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RESEARCH ARTICLE OPEN ACCESS

When the Going Gets Tough, the Females Get Going: Sex-Specific Physiological Responses to Simultaneous Exposure to Hypoxia and Marine Heatwave Events in a Ubiquitous Copepod

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Received: 9 February 2024 | **Revised:** 9 August 2024 | **Accepted:** 30 September 2024

Funding: This work was funded by a FIR UQAR, a Canada Foundation for Innovation (CFI) (36665) and a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grants (RGPIN-2020-05627), all granted to P.C. Thanks are also due for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020), to FCT/MEC through Scientific Employment Stimulus—Individual Call 2018 (CEECIND/01250/2018) and 2022 (2022.00153.CEECIND), all granted to D.M. F.V. is supported by an FRQNT scholarship (274427) and a Vanier Canada Graduate Scholarships (433956). H.G.D. and M.S. were supported by the National Science Foundation - Division Ocean Sciences OCE (1559180) and (1947565).

Keywords: extreme events | metabolic rates | multiple stressors interaction | oxygen | temperature | thermal limits

ABSTRACT

The existence of sex-specific differences in phenotypic traits is widely recognized. Yet they are often ignored in studies looking at the impact of global changes on marine organisms, particularly within the context of combined drivers that are known to elicit complex interactions. We tested sex-specific physiological responses of the cosmopolitan and ecologically important marine copepod *Acartia tonsa* exposed to combined hypoxia and marine heatwave (MHW) conditions, both of which individually strongly affect marine ectotherms. Females and males were acutely exposed for 5 days to a combination of either control (18°C) or a high temperature mimicking a MHW (25°C), and normoxia (100% O_2 sat.) or mild hypoxia (35% O_2 sat.). Life-history traits, as well as sex-specific survival and physiological traits, were measured. Females had overall higher thermal tolerance levels and responded differently than males when exposed to the combined global change drivers investigated. Females also showed lower metabolic thermal sensitivity when compared to males. Additionally, the MHW exerted a dominant effect on the traits investigated, causing a lower survival and higher metabolic rate at 25°C. However, egg production rates appeared unaffected by hypoxia and MHW conditions. Our results showed that MHWs could strongly affect copepods' survival, that combined exposure to hypoxia and MHW exerted an interactive effect only on CT_{max}, and that sex-specific vulnerability to these global change drivers could have major implications for population dynamics. Our results highlight the importance of considering the differences in the responses of females and males to rapid environmental changes to improve the implementation of climate-smart conservation approaches.

Diana Madeira and Piero Calosi contributed equally to this paper.

https://doi.org/10.1111/gcb.17553

Men Are from Mars, Women Are from Venus
John Gray, 1992.

1 | Introduction

Intrinsic differences among sexes across the animal kingdom are widely recognized. Females and males not only differ in biological, anatomical, and hormonal aspects (Ivan, Daniela, and Jaroslava 2023), but they can also differ in their life-history strategies, behavior, and physiology (Ellis et al. 2017; Gissi et al. 2023). Such sex-specific differences can occur both in the mean value of a trait as well as in its variation (Zajitschek et al. 2020; Brand et al. 2023). For example, several taxonomic groups display sex-specific differences in their ability to tolerate starvation (Finiguerra et al. 2013) exposure to dinoflagellate toxins (Avery, Altland, and Dam 2008), cold, and desiccation (Andersen et al. 2010). These differences usually arise from differential selective pressures between sexes, which are typically linked to the differential cost of reproduction (Lambert 1978; Roze and Otto 2012), and sexual selection (Darwin 1888). Nevertheless, most studies looking at marine species' responses to global change drivers neglect to investigate sex-specific responses (Ellis et al. 2017; Pottier et al. 2021; Gissi et al. 2023). Yet, the studies that have explicitly accounted for sex in marine species showed generally that an array of responses are influenced by sex, supporting the idea that sex-specific responses are prevalent in the marine ecosystem (Gissi et al. 2023). For example, differences between males and females have been reported for survival, growth and metabolic rates, thermal tolerance and acclimation potential (e.g., Hall 2001; Cripps, Flynn, and Lindeque 2016; Sasaki et al. 2019; Pottier et al. 2021). However, these differences are not universal. Rather, they appear to be trait-dependent, condition-dependent, and not systematically biased for the same sex in all species (Zajitschek et al. 2020; Pottier et al. 2021; Gissi et al. 2023). Overall, these life-history and physiological differences between females and males define sex-specific vulnerability to global change drivers, with cascading effects on operational sex ratio (Edmands 2021 and references within; Brand et al. 2023), demographic trends, population, communities, and ecosystem dynamics (Ellis et al. 2017; Edmands 2021 and references within; Brand et al. 2023; Gissi et al. 2023). Disregarding sex in studies investigating species responses to global change drivers could therefore bias our understanding of the consequences of these stressors on marine biodiversity. In turn, this could impact how we implement environmental management and conservation policies (Gissi et al. 2023) and climate-smart conservation approaches (Stein et al. 2014). Specifically looking at the sex-specific life-history and physiological responses to extreme events is particularly relevant, as the abrupt nature of these events could lead to differential behavioral or physiological responses in males and females.

In this context, marine heatwaves (MHW—as defined by Hobday et al. 2016) are of particular interest as they have increased over the past century due to human activities (Oliver et al. 2018). They are expected to increase in duration, intensity, and frequency in the coming decades (Frölicher, Fischer, and Gruber 2018; Oliver et al. 2019) and can have more immediate devastating consequences than progressive chronic warming (e.g., Wernberg et al. 2013; Sanz-Lazaro 2016; Smale et al. 2019). The rapid temperature rise during MHW can cause physiological stress, decreased performance, and thermal damages (Pörtner 2012; Leung, Connell, and Russell 2017; Smith et al. 2023). Ultimately, if the rates of temperature change are too abrupt and exceed a species' acclimatization ability, MHWs can act as a strong selective pressure (Wernberg et al. 2013; Filbee-Dexter et al. 2020). Species' range shifts, tropicalization of ecosystems, invertebrates mass mortality, and severe loss of habitat-forming species are only some examples of the drastic consequences of past MHWs (e.g., Wernberg et al. 2016; Hughes et al. 2017; Garrabou et al. 2022), with potentially long-lasting consequences on socio-economic systems (Mills et al. 2013; Smale et al. 2019; Smith et al. 2021).

Despite our growing understanding of MHWs occurrence (e.g., Pearce and Feng 2013; Hobday et al. 2016), our comprehension of their direct biological impacts is rather limited in the context of multiple stressors and compound events (Oliver et al. 2019; Gruber et al. 2021; Smith et al. 2023). Indeed, marine environments are complex habitats where multiple stressors and extreme events can co-occur (Halpern et al. 2019) and interact (Piggott, Townsend, and Matthaei 2015; Côté, Darling, and Brown 2016), eliciting emerging properties (e.g., Dam et al. 2021; Carrier-Belleau et al. 2023). The combined effect of MHWs and hypoxia is of particular interest, as hypoxic regions are spreading rapidly (Diaz and Rosenberg 2008; Vaquer-Sunyer and Duarte 2011) as a result of warming, anthropogenic activity, and natural processes (Levin and Breitburg 2015; Breitburg et al. 2018; Laffoley and Baxter 2019). In addition, evidence of co-occurrence between MHWs and hypoxic zones is emerging (Gruber et al. 2021). This co-occurrence is expected to negatively impact the aerobic metabolism of ectotherms due to the competing effect of temperature and hypoxia on oxygen demand and supply (Fry and Hart 1948; Hochachka and Somero 2002; Pörtner 2010; Rubalcaba et al. 2020). Yet, the potential for extreme acute temperature events (such as MHW) to elicit different effects than gradual warming when co-occurring with hypoxia is poorly documented (Woods et al. 2022; cf. Lucey et al. 2022; Bowering et al. 2023; Tran and Johansen 2023). Additionally, systematic studies looking at sex-specific responses to combined hypoxia and MHW are notably lacking. In this sense, it is paramount to increase our understanding in this area if we are to develop adequate mitigation and conservation strategies, particularly in coastal habitats and estuaries, as these environments could be particularly exposed to combined hypoxia and MHW (Roegner, Needoba, and Baptista 2011; Woods et al. 2022; Safonova, Meier, and Gröger 2024). In this context, zooplankton species, and in particular copepods, are ideal model systems. They constitute the most abundant pelagic metazoans in the marine environment (Turner 2004), and they play pivotal roles in food webs and biogeochemical cycles (Dam et al. 1995; Schiel et al. 2002; Mitra et al. 2014; Steinberg and Landry 2017). High temperature and hypoxia in isolation are known to be selective forces for copepods, usually negatively affecting survival, behavior, and life-history traits (e.g., Marcus et al. 2004; Holste and Peck 2005; Sasaki et al. 2019), with temperature inducing lipid remodeling and upregulation of heat shock proteins (HSPs) (Garzke et al. 2016; Rahlff et al. 2017). Nevertheless, the physiological responses of copepods to combined MHWs and hypoxia are yet to be unraveled.

Therefore, the present study aims to examine the sex-specific life-history and physiological responses of the ubiquitous marine copepod, *Acartia tonsa* (Dana 1849) to simultaneous exposure to hypoxia and MHW under laboratory conditions. Temperature greatly affects oxygen solubility and availability in the marine environment (Verberk et al. 2011; Breitburg et al. 2018; Earhart et al. 2022). Additionally, both temperature and oxygen can strongly influence ectotherm's physiology (Hochachka and Somero 2002; Roman et al. 2019). On this basis, we predict that the combined exposure to hypoxia and MHW will elicit negative synergistic effects on life-history and physiological traits in *A. tonsa*. In addition, based on the existing literature, we predict that *A. tonsa* males will be more sensitive to both the single and combined effects of hypoxia and MHW when compared to females.

2 | Materials and Methods

2.1 | Study Species

The Calanoid copepod *Acartia tonsa* is a dominant species in coastal and estuaries ecosystems worldwide, including in the Northwest Atlantic (Cervetto, Gaudy, and Pagano 1999). This species presents an evident size dimorphism (average size difference of 0.3 mm), with males' length around 0.7 mm and females reaching approximately 1 mm at sexual maturity (Sasaki et al. 2019). This free-spawning species continuously releases eggs directly in the water that hatch 24–48 h after spawning at 20°C (Mauchline 1998; Marcus and Wilcox 2007). Females can reproduce multiple times during their lifespan (Parrish and Wilson 1978). Generation time is temperature-specific, around 14–15 days at 18°C (Berggreen, Hansen, and Kiørboe 1988).

2.2 | Specimens' Collection and Maintenance

Specimens of *A. tonsa* were collected at Esker Point Beach in Groton, CT, USA (41.320725° N, 72.001643° W) in June 2016 and reared for 126 generations under current optimal conditions for *A. tonsa* in the NE Atlantic: temperature = 18°C and pH ~ 8.2, salinity between 31 and 36, see Dam et al. (2021) for further details about laboratory conditions. In June 2022, approximately 200 individuals were sent inside two 500 mL bottles placed in an insulated box to the Marine Ecological and Evolutionary Physiology laboratory (MEEP) at the University of Quebec in Rimouski (UQAR) (Rimouski, QC, Canada). Experimental stock cultures were maintained using the same culturing methods as Dam et al. (2021): see Data S1. Copepods were fed at a food-replete concentration (i.e., $\geq 800\,\mu g$ carbon L^{-1}) with a mixture of the phytoplankters *Tetraselmis sp.*, *Thalassiosira weissflogii*, and *Rhodomonas salina*, cultured semi-continuously in F/2 medium (Guillard 1975) following the long-standing protocols used in the Dam laboratory (Feinberg and Dam 1998). These microalgae cultures were kept in an environmental chamber (MLR-3515 Sanyo Versatile, Sanyo Electric Co., Ltd., Osaka, Japan) at 18°C and a 13 h light:11 h dark photoperiod. To remove potential maternal and environmental variation effects as much as possible (Cournoyer 2013; Pereira, Sasaki, and Burton 2017), the copepod stock culture (F0) was kept for one generation under laboratory conditions before the start of the experiment, for which the F1 generation was used.

2.3 | Experimental Design, System, and Protocol

To investigate sex-specific responses to acute exposure to hypoxia and MHW in *A. tonsa*, females and males were exposed for 5 days to one of the four temperature-oxygen treatments, according to a factorial experimental design (Figure S1): control (C—18°C, 100% O_2 sat. or ~ 9.46 mg L^{-1}), hypoxia (H—18°C and 35% O_2 sat. or ~3.31 mg L^{-1}), marine heatwave (MHW—25°C and 100% O_2 sat. or ~8.25 mg L^{-1}) and combined hypoxia and marine heatwave (HMHW 25°C and 35% O_2 sat. or ~2.89 mg L^{-1}). The control conditions were chosen based on the optimal temperature for recruitment (Dam et al. 2021), as well as the temperature used to rear the copepods since their collection, and the optimal oxygen saturation level. The value of 35% O_2 sat. was chosen as it represents a mild, non-lethal, level of hypoxia commonly encountered in the area of collection during hypoxic events (CTDEEP 2016, 2021). Given the relatively shallow depth of the Long Island Strait and the fact that hypoxia zones can sometimes reach surface water (CTDEEP 2016), copepods are exposed to this condition in their natural environment. Finally, to identify a realistic temperature to mimic a MHW following Hobday et al. (2016) definition, we used the "heatwaveR" package (Schlegel and Smit 2018) in R (R Core Team 2022) to detect past extreme events in the coastal area where copepods were collected, using the NOAA OISST dataset. The highest SST temperatures recorded during previous recent MHWs reached 25°C, hence this temperature was selected for the MHW and HMHW treatments: see for details Data S2.

All treatments were generated and maintained using four independent in-house-built recirculating experimental systems (Figure S1): see Data S3 for details. Briefly, copepods were maintained in 2 L aquaria (Clarity container, Type A, Toronto, ON, Canada) placed inside a 121 L holding tank (Clarity container, Type A) that served as a water bath. Each experimental system contained four aquaria, for a total of 16 aquaria: four per treatment (Figure S1). Each holding tank was continuously supplied with artificial seawater coming from a corresponding 26 L header tank (ClearView, Sterilite Ultra, Portland, OR, USA), that was held at the desired temperature through a feedback system composed of a temperature probe and aquarium heaters: see Data S3. To obtain the desired oxygen levels and ensure a continuous gentle mixing of sea water, each aquarium was bubbled with ambient air using a small flexible tube mounted with a glass pipette connected to an air pump (PT1624, Laguna, Mansfield, MA, USA). Finally, the aquaria hosting the H and HMHW were equipped with an O_2 probe (optical oxygen sensor IKS ODO, IKS Aquastar, Karlsbad, BW, Germany) that continuously monitored the oxygen concentration inside the aquarium. The probe was connected to the feedback system (IKS Aquastar Industrial, IKS aquastar) that regulated the addition of pure gaseous N_2 into each H and HMHW aquarium.

At the beginning of the exposure period, nine females and nine males for each aquarium ($N = 36$ individuals per treatment per sex) were randomly pipetted from the stock culture under the stereomicroscope (MDF41, Leica, Wetzlar, Germany) and placed into 500 mL beakers halfway filled with artificial seawater. Sea water was produced by mixing artificial sea salt (Instant Ocean Sea Salt, United Pet Group Inc., Cincinnati, OH, USA) with distilled water to the desired salinity. Each beaker was placed in a thermal bath (F32 HL, Julabo, Allentown, PA, USA) for 6 h to gradually expose them to their respective treatment until target temperature and O_2 level were reached. This was achieved by decreasing O_2 sat. via adding small amounts of hypoxic water coming from the experimental system and/or by increasing the temperature by 1.2°C h^{-1}: rates between 1 to 2°C h^{-1} are commonly used (e.g., Fernandes et al. 2023; Missionário et al. 2023). At the same time, to standardize any effects linked to the manipulations associated with this gradual exposure period, copepods assigned to the control treatment were also kept inside a thermal bath kept at 18°C for the same duration. At the end of the pre-exposure period, copepods were observed under the stereomicroscope to detect any mortality. None was observed. Then, they were gently transferred to their dedicated aquarium inside the experimental system and maintained under experimental conditions for 5 days (Figure S3).

Sea water parameters were monitored throughout the experiment (Table 1) using the same methods described in Data S1 and water was changed every 48–72 h. Furthermore, copepods were fed ad libitum every 48–72 h, using the procedures followed for the stock culture. No food was added 24 h before the start of the physiological assays to limit the impact of feeding and excretion on the reading while avoiding mortality due to starvation.

2.4 | Copepod Survival

At the end of the exposure period, live females and males were counted individually under a stereomicroscope (MDF41, Leica) to determine survivorship in each aquarium and gently pipetted in a well of a culture plate (12 wells Tissue Culture Plates, VWR, International LLC, Radnor, PA, USA) for physiological measurement. Sex-specific survival (%) was calculated per aquarium as

$$\frac{n_f}{n_i} \times 100 \qquad (1)$$

where n_f represents the number of live males or females at the end of the exposure period and n_i represents the initial number of males or females placed in the aquarium.

2.5 | Egg Production Rates

Mean egg production rate per aquarium was determined by counting under a stereomicroscope (MDF41, Leica) the total number of laid eggs and hatched nauplii produced by the F1 adults. Eggs and nauplii were collected by gently screening the aquarium water through a 41 μm sieve and transferred to small plastic containers to be counted under the stereomicroscope. The daily egg production rate was calculated by dividing the total number of eggs by the number of females (9) and incubation days (5).

2.6 | Metabolic Rates

Oxygen consumption, used as a proxy for metabolic rates (MO_2, Ege and Krogh 1914), was measured individually for an average of four females and four males *per* aquarium (*N* = approximately 16 individual per sex per treatment), using closed non-invasive optical fluorescence-based respirometry described in Köster, Krause, and Paffenhöfer (2008). Briefly, the method uses a 24-channel oxygen meter (SDR SensorDish Reader, PreSens, Regensburg, Germany) to read oxygen concentration in hermetic 2 mL glass vials equipped with an oxygen sensor spot at the bottom of the vials (SensorVial SV-PSt5-2 mL, PreSens). The volume of the vial was chosen as it enabled copepods to swim without constraints, ensuring that the MO_2 measurements during the trials were the closest representation possible of the copepods' routine metabolic rates (RMR) (Harris et al. 2000; Ikeda et al. 2001). Each individual previously isolated in a well of a culture plate (see copepod survival) was gently transferred to a glass vial filled with artificial seawater set to its treatment condition. The vials were placed on the SDR reader and kept in a dark environmental chamber (MLR-3515 Sanyo Versatile, Sanyo Electric Co., Ltd) kept at either 18 (for C and H treatments) or 25°C (for MHW and HMHW treatments). Oxygen concentration in the vial was measured every 3 min for 10 h. Four blank vials per SDR plate, containing water from each treatment, were added to estimate the potential influence of background microbial respiration/oxygen production for each run. At the end of the measurements, copepods were observed under the stereomicroscope (MS5, Leica) to assess any mortality. Only one individual (out

TABLE 1 | Summary of mean values (mean ± SD) for seawater physico-chemical parameters for each treatment, with different subscript letters representing significant differences between mean values for the same parameter among different treatments.

Treatment	Temperature (°C)	O_2 (% sat.)	Salinity	pH_{NBS}
C	18.1 ± 0.3^a	94.3 ± 1.1^a	27.7 ± 0.7^a	8.15 ± 0.04^a
	$n = 20$	$n = 20$	$n = 20$	$n = 10$
H	18.1 ± 0.2^a	34.7 ± 3.8^b	28.0 ± 0.7^a	8.20 ± 0.13^a
	$n = 20$	$n = 19$	$n = 20$	$n = 9$
MHW	24.6 ± 0.5^b	93.3 ± 1.9^a	28.0 ± 0.7^a	8.17 ± 0.05^a
	$n = 20$	$n = 20$	$n = 20$	$n = 10$
HMHW	24.8 ± 0.2^b	35.7 ± 3.8^b	27.8 ± 0.7^a	8.19 ± 0.09^a
	$n = 20$	$n = 19$	$n = 20$	$n = 10$

Note: n refers to the number of measurements.

of 125 individual tested) died during the measurement and was discarded from the analyses. Copepods were left to rest for at least 30 min in their treatment condition before undertaking upper thermal tolerance limit measurements.

Individual RMR (μmol h^{-1}) were obtained by calculating the slope of the decrease in oxygen concentration over time (% air sat. min^{-1}) using the respR package (version 2.2.0; Harianto et al. 2019) in R (version 4.2.1). Data were first processed by removing the first 5 h of measurements, as oxygen profiles during this period were non-linear. This likely reflects the time required for the water to equilibrate to the conditions in the environmental chamber and for the oxygen sensor dots to become saturated (Holmes-Hackerd, Sasaki, and Dam 2023). Then, rolling regressions were calculated for each individual with the "auto_rate" function, using a window width of 0.5 for the rolling slopes. Background respiration was subtracted from the individual rates within the same run using the "concurrent method" in the "adjusted_rate" function. A minr^2 (coefficient of determination) threshold of 0.7 was applied to remove non-linear sections of the slope, using a histogram of r^2 values and the proportion of rejected slopes as function of minr^2 as diagnostic tools to determine the threshold (Chabot, Zhang, and Farrell 2021). On this basis, a total of 15 out of 125 individual tested were thereafter removed from the database. Then, the mean of the slopes above the threshold was calculated for each remaining individual, and rates were converted to μmol h^{-1} to be used in further statistical analyses.

Furthermore, to compare females and males' thermal sensitivity, sex-specific Q_{10} values were calculated for each oxygen saturation level following the van't Hoff's coefficient:

$$Q_{10} = \left(\frac{\mathrm{MR}_2}{\mathrm{MR}_1}\right)^{\frac{10}{T2-T1}} \quad (2)$$

where MR_1 and MR_2 correspond to the mean in the specific metabolic rates in a treatment condition and T_1 and T_2 the two corresponding temperature levels: 18°C and 25°C.

2.7 | Upper Thermal Limit

Copepods' upper thermal limit was determined using the critical thermal maximum approach ($\mathrm{CT}_{\mathrm{max}}$), with the loss of locomotor performance (LLP) as the endpoint (Harada, Healy, and Burton 2019; Healy, Bock, and Burton 2019). Individuals were transferred to a 1 mL glass vial (Clear shell glass, Thermo Fisher Scientific, Waltham, MA, USA), reduced to a volume of 600 μL to facilitate observation. The vials were tightly closed and placed underwater in a thermal bath (F32 HL, Julabo). A stereomicroscope (Nikon SMZ645 Stereo Zoom, Amstelveen, The Netherlands) was placed above the thermal bath to facilitate the observation of the copepods inside their vial. The temperature was then raised by 1°C min^{-1} (ramping slopes $R^2 = 0.99$) until the last individual reached the LLP (Harada, Healy, and Burton 2019; Healy, Bock, and Burton 2019). Copepods' internal body temperature was considered to be equivalent to that in the thermal bath considering their microscopic size and surface-to-volume ratio (González 1974). The loss of locomotor performance was monitored for each individual by gently turning the vial to generate a swimming response in copepods. The endpoint was reached when the swimming response disappeared and the copepod was passively sinking to the bottom of the microtube (Healy, Bock, and Burton 2019). The corresponding temperature was identified as the $\mathrm{CT}_{\mathrm{max}}$ for a given individual Two control vials filled only with sea water and equipped with a thermocouple probe (HH802U, OMEGA, Laval, QC, Canada) were placed next to the vials holding the copepods to accurately monitor the temperature in real-time, without stressing the copepods. As soon as an individual reached its endpoint, it was placed back at its original treatment conditions for 10 min and was then observed under the stereomicroscope to assess if they were still alive. Copepods that did not survive the $\mathrm{CT}_{\mathrm{max}}$ assay (one out of 125 individual used) or for which we did not successfully measure the weight or were not successfully retrieved from MO_2 trials (10 individual) were discarded from statistical analyses.

2.8 | Body Length

Following $\mathrm{CT}_{\mathrm{max}}$ measurements, each copepod was photographed using a microscope camera (Leica IC90E Integrated CMOS, Leica) mounted on a stereomicroscope (M60, Leica). Prosome length (mm) was then determined using the software ImageJ (Schneider, Rasband, and Eliceiri 2012), which was then converted into body mass (μg) using the equation for *A. tonsa* (Kiørboe, Møhlenberg, and Hamburger 1985):

$$W = 13.4\, L^3 \quad (3)$$

where W refers to the dry weight (μg) and L to the prosome length (mm).

2.9 | Statistical Analyses on Life-History and Physiological Traits

All data collected was regrouped into survival, fecundity and physiological datasets (Vermandele et al. 2024) for statistical analyses. To test the effects of simultaneous exposure to hypoxia and a MHW on the life-history and physiological traits in females and males, a generalized linear mixed model (GLMM, family: binomial, link function: logit) was used for survival data, and linear mixed models (LMM) were used for egg production rates, RMR and $\mathrm{CT}_{\mathrm{max}}$, using the lme4 package (Bates et al. 2015). Survival data were converted into a binomial form by assigning a value of 1 if the individual was alive or 0 if it was dead at the end of the experiment. "Temperature" and "Oxygen" were used as fixed factors in the egg production model, while "Sex" was added as a fixed factor for the other traits. In addition, "Experimental system" and "Aquarium" (for RMR and $\mathrm{CT}_{\mathrm{max}}$) were initially added as random factors to the models. As "Experimental system" was found not to exert a significant effect and did not improve the models' fit, it was removed from all the analyses. Finally, "body weight" was included as a covariate in the RMR and $\mathrm{CT}_{\mathrm{max}}$ models. When evidence of effects was observed (sensu Muff et al. 2022), post hoc analyses were performed using multiple comparisons of means with Tukey contrast, using the "glht" function of the multcomp library (version 1.4–20; Hothorn, Bretz, and Westfall 2008). To reduce the number of comparisons in the case of a significant three-way interaction, we selected specific pairwise contrasts that were directly linked to our aims: that is, comparison of the responses of females between treatments, males between treatments, and females versus males within each treatment. All *P* values were adjusted with the Holm method for multiple comparisons.

Assumptions of linearity between "body weight" and RMR or CT_{max}, as well as the homogeneity of the regression slopes were tested visually. In addition, assumptions of normality and homoscedasticity were verified visually (performance library version 0.10.2; Lüdecke et al. 2021) and with a Shapiro–Wilk and Levene's test. Individual RMR and "body weight" were Log_{10} transformed to meet the assumptions. In the case of CT_{max}, the assumptions of normality and homoscedasticity were not met, even after a Log_{10} transformation. Therefore, we decided to use the untransformed data, as we assume that our model would tolerate a deviation from the assumptions due to the high level of replication and experimental design used (Sokal and Rohlf 1995).

Finally, to test for potential bias in size selection between treatments (i.e., bigger individual being involuntarily selected for specific treatment), an LMM with "Temperature," "Oxygen," and "Sex" as main fixed factors and "Aquarium" as a random factor was run on the copepods' length. There was no evidence that specimens' size was different between temperature ($p = 0.9583$), oxygen ($p = 0.4574$), or any of the interactions tested (minimum $p = 0.1477$). However, there was very strong evidence that females were longer than males ($p < 0.0001$), by 0.13 mm on average, which was to be expected considering the known size dimorphism of this species (Sasaki et al. 2019).

2.10 | Trait Variability Analyses

Additionally, to distinguish potential differences in response between females and males, sex-specific trait variability was calculated for survival, RMR and CT_{max}, within each treatment using the coefficient of variation ratio, following the equation used in Missionário et al. (2022):

$$\ln\mathrm{CVR} = \ln\left(\frac{\frac{SD_f}{mean_f}}{\frac{SD_m}{mean_m}}\right) \tag{4}$$

where SD_f and $mean_f$ correspond to the standard deviation and mean calculated in females and SD_m and $mean_m$ correspond to the standard deviation and mean in males.

2.11 | Multistressor Models Calculation

To characterize the nature of the combined effects and interactions (i.e., additive, synergistic, or antagonistic) between oxygen and temperature on survival, RMR, and CT_{max}, multistressor models were calculated using Piggott, Townsend, and Matthaei (2015) definition of synergies and antagonism: see also Côté, Darling, and Brown (2016). Namely, we calculated the magnitude and direction of the effect of each "stressor" (i.e., H, MHW, and HMHW) relative to the control (C) condition. We, then, compared the realized effect of the combined treatment (AB) to a null model corresponding either to a simple additive effect model (A + B) for the RMR and CT_{max} data or a multiplicative model ((A + B) − (A × B)) for survival. Then, for the traits in which no interaction between stressors was found (2 or 3-way interactions not significant in the ANOVA), we looked at whether one stressor had a dominant effect or an additive effect (sensu Piggott, Townsend, and Matthaei 2015). When, however, a three-way interaction term (Temperature × Oxygen × Sex) in a model for a trait was significant, the effect of the combined stressors (HMHW) was compared to a null model, for each sex separately, to identify the antagonistic or synergistic nature of the interactions (Piggott, Townsend, and Matthaei 2015). Raw or transformed data were used in accordance with the input data used in the ANOVA.

All analyses were performed using the R software (version 4.2.1; R Core Team 2022) and results interpretation was conducted using the "language of evidence" guidelines proposed by Muff et al. (2022) to allow for a more nuanced and rigorous way to present the results. In more detail, evidence of effects is described following this range: $0.0001 < p < 0.001$—very strong evidence; $0.001 < p < 0.01$—strong evidence; $0.01 < p < 0.05$—moderate evidence; $0.05 < p < 0.1$ weak evidence; $0.1 < p < 1$—little to no evidence.

3 | Results

3.1 | Survival

Our results show that females and males are differentially affected by exposure to hypoxia, as supported by the very strong evidence for the presence of an interaction between the terms "Oxygen" and "Sex" for survival (Table 2 and Figure 1a). Namely, there was weak evidence that females exposed to hypoxia had a higher survival than their counterparts kept in normoxic conditions ($p = 0.06$), and there was moderate evidence for a higher survival when compared to males also exposed to hypoxia ($p = 0.03$). However, there was little to no evidence of differences in survival between males at the two oxygen levels tested, between males and females in the normoxic condition and between females in the normoxic condition and males in the hypoxic condition (minimum $p = 0.19$ for all comparisons). In addition, we report a 27.08% decline in survival in copepods exposed to 25°C (i.e., MHW and HMHW treatments) in comparison to copepods exposed to 18°C (i.e., C and H treatments) (Figure 1b). This was supported by the very strong evidence that a 5 days exposure to elevated temperature mimicking a MHW leads to a decrease in survival in *A. tonsa* (Table 2 and Table S1). There was, however, little to no evidence that females and males had different survival rates following the exposure to MHW conditions in isolation, or when exposed to the combined effects of hypoxia and a MHW (Table 2 and Table S1).

3.2 | Egg Production Rates

Females produced an average 3–7 eggs $females^{-1}$ $days^{-1}$ under the different tested conditions (Figure 1c and Table S1). Despite the lower survival rates reported at 25°C, there was little to no evidence that egg production rates were affected by exposure to

TABLE 2 | Summary of the results of the analyses of variance (ANOVA type III) for the effect of “Temperature”, “Oxygen”, “Sex” and their interactions on copepods' survival, egg production rates, routine metabolic rates (RMR), and the upper thermal limit (CT_{max}) following a 5-day exposure to the single and combined effects of hypoxia and marine heatwave (MHW) conditions on the marine copepod *Acartia tonsa*.

Survival

Factor	df	χ^2	Pr(>χ^2)	Evidence
Temperature	**1**	**22.17**	**< 0.0001**	**Very strong**
Oxygen	1	0.08	0.78	Little/no
Sex	1	0.47	0.49	Little/no
Temperature: Oxygen	1	2.38	0.12	Little/no
Temperature: Sex	1	0.36	0.55	Little/no
Oxygen: Sex	**1**	**10.91**	**< 0.001**	**Very strong**
Temperature: Oxygen: Sex	1	0.00	0.99	Little/no

Egg production rates

Factor	Sum sq	df	*F* value	Pr(>*F*)	Evidence
(Intercept)	379.82	1	34.27	< 0.0001	
Temperature	16.54	1	1.49	0.25	Little/no
Oxygen	12.88	1	1.16	0.30	Little/no
Temperature: Oxygen	0.07	1	0.01	0.94	Little/no
Residuals	132.98	12			

Routine metabolic rates (RMR)

Factor	df	χ^2	Pr(>χ^2)	Evidence
(Intercept)	1	57.44	0.00	
Log weight	1	0.08	0.78	Little/no
Temperature	**1**	**15.86**	**< 0.0001**	**Very strong**
Oxygen	1	0.06	0.81	Little/no
Sex	1	0.57	0.45	Little/no
Temperature: Oxygen	1	1.38	0.24	Little/no
Temperature: Sex	1	1.19	0.27	Little/no
Oxygen: Sex	1	0.28	0.60	Little/no
Temperature: Oxygen: Sex	1	0.90	0.34	Little/no

Upper thermal limit (CT_{max})

Factor	df	χ^2	Pr(>χ^2)	Evidence
(Intercept)	1	538.97	0.00	
Weight	1	1.81	0.18	Little/no
Temperature	**1**	**8.65**	**< 0.01**	**Strong**
Oxygen	1	1.57	0.21	Little/no
Sex	**1**	**18.34**	**< 0.0001**	**Very strong**
Temperature: Oxygen	1	0.11	0.74	Little/no
Temperature: Sex	1	0.04	0.84	Little/no
Oxygen: Sex	1	0.63	0.43	Little/no
Temperature: Oxygen: Sex	**1**	**4.30**	**0.04**	**Moderate**

Note: Factors for which we have evidence of effect ($p < 0.1$) are highlighted in bold.

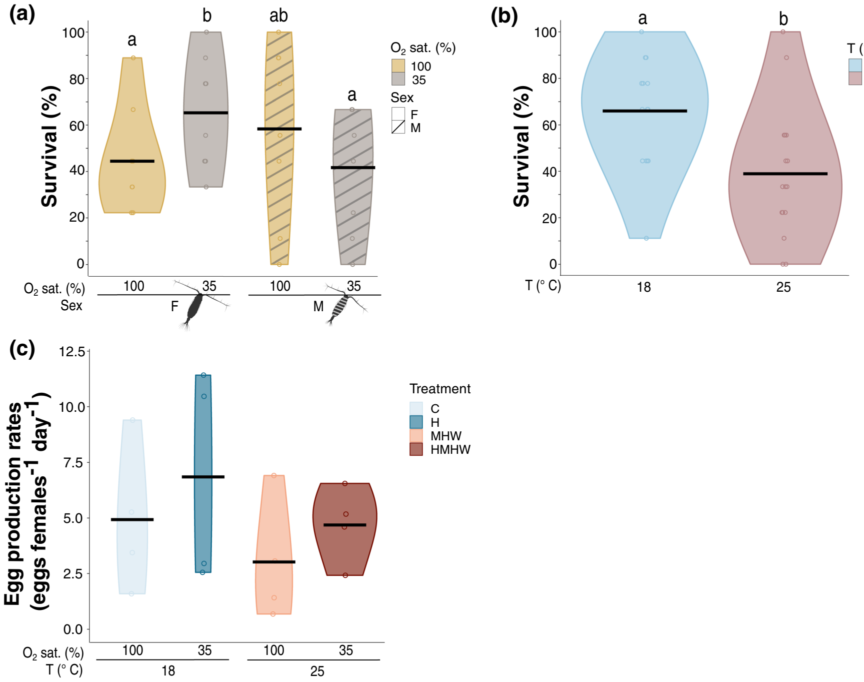

FIGURE 1 | Mean survival and egg production rates following a 5-day exposure to hypoxia and marine heatwave (MHW) in the copepod *Acartia tonsa*. (a) Sex-specific survival rates at the two oxygen saturation levels (O_2 sat.), both temperatures combined. (b) Survival rates between the two temperature levels, all other factors combined. (c) Average egg production rates of *A. tonsa* females, represented by the number of offspring spawned day^{-1} female^{-1}. Violin plots represent the distribution of the data, with the black horizontal line and the small open circles corresponding respectively to the mean and individual data points. Lowercase letters identify evidence of differences between the groups.

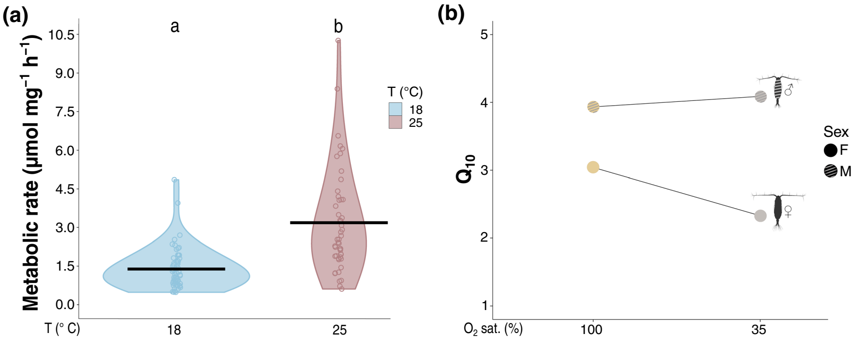

FIGURE 2 | Routine metabolic rates (RMR) and thermal sensitivity (Q_{10}) of females and males of the copepod *A. tonsa* following a 5-day exposure to hypoxia and MHW conditions. (a) Specific RMR (µmol mg^{-1} h^{-1}) between the two temperature levels (18°C in blue and 25°C in rosy mauve), all other factors combined. Violin plots represent the distribution of the data, with the black horizontal line and the small open circles representing respectively the mean and individual data points. Lowercase letters represent evidence of differences between the temperature treatments. (b) Thermal sensitivity of metabolic rate expressed in Q_{10} metric in function of the two O_2 sat. levels (%) for females (full dot) and males (stripped dot).

hypoxia or MHW conditions, in isolation or combination in *A. tonsa* (Table 2).

3.3 | Routine Metabolic Rates (RMR)

RMR was highest at 25°C (3.18 ± 2.01 µmol mg^{-1} h^{-1}) in comparison to 18°C (1.39 ± 0.79 µmol mg^{-1} h^{-1}), regardless of the oxygen levels during the exposure (Figure 2a). This difference was supported by very strong evidence for a positive effect of temperature on metabolic rates (Table 2). By contrast, there was no evidence for an effect of hypoxia or any interaction between factors on RMR. In addition, there was little to no evidence that the 5-day exposure to the single and combined effects of hypoxia and a MHW impacted females' and males' RMR differently (Table 2) as their metabolic rates were comparable across all conditions tested (Table S1).

Q_{10} values calculated for females and males at the two oxygen saturation levels were higher for males than females, with Q_{10} values measured after the 5-day exposure to normoxic and hypoxic conditions being, respectively, 3.93 and 4.09 in males and 3.4 and 2.33 in females (Figure 2b). Males and females' Q_{10} showed different patterns in response to exposure to hypoxia, with males showing only a 4% increase in Q_{10} between normoxic and hypoxic conditions but a 23% decrease in females.

3.4 | Upper Thermal Limit (CT_{max})

Acartia tonsa females had a higher upper thermal limit (average $\text{CT}_{\text{max}} = 34.72 \pm 2.52$) than males (average $\text{CT}_{\text{max}} = 31.99 \pm 2.19$). In contrast to RMR, the combined effects of hypoxia and a MHW on copepod's CT_{max} differed between females and males, as supported by the moderate evidence for the presence of a three-way interaction between temperature, oxygen, and sex (Table 2). Namely, within most treatments, females showed higher mean CT_{max} than males (maximum $p < 0.019$), with females and males showing comparable mean CT_{max} only in the HMHW treatment ($p = 0.26$; Figure 3 and Table S1). As for the single effects of temperature and oxygen on females, there was respectively weak and no evidence that CT_{max} was higher under MHW conditions when compared to the control treatment (C-MHW; $p = 0.09$) or under H conditions when compared to the control (C-H; $p = 1.00$). Moreover, there was no evidence that females' mean CT_{max} changed in the HMWH treatment when compared to control (C-HMHW; $p = 1.00$), or hypoxia (H-HMHW; $p = 1.00$). In addition, females' CT_{max} did not change between MHW conditions occurring under normoxic or hypoxic water (MHW-HMHW; $p = 0.26$). Still, there was moderate evidence of a shift in females' CT_{max} from H to MHW conditions (H-MHW; $p = 0.03$) (Figure 3). In comparison, for males, there was moderate evidence that the mean CT_{max} under the combined condition (HMHW) was higher when compared to the mean CT_{max} measured under H conditions ($p = 0.04$). However, males' CT_{max} was not different between H-C ($p = 1.00$), H-MHW ($p = 0.26$), as well as C-MHW ($p = 1.00$), C-HMHW ($p = 0.30$), and MHW-HMHW ($p = 1.00$) (Figure 3).

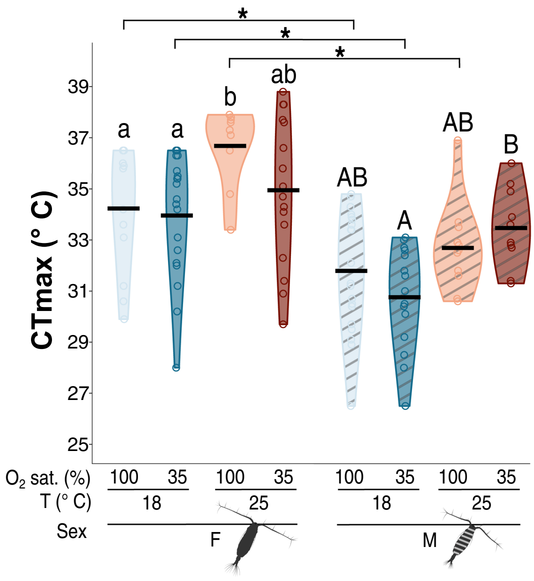

FIGURE 3 | The effect of a 5-day exposure to hypoxia and MHW conditions on the thermal limit (CT_{max}) of females and males of the copepod *A. tonsa*. Violin plots represent the distribution of the data for all combinations of "Temperature", "Oxygen", and "Sex" levels. The black horizontal line and small open circles within the shape correspond respectively to the mean and individual data points. Lowercase letters represent evidence of differences between treatments within females, capital letters showcase evidence of differences between treatments within males, and squared brackets [*] identify evidence of differences between sexes within each treatment.

3.5 | Coefficient of Variation Ratio

Traits' variation between sexes was not found to be consistently biased towards one sex for all traits and treatments, as the lnCVR calculated was not systematically above or below 0 (Figure 4). Namely, while lnCVR for metabolic rates were systematically below 1 in all treatments, indicating male-biased variation, survival was only male-biased for the H, MHW, and HMHW treatments, not the control. As for CTmax, a higher level of variation was observed among females (lnCVR > 0) in the hypoxic and the combined treatments, while males' CTmax was more variable in the control and the MHW treatments (Figure 4).

3.6 | Multistressor Models to Identify the Nature of Combined Stressors Effects

Multistressor models revealed sex-specific differences in the type of interaction for CT_{max} (Figure 5). Namely, in females, the combined effect of temperature and hypoxia on their CT_{max} was lower than the null additive model and lower than the individual effect in the same direction, indicating the presence of a positive antagonistic interaction (sensu Piggott, Townsend, and Matthaei 2015). Conversely, a positive synergistic effect was evident for males, as the combined effects of temperature and hypoxia on males' CT_{max} was higher than the null additive model and higher than any individual effect in the same direction (sensu Piggott, Townsend, and Matthaei 2015). As for copepod's survival and RMR, our models showed an effect of dominance of temperature. Indeed, the effect of the combined stressors was lower than the null multiplicative or additive model used and close to the single effect of temperature (Figure S4).

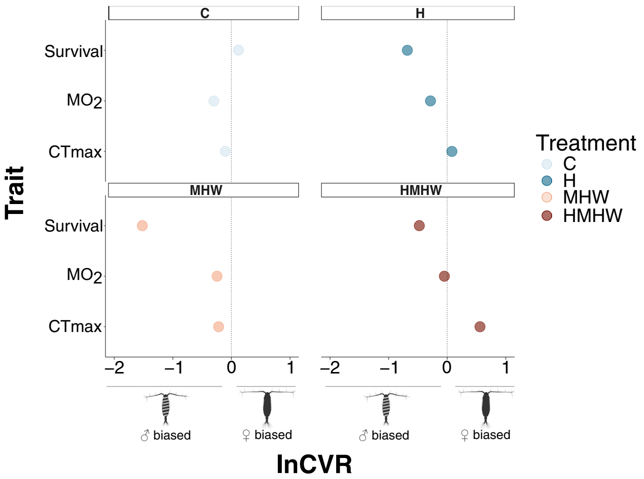

FIGURE 4 | Representation of the level of trait variation for survival, RMR, and CT_{max} in *A. tonsa*'s male and female across the four treatments: Control (C, light blue), hypoxia (H, dark blue), marine heatwave (MHW, orange) and the combined treatment (HMHW, red). A lnCVR > 0 indicates that the trait is more variable in females than males (i.e. female-biased), mean-adjusted.

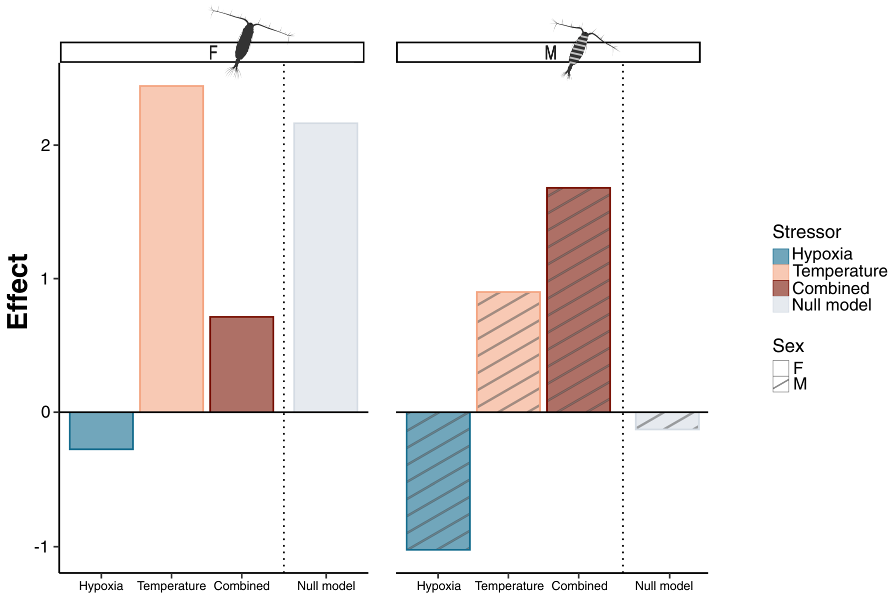

FIGURE 5 | Multistressor models showing antagonistic and synergistic effects of combined hypoxia and a MHW condition on *A. tonsa*' s females (left panel) and males' (right panel) CT_{max}. The bar plot represents the single effect of hypoxia (H, in blue) and temperature (MHW, in orange) and the combined effect of both stressors (HMHW, in red) in comparison with the calculated additive null model (H + MHW, in light grey).

4 | Discussion

Our study provides evidence for the existence of sex-specific responses in the cosmopolitan and ecologically important *Acartia tonsa* following simultaneous exposure to hypoxia and MHW events. Females appear less vulnerable, having higher upper thermal limits and lower metabolic thermal sensitivity than males. Yet, both sexes responded more strongly to the effect of MHW in isolation, with the exposure to combined stressors not yielding interactive effects on most of the traits measured. Overall, our results highlight the relevance of defining the effect of global change drivers on both sexes. In addition, the observed dominant effect of temperature on mortality and metabolic rates in our study supports the idea that MHW events have pervasive implications for this species. Finally, the absence of strong interaction between hypoxia and temperature might indicate that potential cross-protection mechanisms are at play.

4.1 | Sex-Specific Responses Lead to Differential Vulnerability

The complex interplay that temperature, oxygen, and sex play in affecting copepods' ability to tolerate heat strikingly highlights the pivotal influence of sex on physiological traits. Sex differences in thermal tolerance have been documented on several occasions in marine organisms (Bradley 1978; Foley et al. 2019; Sasaki et al. 2019), with a general trend of females possessing higher thermal tolerance than males in arthropods (Edmands 2021; cf. Missionário et al. 2022). The greater thermal tolerance of females combined with their lower metabolic thermal sensitivity (i.e., lower Q_{10}) we report supports the notion of a higher male-biased vulnerability to MHW. Interestingly, Sasaki et al. (2019) also reported higher thermal tolerance in *A. tonsa*'s females (LD_{50}) in two populations living under distinct thermal regimes following developmental exposure to 18°C and 22°C. This implies that *A. tonsa* females could be more thermally tolerant than males, both following developmental and acute temperature exposure.

Several mechanisms have been proposed to explain sex-specific differences, including differences in metabolic rates, body size, and reproductive investments (Edmands 2021). We report no significant differences in metabolic rates between sexes, but males had higher metabolic sensitivity, suggesting that they are less equipped metabolically to face the intense and rapid increase in temperature associated with MHW exposure (Seebacher, White, and Franklin 2014). As for the body size hypothesis, higher thermal tolerance is often correlated to a smaller body size (Leiva, Calosi, and Verberk 2019). While this general trend applies within each sex (Figure S5), *A. tonsa* females are bigger than males, yet have a higher thermal tolerance. Hence, we posit that the differences observed here result from true physiological rather than allometric differences between the two sexes. These physiological differences most likely reflect the "live fast and die young" strategy often adopted by males (Bonduriansky et al. 2008; Ceballos and Kiørboe 2011), making it the "weak sex" in copepods (Ceballos and Kiørboe 2011). Indeed, males have a high mating energy investment (Burris and Dam 2015a) due to the production of spermatophores, a slow and costly process (Burris and Dam 2015b; Bjærke et al. 2016). This high reproductive investment can generate a trade-off between reproduction and maintenance, resulting in the accumulation of oxidative stress (Rodríguez-Graña et al. 2010) and a shorter lifespan with successive mating (Ceballos and Kiørboe 2011; Burris and Dam 2015c). Interestingly, in the copepod *Tigriopus californicus*, males' lower tolerance to various stressors has been associated with their lower proteolytic capacity (Foley et al. 2019) and efficiency in generating cellular stress responses (Li et al. 2020). Consequently, damaged proteins are not properly degraded, leading to cytotoxicity and reduced fitness (Foley et al. 2019). Higher accumulation of oxidative stress in males has also been observed in *A. tonsa* due to their lower protein turnover relative to females (Rodríguez-Graña et al. 2010), but this has not been tested in the context of combined hypoxia and MHW. In addition, male copepods could be subjected to the "mother's curse" (Gemmell, Metcalf, and Allendorf 2004). As mitochondria are inherited from mothers, mutations that are neutral or beneficial for females are kept, even if they are deleterious for males' fitness (Foley et al. 2019; Nagarajan-Radha et al. 2020). Taken together, the high cost of life for males combined with their lower ability to repair temperature-induced cellular damages, could make them particularly vulnerable to future MHWs.

This vulnerability seems to extend to hypoxic and combined conditions. Indeed, the positive antagonistic and synergistic interactions found for females and males, respectively, reveal that the exposure to the combined stressors generates sex-specific non-additive effects on the upper thermal limit. Although counterintuitive, the positive antagonistic interaction found in females meets our prediction: CT_{max} is highest under the MHW condition tested, showing that *A. tonsa* can rapidly increase its thermal limits through acclimation following an acute temperature exposure. Notably, this acute and ecologically realistic 5-day MHW exposure represents more than 40% of the generation time in this species. This phenomenon is well documented in aquatic organisms (e.g., Calosi, Bilton, and Spicer 2008; Sasaki and Dam 2021a; Fernandes et al. 2023). *Acartia tonsa* specifically, can increase the expression of HSPs in a matter of hours following a heat shock (Rahlff et al. 2017). In addition, the non-additive response we report shows a negative effect of hypoxia on the CT_{max} for females, with mean CT_{max} under the combined condition being intermediate between that measured under control and MHW conditions. Considering the effect of combined temperature and hypoxia on thermal tolerance discussed above, this effect was expected. Yet, in males, this pattern is not observed, as CT_{max} under combined conditions was higher than under hypoxia, reaching similar CT_{max} values as females in the combined condition. In theory, this positive synergism in CT_{max} in comparison to the positive antagonism found in females would indicate that males could be less vulnerable to the combined effects of hypoxia and MHWs events. Yet, when analyzed together with the higher survival and stronger decrease in thermal sensitivity observed in females in hypoxic conditions compared with males, we suggest instead that females are more tolerant to the negative effects of hypoxia and MHW in isolation or combined. The fact that traits' variability was mostly male-biased across treatments, but female-biased for CT_{max} under hypoxia and combined conditions, suggests a stronger selective pressure in males under low O_2 conditions (Salinas et al. 2019) and explains why males' CT_{max} was the highest in the combined condition.

4.2 | Marine Heatwaves as the Dominant Stressor

As expected, the 5-day exposure to a MHW event affected both survival and metabolic rates, leading respectively to a decrease of 27% in survival and an increase of 150% in RMR, irrespective of sex. Both lethal and sublethal effects following exposure to MHW have been frequently reported in past laboratory and field studies focusing on copepods (Siegle, Taylor, and O'Connor 2022; Truong et al. 2022; Semmouri et al. 2023). In *A. tonsa* specifically, the higher percentage of mortality suggests that 25°C is beyond its optimal temperature for performance. Our results are coherent with those from Sasaki and Dam (2021b), who reported a 53% survival after 24 h in *A. tonsa*' s females raised under similar conditions. High mortality is expected when the energy imbalance and the cellular damages induced by temperature are so extensive that organisms are not able to recover (Hochachka and Somero 2002; Sokolova 2013; Schulte 2015). Previous studies have shown that *A. tonsa*'s responds to acute heat stress through the upregulation of HSPs and lipid remodeling (Garzke et al. 2016; Werbrouck et al. 2016; Rahlff et al. 2017). We suggest that exposure to a MHW event, singly or combined with hypoxia, led some individuals to undergo rapid upregulation and cellular remodeling, which may have granted survival, while the upregulation may have been too slow to protect other individuals, leading to high mortality. Altogether, these differences in response prevent the complete extinction of our lineages. However, the cellular stress response was not measured here and we cannot confirm this hypothesis. Yet, the strong temperature-induced metabolic responses and high values of Q_{10} we report in comparison to previous studies ($Q_{10} = 1.8–2.1$, Ikeda et al. 2001) suggest that *A. tonsa* is sensitive to MHWs, both under normoxic or hypoxic conditions, and that energy costs for maintenance, cellular adjustments, and reproduction are high. Additionally, behavioral adjustments leading to sex-specific differences in activity levels in each treatment could also have impacted their thermal sensitivity. Indeed, activity levels can highly impact routine metabolic rates, including in copepods (Terry et al. 2024). Hence, measuring how activity levels may differ between sexes and under different treatments is an interesting avenue to investigate in the future.

High energy investment in reproduction could explain why egg production rates were unaffected. Either copepods possess enough energy to allocate into reproduction, or the energy investment into reproduction is at the expense of homeostasis and cellular repair, causing accumulation of oxidative damages that can be lethal for some individuals (Zera and Harshman 2001; Latta, Tucker, and Haney 2019; von Weissenberg et al. 2022). Another non-mutually exclusive hypothesis, given the high inter-individual variability we observe, is that offspring at 25°C were spawned by the surviving individuals: that is, those able to rapidly enhance cellular protection mechanisms or had already high constitutive levels of antioxidants, enabling them to invest energy into reproduction. As we did not measure egg production daily, we cannot exclude this hypothesis. Considering that copepods were fed ad libitum in all treatments, we believe that this non-limiting access to food could have helped them to support the high energetic need imposed by temperature: that is, compensatory feeding (Holmes-Hackerd, Sasaki, and Dam 2023). Still, *A. tonsa* females can transfer part of the oxidative damage into the eggs (Rodríguez-Graña et al. 2010) and egg size can decrease following long-term acclimatization to high temperature (Hansen et al. 2009). Therefore, whether the maintenance of high levels of egg production rates was at the expense of egg size and quality, leading to poor performance in offspring (i.e., carry-over effects), or whether protective parental effects were transmitted to the next generation through the eggs should be further investigated (Marshall and Uller 2007; Bonduriansky, Crean, and Davey 2017; Dinh et al. 2021).

4.3 | Cross-Protection Interaction Between Hypoxia and MHW?

Surprisingly, contrary to our prediction, apart from the upper thermal limit, we report no evidence of an interactive effect between temperature and oxygen on all traits measured. Instead, survival and metabolic rate were more strongly influenced by the MHW, as mentioned above. This might indicate that the mild hypoxic condition used here was above *A. tonsa*'s oxygen threshold (P_{crit}) for sublethal responses and could explain the dominant effect of temperature reported in this study. While we did not measure P_{crit} in this study, exposure to hypoxia alone did not significantly affect the traits we measured, supporting our hypothesis. In fact, according to modelling work by Elliott, Pierson, and Roman (2013) on *A. tonsa*, the level of hypoxia used here ($3.31\,\text{mg}\,\text{L}^{-1}$) was slightly above their P_{crit} ($3.10\,\text{mg}\,\text{L}^{-1}$) at 18°C. However, it should have been within the sublethal range at 25°C. Still, previous studies have also highlighted that oxygen levels may modulate heat tolerance in some species, but not ubiquitously, suggesting species-specific abilities in oxygen uptake regulation (Verberk et al. 2016; Jutfelt et al. 2018). Notwithstanding, the absence of a synergistic interaction between hypoxia and MHW on survival, egg production rates, and metabolic rates could also indicate that a potential "cross-protection" mechanism is possible between the two stressors (Rodgers and Gomez Isaza 2021). Cross-protection develops when prior acclimation to hypoxia or high temperature leads to improved tolerance to the other stressor (Rodgers and Gomez Isaza 2021). It usually requires a period of recovery between stressors (Todgham, Schulte, and Iwama 2005) to enable structural adjustments to improve oxygen uptake (McBryan et al. 2013; Anttila et al. 2015), metabolic depression (Somero, Lockwood, and Tomanek 2017) or trigger the cellular stress response (Ely et al. 2014) to take place. However, we report no metabolic depression in our study, and copepods rely on the passive diffusion of oxygen and must molt to enable morpho-functional changes, which they do not undertake once adult. Thus, they have limited ability to improve oxygen uptake (Roman et al. 2019). Still, exposure to the sub-lethal level of hypoxia could have stimulated the production of reactive oxygen species (ROS) which, in small amounts, act as signaling molecules priming the fast activation of cellular responses (Rodgers and Gomez Isaza 2021). Cross-talk between the hypoxia-inducible transcription factor-1 (HIF-1), which is involved in organisms' responses to hypoxia (Baird, Turnbull, and Johnson 2006; Levesque, Wright, and Bernier 2019), and HSPs have also been suggested as a cytoprotective mechanism leading to cross-protection in some species (Ely et al. 2014). This mechanism should be further investigated in the future, as it should lead to antagonistic effects on the biological responses and such effects were not identified in the present study after 5-day exposure. However, it could explain why the effects we report under combined conditions were lower than the null models and lower than the effect of MHW alone, both for survival and metabolic rate (Figure S4). In addition, behavioral adjustments such as a reduction in swimming speed to prolong tolerance to hypoxic bottom waters, a strategy observed in *Calanus pacificus* under hypoxic conditions (Wyeth, Grünbaum, and Keister 2022), could be employed by *A. tonsa* to reinvest the energy in maintenance instead.

4.4 | Conclusion

We show that *A. tonsa* is vulnerable to the effects of MHW alone, particularly males. Interestingly, synergistic and antagonistic effects of combined stressors were only observed for copepods' upper thermal limits, revealing that potential cross-protection mechanisms could be involved when these stressors occur simultaneously. Nevertheless, the sex-specific differences observed in our study highlight the need to consider sex in future studies. This is particularly relevant to consider in view of the implementation of climate-smart conservation approaches (Stein et al. 2014). The physiological sexual dimorphism observed here is potentially underpinned by different strategies in cellular stress responses, which appear to be dependent on the intensity and duration of the exposure. Therefore, we suggest integrating cellular, physiological and life-history traits measurements in future studies aiming at characterizing the simultaneous effect of hypoxia and MHW events of various intensities and duration to shed light on the mechanism underpinning the sex-specific vulnerability observed here. Still, the higher vulnerability of males to extreme events is particularly concerning, as it could reinforce the existing female-biased sex ratio observed in copepods (Gusmão and McKinnon 2009; Burris and Dam 2015b), with major repercussions for their population dynamics (Gissi et al. 2023). Considering the paramount ecological importance of copepods in marine habitats, such changes can have implications for the functioning of entire ecosystems, as shown for example with the changes in the distribution and abundance of calanoid copepods in the North Sea (Beaugrand et al. 2002; Helaouët, Beaugrand, and Edwards 2013). Given the strong sex-specific differences observed here for *A. tonsa*, the question now remains: will extreme events cause an irreversible shift in the operational sex ratio in this species?

Author Contributions

Fanny Vermandele: conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, visualization, writing – original draft, writing – review and editing. **Matthew Sasaki:** methodology, resources, writing – review and editing. **Gesche Winkler:** resources, supervision, writing – review and editing. **Hans G. Dam:** methodology, resources, supervision, writing – review and editing. **Diana Madeira:** conceptualization, methodology, project administration, supervision, validation, writing – review and editing. **Piero Calosi:** conceptualization, funding acquisition, methodology, project administration, supervision, validation, writing – review and editing.

Acknowledgments

Thanks are due to Laurie Jacob, Tessa Page, Sandrine Picotte, Mizuki Brault-Hori, Haluka Kandachi-Toujas for their help in maintaining the live cultures and the experimental system. We are grateful to André Lucas dos Reis Cuenca and Ellia Roy for their precious help before and during the experiment. We also thank Lydia Norton for her technical advice, Jonathan Coudé for his technical support, Charlotte Carrier-Belleau for her advice on multistressor interactions, Guillaume de

Lafontaine for providing access to his stereomicroscope camera and Madeleine-Zoé Corbeil-Robitaille for the copepod's icons. We would like to acknowledge that UQAR is situated of the traditional and unceded territories of the Wolastoqiyik Wahsipekuk and Mi'kmaqq First Nations. FV, GW, and PC are members of the inter-institutional strategic research network *Québec-Océan*, and this project is a contribution to the *Québec-Océan* research program.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in PANGEA at https://doi.pangaea.de/10.1594/PANGAEA.967352.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.