosystems.

2. Materials and methods

2.1 Pseudodiaptomus annandalei

Pseudodiaptomus annandalei is a dominant calanoid copepod occurring along the central coast of Vietnam and Southeast Asian regions (Truong et al., 2022, Truong et al., 2014, Chew et al., 2012). Adult males and females of *P. annandalei* were collected from an aquaculture pond in the Cam Ranh Centre for Tropical Marine

93 Research and Aquaculture in the summer, using a zooplankton net (mesh size = 200 μm). The sampling site
94 was located at 11°49'26.3"B, 109°07'23.9"E. At the time of sampling, the water temperature and salinity of
95 the pond were 26 °C and 29 PSU, respectively.

96 *2.2 Copper*

97 Copper stock solutions were prepared from $CuSO_4$ (purity >99%, Sigma-Aldrich, Søborg, Denmark) following
98 a method similar to that described by Dinh et al. (, 2021, 2020). Copper concentrations in the control treatment
99 (hereafter referred to as the control) were below the detection limit (1 μg L^{-1}, ICP-MS).

100 *2.3 Experimental design and setup*

101 To test the sensitivity of *P. annandalei* to a range of temperatures and copper exposure, adult copepods were
102 subjected to a fully orthogonal experimental design comprising 20 treatments: 5 copper concentrations
103 (control, 10, 20, 30, and 40 μg L^{-1}) × 4 temperatures (26, 29, 32, and 35°C) (Figure 1). The Cu concentrations
104 varies from ecologically relevant to high levels, but such a range of Cu levels has been found in the coastal
105 waters in Vietnam and worldwide (Jonathan et al., 2011, Teasdale et al., 2003, Marcussen et al., 2014). These
106 temperatures were chosen to cover typical temperature ranges in the shallow coastal water in the South of
107 Vietnam, where the study species is abundant (Doan et al., 2018). Each treatment was replicated ten times,
108 resulting in a total of 100 experimental units (50-ml Falcon tubes).

109 Each replicate consisted of one egg-carrying female and one male *P. annandalei* placed in a 50 mL
110 Falcon tube containing 20 mL of seawater with the designated copper concentration. The tubes were placed in
111 temperature-controlled water baths maintained using aquarium heaters. All copepods were fed with *Isochrysis*
112 *galbana* at a concentration of 30,000–33,000 cells L^{-1} (equivalent to 800–850 μg C L^{-1}; Berggreen et al., 1988;
113 Doan et al., 2018). Throughout the experiment, the salinity, photoperiod, and dissolved oxygen concentration
114 were maintained at 30 PSU, 12 h light : 12 h dark, and 5–6 mg L^{-1}, respectively. The contents of each tube
115 were replaced daily with freshly prepared solutions to maintain consistent experimental conditions. Over seven
116 consecutive days, adult survival, cumulative nauplii, and cumulative faecal pellets were recorded daily.

117 To determine the survival of adult *P. annandalei*, cumulative nauplii production, and cumulative faecal
118 pellets, the contents in each bottle were filtered every day (mesh size = 25 μm). The dead copepods were
119 removed from the tube, and their sex was identified. Alive males and females were then returned to the tube.
120 The remaining contents, containing nauplii and pellets, were transferred to 12-well plates, fixed with Lugol

121 4%. Numbers of nauplii and faecal pellets were counted using a stereo-microscope (SZ51, Olympus, Japan).
122 The number of alive females and males, and the total number of nauplii and faecal pellets of each tube in seven
123 days, were used for statistical analysis.

124 *2.4 Statistical analyses*

125 Statistical analyses were conducted in R (version 4.4.2). The survival of males and females were analysed
126 using generalized linear mixed models (GLMMs) with a binomial error distribution and logit link function.
127 Temperature, copper, and their interaction were fitted as fixed effects. The significance of fixed effects was
128 evaluated using likelihood ratio tests. Cumulative nauplii production and cumulative faecal pellets were
129 analysed using a generalized linear model with a negative binomial error distribution as their counts showed
130 overdispersion under a Poisson model (dispersion ~15). A log link function to ensure positive fitted values
131 appropriate for cumulative count data (Truong et al., 2022). Temperature, copper, and their interaction were
132 fixed factors. Model significance was assessed using likelihood ratio chi-square tests (Truong et al., 2022).
133

134 **3. Results and discussion**

135 *3.1 Effects of temperature on P. annandalei*

136 Survival did not differ among treatments during this short-term exposure (Table 1, Figures 2 and 3). In our
137 previous studies, temperatures of 34 - 35 °C caused reductions in survival when the exposure duration lasted
138 for an entire one or two generations of *P. incisus* a congeneric species (Dinh et al., 2021), suggesting a species
139 specific vulnerability and also the short-term tolerance. In a short-term exposure, copepods may prioritize
140 maintenance metabolism to preserve survival under acute heat stress (Sokolova, 2013). Such prioritization
141 often comes at energetic costs, which are often manifested in energy and resources limitation (Metcalfe, 2025,
142 Sokolova, 2013, Saiz et al., 2022). The reduction in feeding activity at 35 °C suggests (see below) that energy
143 intake did not fully match the elevated metabolic demand associated with warming (Metcalfe, 2025, Sokolova,
144 2013, Saiz et al., 2022). Under these conditions, available energy is preferentially allocated to essential
145 maintenance processes, such as upregulation of heat shock protein, and coping with oxidative damage (Harada
146 et al., 2019, Low et al., 2018), while energetically expensive processes are constrained.

147 Acute heat stress significantly reduced the cumulative nauplii and faecal pellet production of *P.*
148 *annandalei*. Highest cumulative nauplii produced by *P. annandalei* was found at 32 °C, which is approximately

149 11% higher than those at 29 °C, and 26% higher than the lowest ones at 26 °C and 35 °C (Table 1, Fig. 4).
150 Similarly, the cumulative number of pellets per individual was highest at 32 °C, approximately 10% higher
151 than at 35 °C and 16% higher than those at 26 °C and 29 °C (Table 1, Figure 5). Our results support H1 as the
152 unimodal pattern in feeding and nauplii production suggests that 29 - 32 °C approximates the optimal thermal
153 range under the present experimental conditions. As faecal pellet production scales with ingestion and carbon
154 assimilation, it provides a proxy for energy intake (Isla et al., 2008, Dinh et al., 2019), suggesting a higher
155 energy acquisition than at both sup- or supra-temperatures. Temperature of 35 °C likely approaches the upper
156 performance boundary, which is in agreement with general findings of the vulnerability of tropical ectotherms
157 to temperature extremes (Tewksbury et al., 2008, Deutsch et al., 2008, Grønning et al., 2019). A decline in
158 faecal pellet production at 35 °C relative to the peak at 32 °C suggests that feeding activity may begin to
159 decrease as temperatures approach the upper thermal boundary, a pattern has been observed in previous studies
160 (Doan et al., 2018, Doan et al., 2019, Saiz et al., 2022). This creates a double energetic constraint: increased
161 expenditure combined with reduced intake efficiency, that may partly contribute to reduced reproduction
162 (Sokolova and Lannig, 2008, Holmstrup et al., 2010, Jackson et al., 2016, Dinh et al., 2022).
163
164 *3.2 Temperature dependent Cu-toxicity in P. annandalei*
165 Contrary to H2, Cu exposure did not significantly affect the survival of male or female *P. annandalei* during
166 the seven-day experiment (Table 1, Figures 2 and 3). Survival varied considerably among temperature × Cu
167 treatments, and in some cases the lowest survival occurred in the control while the highest survival was
168 observed at the highest Cu concentration (Figures 2 and 3). Such patterns suggest that variability in the
169 physiological condition or age structure may have influenced mortality during the experiment (Saiz et al.,
170 2015, Truong et al., 2020). Age-specific mortality is common in field-collected copepods and can obscure
171 treatment effects when individuals of different physiological states are used. This variability likely masked
172 potential effects of temperature and Cu exposure on survival. In our previous studies, we found the strong
173 effects of both temperature extreme and Cu on the survival of *P. annandalei* and *P. incisus* at comparable
174 temperatures and Cu concentrations (Dinh et al., 2021, Dinh et al., 2020).
175 High variabilities of treatment-specific responses of *P. annandalei* were also observed for cumulative
176 nauplii and faecal pellet production, especially at temperatures of 26 and 29 °C, that may be explained by
177 similar age-specific performance (Saiz et al., 2015, Truong et al., 2020). However, under extreme temperature,

178 the interactive effects of temperature and Cu on these parameters emerged, but only partially supporting H3.
179 At 32°C, Cu-exposed *P. annandalei* had lower cumulative nauplii and faecal pellet production, while at 35 °C,
180 the effects of Cu on reproductive output were highly complicated and concentration-dependent, generating
181 interactive effects of Temperature and Cu (Table 1, Figures 4 and 5). Specifically, at 35 °C, cumulative nauplii
182 production was reduced by 40% at 30 µg Cu L^{-1} compared to the corresponding value at 32 °C, and , and
183 approximately 24% lower than the control treatment at one at 35 °C, yet at 20 µg Cu L^{-1} it was higher than in
184 the control treatment at 35 °C and comparable to that observed at 32 °C (Figure 4, Table 1).

185 For faecal pellet production, *P. anndanalei* showed an average number lower at 35 °C than at 32 °C,
186 yet this difference was largely driven by reduced pellet production in the control treatment while in Cu-exposed
187 copepods, pellet production remained similar between the two temperatures (Figure 5, Table 1). Cu-induced
188 energetic constrains may be even more severe under the combined stressor exposures (Sokolova and Lannig
189 2008, Sokolova, 2013, Holmstrup et al. 2010). At 35 °C, Cu could further increase oxidative damage and
190 demands additional ATP for detoxification processes such as metallothionein synthesis and antioxidant
191 (Harada et al., 2019). Maintenance and detoxification costs could rise simultaneously (Pörtner, 2010, Sokolova
192 et al., 2012, Lushchak, 2011) that could result in the energy demand exceeding the supply, reproduction is
193 compromised before survival. This energetic trade-off is consistent with our previous findings, where
194 combined Cu and MHW exposures reduced fecundity (Dinh et al., 2021, Dinh et al., 2020). We are cautious
195 of a possibility that variability in male survival influenced reproductive output in some treatments (Kiørboe,
196 2007, Gusmao and McKinnon, 2009). For example, the loss of males may reduce mating success and
197 consequently limit nauplii production. However, this mechanism cannot be confirmed with the present data
198 and requires further investigation.

199 Importantly, our results also suggest that Cu did not shift the thermal optimum but narrowed
200 performance breadth by steepening the decline at the upper thermal limit. This supports the principle
201 articulated in recent thermal performance curve-based ecotoxicology studies: contaminant toxicity is minimal
202 near the thermal optimum but increases toward thermal extremes (Verheyen and Stoks, 2023). In tropical
203 systems, where organisms already live close to their upper thermal limits (Deutsch et al., 2008), even small
204 temperature increases may amplify contaminant effects. Reproduction emerged as the most sensitive endpoint.
205 Embryonic development is energetically demanding and particularly vulnerable to oxidative stress from

extreme temperatures and pollutants, increasing embryonic mortality, lowering hatching success and resulting in reduced nauplii production (Grønning et al., 2019, Dinh et al., 2021).

3.4 Implications for ecological risk assessments and conclusions

Our findings have several implications for understanding and managing the impacts of multiple environmental stressors in tropical coastal ecosystems. In particular, they provide empirical support for the growing recognition that contaminant effects cannot be evaluated independently of environmental temperature, a central issue in ecotoxicology under global change (Moe et al., 2013, Sokolova and Lannig, 2008, Holmstrup et al., 2010, Dinh et al., 2022, Noyes et al., 2025). The influence of Cu on reproductive output became evident near the upper thermal boundary, suggesting that contaminant toxicity may only emerge under particular environmental contexts, such as during marine heatwaves or other extreme events. Consequently, ecological risk assessments based on single-temperature toxicity tests may underestimate the vulnerability of tropical marine organisms under future warming scenarios. The copepods of the genus *Pseudodiaptomus* play a central role in tropical coastal food webs by transferring primary production from phytoplankton to higher trophic levels, including fish larvae and juvenile stages (Chew et al., 2012). Changes in feeding activity and reproductive output can therefore propagate through the food web and influence energy transfer in coastal ecosystems (Chew et al., 2012, Turner, 2004).

Finally, the relatively high variability observed among treatments likely reflects the natural heterogeneity of wild populations (Blanda et al., 2015, Grønning et al., 2019). Field-collected copepods may differ in age, reproductive stage, and physiological condition, all of which can influence their responses to environmental stressors (Muyssen and Janssen, 2007, Dinh et al., 2022, Schmid et al., 2023, Rohr et al., 2016). While such variability can obscure treatment effects in short-term laboratory experiments, it may better represent the demographic structure of natural populations. In this context, our results suggest that environmentally realistic population variability may mask stressor effects that could become more pronounced under extreme environmental conditions, further challenging the ecological risk assessments. Therefore, combining controlled laboratory studies with standardised life stages and physiological conditions, with ecologically realistic experiments, and field observations will be necessary to improve predictions of how multiple stressors influence tropical marine ecosystems under ongoing global change (Dinh et al., 2022).

Funding

We thank the support of The Ministry of Education and Training, project number B2024-TSN-15.

Acknowledgments

We thank Nha Trang University for supporting facilities to conduct this study and to implement the project B2024-TSN-15.

Conflict of interest

None.

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430 **Table 1**. The results of the statistical analyses testing effects of heat stress (Temp) and copper concentration

431 (Cu) on survival of males and females, cumulative nauplii, and cumulative fecal pellets of *Pseudodiaptomus*

432 *annandalei*. Significant *P* values are signed in bold.

Effects	Male survival			Female survival			Cumulative nauplii production			Cumulative faecal pellet production		
	df1, df2	F	*P*	df1, df2	*F*	*P*	df	χ^2	*P*	df	χ^2	*P*
Temp	3, 180	2.493	0.075	3, 180	0.226	0.878	3	11.806	**0.008**	3	13.679	**0.003**
Cu	4, 180	0.389	0.610	4, 180	1.637	0.167	4	6.153	0.188	4	3.794	0.434
Temp × Cu	12, 180	1.366	0.184	12, 180	1.254	0.249	12	29.650	**0.003**	12	47.788	**<0.001**

433

434

435 **Figure legends**

436 **Figure 1**. Experimental design used to test the effects of temperature (26, 29, 32, and 35 °C) and copper
437 concentrations (0, 10, 20, 30 and 40 µg L^{-1}) on the tropical copepod *Pseudodiaptomus annandalei*.

438 **Figure 2**. Effects of temperature and copper on male survival of *Pseudodiaptomus annandalei*. Data are
439 visualized as mean ± SEs (n = 10 replications).

440 **Figure 3**. Effects of temperature and copper on female survival of *Pseudodiaptomus annandalei*. Data are
441 visualised as mean ± SEs (n = 10 replications).

442 **Figure 4**. Effects of temperature and copper on cumulative nauplii of *Pseudodiaptomus annandalei*. Data are
443 visualized as mean ± SEs (n = 10 replications).

444 **Figure 5**. Effects of temperature and copper on cumulative fecal pellets of *Pseudodiaptomus annandalei*. Data
445 are visualized as mean ± SEs (n = 10 replications).

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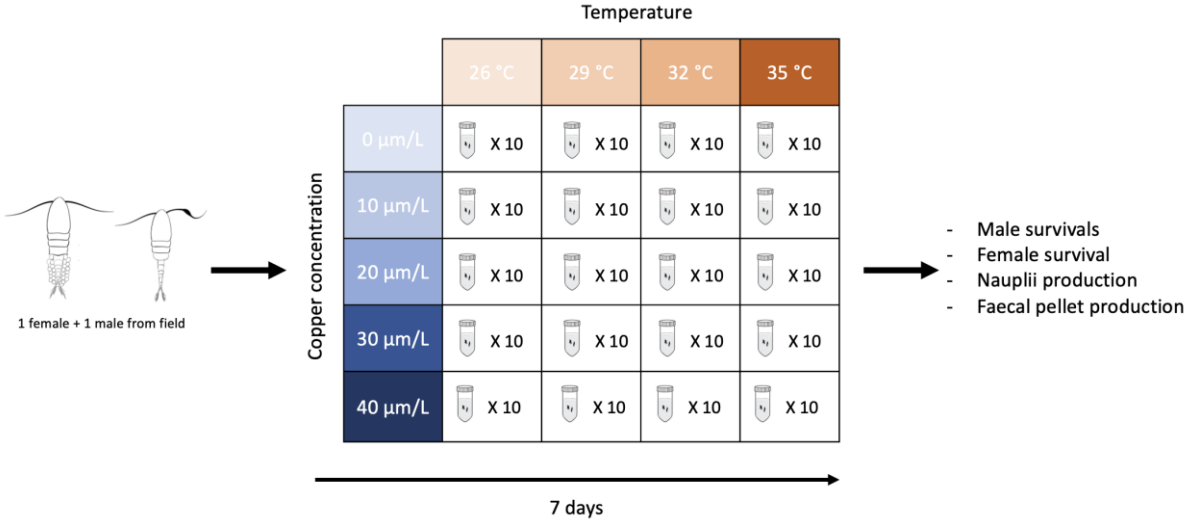

447

448 **Figure 1**

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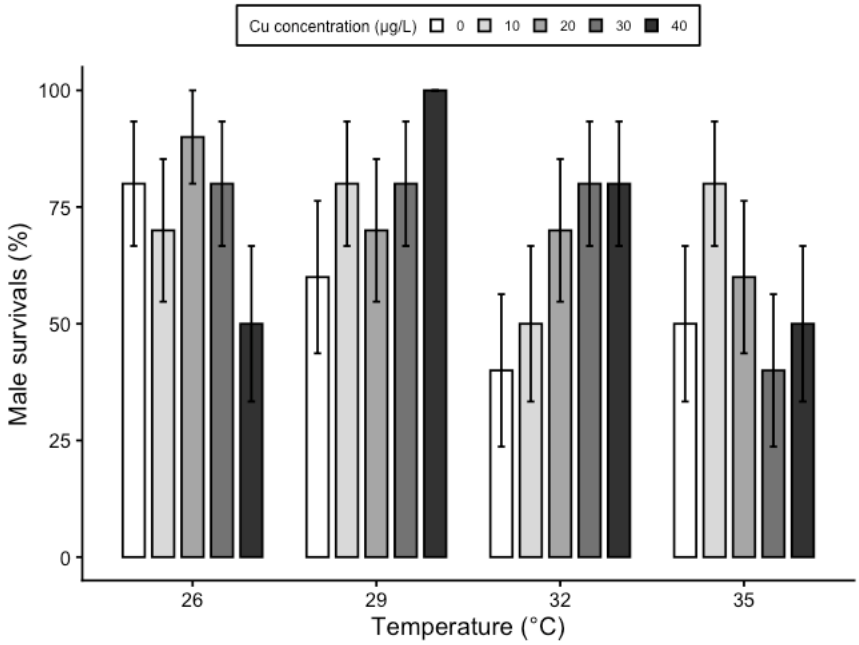

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452 **Figure 2**.

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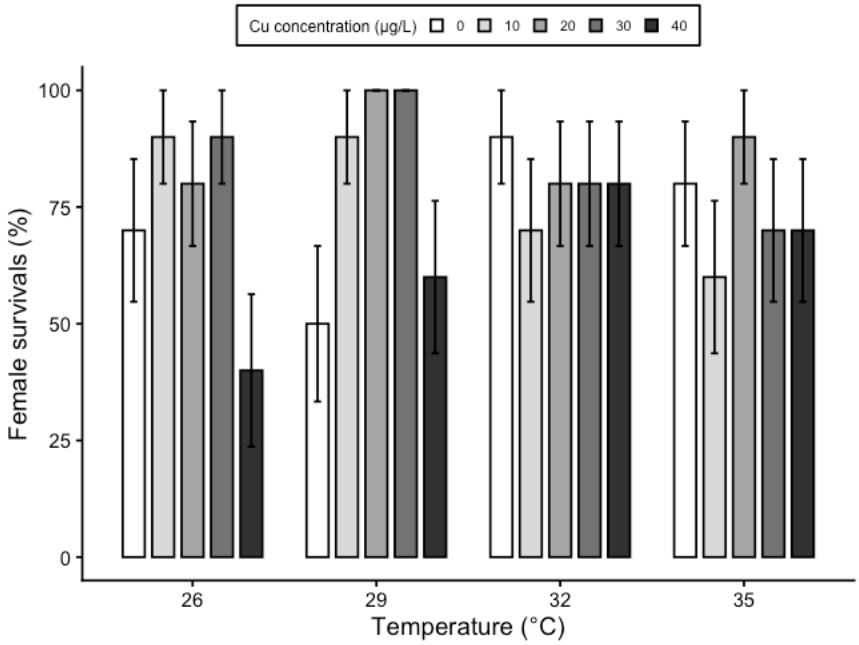

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456 **Figure 3**.

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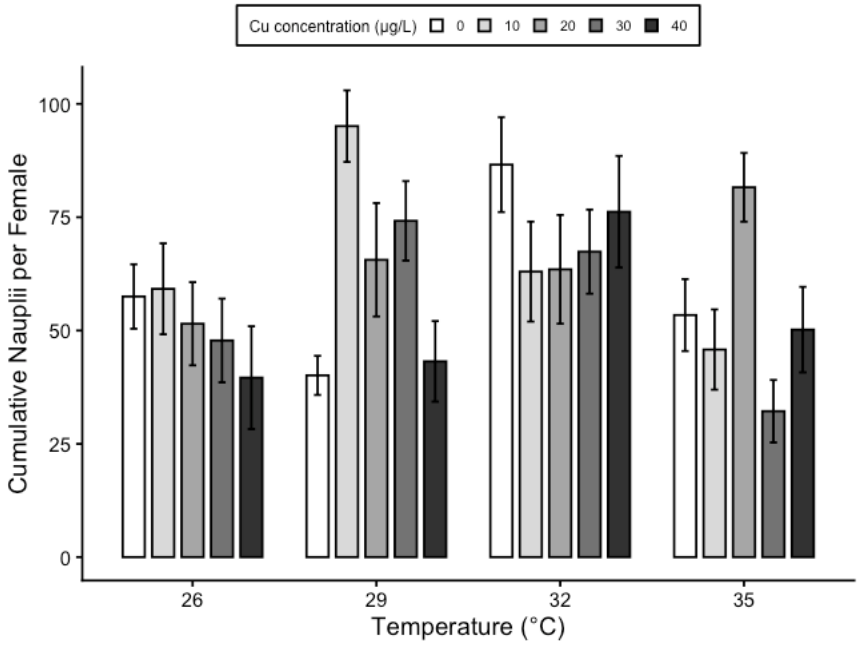

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460 **Figure 4**.

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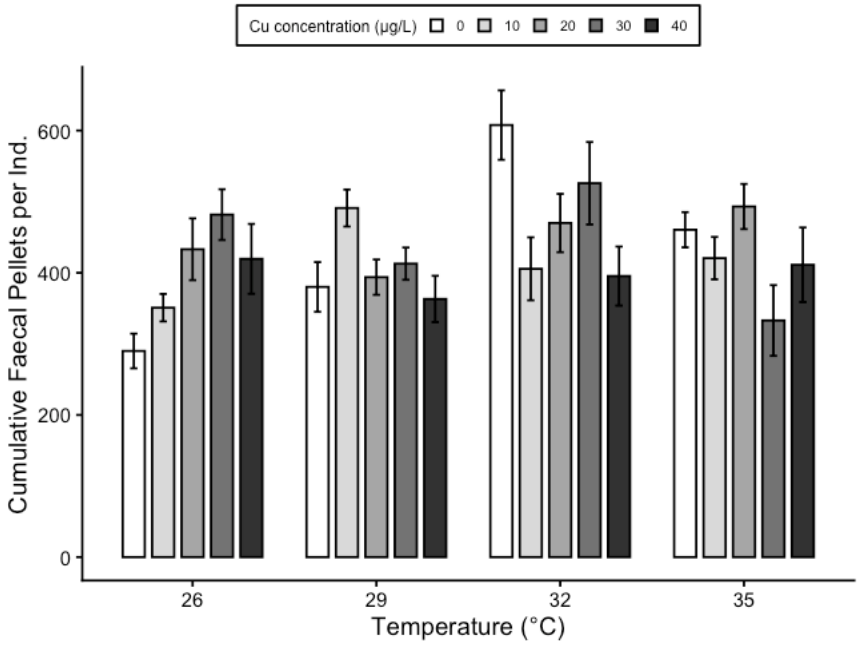

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465 **Figure 5**.