

Marine heatwave exposure alters metabolism and behavioral strategies in juvenile ectotherm crustacean *Maja squinado*

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Abstract

Marine heatwaves (MHWs) are rapidly intensifying in the Mediterranean Sea, posing major threats to ectothermic species, particularly during early life stages. We investigated the physiological and behavioral responses of juvenile spider crab *Maja squinado* (Decapoda) to a simulated MHW (14–26°C), encompassing rapid warming and post-heatwave cooling. We assessed metabolic rate, hemolymph osmotic pressure, and behavioral traits including locomotion, refuge use, and feeding. During warming, juveniles exhibited a marked shift in survival strategy. Oxygen consumption doubled, feeding activity increased, exploration intensified, and refuge use declined. At the heatwave peak (26°C), individuals adopted a bolder behavioral phenotype, likely driven by elevated energetic demands. This shift coincided with increased predation risk, rising hemolymph osmotic pressure, and peak mortality during warming and sustained exposure. Following the heatwave, juveniles entered a state of metabolic depression consistent with organismal exhaustion. Behavioral activity recovered only later at lower temperatures and remained decoupled from metabolic recovery, suggesting active replenishment of depleted energy reserves. Overall, MHW exposure caused substantial cumulative mortality (~ 20%) and persistent physiological and behavioral alterations. These findings demonstrate that the impacts of MHWs extend beyond peak temperatures and emphasize the importance of considering the complete heatwave cycle, including recovery, when predicting ectotherm resilience to climate change.

1. Introduction

The Mediterranean Sea, and particularly the coasts of the western basin, rank among some of the marine ecosystems most exposed to climate-related risks worldwide ^[1]. Of these risks, marine heatwaves (MHWs) are defined as episodes of rapid, transient, and abnormally high increases in sea temperature (exceeding the 90th percentile of the 30-year climatological mean) lasting more than five consecutive days in a given area ^[2]. The events are becoming increasingly frequent and intense due to climate change ^[3–5]. Over the past decade, no summer period (June - September) in the Mediterranean has been spared from MHWs, with approximately two-thirds of days exhibit sea surface temperatures between 20 and 26°C, while the remaining third reach or exceed 26°C (ranging from 29 days in 2017 to 67 days out of 122 days in 2022) (Fig. 1). For instance, 107 MHW were recorded during summer 2022 ^[6]. Although variability in intensity, duration, and frequency has been observed, all MHW events have been associated with significant disruptions to marine ecosystems ^[4]. These anomalies can persist for several months or even an entire summer, as observed in the Mediterranean Sea during summer 2022 (Fig. 1). Such extreme events, and the acute thermal stress they generate, can lead to major ecological consequences, affecting biological organization from cellular processes to interspecific interactions, and impacting a wide range of organisms. The resulting cascading effects may include alterations to trophic networks, shifts in species distributions, mass mortalities, and even local extinctions ^[5, 7–9].

Ectothermic organisms, whose body temperature depends on the external environment, are particularly vulnerable to MHWs ^[7, 8, 10, 11]. MHWs can have devastating consequences for their populations, with

significant impacts on local economies, as illustrated by the blue crab *Portunus armatus* following the 2011 MHW in Western Australia, where the fishery had to be closed for 18 months to preserve the populations^[7]. Consequently, MHWs can lead to a substantial reduction in fishing activity, affecting effort, catch levels, and overall income^[3, 4, 8]. This raises important questions about the capacity of ectothermic organisms for rapid acclimation to warming and, consequently, their ability to sustain future generations^[12].

These adjustments in response to thermal stress may include changes in osmoregulatory capacity, reflected by the difference in osmotic pressure between the hemolymph and the external medium in crustaceans, which serves as a reliable indicator of physiological state and stress response^[13]. Measuring metabolic rate through respirometry is also a key tool for quantifying the energetic cost of temperature fluctuations, revealing physiological limits imposed by rising water temperatures^[14–16], and can even provide valuable insights into future organismal ecology, such as species distributions^[17]. Together, these two measures of major physiological functions (osmoregulation and oxygen consumption), assessed at the whole-organism level, highlight global metabolic responses to thermal stress.

Beyond physiology, thermal fluctuations also strongly influence individual behavior of ectotherms^[11, 18]. In most marine species, internal heat regulation is nearly absent, making behavioral acclimation a key mechanism for dealing with extreme conditions and therefore a particularly relevant indicator to study. Temperature fluctuations thus induce a wide range of behavioral responses such as avoidance, changes in locomotor activity or trajectories, and more broadly behavioral strategies such as boldness or foraging^[10, 16, 19, 20]. These responses can occur over different time scales, ranging from immediate and transient effects to more delayed adjustments^[11].

Species with restricted distributions are among the most vulnerable to MHWs, as they have developed thermal tolerances specific to their local environments^[9]. Among endemic marine ectotherms, the spider crab *Maja squinado*, endemic of the Mediterranean Sea, holds both ecological and economic importance^[21–24]. Although it is not currently listed by the IUCN (International Union for Conservation of Nature), this species is experiencing marked declines and even local extinctions, as observed in the Balearic Islands^[21]. In Corsica, CPUE (“Catch Per Unit Effort”) dropped fivefold between 2011 and 2020^[22]. This decline may be partly attributed to stressors affecting critical developmental stages. Indeed, larval and juvenile stages are among the first to be exposed to temperature disturbances and generally exhibit lower thermal tolerance than adults, partly due to their higher energetic demands for growth and molting^[8, 24]. Larval dispersal of *M. squinado* species can be affected by temperature variations, which partly drive the intensification of turbulent activity^[23], and also affect larval drift duration, as the length of this developmental phase is closely determined by temperature (13 to 20 days at 18–20°C, ^[21, 25]). Moreover, juvenile *M. squinado* inhabit shallow coastal waters, between 3- and 15-meters depth, during a

two-year growth phase^[26, 27], making them particularly exposed to temperature fluctuations during this period.

In this context, it is essential to understand how juvenile ectotherm species respond to MHWs and the mechanisms they deploy to cope with these extreme thermal disturbances, using *M. squinado* as a relevant ecological model. These physiological and behavioral adjustments are key to assessing the species' resilience and acclimation potential in degraded habitats.

The present study aims to analyze the effects of a simulated MHWs on juvenile *M. squinado* by examining their physiological and behavioral responses throughout both the warming and subsequent cooling phases of a heatwave, with a temperature range from 14 to 26°C. The objective is to identify key functional traits, including metabolic rate, osmoregulation and behavior strategies, that may be affected by MHWs.

Understanding the phenotypic responses of juvenile *M. squinado* to MHWs is therefore critical for informing stock enhancement strategies and promoting the long-term sustainability of Mediterranean crustacean populations in the face of climate change.

2. Material and methods

2.1. Recent MHW dynamics in the western Mediterranean basin (2016–2025)

Marine heatwaves (MHWs) occurring over the past decade (2016–2025), between June 1 and September 30, were assessed to provide an estimate of the thermal conditions that juvenile spider crabs may experience in their natural habitat, and to place the experimental results in an ecologically relevant context. To this end, daily sea surface temperature (SST) data for the western Mediterranean basin were obtained using E.U. Copernicus Marine Service Information^[6]. MHWs were identified following Hobday *et al.* (2016)^[2] as periods of at least five consecutive days during which SST exceeded the seasonally varying 90th percentile threshold derived from the 30-year baseline.

2.2. Animal collection and rearing conditions

In May 2024, four ovigerous female *M. squinado* (average weight: 1.445 ± 0.108 kg) were captured in the Gulf of Saint-Florent, on the western coast of Cap Corse (42°45.441'N 09°12.713'E) by a local fisherman, and then brought to the Stella Mare marine platform in Biguglia, Corsica. Egg hatching, larval development, and juvenile rearing were carried out in captivity, in tanks supplied with filtered and disinfected seawater at a temperature of $19.15 \pm 0.55^\circ\text{C}$, a salinity of $37.5 \text{ g}\cdot\text{kg}^{-1}$, and a pH of 8.02 ± 0.10 . Once the individuals reached the third crab stage, they were transferred to an outdoor 75 m³ tank with continuous partial water renewal (20% per hour with natural filtered and disinfected seawater), and fed every other day with a mixture of fish, mussels, and shrimp.

2.3. Ethics statement

The capture of berried females was authorized under Order No. 001/DLMC/2024, issued on 30 January 2024 by the Direction of the Sea and Corsican Coast, permitting personnel of the UAR 3514 Stella Mare (University of Corsica–CNRS, France) to collect live biological samples for scientific purposes. Under the applicable French regulations, no specific ethical approval was required for experimental procedures involving the decapod crustacean *M. squinado*. However, all animals maintained alive in captivity were covered by a European animal health approval and an animal experimentation authorization (no. 2B.037.001) issued by the French state authorities. All methods were carried out in accordance with the relevant institutional, national, and European guidelines and regulations.

2.4. Experimental design

Acclimation procedure

For this study, 80 8-month-old juvenile *M. squinado* were randomly selected (36 females, 44 males, mean weight: 6.38 ± 3.00 g; mean cephalothorax length: 32.6 ± 5.94 mm) and individually placed in 2-L boxes. These boxes were distributed across 10 tanks of 180L (8 individuals per tank), supplied by a recirculating aquaculture system (RAS) using 1- μ m filtered and UV-disinfected seawater. Individuals were randomly assigned to tanks while maintaining a male/female sex ratio of 1.22. The entire setup was installed in a climate-controlled room. The physicochemical parameters of the water (dissolved oxygen, ammonia, nitrites, nitrates) were kept within optimal ranges using water renewal and monitored daily. The tanks were maintained at a temperature of $13.62 \pm 0.46^\circ\text{C}$, a salinity of 36.23 ± 0.62 g·kg⁻¹, and an oxygen saturation of $96.59 \pm 1.11\%$. The individuals were fed twice a week with mussels and fish.

Photoperiod inversion

As *M. squinado* is a predominantly nocturnal species, a photoperiod inversion was implemented to synchronize activity peaks with laboratory working hours. The initial light cycle (7:30 a.m.-5:30 p.m.) was gradually reversed over 9 days, with a shift of 1.5 hours per day, to reach an inverted light cycle of 7:30 p.m.-5:30 a.m. Artificial darkness was then maintained during the day, allowing measurements to be carried out during the animals' active phase. A two-week acclimation period followed this manipulation before the start of thermal experiments.

Experimental marine heatwave simulation (Fig. 2)

Figure 2. Graph of temperature variations for *M. squinado* individuals: groups of individuals (CTRL: control; WP: warming phase; CP: cooling phase), inversion of photoperiod, acclimation time, and measurement periods (dashed lines) are represented throughout the weeks.

Behavioral, respiratory and osmometry measurements were assessed at three key temperatures: 14°C, 20°C, and 26°C. These temperatures represent a broad thermal range to which juvenile *M. squinado* are likely to be exposed during their first two years of life. Measurements were conducted in two successive

phases: first a warming phase ('WP', 14°C → 20°C → 26°C), followed by a cooling phase ('CP', 26°C → 20°C → 14°C). The mean temperatures ± standard deviation at each stage of the heatwave were: 13.82 ± 0.46°C (CTRL); 19.85 ± 0.46°C and 26.10 ± 0.44°C (WP); and 19.94 ± 0.47°C and 13.88 ± 0.45°C (CP). The 80 individuals were divided into five thermal groups (16 individuals each), with each temperature assigned to a pair of tanks for measurements (e.g., tanks 1 and 2: 14°C, tanks 3 and 4: 20°C, etc.). Temperature transitions were carried out at a controlled rate of 0.5°C/h over 12 hours. Once the target temperature was reached, it was maintained for seven days (five days of acclimation followed by two days of measurements). For each temperature level, the corresponding individuals underwent a 7-day fasting period before behavioral, respirometry, and osmometry measurements were conducted. After each measurement session, the tested individuals were returned to external tanks. For the remaining experimental groups, temperature was increased (or decreased) for the next measurements.

Temperature treatments were coded as follows: 14CTRL for the 14°C control condition; 20WP and 26WP for 20°C and 26°C during the warming phase, respectively; and 20CP and 14CP for the 20°C and 14°C treatments during the cooling phase, respectively.

2.5. Mortality assessment

Mortality was monitored throughout the experiment, and any dead individuals were removed from the tanks each day. For each experimental period (acclimation or temperature variation), mortality was calculated as the number of dead individuals during the considered period divided by the number of individuals still included in the study at that time. Individuals that had been removed after completing the final measurements and returned to the outdoor tank were excluded from the counting. This approach therefore provides a conservative estimate of mortality, as additional mortality would likely have occurred if all individuals had remained exposed throughout the entire MHW simulation.

2.6. Behavioral analysis (Fig. 3)

The behavior of the juveniles was observed in a 60-L aquarium with the walls covered with opaque PVC panels to prevent mirror effects. The aquarium was divided into two watertight compartments to prevent any interaction between individuals and the diffusion of chemical cues. This setup allowed for the simultaneous observation of two individuals. The observations were conducted in darkness using an infrared table on which the aquarium was placed. The behavior of the individuals was recorded using a high-definition infrared video camera (Basler acA1440-220um, Germany) positioned above the aquarium and connected to a computer equipped with the Pylon Viewer software. Each individual was placed in one of the two compartments of the aquarium at a specific temperature (14°C, 20°C, or 26°C).

Preliminary tests using HOBO TidbiT MX2203 Temp 400 data loggers (Onset Computer Corporation, Bourne, MA, USA) confirmed that temperature was homogeneous within the tank.

First, the juveniles were left for 10 minutes for free exploration. Then, for the first behavioral phase, they were filmed for 30 minutes to analyze their activity in the absence of external stimuli (Fig. 3.A). The videos were analyzed and behaviors quantified using DeepLabCut (Nath et al., 2019), a deep learning-based software that performs markerless pose estimation of user-defined body parts. Locomotor

trajectories were reconstructed from the centroid coordinates of each individual (point on the center of the carapace) extracted from video recordings (5 frames.s⁻¹). Frame-to-frame displacements were computed using Euclidean distance between successive positions. Six behavioral parameters were extracted: immobility, total distance travelled, explored area, path tortuosity, mean and maximum speed.

Immobility was quantified using non-overlapping 1-s windows (5 frames); a window was classified as immobile when the cumulative displacement within the window was < 5 pixels.s⁻¹, and the percentage of immobility was calculated as the proportion of immobile windows. Total distance travelled was obtained by summing all step lengths and converted from pixels to cm using the spatial calibration (1 cm = 14.42 pixels). The explored area was estimated as the area of the convex hull encompassing all recorded positions. Tortuosity was quantified as the cumulative sum of turning angles between successive movement vectors calculated from smoothed coordinates (1-s rolling median). To limit the influence of tracking jitter, turning angles were only computed when both adjacent step lengths were ≥ 2 pixels. Tortuosity was expressed in degrees and normalized by total distance travelled (°·cm⁻¹), allowing comparisons among individuals independently of trajectory length, with higher values indicating more sinuous trajectories. Instantaneous speeds were calculated by dividing step length by the inter-frame interval (0.2 s), and mean speeds were then derived for each individual. Peak speed was defined as the 95th percentile of instantaneous speeds.

In a second phase, survival strategy was assessed by introducing two stimuli placed at opposite ends of the aquarium: a food source (mussel, following a 7-day fasting period) at the right end, and a shelter (made of dark grey PVC for better camouflage, with an opening allowing the individual to enter) at the left end (Fig. 3.B). The individuals were manually placed at each end in turn to familiarize them with the presence of the new stimuli, then returned to the center of the aquarium where they could choose to either seek refuge in the dark shelter or move toward the food. This phase was recorded for 30 minutes. The videos were analyzed using BORIS (Behavioral Observation Research Interactive Software, Friard & Gamba, 2016), an open-source software package that allows the systematic coding and quantification of behavioral events from video recordings. The observed variables included the individuals' initial choice, as well as the proportions of time spent: feeding, within the shelter or exploring.

2.7. Indirect flow-through respirometry

Once the behavioral observations were completed, oxygen consumption rates were carried out following the protocol described by Raffalli et al. (2025). The volume capacity for the metabolic chambers was 300 mL, ensuring individuals to move only slightly. Therefore, the measured routine metabolic rate (RMR) should be as close as possible to the standard metabolic rate (SMR). After each measurement, biomass weight in each metabolic chamber was measured using VWR LA 614i weighing scale (VWR, West Chester, United States). The final oxygen consumption was calculated as follows:

$$O_2 \text{ consumption} = \frac{([O_2]_{FINAL} - [O_2]_{INITIAL}) \times V \times MO_2}{W \times t} - \text{Blank consumption}$$

- V is the volume of the MC (in L)
- W is the weight of fresh material (in g)
- $[O_2]$ is the oxygen concentration in the MC (in $\mu\text{mol.L}^{-1}$)
- T is the time spent in the MC (in s)
- M_{O_2} is the molar mass of oxygen (in g.mmol^{-1})
- Final oxygen consumption in $\text{mgO}_2.\text{g}^{-1}.\text{h}$

2.8. Hemolymph osmolarity and ions analysis

To ensure that handling stress did not interfere with behavioral or respirometric data, hemolymph sampling for osmotic pressure (OP) was performed only after these measurements were completed. Hemolymph was sampled with a 1-mL hypodermic syringe by inserting the needle through the joint between the Coxa and Basis articles in the right or left fourth leg (Lignot et al., 2000). OP was immediately quantified by freezing-point depression osmometry using a Model 3320 osmometer (Advanced Instruments, Norwood, MA, USA).

2.9. Statistical analysis

All statistical analyses were performed using R (R Core Team, 2021).

Among the 65 individuals analyzed, 12 molted during the experimental weeks. As no influence of molting was detected on behavioral parameters (Kruskal-Wallis tests), all individuals were pooled for the analysis. In contrast, because molting status significantly influenced the physiological parameters associated with respiration and osmoregulation (see Results section 3.3), the 12 post-molt individuals were excluded from these analyses.

Behavioral data of the first phase (proportion of immobile time, total distance travelled, explored area, directionality, tortuosity, mean and peak speed) were not normally distributed (Shapiro–Wilk test) and did not meet the assumption of homogeneity of variances (Bartlett test). Therefore, Kruskal–Wallis tests were used to assess the effect of thermal conditions on each behavioral parameter, followed by Dunn post-hoc tests.

For the second phase of behavioral trials, initial behavioral choice (orientation toward shelter or food) was analyzed using a chi-square test of independence to assess the effect of temperature on the distribution of behavioral choices. Then, we tested whether the overall behavioral composition differed among experimental conditions. Because the three behavioral categories (feeding, sheltering, and exploring) are not independent, and since their proportions sum to one for each individual (i.e., compositional data), behavioral time budgets were analyzed using Dirichlet regression. Zero proportions were replaced by a small constant ($\epsilon = 10^{-4}$), and proportions were subsequently rescaled to sum exactly to one prior to model fitting. Thereafter, to compare behaviors within each temperature condition, pairwise log-ratios between behavioral categories were computed following Aitchison's compositional

approach (e.g., log feeding/sheltering, log sheltering/exploring). These log-ratios were analyzed using linear models to test condition effects on specific behavioral balances.

For physiological data, one-way ANOVAs were conducted, followed by Tukey’s post-hoc tests to identify significant differences between conditions. The effect of molting on physiological parameters was tested using Wilcoxon tests by comparing post-molt individuals with intermolt or pre-molt individuals.

Finally, to explore relationships between metabolic and behavioral variables, a heatmap was generated for each temperature, displaying pairwise Spearman correlation coefficients for all variable combinations.

3. Results

3.1. Experimental survival rate (Table 1)

Table 1

Experimental setup. Water temperature and duration for each of the experimental conditions are presented. Mortality rates for each experimental phases and number of remaining individuals are also recorded.

Temperature condition	Withdrawn individuals	Experimental phase	Phase duration	Dead individuals	Mortality rate	Remaining individuals
14°C (14CTRL)	--	Acclimation	2 weeks	2 (/80)	2.50%	78
20°C (20WP)	16 (14CTRL)	Warming phase from 14 to 20°C	12 hours	2	2.56%	60
20°C (20WP)	--	Acclimation	1 week	2	3.33%	58
26°C (26WP)	15 (20WP)	Warming phase from 20 to 26°C	12 hours	3	5.17%	40
26°C (26WP)	--	Acclimation	1 week	3	7.50%	37
20°C (20CP)	12 (26WP)	Cooling phase from 26 to 20°C	12 hours	1	2.70%	24
20°C (20CP)	--	Acclimation	1 week	1	4.17%	23
14°C (14CP)	12 (20CP)	Cooling phase from 20 to 14°C	12 hours	0	0.00%	11
14°C (14CP)	--	Acclimation	1 week	1	9.09%	10

During the experiment, 15 mortalities in total were recorded out of 80 individuals (19%). At 26°C, mortality rates were higher than at the other temperatures: 5.17% during the rapid warming phase (12 h)

and 7.50% during the one-week acclimation at this temperature. Additionally, at 14°C at the cooling phase, the mortality rate reached 9.09%, the highest observed, but this corresponded to the death of only one individual out of the 11 remaining in the experiment. For the other temperature treatments, mortality rates ranged between 0 and 4.17%.

3.2. Behavioral analysis

Baseline activity (without stimuli)

The proportion of time spent immobile was generally high across all conditions (Fig. 4.A.). It was maximal at the beginning of the experiment ($79 \pm 3\%$) under the 14CTRL condition and minimal at the same temperature at the end of the heatwave ($59 \pm 6\%$ under the 14CP condition), although this trend was not significant ($p = 0.08$). Total distance travelled and explored surface area followed the same pattern (Figs. 4.B. and C.), significantly for total distance ($p < 0.05$) but not for explored surface ($p = 0.26$), with minimum values at the beginning of the experiment ($314 \pm 42\text{cm}$ and $166 \pm 49\text{cm}^2$, respectively, under the 14CTRL condition) and maximum values after the heatwave ($568 \pm 80\text{cm}$ and $334 \pm 59\text{cm}^2$, respectively, under the 14CP condition).

Normalized tortuosity (cumulative turning angle standardized by total distance travelled) was significantly affected by temperature variation ($p < 0.01$) (Fig. 4.D). It increased during the warming phase, rising from $4.156 \pm 0.719^\circ.\text{cm}^{-1}$ under the 14CTRL condition to $7.917 \pm 1.445^\circ.\text{cm}^{-1}$ under the 20WP condition. During the cooling phase, tortuosity dropped to its lowest value at 20CP ($3.572 \pm 0.591^\circ.\text{cm}^{-1}$) and then sharply increased, reaching its highest level at 14CP ($9.002 \pm 1.181^\circ.\text{cm}^{-1}$). These differences were significant between 14CTRL and 14CP and between 20CP and 14CP conditions ($p < 0.05$).

Mean and peak (P95) locomotor speeds increased throughout the heatwave in a non-significant manner ($p = 0.112$ and 0.148 respectively) (Figs. 4.E. and F), with minimum values under the 14CTRL condition (0.246 ± 0.031 and $1.095 \pm 0.113\text{cm.s}^{-1}$, respectively) and maximum values at the same temperature at the end of the heatwave (14CP: 0.44 ± 0.062 and $1.786 \pm 0.212\text{cm.s}^{-1}$, respectively).

The density maps illustrating all individuals' trajectories (Fig. 4.G) reveal distinct patterns depending on thermal conditions. Individuals exhibited a pronounced edge effect, remaining predominantly near the walls of the aquarium, particularly in the corners, across all temperature treatments. However, at the peak temperature of 26°C, a tendency towards increased central exploration was observed. While large central areas with zero density were visible at all other temperatures (14CTRL, 20WP, 20CP, and 14CP), no area of the aquarium displayed zero density at 26°C, and many trajectories crossed through the center of the arena.

Activity under enriched conditions (food and shelter)

Initial behavioral choice, whether toward shelter or food, was not significantly influenced by temperature (Fig. 5.A, $p = 0.31$). However, under the 14CTRL, 20WP, and 20CP conditions, nearly 75% of individuals

initially chose the shelter. At 26WP, this proportion decreased to 60%, and dropped to only 30% at 14CP, meaning that 70% of individuals first selected the food under this condition.

Considering the entire duration of the second phase of the behavioral trials (Fig. 5.B), the overall behavioral composition differed between conditions ($p = 0.047$), suggesting a behavioral reallocation. The model showed a difference in feeding time proportion between 14CTRL and 26WP ($p = 0.0038$). Indeed, it rises from 0.311 at 14CTRL, 0.52 at 20WP, reaching a maximum of 0.695 at 26WP, and then decreasing to 0.303 at 20CP during the cooling phase. As expected, the proportion of time spent inside the shelter followed the opposite pattern: it was highest under the control condition (14CTRL: 0.652), decreased to 0.423 at 20WP and further declined to 0.127 at 26WP, before returning to its initial level during the cooling phase (0.666 at 20CP). At the final temperature condition (14CP), time allocation was more balanced, with individuals spending 0.486 and 0.373 of their time feeding and in shelter, respectively.

Considering the behavioral composition within conditions, the log-ratio analysis showed significant effect of the temperature on the Sheltering/Exploring and the Feeding/Sheltering ratios ($p = 0.027$ and 0.041 respectively). While sheltering predominated over exploration and feeding in 14CTRL and 20CP conditions, this balance was reversed in 26WP condition, where exploratory and feeding behavior became relatively more prevalent. Indeed, the time allocated to exploration was negligible (< 0.06) under the 14CTRL, 20WP, and 20CP conditions, but increased markedly under the peak (26WP) and the final condition (14CP) of the MHW, rising 0.178 and 0.141 respectively.

3.3. Metabolic rate and osmotic pressures analysis

Molting had a significant effect on O_2 consumption (i.e. metabolic rate) (Fig. 6.A; $p = 0.03$): individuals that molted between acclimation and measurements showed a lower average oxygen consumption ($0.043 \pm 0.009 \text{ mgO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) compared to those that did not molt ($0.075 \pm 0.014 \text{ mgO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). Molting also had a significant effect on osmotic pressure (Fig. 6.C; $p = 0.02$): molted individuals exhibited lower osmolarity ($1033.2 \pm 5.559 \text{ mOsm} \cdot \text{L}^{-1}$) than non-molted individuals ($1049 \pm 8.308 \text{ mOsm} \cdot \text{L}^{-1}$).

Since post-molting stage significantly affects both metabolic rate and hemolymph osmolarity, the following analyses were restricted to individuals that did not molt between acclimation and measurement (i.e. inter or premolt individuals).

Temperature has a significant effect on metabolic rate (Fig. 6.B; $p < 0.001$) with O_2 consumption increasing progressively with rising temperature: starting at $0.054 \pm 0.005 \text{ mgO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ at 14CTRL condition, it rises $0.104 \pm 0.006 \text{ mgO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ at 20WP and peaked at $0.113 \pm 0.020 \text{ mgO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ at 26WP. In contrast, during the cooling phase, a marked decrease in O_2 consumption was observed: it dropped to $0.046 \pm 0.003 \text{ mgO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ at 20CP and reached $0.058 \pm 0.004 \text{ mgO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ at 14CP. These last values were significantly lower than those recorded during warming (20WP vs. 20WP: $p < 0.001$; 20WP vs. 14CP: $p = 0.01$; 26WP vs. 20CP: $p < 0.001$; 26WP vs. 14CP: $p < 0.01$).

Osmotic pressure of the hemolymph followed a similar trend to that of O_2 consumption (Fig. 6.D; $p = 0.013$), with osmotic pressure of the hemolymph rising from $1039 \pm 6.172 \text{ mOsm}\cdot\text{L}^{-1}$ at 14CTRL condition to $1076.7 \pm 9.790 \text{ mOsm}\cdot\text{L}^{-1}$ at 26WP condition ($p = 0.04$), followed by a sudden decrease at 20CP condition (26WP vs. 20CP: $p = 0.01$), with a value of $1028.6 \pm 2.430 \text{ mOsm}\cdot\text{L}^{-1}$. The measured seawater osmolarity was $1026.33 \pm 7.17 \text{ mOsm}\cdot\text{L}^{-1}$. Thus, individuals, initially hyperosmotic relative to their environment, became temporarily iso-osmotic at 20CP condition, before returning to a slightly hyperosmotic state at the 14CP condition ($1046.5 \pm 12.254 \text{ mOsm}\cdot\text{L}^{-1}$).

3.4. Correlation's analysis of physiological and behavioral metrics (Fig. 7)

The five temperature-specific heatmaps revealed both shared and condition-specific correlation patterns. All correlations reported below are significant ($p < 0.05$).

Across all temperatures, a consistent set of positive correlations is observed among tortuosity, explored surface area, total distance traveled, mean speed, and peak speed. These variables are all negatively correlated with the proportion of time spent immobile, together reflecting overall locomotor activity. Except under the 14CP condition, the proportion of time spent feeding is consistently negatively correlated with the proportion of time spent in shelter. Beyond these general patterns, condition-specific relationships emerge. Under the 20WP condition, feeding time is negatively correlated with time spent immobile, whereas camouflage remains positively correlated. Regarding physiological variables, metabolic rate shows positive correlations with exploration time under the 26WP, and 14CP conditions. It is also negatively correlated with hemolymph osmotic pressure under the 20CP condition. Interestingly, at 26WP, metabolic rate is negatively correlated with feeding time proportion and positively correlated with time spent in shelter. Similarly, under the 14CP condition, metabolic rate is negatively correlated with general locomotor activity and positively correlated with time spent inactive. A comparable pattern is observed for hemolymph osmotic pressure, which is negatively correlated with both mean and peak speeds at 14CP.

4. Discussion

4.1. Warming phase of a MHW: increased energetic demand and shift in survival strategy

During the warming phase of the MHW, from 14°C (14CTRL condition) to 26°C , spider crab *M. squinado* juveniles exhibit marked behavioral changes. Their movement trajectories become significantly more tortuous and, whereas their primary survival strategy at lower temperatures relies on refuge use and crypsis behavior for predator avoidance. Therefore, individuals adopt a much bolder behavior at the peak of the heatwave.

At all temperatures except for the thermal peak, juveniles primarily locate along the edges of the aquarium, especially in the corners, with the central area showing a relative density of zero (i.e., no individuals entering the center). This pattern is consistent with the known ecology of the species and direct observations from individuals kept in captivity^[24]. Typically, juveniles shelter in crevices, remain largely immobile (here, 60–80% of the time), rely on crypsis, and feed opportunistically within their immediate surroundings. In contrast, at the MHW peak (26°C), the central area is no longer devoid of individuals: numerous trajectories cross the aquarium, and although density hotspots remain in the corners, they expand considerably. This reflects a substantial increase in environmental exploration and a shift toward a bold behavioral phenotype, defined here as increased risk-taking and reduced predator avoidance in favor of active movement and foraging^[16]. This type of behavior has already been demonstrated in ectotherms, for example in the fish *Pomacentrus moluccensis*, which increases its overall activity and aggression, and reduces its latency to emerge from shelter following a threat even under relatively small temperature increases (on the order of 1 to 3°C)^[19].

Several mechanisms may explain this shift^[11, 18]: (i) the search for cooler microhabitats, (ii) temperature-driven metabolic activation leading to intrinsically higher activity levels, and (iii) active foraging to meet increased energetic demands associated with elevated metabolic rates. The latter two hypotheses are strongly supported by the results of this study. As an ectothermic organism, *M. squinado* has a body temperature entirely determined by environmental conditions. Since metabolic rate is directly temperature-dependent, increasing temperature mechanically leads to higher metabolic rates^[28]. Moreover, increasing temperatures reduce dissolved oxygen concentration at saturation, thereby decreasing O₂ availability. This constrains the oxygen partial pressure gradient, limiting passive diffusion to the tissues. Consequently, organisms must increase their ventilatory effort to maintain equivalent oxygen uptake^[29], inevitably increasing energy expenditure and, in turn, metabolic rate. This rise necessarily increases energetic demands, requiring greater nutrients intake through feeding^[30]. A positive feedback loop may then develop, whereby the energy required to search for and digest food further increases the metabolic rate^[14, 16, 18]. In this study, O₂ consumption doubled between 14°C (14CTRL) and 20°C (20WP), then remained elevated at 26°C. Similarly, the proportion of time spent feeding increased from 31% at 14CTRL condition to 54% at 20WP condition and reached 70% at 26°C. At 20WP condition, a positive correlation is observed between metabolic rate and both exploration time and feeding activity, as well as with all locomotor metrics (explored surface, distance traveled, and mean and maximum speeds). This supports the hypothesis of a global metabolic activation aimed at increasing energy acquisition during the warming phase of the MHW. Together, these results highlight a complete shift in survival strategy at the peak of the MHW. Individuals transition from a low-activity strategy based on refuge use and crypsis (65% of time spent in refuge at 14CTRL) to a much bolder strategy (only 13% at 26°C), primarily driven by active foraging to meet metabolic demands, and likely also by the search for cooler microhabitats.

Temperature variation has complex effects on the diet of organisms^[18, 30], acting through several pathways: (i) direct effects on food resources, as temperature alters food quality by modifying lipid,

carbohydrate and protein content; (ii) effects on nutritional requirements, depending on physiological priorities such as locomotion (carbohydrate demand), growth (protein demand) or membrane fluidity regulation (lipid demand); and (iii) effects on oxidative stress, which can be modulated through dietary antioxidant intake. Consequently, organisms may adjust their diet in ways that increase tolerance to approaching their thermal limits, i.e. the range of extreme temperatures they can tolerate over short periods. This behavioral and physiological shift may have multiple direct and indirect consequences. Directly, increased exploration and foraging expose individuals to higher predation risk^[16, 20]. This risk is likely exacerbated under MHW conditions, as other species may also experience elevated energetic demands, intensifying both competition and predation pressure. Indirectly, the energy allocated to foraging and to detoxification and antioxidant defenses during warming^[31] may come at the expense of growth and gonadal development, potentially affecting future reproductive capacity and population renewal^[12]. Moreover, elevated activity levels do not necessarily reflect high performance capacity. In the freshwater crab *Aegla longirostri*, for example, although daytime activity increases with temperature, the time required to regain equilibrium after being turned upside down also increases, indicating substantial exhaustion as well as a reduced escape performance in response to predators .

A particularly interesting negative correlation was observed at the peak of the MHW: the higher the metabolic rate of individuals, the less time they spend feeding, relative to time spent exploring and remaining in shelter. This is not necessarily surprising, given that the nutritional requirements of ectotherms do not vary linearly with temperature, unlike metabolic rate^[30]. Rather, they depend strongly on the interactions among different metabolic processes. Thus, beyond a certain threshold of temperature-induced metabolic increase, it becomes more advantageous for an ectothermic organism to engage in behavioral thermoregulation (i.e. seeking cooler microhabitats) rather than increasing food intake^[18]. Moreover, correlations showed both tortuosity and directionality positively associated with locomotor activity, suggesting a generalized increase in movement intensity rather than a shift toward a specific movement strategy. Altogether, these patterns likely reflect a combination of heightened metabolic activation and reduced behavioral control under thermal stress.

In our study, the metabolic rate is not the only global marker that is altered under thermal stress. Hemolymph osmotic pressure also increases progressively during the MHW, rising from 1040 mosm·L⁻¹ at 14CTRL to 1060 and 1080 mosm·L⁻¹ at 20WP and 26WP, respectively, with individuals becoming slightly hyperosmotic. The relationship between temperature and osmotic pressure has previously been demonstrated in *M. squinado*^[31] and reflects a significant physiological disturbance, as hemolymph osmotic pressure is a well-established integrative indicator of organismal health^[13]. This is consistent with the survival patterns observed in this study, where mortality peaks at the highest temperatures. The most critical periods correspond to the rapid temperature increase from 20 to 26°C (12-hour ramp; 5.17% mortality) and the subsequent week of acclimation at 26°C (1 week; 7.50% mortality). These mortalities could result from dysfunctional activation of antioxidant and detoxification mechanisms during acute thermal stress^[31]: when these mechanisms fail to sufficiently counteract cellular and tissue damage, mortality occurs.

4.2. Cooling phase of a MHW: organismal exhaustion and delayed recovery

Immediately following the peak of the marine heatwave, during the cooling phase at 20°C (20CP condition), both behavior and metabolism are profoundly altered in *M. squinado* juveniles. Considering the behavioral analysis, while trajectory tortuosity reaches its maximum at 26°C, it drops to its lowest level at 20CP, and the survival strategy shifts back toward one dominated by refuge use and crypsis behavior, relative to the time spent feeding and exploring the environment. The shift toward a shyer behavioral phenotype was more pronounced at 20CP than at the corresponding temperature during the warming phase (20WP), although this difference is not statistically significant. Metabolic responses show an even more pronounced and significant shift. Metabolic rate at 20CP collapses compared to the initial temperature (20WP), the warming phase, and MHW peak (26°C). A similar pattern is observed for hemolymph osmotic pressure, which returns to values comparable to seawater: individuals shift from slightly hyperosmotic under the previous conditions to iso-osmotic at 20CP. Altogether, these behavioral and physiological indicators reach their maximum values at the MHW peak and drop to their lowest levels during the cooling phase (26WP → 20CP). This pattern likely reflects organism exhaustion, with individuals entering a phase of reduced activity following the substantial energetic costs endured during the heatwave peak. These alterations may have important consequences, particularly given the combination of reduced oxygen consumption and low feeding activity, which could, over longer periods, fail to meet the energetic requirements associated with this temperature. It has already been shown that, in ectotherms exposed to cold thermal stress, short periods of rewarming between stress events can enhance long-term survival. During these recovery phases, organisms can activate physiological mechanisms involved in damage repair, such as detoxification pathways, antioxidant defenses, and restoration of ionic homeostasis^[11]. A similar process may also occur under heat stress. Following a heatwave, individuals may enter a state of generalized metabolic depression, allowing repair processes to predominate.

During the second phase of cooling (20CP → 14CP), a form of physiological and behavioral reactivation emerges. At 14CP, and in contrast to both 20CP and the pre-heatwave condition at the same temperature (14CTRL), trajectory tortuosity and total distance traveled reach their highest observed values. Non-significant trends are also observed for explored surface area as well as mean and maximum speeds. Conversely, and consistently, the proportion of time spent immobile shows a decreasing trend, reaching its lowest values at 14CP, although this observation is non-significant. Similarly, while 75% of individuals initially select refuge under control conditions (14CTRL), this proportion drops to 40% at 14CP, with 60% of individuals preferentially selecting food as their first choice. All together, these results indicate a form of behavioral reactivation, contrasting with the persistently low metabolic rate observed at this temperature. This apparent paradox is further supported by significant negative correlations between metabolic rate and behavioral metrics (explored surface, total distance traveled, and locomotor speeds), suggesting a decoupling between behavioral activity and metabolic capacity. This hypothesis is supported by the unexpected negative correlation between general locomotor activity and metabolic rate

at this specific temperature. These observations are consistent with the hypothesis of a recovery phase^[11]. Although this temperature (14°C) typically corresponds, for this species, to a state of metabolic quiescence (supported here by the low metabolic rate), the energy reserves depleted during the marine heatwave likely need to be replenished. To achieve this, individuals may either seek cooler microhabitats to reduce energetic expenditure or increase foraging activity to enhance energy intake.

Data obtained during the cooling phase highlight that the effects of a MHW extend well beyond the warming period and cannot be reduced to temperature increase alone. While many studies have focused on the impacts of rising temperatures, far fewer have investigated the consequences of subsequent cooling. These results emphasize the importance of considering the full thermal cycle of MHWs, including post-heatwave recovery dynamics, to better understand their ecological and physiological impacts.

4.3. Overall impacts of a MHW on juvenile *M. squinado*

Beyond behavioral and metabolic responses, MHWs can have direct consequences on individual survival and even drive mass mortalities^[5, 7]. In our study, cumulative mortality reached 19% over the full 7-week experimental period (including acclimation, warming, and cooling phases), and 22% when considering only the onset of the heatwave (i.e., from the temperature increase between 14 and 20°C). Cerezer et al. (2020)^[10] also reported high mortality in the freshwater crab *A. longirostri*, with mortality increasing from 0% at 18°C to 30% at 26°C under a warming rate of 1°C per day. However, the mortality obtained in our study is likely underestimated, as individuals subjected to physiological measurements were progressively removed from the experiment (e.g., individuals measured at 20WP were removed prior to the heatwave peak at 26°C). Moreover, mortality rates are expected to be even higher under natural conditions, where additional stressors come into play, including limited food availability, increased competition, predation pressure, and environmental degradation associated with MHWs (e.g., fluctuations in salinity, pollution). A mortality rate approaching as high as 20% of the population, or even more, could be considered, especially given that multiple MHWs can occur within a single year (Fig. 1), and that *M. squinado* juveniles typically spend at least two consecutive summers at depths of 3–15 m before reaching adult stage. These findings are particularly concerning given the central ecological role of decapod crustaceans. The latter occupy an intermediate position in the trophic web both as prey for numerous fish, marine mammals, and seabirds, and simultaneously, as predators contributing to the regulation of herbivory^[32]. Their ecological importance is further reinforced by their major economic value, representing a substantial proportion of global fisheries production and accounting for more than 20% of aquaculture-derived income worldwide^[33]. Yet, despite these key roles, the impact of MHWs on decapod crustaceans remains surprisingly understudied, with most existing work focusing exclusively on commercially exploited adult stages^[8]. Thus, *M. squinado* juveniles provide a particularly informative model for investigating thermal stress response in decapods and, more broadly, in marine ectotherms.

In this study, the thermal amplitude of the MHW was particularly high (12°C overall, with two successive 6°C increases). However, individuals were exposed to each temperature level for only one week, whereas

natural MHWs can persist for several weeks to months ^[2–5]. Although some degree of acclimation to prolonged high temperatures may occur ^[11], ectothermic organisms lack the ability to regulate their internal body temperature. As a result, their metabolic rates cannot fully decrease under sustained thermal stress. Consequently, energetic demands are likely to remain elevated, suggesting that the metabolic and behavioral patterns observed in this study could persist during prolonged heatwave events.

It would nevertheless be valuable to investigate these processes over longer timescales, in order to determine whether cumulative exposure to high temperatures leads to progressive physiological exhaustion or, conversely, to partial acclimation ^[11, 34]. Such studies would be essential to better understand the long-term impacts of increasingly frequent and intense MHWs on the ecology and population dynamics of this species.

5. Conclusion

The effects of a marine heatwave (MHW) on ectothermic organisms, was assessed using the Mediterranean spider crab *M. squinado* as a model species to identify key physiological and behavioral responses to thermal stress. MHW induces profound and dynamic shifts in the behavior, physiology, and survival of this model. However, the results suggest a certain degree of ecological resilience in juvenile *M. squinado*. Ecological resilience can be broadly defined as the capacity of a system or organism to withstand disturbance, adjust to changing conditions, and maintain its essential functions and integrity ^[35]. In the present study, juveniles appeared to remodel both their survival strategy and metabolic functioning to cope with temperature changes, supporting the idea of an acclimation response to thermal stress.

During the warming phase, individuals exhibit a clear transition from a low-activity, risk-averse strategy based on refuge use and crypsis behavior to a bold, energetically driven strategy characterized by increased exploration and feeding. This shift is tightly linked to temperature-driven metabolic activation, resulting in elevated oxygen consumption and energetic demands. While this strategy likely supports short-term survival under thermal stress, it comes at the cost of increased exposure to predation and potential trade-offs with growth and future reproduction.

In contrast, the cooling phase reveals a marked state of physiological exhaustion. Immediately following the thermal peak, both metabolic rate and activity collapse, suggesting a recovery phase after intense energy expenditure. Although a partial behavioral reactivation occurs at lower temperatures, the decoupling between activity and metabolism highlights lingering physiological constraints beyond the heatwave itself.

At a broader scale, MHWs represent a significant threat to juvenile survival, with substantial mortality rates observed even under controlled conditions. Because of climate change and given the increasing frequency, intensity, and duration of MHWs in the western Mediterranean basin, these impacts are likely

to be amplified in natural environments, where additional stressors such as predation, competition, and environmental variability are also at play.

Overall, this study underscores that the effects of MHWs extend beyond immediate thermal exposure, influencing organismal performance across both warming and cooling phases. Understanding these cumulative and delayed responses is essential for predicting the resilience of ectothermic species under global warming scenarios.

Declarations

Consent to Publish

All authors give their consent to publish this manuscript.

Additional Information

The authors have no relevant financial or non-financial interest to disclose.

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Author Contribution

Raffalli: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing: review and editing; Favaro: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing: review and editing; Durieux & Lignot: Conceptualization, Methodology, Supervision, Writing – review and editing; Filippi: Funding acquisition, Project administration, Methodology, Writing – review and editing; Bracconi, Crescioni, Gattacceca & Ronchi-Perfetti: Resources (M. squinado broodstock maintenance and larval rearing), Writing – review and editing.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on request.

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Figures

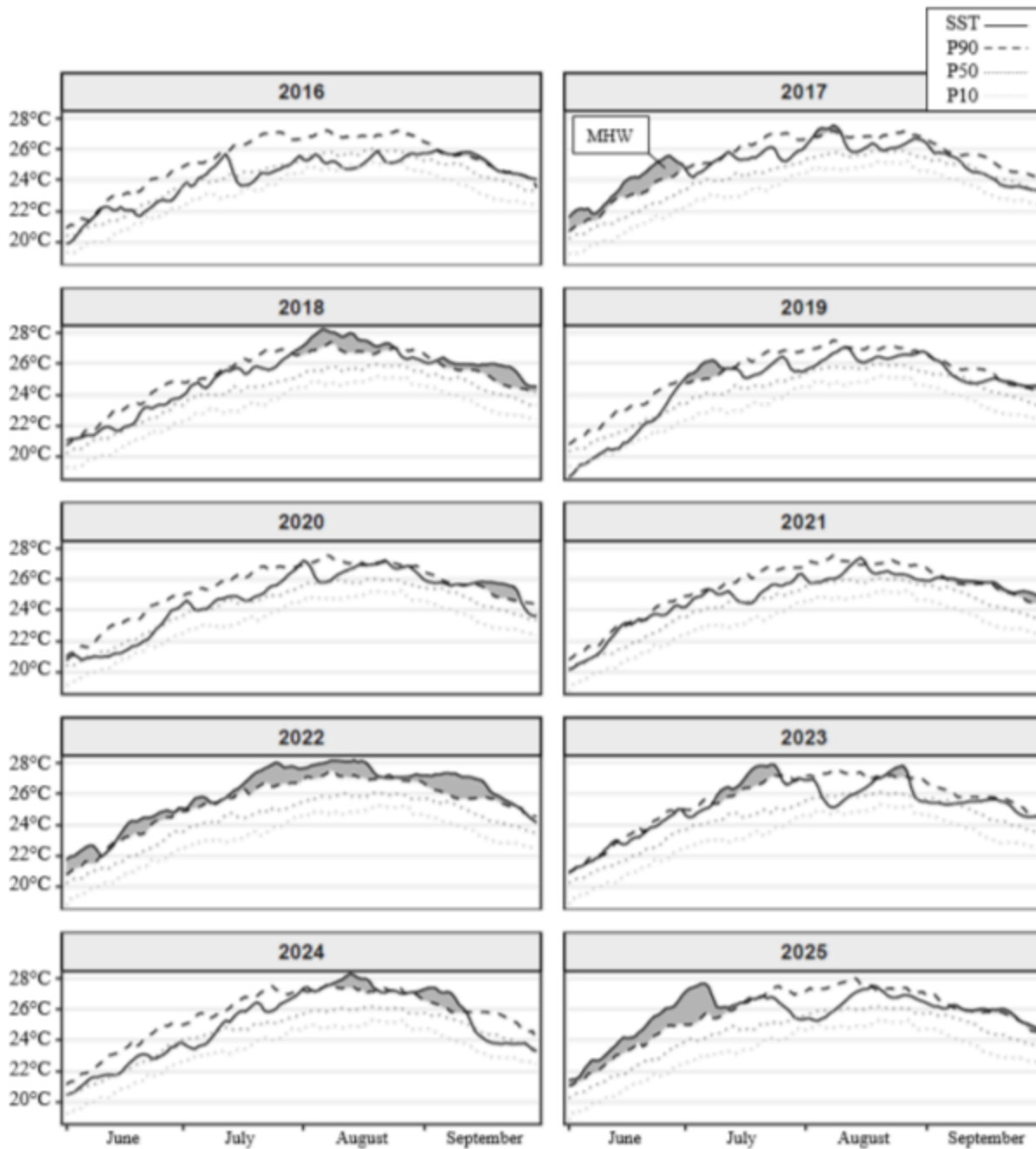


Figure 1

Daily sea surface temperature in the western Mediterranean Sea from 1 June to 30 September for the past ten years (2016-2025) based on Copernicus data (1993-2025). Solid line: observed SST for each year. Black dashed line: 90th percentile of the area averaged temperature over the previous 30 years (P90). Grey dotted line: 50th percentile (P50). Light grey dotted line: 10th percentile (P10). Shaded areas: marine heatwaves (MHWs) according to Hobday et al. (2018) definition.

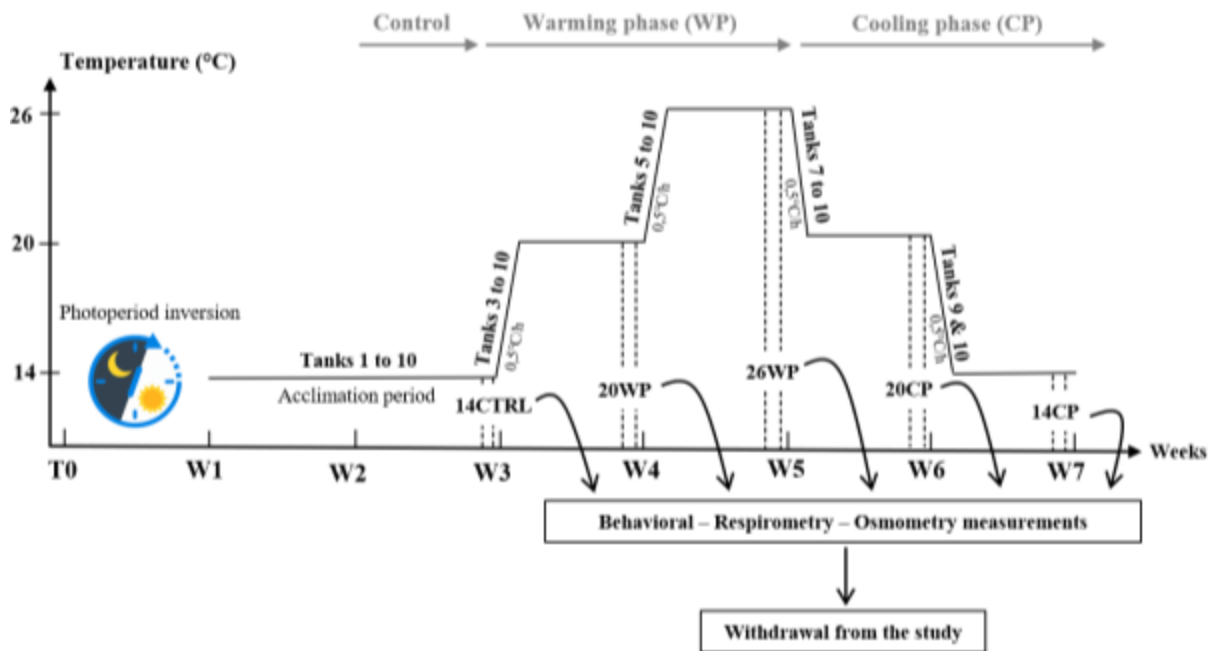


Figure 2

Graph of temperature variations for *M. squinado* individuals: groups of individuals (CTRL: control; WP: warming phase; CP: cooling phase), inversion of photoperiod, acclimation time, and measurement periods (dashed lines) are represented throughout the weeks.

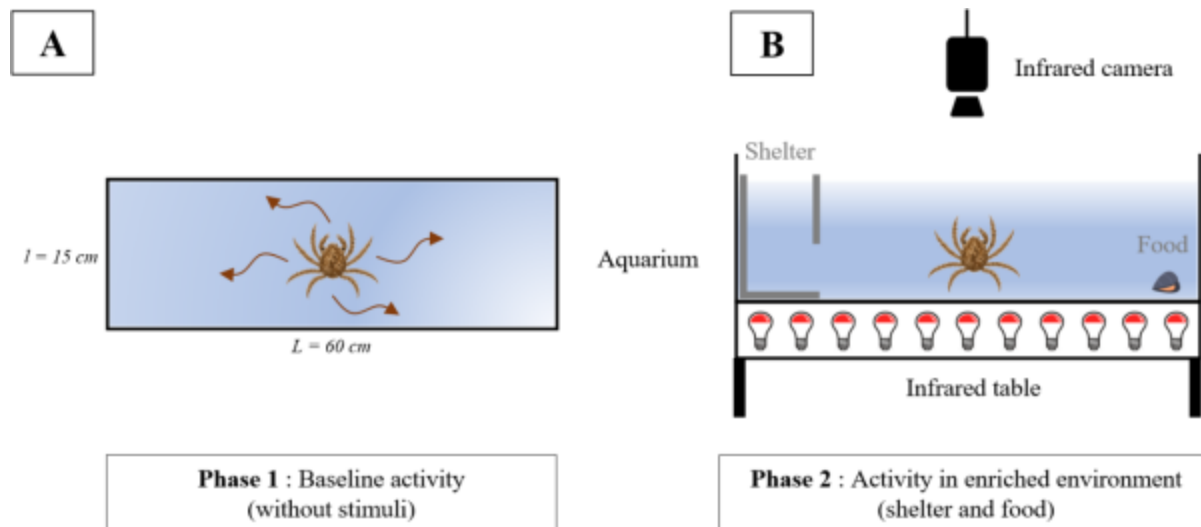


Figure 3

Experimental setup used for behavioral measurements from **A.** top view and **B.** side view.

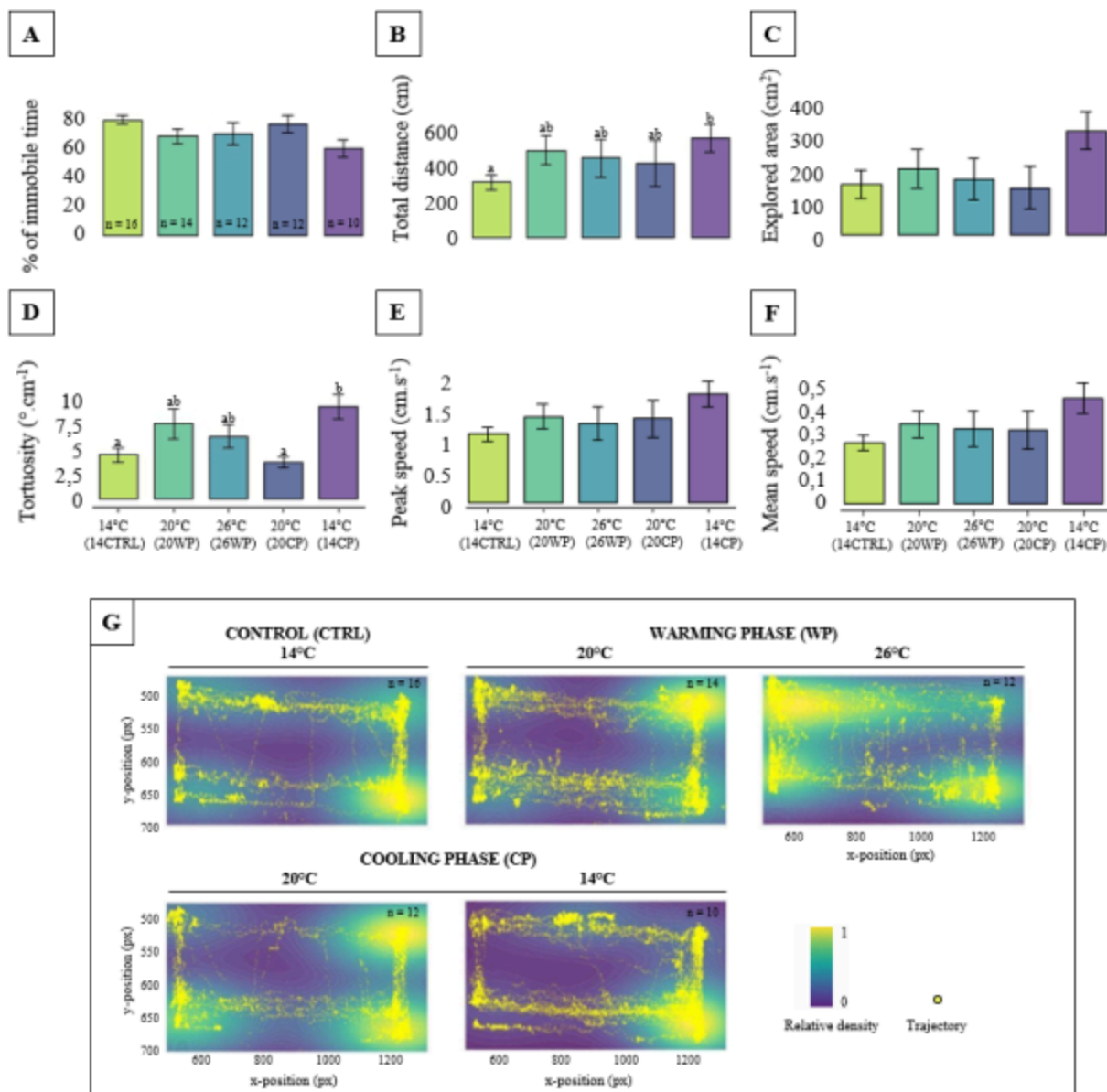


Figure 4

Behavioral responses of juvenile *M. squinado* during a 30 min exploration period without external stimuli (first phase of the behavioral experiment) under different temperature treatments (14CTRL, 20WP, 26WP, 20CP and 14CP). Bar plots show **A.** Proportion of immobile time, **B.** Total distance travelled **C.** Explored area, **D.** Tortuosity, **E.** Peak speed and **F.** Mean speed. Values are presented as mean ± SE. **G.** Relative density maps illustrating spatial occupancy patterns under each temperature condition. Warmer colors indicate areas more frequently occupied by individuals, whereas cooler colors indicate areas less frequently occupied.

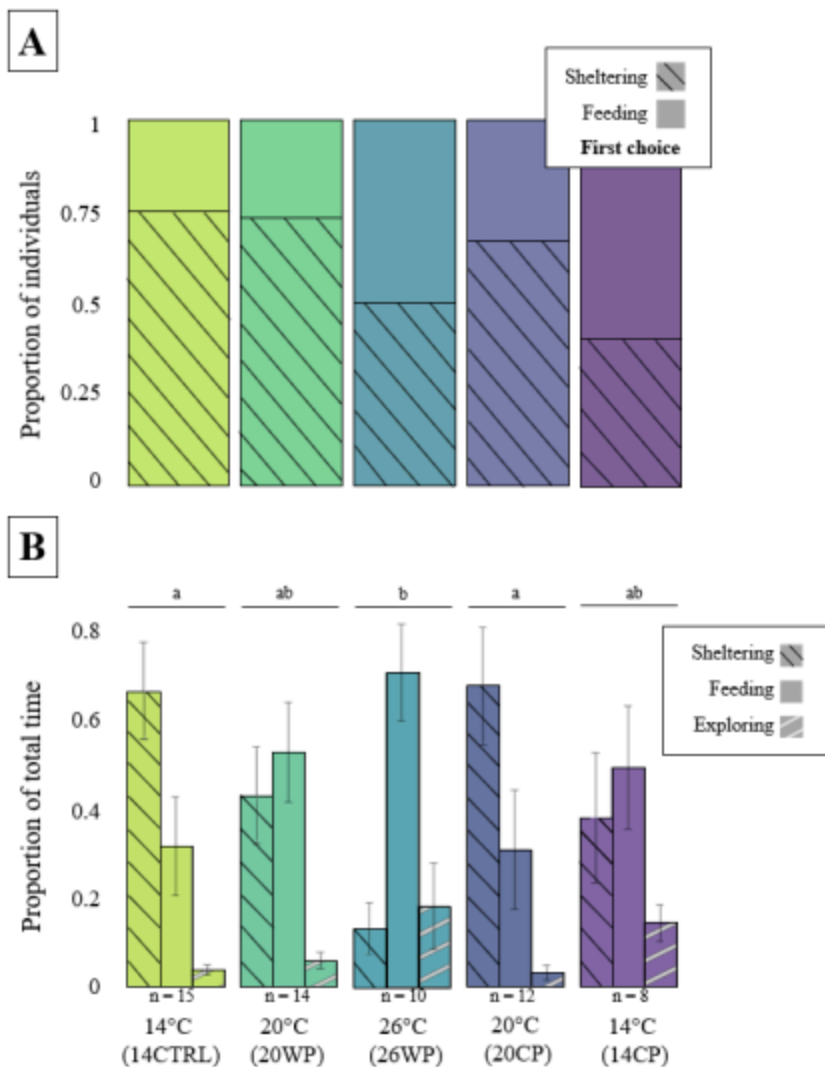


Figure 5

Behavioral responses of juvenile *M. squinado* during a 30 min period with external stimuli (second phase of the behavioral experiment: shelter or food) under different temperature treatments (14CTRL, 20WP, 26WP, 20CP and 14CP). Solid bars represent feeding behavior, bars with black diagonal hatching (\) represent sheltering behavior, and bars with white diagonal hatching (/) represent exploring behavior. **A.** First choice represented by the proportion of individuals that initially moved toward the shelter or the food. **B.** Bar plots showing the total proportion of time spent feeding, sheltering and exploring, with values presented as mean $\pm$ SE. Letters: effect of condition on the Sheltering/Exploring and Feeding/Sheltering balances.

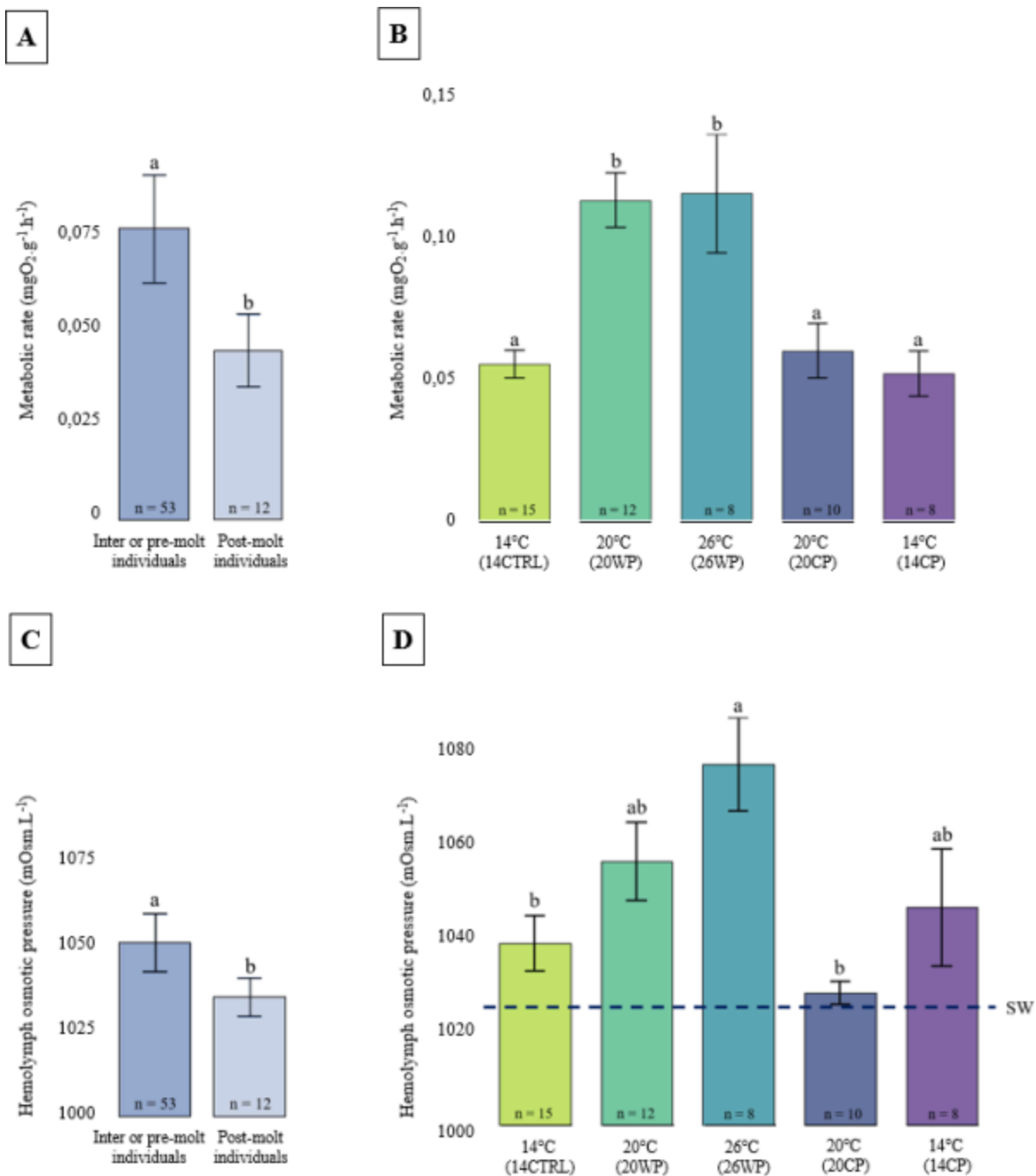


Figure 6

Effects of molting stage and temperature on metabolic rate and hemolymph osmotic pressure in *M. squinado*. Bar plots show metabolic rate according to **A.** molting stage (inter-/pre-molt vs. post-molt individuals) and **B.** temperature treatment (14CTRL, 20WP, 26WP, 20CP and 14CP). Hemolymph osmotic pressure is shown according to **C.** molting stage and **D.** temperature treatment. Values are presented as mean $\pm$ SE. Dashed line: seawater osmotic pressure measured during the experiment.

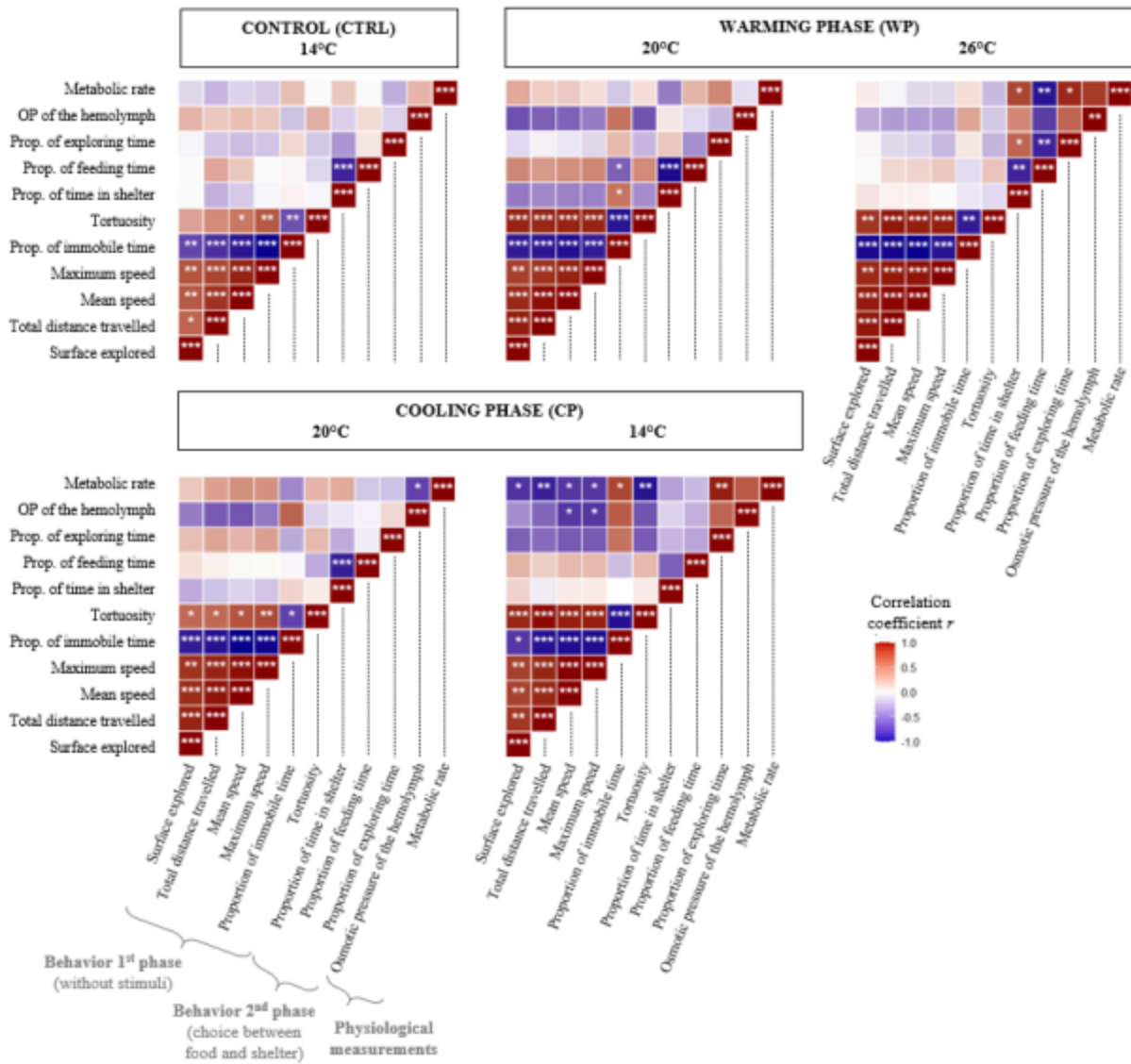


Figure 7

Heatmaps of correlations (Spearman coefficient) among all variables measured during the experiment, for each temperature conditions (14CTRL, 20WP, 26WP, 20CP and 14CP): surface explored, total distance travelled, mean speed, maximum speed, proportion of immobile time, and tortuosity (first phase of the behavioral analysis); proportions of feeding, sheltering, and exploring time (second phase of the behavioral analysis); hemolymph osmotic pressure and metabolic rate (physiological analysis). Blue color indicates a correlation coefficient $r = -1$, white $r = 0$ and red $r = 1$. Asterisks denote significance levels * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.