

Transgenerational exposure to marine heatwaves ameliorates the lethal effect on tropical copepods regardless of predation stress

Kiem Truong¹, Ngoc-Anh Vu¹, Nam Doan², Canh Bui², Minh Hoang Le², Minh Vu³, and Khuong Dinh³

¹Vietnam National University Hanoi

²Nha Trang University

³University of Oslo Department of Biosciences

April 14, 2022

Abstract

Marine heatwaves (MHWs) emerge as a severe stressor in marine ecosystems. Extreme warm sea surface temperatures during MHWs are often beyond the optimal thermal range and beyond one generation of tropical coastal zooplankton. However, it is relatively unknown whether transgenerational MHW effect may shape the offspring fitness, particularly in an ecologically relevant context with biotic interactions such as predation stress. We addressed these novel research questions by quantifying the reproductive success, grazing, and survival of copepod *Pseudodiaptomus incisus* exposed to MHW and fish predator cues (FPC) for two generations (F1 and F2). There were four F1 treatments [(control or F1-MHW) \times (no FPC or F1-FPC)] and 16 F2 treatments [(control or F1-MHW) \times (no F1-FPC or F1-FPC)] \times [(control or F2-MHW \times no F2-FPC or F2-FPC)]. In both generations, *P. incisus* performance was substantially lowered in MHW, but slightly higher in FPC, particularly in control temperature. F2 reproductive success and cumulative faecals were reduced by 20-30% in F1-MHW, but increased by ~2% in F1-FPC. Strikingly, direct MHW exposure strongly reduced survival, but transgenerational MHW exposure ameliorated its lethal effect and was independent of FPC. The increased survival came with a cost of reduced reproductive success, constrained by reduced grazing. The rapid transgenerational MHW acclimation and its associated costs are likely widespread and crucial mechanisms underlying the resilience of coastal tropical zooplankton to MHWs under high predation pressure in the tropical coastal marine ecosystems.

Introduction

Marine heatwaves (MHWs) are discrete events of abnormally high temperature periods ([?] 5 days) above the 90th percentile of 30-years sea surface temperatures measured at the locality (Hobday et al., 2016; Oliver et al., 2018). In the last decade, MHWs have emerged as one of the greatest drivers reshaping the marine biodiversity, including the tropicalisation of the temperate coastal ecosystems, mass coral bleaching worldwide, mass mortality of coastal invertebrates (Garrahou et al., 2009; 2019; Hughes et al., 2017; Smale et al., 2019). The effects of MHWs on marine ecosystems and biota are becoming more severe under ongoing climate change (Smale et al., 2019). In the Southeast Asian region, coastal marine organisms are also increasingly being exposed to episodes of MHWs (Feng et al., 2022; Yao, Wang, Yin, & Zou, 2020). In 1982 – 2018, the duration of MHWs in the Southeast Asian Seas had increased from 5 to 9 days per decade (Yao et al., 2020), and the number of days that the sea surface temperature exceeded 34°C is about 40-60 days per year (Dinh K.V., Doan X.N and Pham Q.H., unpublished data), which are often beyond one generation of tropical coastal invertebrates. A few studies have investigated the effects of MHWs on marine organisms such as copepods and fish in the Southeast Asian region (e.g., Doan et al., 2019; Le, Dinh, Nguyen, & Rønnestad, 2020; Nguyen et al., 2020), yet, most of these studies have limited the MHW effects on one generation. It

remains to explore how transgenerational acclimation to MHWs may ameliorate the effects of warming on offspring generation (see, e.g., in coral fish, Donelson, Munday, McCormick, & Pitcher, 2012).

Recent advancements in eco-evolutionary studies on adaptations of organisms to warming, ocean acidification, and contaminants have revealed the critical role of transgenerational plasticity that the environment experienced by parental generation may improve the offspring performance in the same environment (Donelson et al., 2012; Donelson, Salinas, Munday, & Shama, 2018; Fox, Donelson, Schunter, Ravasi, & Gaitán-Espitia, 2019; Guillaume, Monro, & Marshall, 2016; Krause, Dinh, & Nielsen, 2017; Munday, 2014; Thor & Dupont, 2015). For example, transgenerational exposure to ocean acidification may alleviate the fecundity loss in marine copepod *Pseudocalanus acuspes* (Thor & Dupont, 2015). Parental exposure to pyrene also resulted in higher performance of copepod *Acartia tonsa* at 100 nM pyrene (Krause et al., 2017). The transgenerational effect may occur through maternal provisioning, phenotypic plasticity, epigenetic changes, and genetic selections (Dinh, Dinh, Pham, Selck, & Truong, 2020; Donelson et al., 2012; Ryu, Veilleux, Donelson, Munday, & Ravasi, 2018).

In the shallow tropical coastal ecosystems such as seagrasses, mangroves, and coral reefs, the predation stress is typically high as these ecosystems are the spawning and nursery ground of marine species. To survive, prey species such as zooplankton will have to adjust their physiology to cope with thermal stress during MHW and the stress caused by natural predators such as voracious fish larvae and juveniles. Predators can significantly influence their prey's morphology, behaviour, physiology, growth, and reproduction (e.g., Bjærke, Andersen, & Titelman, 2014; Lasley-Rasher & Yen, 2012; Truong et al., 2020). Investigations of the transgenerational effect of MHWs in an ecologically relevant context, such as the presence of fish predator cues (FPC) on key zooplankton species such as copepods are relevant and timely because these species have to cope with both stressors from one to another generation. The vulnerability or resilience of copepods to MHWs in an ecologically relevant context such as predation stress is a key pathway for the transfer of energy and resources from photosynthesis organisms to higher trophic levels, hence the productivity of the coastal ecosystems (Chew, Chong, Tanaka, & Sasekumar, 2012). However, the combined effects of heatwaves and non-consumptive predation risk on the prey are still a major knowledge gap in current ecology, especially across generations.

Our previous study shows that fish predator cues induced a higher individual performance of the calanoid copepod *Pseudodiaptomus incisus* under control temperature, but it magnified the impacts of MHW on grazing and reproductive success (Truong et al., 2020). In this study, we fundamentally increase the realism in the experiment by investigating the role of transgenerational exposure to MHW and/or fish predator cues in shaping the fitness of the offspring generation. We tested the susceptibility of *P. incisus* to MHW and FPC by following four hypotheses:

- (1) FPC strengthens the MHW effect on *P. incisus* (see Truong et al., 2020).
- (2) Parental MHW exposure increases the MHW tolerance of *P. incisus* offspring (see Donelson et al., 2012).
- (3) Parental FPC exposure increases the FPC performance of *P. incisus* offspring (see Sentis et al., 2018).
- (4) Transgenerational FPC effect strengthens the transgenerational MHW effect.

Materials and methods

The tropical calanoid Pseudodiaptomus incisus

Pseudodiaptomus incisus were collected from a coastal aquaculture pond (11.82397°N, 109.1233°E) in Cam Ranh Bay, July 2020, using a zooplankton net (mesh size = 200 µm). Copepods were transferred to the Copepod Laboratory at Cam Ranh Centre for Tropical Marine Research and Aquaculture, Nha Trang University. The pond water salinity and temperature were 27 - 28°C and 35 PSU, respectively. Healthy adult copepods (F0) were sorted and then divided into 5-L bottles, approximately 1200 individuals per bottle, for acclimation. Adult males and females were acclimated in water baths to 30°C or 34°C for three days. The temperature was increased by 1°C every 12 hours until reaching the experimental temperatures. During the acclimation, the salinity, light : dark cycle, and dissolved oxygen concentration were kept at 30 PSU, 12L:12D, and >5 mg L⁻¹ by aerations, respectively (see also Truong et al., 2020). *P. incisus* were fed

two times a day with *Isochrysis galbana* at 30,000-33,000 cell L⁻¹ (~800 – 850 µg carbon per liter, Doan et al., 2018).

Fish predator cue preparation

Barramundi larvae *Lates calcarifer* (15 individuals with a total length of 14 ± 1 mm) were reared in a 1-L bottle. Fish larvae were fed with ~100 *P. incisivus* twice a day. After three rearing days, we removed barramundi larvae and filtered the rearing water containing fish predator cues (FPC) through a 0.5-µm filter paper. The filtered water with PFC was divided into aliquots and subsequently frozen at -20°C. FPC was thawed before being used in the experiment, as predator cue effects on the prey still remain after being frozen (Lüring & Scheffer, 2007).

Transgenerational experiment

To test the transgenerational effect of MHW and FPC on the performance of tropical copepod *Pseudodiaptomus incisivus*, *P. incisivus* were studied over two generations: F1 and F2. F1 *P. incisivus* were exposed to one of four treatments, including 2 thermal treatments (30 or 34°C) × 2 FPC (presence or absence) × 10 replicates (Fig. 1). The control temperature of 30°C was chosen as it is the mean coastal water temperature in the coastal water in southern Vietnam (see Appendix S1, Doan et al., 2018). We manipulated an experimental MHW condition with a temperature of 34°C, which is about ~2°C higher than the 90% temperature variations measured in the Cam Ranh Bay (Doan X.N., Pham, Q.H. and Dinh K.V., unpublished data).

To start the experiment, acclimatized F0 *P. incisivus* carrying egg sacs (prosomal length = 797.43 ± 2.17 µm, clutch size = 16 ± 2 eggs) were assigned to 1.2-L plastic bottles (15 females each bottle) and fed with *I. galbana* for acclimation. After 30 h, 180 - 240 F1 nauplii were assigned to each experimental bottle; those bottles were pre-acclimated to experimental temperatures. FPC solution or filtered seawater (1 ml) was added to each experimental bottle. The rearing medium and FPC were renewed daily to minimize the change in the FPC concentration (Truong et al., 2020).

To generate F2, 20 F1 females carrying egg sacs in 10 bottles of each treatment (200 individuals per F1 treatment) were transferred into 20 bottles (10 F1 females per bottle) and incubated for 30 h. F2 offspring in every F1 treatment was divided into four groups, corresponding to four experimental conditions 30°C – no FPC, 30°C – FPC, 34°C – no FPC and 34°C - FPC, resulting in 16 treatments (Fig. 1).

In both generations, we analysed clutch size, hatching success, survival of males and females, cumulative nauplii and faecals of *P. incisivus* over five days using the same procedure in our previous study (Truong et al., 2020).

Cumulative nauplii and faecals

To evaluate the reproductive output and the grazing rate of *P. incisivus*, we quantified the cumulative nauplii and faecals in five days. Ten adults of both sexes were transferred to a separated 1-L bottle (10 replicates per treatment). Bottles were daily filtered using the same filtering net (see above) to collect the nauplii and faecals. Alive adults were returned to the bottle while the dead ones were removed. The content containing nauplii and faecals was transferred to a petri dish, fixed with Lugol (4%). The number of nauplii and faecals was counted using a stereo-microscope (SZ51, Olympus, Japan).

Statistical analyses

We ran generalized linear mixed models (GLMMs, Statistica v12, StatSoft Inc., Tulsa, OK, United States) to test MHW, FPC effect, and their interaction on reproductive parameters, cumulative faecals and survival. We converted the data of the percentage of females with hatched eggs and survival of males and females to logit form $\log(x+1)$ following the recommendation in Warton and Hui (2011). In F2 generation, we ran GLMMs with four fixed effects of F1-MHW, F1-FPC, F2-MHW and F2-FPC and their two-, three- and four-way interactions. Significance is considered with $P < 0.5$.

Results

F1 generation

Exposure to MHW strongly reduced the survival of both males and females (MHW, Table 1, Fig. 2). The lethal effect of MHW was independent of FPC exposure, as indicated by an insignificant interaction of MHW \times FPC (Table 1, Fig. 2).

The reproductive success and grazing of *P. incisus* were overall affected negatively by MHW (MHW, Table 1, Fig. 3a-e). The hatched nauplii per clutch dropped by 62% at 34°C (Table 1, Fig. 3c). The cumulative nauplii and faecals were 27% and 28% lower in MHW than in the control temperature (Table 1, Fig. 3d,e). The FPC effect on the percentage of females with hatched eggs was insignificant (Table 1, Fig. 3b). The number of hatched nauplii per clutch, and cumulative faecals of *P. incisus* increased in the presence of FPC, but the FPC effect was several times higher in the control temperature than in MHW (MHW \times FPC, Table 1, Fig. 3c,e). For the clutch size, the effect of FPC was statistically insignificant in both temperatures (MHW \times FPC, Table 1, Fig. 3a). *P. incisus* produced approximately 15% more cumulative nauplii in the presence of FPC only in control temperature, but not in MHW (MHW \times FPC, Table 1, Fig. 3d). The correlation of cumulative nauplii production and cumulative faecals was insignificant ($F_{1, 36} = 2.65$, $P = 0.11$, slope ± 1 SE = 0.38 ± 0.24).

F2 generation

Survival of F2 *P. incisus* females and males was reduced under direct exposure to MHWs (F2-MHW, Table 2, Fig. 4). FPC did not affect F2 female and male survival as the F2-FPC effect was insignificant (Table 2, Fig. 4). F2-FPC did not alter the lethal effect of F2-MHW, as indicated by an insignificant interaction of F2-MHW \times F2-FPC (Table 2, Fig. 4). Strikingly, transgenerational MHW exposure (F1-MHW \times F2-MHW) resulted in a similar female and male survival compared to the control temperature; all were ~10% higher survival than F2-MHW exposed females and males (F1-MHW \times F2-MHW, Table 2, Fig. 4), suggesting that lethal F2-MHW effect was ameliorated. This pattern was independent of F1-FPC and F2-FPC (Table 2, Fig. 4). The lethal MHW effect in the parental generation was no longer present in F2 generation when F2 copepods were returned to the control temperature (F1-MHW, Table 2, Fig. 4).

The clutch size, % females with hatched eggs and hatched nauplii from a clutch of F2 *P. incisus* were 42%, 5%, and 22% reduced in F2-MHW (F2-MHW, Table 2, Fig. 5a-c). The F2-MHW effect was independent of the F2-FPC as F2-MHW \times F2-FPC was insignificant. Importantly, F2-MHW was generally less intense when F1 was also exposed to MHW (F1-MHW \times F2-MHW, Table 2, Fig. 5a-c). Parental exposure to MHW (F1-MHW) also decreased 22% and 33% the size of clutches and hatched nauplii from a clutch of F2 *P. incisus*. No F1-MHW effect on % F2 females with hatched eggs as it was similar to the control level (F1-MHW, Table 2, Fig. 5a-c). Overall, F2-FPC increased ~4% the clutch size of F2 females, but this pattern was mainly driven by large clutches of control F2-females that their parental generation was also exposed to both MHW and FPC (F1-MHW \times F1-FPC), resulting in two-, three- and four-way interactions (Table 2, Fig. 5a). The F2-FPC effect on other reproductive parameters was absent or minimal (F2-FPC, Table 2, Fig. 5b-d).

Overall, cumulative nauplii and faecals of F2 copepods decreased by 40 and 32% in MHW, respectively (F2-MHW, Table 2, Fig. 5d,e). Parental exposure to MHW (F1-MHW) also resulted in lower cumulative nauplii and faecals than the control copepods (F1-MHW, Fig. 5b-e). F2-MHW induced reductions in cumulative nauplii, and faecals were ~12% less strong in F2 copepods whose parental generation was also exposed to MHW (F1-MHW) (transgenerational effect F1-MHW \times F2-MHW, Table 2, Fig. 5d,e). The F2-MHW effect was also 3-4% less strong in F1-FPC (F1-FPC \times F2-MHW), but F1-FPC did not influence the cumulative nauplii and faecals of copepods that were exposed to MHW for both generations (F1-MHW \times F1-FPC \times F2-MHW, Table 2, Fig. 5d,e). F2-FPC caused a minor increase (~3-4%) in both cumulative nauplii and faecals of F2 *P. incisus*, but only in control temperature and in F1-FPC (F2-MHW \times F2-FPC, F1-FPC \times F2-FPC, F1-FPC \times F2-MHW \times F2-FPC, Table 2, Fig. 5e). F2-FPC caused a slightly (~3%) stronger reduction in cumulative faecals in F2-MHW only in copepods that were not exposed to F1-MHW (F1-MHW \times F2-MHW \times F2-FPC, Table 2, Fig. 5e). Cumulative nauplii covaried positively with the cumulative faecals

($F_{1, 64} = 30.3$, $P < 0.001$, slope ± 1 SE = 0.68 ± 0.12).

Discussion

Interactive effects of marine heatwave and fish predator cues on F1 generation

Results in the F1 generation mostly confirmed and strengthened major findings of severe MHW impacts on *P. incisus* performance which were observed in our previous study focusing on the interactive effects of MHW and FPC on the life history traits of *P. incisus* for an entire one generation (Truong et al., 2020). Indeed, MHW exposure increased mortality, lowered hatching success, and cumulative nauplii and faecals. MHW increased mortality, which may be a result of physiological dysfunctions such as the collapse of ATP synthesis (Harada, Healy, & Burton, 2019), the damage of macromolecules (Somero, 2010), the higher cellular oxygen demand than the capacity of oxygen delivery (Pörtner, 2010). The reduced performance of *P. incisus* was particularly strong for reproductive success with ~30-60% reduced the size of egg clutches, the percentage of females produced hatched eggs, hatched nauplii from a clutch, and cumulative nauplii under direct exposure to MHW are widespread in tropical copepods (Doan et al., 2019; Nguyen et al., 2020). The lower reproductive outputs are generally related to reduced grazing, thereby energy intake. While we observed a lower cumulative faecals of F1 *P. incisus*, the correlation of cumulative faecals and nauplii was insignificant, but a positive correlation of these two parameters was observed in F2 *P. incisus*.

The FPC presence caused a small increase in females with successfully hatched eggs, the number of nauplii hatched per clutch of *P. incisus*, and the FPC effect was stronger at 30°C than at 34°C, which may be an adaptive response to predators. For example, predation can cause 50-75% mortality in marine copepods, and increased reproductive outputs are a general mechanism to compensate for consumptive mortality (Hirst & Kiorboe, 2002). Importantly, the FPC-induced increase in nauplii production only occurred at the control temperature, but not under MHW may result from the reduced grazing as indicated by the MHW-induced lower cumulative faecals and higher energy demand for metabolism (Low et al., 2018). Stronger MHW-induced reductions in cumulative nauplii and faecals were observed in FPC-exposed *P. incisus* in our previous study (Truong et al., 2020), in which we could quantify both parameters for an entire adult lifespan.

Parental effect of the marine heatwave and fish predator cues on F2 generation

One of our important findings was that parental exposure to MHW (F1-MHW) reduced reproductive success and grazing, but not survival of F2 *P. incisus* when the F2 was returned to the control temperature. The reduced F2 reproductive success and cumulative faecals may result from reduced energy and resource investments for F1 reproduction, further limited by the lower F1 grazing. Poor maternal provisioning was likely the reason for the lower performance of F2 copepods whose parental generation was exposed to MHW. A similar result has been observed in a previous study on the same copepod species (Dinh et al., 2021).

Across treatments, the main parental effect of FPC accounted for only around ~2% positive effect of the cumulative nauplii and faecals. The positive effect of parental exposure to FPC on the reproductive outputs of offspring is common in zooplankton and this effect may last for several generations after removing the predation stress (see e.g. in *Daphnia ambigua*, Walsh, Cooley IV, Biles, & Munch, 2015). However, the positive parental FPC effect on *P. incisus* was at least an order of magnitude smaller than the negative parental MHW effects on these life history traits and was unlikely to shape the overall sensitivity of *P. incisus* to MHW. Both types of non-consumptive predation stress, including direct and parental exposure, had a generally small effect on *P. incisus*. *Pseudodiaptomus* copepods account for the majority of the diet of fish predator *Lates calcarifer* (Davis, 1985). MHW-induced strong reduction in reproductive success of *P. incisus* may directly affect the food availability for fish larvae in the coastal environment (Chew et al., 2012).

Transgenerational MHW effect was independent of fish predator cues

There is mixed evidence of transgenerational effects of multiple stressors on the vulnerability of aquatic species. On the one hand, there is evidence that corals (Torda et al., 2017) coral fishes (Donelson et al., 2012) show rapid transgenerational acclimation to warming, and that can be linked to a complete compensation

in aerobic scope (Donelson et al., 2012), the epigenetic changes (Ryu et al., 2018) or the associated microbes (Torda et al., 2017). On the other hand, there is evidence that transgenerational exposure to extreme warming, metal and fish predator cues (Pham, Dinh, Nguyen, & Quoc, 2020), pesticides (Tran, Janssens, Dinh, & Stoks, 2018) may reduce the offspring performance in same stressors as the cumulative impacts of stressors across generations. This study found the different levels of transgenerational acclimation of *P. inciscus* to MHW in shaping the F2 performance. Indeed, *P. inciscus* showed a rapid transgenerational acclimation to the lethal MHW effect as indicated by increased survival in F1-MHW \times F2-MHW exposed males and females to the control temperature. As MHW induced mortality in F1 which may result in some genetic selection for higher thermal tolerance. Interestingly, FPC played a minor role in MHW transgenerational acclimation as indicated by no or minor variation in the effect size of the transgenerational acclimation to MHW in the presence or absence of FPC, suggesting the dominant effect of MHW.

At the sublethal effect, the magnitude of transgenerational MHW acclimation was much smaller than survival. Indeed, we found consistent and clear evidence that transgenerational effects of MHWs (F1-MHW \times F2-MHW) resulted in 5-10% better performance of F2 *P. inciscus* than the direct MHW effect (F2-MHW alone); all were substantially lower than the performance of *P. inciscus* in the control temperature. The transgenerational MHW effects caused ~50% reductions in cumulative nauplii and faecals; both parameters were significantly and positively correlated, suggesting that the lowered energy intake constrained the reproduction. Furthermore, the lower number of nauplii hatched per F1-MHW \times F2-MHW exposed females further contributed to the lowered cumulative nauplii and suggested the lethal transgenerational MHW effect on embryonic development (see also Grønning, Doan, Dinh, Dinh, & Nielsen, 2019 for *Pseudodiaptomus annandalei*). Similar lower cumulative nauplii and faecals have been previously observed in *P. inciscus* that was exposed to MHW for two generations (Dinh et al., 2021). The reduced F2 *P. inciscus* performance was likely a direct cost of maintaining a high survival and the selection for tolerant genotypes from the F1 generation.

Similar to the survival, the presence of FPC in F1 (F1-FPC) or F2 (F2-FPC) generally only played a minor role in shaping the parental effect of MHW (F1-MHW) or direct effect of MHW (F2-MHW) on the F2 generation as indicated by the change in the effect size of parental and transgenerational MHW effects was typically smaller 5% of the total effects of both MHW and FPC. The dominant effect of MHW is likely widespread for most marine invertebrates, as indicated by massive ecological consequences during MHWs (Hughes et al., 2019; Smale et al., 2019), even mass mortality of various groups of coastal aquatic taxa (Garrabou et al., 2009; 2019).

Conclusions and perspectives

There is a great concern that the hyperdiverse tropical ecosystems may be collapsed under the cumulative impact of multiple stressors (Barlow et al., 2018; Dinh, 2019; Worm et al., 2006). Among others, MHWs are becoming a new but severe threat, which sometimes causes massive and widespread mortality across taxa (Garrabou et al., 2009; 2019). However, we know much less about how MHW may affect key grazers and intermediate prey in the coastal marine ecosystems such as copepods, particularly in an ecologically relevant context with biotic interactions (Truong et al., 2020). Our results contribute to this by highlighting the severe direct effect of MHWs on reproduction and grazing *P. inciscus*, a common copepods species in the tropical coastal ecosystems of Southeast Asia. Strong mortality together with reduced reproductive success are important mechanisms underlying the reduced copepod abundance under MHWs (Evans, Lea, Hindell, & Swadling, 2020). Consequently, MHWs may substantially reduce the secondary biomass production of the zooplankton, which fuels resources and energy to the vast majority of other marine predators such as corals, crustaceans, and fish (Arimitsu et al., 2021; Chew et al., 2012). Most importantly, our results suggest that *P. inciscus* may evolve in a rapid transgenerational acclimation to MHW, which fully ameliorated its lethal effect in the second generation. The thermal acclimation comes with a cost of reduced reproduction, and grazing may be a crucial and widespread mechanism for invertebrates coping with the transient effects of heatwaves (Cavieres et al., 2020; Dinh et al., 2020; Doan et al., 2019). Interestingly, fish predator cues played a minor role in shaping both direct and transgenerational effects of MHWs, highlighting the dominant MHW effects on *P. inciscus*. Given the widespread and severe effects of MHWs on coastal invertebrates,

the dominant effect of MHWs is likely widespread with implications for ecological risk assessment under the rapidly changing environment of the tropical coastal ecosystems.

Acknowledgements

This research was financially supported by the International Foundation for Science, Stockholm, Sweden, through a grant to Nam X. Doan (Grant I-2-A-6347-1).

References

- Arimitsu, M. L., Piatt, J. F., Hatch, S., Suryan, R. M., Batten, S., Bishop, M. A., . . . von Biela, V. R. (2021). Heatwave-induced synchrony within forage fish portfolio disrupts energy flow to top pelagic predators. *Global Change Biology*, 27 (9), 1859-1878. doi:10.1111/gcb.15556
- Barlow, J., Franca, F., Gardner, T. A., Hicks, C. C., Lennox, G. D., Berenguer, E., . . . Graham, N. A. J. (2018). The future of hyperdiverse tropical ecosystems. *Nature*, 559 (7715), 517-526. doi:10.1038/s41586-018-0301-1
- Bjærke, O., Andersen, T., & Titelman, J. (2014). Predator chemical cues increase growth and alter development in nauplii of a marine copepod. *Marine Ecology Progress Series*, 510 , 15-24.
- Cavieres, G., Rezende, E. L., Clavijo-Baquet, S., Alruiz, J. M., Rivera-Rebella, C., Boher, F., & Bozinovic, F. (2020). Rapid within-and transgenerational changes in thermal tolerance and fitness in variable thermal landscapes. *Ecology and Evolution*, 10 (15), 8105-8113.
- Chew, L. L., Chong, V. C., Tanaka, K., & Sasekumar, A. (2012). Phytoplankton fuel the energy flow from zooplankton to small nekton in turbid mangrove waters. *Marine Ecology Progress Series*, 469 , 7-24. doi:10.3354/meps09997
- Davis, T. L. O. (1985). The food of barramundi, *Lates calcarifer*(Bloch), in coastal and inland waters of Van Diemen Gulf and the Gulf of Carpentaria, Australia. *Journal of Fish Biology*, 26 (6), 669-682. doi:10.1111/j.1095-8649.1985.tb04307.x
- Dinh, K. V. (2019). Vietnam's fish kill remains unexamined. *Science*, 365 (6451), 333-333. doi:10.1126/science.aay6007
- Dinh, K. V., Dinh, H. T., Pham, H. T., Selck, H., & Truong, K. N. (2020). Development of metal adaptation in a tropical marine zooplankton. *Scientific Reports*, 10 (1), 10212. doi:10.1038/s41598-020-67096-1
- Dinh, K. V., Doan, K. L. U., Doan, N. X., Pham, H. Q., Le, T. H. O., Le, M.-H., . . . Truong, K. N. (2021). Parental exposures increase the vulnerability of copepod offspring to copper and a simulated marine heatwave. *Environmental Pollution*, 287 , 117603. doi:https://doi.org/10.1016/j.envpol.2021.117603
- Doan, N. X., Vu, M. T. T., Nguyen, H. T., Tran, H. T. N., Pham, H. Q., & Dinh, K. V. (2018). Temperature- and sex-specific grazing rate of a tropical copepod *Pseudodiaptomus annandalei* to food availability: implications for live feed in aquaculture. *Aquaculture Research*, 49 (12), 3864-3873. doi:10.1111/are.13854
- Doan, X. N., Vu, M. T. T., Pham, H. Q., Wisz, M. S., Nielsen, T. G., & Dinh, K. V. (2019). Extreme temperature impairs growth and productivity in a common tropical marine copepod. *Scientific Reports*, 9 , 4550. doi:10.1038/s41598-019-40996-7
- Donelson, J. M., Munday, P. L., McCormick, M. I., & Pitcher, C. R. (2012). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, 2 , 30-32.
- Donelson, J. M., Salinas, S., Munday, P. L., & Shama, L. N. S. (2018). Transgenerational plasticity and climate change experiments: Where do we go from here? *Global Change Biology*, 24 (1), 13-34. doi:10.1111/gcb.13903

- Evans, R., Lea, M. A., Hindell, M. A., & Swadling, K. M. (2020). Significant shifts in coastal zooplankton populations through the 2015/16 Tasman Sea marine heatwave. *Estuarine Coastal and Shelf Science*, 235 , 11. doi:10.1016/j.ecss.2019.106538
- Feng, Y., Bethel, B. J., Dong, C., Zhao, H., Yao, Y., & Yu, Y. (2022). Marine heatwave events near Weizhou Island, Beibu Gulf in 2020 and their possible relations to coral bleaching. *Science of The Total Environment* , 153414. doi:<https://doi.org/10.1016/j.scitotenv.2022.153414>
- Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitan-Espitia, J. D. (2019). Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374 (1768), 20180174. doi:doi:10.1098/rstb.2018.0174
- Garrabou, J., Coma, R., Bensoussan, N., Bally, M., ChevaldonnE, P., Cigliano, M., . . . Cerrano, C. (2009). Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. *Global Change Biology*, 15 (5), 1090-1103. doi:10.1111/j.1365-2486.2008.01823.x
- Garrabou, J., Gomez-Gras, D., Ledoux, J. B., Linares, C., Bensoussan, N., Lopez-Sendino, P., . . . Harmelin, J. G. (2019). Collaborative database to track mass mortality events in the Mediterranean Sea. *Frontiers in Marine Science*, 6 , 707. doi:10.3389/fmars.2019.00707
- Gronning, J. B., Doan, N. X., Dinh, T. N., Dinh, K. V., & Nielsen, T. G. (2019). Ecology of *Pseudodiaptomus annandalei* in tropical aquaculture ponds with emphasis on the limitation of production. *Journal of Plankton Research*, 41 (5), 741-758.
- Guillaume, A. S., Monro, K., & Marshall, D. J. (2016). Transgenerational plasticity and environmental stress: do paternal effects act as a conduit or a buffer? *Functional Ecology*, 30 (7), 1175-1184. doi:<https://doi.org/10.1111/1365-2435.12604>
- Harada, A. E., Healy, T. M., & Burton, R. S. (2019). Variation in thermal tolerance and its relationship to mitochondrial function across populations of *Tigriopus californicus* . *Frontiers in Physiology*, 10 . doi:21310.3389/fphys.2019.00213
- Hirst, A. G., & Kiorboe, T. (2002). Mortality of marine planktonic copepods: global rates and patterns. *Marine Ecology Progress Series*, 230 , 195-209. doi:10.3354/meps230195
- Hobday, A. J., Alexander, L. V., Perkins, S. E., Smale, D. A., Straub, S. C., Oliver, E. C. J., . . . Wernberg, T. (2016). A hierarchical approach to defining marine heatwaves. *Progress in Oceanography*, 141 , 227-238. doi:10.1016/j.pocean.2015.12.014
- Hughes, T. P., Kerry, J. T., Alvarez-Noriega, M., Alvarez-Romero, J. G., Anderson, K. D., Baird, A. H., . . . Wilson, S. K. (2017). Global warming and recurrent mass bleaching of corals. *Nature*, 543 (7645), 373-+. doi:10.1038/nature21707
- Hughes, T. P., Kerry, J. T., Connolly, S. R., Baird, A. H., Eakin, C. M., Heron, S. F., . . . Torda, G. (2019). Ecological memory modifies the cumulative impact of recurrent climate extremes. *Nature Climate Change*, 9 (1), 40-43. doi:10.1038/s41558-018-0351-2
- Krause, K. E., Dinh, K. V., & Nielsen, T. G. (2017). Increased tolerance to oil exposure by the cosmopolitan marine copepod *Acartia tonsa* . *Science of The Total Environment*, 607-608 , 87-94. doi:<https://doi.org/10.1016/j.scitotenv.2017.06.139>
- Lasley-Rasher, R. S., & Yen, J. (2012). Predation risk suppresses mating success and offspring production in the coastal marine copepod, *Eurytemora herdmanni* . *Limnology and Oceanography*, 57 (2), 433-440. doi:10.4319/lo.2012.57.2.0433
- Le, M.-H., Dinh, K. V., Nguyen, M. V., & Ronnestad, I. (2020). Combined effects of a simulated marine heatwave and an algal toxin on a tropical marine aquaculture fish cobia (*Rachycentron canadum*). *Aquaculture Research*, 51 (6), 2535-2544. doi:10.1111/are.14596

- Low, J. S., Chew, L. L., Ng, C. C., Goh, H. C., Lehet, P., & Chong, V. C. (2018). Heat shock response and metabolic stress in the tropical estuarine copepod *Pseudodiaptomus annandalei* converge at its upper thermal optimum. *Journal of Thermal Biology*, 74 , 14-22.
- Lurling, M., & Scheffer, M. (2007). Info-disruption: pollution and the transfer of chemical information between organisms. *Trends in Ecology & Evolution*, 22 (7), 374-379. doi:10.1016/j.tree.2007.04.002
- Munday, P. L. (2014). Transgenerational acclimation of fishes to climate change and ocean acidification. *F1000prime reports*, 6 .
- Nguyen, T. T., Le, M. H., Doan, N. X., Pham, H. Q., Vu, M. T. T., & Dinh, K. V. (2020). Artificial light pollution increases the sensitivity of tropical zooplankton to extreme warming. *Environmental Technology & Innovation*, 20 , 101179. doi:https://doi.org/10.1016/j.eti.2020.101179
- Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., . . . Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature Communications*, 9 (1), 1324. doi:10.1038/s41467-018-03732-9
- Pham, H. T., Dinh, K. V., Nguyen, C. C., & Quoc, L. B. (2020). Changes in the magnitude of the individual and combined effects of contaminants, warming, and predators on tropical cladocerans across 11 generations. *Environmental Science & Technology*, 54 (23), 15287-15295. doi:10.1021/acs.est.0c05366
- Portner, H. O. (2010). Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology*, 213 (6), 881-893. doi:10.1242/jeb.037523
- Ryu, T., Veilleux, H. D., Donelson, J. M., Munday, P. L., & Ravasi, T. (2018). The epigenetic landscape of transgenerational acclimation to ocean warming. *Nature Climate Change*, 8 (6), 504-+. doi:10.1038/s41558-018-0159-0
- Sentis, A., Bertram, R., Dardenne, N., Ramon-Portugal, F., Espinasse, G., Louit, I., . . . Danchin, E. (2018). Evolution without standing genetic variation: change in transgenerational plastic response under persistent predation pressure. *Heredity*, 121 (3), 266-281. doi:10.1038/s41437-018-0108-8
- Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., . . . Moore, P. J. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change*, 9 , 306-312. doi:10.1038/s41558-019-0412-1
- Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology*, 213 (6), 912-920. doi:10.1242/jeb.037473
- Thor, P., & Dupont, S. (2015). Transgenerational effects alleviate severe fecundity loss during ocean acidification in a ubiquitous planktonic copepod. *Global Change Biology*, 21 (6), 2261-2271. doi:10.1111/gcb.12815
- Torda, G., Donelson, J. M., Aranda, M., Barshis, D. J., Bay, L., Berumen, M. L., . . . Munday, P. L. (2017). Rapid adaptive responses to climate change in corals. *Nature Climate Change*, 7 (9), 627-636. doi:10.1038/nclimate3374
- Tran, T. T., Janssens, L., Dinh, K. V., & Stoks, R. (2018). Transgenerational interactions between pesticide exposure and warming in a vector mosquito. *Evolutionary Applications*, 11 (6), 906-917. doi:doi:10.1111/eva.12605
- Truong, K. N., Vu, N.-A., Doan, N. X., Le, M.-H., Vu, M. T. T., & Dinh, K. V. (2020). Predator cues increase negative effects of a simulated marine heatwave on tropical zooplankton. *Journal of Experimental Marine Biology and Ecology*, 530-531 , 151415. doi:https://doi.org/10.1016/j.jembe.2020.151415
- Walsh, M. R., Cooley IV, F., Biles, K., & Munch, S. B. (2015). Predator-induced phenotypic plasticity

within-and across-generations: a challenge for theory? *Proceedings of the Royal Society B: Biological Sciences*, 282 (1798), 20142205.

Warton, D. I., & Hui, F. K. C. (2011). The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, 92 (1), 3-10. doi:10.1890/10-0340.1

Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., . . . Watson, R. (2006). Impacts of biodiversity loss on ocean ecosystem services. *Science*, 314 (5800), 787-790. doi:10.1126/science.1132294

Yao, Y., Wang, J., Yin, J., & Zou, X. (2020). Marine heatwaves in China’s marginal seas and adjacent offshore waters: past, present, and future. *Journal of Geophysical Research: Oceans*, 125, (3), e2019JC015801.

Table 1. The results of the generalized mixed models testing effects of marine heatwave (MHW) and fish predator cues (FPC) on survival, reproductive parameters and cumulative faecals of F1 *Pseudodiaptomus incisus* . Significant *P* values are signed with *.

Effects	Survival males	Survival males	Survival males	Survival females
	df1, df2	F	<i>P</i>	df1, df2
MHW	1, 36	22.79	<0.001*	1, 36
FPC	1, 36	0.66	0.422	1, 36
MHW × FPC	1, 36	0.01	0.941	1, 36
Effects	Hatched nauplii clutch ⁻¹	Hatched nauplii clutch ⁻¹	Hatched nauplii clutch ⁻¹	Cumulative nauplii female
	df1, df2	F	<i>P</i>	df1, df2
MHW	1, 36	267.39	<0.001*	1, 36
FPC	1, 36	18.54	<0.001*	1, 36
MHW × FPC	1, 36	6.27	0.017*	1, 36

Table 2. The results of generalized mixed models testing the direct and transgenerational effects of marine heatwave (MHW) and fish predator cues (FPC) on survival, reproductive parameters and cumulative faecals of F2 *Pseudodiaptomus incisus* . Significant *P* values are signed with *.

Effects	Survival males	Survival males	Survival males
	df1,df2	F	P
F1-MHW	1, 64	20.35	<0.001*
F1-FPC	1, 64	3.65	0.061
F2-MHW	1, 64	24.17	<0.001*
F2-FPC	1, 64	0.84	0.364
F1-MHW × F1-FPC	1, 64	0.71	0.402
F1-MHW × F2-MHW	1, 64	35.81	<0.001*
F1-FPC × F2-MHW	1, 64	0.10	0.755
F1-MHW × F2-FPC	1, 64	0.97	0.328
F1-FPC × F2-FPC	1, 64	0.09	0.762
F2-MHW × F2-FPC	1, 64	3.69	0.059
F1-MHW × F1-FPC × F2-MHW	1, 64	0.15	0.701
F1-MHW × F1-FPC × F2-FPC	1, 64	0.10	0.755
F1-MHW × F2-MHW × F2-FPC	1, 64	0.73	0.397
F1-FPC × F2-MHW × F2-FPC	1, 64	0.10	0.755
F1-MHW × F1-FPC × F2-MHW × F2-FPC	1, 64	0.06	0.809
Effects	Hatched nauplii clutch ⁻¹	Hatched nauplii clutch ⁻¹	Hatched nau
	df1,df2	F	P
F1-MHW	1, 64	68.28	<0.001*

Effects	Survival males	Survival males	Survival males
F1-FPC	1, 64	1.05	0.309
F2-MHW	1, 64	102.73	<0.001*
F2-FPC	1, 64	0.32	0.574
F1-MHW × F1-FPC	1, 64	0.32	0.574
F1-MHW × F2-MHW	1, 64	94.76	<0.001*
F1-FPC × F2-MHW	1, 64	0.06	0.813
F1-MHW × F2-FPC	1, 64	0.27	0.605
F1-FPC × F2-FPC	1, 64	7.00	0.010*
F2-MHW × F2-FPC	1, 64	0.05	0.824
F1-MHW × F1-FPC × F2-MHW	1, 64	0.67	0.417
F1-MHW × F1-FPC × F2-FPC	1, 64	0.16	0.690
F1-MHW × F2-MHW × F2-FPC	1, 64	0.35	0.554
F1-FPC × F2-MHW × F2-FPC	1, 64	0.24	0.626
F1-MHW × F1-FPC × F2-MHW × F2-FPC	1, 64	4.58	0.036*

Figure legends

Figure 1. The schematic overview of the transgenerational experiment for the direct and transgenerational MHW and FPC effects on *Pseudodiaptomus incisus* (CT = control temperature).

Figure 2. Effects of the marine heatwave (MHW) and fish predator cues (FPC) on the survival (mean ± SE) of males (a) and females (b) in F1 *Pseudodiaptomus incisus* males (a) and females (b).

Figure 3. Effects of marine heatwave (MHW) and fish predator cues (FPC) on the number of eggs per clutch (a), % females produced hatched eggs (b), hatched nauplii hatched from a clutch (c), cumulative nauplii per female (d) and faecals per individual (e) of F1 *Pseudodiaptomus incisus*. Data are visualized as mean ± SEs.

Figure 4. Direct and transgenerational effects of marine heatwave (MHW) and fish predator cues (FPC) on the survival (mean ± SE) of F2 *Pseudodiaptomus incisus* males (a) and females (b). *CT: control temperature.

Figure 5. The number of eggs per clutch (a), % females produced hatched eggs (b), hatched nauplii egg clutch (c), cumulative nauplii per female (d) and faecals per individual (e) of F2 *Pseudodiaptomus incisus*. Data are visualized as mean ± SEs. *CT: control temperature.

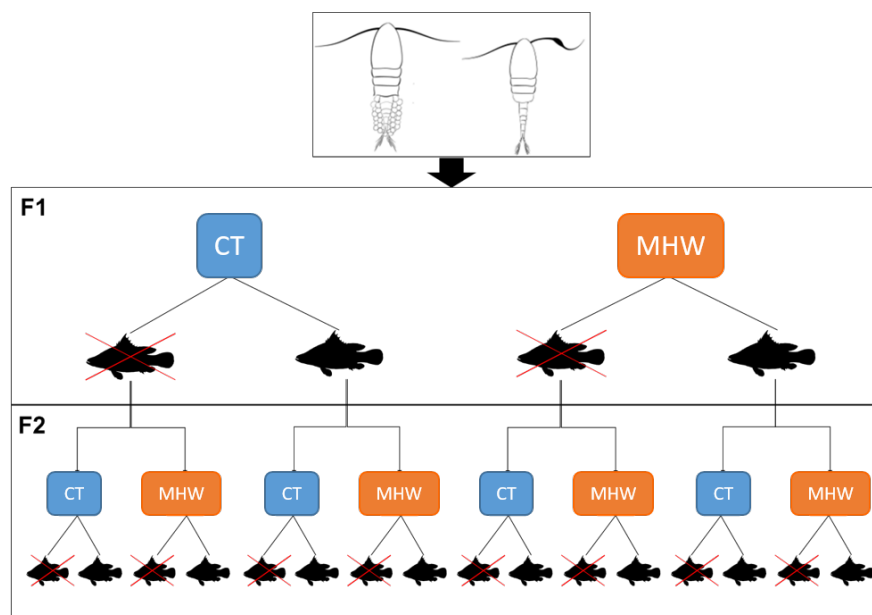


Figure 1

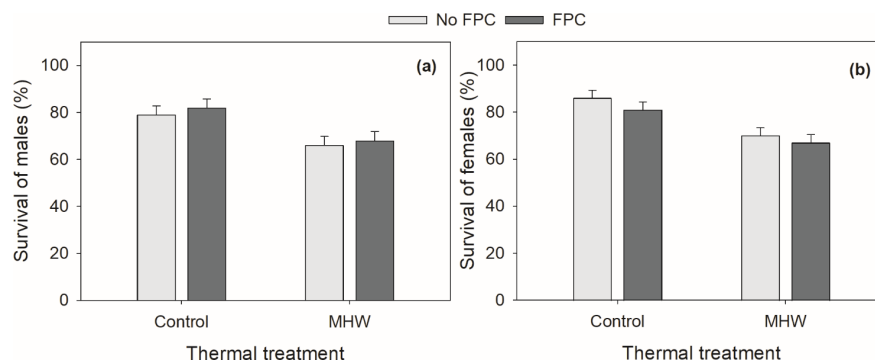


Figure 2

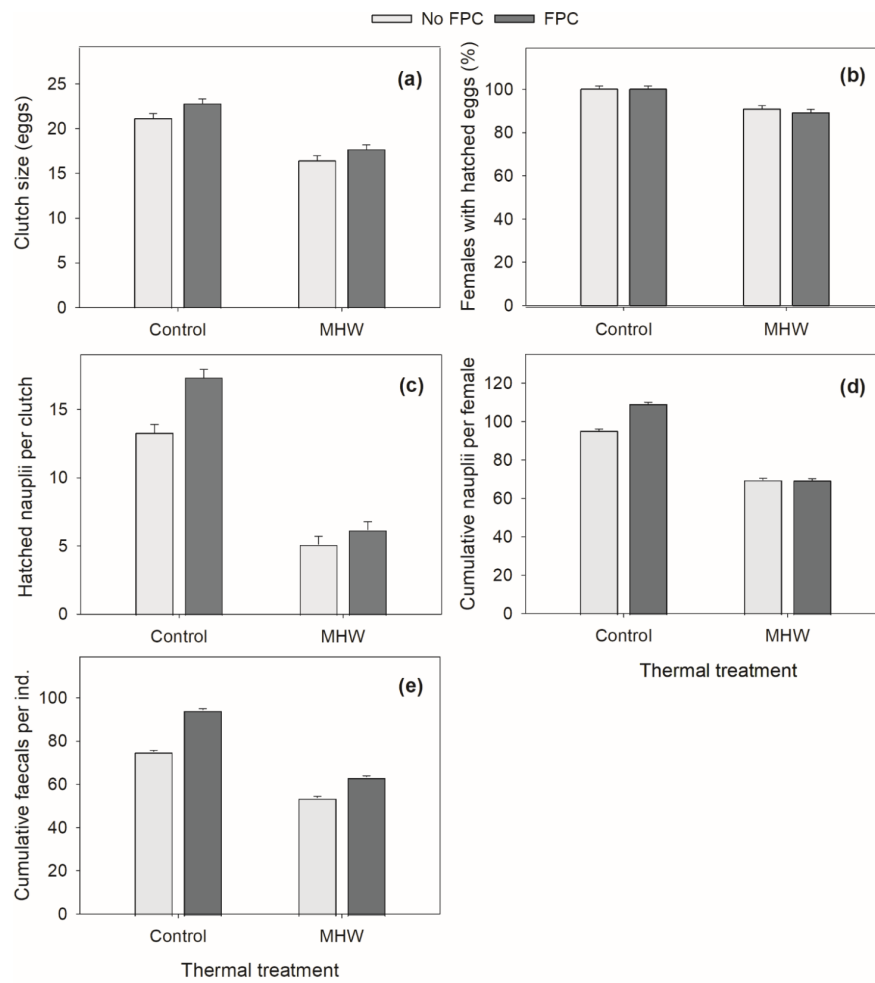


Figure 3

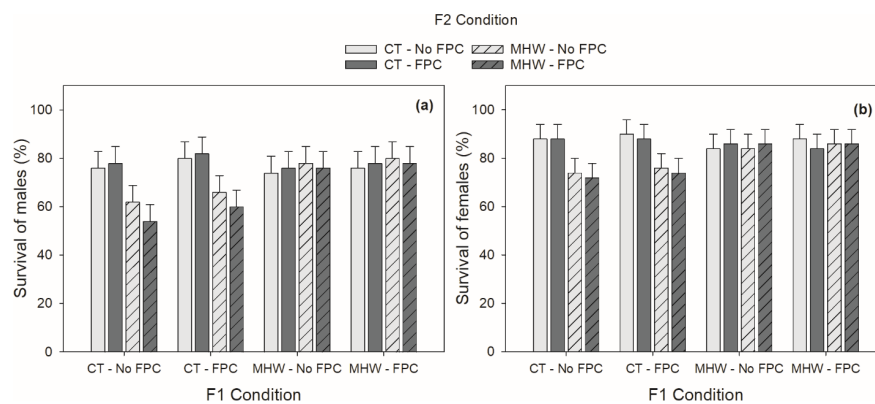


Figure 4

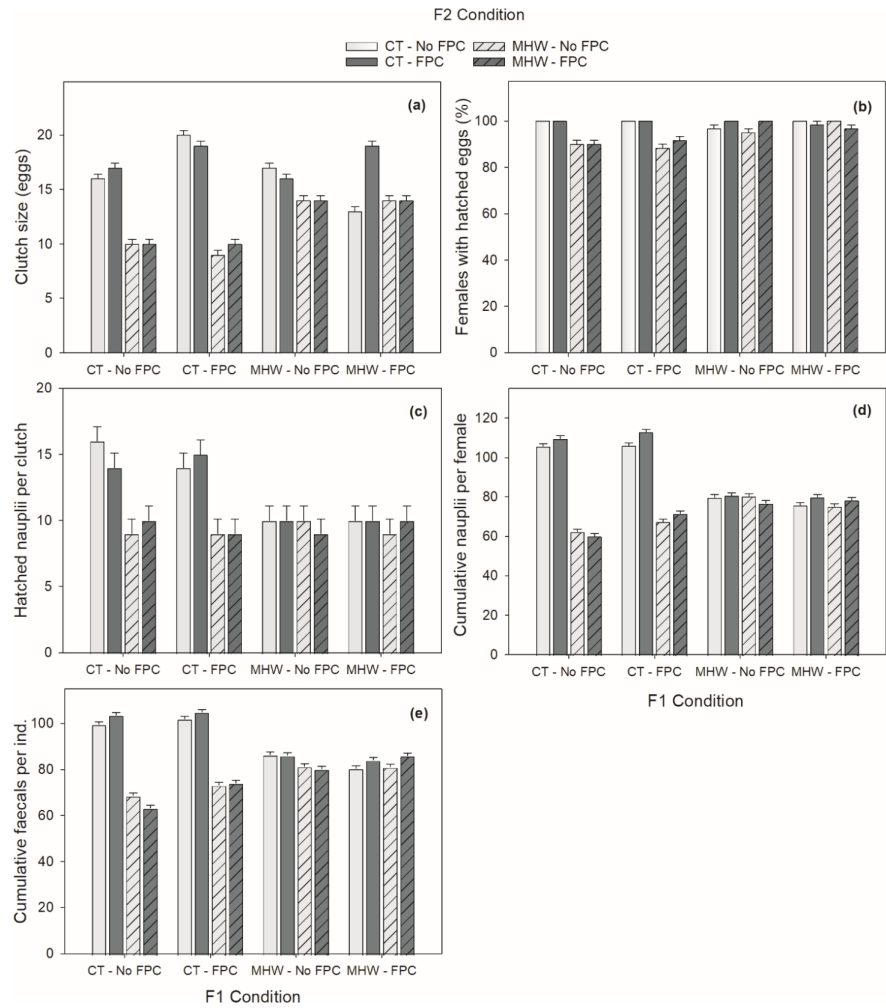


Figure 5