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Spawning is accompanied by increased thermal performance in blue mussels

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A B S T R A C T

Climate change is causing extreme short-term warming with greater intensity and more frequent occurrence. Reproduction and subsequent recruitment of coastal ecosystem engineers, such as the blue mussel, may be impacted by the extreme temperatures because these vital functions are sensitive to the timing of short-term changes in abiotic factors. We exposed intertidal blue mussels, *Mytilus edulis,* to a thermal challenge from 10 to 29 °C using an ecologically relevant heating rate of 4 °C/h. We assessed their reproductive status by observing spawning activity and by analyzing histological sections of their gonad tissue. In addition, we monitored their heart rates and valve gaping behavior to determine their thermal performance. We identified three spawning groups: non-spawners that had mature gonads but did not release gametes, post-spawners that released mature gametes prior to the thermal challenge, and active spawners that released mature gametes during the thermal challenge. Across temperatures, active spawners had significantly higher heart rates and their heart rate peaked at the temperatures 3.9 and 3.2 °C higher compared to those of non-spawners or post-spawners, respectively. Post-spawners had significantly narrower valve gapes across temperatures compared to both other spawning groups. Hence, the metabolic response to warming strongly depends on the reproductive status, with active spawners experiencing increased thermal stress due to heightened metabolism, non-spawners showing heat-induced metabolic depression, and post-spawners adopting an energy-conserving strategy indicated by reduced gaping. Considered together, spawning during recurring short-term warming events may elevate mortality risk with potential consequences for the local biodiversity in a future climate.

1. Introduction

Intertidal species are naturally exposed to extreme variability in abiotic factors due to the interaction of cycling tides, weather and ocean conditions. In temperate regions, temperature is a key factor that shows strong tidal, diurnal and inter-annual variability. The amplitude and rate of warming greatly varies between the seasons. Particularly in the warmer times of the year, such as spring and summer, organisms must cope with rapid warming and cooling. For example, in the worlds' largest tidal flat system, the Wadden Sea, the average sea surface and air temperatures increase by approx. 8 °C from late March to late June. Yet, during the same time sea surface and air temperature extremes can range from 2 to 23 °C and from −4 to 31 °C, respectively (Database Royal Netherlands Institute for Sea Research: www.dataverse.nioz.nl, database Royal Netherlands Meteorological Institute for stations de Kooy and Vlieland: www.daggegevens.knmi.nl; years 2001–2021). In a future climate, these extreme temperatures are projected to occur more frequently and in even larger amplitude (Russo et al., 2015). Thus, a question arises: how will species that inhabit the intertidal zone cope with increased temperature extremes? This question is particularly important as spring and summer coincide with the reproductive season in temperate marine organisms. Reproduction and subsequent recruitment may be subject to the timing of short-term and seasonal changes in abiotic factors, and reproductive success is sensitive to changes in temperature (Pincebourde et al., 2012; Normand et al., 2008). Temperature shifts can disrupt spawning cues, leading to mistimed spawning events outside the optimal conditions for larval survival and development, and cause metabolic stress and mortality in adult mussels (Lawrence and Soame, 2004; Masanja et al., 2023). Additionally, the energy demands of spawning can weaken the organism, impairing immune defenses and reducing thermal protection, thereby creating a physiological basis for synergistic temperature-reproduction interactions (Li et al., 2007; Mredul et al., 2024).

In ectotherms, temperature strongly affects metabolic performance,

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leading to significant consequences for their growth, reproduction, and survival. Starting from the critical thermal minimum (CT_{min}), the metabolic performance of ectotherms typically increases exponentially with rising temperature until reaching a peak at the optimal temperature for metabolism. Beyond this optimal point, performance rapidly declines until it reaches the critical thermal maximum (CT_{max}), where the neuromuscular dysfunction and mortality set in (Martin and Huey, 2008). In a thermally variable environment, the performance of individuals is dependent on the magnitude of the thermal fluctuation but also the mean temperature around which the fluctuation occurs and can be quite different to the performance of individuals exposed to a stable mean temperature on which the vast majority of knowledge has been generated thus far (Martin and Huey, 2008; Bozinovic et al., 2011; Verheyen and Stocks, 2019). Also the life-stage influences the thermal performance of the species whereby spawning adults and embryos have narrower thermal tolerance ranges than larvae and non-spawning adults, as demonstrated for fish (Dahlke et al., 2020). Compared to non-spawning adults, spawning adults have an increased energy demand for gamete production and biomass. The concomitant increased oxygen demand may result in a net decrease in aerobic capacity and thus a narrower thermal tolerance (Dahlke et al., 2020). Such data are scarce for marine invertebrates, limiting our predictive capacity on their future distributions and vulnerability to climate change as this depends on the most sensitive life stage (Dahlke et al., 2020; Herrando-Pérez et al., 2023).

In the intertidal zones, mussels can be a dominant species by building ecologically important mussel beds that function as structural habitats for other benthic species and increase local biodiversity. Because mussels have limited relocation capabilities, their ability to react to adverse environmental conditions is restricted. They may react, for example, by withdrawing into their shells and by modifying their valve gape in order to isolate their soft tissue from the unfavorable conditions (Reimer and Tedengren, 1997, Kobak et al., 2012; Naddafi and Rudstam, 2013, Jakubowska and Normant, 2015). Yet, prolonged valve closure can be detrimental for their survival because many mussel species rely on frequent water pumping activity for feeding, oxygenation, and reproduction (Gosling, 2003). While valve closure can be used as a behavioral sign for unfavorable conditions, cardiac activity is often used to determine sublethal and lethal thermal limits of bivalves (Braby and Somero, 2006; Logan et al., 2012). During exposure to a short term temperature increase, a sudden decrease after the performance maximum is indicative for cumulative cell damage caused by the foregone thermal stress and indicates the individuals' sublethal limit. Ongoing exposure to further temperature increase will result in cardiac arrest, which if upheld for too long, will ultimately cause death (Moyen et al., 2019) with potential consequences for the local biodiversity.

In this study, we exposed acclimatized blue mussels, *Mytilus edulis,* collected from the high intertidal, to a thermal challenge using a naturally occurring heating rate of 4 °C/h and measured their heart rates and valve gaping behavior. In addition, we assessed their reproductive status by observing spawning activity during the thermal challenge and by analyzing histological sections of gonad tissue. We hypothesized that the high energy expenditure during spawning in mussels (Mredul et al., 2024) would decrease the ability of spawning individuals to withstand increasing temperatures compared to non-spawning individuals.

2. Material and methods

2.1. Temperature field measurements

In late March 2023, temperature loggers (EnvLoggers T7.3, ElectricBlue, Portugal, n = 4) were placed within the high intertidal zone of the Wadden Sea coast off the island Texel, the Netherlands (N53°00614, E4°784917). Temperature was logged continuously at a rate of one reading per 5 min for a total of one month.

2.2. Animal preparation and experimental design

In late April 2023, blue mussels*, Mytilus edulis* Linnaeus, 1758 (n = 20) were collected from the high intertidal zone close to the position of the temperature loggers and brought to the laboratory. *Mytilus trossulus* A. Gould, 1850 and *Mytilus galloprovincialis* Lamarck, 1819 rarely inhabit Dutch water; however, the possibility of the collection of *M. trossulus*, *M. galloprovincialis* and *M. edulis* hybrids cannot be excluded because of their morphological similarities (Daguin et al., 2001; Väinölä and Strelkov, 2011). Upon arrival at the laboratory, individuals were brushed clean, the epifauna of the shell was removed and the shell was dried with paper towels. Then, a wired heart rate sensor (Pulse V2, Electric Blue, Portugal) and a custom built valve gape sensor (Ballesta-Artero et al., 2017) were attached onto the shell above the heart and onto the posterior margin of both valves, respectively, using cyanoacrylate (Colle SecondeLijm, Bison, the Netherlands). The heart rate sensor recorded at a sampling rate of 10 Hz and stored in a log file (Pulse V2, Electric Blue, Portugal). The valve sensor consisted of paired electromagnetic coils between which an electromagnetic field existed that changed in strength according to the distance between the two coils. The field was recorded at a sampling rate of 1 Hz and stored in a log file. Mussels were kept in individual chambers (500 ml beakers, Duran, Germany) containing filtered (0.1 μm) and UV-treated seawater at 10 °C and 29 PSU salinity corresponding to the local abiotic conditions. Gentle aeration was provided to each individual chamber via a glass tube. In seven chambers, temperature loggers (Hobo Pendant Mx Temp) were attached to the lower tip of the glass tube and used to measure the temperature (one reading per minute) near the bottom of the chamber. Chambers were placed in three water baths (Lauda Alpha RA24, Lauda-Brinkmann, Germany) and kept for 18 h in darkness without food supply until the next morning. Salinity (range 28.8–29.7) and oxygen level (>95% air saturation), were measured using WTW Multi 3620 IDS (Germany) attached to TetraCon 925 or FDO 925 probes, respectively, at time of transfer of the mussels to their individual chamber and at the termination of the experiment.

The next morning, mussels were transferred to clean chambers with fresh sea water at the same abiotic conditions. Thereby, the attachment of the byssal threads to the glass wall were broken. It was noted which individual had released gametes during the night. Two hours after the water exchange, the temperature challenge trial commenced. Pre-experiments had shown that 2 h is sufficient time for heart rates and valve gapes to return back to baseline levels after a disturbance. During the temperature challenge trial, temperatures in two of the water baths (n = 9 chambers each) were raised stepwise by 1 °C every 15 min (4 °C/h) until 30 °C was reached. The temperature of the third water bath was kept constant at 10 °C and mussels within these chambers (n = 2) provided a reference point that any observed changes in physiology and behaviour during the thermal ramping test were indeed attributable to temperature changes rather than the duration of exposure. In these individuals, the heart rate remained stable and the valve gape remained open throughout the duration of the experiment (Fig. S1). Heart rate, valve gape and temperature were measured continuously as described above. The trial was conducted between 1 and 7pm in light conditions and without food supply. After the trial was terminated, it was noted which individual had released gametes. Length, height and width of the shell (Mitutoyo, Japan, ±0.01 mm) of each individual was determined. Approx. 0.25 cm^2 of gonad tissue was sampled from the right valve and stored in 4% formalin for later histological analysis of the reproductive status. Wet weight of the remaining tissue and shell (Delta Tange, Mettler Toledo, USA, ±0.1 mg) was determined and the condition index (CI) was calculated according to the formula:

$$CI = \frac{total\ wet\ weight\ (mg)}{shell\ length\ (mm)^3}$$

2.3. Histological analysis of gonad tissue

Fixed gonad tissues of each mussel were embedded in paraffin, sectioned at 4–5 μm and stained with haematoxylin and eosin. The stained slides were examined under the microscope at 5x to 40x magnification for determination of the gonadal developmental stage. Samples were divided by sex and classified into the following categories: resting or empty stage, advanced gametogenesis, mature, spawning and post-spawning. Images were taken with a camera (CTR5000, Leica, Germany) connected to a microscope (DM500B, Leica, Germany).

2.4. Analyses

Temperatures measured in the field were used to determine the thermal history of the mussels (the average, minimum, and maximum field temperature and minimum and maximum daily temperature change) in one month preceding the experiments. They were also used to inform on naturally occurring heating rates of which one was chosen for the study. The temperatures measured within the experimental chambers were averaged every 5 min throughout the temperature challenge trial.

The heart rate data were visually inspected because the shape of the frequency curve may feature multiple peaks per heartbeat (Burnett et al., 2013). The average heart rate (in bpm) was then calculated for every 5 min interval using R (version 4.2.2). Thermal performance curves were fitted to the heart rate data to determine the temperature at which the heart rate was maximal. Five thermal performance curves ("Gaussian_1987", "Joehnk_2008", "Oneill_1972", "Pawar_2018", "Weibull_1995") were fitted (packages *rTPC*, *nls.multstart*) using nonlinear least squares regression according to Padfield et al. (2021). Subsequently, the five models were weighted based on the smallest Akaike information criterion with correction for small sample size and the temperature at which heart rate was maximal was estimated using the weighted model (Padfield et al., 2021). In addition, the natural log of heart rate and the inverse temperatures (1000 K^{-1}) were calculated and used to fit broken stick regressions (package *respirometry*, v1.3.0, Birk, 2021) to determine the Arrhenius breakpoint temperature (ABT).

Q_{10} values were calculated using the heart rate data of each individual according to the formula:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}$$

where R_2 is the rate at high temperature (T_2, 20 °C) and R_1 is the rate at low temperature (T_1, 10 °C).

For valve gape data, the thickness of each mussel and the placement of the valve gape sensors on the margins of both valves influenced the strength of the electromagnetic field and, hence, the absolute distance between the two coils. The relationship between the strength of the field and the distance was calibrated using the formula:

$$VC_t = \sqrt{\frac{1}{VR_t}}$$

where VC_t is the calibrated value at time t and VR_t is the raw value of the strength of the electromagnetic field. Then, VC_t data were converted into fraction openness, where 0 indicated a closed mussel and 1 was the maximum measured distance between the coils measured during the initial night and the thermal challenge trial (Ballesta-Artero et al., 2017). Then, data (fraction open) were averaged for every 5 min interval throughout the temperature challenge trial and broken stick regressions were fitted in R (package *respirometry*, v1.3.0, Birk, 2021) to determine the temperature at which individuals were closing their shell, i.e. the breakpoint temperature (BPT).

2.5. Statistical analyses

The heart rate during ramping temperature was tested by linear mixed model, with spawning groups and temperature (quadratic term via 'poly-'function) as fixed effects, including their interaction, and individual mussel as random effect. The thermal performance curves for valve gape (fraction open) was tested by a beta regression model applying a logit link, using the same structure as the heart rate model (Table S1). Heart rate at field temperature, condition index, ABT of heart rate, breakpoint temperature for valve gape, valve gape at temperatures below the breakpoint temperature, Q_{10} of heart rate, and difference in temperature at breakpoint temperature of valve gape and thermal optimum of heart rate were tested by linear models and pairwise post-hoc comparisons, using spawning group as fixed effect with three levels (non-spawners, post-spawners, and active spawners) (Table S2). The total wet mass was included as a covariate in the models for heart rate at field temperature and ABT of heart rate to account for the mussel size (Tables S2A–B). The valve gape before breakpoint temperature was analyzed in a beta regression model applying a logit link, with spawning groups as a fixed effect, followed by post-hoc comparisons (Table S2D).

Linear models and linear mixed models (LM, LMM, package *lme4* v1.1–35.3, Bates et al., 2022) and beta regression models (package *glmmTMB*, v1.1.9) have been run, followed when necessary by pairwise post-hoc comparisons (p-value adjusted by Tukey method, package *emmeans* [AB2] v1.10.1, Lenth, 2024). The R^2 values of the models have been reported (package *MuMIn* v1.47.5, Bartón, 2023), and data were graphically displayed with *ggplot2* (v3.5.1, Wickham, 2016). When results are provided, *est* stands for an estimate of the model, and *SE* for the standard error. All statistical analyses were performed in R (R Core Team, v. 4.4.0), with significance set at $\alpha = 0.050$.

3. Results

3.1. Field temperature

The average temperature that animals experienced one month prior to the temperature challenge trial was 8.7 ± 1.5 °C (mean ± SD) with minimum and maximum temperatures of 3.5 and 16.2 °C, respectively (Fig. 1). Daily temperature differences ranged from 0.5 °C (30th March) to 12.5 °C (4th April) and maximum daily increases in temperature ranged from 0.2 °C/h (30th March) to 5.6 °C/h (4th April).

3.2. Histology and gamete release

70% of the samples (14 individuals) were females and 30% (six individuals) were males. Eight females showed advanced gametogenesis and/or maturity. In most cases, tissue heterogeneity was observed with different sized acinis, some of which began to fuse. Most acini contained oocytes with beginning vitellogenesis and attached to the acini walls (Fig. 2A), while others were already mature with yolk granules and vitelline envelopes (Fig. 2B). Free oocytes and empty spaces were also observed within some follicles, which indicates partial spawning. A large area with atretic follicles was observed in three of those eight females (Fig. 2C), suggesting disturbances during the maturation process. The remaining four females were in a spawning stage (Fig. 2D) with detached oocytes and larger empty spaces in the follicular lumen of most follicles. Two females were not reproductively active. One was in a post-spawning state with atrophied follicles with residual oocytes (Fig. 2E), and the other one individual was in a resting stage with the mantle dominated by storage tissue and with little to no gonadal tissue (Fig. 2F).

Male gonadal development was classified by gametogenesis and maturation of spermatozoa of the male germinal acini. 50% of the males (three individuals) were in advanced gametogenesis (Fig. 3A), with immature sperm cells occupying at least half of the acini. The other half of the males were mature (Fig. 3B), with acini filled with mature spermatozoa. Some of the acini appeared partially empty, suggesting partial

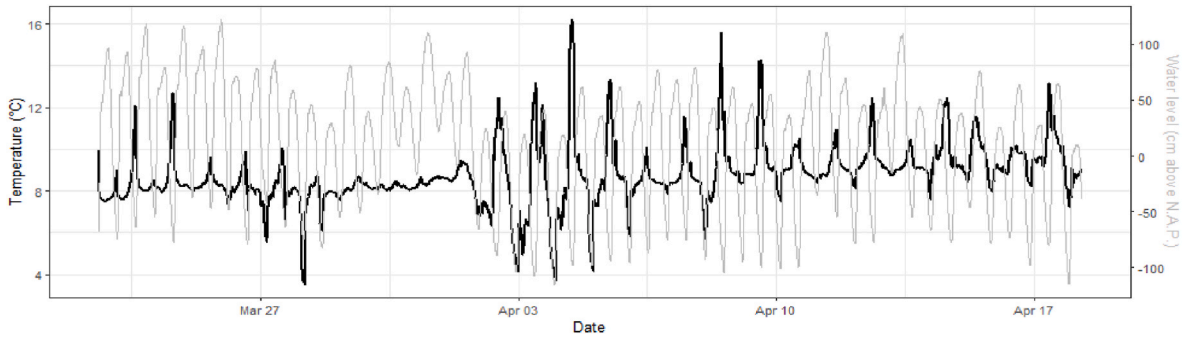

Fig. 1. Thermal history (black line, °C) of *Mytilus edulis* across four weeks prior to the experiments. The water level (cm above N.A.P.) is indicated in gray and was obtained from the Royal Netherlands Institute for Sea Research jetty data (van Leeuwen et al., unpublished data).

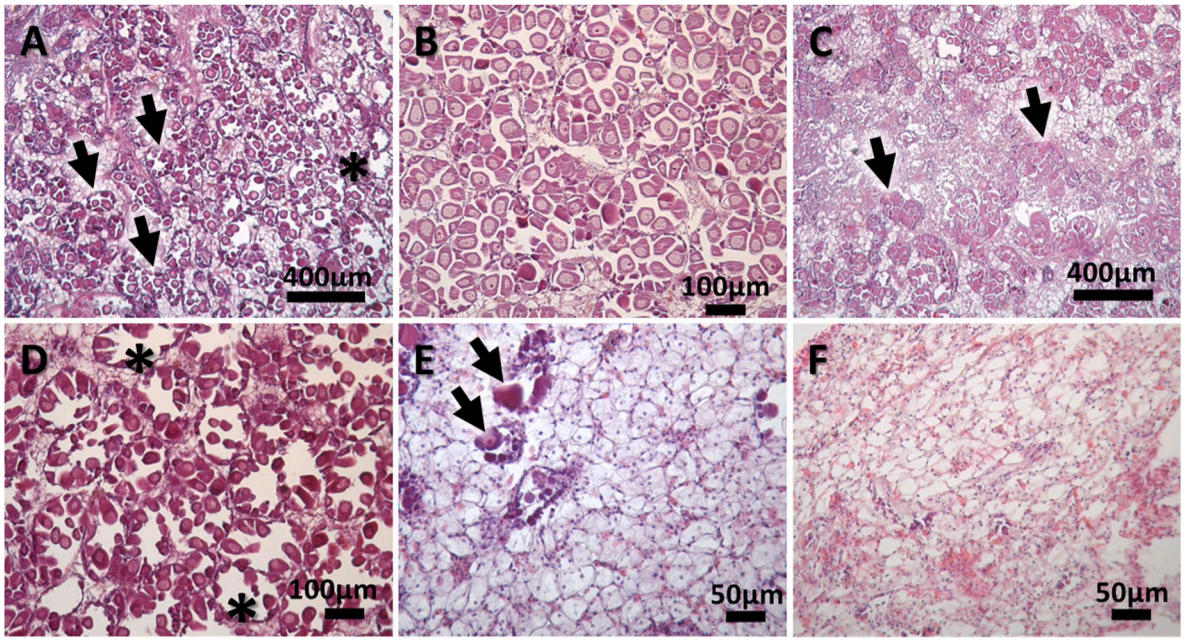

Fig. 2. Histological sections of the female gonad. A) Advanced gametogenesis and/or mature stage with follicles of different sizes, some of which were already fused (*), and most oocytes attached to the follicle wall (arrows). B) Mature stage with yolk granules and vitelline envelope. C) Mature stage with large areas of atretic follicles (arrows). D) Spawning stage with detached oocytes and large empty spaces in the follicular lumen (*). E) Post-spawning stage with atrophied follicles and some residual oocytes (arrows). F) Resting stage dominated by connective tissue and little or no gonadal tissue.

spawning.

During the temperature challenge, 50% of the individuals (9 individuals, 4 females, 5 males) did not release gametes. Of the other nine individuals, three females released gametes the night before but not during the thermal ramping and six individuals (5 females, 1 male) released gametes during the ramping. Among the two individuals kept at control temperature, one did not release gametes while the other one released eggs during the time of thermal ramping.

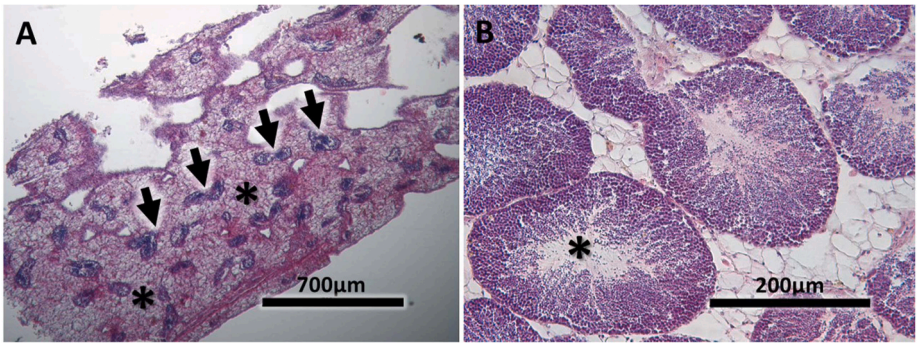

Fig. 3. Histological sections of the male gonad. A) Advanced gametogenesis stage with large areas of connective tissue and most of the acini with immature sperm cells (arrows). B) Clearly mature stage with mature spermatozoa within most of the acini. Some of the acini appeared partially empty (*).

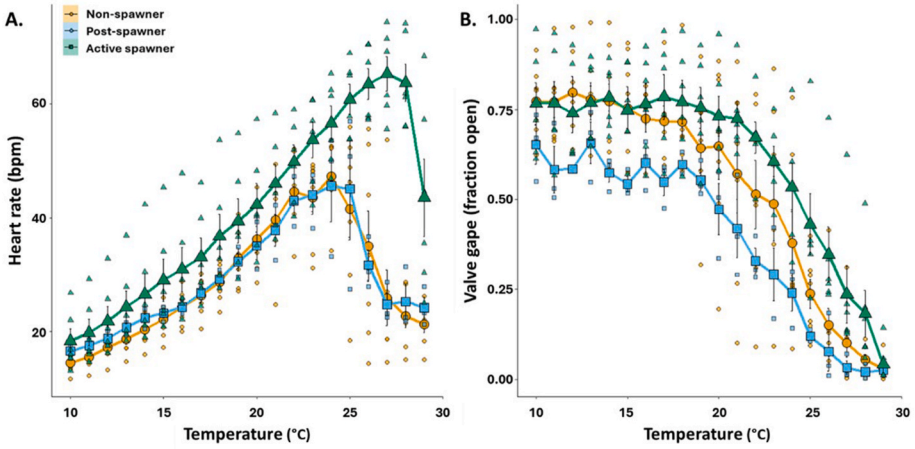

Fig. 4. Thermal performance curves of A) heart rates (bpm) and B) valve gape (fraction open) of blue mussels *Mytilus edulis* at temperatures between 10 and 29 °C. Spawning activity of individuals was not observed (non-spawner, orange circles, n = 7) or observed either the night before the thermal ramping (post-spawner, blue squares, n = 3) or also during the thermal ramping (active spawner; green triangles, n = 6). Large symbols are mean ± SE. Small symbos are raw data. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Based on the histological assessments and spawning activity, individuals were grouped into four maturity stages: 1) able to spawn but no gamete release observed (2 females, 5 males, hereafter "non-spawner"), 2) able to spawn with gamete release the night before but not during the thermal ramping (3 females, hereafter "post-spawners"), 3) able to spawn with gamete release during the thermal ramping (5 females, 1 male, hereafter "active spawners"), 4) not able to spawn (2 females). The two females in the last category were excluded from statistical analyses due to insufficient replicates.

3.3. Biometric characteristics

All measured biometric characteristics (the total wet mass, tissue wet mass, shell length and condition index) were similar among the three spawning groups (Table 1, LM, post hoc, $p > 0.05$, Table S2B).

3.4. Thermal performance curves (TPC)

Across exposure temperatures, heart rate of active spawners was significantly higher than that of non-spawners or post-spawners (LMM, post-spawner: est = 0.501, SE = 3.574, p = 0.089; active spawner, est = 12.358, SE = 2.882, $p < 0.001$, Table S1A, Fig. 4A). Yet, the average Q_{10} of heart rate (2.4 ± 0.1) (mean ± SE) was similar among spawning groups (LM, post hoc, $p > 0.05$, Table S2F).

The TPCs for valve gape of post-spawners and active spawners were different to that of non-spawners (betareg, post-spawner: est = −0.695, SE = 0.234, p = 0.004; active spawners, est = 0.391, SE = 0.192, p = 0.042, Table S1B, Fig. 4B). The temperature-dependent valve gape displayed a positive quadratic pattern, being highest between 15 and 20 °C and rapidly decreasing thereafter. The significant interaction showed that the valve gape of non-spawners and active spawners was similar at temperatures below 20 °C, but at temperatures above 20 °C the valve gape of the latter is wider open (betareg, est = −6.873, SE = 1.583, $p < 0.001$; Fig. 4B).

The routine heart rate of individuals before the thermal ramping (at field temperature) was similar between spawning groups and on average 16.4 ± 0.9 bpm (mean ± SE) (LM, post hoc, $p > 0.05$, Table S2A, Fig. 5A). The ABT of heart rate of active spawners was 3.9 and 3.2 °C higher compared to those of non-spawners or post-spawner, respectively (LM, post hoc, post-spawner: est = −3.128, SE = 1.034, p = 0.027; non-spawner: est = −3.778, SE = 0.816, p = 0.002, Table S2C, Fig. 5B). The maximum numerical value of heart rate of active spawners was also 1.3-fold higher compared with both other spawning groups. There was a strong correlation between the ABT and the maximum numerical value of heart rate of mussels ($r^2 = 0.81$, $p < 0.001$). Hence no additional analyses were run comparing numerical heart rate values among spawning groups.

The breakpoint temperature for valve gape was similar among spawning groups and on average 21.0 ± 0.7 (mean ± SE) °C (LM, post hoc, all $p > 0.05$, Table S2D, Fig. 5C).

The valve gape at temperatures below the breakpoint temperature was 1.3-fold smaller in post-spawners compared to non-spawners (LM, post hoc, est = 0.884, SE = 0.375, p = 0.048), yet the difference between post-spawners and active spawners was not significant (LM, post hoc, est = −0.788, SE = 0.383, p = 0.09, Table S2E, Fig. 5D).

No significant difference in the ratio between ABT of heart rate and breakpoint temperature of valve gape was found between spawning groups (LM, post hoc, $p > 0.05$, Table S2G).

4. Discussion

Actively spawning *M. edulis* had overall higher heart rates and a 3–4 °C higher temperature for the maximum cardiac performance than those of non-spawners and post-spawners. The elevated heart rates of active spawners indicate an increased aerobic metabolism as heart rate correlates strongly with oxygen consumption (Bakhmet, 2017). Similarly, spawning-induced increase in the rates of aerobic metabolism were observed at the cellular level, shown by elevated electron transport system activities (a proxy for the mitochondrial activity) in spawning blue mussels (Mredul et al., 2024). In bivalves, the high energy demands of reproduction deplete tissue energy reserves, including those in non-spawning somatic tissues, and negatively impact cellular energy balance, as indicated by a lower adenylate energy charge (Li et al., 2007, 2009; Mredul et al., 2024).

During spawning, bivalves allocate the aerobic energy towards reproduction rather than maintenance, which may impair cellular protection and defense mechanisms (Brokordt et al., 2019; Kim et al., 2022). The drastic decrease in heart rate of mussels after its thermal performance maximum during a thermal challenge could suggest cell damage due to heat stress. Studies have shown that tissue levels of cellular protective proteins such as molecular chaperones (heat shock proteins) are upregulated several degrees below the thermal maximum for cardiac performance as a mechanism to safeguard the cellular proteome from damage (Roberts et al., 1997; Gracey et al., 2008; Han et al., 2013; Zhang et al., 2014; Moyen et al., 2019). Spawning may interfere with the expression of the cellular protection systems. For instance, in oysters (*Crassostrea gigas*), spawned individuals demonstrated a reduced ability to upregulate both constitutive and inducible HSP70 isoforms in

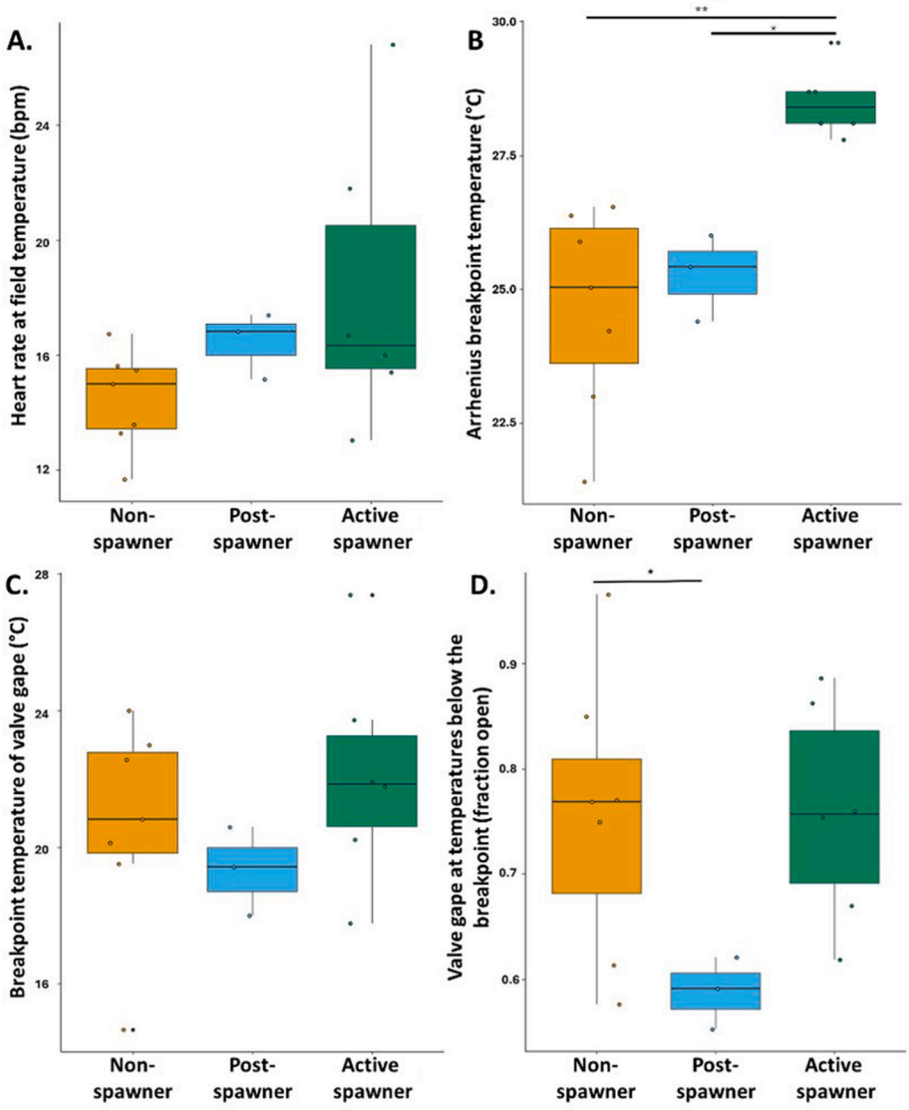

Fig. 5. A) Routine heart rate before thermal ramping (bpm, at field temperature), B) Arrhenius breakpoint temperature of heart rate (°C), C) breakpoint temperature (BPT) of valve gape (°C), and D) routine valve gape at temperatures below the BPT (fraction open) of adult *Mytilus edulis* for which spawning activity was not observed (non-spawners, orange box, n = 7) or observed either the night before the thermal ramping (post-spawners, blue box, n = 3) or during the thermal ramping (active spawners, green box, n = 6). In the boxplots, black horizontal lines represent the median, boxes represent 50% of the values, and upper and lower whiskers represent values > 75th and <25th percentiles. Significant differences between groups are indicated in the figures with an asterisk, following *p < 0.05, **p < 0.01. Colored circles are raw data. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Biometric characteristics of blue mussels *Mytilus edulis* that did not spawn (non-spawner), spawned only before (post-spawner) or during a thermal ramping (active spawner). *n* = number of replicates. Data are mean ± SE.

	non-spawner	post-spawner	active spawner
Total wet mass (WM) (g)	6.1 ± 0.8	5.9 ± 1.3	6.9 ± 0.9
Tissue WM (g)	1.5 ± 0.2	1.3 ± 0.2	1.8 ± 0.2
Shell length (SL) (mm)	38.4 ± 1.4	39.0 ± 2.7	42.0 ± 1.7
CI (WM/SL^3) (mg/mm)	0.104 ± 0.006	0.095 ± 0.005	0.092 ± 0.003
n	7	3	6

response to heat stress compared to their sexually mature, non-spawned counterparts (Li et al., 2007). Taken together, these data suggest that despite a higher maximum temperature for cardiac performance in active spawners, their potentially reduced heat stress protection—due to the diversion of energy resources from maintenance and suppressed cellular protection mechanisms—may make them more vulnerable to thermal damage. Investigating the cellular responses of different spawning groups under heat stress could provide insights into the potentially reduced stress protection and increased cellular damage in reproductively active individuals.

To our knowledge, this is the first study that investigated TPCs during active spawning of mussels so that no comparison with other studies was possible. However, heart rate TPCs were determined for non-spawning and post-spawning scallops, *Chlamys farreri*. In contrast to our results, post-spawning scallops had overall higher heart rates and a 3.5 °C lower performance maximum compared to non-spawning individuals (Xing et al., 2016). It is unclear how much time had passed between spawning and thermal challenge in the study on scallops. The heart rate increase and reduced thermal tolerance may be indicative for elevated metabolic costs to reestablish homeostasis (Mredul et al., 2024). Measuring TPCs at different time points after spawning could shed light on if *M. edulis* behaves similarly or if species-specific differences in thermal sensitivity of spawning individuals exist.

Post-spawning mussels were less wide open during the thermal ramping compared to both other spawning groups. A less wide open valve gape may indicate an energy conserving strategy although measurements that directly link valve gape and energy expenditur are lacking (Cheng et al., 2024; Bertolini et al., 2022; Tang and Riisgård, 2018; Riisgård and Larsen, 2015). Gametogenesis as well as spawning itself are energetically costly processes (Mendo et al., 2016; Muller et al., 2019; Mredul et al., 2024). Bivalves deplete their energy reserves during spawning and potentially require weeks to restore them (Lodeiros et al., 2001; Li et al., 2009; Mredul et al., 2024). Hence, the reduced valve gape in post-spawning mussels during the thermal challenge may conserve energy of the exhausted budget from recent spawning. Decreased energy expenditures can decrease the sensitivity of mussels to environmental stress (Sokolova et al., 2012). During the thermal challenge this strategy may have ensured the stress tolerance window and hence survival. However, this strategy may not be sufficient to maintain thermal tolerance during prolonged or repeated heat exposure. Prolonged exposure to a 6 °C increase above control temperature within the thermal window of the mussel, *Mytilus trossulus*, for example, lead to increased mortality in post-spawning individuals (Tomanek and Zuzow, 2010; Clements et al., 2018). Similarly, the mortality rate of *M. edulis*, *Mytilopsis leucophaeata* and *Dreissena polymorpha* was 40–45% higher in breeding individuals compared to non-breeding individuals (Rajagopal et al., 2005). Yet, experiments with spawners were conducted between June and October while those with non-spawners were conducted between November and April (Rajagopal et al., 2005). Hence, seasonal differences cannot be excluded. Also Li et al. (2007) assessed the impacts of heat exposure on bivalves by exposing the oyster *C. gigas* to repeated heat shocks. While mortality was negligible in both pre- and post-spawning oysters after the first heat shock (37 °C), exposure to a second heat shock (44 °C) caused severe mortalities (80%) in post-spawning individuals while those of pre-spawning individuals remained comparatively low (10%, Li et al., 2007). All of the above suggests that spawning contributes to summer mass mortality events of *M. edulis*, as those observed for example in the Netherlands and France (Seuront et al., 2019; Capelle et al., 2021).

5. Conclusion

Active spawners had an increased thermal stress due to their active metabolism at temperatures that cause metabolic depression in non-spawners. Once spawned, individuals conserve energy as indicated by reduced valve gapes in post-spawners. Considered together, these results suggest increased vulnerability to mortalities when spawning coincides with recurring short-term warming events. *Mytilus edulis* is an important ecosystem engineer of the intertidal zone as well as an important aquaculture resource. Increased mortalities may have potential consequences for the local biodiversity in a future ocean, in which extreme temperatures are projected to occur more frequently and in larger amplitude.

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CRediT authorship contribution statement

Katharina Alter: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Maria Constenla:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Francesc Padrós:** Writing – review & editing, Investigation. **Inna M. Sokolova:** Writing – review & editing, Funding acquisition. **Ana Born-Torrijos:** Writing – review & editing, Visualization, Investigation, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2024.104018.

Data availability

The data generated and analysed as well as the code used in the present study are available from Zenodo Digital Repository, DOI: 10.5281/zenodo.14055288.

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