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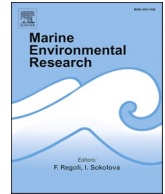
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Are clam-seagrass interactions affected by heatwaves during emersion?

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ABSTRACT

The increased frequency of heatwaves expected in the context of global warming will affect socio-ecological systems such as shellfish beds at intertidal seagrass meadows. A mesocosm experiment was performed to assess the effects of a simulated atmospheric heatwave during low tide on the bioturbation indicators and growth of the commercial juvenile native *Ruditapes decussatus* and the introduced clam *R. philippinarum*, and on their interactions with the seagrass *Zostera noltei*. Under the heatwave, heat dissipation at 5 cm depth was significantly greater in the sediments below *Z. noltei* than below bare sand, the photosynthetic efficiency (F_v/F_m) of *Z. noltei* decreased and the clams tended to grow less. Furthermore, after the heatwave clams below bare sand tended to burrow deeper than those below *Z. noltei*, indicating that seagrass provided a refuge for clams. *Ruditapes philippinarum* grew less, and did not burrow as deeply as *R. decussatus*, which may imply greater vulnerability to desiccation and heat at low tide. The particle displacement coefficient (PDC) of *R. philippinarum* indicated lower bioturbation values in *Z. noltei* than in bare sand and was a suitable bioturbation indicator for juvenile *Ruditapes* spp. clams. In *Z. noltei* coexisting with *R. philippinarum*, the F_v/F_m values were higher than without clams after a recovery period, which may be linked to the assimilation of phosphate excreted by the clams and suggests a facilitative interaction. No such interaction was observed with *R. decussatus*, probably because of its deeper burrowing depth. The findings suggest reciprocal facilitative interactions between *R. philippinarum* and *Z. noltei* and the potential contribution of *Z. noltei* to the sustainability of clams under global warming scenarios, which may support management actions aimed at enhancing the coexistence between shellfishing activities and seagrass conservation.

1. Introduction

In the current scenario of global warming, an increase in the intensity and frequency of heatwaves is expected (Koffi and Koffi, 2008; IPCC, 2019), what will have several concerning consequences on the ecosystems and human communities (Stillman, 2019). In the marine realm, marine heatwaves have negative effects on the survival and physiological performance of species (Harvey et al., 2022). Although less well studied, atmospheric heatwaves, i.e. prolonged periods where air temperatures are substantially hotter than normal (see in Hobday et al., 2016), represent an additional source of stress to intertidal organisms during emersion (Mieszekowska, 2009; Stillman, 2019). The thresholds for defining atmospheric heatwaves are broad and depend on the study

region. For instance, Perkins and Alexander (2013) and the Spanish meteorological agency defined the duration of atmospheric heatwaves as three or more days above a defined percentile (90th and 95th, respectively). Accordingly, several studies about effects of atmospheric heatwaves on intertidal species applied a four-day period (Olabarria et al., 2016; Hereward et al., 2019; Román et al., 2020; Domínguez et al., 2021; Vázquez et al., 2021).

During these events, sessile or slow-moving organisms experience heat stress and desiccation, provoking changes in their behaviour, physiological performance and ultimately mortality (Helmuth et al., 2006; Gosling, 2015; Stillman, 2019). These processes can modify community structure and functioning, change the vertical zonation patterns and alter the geographical distribution of communities at

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different spatial scales through migrations towards thermal refuges or cooler latitudes (Harley et al., 2006; Mieszkowska, 2009). This leads to species range shifts and changes in the interactions (Helmuth et al., 2006; Stillman, 2019; Harvey et al., 2022). The warming coastal regions can experience both ecological and socio-economic impacts if food provisioning, employment and/or income rely on the ecosystems impacted (Harley et al., 2006).

Socio-economic activities carried out in coastal areas, such as shellfishing and aquaculture, are vulnerable to the synergistic effects of global warming and anthropogenic physical disturbance (Allison et al., 2011; Villasante et al., 2021), especially if the activities occur in intertidal areas, which are subjected to the effects of heat stress events (Helmuth et al., 2006). A noteworthy example of such interactions is observed in NW Spain, where the intertidal *Zostera noltei* Hornemann meadows are historically used as areas for seeding and harvesting infaunal bivalves, including the venerid native clam *Ruditapes decussatus* (Linnaeus, 1758) and its congeneric introduced species *R. philippinarum* (A. Adams & Reeve, 1850), which together yielded 32% (2320 tons) of the first sales of bivalves in 2021 (<https://www.pescadegalicia.gal/gl/descargas>). *Ruditapes philippinarum* was introduced with aquaculture purposes in the 1980s (Flassch and Leborgne, 1992) and it is seeded in shellfishing beds to warrant annual stocks (Frangoudes et al., 2008). Its resistance and good performance for aquaculture favoured the settlement of natural populations (Bidegain and Juanes, 2013) that currently yield a greater productivity than that of the native *R. decussatus* in Galicia (<https://www.pescadegalicia.gal/gl/descargas>). In this region, shellfishing on foot contributes to employment, especially of women (Surís-Regueiro and Santiago, 2014; Villasante et al., 2021). Seagrasses are associated with these marine activities, performing an important socio-ecological role (Bas Ventín et al., 2015). However, global warming can threaten these functional groups and, consequently, the whole ecosystem.

Heat stress during low tide alters the activity and burrowing behaviour of *R. decussatus* and *R. philippinarum* (Gosling, 2015; Macho et al., 2016; Nerlović et al., 2016), and provokes changes in the reproduction, scope for growth and survival (Sobral and Widdows, 1997; Domínguez et al., 2021; Vázquez et al., 2021). Likewise, the exposed *Z. noltei* canopies experience tissue desiccation (Azevedo et al., 2013), decreased photosynthetic performance and shoot survival (Massa et al., 2009), and reduction in biomass (Cardoso et al., 2008). An additional impact on *Z. noltei* is the anthropogenic physical disturbance, as dense patches are perceived as unsuitable for seeding and recruitment in areas of intensive clam harvesting (Bas Ventín et al., 2015; Guimarães et al., 2012) and the clam gathering is easier in bare sediment than in seagrass. Therefore, canopies are often uprooted with tractors before the clam seeding, and then are manually dug with rakes and hoes to collect adults. This apparent incompatibility between clam shellfisheries and seagrass conservation may be due to the fact that both functional groups act in opposite ways as ecosystem engineers. Seagrasses stabilize the sediment, enhance the deposition of fine particles and organic matter and promote anoxia (Hemminga and Duarte, 2000; Miyajima and Hamaguchi, 2019), although plants exude oxygen through the roots, oxidizing its surrounding sediments (Jensen et al., 2005). By contrast, bivalves are responsible for bioturbation, destabilizing, eroding, aerating and draining sediment by reworking particles (Norkko and Shumway, 2011; Kristensen et al., 2012).

Nevertheless, interactions between filter-feeding bivalves and seagrasses may be positive, suggesting a facultative mutualistic interaction (Peterson and Heck, 2001; Norkko and Shumway, 2011). Bivalves reduce turbidity in the water (Peterson and Heck, 2001; Wall et al., 2008; Maxwell et al., 2017), prevent sulphide toxicity and increase the concentrations of pore water nutrients in the sediment (Peterson and Heck, 1999; Wall et al., 2008) especially of NH_4^+ (Cardini et al., 2019), and favour plant growth and productivity (Cardini et al., 2022). In addition, they enhance the retention of seagrass propagules (Meysick et al., 2019) and increase the efficiency of burial of seagrass seeds by

reworking the sediment (Li et al., 2017). In turn, seagrasses provide a suitable habitat for the bivalves (Sanmartí et al., 2018), increase bivalve survival and growth, reduce predation (Irlandi and Peterson, 1991; Peterson and Heck, 2001), enhance larval deposition (Orth, 1992) and buffer temperature increases in the sediment during warming events (Crespo et al., 2017; Román et al., 2022).

It is not clear how atmospheric heatwaves affect these facilitative interactions. Under harsh environmental conditions, positive interactions may prevail (Bertness and Callaway, 1994) through the enhancement of seagrass productivity by lucinid bivalves (Chin et al., 2021), or the reduction of environmental stress effects and mortality of bivalves by the seagrass (Gagnon et al., 2020; Cardini et al., 2022). The potential mechanisms involved range from the presence of a seagrass-lucinids-chemoautotrophic bacteria mutualism, to the fertilization of the sediment by bivalves through excretion and bioturbation, or the shelter against abiotic stress provided by the seagrass. However, after reaching a critical threshold, these interactions may be disrupted accelerating ecosystem degradation (Mieszkowska, 2009; de Fouw et al., 2016; Sanmartí et al., 2018). Although the negative interactions between the infaunal bivalves and intertidal seagrasses tend to increase as seawater temperature increases (Gagnon et al., 2020), the responses to increased air and sediment temperatures have been rarely studied (but see de Fouw et al., 2016).

In NW Spain, the days per year with a maximum atmospheric temperature above the 90th percentile has increased at a rate of 3–6 days decade⁻¹ since 1974 (Gómez-Gesteira et al., 2011). According to CIMP6 climate models, the number of hot days (mean surface temperature >30 °C) per year will continue to increase by the end of this century under the “business as usual” scenario SSP5-8.5 (Carvalho et al., 2021). Therefore, the experimental assessment of the effects of heat stress during low tide on the interactions between *R. decussatus* and *R. philippinarum* and *Z. noltei* would help to provide a better understanding of the mechanisms that will prevail in intertidal shellfisheries in a future warming scenario.

The general aim of this study was to test if the positive interactions between *Z. noltei* and the clams *R. decussatus* and *R. philippinarum* buffer the impacts of an atmospheric heatwave. The data were used to test the hypotheses that 1) the negative effects of an atmospheric heatwave on the photosynthetic efficiency of *Z. noltei* are ameliorated by the presence of clams, 2) the negative effects on the growth and activity of the two clam species in sediment are reduced by the presence of *Z. noltei* and, 3) the effects of the atmospheric heatwave on pore water nutrients vary depending on the presence of clams and/or *Z. noltei*. We expected a better physiological performance of the clams and the seagrass under heat stress when they coexisted than when they were alone.

2. Materials and methods

2.1. Mesocosm experiment

A mesocosm experiment was performed between 21 September and 5 October 2020. The experimental design included Habitat (2 levels: bare sand and *Z. noltei*), Clam (3 levels: *R. decussatus*, *R. philippinarum* and no clam) and Temperature during low tide (2 levels: high and control) as fixed orthogonal factors ($n = 6$).

A *Z. noltei* canopy (of approximately 1 m²) and the underlying sediment (to a depth of 25 cm) were sampled on 4 September 2020 from the *A Seca* shellfish bed, located in an intertidal flat in Combarro, Ría de Pontevedra, NW Spain (Coordinates: 42° 26' 12.4" N, 8° 41' 25.3" W). The mean temperatures recorded by Envloggers™ (Electric blue CRL) deployed at the sampling site were 20.96 ± 3.65 °C in sediment (3 cm) during summer time.

The sediment was sieved in the field through a 5 mm mesh to remove macrofauna and taken with the seagrass canopy to a controlled temperature room (23 °C) in the Estación de Ciencias Mariñas de Toralla (ECIMAT, <https://cim.uvigo.gal/>), of the Universidade de Vigo, where

80 PVC cores of 10 cm diameter and 25 cm length were each filled with a column of sediment (length 18 cm). The sediment collected was sand according to the Udden-Wentworth scale, with a mean size (\pm S.E.) of $325 \pm 14 \mu\text{m}$ ($n = 4$). Two holes (1 cm diameter) were drilled in opposite sides of the cores (1 cm above the bottom), to ease the flow of water through the sediment, and covered with $500 \mu\text{m}$ mesh. *Zostera noltei* plants were placed in each of the 40 cores emulating the canopy of the sampling site (shoot density in the field: $4892 \pm 1342 \text{ shoots m}^{-2}$), and the other 40 cores were left with bare sand. Mean (\pm SD) fresh weight of plants per core was of $\sim 20.50 \pm 0.81 \text{ g}$.

There were a total of 80 cores distributed in two replicated immersion tanks (height x length x width: 60 x 180 x 90 cm). Forty cores were placed in each immersion tank; 36 of these ($n = 3$ per combination of the factors Habitat, Clam and Temperature during low tide) were used for the biological and geochemical measurements of response variables in seagrass, clams and sediment, and 4 cores (2 with bare sediment and 2 with *Z. noltei*) were left without clams and used for the environmental

measurements with data loggers (Fig. 1). Running seawater from the surroundings of the ECIMAT facility was continuously pumped through the immersion tanks that flushed through their drainpipes in an open circuit, and the tanks were lit by a combination of indirect natural light and cool white LED lamps under a 12:12 h photoperiod. The cores were stabilized and acclimatized to these conditions for 17 days (Fig. 2). Cores were submerged over the entire acclimation period to provide the most favourable and less stressful environmental conditions for the seagrass.

Three days before the beginning of the experiment (D-3), 100 juvenile clams of each species were measured (mean length \pm SD: *R. decussatus* = $19.98 \pm 1.05 \text{ mm}$, *R. philippinarum* = $20.67 \pm 1.16 \text{ mm}$) and submerged in a calcein solution of 250 mg L^{-1} (Sigma, CAS 1461-15-0) in seawater for 2 h, to mark the edges of the shells. The clams filtered the diluted calcein, which was incorporated into their shells, leaving a mark visible under UV light that allowed tracking their growth from that day (van der Geest et al., 2011). From this batch, 96 dyed individuals were selected for seeding: 24 cores (12 with *Z. noltei* and 12

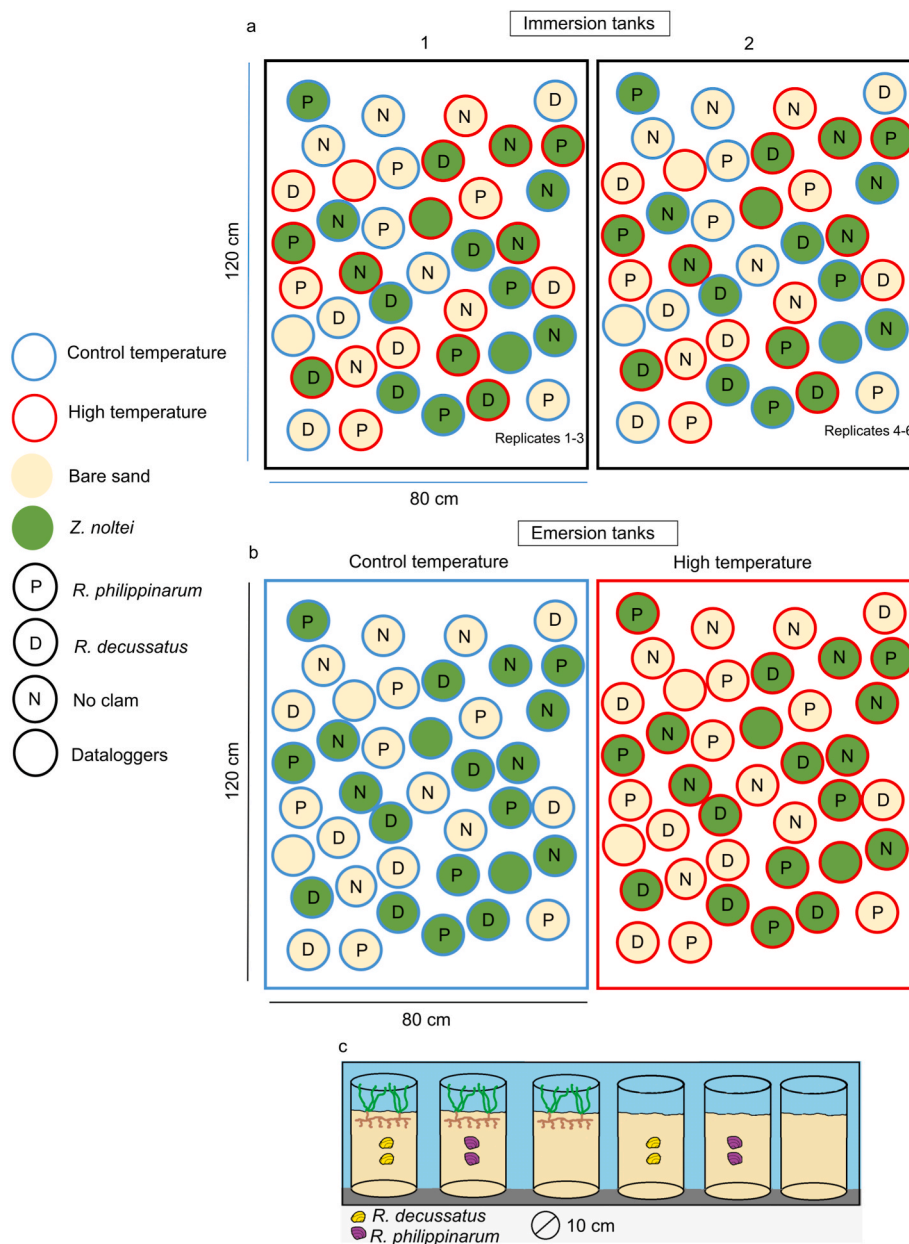


Fig. 1. Distribution of experimental units (cores) in (a) the duplicate seawater tanks, (b) the emersion tanks, and (c) detail of the cores with each combination of habitat and clam treatments.

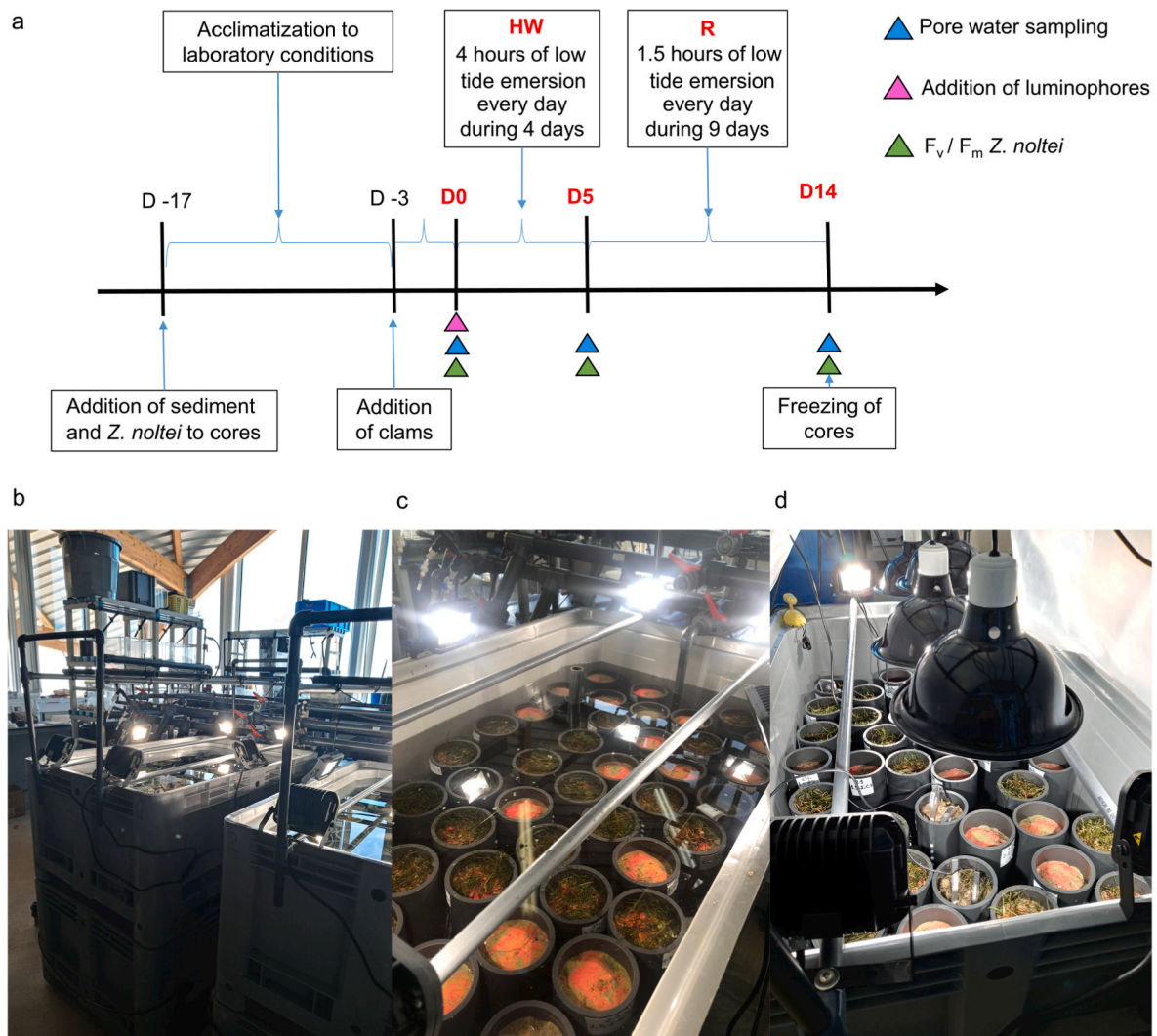


Fig. 2. a) Timing of the experiment, HW: Heatwave, R: Recovery, b) the immersion tanks under natural indirect sunlight and artificial cool white light, c) cores in the immersion tanks after addition of the luminophore tracers, d) cores in the emersion tanks exposed to the heatwave conditions.

with bare sediment) were seeded with two individuals of *R. decussatus*, and following the same system (half of the cores for each habitat), 24 cores were seeded with two individuals of *R. philippinarum* and 24 cores were left without clams. Two individual clams per core is equivalent to a density of 254 individuals m^{-2} , which is within the range of juvenile seeding densities in shellfish beds in NW Spain (from 120 ind m^{-2} to 300 ind m^{-2} ; Navajas et al., 2003). The clams were acclimated to the cores and fed daily during the experiment with a mixture of *Tetraselmis suecica*, *Chaetoceros gracilis*, *Phaeodactylum tricorutum*, *Rhodomonas lens* and *Isochrysis* spp. (diet based on the 3% of the mean dry weight of clams), which was daily provided by the technical staff of ECIMAT.

The day before applying the heatwave treatment (D0), a low tide was simulated, and the cores with *Z. noltei* were dark adapted for 30 min to measure the quantum efficiency of photosystem II photochemistry, as an indicator of photosynthetic efficiency (F_v/F_m), at 6 shoots per core, with a Pulse-Amplitude Modulation fluorometer (Junior PAM, Walz-Germany). The pore water at 2 cm depth (the depth within the rhizosphere of *Z. noltei*) was extracted from each core by inserting a Rhizon™ sampler attached to a syringe (Rhizosphere research products) into a hole previously fitted with a plastic screw. The water samples were extracted through filters with a pore size of 0.15 μm incorporated in the rhizons and were frozen until further analysis. After the D0 measurements, 2.5 g of a mixture of pink (63–125 μm) and green (300–500 μm) Partrac™ (Glasgow, Scotland, UK) luminophores (i.e. fluorescent

particulate tracers) were hydrated with tap water and added evenly to the surface of sediment cores in a single pulse. The luminophores are used to track and quantify bioturbation, more especially its sediment reworking component, which is the transport of particles by animals in a sediment matrix (Gerino et al., 1998; Kristensen et al., 2012). Modelling the luminophore distribution in the sediment column allows to obtain comparable sediment reworking coefficients (Majdi et al., 2014; Capowiez et al., 2021), which were used in this study as indicators of the activity of clams exposed to the different experimental treatments.

Between days 1 and 4 (D1–D4), heatwave conditions were applied daily during the simulated low tide (HW), for 4 h, from 10:00 to 14:00, thus mimicking conditions that frequently occur in the shellfish beds during summer, when low spring tides often occur at noon. Every day, 6 cores for each combination of Clam and Habitat were placed in each of the emersion tanks, and one tank was subjected to the control temperature (20 °C) and the other to the high temperature (periodical heatwave). Half of the cores from each immersion tank were placed in each emersion tank, so that cores from both immersion tanks were mixed together during the low tide period (Figs. 1 and 2). The tanks were filled with a ~8 cm layer of running seawater, so that the cores were emerged, and water was allowed to move through the deepest sediment layers through the holes in the cores, to mimic the fall of the phreatic layer. The emersion tanks were each placed below 4 infrared ceramic heaters (Exo Terra) and were covered by greenhouse plastic, to prevent heat loss, and

equipped with air fans, to homogenize the air temperature. The heaters in the control temperature tank were permanently switched off, whereas in the high temperature tank they were used to increase the temperature gradually and were controlled by a temperature sensor inserted at 2 cm depth in a core for data loggers. The target temperatures at 2 cm depth were 20 °C at 10:00, 25 °C at 12:00, and 30 °C between 13:00 and 14:00. The high target temperature of 30 °C at 2 cm depth was recorded in the shellfish beds in the region during low tide in hot weather in summer (Macho et al., 2016). This temperature (30 °C) affected the activity of *R. decussatus* and *R. philippinarum* without being lethal, whereas the control temperature of 20 °C was within the optimal range of temperature for performance of the clams (Macho et al., 2016 and refs. therein; Domínguez et al., 2021).

The cores were randomly placed in different positions of the emersion tanks every day of the daily low tide during the simulated heatwave. After each simulated daily low tide the cores were returned to the immersion tanks and randomly placed. Twenty-four hours after the end of the simulated heatwave, on day 5 (D5), the F_v/F_m of *Z. noltei* was measured and the pore water was sampled. The cores were then allowed to recover (R) for 9 days in the immersion tanks, with exposure to daily neap tide conditions during 1:30 h at a temperature of 20 °C at 2 cm depth in the sediment. Twenty-four hours after the recovery period, on day 14 (D14), the F_v/F_m was measured, the pore waters were extracted, and the cores were frozen until sample processing (Fig. 2).

During the experiment, seawater temperature inside each immersion tank was recorded every 30 min with a HOBO® Pendant data logger (Onset Computer Corp., Bourne, MA, USA; accuracy range of ± 0.53 °C and resolution of 0.14 °C) glued to the top of one of the cores used for environmental measurements. The photosynthetically active radiation (PAR, 400–700 nm) on the water surface at different positions of the immersion tanks was measured with a Delta Ohm HD2102 photo radiometer over the experimental period. Throughout the simulated heatwave, the air temperature and humidity and the sediment temperatures (at 0 cm, 2 cm and 5 cm depth) were recorded every 10 min with Ibutton® data loggers (Maxim Integrated Products, Dallas Semiconductor, USA; resolution of 0.06 °C and 0.6% for temperature and humidity, respectively) in methacrylate plates inserted in the 4 cores (2 with *Z. noltei* and 2 with bare sediment) in each emersion tank (Figs. 1 and 2).

2.2. Sample processing

In order to determine the repartition of luminophore tracers due to clams activity and to further calculate corresponding sediment reworking coefficients, the cores were defrosted and cut in half longitudinally. In both halves, the sediment was sliced in 0.5-cm thick layers from the surface to 5 cm depth, and in 1-cm thick layers from 5 to 18 cm. Subsamples from each layer were homogenized and placed in 24-well microplates (BD Falcon™, Franklin Lakes, NJ, USA). The luminophores were then quantified by measuring the fluorescence (bottom reading fluorescence; λ excitation = 565 nm; λ emission = 602 nm; area scan: 9 x 9) in a microplate reader (Synergy Mx, Biotek, Winooski, VT, USA) in the Laboratoire Écologie Fonctionnelle et Environnement from the Université Toulouse III-Paul Sabatier (France).

The burrowing depth (cm) of the clams was measured in the open cores as an indicator of their position after the recovery period. The clam shells were separated from the flesh and dried in an air oven (60 °C, 48 h). The right valves were submerged in a solution of H₂O₂ (35%) for 24 h, to remove the periostracum, and were then dried at ambient temperature and observed under a binocular microscope with a UV light source, to detect the calcein mark. Three measurements were made per clam shell: one in the centre of the shell edge, aligned with the position of the umbo, and one each at 4 mm above and below the first measurement. The three measurements were averaged in order to determine the shell growth (mm) in each clam, calculated as the difference between the calcein mark and the shell edge.

The concentrations of nitrate (NO₃⁻), ammonium (NH₄⁺), and phosphate (PO₄³⁻) ($\mu\text{mol L}^{-1}$) in the sediment pore waters were analysed through colorimetry in a continuous segmented flow autoanalyzer (Bran + Luebbe, mod. AA3) at the CACTI facilities of the Universidade de Vigo.

2.3. Data analysis

The distribution of the luminophores in the sediment column was used to calculate the reworking coefficients. The maximum penetration depth of the tracers (MPD, cm) was the deepest layer where the luminophores were detected, and it was considered an indicator of the maximum depth reached by the clams during the experiment. The difference between the MPD and the burrowing depth was calculated as an indicator of the vertical displacement of the clams (i.e. VD, cm), between the most stressful conditions during the experiment (i.e. days 1–4, with 4 h of simulated low tides) and the end of the recovery period. The biodiffusion coefficient (D_b , $\text{cm}^{-2} \text{year}^{-1}$) quantifies the omnidirectional, short distance and step-by-step displacement of particles by taking into account the relative concentration of luminophores at a given depth during a defined time, neglecting the effects of advection, reaction and sedimentation. It is specific to the reworking functional group of biodiffusers to which *Ruditapes* spp. belong (Kristensen et al., 2012) and was calculated according to Majdi et al. (2014). The particle displacement coefficient quantifies the cumulative proportion of particles displaced upwards and downwards and the distance of displacement during a defined time (PDC, $10^{-3} \text{cm year}^{-1}$). This non-specific coefficient, contrary to the D_b coefficient, is suitable for any mode of sediment reworking and was calculated according to Capowiez et al. (2021).

The heat dissipation in the sediment below *Z. noltei* and bare sand during the heatwave was estimated by subtracting the temperatures at 5 cm depth from the temperatures at 0 cm. The differences of temperature between *Z. noltei* and bare sand in air, 0, 2 and 5 cm depth, heat dissipation and humidity in each temperature treatment of the simulated heatwave were tested with Generalized estimating equations (GEEs). The GEEs estimate regression parameters of data collected in clusters taking into account their correlation and are useful for environmental measurements (Halekoh et al., 2006). The GEEs were fitted with core as a cluster variable and an autoregressive correlation structure, which assumed that there was more correlation between responses taken closer within each cluster than between those of different clusters.

The effect of the fixed factors (Habitat, Clam and Temperature) on the biological and geochemical response variables was tested by generalized least squares (GLS) models with an auto-correlation compound symmetry structure determined by Immersion tank; the correlation coefficient (ρ) was below 10^{-7} for all the models. The effects on the F_v/F_m in *Z. noltei* and on the concentrations of nitrate, ammonium and phosphate in sediment pore water were tested before and after the heatwave and after the recovery period, whereas the effects on the growth, burrowing depth, MPD, vertical displacement, D_b , and PDC of clams were tested after the recovery period.

The replicate measurements of F_v/F_m of *Z. noltei* and of the growth and burrowing depth of clams were averaged per core. Outliers (i.e. values three times the inter-quartile range above the third quartile or below the first quartile) were removed to increase the confidence of the statistical tests (Crawley, 2013). The number of outliers removed represented less than the 8% of the data. The normality of the residuals was inspected in Q-Q plots and the homogeneity of the variances was checked in plots of standardized residuals vs. fitted values. When the variances were not homogeneous for each combination of fixed factors, the heteroscedasticity structure was defined in the GLS models through the “VarIdent” function. Post-hoc pairwise multiple comparisons for the significant terms of the models were calculated through general linear hypothesis test through the “glht” function and the p -values were adjusted by Tukey’s correction to minimize the family-wise error rates

(Crawley, 2013). The null hypotheses were rejected and thus differences between experimental treatments were considered statistically significant when p -values were below $\alpha = 0.05$, whereas when p -values ranged between 0.05 and 0.1 the null hypotheses were accepted, but differential trends in patterns were acknowledged. All data are reported as mean \pm S.E. Statistical analyses were performed with R software (R core Team, 2021) by using the “geepack” (Halekoh et al., 2006), “nlme” (Pinheiro et al., 2014), “multcomp” (Hothorn et al., 2016) and the default system packages.

3. Results

3.1. Environmental conditions

The mean water temperature in the immersion tanks was 17.09 ± 0.02 °C ($n = 1969$). The mean PAR recorded on the water surface was 126 ± 8 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ($n = 120$), equivalent to 5.6 photons $\text{m}^{-2} \text{d}^{-1}$, which is between the compensation and saturation irradiances for growth of *Z. noltei* (Peralta et al., 2002). Throughout the experiment, the mean concentrations of nitrate, ammonium and phosphate in seawater between 0 and 15 m where the ECIMAT seawater intake is placed (~ 1 km from ECIMAT) were 4.55 ± 0.99 , 1.43 ± 0.22 and 0.43 ± 0.06 $\mu\text{mol L}^{-1}$, respectively ($n = 3$). Temporal trends showed that the concentrations of nitrate increased, whereas those of ammonium decreased and those of phosphate remained stable (Fig. S1, www.intecmar.gal).

Throughout the heatwave during low tide the temperatures and the humidity were stable in the control temperature tank, whereas in the high temperature tank they gradually increased and decreased, respectively, until reaching the highest value of temperature and the lowest of humidity, between 13:00 and 14:00 (Figs. S2–S3, Table S1). In this final emersion hour, mean air temperatures under the high temperature treatment exceeded 37 °C. This temperature was above the threshold defined for atmospheric heatwaves, i.e. 32.7 °C, at the study region (Román et al., 2020). During the emersion period, the mean temperatures, especially in the heatwave treatment, tended to decrease with increasing sediment depth (Fig. S2, Table S1). The dissipation of heat in the sediment (°C) between 0 cm and 5 cm was significantly greater in *Z. noltei* than in bare sand under high temperature treatments (2.37 ± 0.09 vs. 1.98 ± 0.80 , $n = 175$; GEE: $df = 1, 348$, $\chi^2 = 6.12$, $p = 0.01$). The ambient humidity (%) in *Z. noltei* was significantly greater than in bare sand in the control temperature treatment (78.66 ± 0.32 , $n = 175$ vs. 76.75 ± 0.27 , $n = 200$; GEE: $df = 1, 373$, $\chi^2 = 289$, $p < 0.001$), and tended to be greater than in bare sand in the high temperature treatment (56.60 ± 0.90 , $n = 175$ vs. 54.70 ± 0.81 , $n = 200$) (Table S2). Mean differences between *Z. noltei* and bare sand were about a 16% increase in heat dissipation under high temperatures, and a 2% increase in humidity under control temperature.

3.2. Photosynthetic efficiency of seagrass

Before the heatwave, the F_v/F_m values for *Z. noltei* tended to be higher in cores with *R. philippinarum* (0.71 ± 0.01 , $n = 12$) than in cores without clams (0.67 ± 0.02 , $n = 12$). (Fig. 3, Table 1). The F_v/F_m decreased after the heatwave in all *Z. noltei* plants and the values were significantly lower in the high (0.34 ± 0.04) than in the control temperatures (0.55 ± 0.02) ($n = 18$) (Fig. 3, Table 1). After the recovery period, the F_v/F_m values increased under both conditions, although they did not reach the values of D0, and there were still significant differences between high (0.41 ± 0.02) and control (0.60 ± 0.01) temperature treatments ($n = 18$) (Fig. 3, Table 1). After the recovery period, the F_v/F_m values in cores with *R. philippinarum* (0.55 ± 0.04) were significantly higher than in cores without clams (z -value = 3.12, $p < 0.01$) and with *R. decussatus* (z -value = 4.13, $p < 0.001$) (Fig. 3, Table 1), which showed mean F_v/F_m values of 0.49 ± 0.03 and 0.47 ± 0.04 , respectively ($n = 12$). No differences were found between cores with *R. decussatus* and

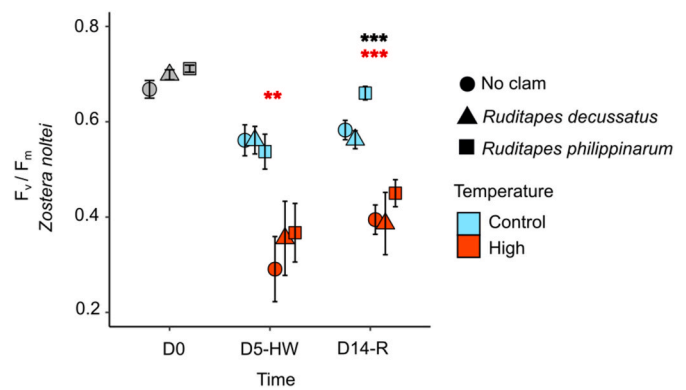


Fig. 3. Mean (\pm S.E., $n = 12$ in D0, $n = 6$ in HW and R) F_v/F_m values for *Z. noltei* leaves in cores without clams, with *R. decussatus*, and with *R. philippinarum*, exposed to control and high temperatures. D0: before the heatwave, D5-HW: after the heatwave, and D14-R: after the recovery period. Red and black asterisks indicate significant differences between temperature and clam treatments, respectively (*** = $p < 0.001$, ** = $p < 0.01$).

without clams (z -value = -0.02 , $p = 0.742$). After the heatwave and after the recovery period, the F_v/F_m in *Z. noltei* showed greater variability under the high than under the control temperature (Fig. 3).

3.3. Clam growth, burrowing depth and sediment reworking

Ruditapes decussatus grew significantly greater than *R. philippinarum* (0.62 ± 0.05 mm, $n = 24$ vs. 0.29 ± 0.03 mm, $n = 20$) and the growth of clams, irrespective of the species, tended to be smaller under the high (0.41 ± 0.05 mm, $n = 21$) than under control temperature (0.52 ± 0.06 mm, $n = 23$) (Fig. 4a, Table 2). Although heat stress negatively affected both functional groups, the F_v/F_m of *Z. noltei* was not correlated with the shell growth of any clam species (Fig. S4).

Ruditapes decussatus burrowed deeper than *R. philippinarum* (4.54 ± 0.15 cm vs. 2.96 ± 0.21 cm, $n = 24$, Table 2). The Habitat \times Temperature interaction tended to have an effect on the burrowing depth (Fig. 4b, Table 2). Clams exposed to high temperatures burrowed on average 1.1 cm deeper in bare sand (4.21 ± 0.41 cm) than in *Z. noltei* (3.11 ± 0.29 cm) ($n = 12$), whereas clams exposed to control temperatures showed similar burrowing depths in both habitats, with a mean difference of 0.5 cm (bare sand: 4.08 ± 0.32 cm, *Z. noltei*: 3.60 ± 0.28 cm, $n = 12$) (Fig. 4b, Table 2).

The profiles showed an exponential decrease in luminophores with depth in all treatments (Fig. S5). On average, the percentage of tracers below 2 cm depth was 1.22% in cores without clams, 4.50% in cores with *R. decussatus* and 3.48% in cores with *R. philippinarum*. The luminophores depth (indicated by MPD) showed a similar pattern to the burrowing depth in all the treatments (Fig. 4b and c) and these variables were positively correlated (Spearman correlation coefficient: $\sigma = 0.66$, $p < 0.001$, $n = 48$). No significant differences were detected in D_b , MPD or vertical displacement (VD) between habitats, temperatures or clams (Fig. 4c–e, Table 3). Nevertheless, the Habitat \times Clam interaction tended to have an effect on the PDC (Table 3). The PDC values (10^{-3} cm year $^{-1}$) of *R. philippinarum* tended to be lower in *Z. noltei* (19.34 ± 1.99) than in bare sand (26.57 ± 3.92) ($n = 12$), whereas the PDC of *R. decussatus* showed similar values between habitats (*Z. noltei*: 26.94 ± 3.38 ; bare sand: 26.82 ± 3.21 , $n = 12$) (Fig. 4f).

3.4. Sediment pore water nutrients

Before the simulated heatwave, the cores with *Z. noltei* had significantly greater concentrations of ammonium and phosphate than cores with bare sand (Fig. 5, Table 4). After the simulated heatwave, in the cores with *Z. noltei* the concentration of nitrate decreased slightly and

Table 1

Fv/Fm values in *Z. noltei*. Summarized results of the generalized least squares models used to test the effects of the fixed factors Clam (3 levels: no clam, *R. decussatus* and *R. philippinarum*), Temperature (2 levels: control and high), and their interaction, with Immersion Tank (2 levels: 1 and 2) as correlation variable. D0: before the heatwave, D5-HW: after the heatwave, and D14-R: after the recovery period. Significant effects are in bold.

Fv/Fm		D0			D5-HW			D14-R		
		df	F	p	df	F	p	Df	F	p
Fixed effects	Clam	2, 33	2.921	0.068	2, 30	0.064	0.938	2, 30	10.160	<0.001
	Temperature	–	–	1, 30	12.358	<0.01	1, 30	25.954	<0.001	
	Temperature x Clam	–	–	2, 30	0.436	0.651	2, 30	0.160	0.853	
Correlation (ρ)	Immersion Tank	3.7·10 ⁻⁸			6.7·10 ⁻¹⁸			2.1·10 ⁻¹⁰		

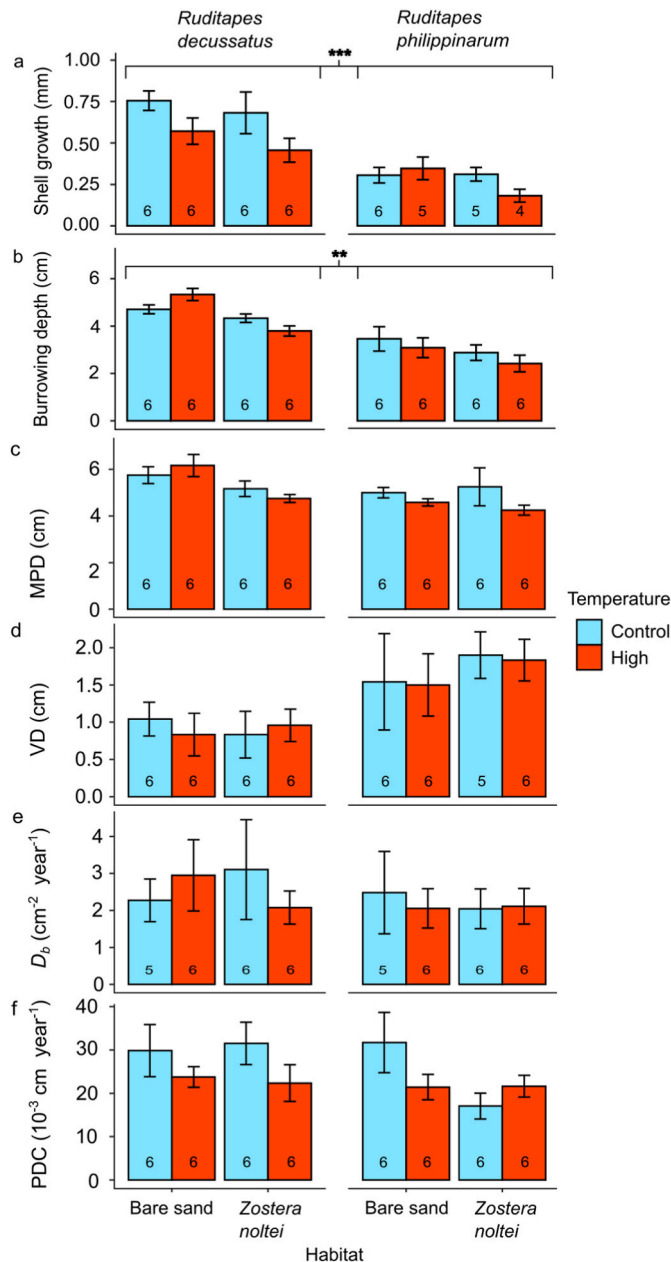


Fig. 4. Mean (±S.E) (a) shell growth, (b) burrowing depth, (c) maximum penetration depth of the luminophores (MPD), (d) vertical displacement (VD), (e) biodiffusion coefficient (D_b), and (f) particle diffusion coefficient (PDC) of *R. decussatus* and *R. philippinarum* in bare sand and in *Z. noltei*, exposed to control and high temperature treatments measured at the end of the experiment (HW + R). The numbers within the bars indicate the number of replicates. Asterisks indicate significant differences between clam species (*** = $p < 0.001$, ** = $p < 0.01$).

the concentration of ammonium also decreased, so that the initial differences between habitats disappeared, whereas the concentration of phosphate was still significantly greater in cores with *Z. noltei* than in cores with bare sand (Fig. 5, Table 4). The presence of clams had a significant effect on the concentration of phosphate, which was greater in cores with *R. philippinarum* than in cores with *R. decussatus* (z-value = 2.77, $p = 0.015$) (Fig. 5). After the recovery period, the concentrations of nitrate and ammonium continued to decrease, especially in *Z. noltei*, whereas the concentrations of phosphate were still significantly greater in cores with *Z. noltei* than in cores with bare sand (Table 4). The factor Clam had a significant effect on the phosphate concentrations, which were greater in cores with *R. philippinarum* than in cores with *R. decussatus* (z-value = 4.95, $p < 0.001$) or cores without clams (z-value = 3.99, $p = 0.002$).

4. Discussion

The study showed that some of the negative effects caused by a heatwave during emersion on the seagrass *Z. noltei* and the clams *R. decussatus* and *R. philippinarum* were ameliorated by the positive interactions driven by the coexistence of both functional groups. After the recovery, the presence of the introduced clam *R. philippinarum* enhanced the photosynthetic efficiency of *Z. noltei* canopies and increased the concentrations of phosphate in the pore water within the seagrass rhizosphere, whereas the native clam did not have any effect. Moreover, the clams below *Z. noltei* tended to burrow shallower than the clams below bare sand, which implies less energy expenditure (Haider et al., 2018). However, such interactions did not prevent the heatwave to cause overall decreasing patterns of growth in the two clam species and a significant decrease of the photosynthetic efficiency in the seagrass. Such effects might be exacerbated in the field where emersion times of *Z. noltei* meadows can be longer than those of our experiment, with a mean of 5 h in Combarro shellfishing bed (unpublished results) and up to 6 h in other regions (Leuschner et al., 1998). There, the occurrence of multiple consecutive heatwave events is also expected, given their predicted increasing frequency in future global warming scenarios (Gómez-Gesteira et al., 2011; Carvalho et al., 2021). However, changes in the interactions between species may happen after longer time periods than the 14 days of our experiment. For example, the growth of *R. decussatus* was smaller in bare sand than below *Z. noltei* after one month under a slight increase of temperature (Román et al., 2022), showing opposite results than those reported in our study. Moreover, previous studies have evidenced seagrass-clams mutualisms under adverse environmental conditions (Cardini et al., 2022; Chin et al., 2021). Therefore, the negative effects of heatwaves during low tide for infaunal bivalves at intertidal seagrass meadows should not be underestimated and need further study.

The photosynthetic efficiency of *Z. noltei* was enhanced by the presence of the clam *R. philippinarum* after the recovery period. The shallower burrowing depth of *R. philippinarum* than of *R. decussatus* may imply a closer interaction with the *Z. noltei* rhizosphere in the former. Indeed, the greater concentrations of PO_4^{3-} in sediment with *R. philippinarum* after the heatwave was probably caused by remineralization of clam excretions (Peterson and Heck, 1999), which could be

Table 2

Shell growth and burrowing depth of clams. Summarized results of the generalized least squares models used to test, at the end of the experiment (i.e. after the recovery period), the effects of the fixed factors Habitat (2 levels: bare sand and *Z. noltei*), Clam (2 levels: *R. decussatus* and *R. philippinarum*), Temperature (2 levels: control and high) and their interactions, with Immersion Tank (2 levels: 1 and 2) as correlation variable. Significant effects are in bold.

		Shell growth			Burrowing depth		
		df	F	p	Df	F	p
Fixed effects	Habitat	1, 36	0.512	0.479	1, 40	0.657	0.422
	Clam	1, 36	19.248	<0.001	1, 40	7.329	<0.01
	Temperature	1, 36	3.217	0.081	1, 40	1.832	0.184
	Habitat x Clam	1, 36	0.283	0.598	1, 40	0.102	0.751
	Habitat x Temperature	1, 36	0.082	0.775	1, 40	3.192	0.082
	Clam x Temperature	1, 36	2.298	0.138	1, 40	2.345	0.134
	Habitat x Clam x Temperature	1, 36	0.356	0.555	1, 40	1.376	0.248
	Correlation (ρ)	Immersion tank		3.4·10 ⁻¹⁸			3.4·10 ⁻¹⁸

Table 3

Maximum penetration depth of the luminophores (MPD), the vertical displacement (VD), the biodiffusion coefficient (D_b) and the particle displacement coefficient (PDC). Summarized results of the generalized least squares models used to test, at the end of the experiment (HW + R), the effects of the fixed factors Habitat (2 levels: bare sand and *Z. noltei*), Clam (2 levels: *R. decussatus* and *R. philippinarum*), Temperature (2 levels: control and high) and their interactions, with Immersion Tank (2 levels: 1 and 2) as correlation variable.

		MPD			VD			D_b			PDC		
		df	F	p	df	F	p	df	F	p	df	F	p
Fixed effects	Habitat	1, 40	1.064	0.309	1, 39	0.167	0.685	1, 38	0.498	0.485	1, 40	0.071	0.792
	Clam	1, 40	1.759	0.192	1, 39	0.960	0.333	1, 38	0.029	0.867	1, 40	0.089	0.768
	Temperature	1, 40	0.542	0.466	1, 39	0.167	0.685	1, 38	0.330	0.569	1, 40	0.950	0.336
	Habitat x Clam	1, 40	1.086	0.304	1, 39	0.587	0.448	1, 38	0.579	0.451	1, 40	3.423	0.072
	Habitat x Temperature	1, 40	1.086	0.304	1, 39	0.213	0.647	1, 38	1.095	0.302	1, 40	0.122	0.729
	Clam x Temperature	1, 40	1.086	0.304	1, 39	0.053	0.819	1, 38	0.437	0.512	1, 40	0.227	0.637
	Habitat x Clam x Temperature	1, 40	0.049	0.826	1, 39	0.120	0.731	1, 38	0.910	0.346	1, 40	2.070	0.158
	Correlation (ρ)	Immersion tank		3.4·10 ⁻¹⁸		3.4·10 ⁻¹⁸		2.2·10 ⁻⁸		3.4·10 ⁻¹⁸			

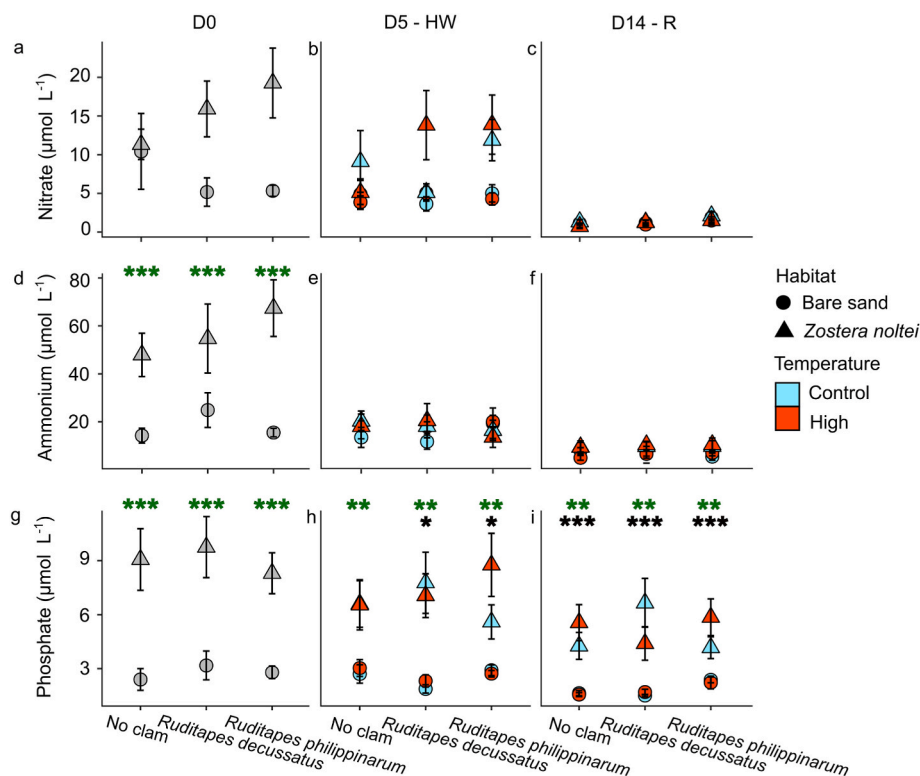


Fig. 5. Mean (\pm S.E, $n = 8-12$ in D0, $n = 4-6$ in HW and R) concentrations of (a - c) nitrate, (d - f) ammonium and (g - i) phosphate measured in cores with bare sand and *Zostera noltei*, without clams, with *Ruditapes philippinarum* and *Ruditapes decussatus*, under control and high temperatures. D0: before the heatwave, D5-HW: after the heatwave, and D14-R: after the recovery period. Green and black asterisks indicate significant differences between habitat and clam treatments, respectively ($*** = p < 0.001$, $** = p < 0.01$).

further assimilated through the seagrass rhizosphere, thus enhancing the plant productivity (Pérez-Lloréns and Niell, 1995; Peterson and Heck, 2001). After the simulated heatwave, the large decrease in F_v/F_m of *Z. noltei* subjected to warming was probably caused by the joint effect of

heat and desiccation, as already observed in *Zostera* spp. (Adams and Bate, 1994; Massa et al., 2009).

The trend of lower shell growth of the clams subjected to high temperatures was probably related to the increasing metabolic costs

Table 4

Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) in pore water at 2 cm depth. Summarized results of the generalized least squares models used to test the effects of the fixed factors Habitat (2 levels: bare sand and *Z. noltei*), Clam (3 levels: no clam, *R. decussatus* and *R. philippinarum*), Temperature (2 levels: control and high) and their interactions, with Immersion Tank (2 levels: 1 and 2) as correlation variable. D0: before the heatwave, D5-HW: after the heatwave, and D14-R: after the recovery period. Significant effects are shown in bold.

			D0			D5-HW			D14-R		
			df	F	p	df	F	P	df	F	p
NO_3^-	Fixed effects	Habitat	1, 59	0.030	0.862	1, 57	0.901	0.347	1, 57	0.320	0.574
		Clam	2, 59	0.538	0.587	2, 57	0.497	0.611	2, 57	1.264	0.290
		Temperature	1, 59	–	–	1, 57	0.238	0.627	1, 57	0.027	0.871
		Habitat x Clam	2, 59	1.842	0.168	2, 57	1.461	0.240	2, 57	0.036	0.965
		Habitat x Temperature	1, 59	–	–	2, 57	0.383	0.538	2, 57	0.784	0.380
		Clam x Temperature	2, 59	–	–	2, 57	0.836	0.439	2, 57	0.181	0.835
		Habitat x Clam x Temperature	2, 59	–	–	2, 57	1.157	0.322	2, 57	0.307	0.737
		Correlation (ρ)	Immersion tank			$-1.1 \cdot 10^{-8}$			$3.2 \cdot 10^{-9}$		
	NH_4^+	Fixed effects	Habitat	1, 62	12.469	<0.001	1, 55	1.370	0.247	1, 55	0.368
Clam			2, 62	0.925	0.402	2, 55	0.958	0.390	2, 55	0.533	0.590
Temperature			1, 62	–	–	1, 55	0.712	0.403	1, 55	1.272	0.264
Habitat x Clam			2, 62	0.898	0.412	2, 55	0.902	0.412	2, 55	0.313	0.733
Habitat x Temperature			1, 62	–	–	1, 55	0.722	0.399	1, 55	0.576	0.451
Clam x Temperature			2, 62	–	–	2, 55	0.255	0.776	2, 55	0.773	0.467
Habitat x Clam x Temperature			2, 62	–	–	2, 55	0.056	0.945	2, 55	0.264	0.769
Correlation (ρ)			Immersion tank			$1.0 \cdot 10^{-8}$			$5.1 \cdot 10^{-18}$		
PO_4^-		Fixed effects	Habitat	1, 66	13.446	<0.001	1, 58	7.840	<0.01	1, 58	11.886
	Clam		2, 66	0.316	0.730	2, 58	4.180	0.020	2, 58	12.268	<0.001
	Temperature		1, 66	–	–	1, 58	0.224	0.638	1, 58	0.221	0.640
	Habitat x Clam		2, 66	0.199	0.820	2, 58	1.343	0.269	2, 58	2.563	0.086
	Habitat x Temperature		1, 66	–	–	1, 58	0.036	0.850	1, 58	1.192	0.279
	Clam x Temperature		2, 66	–	–	2, 58	0.751	0.476	2, 58	0.767	0.469
	Habitat x Clam x Temperature		2, 66	–	–	2, 58	1.383	0.259	2, 58	2.376	0.102
	Correlation (ρ)		Immersion tank			$-7 \cdot 10^{-10}$			$-2.3 \cdot 10^{-9}$		

caused by emersion and heat. When the substrate is emerged, clams close their valves to maintain water within the mantle cavity and reduce their activity and metabolism to minimize oxygen consumption (Ali and Nakamura, 1999; Yin et al., 2017). However, these mechanisms increase hypoxia and decrease the energy reserves in *Ruditapes* spp. (Yin et al., 2017). Additionally, heat stress increases oxygen consumption (Shick et al., 1988; Ali and Nakamura, 1999; Zhou et al., 2022), which reduces the scope for growth of bivalves (Shick et al., 1988; Sobral and Widdows, 1997; Domínguez et al., 2021), and may cause oxidative stress, as observed in intertidal mussels (Olabarria et al., 2016). We did not observe a positive effect of *Z. noltei* on the growth of clams under heat stress, given the short duration of the experiment. However, such effect may be expected in the longer term, as has been already shown (Román et al., 2022).

The lower growth of *R. philippinarum* than of *R. decussatus* contrasted with the growth patterns of adults observed in the field in the longer term (Bidegain and Juanes, 2013). Surprisingly, in bare sand *R. philippinarum* showed a trend towards greater growth at high than at control temperature. The shallower burrowing depth of *R. philippinarum* makes it more vulnerable to emersion stress and anoxia, especially under heat stress (Ali and Nakamura, 1999; Yin et al., 2017; Domínguez et al., 2021). *Ruditapes philippinarum* burrows to shallower depths than *R. decussatus* because its siphon is shorter (3 cm vs. 8 cm, Macho et al., 2016; Nerlović et al., 2016) and it must therefore remain in the upper layers of the substrate to be able to filter-feed (Gosling, 2015). Thus, more frequent heatwaves in a future warmer world may be detrimental for the achievement of commercial size of *R. philippinarum* clams, potentially giving a competitive advantage to the native species, which has experience a sharp drop in catches in recent years (www.pescadega Galicia.gal).

The clams exposed to high temperatures tended to burrow deeper in bare sand than in *Z. noltei* canopies, which may be due to the greater heat attenuation and/or moisture retention below the seagrass, which provided a refuge for clams (Crespo et al., 2017; Román et al., 2022). In fact, the heat dissipation of *Z. noltei* canopies at 5 cm depth in the sediment was 0.4 °C greater than in bare sand, which is consistent with patterns

observed in the rhizosphere of seagrasses (Campbell et al., 2018) and may be driven by their greater water retention capacity (Crespo et al., 2017; Maxwell et al., 2017). A similar effect was also noted for cockles (*Cerastoderma edule*) exposed to simulated heatwaves during periods of emersion and which burrowed deeper below bare sand than below a water layer during low tide (Zhou et al., 2022). The effects of temperature on the burrowing depth observed in the present study could be due to the fact that the response of clams integrated the effect of a heatwave and 9 days of recovery, during which they could move upwards for feeding. These results agree with the lack of effects of temperature in burrowing depths of adult clams after a heatwave with 2 days of recovery (Domínguez et al., 2021).

The values of PDC reworking coefficient tended to be lower in *R. philippinarum* clams below *Z. noltei* than in those below bare sand indicating less lateral displacement of particles and/or less frequent reworking events (Bernard et al., 2014; Capowiez et al., 2021). This is related to the capacity of *Z. noltei* to stabilize the sediment and reduce the bioturbation of benthic communities (Bernard et al., 2014). The luminophores profiles for both clam species evidenced their biodiffusive behaviour, and their D_b coefficients under control temperatures were within the range of values previously measured in e.g. *R. decussatus*, *Venerupis aurea* and *C. edule* (François et al., 1999; Mermillod-Blondin et al., 2004). Nonetheless, the generalist PDC coefficient was a more sensitive indicator of bioturbation in this experiment and can be suitable for future studies assessing the responses of the activity and/or behaviour of juvenile commercial venerid clams to changes in environmental conditions.

The greater concentrations of ammonium and phosphate in pore water in the presence of *Z. noltei* than in bare sand at the start of the experiment is a characteristic feature of seagrass meadows, where the sediments below the canopies accumulate nutrients and prevent their release to the seawater (Maxwell et al., 2017). The temporal decreases in the pore water concentrations of nitrate and ammonium below *Z. noltei* throughout the experiment can be influenced by their uptake by the seagrass, especially of ammonium (Touchette and Burkholder, 2000), and by the denitrification occurring in the increasingly anoxic sediment

during the experiment (see Fig. S6) (Hemminga and Duarte, 2000; Libes, 2009). By contrast, the concentration of phosphate remained moderately stable throughout the experiment because its reactivity in the sediment is only regulated by mineral solubility (Libes, 2009). The temporal variability in pore water and seawater nutrient concentrations were uncoupled throughout the experiment, so that the influence of seawater sources in the pore water patterns was ruled out (Fig. 5, S1).

The lack of any effect of the heatwave treatment on the concentrations of nutrients in pore water is consistent with previous findings of no effects in the water overlying the sediments with *Ruditapes* spp. subjected to a marine heatwave (Crespo et al., 2021) and with natural benthic intertidal communities exposed to mild increases of temperature (Crespo et al., 2017). In addition, the presence of clams only slightly altered the concentrations of nutrients in the sediment, with the exception of the greater phosphate concentrations in sediments with *R. philippinarum*. The lack of a general influence of clams on nitrate and ammonium concentrations in pore water was unexpected, given that the process of bioturbation transports solutes and alters the chemical gradients in marine sediments (Mermillod-Blondin et al., 2004; Norkko and Shumway, 2011; Kristensen et al., 2012). Our findings may be explained by the relatively shallow sampling depth (2 cm), which was less influenced by the excretions and bioturbation of clams, especially of *R. decussatus*.

Given that the effects on the seagrass-bivalve interaction were not particularly strong under the experimental conditions used in this study, similar experiments should incorporate additional improvements. On one hand, sampling the pore waters at deeper depths could reveal changes in the biogeochemistry linked to different burrowing depths of each bivalve species. On the other hand, biochemical analyses of seagrass might help to understand patterns of nutrient assimilation in the presence of clams under heating conditions. As well, the increase of the experimental recovery period would allow testing if past heat stress during emersion had negative legacy effects on the growth and bioturbation of clams or on the photosynthetic efficiency of *Z. noltei*, and if these effects could be buffered by the interactions between clams and seagrass.

5. Conclusions

Our results showed that atmospheric heatwaves can reduce growth in juvenile stages of *R. decussatus* and *R. philippinarum*, what may decrease the densities of adult size commercial clams, and decreased photosynthetic efficiency in intertidal *Z. noltei* canopies, what may lead to smaller growth and cover, with consequent thermal stress in the infauna and release of nutrients into the water column. These ecological effects will be detrimental to the functions and services provided by this socio-ecological system, ranging from decreased landings of commercial size clams and other shellfish species to a degradation of the habitat provided by seagrass. Such processes have important social and economic consequences in NW Spain, especially for women who are the majority of shellfishers on foot in the intertidal shellfish beds, whereas men are the majority of shellfishers on boat in the subtidal. Positive interactions under heat stress included shallower burrowing depths for those clams below *Z. noltei*, which implies less energy expenditure. After recovery, a greater photosynthetic efficiency was observed in *Z. noltei* with *R. philippinarum*, but not with *R. decussatus*. By maintaining or restoring positive interactions between these two functional groups the ecological impacts of thermal stress could be ameliorated and, therefore, its socio-economic consequences mitigated. These findings may encourage further research on the topic and support the development of management actions aimed at enhancing the sustainable coexistence between both functional groups in shellfish beds under global warming scenarios.

CRedit authorship contribution statement

Marta Román: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Franck Gilbert:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – review & editing. **Rosa M. Viejo:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing. **Salvador Román:** Methodology, Investigation. **Jesús S. Troncoso:** Methodology, Investigation. **Elsa Vázquez:** Methodology, Resources, Writing – review & editing, Funding acquisition. **Celia Olabarria:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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