Temperature induced changes in hatching size of a tropical snail occur during oogenesis and can persist for several weeks

Short title: **Temperature effect on hatching size**

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ABSTRACT

It is accepted that temperature affects offspring size in ectotherms. However, the processes that result in temperature induced changes are not well understood. We sought to determine when temperature changes during development induce changes in hatching size, and how long hatchlings reflect the thermal experiences of their mother. Juveniles of the common tropical slipper snail, *Crepidula* cf. *marginalis* were collected at Playa Venado, Panama and raised in the laboratory at either 24°C or 28°C, temperatures experienced in nature, and were reciprocally moved between the two temperatures. In the first experiment, the animals were moved immediately after oviposition to determine whether temperatures experienced during oogenesis or embryogenesis contribute to differences in hatching size. The second experiment transplanted animals between the same two temperatures after the first brood hatched. The subsequent three broods were measured to determine how long the legacy of the first temperature persists. We found that (i) the temperature the mother experienced during oogenesis significantly affects hatching size, whereas the temperature experienced during embryogenesis does not; and (ii) hatching size is impacted for at least two broods after a change in temperature (≥17 days). These results show hatching size is a legacy of temperatures experienced prior to oviposition and this legacy does not persist for more than two brooding cycles. It remains unclear if this rapid response to environmental temperature is adaptive, or the result of a physiological constraint on oogenesis. Understanding the process whereby temperature influences offspring size will provide insight into the potential for organisms to respond to temperature changes, and ultimately, climate change.

INTRODUCTION

As more published reports demonstrate rising environmental temperatures, warming sea surface temperatures heighten concern for marine life and add urgency to understanding organismal responses to warming. One well known organismal response to temperature is the Temperature-Size Rule (TSR). The TSR refers to the general pattern in which ectotherms mature at smaller sizes with increasing temperature (Atkinson, 1994; Gillooly *et al.,* 2011) and its corollary, that offspring size is inversely related to temperature (Atkinson *et al*., 2001). Not all ectotherms follow the TSR, with some showing no relationship between temperature and size and others showing the reverse pattern, with animals maturing at larger sizes with increasing temperatures (Bernays, 1972; Atkinson, 1994, 1995). However, vastly more organisms conform to the TSR than do not, as demonstrated by a meta-analysis of 61 studies of aquatic ectotherms in which 90% of the studies showed warmer temperatures reduced adult body size (Atkinson, 1995).

Less is known about how the TSR operates in offspring, with the most recent review finding that only 58% of 32 studies demonstrated inverse relationships between maternal temperature and offspring size once maternal size was accounted for (Atkinson *et al*., 2001). There are few studies of offspring TSR in marine invertebrates, although additional recent studies seem to support the pattern. These include significant decreases in egg size with increasing temperatures in the green sea urchin, *Stronglyocentrotus droebachiensis* (Garrido and Barber, 2001) and in various species of slipper limpets (Collin, 2012; Collin and Salazar, 2010; Collin and Spangler, 2012), as well as significant decreases in offspring size with increasing temperatures (Collin, 2012; Collin and Spangler, 2012; Camargo-Cely and Collin, 2019). Field studies conducted in the Bay of Panama, show a consistent pattern with intertidal crabs, intertidal gastropods, and reef fishes producing larger eggs or hatchlings during the colder upwelling season compared to the warmer temperatures of the non-upwelling season. As other environmental conditions co-vary seasonally with temperature, this difference may not be entirely due to seasonal changes in temperature (Robertson and Collin, 2015; Collin and Ochoa, 2016; Collin *et al.,* 2018).

The overall consensus is both offspring size and adult size are negatively correlated with temperature, and this pattern unites organisms from different taxa and environments. Yet, the physiological processes that underlie the TSR are still not well understood. Individual studies

often explain their results in terms of specific adaptation to environmental conditions, but the near universality of the TSR suggests that there might be a common underlying physiological process or mechanism (Blanckenhorn and Llaurens, 2005; Forster *et al*., 2011), and the de-coupling of growth rate from development rate seems to be a likely candidate (van der Have and de Jong, 1996; Zuo *et al*., 2011; Forster *et al.*, 2011; Ernsting and Isaaks, 2000; Steigenga and Fischer, 2007; Geister *et al.*, 2009; Forster *et al*., 2011; Forster and Hirst, 2012). A first step in understanding the physiological process underlying the effect of temperature on offspring size is to (i) identify the developmental period or stage during which temperature induces differences in offspring size and (ii) determine for how long this influence lasts. We took this approach to determine the relative contributions of temperature experienced during oogenesis and temperature experienced during embryogenesis on the hatching size of a tropical marine snail, *Crepidula* cf. *marginalis*. A second experiment was designed to document how long the effect persists on hatching size after a change in temperature. Such lasting effects of temperature have been reported in some insects (e.g., Blanckenhorn, 2000; Fischer *et al*., 2003).

Crepidula cf. *marginalis*, a protandrous, sedentary filter-feeding gastropod, follows the TSR in both adult size and offspring size (Collin, 2012; Camargo-Dely and Collin, 2019). Both its egg size and hatching size are also larger at cooler temperatures (Collin, 2012). These snails are common in the low and mid intertidal in the Bay of Panama, where they are subject to changes in seawater temperature between the upwelling and non-upwelling seasons. The upwelling season is characterized by strong northern wind-jets that displace surface water with deep, colder (23-25°C) water (Wellington and Dunbar, 1995; D'Croz and Robertson, 1997), while the non-upwelling season consists of weak winds, resulting in warmer (27-29°C) water (D'Croz and Robertson, 1997). During non-upwelling, the average water temperature varies very little, but during the transition between upwelling and non-upwelling, and during some periods of the upwelling season, daily average temperatures can change from 23 to 28°C over the course of only a few days. As *C.* cf. *marginalis* reproduce year-round (Collin *et al*., 2017), mothers and developing offspring can experience a range of temperatures.

METHODS

Experimental design

In order to determine the period of development during which hatching size plasticity can be induced by temperature in *C.* cf. *marginalis*, we conducted two experiments by switching snails between two temperature-controlled incubators that were maintained at temperatures within the optimal thermal range (Walczyńska *et al.*, 2016) of *C.* cf. *marginalis*. Both experiments used reciprocal transplants between the same two temperatures, 24°C and 28°C, but changes were implemented at a different stage in the brooding cycle. Each experiment used different animals in the reciprocal temperature change treatments, but due to limitations in incubator space, both experiments shared the same control group of 40 females, half of which were maintained constantly at 24°C and the other half at 28°C. As the response variables did not overlap between the two experiments, this experimental design should not impact the statistical tests.

In the first experiment, 30 experimental females were placed in each starting temperature. Immediately after she laid her first brood, each female was moved to the other temperature, so that oogenesis took place at one temperature while embryogenesis occurred at the other temperature. This experimental design allowed us to determine the relative contribution to hatching size of temperature experienced during oogenesis and temperature experienced during embryogenesis. In the second experiment, we transplanted 80 animals between the two temperatures (40 in each direction) after the first brood hatched. They remained at the new temperature until they had produced four broods in total. This design for the second experiment demonstrated how many broods must be laid before the hatching size matches the hatching size of animals that had not been subjected to a change in temperature.

Experimental procedures

To ensure that previous female mating history did not impact our results, we collected three hundred juvenile or male *Crepidula* cf. *marginalis* (~8-11mm in length) snails in mid-July 2018. Previous work has shown that these sequential hermaphrodites will change sex when grown individually in the laboratory (Collin *et al*., 2005). The snails were collected from the intertidal at Playa Venado, Panama (8.892°N, 79.595°W) near the Pacific coastal town of Veracruz, under permit #SE/APHBO-9-18 issued by the Ministry of Environment of the Republic of Panama. In the laboratory, each animal was placed in a 350 ml plastic cup, filled with UV-treated filtered seawater, and fed 38.6 x 10^6 cells/ml *Isochrysis galbana* strain *T. iso*

five times per week. The water in the cups was changed 3 times per week. The animals were acclimated to laboratory conditions at ~23°C for one month, and then 180 animals were randomly assigned to a treatment and placed in a Thermo Scientific precision low temperature refrigerated incubator set at 24°C or 28°C. After the animals experienced a month at the experimental temperature, a male or juvenile, which had been collected three weeks previously, was added to each cup.

The temperature in each incubator was measured twice daily with an Omega high precision thermocouple. Realized temperatures of the water in the cups over the four months of the experiment were 23.87°C (S.D. = 0.29°C; N = 970) and 28.40°C (S.D. = 0.45°C; N = 970). To minimize temperature fluctuations, we adjusted the temperature of the new water before the water changes, with temperatures averaging 24.41 ± 0.55°C and 28.00 ± 0.65°C. As the entire experiment occurred during the wet season, salinity was around 30 ppt and was not adjusted. Two Thermochron temperature logging iButtons (Maxim Integrated Products) were placed in each incubator to both monitor temperature stability and determine that the incubators did not malfunction over the course of the experiment.

During the experiment, animals were fed a mixed diet of 20 x 10^6 cells/ml *Isochrysis galbana* strain *T. iso* and 3.33 x 10^6 cells/ml *Tetraselmis* sp. daily (following Camargo-Cely and Collin, 2019) and the water was changed 3 times per week. The presence of eggs or the release of larvae was noted every morning and afternoon. When egg masses were expelled, that brood was not counted. Upon hatching, larvae were collected via reverse filtration and preserved in 70% ethanol and the maternal shell length was measured. 15 larvae from each brood were photographed in lateral view using ProgRes CapturePro 2013 at 10x magnification with the Nikon E600 compound microscope. The longest distance across the shell was measured for each larva using Image J. When we were not able to image 15 intact larvae, as many larvae as could be imaged were measured.

Statistical analyses were conducted in JMP 14. Each experiment was analyzed with a single standard least squares ANOVA of hatching size conducted on individual larval size measurements, with female included as a random effect nested inside treatment. Due to the random nested effect, the model was fitted with restricted maximum likelihood (REML). Residuals were checked for approximate normality. Analyses of time to hatching and time between broods were conducted using non-parametric statistics, as these are count data with little

variability that do not approximate a continuous distribution and did not fit the assumptions for linear analyses.

RESULTS

Experiment 1

A fully factorial ANOVA model with the first and second temperatures the female experienced as the factors and with female as a random effect nested within both temperatures was a significant fit to the hatching size data ($R^2 = 0.79$; N = 997; $p<0.0001$; Table 1). 58% of the total variation was attributed to the random effect of female. The first temperature, that is the temperature experienced prior to oviposition, was the only significant factor ($p<0.0001$; Table 1; Figure 1), with females exposed to the cooler temperature during oogenesis producing larger hatchlings (301.5 ± 2.7μm) than those exposed to the warmer temperature (269.9 ± 2.2μm). Females that were raised at 24°C first were significantly larger (19.05 ± 0.26 mm) than those first raised at 28°C (17.58 ± 0.23 mm). Because differences in maternal size is to some extent accounted for in the random effect term, we did not include maternal size in the model. But, post-hoc tests showed that maternal size did not contribute significantly to hatching size when maternal identity was included as a random effect in the model, nor did including it as a factor alter the effect tests reported in Table 1. Treatment also had a significant impact on the time to hatching (Wilcoxon/Kruskal-Wallis test, $\chi^2 = 19.53$, df = 3, $p = 0.0002$; Figure 2). Time to hatching, and therefore development rate, was significantly faster in animals that were at the warmer temperature for the entire experiment (mean = 7.9 ± 0.2 days) compared to the other three treatments (means = 9.5 ± 0.3, 9.0 ± 0.2, 9.1 ± 0.2 days). Pair-wise Wilcoxon non-parametric comparisons were $p<0.005$ for all three comparisons including the 28°C treatment and $p>0.1$ for the other 3 comparisons.

Experiment 2

We conducted a REML ANOVA analysis of the effects on hatching size of treatment (28-28°C, 24-24°C, 28-24°C and 24-28°C), brood number, and their interaction, with female as a random effect nested within treatment. There was a significant random effect of female, which explained 42% of the variation in hatching size, and a significant interaction between treatment and brood number (Table 3; Figure 3; $r^2 = 0.56$; n = 4702). Inclusion of the non-significant

covariate, maternal length, did not alter the conclusions of the effects test. Post-hoc Tukey HSD tests showed that the average hatching size from females raised at constant temperatures did not differ significantly between the four successive broods within each treatment (Table 3; Figure 3). Hatchlings of females raised at 24°C were larger (mean = 298.78 ± 3.75 μm) than those raised at 28°C (mean = 274.58 ± 3.00 μm). The significant interaction was due to changes in hatching size in the two treatments where females were moved between temperatures. The females who moved from the warmer to the cooler temperature after the first brood hatched produced hatchlings from the first and second broods that were the same size (274.77 ± 2.76 μm; 275.70 ± 2.9 μm) as those maintained entirely at 28°C. Hatchlings from the third and fourth broods were significantly larger (295.78 ± 2.98 μm; 300.00 ± 3.5 μm) than the first two broods and did not differ significantly from the hatching size of females maintained at 24°C (Figure 3). Likewise, the females who started at the cooler temperature and were moved to the warmer temperature showed significant changes in hatching size. For the first two broods, the average hatching size (299.50 ± 2.94 μm and 299.59 ± 3.11 μm, respectively) did not differ significantly from the hatching size of the 24°C control. Hatching size from the third brood was significantly smaller than the first two in this treatment (Figure 3). By the fourth brood, hatching size (275.70 ± 3.9 μm) did not differ significantly from those produced by the 28°C control and did differ significantly from the first two broods in the same treatment.

Non-parametric ANOVA of the time to hatching also showed a significant impact of the temperature treatments. The time to hatch did not differ significantly across the four sequential broods in the control 24°C or the control 28°C treatments (9.6 days and 8.1 days, respectively). For each of the two treatments that changed temperature, the time to hatch was significantly different for the first brood at the original temperature compared to each of the subsequent broods that were produced at the second temperature ($p<0.02$ pair-wise Wilcoxon tests), while the broods produced at the second temperature did not differ significantly from each other (T24-28: 9.6, 9.0, 8.6, 8.9 days, respectively; and T28-24: 8.3, 9.3, 9.7, 9.5 days, respectively; Figure 4). In both treatments, the time to hatch was faster for the broods developing at the warmer temperatures. There was no significant difference in the time period between broods across the treatments ($p>0.1$).

DISCUSSION

Our results show that despite faster rates of embryological development at warmer temperatures, hatching size in *C.* cf. *marginalis* is largely the result of temperatures experienced during oogenesis. Experiment 1 demonstrated that temperature-dependent differences in hatching size are due to the temperature experienced by the mother during oogenesis and that the temperature embryos experienced during embryogenesis does not contribute to this difference. This finding appears contrary to many models that suggest the TSR results from differences in the temperature dependence of growth rate and metabolism during embryogenesis (Angilletta *et al.*, 2004; Walters and Hassall, 2006; Zuo *et al.*, 2011). Although temperature-mediated plasticity in *C.* cf. *marginalis* is induced during oogenesis, this finding may be consistent with such models if the earlier induction, rather than the embryonic temperature, results in subsequent changes in growth rate and metabolic rate during embryogenesis. Data from Experiment 2 support the idea that growth rate reflects conditions experienced by the embryos, as the second broods had development rates statistically indistinguishable from the subsequent broods, despite being derived from eggs that developed at the other temperature. However, our data are not adequate to determine if earlier induction causes growth and metabolic rates to change during later embryogenesis, which may also receive some support from Experiment 1 where only broods that were entirely from high temperatures hatched after a shorter time than in the other three combinations. The small differences relative to our observation rates limit our ability to detect very fine scale changes.

Our results are nonetheless consistent with previous observations on several species of *Crepidula* in that egg size in this genus generally conforms to the TSR (Collin, 2012; Collin and Salazar, 2010; Collin and Spangler, 2012) and that in *C.* cf. *marginalis*, specifically, egg size varies from 178 μm at 23°C to 158 μm at 28°C (Collin, 2012). Similar results have been obtained by a number of studies on insect egg size (e.g., Ernsting and Isaaks, 1997; Blanckenhorn, 2000; Fischer *et al.*, 2003; Steigenga and Fischer, 2007). This is the first time that the effects of temperature on oogenesis and embryogenesis have been distinguished in a slipper snail. Few studies have taken this approach, however Geister *et al.* (2009) has demonstrated the significant effects of temperatures experienced during both oogenesis and embryogenesis on the hatching size and biochemical composition in the butterfly *Bicyclus anynana*. In this species, as in many other insects (Ernsting and Isaaks, 1997; Blanckenhorn, 2000; Fischer *et al.*, 2003;

Steigenga and Fischer, 2007), fewer larger eggs are produced at cooler temperatures. Geister *et al.* (2009) concluded that in addition to the effect of temperature on egg size, embryonic development was more efficient at the cooler temperature, resulting in larger, higher quality offspring as compared to smaller offspring with more reserves at the warmer temperature. In contrast, our study found significant effects of temperature during oogenesis but not during embryogenesis, suggesting that differences in hatching size in *C.* cf. *marginalis* can most likely be attributed to differences in egg size and are not modulated by temperature-dependent differences in growth or metabolism of the embryo. However, using egg size or hatching size as a proxy for offspring quality or energy content has its limitations, and a more detailed analysis like that of Geister *et al.* (2009) could provide further a more nuanced view of the impact of temperature on offspring quality.

In contrast to our results and the findings in insect research, the literature on crustaceans show that, in general, egg size exhibits little temperature-mediated plasticity and that differences in size consistent with the TSR appear later in development (Forster and Hirst, 2012). This finding was the case in the *Artemia franciscana*, which showed an inverse TSR early in development which subsequently changed to a postitive TSR in in adults (Forster and Hirst, 2012). In a literature review of crustaceans, they found that this was also generally the case in the ten other species for which they found relevant data. Forster and Hirst (2012) concluded that arthropod groups differ in the developmental stage that is most responsive to temperature, and they infer that this finding demonstrates the TSR is generated by different underlying processes or mechanisms across groups. Differences like these among taxonomic groups demonstrate the importance of studying a variety of taxa and broadening studies to include more non-arthropods like gastropods. Our study of *C.* cf. *marginalis*, the first such study of a marine gastropod, shows a pattern different from crustaceans with both egg size and adult size following the TSR, and also shows the temperature experienced during embryonic development showing no detectable impact on hatching size but a detectable impact on developmental rate.

Our second experiment showed that the response of hatching size to changes in temperature occurs rapidly, and that temperature may have an almost immediate effect on eggs undergoing oogenesis. When females were transplanted from 28°C to 24°C after their first brood hatched, the hatching size had changed by the second brood produced after the transition. In this species, broods are laid shortly after the previous brood hatches, sometimes even the day after

the brood hatches. In our experiment, it took a median of 3 days from when the first brood hatched and the temperature was changed for the second brood to be laid, demonstrating that exposure to a cooler temperature for 3 days in late oogenesis is insufficient to cause a significant change in hatching size. However, 17 days, the median time from hatching of the first brood until deposition of the third brood, was sufficient for hatchlings to attain temperature-specific size. An interesting finding was that the response to a temperature increase seemed to be slower. For females who were moved from the cooler to the warmer temperature, hatching size was fully adjusted to the new temperature by the third brood (or 33 days) after the temperature change, while for females who were moved from the warmer to the cooler temperature, hatching size was fully adjusted by the second brood (or 17 days) after the change. A longer lag in adjustment for mothers moving from the cooler to the warmer conditions may indicate a more stressful adjustment period than when the mothers were moved to the cooler temperature. By using this reciprocal experimental design, our results indicate that changes in hatching size due to temperatures experienced at oogenesis are reversible and can occur quickly (<17 days). This result is consistent with previous insect studies where adult females were found to alter their egg size rapidly after changes in temperature. For example, Fischer *et al*. (2003) found that female butterflies could alter egg size and produce the typical temperature-specific egg size after only ten days in the new temperature.

We do not know if temperature-mediated plasticity in hatching size is adaptive or the result of physiological constraint. Previous studies, primarily on insects and aquatic arthropods, have provided evidence that in some taxa, the TSR may be the result of adaptive trade-offs in life-history characteristics, a response to oxygen limitation (Hoefnagel and Verberk, 2015) or the result of differences in the rates of growth and differentiation. Further study is necessary to determine which, if any, of these play a role in the TSR in *C.* cf. *marginalis*. However, it is clear that hatching size in *C.* cf. *marginalis* can respond to temperature changes like those experienced during the transition between upwelling and non-upwelling seasons. This finding that *C.* cf. *marginalis* hatching size is responsive to temperature changes experienced during oogenesis provides further evidence that temperature likely plays a role in the 7.9 µm difference in shell length between field-collected broods produced during the upwelling and non-upwelling seasons (Collin and Ochoa, 2016). It does not, however, explain why the seasonal increase in hatching size in response to upwelling (cooling temperatures) happens gradually over four months, while

the decrease in hatching size at the end of upwelling (warming temperatures) takes less than two months (Collin and Ochoa, 2016), nor does it demonstrate if these size differences have fitness consequences. Our results showed a slower response time to warming than to cooling, the opposite of the pattern in the field, suggesting that other covarying environmental factors may also play a role in determining offspring size in the field. Ultimately, it may be necessary to understand the cellular mechanisms that control egg size and quality to fully comprenhend how changes in environmental conditions result in differences in offspring size.

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TABLES

Table 1: Test for Fixed Effects on Average Hatching Size from Experiment 1

Source	NParm	DF	DFDen	F ratio	Prob > F
First Temperature	1	1	64.03	80.91	**<0.0001**
Final Temperature	1	1	64.03	2.72	0.10
First Temperature * Final Temperature	1	1	64.03	0.43	0.52
Female [First and Final Temperature] Random	NA	NA		NA	**<0.0001**

ANOVA table showing the effect of the first and second temperatures experienced and their interaction on the average hatching size of offspring for a single brood from each female. Significant effects are highlighted in bold.

Table 2: Test for Fixed Effects on Standard Deviation of Hatching Size from Experiment 1

Source	Nparm	DF	Sums of Squares	F ratio	Prob > F
First Temperature	1	1	131.62	8.33	**0.0053**
Final Temperature	1	1	0.26	0.017	0.90
First Temperature * Final Temperature	1	1	11.51	0.73	0.40

ANOVA table showing the effect of the first and second temperatures experienced and their interaction's standard deviation of hatching size for a single brood from each female. Significant effects are highlighted in bold.

Table 3: Test for Fixed Effects on Average Hatching Size from Experiment 2

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Brood #	3	3	4612	11.97	**<0.0001***
Treatment	3	3	91.84	8.57	**<0.0001***
Treatment*Brood #	9	9	4615	132.54	**<0.0001***
Female [Treatment] Random	NA	NA	NA	NA	**<0.0001***

ANOVA table showing the effects of brood number, treatment, the interaction between treatment and brood number, with the female as a random effect nested within treatment on the average hatching size for each brood. Significant effects are highlighted in bold. NA = Not applicable as the REML ANOVA used a maximum likelihood approach rather than a sums of squares approach to assess the nested random effects.

FIGURE CAPTIONS

Figure 1. The effect of temperature treatment on mean *Crepidula* cf. *marginalis* hatching size, based on the brood means of 15 larvae from each female from Experiment 1 (temperatures changed after the brood was laid). Error bars represent the standard error values (ANOVA, $R^2 = 0.12$; N = 68; p = 0.04).

Figure 2. Box plots comparing the overall time to hatching for the brood of *Crepidula* cf. *marginalis* across the four temperature treatments in Experiment 1. Temperatures were changed the day the brood was first observed under the female. The time to hatching for the temperature treatment that remained at 28°C is significantly different from the other three treatments, as denoted by the asterisk (n = 68).

Figure 3. The effect of brood number and temperature treatment on *Crepidula* cf. *marginalis* hatching size, based on the brood means of 15 larvae from each female in Experiment 2. Temperatures were changed after the first brood hatched. Error bars represent the standard error values (REML ANOVA, $r^2 = 0.71$; n = 319).

Figure 4. Box plots comparing the time to hatching for the four broods of *Crepidula* cf. *marginalis* across the four temperature treatments, based on the results from Experiment 2 (n = 322). Temperatures were changed after the first brood hatched. The first brood in the treatments that experienced temperature change were significantly different from the subsequent broods in the same treatment at $p<0.01$, which is denoted by the asterisk. In the

24-28°C treatment, the time to hatching of the second brood was marginally significantly different from the subsequent broods at $p = 0.03$, which is denoted by the pound symbol.

Figure 1

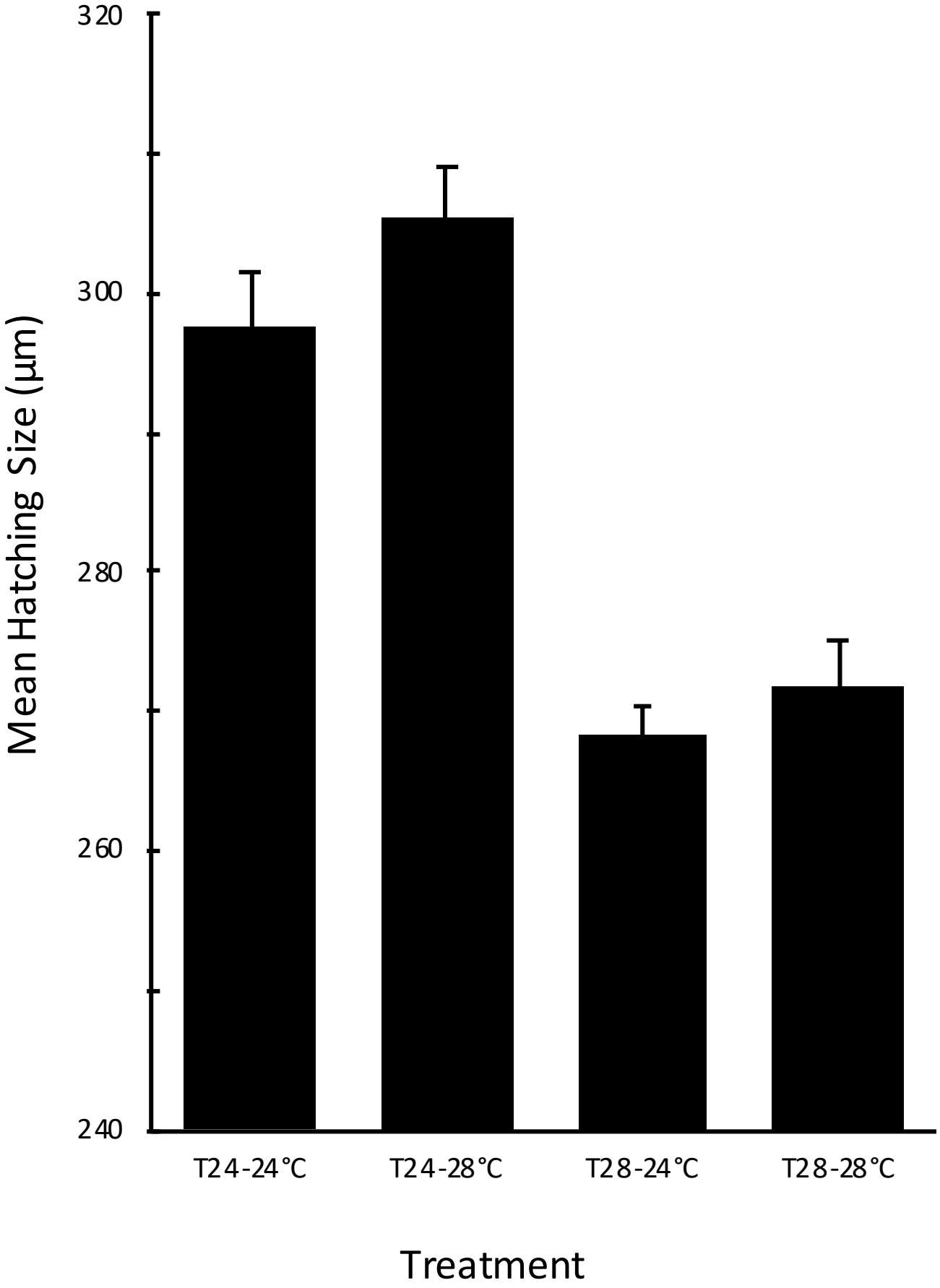

320
300
280
260
240
Mean Hatching Size (µm)
T24-24°C
T24-28°C
T28-24°C
T28-28°C
Treatment

Figure 2

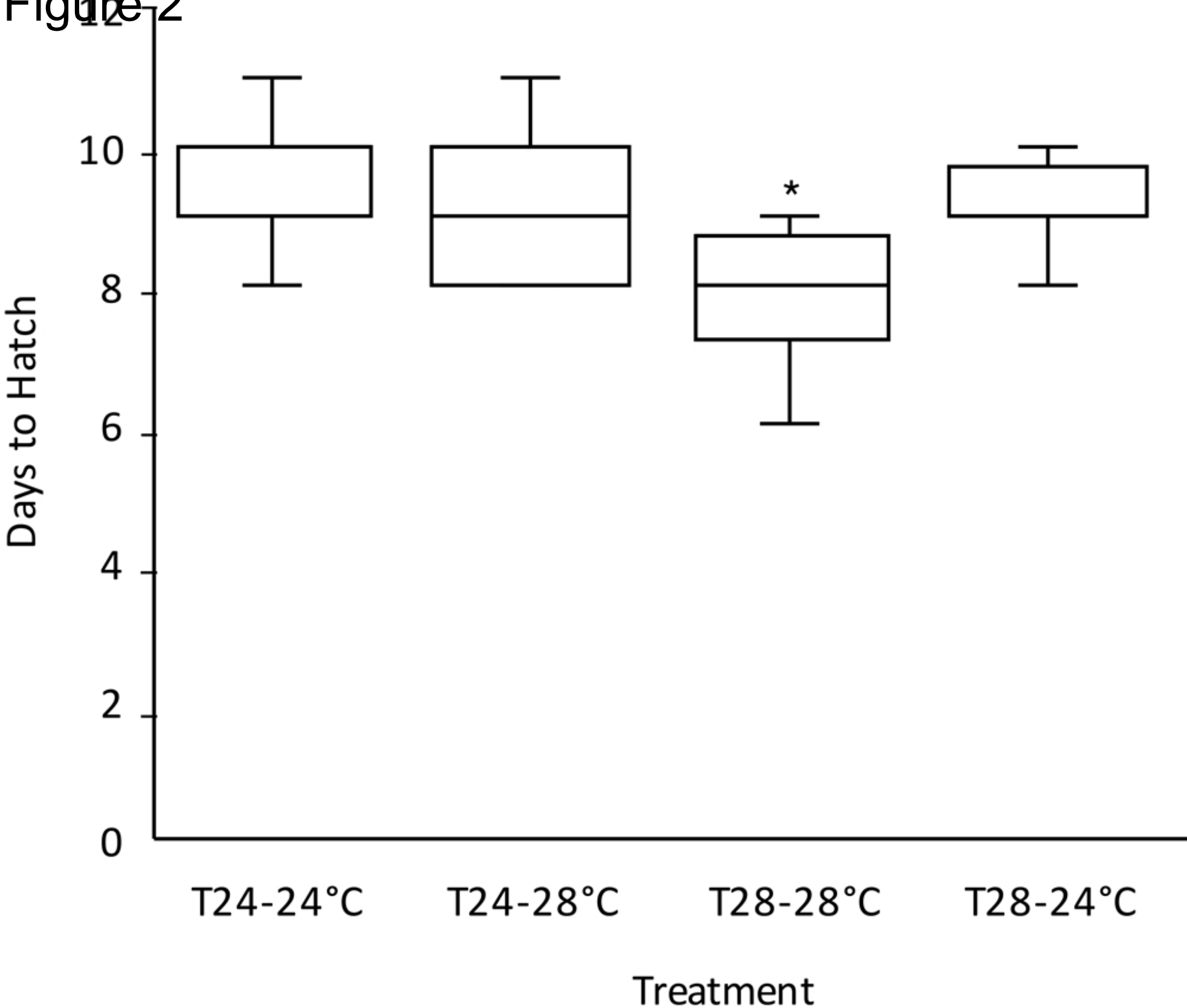

12
10
8
6
4
2
0
Days to Hatch
*
T24-24°C
T24-28°C
T28-28°C
T28-24°C
Treatment

Figure 3

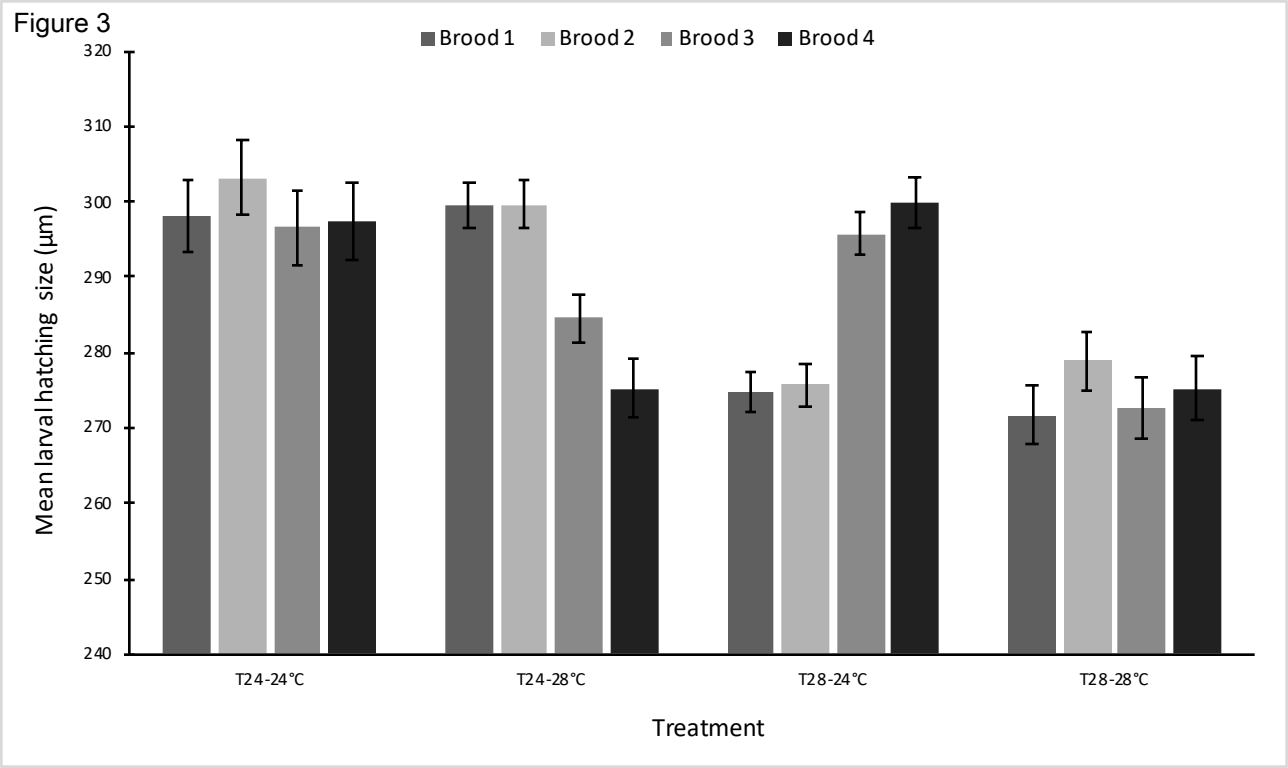
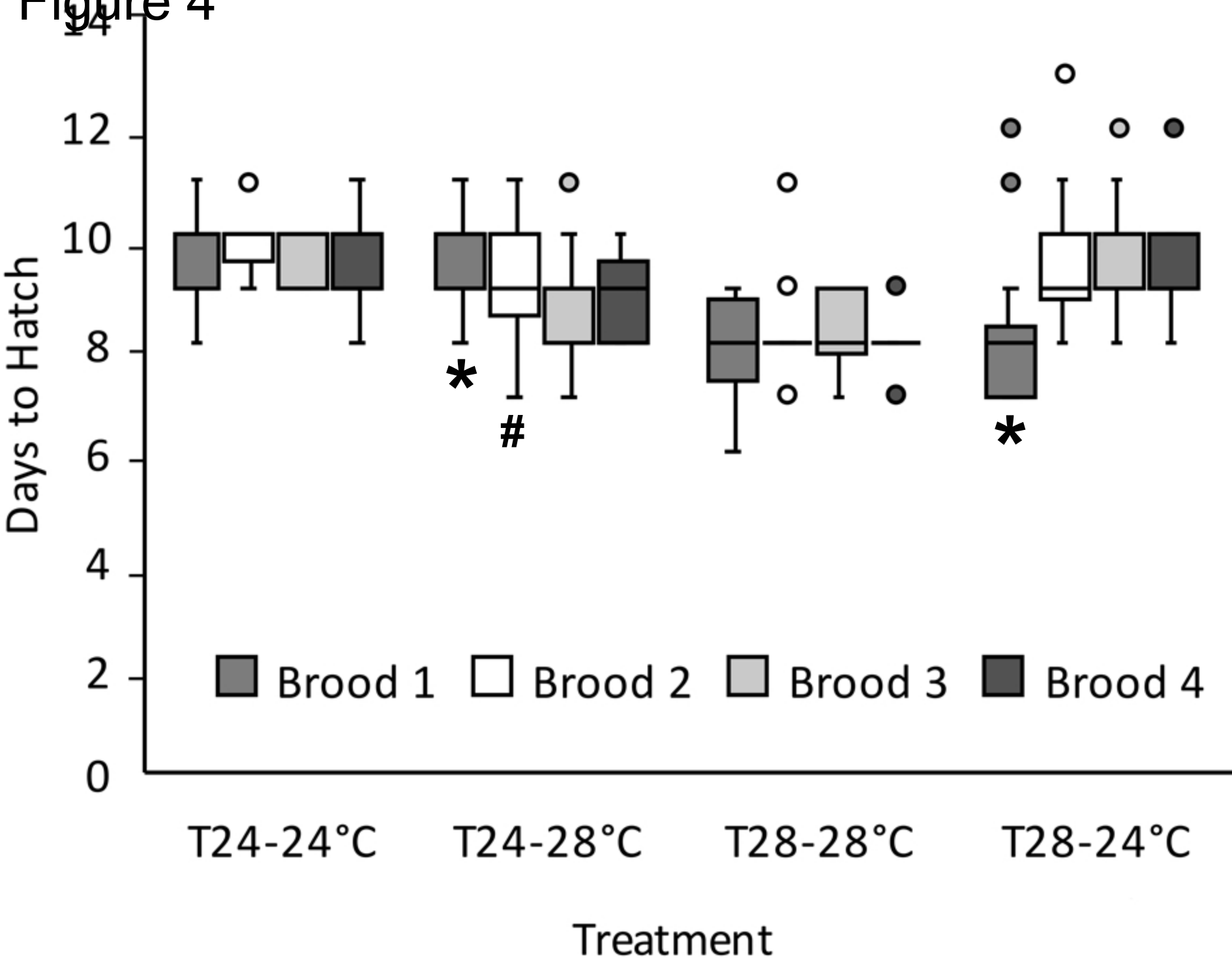

Brood 1
Brood 2
Brood 3
Brood 4
320
310
300
290
280
270
260
250
240
Mean larval hatching size (μm)
T24-24℃
T24-28℃
T28-24℃
T28-28℃
Treatment

Figure 4


14
12
10
8
6
4
2
0
Days to Hatch
*
#
*
Brood 1
Brood 2
Brood 3
Brood 4
T24-24°C
T24-28°C
T28-28°C
T28-24°C
Treatment