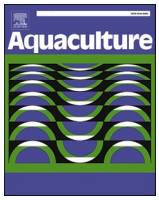
Aquaculture 576 (2023) 739891

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Experimental determination of differential seasonal response in seed of the Manila clam, *Ruditapes philippinarum*, in context of climate change

Eve Galimany [a,*], Arturo Lucas [a,b], Francesc Maynou [a], Montserrat Solé [a], Carles Pelejero [a,c], Montserrat Ramón [a]

[a] Institut de Ciències del Mar, ICM-CSIC, Barcelona, Spain
[b] Institut de Ciència i Tecnologia Ambientals (ICTA-UAB), Universitat Autònoma de Barcelona, 08193, Cerdanyola del Vallès, Barcelona, Spain
[c] ICREA, Institució Catalana de Recerca i Estudis Avançats, Barcelona, Catalonia, Spain

ARTICLE INFO

Keywords:
Bivalve
Temperature
pH
Physiology
Biochemistry

ABSTRACT

Marine bivalves are found as key components of coastal habitats and have several important roles, such as serving as a food source for human beings and aquatic organisms. In fact, as the world's population continues to grow, bivalve aquaculture is expected to increase in importance as a means of addressing demands for animal protein; however, its growth may be possibly compromised by unfavourable climatic conditions. Thus, we assessed the effects of increased water temperature and acidification on the seeds of a bivalve of commercial importance, the Manila clam, *Ruditapes philippinarum*, in order to understand how this species may be affected by climate change at its early life stages. We examined the expected response of clams by experimentally mimicking seasonal conditions that could be forecasted to occur at the end of the twenty-first century. Different physiological responses were measured including growth rates, clearance rate, burrowing time and different biochemical biomarkers of metabolic stress. The results showed that growth decreased in acidic experimental conditions in spring, with a weak interaction with temperature. Clearance rate was negatively affected by a lower pH in spring and summer but, under extreme summer conditions, the effect of pH was overridden by the negative impact of a higher temperature. Burrowing rates were reduced by half under warm temperature conditions in spring and summer. In contrast, biochemical biomarkers were only significantly depicted under climate change conditions in autumn. Overall, this study demonstrates the need to consider seasonality when evaluating the potential effects of climate change on clam aquaculture in order to forecast consequences for biological production.

1. Introduction

Marine bivalves are found as key components of coastal habitats and have many important roles with regard to maintaining a healthy ecosystem including functions related to nutrient coupling and habitat-forming capacity, enhancing coastal resilience. Additionally, bivalves yield a wide array of natural products that are based on the use of meat as well as shells (Smaal et al., 2019). All of these functions have worldwide economic impacts. For example, added-value goods obtained from bivalves are estimated to have an annual market value of $23.9 billion globally, including not only meat destined for consumption, but also ornamental pearls and shells, and components of poultry grit (van der Schatte Olivier et al., 2020). On the other hand, non-food bivalve aquaculture products are worth $6.47 billion ($2.95–9.99 billion) per annum around the globe (van der Schatte Olivier et al., 2020). However, the ability to derive such products from bivalve aquaculture may be compromised by unfavourable environmental conditions occurring as a result of increases in water temperature and acidification of ocean waters. It has been estimated that the detrimental costs of ocean acidification on bivalves could reach over $100 billion by 2100; this value is projected based on the assumption that there will be an increasing demand for cultured molluscs (Narita et al., 2012).

During the twenty-first century, oceans around the world are predicted to increase in water temperature and acidification with the progression of land-based human activities (Bindoff et al., 2019). The trends already observed over the past few decades will be strengthened by future changes; these trends include a global sea surface temperature increase of ~0.9 °C since the beginning of the 20th century (Arias et al.,

* Corresponding author.
E-mail address: galimany@icm.csic.es (E. Galimany).

https://doi.org/10.1016/j.aquaculture.2023.739891
Received 3 March 2023; Received in revised form 11 July 2023; Accepted 12 July 2023
Available online 17 July 2023

2021) and a pH decrease of 0.017–0.027 units per decade across a range of a 15 years-time series of pH observations (Bindoff et al., 2019). However, water temperature increases and pH decreases will reach different values in each area. For example, the Mediterranean region, which represents a hot spot of climate change, temperature is projected to highly rise and precipitation considerably decrease by the end of the 21st century (Calvo et al., 2011; Durrieu de Madron et al., 2011; Paeth et al., 2017). All these environmental changes could hamper the physiological processes of bivalves, which may be related to shell calcification, growth, and immunological responses, also making them more vulnerable to parasites and diseases (Fernández Robledo et al., 2018; Fitzer et al., 2018; Matozzo and Marin, 2011; Tan and Zheng, 2020). The metabolic processes of bivalves may be further compromised by the combination of high temperature and low pH with other stressors such as marine pollution (Maynou et al., 2021).

The Manila clam, *Ruditapes philippinarum* (Adams & Reeve, 1850), is a commercial bivalve species reported to be affected by climate change. This species is native to the Indo-Pacific area but it was brought to Europe for commercial purposes in the 1970s; today it is found all over the world (Coelho et al., 2021). It is a bivalve species with high commercial value and it is by far the most common cultured clam species in the world, with a total catch >4200 t in 2020 (FAO, 2022). *R. philippinarum* may be affected by climate change in different ways. For example, ammonia excretion rate increased when seawater pH was experimentally reduced from 8.0 to 7.7, whereas clearance rate and the ratio of oxygen consumption to nitrogen excretion significantly decreased (Xu et al., 2016). Similarly, *R. philippinarum* reduced the clearance, ingestion and respiration rates and increased the ammonia excretion rate at pH 7.7 and 7.4 (Fernández-Reiriz et al., 2011). However, other studies have reported contradicting results. While Velez et al. (2016) reported that clams were able to maintain their physiological status and biochemical performance at pH 7.8, Range et al. (2011) reported no differences among pH treatments in net calcification or clam growth and weight for *R. decussatus*. These contradicting findings suggest that other factors, such as seasonality, may have affected the physiology of the clams. In this sense, *R. philippinarum* biochemical performance did not change as a result of low contamination levels but as a result of seasonality (Costa et al., 2020a).

The projected temperature increase in the context of global climate change scenarios will be an additional stressing factor to bivalves. When exposed to temperatures 3 °C to 5 °C higher than in ambient temperatures, clams may suffer from thermal stress and reduce scope for growth (Han et al., 2008; Zhang et al., 2019); however, physiological and metabolic adaptative responses may lessen the impact in some species (Velez et al., 2017). When no interactions with other deleterious factors, like pH or emerging contaminants, are present, moderate temperature increases may lead to enhanced growth and reproduction rates in clams and other bivalves, according to the results from mathematical models applied to physiological traits (Costa et al., 2020a; Maynou et al., 2021; Steeves et al., 2018). On larval stages of bivalves, the negative impact of increased temperature may be even more acute than that on juveniles and adults, but evidence from experimental research is still limited (Prado et al., 2016).

The main goal of this study was to understand the potential influence of seasonality on several biological processes related to juvenile clam (*R. philippinarum*) performance under forecasted climate change conditions. For this, the growth, clearance rate, burrowing activity, energy metabolism and detoxification defences of juvenile clams were compared throughout the seasons of the year under actual conditions and those that could be influenced by global and climate change, such as pH decrease and temperature increase.

2. Materials and methods

2.1. Experimental animals

Approximately 1000 seed-size *Ruditapes philippinarum* clams of 6–19 mm total length (TL) and 6–30 mg dry weight (DW) were purchased from Satmar, the Marennes nurseries, France, before each seasonal experiment. Clams were maintained in an open seawater system at the Aquaria and Experimental Chambers (ZAE) of the Institute of Marine Sciences (ICM-CSIC). They were acclimated to each seasonal in situ seawater temperature for one week before the start of each experiment and fed daily a mixture (1:1 in volume) of the phytoplankton species *Tetraselmis* sp. and *Isochrysis galbana* at a concentration of 4×10^3 cells mL^{-1}, similar to other bivalve studies (Peteiro et al., 2018; Velasco, 2007). The phytoplankton species were cultured at an 18:6 h day:night cycle at 23 °C using standard protocols and F/2 media (Creswell, 2010). Mortality was scarce throughout the experiments, as detailed in Table S1.

2.2. Experimental design

The experiments were performed in a controlled temperature chamber at the ZAE. Four experiments lasting four weeks each over different seasons were undertaken, in which clams were exposed to a combination of two temperatures, ambient in each season (corresponding to the coastal mean sea surface temperature in a farming aquaculture area of the Ebre Delta, Western Mediterranean: spring 14.5 °C, summer 20 °C, autumn 18.8 °C and winter 14.4 °C) and ambient in each season +3 °C, and three different pH levels, 8.1, 7.7, and 7.3 (Fig. 1, Table 1). The warming scenario attempted to mimic the expected conditions for sea surface waters under the intensive-emission Representative Concentration Pathway (RCP) 8.5 scenario by 2100 (Bopp et al., 2013; Van Vuuren et al., 2011). The three pH levels were chosen as representatives of surface global oceans at present, at the end of 2100 and at the end of 2300, respectively, also under RCP 8.5 (Bopp et al., 2013; Hartin et al., 2016). We note, however, that pH values between 7.7 and 7.3 may be temporarily reached earlier in coastal areas and shallow tide estuaries, which are typical environments for bivalve cultivation that exhibit much larger pH variability than the open sea (Lowe et al., 2019).

A total of 20 to 50 seed clams were distributed in each 20-L glass aquarium, with three replicates per treatment, and they were fed a phytoplankton mixture of 1:1 *Tetraselmis* sp. and *Isochrysis galbana* (in volume). A peristaltic pump continuously introduced the phytoplankton into the head tank at 3 L h^{-1} to reach a concentration of 4×10^3 cells mL^{-1}. The clams were slowly acclimated to the targeted experimental conditions over 13–15 days by lowering the pH 0.05 units daily. Heaters were used to increase the temperature 1 °C every 4–5 days until the desired final experimental conditions were achieved. Then, the seed clams were exposed for four weeks to ambient seasonal temperatures (control) and + 3 °C (treatment) over the four seasons (Table 1).

HOBO data loggers were used to keep track of the temperature in each treatment, which was controlled and manipulated by means of two temperature baths in which the experimental aquaria were placed, with two electronic Pt100 regulators (Delta Ohm HD9022) connected to 300 W heaters in the +3 °C treatment (Fig. 1). Seawater pH was adjusted by bubbling CO_2 into two overhead tanks that delivered seawater at each of the two acidic pH levels to all aquaria (Fig. 1). This CO_2 delivery was performed in a controlled way by means of an automated system with glass pH electrodes (Ecotrode Plus 6.0262.100, Metrohm) connected to two pH controllers (Consort R362), which opened and closed the solenoid valves of the CO_2 line when needed. To avoid drifts in the pH measurements, glass electrodes were calibrated on a daily basis with TRIS buffer, following standard procedures (SOP6a of Dickson et al., 2007). For further details on the experimental setup for pH and temperature manipulation, see Movilla et al. (2016) and Movilla et al.

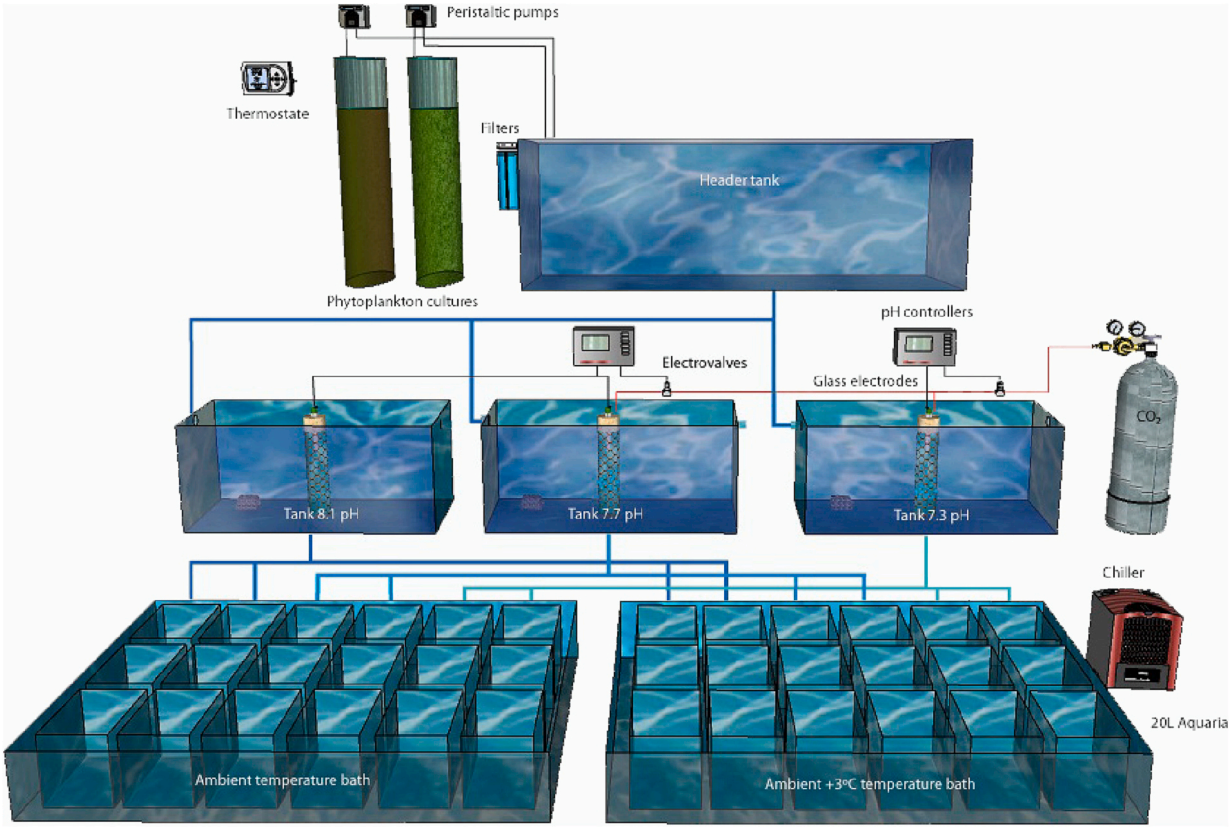


Fig. 1. Diagram of the experimental setup. An open circulating water system was installed in a thermostatic room at the Experimental Aquarium Zone (ZAE) of the ICM-CSIC. Seawater with a concentration of 4×10^3 phytoplankton cells mL^{-1} mixed in the header tank was continuously supplied to 20-L aquaria containing clams. This water was then circulated to three other head tanks where the pH was adjusted using a system of glass electrodes connected to a pH controller, which automatically opened and closed the solenoid electrovalves of the CO_2 line when needed. Water in all aquaria overflowed in the temperature baths. The ambient temperature bath was adjusted to room temperature, and the warm (ambient +3 °C) bath was controlled with an electronic regulator. Note that this set up was designed to study the response of another species in addition to the clams in the experiment presented here, so only half of the aquaria were used in the experiments with *Ruditapes philippinarum*.

(2012). In addition to the pH control using glass electrodes, approximately once a week, seawater samples from each treatment were taken to the laboratory for total alkalinity analyses by potentiometric titration (Perez and Fraga, 1987; Pérez et al., 2000) and determination of pH by spectrophotometry (Clayton and Byrne, 1993), which provides more precise pH measurements than glass electrodes. At the same time, the temperature and salinity of each treatment aquarium were also monitored using a YSI-30 M probe and used to calculate the rest of the parameters of the carbonate system in seawater using the CO2SYS Excel add-in (Pierrot et al., 2006) with dissociation constants for carbonate determined by Mehrbach et al. (1973) and refit by Dickson and Millero (1987). The physicochemical parameters of both treatments during the experiment are shown in Table 1. To minimize potential pseudoreplication issues, weekly shifts in the tubes and the three head tanks delivering seawater from each of the pH treatments were performed, so all aquaria received sequential seawater from all tanks throughout the experiment.

For these four experiments, the following variables were measured: growth, clearance rate and burrowing behaviour as physiological variables and some selected biochemical biomarker determinations related to oxidative stress and metabolism. All clams were measured at the beginning and end of the experiments. Then, each clam was only used to obtain measurements for one of the remaining variables, i.e., clearance rate, burrowing or biomarker determinations.

2.3. Growth

All clams were measured (TL) with a Vernier calliper and weighed (± 0.001 g) before the seasonal experiments to ensure that sizes were equally distributed among the replicated aquaria in each treatment. At the end of the 4-week experiments, all clams, that is, between 20 and 50 clams in each aquarium x 3 replicates per treatment x 6 treatments × 4 seasons, were measured and weighed again to compare the increase in mean size among treatments.

2.4. Clearance rate

The clearance rate, i.e., the volume of water cleared by clams per unit of time (Lh^{-1}) was estimated by quantifying the decrease in algal cell density from the experimental water. Three clams, one from each replicate aquaria corresponding to the same combination of temperature and pH, were placed at the bottom of 50-mL test tubes, which were previously perforated with three holes on the base and three longitudinal openings all around the tube, allowing water to pass through (Fig. S1). The clams in the tube were immediately placed in a 250-mL glass bottle with 200 mL of experimental water from the aquaria. The test tubes with clams were maintained elevated and stable in the glass bottle using tin wire to allow the placement of a 2-cm magnet underneath the test tube. The bottles were then placed on a multi-position magnetic stirring plate to ensure resuspension of particles. Five bottles containing three clams each were used for each treatment, i.e., the combination of each of the two temperatures with each of the three pH

Table 1
Parameters of the seawater carbonate system in aquaria for each treatment corresponding to spring (A), summer (B), autumn (C) and winter (D).

Spring treatment		Measured parameters			Calculated parameters						
Temperature	pH	T	pH_T	Alk	pCO_2	DIC	$[CO_2]_{aq}$	$[HCO_3^-]$	$[CO_3^{2-}]$	Ω_C	Ω_A
A											
Ambient (natural cycle)	8.1	14.5 ± 0.4	8.042 ± 0.022	2550 ± 1	441 ± 27	2299 ± 11	16.4 ± 0.9	2101 ± 16	182 ± 7	4.24 ± 0.15	2.73 ± 0.10
	7.7	14.5 ± 0.4	7.722 ± 0.036	2547 ± 1	1020 ± 91	2444 ± 14	38.0 ± 3.3	2310 ± 19	96 ± 7	2.24 ± 0.17	1.44 ± 0.11
	7.3	14.5 ± 0.4	7.393 ± 0.059	2548 ± 3	2315 ± 350	2563 ± 21	86 ± 12	2429 ± 14	48 ± 6	1.12 ± 0.13	0.72 ± 0.09
Warm (+3 °C)	8.1	17.9 ± 0.5	8.015 ± 0.004	2547 ± 1	473 ± 6	2282 ± 2	15.9 ± 0.1	2074 ± 4	192 ± 2	4.48 ± 0.04	2.90 ± 0.03
	7.7	17.9 ± 0.5	7.736 ± 0.044	2557 ± 1	1000 ± 110	2427 ± 21	33.9 ± 4.2	2281 ± 29	112 ± 12	2.62 ± 0.28	1.70 ± 0.18
	7.3	17.8 ± 0.7	7.352 ± 0.063	2564 ± 2	2624 ± 430	2579 ± 22	88.3 ± 13	2441 ± 15	50 ± 6	1.16 ± 0.14	0.75 ± 0.09
B											
Ambient (natural cycle)	8.1	20.2 ± 0.5	8.034 ± 0.007	2523 ± 10	441 ± 10	2229 ± 16	14.1 ± 0.5	2004 ± 20	210 ± 5	4.92 ± 0.12	3.21 ± 0.08
	7.7	20.0 ± 0.5	7.728 ± 0.028	2542 ± 1	1014 ± 70	2404 ± 10	32.2 ± 2.0	2255 ± 14	117 ± 6	2.73 ± 0.14	1.78 ± 0.09
	7.3	20.0 ± 0.5	7.373 ± 0.006	2533 ± 6	2431 ± 33	2531 ± 7	77.2 ± 1.8	2399 ± 7	55 ± 1	1.28 ± 0.03	0.84 ± 0.02
Warm (+3 °C)	8.1	22.8 ± 0.6	7.996 ± 0.006	2526 ± 13	492 ± 11	2230 ± 19	14.5 ± 0.5	2004 ± 23	212 ± 5	4.97 ± 0.12	3.27 ± 0.08
	7.7	23.1 ± 0.5	7.650 ± 0.005	2534 ± 6	1236 ± 18	2412 ± 9	36.2 ± 1.0	2266 ± 11	110 ± 3	2.56 ± 0.07	1.69 ± 0.05
	7.3	23.0 ± 0.5	7.348 ± 0.018	2551 ± 24	2642 ± 130	2545 ± 32	77.6 ± 5.0	2409 ± 30	58 ± 3	1.36 ± 0.07	0.89 ± 0.05
C											
Ambient (natural cycle)	8.1	18.7 ± 0.7	8.072 ± 0.001	2562 ± 5	406 ± 1	2254 ± 3	13.4 ± 0.3	2021 ± 8	220 ± 5	5.13 ± 0.13	3.33 ± 0.09
	7.7	18.8 ± 0.8	7.770 ± 0.014	2547 ± 7	907 ± 37	2398 ± 9	29.8 ± 0.6	2247 ± 8	122 ± 1	2.84 ± 0.01	1.85 ± 0.01
	7.3	18.8 ± 0.9	7.403 ± 0.018	2544 ± 12	2257 ± 80	2536 ± 2	74.3 ± 4.3	2405 ± 2	56 ± 4	1.32 ± 0.10	0.86 ± 0.06
Warm (+3 °C)	8.1	22.2 ± 0.1	8.019 ± 0.007	2561 ± 24	469 ± 14	2253 ± 21	14.0 ± 0.2	2019 ± 18	221 ± 3	5.17 ± 0.08	3.39 ± 0.06
	7.7	21.7 ± 0.6	7.731 ± 0.001	2562 ± 11	1015 ± 32	2411 ± 11	30.8 ± 0.5	2256 ± 10	125 ± 1	2.92 ± 0.01	1.91 ± 0.01
	7.3	21.9 ± 0.3	7.373 ± 0.018	2556 ± 6	2478 ± 101	2546 ± 2	74.9 ± 3.6	2412 ± 1	59 ± 3	1.38 ± 0.07	0.90 ± 0.05
D											
Ambient (natural cycle)	8.1	14.4 ± 0.2	8.055 ± 0.004	2570 ± 11	428 ± 4	2311 ± 8	16.0 ± 0.1	2108 ± 7	187 ± 2	4.37 ± 0.04	2.81 ± 0.03
	7.7	14.4 ± 0.2	7.686 ± 0.034	2579 ± 10	1129 ± 92	2489 ± 2	42.1 ± 3.2	2357 ± 5	90 ± 6	2.10 ± 0.15	1.35 ± 0.09
	7.3	14.4 ± 0.2	7.353 ± 0.015	2576 ± 14	2530 ± 100	2606 ± 19	94.4 ± 3.8	2468 ± 16	44 ± 1	1.02 ± 0.03	0.65 ± 0.02
Warm (+3 °C)	8.1	17.8 ± 0.3	8.010 ± 0.004	2559 ± 4	482 ± 5	2296 ± 6	16.3 ± 0.1	2089 ± 6	190 ± 1	4.45 ± 0.02	2.88 ± 0.01
	7.7	17.8 ± 0.4	7.638 ± 0.037	2565 ± 17	1280 ± 110	2475 ± 5	43.1 ± 3.2	2341 ± 3	91 ± 6	2.13 ± 0.15	1.38 ± 0.09
	7.3	17.8 ± 0.4	7.314 ± 0.012	2558 ± 12	2805 ± 93	2587 ± 17	94.8 ± 3.4	2447 ± 15	45 ± 1	1.05 ± 0.03	0.68 ± 0.02

T (temperature, °C), pH_T (pH, in total scale), Alk (total alkalinity, μmol/kg-SW) and salinity (which was always 38.0) were used to calculate pCO_2 (partial pressure of CO_2, μatm), DIC (dissolved inorganic carbon, μmol/kg-SW); $[CO_2]$aq (CO_2 concentration in seawater, μmol/kg-SW), $[HCO3^-]$ (bicarbonate ion concentration, μmol/kg-SW), $[CO_3^{2-}]$ (carbonate ion concentration, μmol/kg-SW), Ω_C (calcite saturation state) and Ω_A (aragonite saturation state), using the CO2SYS Excel add-in (Pierrot et al., 2006) with dissociation constants for carbonate determined by Mehrbach et al. (1973) and refit by Dickson and Millero (1987). All parameters are expressed as the mean ± SE.

values. Before the beginning of the experiments, 2 mL of 1:1 *Tetraselmis* sp.:*Isochrysis galbana* was added to each glass bottle to obtain a final concentration of 4×10^3 cells mL^{-1}. One extra bottle was left as a control to detect changes in the water particle concentration. Immediately after phytoplankton addition, samples (1.8 mL) were pipetted every 5 min, starting at time 0 and continuing for 30 min, after which time samples were taken every 10 min until 1 h had passed. All collected samples were preserved in 1% formalin (final concentration) until analysis. At the end of the experiment, bivalves were dissected, and their soft tissue was dried at 60 °C for 48 h to determine individual dry tissue weight. To calculate the phytoplankton remaining in the bottles, water samples were run on a BD Biosciences Flow Cytometer (San Jose, California) for 2 min on the fast flow rate setting (35 μl min^{-1}). The phytoplankton species were identified and quantified using the contrasting side scatter vs. green fluorescence plots produced by the flow cytometer. Then, the clearance rate was determined using the formula

(Riisgård, 2001):

$$CR = (V/n_t)\ ln\ (C_0/C_t)$$

where C_0 and C_t are the algal concentrations (cells mL^{-1}) at time 0 and time t, respectively, V is the volume of water, and n_t is the number of individual clams (three per bottle). All CR values were standardized to 1 g of dried clam flesh using the following equation:

$$CRs = CR\ x\ (1/W_e)^b$$

where CRs is the standardized clearance rate, CR is the experimentally determined clearance rate, W_e is the average dry body mass measured for the clams in each bottle, and b is a predetermined constant feeding rate. We used a value of b = 0.8 determined by Riisgård (1988) for the clam *Mercenaria mercenaria*, as no estimates for *R. philippinarum* were found in the literature.

For the clearance rate parameters, an additional fifth experimental setup was included to assess the influence of extreme summer conditions, mimicking temperatures (> 28 °C) that have actually been recorded in the area (Fernández-Tejedor et al., 2022; Galimany et al., 2011). This is a suspected temperature where the physiology of bivalves might be greatly compromised (Galimany et al., 2011). Thus, this relevant physiological parameter was studied under the temperatures of 25 °C (as ambient) and 28 °C (extreme summer) for the three different pH levels (8.1, 7.7, and 7.3) as for the other experiments.

2.5. Burrowing behaviour

To assess the burrowing activity, aquaria were filled with 500 g of dry sediment (depth 3–4 cm) collected from a clam bed. The sediment was previously calcinated to eliminate all organic matter. When the sediment settled and no resuspension was observed in the aquaria (approximately 24 h later), burrowing bioassays were conducted by placing 15 clams of similar shell lengths over the sediment at each aquarium. Three replicate aquaria were used for each combination of temperature and pH. Clam behaviour was continuously recorded by an observer per aquarium, and the burrowing time was determined as the time that elapsed from the start of digging until the time the clam fully disappeared from the sediment surface.

2.6. Biomarker determinations

Biochemical determinations were conducted with the whole soft tissue of two sets of three clams for each aquarium for a total of 6 individuals. Soft tissue was immediately and individually frozen at −80 °C until analyses. Before biomarker determinations, soft tissue was homogenized at a 1:5 ratio (weight:volume) using a 50 mM phosphate buffer at pH 7.4 containing 1 mM ethylenediaminetetraacetic acid (EDTA). Homogenization was carried out using an electrically driven Polytron® homogenizer. All analytical determinations were carried out on the supernatant resulting from a 10,000 *g* centrifugation for 30 min at 4 °C, corresponding to the post-mitochondrial fraction (S10) and containing most of the cytosol soluble enzymes.

All assays were carried out in triplicate at 25 °C in 96-well plates using a TECAN Infinite M200 microplate reader, and blanks (sample-free) accompanied the sample batches to correct for non-enzymatic reactivity of the substrates. Some purified proteins were included in the readings as methodological controls (Solé et al., 2020). All enzymatic activities are expressed in nmol min^{-1} mg^{-1} of protein.

2.6.1. Esterase activities

Carboxylesterase (CE; EC 3.1.1.1) activity was measured using the commercial colorimetric substrates *p*-nitrophenyl acetate (pNPA) and *p*-nitrophenyl butyrate (pNPB). The hydrolysis rate of pNPA and pNPB was determined by a continuous spectrophotometric enzyme assay following Hosokawa and Satoh (2005).

The kinetic assay was performed in a 50 mM phosphate buffer (pH = 7.4) containing 1 mM (final concentration) of the respective substrates and 25 μL of sample (S10). The formation of 4-nitrophenolate was monitored spectrophotometrically at 405 nm at 25 °C for 5 min. An extinction coefficient of 18 mM^{-1} cm^{-1} was used to express the hydrolysis of these nitrophenyl esters (Hosokawa and Satoh, 2005).

2.6.2. Antioxidant defences

Glutathione reductase (GR; EC 1.8.1.10) activity was measured using 1 mM glutathione disulphide (GSSG), 0.5 mM NADPH and 20 μL of sample. The absorbance decrease was measured using a microplate reader for 3 min at 340 nm ($\varepsilon = 6.22\ mM^{-1}\ cm^{-1}$) following the protocol of Carlberg and Mannervik (1985).

Glutathione S-transferase (GST; EC 4.4.1.20) activity was quantified using a reaction mixture containing 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), 1 mM reduced glutathione (GSH) and 25 μL of sample. Measurements were carried out for 3 min at 340 nm ($\varepsilon = 9.6\ mM^{-1}\ cm^{-1}$) following the protocol of Habig et al. (1974).

2.6.3. Metabolic activity

Electron transport system (ETS) activity was measured following the method of King and Packard (1975) using p-iodonitrotetrazolium chloride as a substrate (0.6 mM). The absorbance was read at 490 nm for 5 min. The amount of formazan formed was calculated using $\varepsilon = 15.9\ mM^{-1}\ cm^{-1}$.

2.6.4. Protein determination

The total protein content was determined by the Bradford method (Bradford, 1976) adapted to microplate readers using the Bradford Bio-Rad Protein Assay reagent and bovine serum albumin (BSA) as a standard (0.05–1 mg mL^{-1}). Absorbance was read at 595 nm.

2.7. Statistical analysis

Values of growth (log transformed), standard clearance rate and biomarker determinations were analysed seasonally using a generalized linear model (GLM) against the environmental variables temperature, pH, and the interaction temperature × pH. Burrowing behaviour was analysed statistically with a Kaplan–Meier survival model for censored data, where individuals were assigned a value of 0 while on the surface or 1 when burrowed. The survival curves determined under each experimental condition were tested for differences with the G-rho statistic. Analyses of the burrowing data were carried out with the R library "survival" (Therneau, 2016).

3. Results

3.1. Growth

The growth of clams showed different trends throughout the studied period. The initial average (± SE) length and wet weight of the shell of clams per season were 8.94 ± 0.72 mm and 0.17 ± 0.04 g in spring, 14.14 ± 1.29 mm and 0.61 ± 0.16 g in summer, 13.54 ± 0.85 mm and 0.48 ± 0.10 g in autumn, and 13.74 ± 0.72 mm and 0.50 ± 0.08 g in winter, respectively.

Clam length and weight at the end of the experiment did not differ among treatments in summer, autumn, and winter (Table S1). The average (± SE) lengths and wet weights of the shells were 14.11 ± 0.07 mm and 0.63 ± 0.01 g, 13.56 ± 0.05 mm and 0.51 ± 0.01 g, and 14.52 ± 0.08 mm and 0.59 ± 0.01 g in summer, autumn, and winter, respectively. However, in spring, both final length and weight were significantly higher in the experimental tanks at pH 8.1 than at pH 7.7 and 7.3 (lengths: 9.77 ± 0.06 mm, 9.57 ± 0.06 mm and 9.47 ± 0.06 mm, respectively, $p < 0.0001$; weights: 0.222 ± 0.013 g, 0.209 ± 0.012 g, 0.205 ± 0.012 g, respectively, $p < 0.0001$) (Fig. 2). There was also a significant effect of the temperature × pH interaction ($p = 0.053$ for

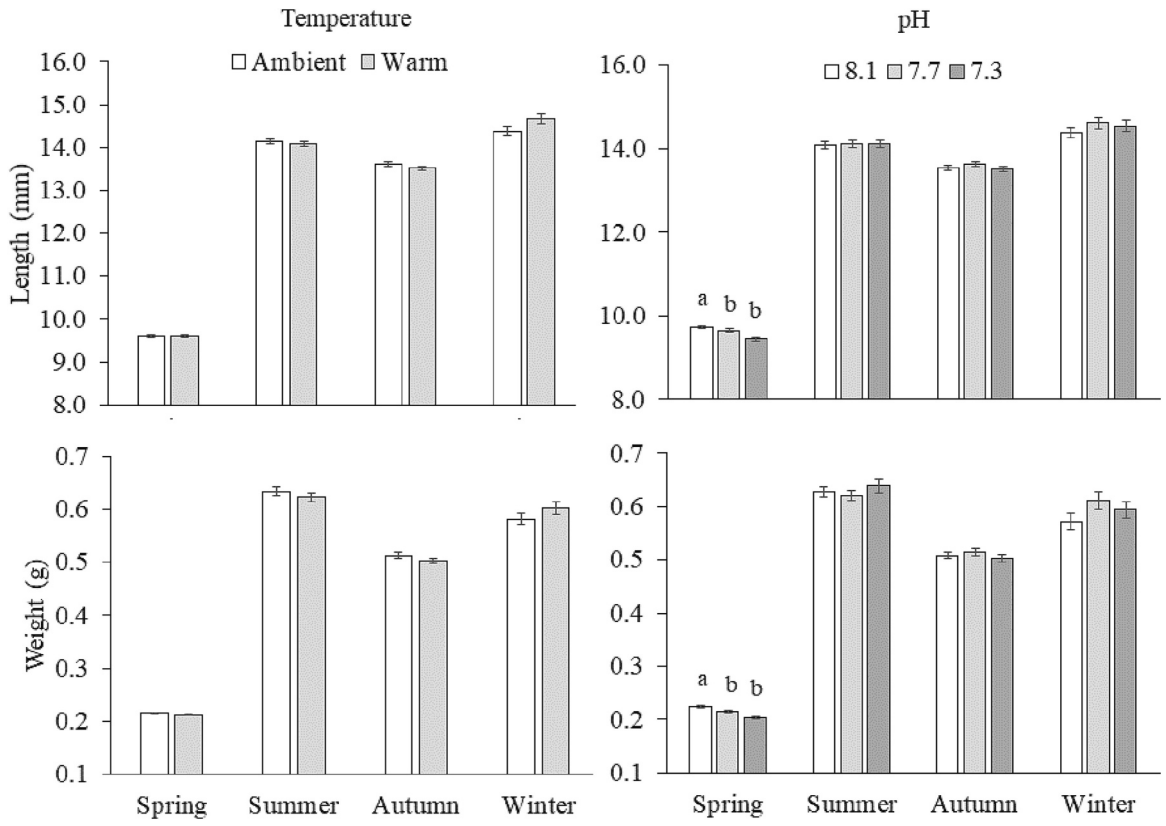


Fig. 2. Average length and weight of clams at the end of each seasonal experiment plotted by temperature and pH separately. Letters denote significant differences ($p < 0.05$). The experimental ambient temperature conditions were as follows: spring 15 °C, summer 23 °C, autumn 18 °C, and winter 14 °C, with the "warm" treatment consisting of increasing the ambient temperature by 3 °C.

length; $p = 0.025$ for weight) in spring, indicating that growth at pH 7.7 was greater at ambient temperature than under warmer water conditions.

3.2. Clearance rate

Clearance rates varied from 0.4 to 1.9 L h^{-1} throughout all the experiments, as detailed in Table 2. When comparing clearance rates between the two different temperatures tested in each season, no significant differences were found; thus, clearance rates did not vary according to temperature throughout the four seasons. However, there were significant differences between pH levels in spring ($F_{2,24} = 3.839$, $p = 0.036$) and summer ($F_{2,24} = 6.711$, $p = 0.006$), showing higher clearance rates at pH 8.1 and lower clearance rates at pH 7.3 (Fig. 3). For the summer season, there was also an interaction between temperature and pH ($F_{2,24} = 3.640$, $p = 0.044$); a main effects test revealed that clearance rates at pH 8.1 and warm treatment were higher than those at the same temperature but in more acidic water. No differences among treatments were recorded for clearance rates in autumn and winter ($p > 0.05$). Nonetheless, when clams were exposed to extreme summer temperatures (i.e., 24 °C and 27 °C), clearance rates were lower at the highest temperature ($F_{1,24} = 7.485$, $p = 0.012$), while no differences were found among pH values or for the interaction of both parameters (Fig. 4).

Table 2
Average standard clearance rates (± SE) for clams during each season, as observed under extreme temperature conditions and experimental conditions, e.g., two water temperatures and three pH values. Ambient temperature and pH 8.1 are considered to be current (control) conditions.

	pH 8.1		pH 7.7		pH 7.3	
	Ambient	Warm	Ambient	Warm	Ambient	Warm
Spring	1.80 ± 0.43	1.91 ± 0.36	1.28 ± 0.17	1.35 ± 0.16	1.09 ± 0.10	1.27 ± 0.13
Summer	1.50 ± 0.13	1.63 ± 0.19	1.50 ± 0.08	0.89 ± 0.09	1.13 ± 0.18	0.98 ± 0.12
Autumn	1.05 ± 0.10	1.46 ± 0.19	1.99 ± 0.60	1.74 ± 0.38	1.09 ± 0.17	1.41 ± 0.27
Winter	0.96 ± 0.30	0.70 ± 0.10	0.64 ± 0.06	0.40 ± 0.07	0.43 ± 0.16	0.49 ± 0.16
Extreme summer	1.55 ± 0.20	1.39 ± 0.29	1.89 ± 0.35	0.96 ± 0.17	1.47 ± 0.21	0.86 ± 0.27

3.3. Burrowing behaviour

Burrowing activity was always faster under ambient temperature in spring and summer. The time needed for 50% of the clams to burrow (Table 3) ranged between 11.30 and 16.20 min in spring and 8.67 and 11.75 min in summer under ambient temperature. In both seasons, increasing 3 °C significantly slowed the time to 50% of the clams to burrow (17.90–21.50 min in spring and 12.67–21.67 min in summer), with G-rho values of 21 (1 d.f.) and 24 (1 d.f.) in spring and summer, respectively, with $p < 0.001$. In both seasons, pH was not significant (G-rho with $p > 0.05$). The burrowing performance showed no significant differences among treatments in autumn and winter ($p > 0.05$), neither for temperature nor pH (Table 3).

3.4. Biomarker determinations

All biomarker determinations showed no significant differences among treatments in spring, summer, or winter ($p > 0.05$) (Supplemental Figs. S2, S3, S4). However, in autumn, CE activities were different among pH values for pNPA-CE measures ($F_{2,30} = 4.445$, $p = 0.020$) and between temperatures when using pNPB as substrate ($F_{1,30}$

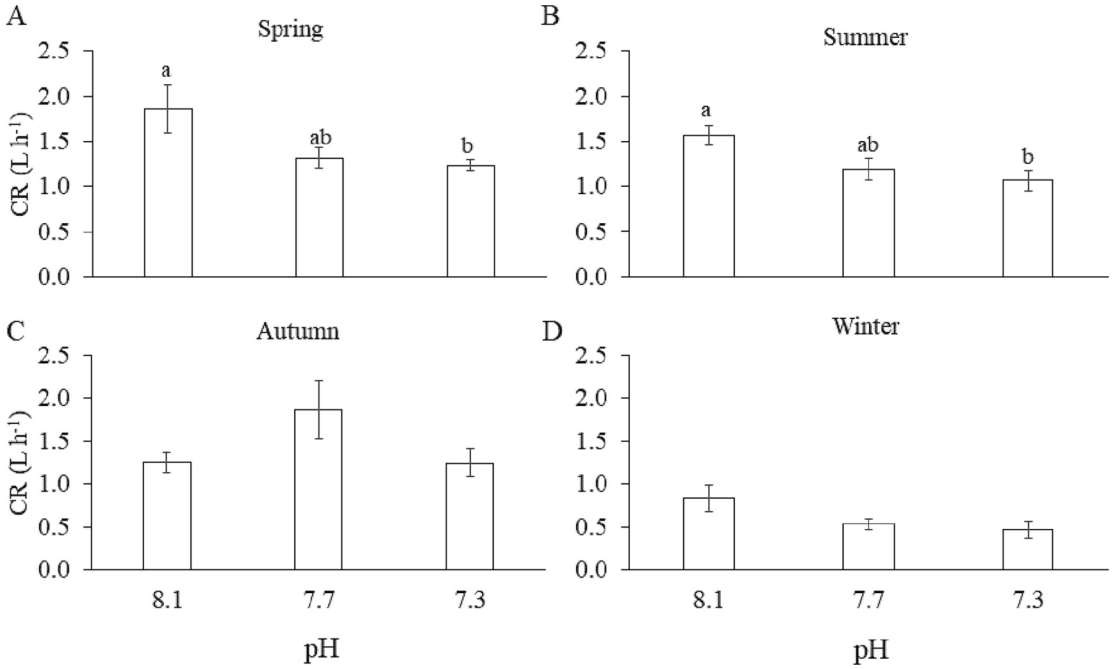


Fig. 3. Seasonal clearance rates (average CR ± SD) estimated at each different experimental pH value (horizontal axis), pooling temperature treatment data. A) spring; B) summer; C) autumn; D) winter. Lowercase letters denote significant differences ($p < 0.05$).

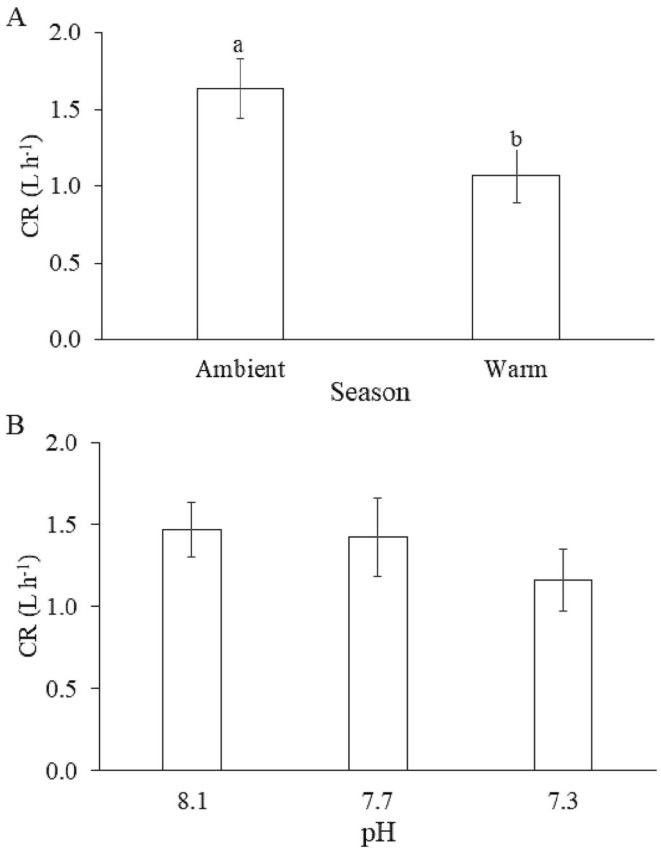


Fig. 4. Clearance rates (average CR ± SD) estimated under extreme summer conditions for both parameters measured, temperature (A) and pH (B). Lowercase letters denote significant differences between the ambient and warm treatments.

Table 3
Time to complete burrowing of 50% of the clam population (in minutes, with 95% confidence intervals) under experimental conditions.

Season	Temperature	pH	50%	95% CI
Spring	ambient	8.1	11.30	(09.00–16.50)
		7.7	16.10	(14.20–20.70)
		7.3	16.20	(11.30–18.30)
	warm	8.1	20.60	(16.70–28.70)
		7.7	21.50	(17.20–29.20)
		7.3	17.90	(16.00–20.70)
Summer	ambient	8.1	11.75	(07.83–17.70)
		7.7	8.67	(07.00–13.80)
		7.3	10.67	(08.50–16.80)
	warm	8.1	19.50	(16.33–24.70)
		7.7	21.67	(18.83–25.60)
		7.3	12.67	(12.00–21.00)
Autumn	ambient	8.1	17.00	(11.50–27.84)
		7.7	12.90	(11.20–26.00)
		7.3	16.20	(11.50–23.80)
	warm	8.1	19.30	(15.00–29.20)
		7.7	11.70	(09.50–28.80)
		7.3	20.30	(12.20–28.30)
Winter	ambient	8.1	15.10	(10.80–21.50)
		7.7	26.80	(21.50–33.50)
		7.3	15.80	(13.00–22.20)
	warm	8.1	17.80	(16.00–28.20)
		7.7	16.80	(14.00–23.80)
		7.3	17.20	(14.30–29.20)

= 9.526, $p = 0.004$). The warmer and more acidic treatments displayed higher enzymatic values (Fig. 5). Both antioxidant defences, GR and GST activities, were also higher in autumn at low pH ($F_{2,30} = 7.940$, $p = 0.002$ and $F_{2,30} = 4.059$, $p = 0.028$, respectively) (Fig. 5). In contrast, the metabolic activity, i.e., ETS, indicative of mitochondrial activity, displayed the opposite response.

A good Pearson correlation coefficient was found among biomarker responses considering the totality of the data ($n = 143$). That is, the antioxidant GR was positively correlated with GST ($r = 0.560$; $p < 0.001$), pNPA-CE ($r = 0.336$; $p < 0.001$) and pNPB-CE ($r = 0.277$; $p = 0.001$). Likewise, GST was correlated with CE measures with pNPA ($r =$

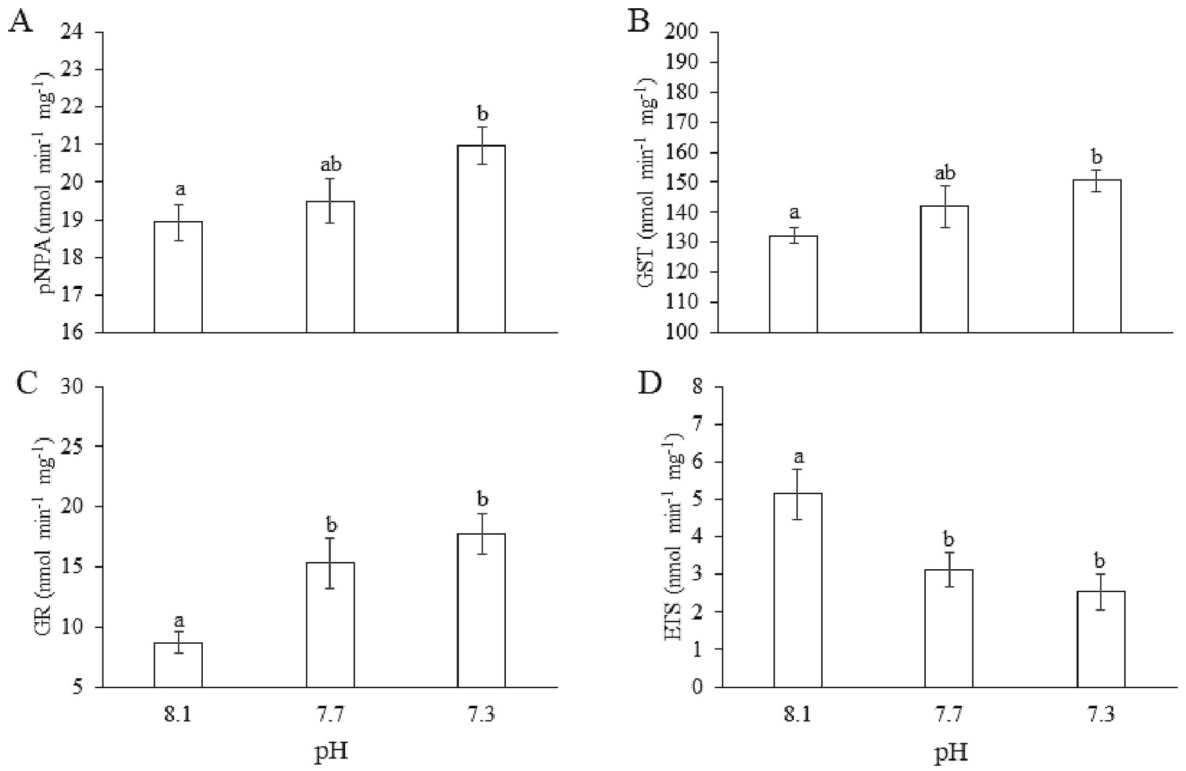


Fig. 5. Enzymatic activities (in nmol min^{-1} mg^{-1} prot) of whole clam (*R. philippinarum*) soft tissue during the autumn experiment as a function of water pH. A) Carboxylesterase phase I measures with the substrate pNPA, B) conjugative phase II glutathione S-transferase (GST) activity, C) antioxidant enzyme glutathione reductase (GR) activity and D) mitochondrial electron transport system (ETS) as an energy metabolism activity marker. The horizontal axis indicates pH treatments.

0.456; $p < 0.001$) and pNPB ($r = 0.397$; $p < 0.001$). In turn, pNPA-CE and pNPB-CE were highly correlated ($r = 0.796$, $p < 0.001$). In contrast, ETS activity was negatively correlated with most parameters: GR ($r = -0.718$; $p < 0.001$) and GST ($r = -0.204$; $p = 0.15$) as well as with pNPA-CE ($r = 0.252$; $p = 0.002$) and pNPB-CE ($r = 0.232$; $p = 0.005$).

4. Discussion

The expansion of aquaculture is needed to feed the growing world's population, but the sector is currently concerned about sustainability and the effects of climate change on aquaculture production (Maulu et al., 2021). In recent decades, many efforts have been made to understand the effects of climate change on aquatic populations, but seasonality is not included in most of the studies (Kwiatkowski and Orr, 2018). The life cycle of most marine organisms in temperate climates is strongly influenced by seasonality, which affect the reproductive cycle and the availability of the trophic resources (Jung et al., 2019). Moreover, seasonality has been determined to influence clam (*R. philippinarum*) performance under physical and chemical exposures (Costa et al., 2020a). In this context, our study provides a novel comprehensive experimental approach aimed at investigating the response of this clam species to forecasted temperature and pH changes brought on by climate change. By focusing on clam seeds reared under different seasonal conditions, we were able to assess the extent of the changes seen throughout the year and pinpoint the most sensitive period. Changing environmental parameters have the potential to greatly influence physiological responses associated with feeding behaviour in bivalves. Growth rates, which are of key importance in aquaculture operations, were only significantly decreased in the spring experiment, due to decreases in pH, while a 3 °C change in temperature was found to be not relevant. Likewise, clearance rates decreased in clams exposed to pH 7.4 (Fernández-Reiriz et al., 2011; Xu et al., 2016) similar to what was seen in our study during spring and summer. Even though the temperatures were similar, other seasons (summer and autumn) did not follow this pattern. This may be because spring and summer are particularly vulnerable periods for clams, which exhibit higher growth rates and adult animals may develop their gonads (Ojea et al., 2004). The experimental clams may have been ripe despite their small size, as previously observed (Range et al., 2011). In *R. philippinarum*, temperature may also cause variation in clearance rates, which typically rise with increasing temperatures (Han et al., 2008; Kang et al., 2016). However, the 3 °C temperature difference between the ambient and warm treatments used in our experiments might not be enough to allow for the detection of changes in this physiological parameter. The clearance rates of clams from the ambient temperature and pH 8.1 treatment were higher in spring, slightly lower in summer, and they were the lowest in winter, the coldest season. This pattern may be associated with the temperature gradient, given that clearance, filtration and absorption rates for *R. philippinarum* increase with temperature up to the threshold maximum (Bo et al., 2000). Clams may adapt physiologically to low pH within limited bounds; for example, despite temperature increases of up to 20 °C and pH values as low as 7.4 may alter clearance rates, food absorption efficiency may remain constant (Xu et al., 2016). The clams may, however, be physiologically compromised by temperatures above their thermal optimum, which are estimated to be between 20 °C and 22 °C (Bo et al., 2000; Han et al., 2008). In our experiments, under extreme summer conditions (28 °C), the effect of temperature was found to override any influence of the pH decrease, when food absorption may have been altered.

The ecological relevance of the effects of climate change on bivalve burrowing activities have not been studied much. Burrowing provides protection from both predators and adverse environmental and temporal conditions, improving survivorship (Takeuchi et al., 2015; Zaklan and Ydenberg, 1997). In the current study, burrowing was always faster under controlled conditions than it was in warmer temperatures. Then,

clams would probably be more vulnerable to predation under predicted climate conditions of increasing temperatures during those seasons (spring and summer). Although *R. philippinarum* is known to burrow quicker under higher temperature regimes (Takeuchi et al., 2015), clams may require more energy to manage the higher metabolic needs, or the overall energy available for muscle movement may be reduced, resulting in longer burrowing times and impaired burrowing (Peng et al., 2017). Larger clams require longer time to burrow because this activity is positively correlated with body mass (Takeuchi et al., 2015); thus, as was also observed for the clam *Mulinia edulis* (Abarca et al., 2019), burrowing is a feature that may be adversely impacted by climate change and compromise seasonal clam production. In our experiments, the were no effects of pH on burrowing activity. However, an overall protective role of sediments against acidification was reported in another study using the same clam species and at the same pH levels, in which comparisons were done between cultures with and without sediments (Yuan et al., 2020). This contrasts with former studies that reported a tendency of bivalves to burrow less in more acidic waters considering that sediment pH tends to be lower than that of the nearby seawater due to microbial activity in the sediment (Clements and Hunt, 2017). A similar pattern was found in our experiments during spring for ambient temperatures, while it was not statistically significant, longer burrowing times were recorded under acidified treatments.

It is important to note that the biochemical responses of the clams were only detectable in the autumn specimens, which also happened to be the season in which no physiological changes in energy use (growth and clearance rate) or burrowing activity were found. The biomarkers related to detoxification capacity, such as CE (phase I), GST (phase II) and GR (antioxidant defence) activities, gradually increased under the most unfavourable conditions (lower pH and higher temperature), with antioxidant enzyme GR being the most sensitive to pH fluctuations. A "preparation for stress" biochemical challenge was corroborated with the lowest ETS values, pinpointing to a metabolic shift towards an anaerobic pathway that is energetically less efficient. Therefore, the clams may reduce growth under longer-term exposures. Seasonal differences in ETS activity were found in adult clams (Costa et al., 2020a). Despite that ETS activity is typically reduced in adverse conditions, the same clam species were not affected when both physical and chemical stressors were present (Costa et al., 2020b). The fact that these biochemical changes from the autumn experiment did not result in a reduction of the clams' physiological performance (lower filtration rates or reduced growth) highlights their efficiency to overcome adverse conditions under moderate physical stress. When adult clams were exposed to warm temperatures, low pH and additional chemicals, biochemical responses led to physiological changes such as reduced respiration rates, which became significant for diclofenac (Costa et al., 2020b) but not for triclosan (Costa et al., 2020c). In the present work, the fact that CE measures using pNPA substrate were more responsive to pH reductions but when using pNPB substrate they were more sensitive to temperature variations, may support a different isoenzyme composition in clams, responding in a stressor-dependent manner. This occurred in spite of the high overlap in substrate specificity described for this enzyme family in bivalves (Solé and Sanchez-Hernandez, 2018) and the significant correlation between both substrate measures.

Overall, our experimental results indicate that the clams were more impacted by a pH decrease than by a 3 °C temperature increase over the tested pH and temperature ranges. In fact, bivalves have often been found to be more sensitive to acidification than other calcifying organisms, which have been observed to decrease survival, calcification, growth and general development (Kroeker et al., 2013). The construction and stability of bivalve shells are hampered by the decrease in carbonate ion concentration in seawater brought on by ocean acidification, which has biological implications for the organisms (Matozzo and Marin, 2011; Range et al., 2011). More broadly, there will be a variety of effects of climate change on aquaculture. For example, seed availability and farming practice may negatively be affected by the timing and success of larval settlement (Ghezzo et al., 2018; Maulu et al., 2021). However, each life cycle of bivalves occurs in a different environment (planktonic larva and benthic adults) and has different physiological requirements (Byrne et al., 2020). Given that the responses to increased temperatures and acidification seem to be season-specific, it is important to investigate the effects of climate change throughout the course of the year, as we did in our study. Thus, year-round comprehensive studies are required as a basis to develop management strategies in the near future for climate change mitigation to enhance clam aquaculture production, including the development of integrated multitrophic aquaculture (IMTA) as well as genetic breeding techniques to increase the resilience of bivalves to climate change (Byrne et al., 2020; Tan and Zheng, 2020).

5. Conclusions

Different climate change-related factors can negatively affect the biology of Manila clams. This study indicates that clam seed seems to be more affected by the anthropogenic-derived pH decrease than by the 3 °C increase in temperature. That is, low pH water values decreased clearance rates and altered biomarker responses in a seasonally dependent manner. However, high temperatures only increased the burrowing time with spring and summer being the most vulnerable seasons. Additionally, an extreme summer scenario was experimentally added, in which the thermal optimum range of the clams was exceeded. In this case, clearance rates decreased at the highest temperature as opposed to responding to acidification, as observed throughout the year, but within the clams' optimal range. Considering that the physiological and biological responses of seed clams are influenced by seasonality, it is essential to develop strategies to improve aquaculture production globally while taking this variable into account.

CRediT authorship contribution statement

Eve Galimany: Investigation, Formal analysis, Writing – original draft. **Arturo Lucas:** Writing – review & editing. **Francesc Maynou:** Funding acquisition, Formal analysis, Writing – review & editing. **Montserrat Solé:** Methodology, Writing – review & editing. **Carles Pelejero:** Writing – review & editing. **Montserrat Ramón:** Conceptualization, Investigation, Writing – review & editing.

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.

Acknowledgements

This research is part of the CERES project (H2020, EU 678193). We are grateful to the staff of the Aquaria and Experimental Chambers (ZAE) of the Institute of Marine Sciences (ICM-CSIC) and the institutional support of the 'Severo Ochoa Centre of Excellence' accreditation (CEX2019-000928-S). We also thank the following graduate students for their help with the experimental setup and sample processing: Pere Forteza Joy, Alizée Desrousseaux, Mireia Sierra, Alicia Paniego Gómez, and Ainoa Cubero Miró.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.739891.

References

Abarca, A., Oliva, D., Toledo, P., 2019. Effect of transfer time, temperature, and size on burrowing capacity of juvenile clams, *Mulinia edulis*, from hatchery. J. World Aquacult. Soc. 50, 774–788.

Arias, P.A., Bellouin, N., Coppola, E., Jones, R.G., Krinner, G., Marotzke, J., Naik, V., Palmer, M.D., Plattner, G.-K., Rogelj, J., Rojas, M., Sillmann, J., Storelvmo, T., Thorne, P.W., Trewin, B., 2021. Technical summary. In: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J.B.R., Maycock, T.K., Waterfield, T., Yelekçi, O., Yu, R., Zhou, B. (Eds.), Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 33–144.

Bindoff, N.L., Cheung, W.W.L., Kairo, J.G., Arístegui, J., Guinder, V.A., Hallberg, R., Hilmi, N., Jiao, N., Karim, M.S., Levin, L., O'Donoghue, S., Purca Cuicapusa, S.R., Rinkevich, B., Suga, T., Tagliabue, A., Williamson, P., 2019. Changing ocean, marine ecosystems, and dependent communities. In: Pörtner, H.-O., Roberts, D.C., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E., Mintenbeck, K., Alegría, A., Nicolai, M., Okem, A., Petzold, J., Rama, B., Weyer, N.M. (Eds.), IPCC Special Report on the Ocean and Cryosphere in a Changing Climate, pp. 447–587.

Bo, D., Qinzhao, X., Jun, L., 2000. The effect of temperature on the filtration rate, clearance rate and absorption efficiency of Manila clam, *Ruditapes philippinarum*. Mar. Fish. Res. 21, 37–42.

Bopp, L., Resplandy, L., Orr, J.C., Doney, S.C., Dunne, J.P., Gehlen, M., Halloran, P., Heinze, C., Ilyina, T., Séférian, R., Tjiputra, J., Vichi, M., 2013. Multiple stressors of ocean ecosystems in the 21st century: projections with CMIP5 models. Biogeosciences 10, 6225–6245.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Byrne, M., Foo, S.A., Ross, P.M., Putnam, H.M., 2020. Limitations of cross- and multigenerational plasticity for marine invertebrates faced with global climate change. Glob. Chang. Biol. 26, 80–102.

Calvo, E.M., Simó, R., Coma, R., Ribes, M., Pascual, J., Sabatés, A., Gili, J.M., Pelejero, C., 2011. Effects of climate change on Mediterranean marine ecosystems: the case of the Catalan Sea. Clim. Res. 50, 1–29.

Carlberg, I., Mannervik, B., 1985. Glutathione reductase. In: Methods in Enzymology. Academic Press, pp. 484–490.

Clayton, T.D., Byrne, R.H., 1993. Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results. Deep Sea Res. Part I Oceanogr. Res. Pap. 40, 2115–2129.

Clements, J.C., Hunt, H.L., 2017. Effects of CO_2-driven sediment acidification on infaunal marine bivalves: a synthesis. Mar. Pollut. Bull. 117, 6–16.

Coelho, P., Carvalho, F., Goulding, P., Chainho, P., Guerreiro, J., 2021. Management models of the Manila clam (*Ruditapes philippinarum*) fisheries in invaded European coastal systems. Front. Mar. Sci. 8, 685307.

Costa, S., Coppola, F., Pretti, C., Intorre, L., Meucci, V., Soares, A.M.V.M., Freitas, R., Solé, M., 2020a. The influence of climate change related factors on the response of two clam species to diclofenac. Ecotox. Environ. Safe. 189, 109899.

Costa, S., Coppola, F., Pretti, C., Intorre, L., Meucci, V., Soares, A.M.V.M., Solé, M., Freitas, R., 2020b. Biochemical and physiological responses of two clam species to Triclosan combined with climate change scenario. Sci. Total Environ. 724, 138143.

Costa, S., Lopes, J., Coppola, F., Correia, S., Henriques, B., Leite, C., Soares, A.M.V.M., Zengjie, J., Pereira, E., Chiesa, S., Freitas, R., 2020c. Bioaccumulation and biochemical patterns of *Ruditapes philippinarum* clams: responses to seasonality and low contamination levels. Estuar. Coast. Shelf Sci. 243, 106883.

Creswell, L., 2010. Phytoplankton Culture for Aquaculture Feed. SRAC Publication. Southern Regional Aquaculture Center, p. 16.

Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Res. Part A Oceanogr. Res. Pap. 34, 1733–1743.

Durrieu de Madron, X., Guieu, C., Sempéré, R., Conan, P., Cossa, D., D'Ortenzio, F., Estournel, C., Gazeau, F., Rabouille, C., Stemmann, L., Bonnet, S., Diaz, F., Koubbi, P., Radakovitch, O., Babin, M., Baklouti, M., Bancon-Montigny, C., Belviso, S., Bensoussan, N., Bonsang, B., Bouloubassi, I., Brunet, C., Cadiou, J.-F., Carlotti, F., Chami, M., Charmasson, S., Charrière, B., Dachs, J., Doxaran, D., Dutay, J.-C., Elbaz-Poulichet, F., Eléaume, M., Eyrolles, F., Fernandez, C., Fowler, S., Francour, P., Gaertner, J.C., Galzin, R., Gasparini, S., Ghiglione, J.-F., Gonzalez, J.-L., Goyet, C., Guidi, L., Guizien, K., Heimbürger, L.-E., Jacquet, S.H.M., Jeffrey, W. H., Joux, F., Le Hir, P., Leblanc, K., Lefèvre, D., Lejeusne, C., Lemé, R., Loÿe-Pilot, M.-D., Mallet, M., Méjanelle, L., Mélin, F., Mellon, C., Mérigot, B., Merle, P.-L., Migon, C., Miller, W.L., Mortier, L., Mostajir, B., Mousseau, L., Moutin, T., Para, J., Pérez, T., Petrenko, A., Poggiale, J.-C., Prieur, L., Pujo-Pay, M., Pulido-Villena, Raimbault P., Rees, A.P., Ridame, C., Rontani, J.-F., Ruiz Pino, D., Sicre, M. A., Taillandier, V., Tamburini, C., Tanaka, T., Taupier-Letage, I., Tedetti, M., Testor, P., Thébault, H., Thouvenin, B., Touratier, F., Tronczynski, J., Ulses, C., Van Wambeke, F., Vantrepotte, V., Vaz, S., Verney, R., 2011. Marine ecosystems' responses to climatic and anthropogenic forcings in the Mediterranean. Prog. Oceanogr. 91, 97–166.

Dickson, A.G., Sabine, C.L., Christian, J.R., 2007. Guide to best practices for ocean CO 2 measurements. PICES Special Publication, p. 191.

FAO, 2022. In: FAO (Ed.), The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation, p. 236. Rome.

Fernández Robledo, J.A., Marquis, N.D., Countway, P.D., Record, N.R., Irish, E.L., Schuldt, M.M., Kingston, S.E., Bishop, T.J., Messerman, N.A., Bowden, T.J., 2018. Pathogens of marine bivalves in Maine (USA): a historical perspective. Aquaculture 493, 9–17.

Fernández-Reiriz, M.J., Range, P., Álvarez-Salgado, X.A., Labarta, U., 2011. Physiological energetics of juvenile clams *Ruditapes decussatus* in a high CO_2 coastal ocean. Mar. Ecol. Prog. Ser. 433, 97–105.

Fernández-Tejedor, M., Velasco, J.E., Angelats, E., 2022. Accurate estimation of chlorophyll-a concentration in the coastal areas of the Ebro Delta (NW Mediterranean) using sentinel-2 and its application in the selection of areas for mussel. Remote Sens. 14, 5235.

Fitzer, S.C., Torres Gabarda, S., Daly, L., Hughes, B., Dove, M., O'Connor, W., Potts, J., Scanes, P., Byrne, M., 2018. Coastal acidification impacts on shell mineral structure of bivalve mollusks. Ecol. Evol. 8, 8973–8984.

Galimany, E., Ramón, M., Ibarrola, I., 2011. Feeding behavior of the mussel *Mytilus galloprovincialis* (L.) in a Mediterranean estuary: a field study. Aquaculture 314, 236–243.

Ghezzo, M., Pellizzato, M., De Pascalis, F., Silvestri, S., Umgiesser, G., 2018. Natural resources and climate change: a study of the potential impact on Manila clam in the Venice lagoon. Sci. Total Environ. 645, 419–430.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Han, K.N., Lee, S.W., Wang, S.Y., 2008. The effect of temperature on the energy budget of the Manila clam, *Ruditapes philippinarum*. Aquac. Int. 16, 143–152.

Hartin, C.A., Bond-Lamberty, B., Patel, P., Mundra, A., 2016. Ocean acidification over the next three centuries using a simple global climate carbon-cycle model: projections and sensitivities. Biogeosciences 13, 4329–4342.

Hosokawa, M., Satoh, T., 2005. Measurement of carboxylesterase (CEs) activities. In: Costa, L.G., Hodgson, E., Lawrence, D.A., Ozolins, T.R., Reed, D.J., Greenlee, W.F. (Eds.), Current Protocols in Toxicology. John Wiley & Sons, New York, pp. 717–730.

Jung, A.S., Van der Veer, H.W., Van der Meer, M.T.J., Philippart, C.J.M., 2019. Seasonal variation in the diet of estuarine bivalves. PLoS One 14, e0217003.

Kang, H.Y., Lee, Y.-J., Choi, K.-S., Park, H.J., Yun, S.-G., Kang, C.-K., 2016. Combined effects of temperature and seston concentration on the physiological energetics of the Manila clam *Ruditapes philippinarum*. PLoS One 11, e0152427.

King, F.D., Packard, T.T., 1975. Respiration and activity of respiratory electron-transport system in marine zooplankton. Limnol. Oceanogr. 20, 849–854.

Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.-P., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob. Chang. Biol. 19, 1884–1896.

Kwiatkowski, L., Orr, J.C., 2018. Diverging seasonal extremes for ocean acidification during the twenty-first century. Nat. Clim. Chang. 8, 141–145.

Lowe, A.T., Bos, J., Ruesink, J., 2019. Ecosystem metabolism drives pH variability and modulates long-term ocean acidification in the Northeast Pacific coastal ocean. Sci. Rep. 9, 963.

Matozzo, V., Marin, M.G., 2011. Bivalve immune responses and climate changes: is there a relationship? Invertebr. Surviv. J. 8, 70–77.

Maulu, S., Hasimuna, O.J., Haambiya, L.H., Monde, C., Musuka, C.G., Makorwa, T.H., Munganga, B.P., Phiri, K.J., Nsekanabo, J.D., 2021. Climate change effects on aquaculture production: sustainability implications, mitigation, and adaptations. Front. Sustain. Food Syst. 5, 609097.

Maynou, F., Costa, S., Freitas, R., Solé, M., 2021. Effects of triclosan exposure on the energy budget of *Ruditapes philippinarum* and *R. decussatus* under climate change scenarios. Sci. Total Environ. 777, 146068.

Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18, 897–907.

Movilla, J., Calvo, E., Pelejero, C., Coma, R., Serrano, E., Fernández-Vallejo, P., Ribes, M., 2012. Calcification reduction and recovery in native and non-native Mediterranean corals in response to ocean acidification. J. Exp. Mar. Biol. Ecol. 438, 144–153.

Movilla, J., Calvo, E., Coma, R., Serrano, E., López-Sanz, A., Pelejero, C., 2016. Annual response of two Mediterranean azooxanthellate temperate corals to low-pH and high-temperature conditions. Mar. Biol. 163, 135.

Narita, D., Rehdanz, K., Tol, R.S.J., 2012. Economic costs of ocean acidification: a look into the impacts on global shellfish production. Clim. Chang. 113, 1049–1063.

Ojea, J., Pazos, A.J., Martinez, D., Novoa, S., Sánchez, J.L., Abad, M., 2004. Seasonal variation in weight and biochemical composition of the tissues of *Ruditapes decussatus* in relation to the gametogenic cycle. Aquaculture 238, 451–468.

Paeth, H., Vogt, G., Paxian, A., Hertig, E., Seubert, S., Jacobeit, J., 2017. Quantifying the evidence of climate change in the light of uncertainty exemplified by the Mediterranean hot spot region. Glob. Planet. Chang. 151, 144–151.

Peng, C., Zhao, X., Liu, S., Shi, W., Han, Y., Guo, C., Peng, X., Chai, X., Liu, G., 2017. Ocean acidification alters the burrowing behaviour, Ca2+/Mg2+-ATPase activity, metabolism, and gene expression of a bivalve species, *Sinonovacula constricta*. Mar. Ecol. Prog. Ser. 575, 107–117.

Perez, F.F., Fraga, F., 1987. A precise and rapid analytical procedure for alkalinity determination. Mar. Chem. 21, 169–182.

Pérez, F.F., Ríos, A.F., Rellán, T., Álvarez, M., 2000. Improvements in a fast potentiometric seawater alkalinity determination. Cienc. Mar. 26, 463–478.

Peteiro, L.G., Woodin, S.A., Wethey, D.S., Costas-Costas, D., Martínez-Casal, A., Olabarria, C., Vázquez, E., 2018. Responses to salinity stress in bivalves: evidence of ontogenetic changes in energetic physiology on *Cerastoderma edule*. Sci. Rep. 8, 8329.

Pierrot, D., Lewis, E., Wallace, D.W.R., 2006. MS excel program developed for CO2 system calculations. In: ORNL/CDIAC-105, Carbon Dioxide Information Analysis Center, oak Ridge National Laboratory. U.S. Department of Energy, Oak Ridge, TN.

Prado, P., Roque, A., Pérez, J., Ibáñez, C., Alcaraz, C., Casals, F., Caiola, N., 2016. Warming and acidification-mediated resilience to bacterial infection determine mortality of early *Ostrea edulis* life stages. Mar. Ecol. Prog. Ser. 545, 189–202.
Range, P., Chícharo, M.A., Ben-Hamadou, R., Piló, D., Matias, D., Joaquim, S., Oliveira, A.P., Chícharo, L., 2011. Calcification, growth and mortality of juvenile clams *Ruditapes decussatus* under increased pCO_2 and reduced pH: variable responses to ocean acidification at local scales? J. Exp. Mar. Biol. Ecol. 396, 177–184.
Riisgård, H.U., 1988. Efficiency of particle retention and filtration rate in 6 species of northeast American bivalves. Mar. Ecol. Prog. Ser. 45, 217–223.
Riisgård, H.U., 2001. On measurement of filtration rates in bivalves — the stony road to reliable data: review and interpretation. Mar. Ecol. Prog. Ser. 211, 275–291.
van der Schatte Olivier, A., Jones, L., Le Vay, L., Christie, M., Wilson, J., Malham, S.K., 2020. A global review of the ecosystem services provided by bivalve aquaculture. Rev. Aquac. 12, 3–25.
Smaal, A.C., Ferreira, J.G., Grant, J., Petersen, J.K., Strand, Ø., 2019. Goods and Services of Marine Bivalves. Springer Open, Switzerland.
Solé, M., Sanchez-Hernandez, J.C., 2018. Elucidating the importance of mussel carboxylesterase activity as exposure biomarker of environmental contaminants of current concern: an in vitro study. Ecol. Indic. 85, 432–439.
Solé, M., Freitas, R., Viñas, L., Rivera Ingraham, G., 2020. Biomarker considerations in monitoring petrogenic pollution using the mussel *Mytilus galloprovincialis*. Environ. Sci. Pollut. Res. 27, 31854–31862.
Steeves, L.E., Filgueira, R., Guyondet, T., Chassé, J., Comeau, L., 2018. Past, present, and future: performance of two bivalve species under changing environmental conditions. Front. Mar. Sci. 5, 184.
Takeuchi, S., Yamada, F., Shirozu, H., Ohashi, S., Tamaki, A., 2015. Burrowing ability as a key trait in the establishment of infaunal bivalve populations following competitive release on an extensive intertidal sandflat. J. Exp. Mar. Biol. Ecol. 466, 9–23.
Tan, K., Zheng, H., 2020. Ocean acidification and adaptive bivalve farming. Sci. Total Environ. 701, 134794.
Therneau, T., 2016. A Package for Survival Analysis in S. version 2.39-5. https://CRAN.R-project.org/package=survival.
Van Vuuren, D.P., Isaac, M., Kundzewicz, Z.W., Arnell, N., Barker, T., Criqui, P., Berkhout, F., Hilderink, H., Hinkel, J., Hof, A., Kitous, A., Kram, T., Mechler, R., Scrieciu, S., 2011. The use of scenarios as the basis for combined assessment of climate change mitigation and adaptation. Glob. Environ. Chang. 21, 575–591.
Velasco, L.A., 2007. Energetic physiology of the Caribbean scallops *Argopecten nucleus* and *Nodipecten nodosus* fed with different microalgal diets. Aquaculture 270, 299–311.
Velez, C., Figueira, E., Soares, A.M.V.M., Freitas, R., 2016. Combined effects of seawater acidification and salinity changes in *Ruditapes philippinarum*. Aquat. Toxicol. 176, 141–150.
Velez, C., Figueira, E., Soares, A.M.V.M., Freitas, R., 2017. Effects of seawater temperature increase on economically relevant native and introduced clam species. Mar. Environ. Res. 123, 62–70.
Xu, X., Yang, F., Zhao, L., Yan, X., 2016. Seawater acidification affects the physiological energetics and spawning capacity of the Manila clam *Ruditapes philippinarum* during gonadal maturation. Comp. Biochem. Physiol. 196, 20–29.
Yuan, H., Xu, X., Yang, F., Zhao, L., Yan, X., 2020. Impact of seawater acidification on shell property of the Manila clam *Ruditapes philippinarum* grown within and without sediment. J. Oceanol. Limnol. 38, 236–248.
Zaklan, S.D., Ydenberg, R., 1997. The body size–burial depth relationship in the infaunal clam *Mya arenaria*. J. Exp. Mar. Biol. Ecol. 215, 1–17.
Zhang, P., Zhao, T., Zhou, L., Han, G., Shen, Y., Ke, C., 2019. Thermal tolerance traits of the undulated surf clam *Paphia undulata* based on heart rate and physiological energetics. Aquaculture 498, 343–350.