Aquaculture 603 (2025) 742422

Contents lists available at ScienceDirect

Aquaculture

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

Effect of simulated prolonged heat wave on biometric performance and *HSP70* synthesis in the striped venus clam *Chamelea gallina*

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ARTICLE INFO

Keywords:
Heat wave
Thermal stress
Clam
Chamelea gallina
Air exposure
Isoform
Heat shock protein

ABSTRACT

In the current scenario of climate change and ocean warming, it is crucial to understand how the fishing and shellfish industries will be affected by extreme weather events. This study simulated a 30-day heat wave at 28 °C to better understand the response of one particular breed of clam (*Chamelea gallina*) to future stress from extreme heat waves, and how such stress might affect its feeding ability. Biometric parameters, physiological indices, and *HSP70* expression in the adductor muscle were analyzed. Results showed no significant changes in condition index. They revealed a stabilized hepatosomatic index decrease after 10 days of exposure, and an initial increase in resistance to air exposure and stable clearance rate. The study demonstrates that heat waves have a minimal impact on overall clam physiology, confirmed by an unchanged expression of *HSP70. C. gallina* proves resistant to prolonged thermal stress, possibly due to an adaptation to its native Mediterranean habitat. These findings suggest that the striped venus clam fishing sector may not be significantly threatened by similar extreme heating events in future.

1. Introduction

Eurostat (2023) data on European aquaculture in 2021 shows production to be primarily concentrated on finfish species and mollusks. Among mollusks, clams ranked third in production volume after mussels and oysters, with an estimated catch of around 32,000 tons. The striped venus clam (*Chamelea gallina*) was one of the most heavily harvested clams in Europe, but its numbers have collapsed over recent years, especially in the Atlantic part of Spain and the Northern Adriatic (FAO, 2020). This decline seems to be the combination of several factors, including overfishing, short lifespan, reduced fertility and, above all, climate change and high temperatures, which affect the status of this particular species, as well as causing a reduction in the number of clam beds and even its death. The study by Monari et al. (2007) showed that exposure to water at 30 °C for seven days altered the immune parameters in clams: while THC (total hemocyte count) increased, the phagocytic activity of the hemocytes decreased, as did the haemocyte population of about 8^{e10} mm. The researchers also found a change in antioxidant defenses, with a significant decrease in total superoxide dismutase (SOD) activity. Using the same exposure condition and species, Monari et al. (2011) evaluated the *Heat Shock Protein 70* (*HSP70)* levels in the digestive gland and gills, discovering an increased *HSP70* expression rate in the digestive gland, but no significant variation in the gills. In the study by Jahan et al. (2023), *HSP70*, *HSP90* and *HSF* (heat shock factor) expressions were analyzed in a different type of tissue - the mantle of clams exposed to a high temperature of 28 °C for 48 h. Here the authors discovered a difference in gene regulation depending on where the animal had been harvested (north or south), and a weak correlation between *HSP* and *HSF* expression: greater *HSP70* down-regulation in southern clams compared to northern, with similar results for *HSP90,* while *HSF1.a* showed an upregulation in both northern and southern clams. Lin et al. (2018) detected *HSP90* regulation also in the mantle, adductor muscle and hemocytes of clams stressed for two hours at 32 °C, where the gene was essentially upregulated.

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https://doi.org/10.1016/j.aquaculture.2025.742422
Received 3 December 2024; Received in revised form 11 February 2025; Accepted 10 March 2025

In this study, the animals were exposed to the stress treatment of 28 °C for 30 days, a condition that had been tested on other species, such as the concave oyster (De Marco et al., 2023), but never on *C. gallina.* The aim to conduct such a test, at this temperature and with this duration, was prompted by the need to recreate the potential future scenario predicted in several studies and models on heat waves (HWs) in the northern Adriatic Sea over the coming decades (Furlan et al., 2019; Kubin et al., 2023; Moulin et al., 2024).

Defined as a situation where the warming of sea water exceeds certain thresholds and extends over time and space, HWs have accelerated in the Mediterranean over the last two decades, increasing in frequency, intensity and duration (Hobday et al., 2016). Together with the French coast (Pastor and Khodayar, 2023), the Adriatic is the area where the hottest and most frequent HWs have been recorded, and the phenomenon is predicted to increase here in the near future, between 2041 and 2050, with temperatures reaching 28 °C for periods of up to a month, with highly negative consequences for marine ecosystems and bivalve production (Galli et al., 2017).

The clam's physiological response to the simulated future heat wave scenario was analyzed through biometric parameters, hemolymphatic analyte values, *HSP70 expression* at different time points, and indices of clearance rate and resistance to air exposure, to define a limit time of high temperature beyond which this mollusk could suffer irreversible damage, with negative consequences for the clam fishing sector.

2. Materials and methods

2.1. Animals and experimental design

Clams (*Chamelea gallina*) harvested by the Italian hatchery *NaturEdulis* in the Goro lagoon in the northern Adriatic Sea were immediately transferred to the aquaculture laboratory at the Department of Veterinary Medical Sciences of the University of Bologna. The animals had an average body weight of 5.36 ± 1.14 g, and a shell length of 26.03 ± 2.2 mm. 240 specimens were randomly distributed into six 70-l aquaria filled with recirculating natural Adriatic seawater, partially replaced by 40 % every four days for water exchange purposes. The animals were acclimatized for one week at a salinity of 25–30 ppt and a temperature of 20 ± 0.5 °C, and fed daily with a maintenance ration of live *Isochrysis galbana* microalgae, consisting of 3 % of the mean dry-meat weight of the animals (determined removing soft tissues from 12 specimens and dried to constant mass in an oven at 80 °C for 72 h) in dry weight of algae feed per day (Helm et al., 2004). Following acclimatization, the aquaria were divided in two groups. The control group was maintained at a temperature of 20 ± 0.5 °C, while the heat wave-exposed group was brought to 28° ± 0.5 °C by warming the water at a rate of 0.5 °C per hour. The water temperatures were kept constant using electronic thermostats and heat exchangers for 30 days, and seawater parameters (temperature, salinity and pH) recorded daily (De Marco et al., 2023).

2.2. Biometric and physiological parameters

Three clams from the control group and three from the treatment group were sampled at different time points,3, 5, 10 and 30 days following heat wave exposure, to determine the most significant biometric indices. The choice of four different time points is based on the objective to understand at what time the clams manage to modulate a response that does not profoundly alter the general homeostasis in a future scenario of prolonged high sea water temperatures.

In detail, timepoints of 3, 5, and 10 days were selected to monitor the expression of Heat Shock Protein 70, which have been previously documented in other bivalves and identified as early-response genes that serve as sensitive indicators of environmental stress and health status in shellfish (De Marco et al., 2023). The animals were individually weighed, then the soft body separated from the shell to weigh the valves and wet meat to calculate the condition index (CI = wet soft tissue weight (g)/ whole specimen weight (g)* 100). At each sampling time point, the hepatopancreas of the same three specimens per group was dissected and weighed to calculate the hepatosomatic index (HSI = hepatopancreatic weight (g)/ whole specimen weight (g) * 100). Clam mortality was monitored daily to determine the survival rate.

A further three clams per group were selected at random to evaluate the physiological index of clearance rate (CR = $(\ln C_0 - \ln C_1)$ V / t, where C0 and C1 were the algal cell concentrations at the beginning and end of each time increment ($t = 0.5$ h), V was the beaker volume of 1 L). The CR of an individual specimen was based on the average rate over the two-hour period. Clams were individually placed in beakers containing 1-L of filtered seawater at the experimental temperatures, and algal cells (*Isochrysis galbana*) added to each beaker at a mean initial concentration of $5 \bullet 10^5$ cell.mL-1. 10–15 mL aliquots were then sampled from each beaker at 30 min intervals over a two-hour period, to measure the decline in algal cell concentration due to calm filtration, using a Bürker cell counting chamber. Six clams per group were sacrificed at each sampling time point to collect 0.2–0.6 mL of hemolymph from the adductor muscle using a 1 mL syringe with 25 gauge needle. The hemolymph was then centrifuged for three minutes at 1000 ×*g*, the cellular fraction removed, and the supernatant stored at −80 °C. The hemolymph supernatant samples were then analyzed by automated analyzer (AU 480; Olympus/Beckman Coulter, Brea, CA, United States) to measure aspartate transaminase (AST) activity (Kinetic UV test, OSR6109, Beckman Coulter, Brea, CA, United States), and concentrations of glucose (Enzymatic UV test, OSR6121, Beckman Coulter, Brea, CA, United States), magnesium (Photometric colour test, OSR6189, Beckman Coulter, Brea, CA, United States), total calcium (Photometric colour test, OSROSR60117, Beckman Coulter, Brea, CA, United States), potassium and sodium (Indirect quantitative determination, ISE module: crown ether membrane electrode, 66,317 ISE Low Serum Standard; 66,316 ISE High Serum Standard, Beckman Coulter, Brea, CA, United States).

A further test was carried out to evaluate the specimens' response (both stressed and control groups) to the additional stress input of exposure to air. Four animals were taken from each experimental group at each sampling point and placed in an airtight container at 18 ± 1 °C and 80–90 % humidity. Survival was monitored daily until 100 % mortality was reached. The LT_{50} value was recorded, indicating the time at which 50 % of the tested bivalves died. The clams were considered dead when found with valves open and unresponsive to an external stimulus (Eertman and de Zwaan, 1993).

2.3. Protein extraction and Western blot

No complete sequenced genome is available for the *Chamelea gallina* species, and data on the expression of *heat shock protein* genes are lacking in the literature, making an accurate and robust analysis of gene expression impossible. We therefore chose to assess the presence of *HSP70* protein using the Western blot method on muscle tissue. We focused on muscle due to its general sensitivity to temperature stress: the thermal stress alters the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump present in the muscle and *Hsp70* binds with the SERCA preventing its inactivation, stabilizing muscle structure and function. So *Hsp70* involvement in muscle is very crucial during this environmental condition (Kumar et al., 2023). Moreover, we used muscle as a detector of thermal resistance/tolerance capability in clams. Infact, De Marco et al., 2023, showed that the muscle in other bivalve, the Pacific oyster, had a higher *Hsps* long-term overexpression than the gills during heat waves: it was more sensitive and more responsive compared to gills. Adductor muscle tissues (50–100 mg) were collected from six clams per group at 3, 5, 10 and 30 days after exposure and stored at −80 °C until use, and a total of 48 muscle samples (24 exposed and 24 unexposed) were homogenized in RIPA buffer at a ratio of 1:8 (mg: μl). The RIPA buffer was composed as follows: 150 mM Sodium Chloride, 1 % Triton X-100, 0.5 % Sodium deoxycholate, 0.1 % Sodium Dodecyl Sulfate, and 50 mM Tris pH 8.0.

At this point, the sample was ready for electrophoresis, and both protease (Sigma Aldrich, code P2714) and phosphoprotease (Roche, code 04–906–837-001) inhibitors were added to the RIPA buffer. After a centrifugation at 12.000 ×*g*, 20 min at 4 °C, the supernatants were used to perform Western blot, using stain-free methods for visualizing the total proteins. Immediately after the run, the gels were photoactivated with ultraviolet (UV) light for five minutes.

This step, known as gel activation, is used to visualize all the proteins loaded in the well. The signal remains visible even after membrane transfer, and is used as a normalizer in place of the housekeeping protein.

The experiment was conducted as follows:

- Electrophoretic run: proteins were separated according to their molecular weight in a 4–20 % polyacrylamide gradient gel (4–20 % Mini-PROTEAN TGX Stain-Free Protein Gels, ten wells, 30 μl. Bio-Rad, code 4568093). For each sample, we loaded 20 μg of total proteins in 16 μl of a mix prepared as follows: ¼ volume of Laemli/β-mercaptoethanol (Bio-Rad, code 161–0747) and ¾ volume of supernatants. The run was performed in a buffer consisting of: TGS running buffer 1× (prepared by dilution from 10× Bio-Rad, code 161–0732), and Rated Perceived Exertion (RPE) water (Carlo Erba code 307584), stored at 4 °C at a constant voltage (200 V) for 40 min. Three exposed and three unexposed samples from the same time point were loaded into each gel.
- Gel Activation: gels were gently moved onto the tray of the ChemiDoc MP Imaging System for five minutes UV photoactivation.
- Transfer: the previously separated proteins were transferred from the gel to a nitrocellulose membrane (AmershamTM ProtranTM, 0.45 μm Nitrocellulose blotting membrane, 150 mm × 4 m, GE Healthcare) to allow the protein to interact with the antibody. Transfer was conducted in a constant voltage cell (100 V for 1 h) with a buffer consisting of: Tris/glycine premixed buffer 1× (prepared by dilution from 10× Bio-Rad, code 161–0734), 20 % Methanol (Sigma Aldrich, code 32213) and RPE water. At the end of the transfer, the membranes were transferred on to the ChemiDoc MP Imaging System tray once more, and the total protein image acquired using the Stain Free application. Following this exposure, nonspecific interaction sites were blocked by incubating the membranes for 1 h at RT in 5 % low fat milk powder diluted in 0.1 % TBST.
- Recognition by antibodies: after blocking, the membranes were incubated overnight at 4 °C with the primary anti-HSP70 antibody (Invitrogen, Mouse, 1:2.000) diluted in 5 % milk powder in 0.1 % TBST. They were then left to incubate for 1 h at RT with the secondary antibody (Bio-Rad, Goat Anti-Mouse, 1:3.000) diluted in 2.5 % milk powder in 0.1 % TBST.
- Detection: ClarityTM Western ECL Substrate (Bio-Rad, code 1705061) was used for detection, and the image of the chemiluminescent signal digitally acquired using the ChemiDoc MP Imaging System.
- Densitometric analysis: densitometric analysis was performed using the software Bio-Rad Image Lab, version 6.1.0 build 7, standard edition. The total protein image acquired after the transfer was used as a normalizer. Results are expressed as the ratio of arbitrary densitometric units (AU) between the protein of interest and the total protein.

To assess potential changes in *HSP70*, we evaluated the expression of both the monomeric protein (70 kDa *HSP70*, low molecular weight band) and all observed isoforms of *HSP70* (tot*HPS70*, high and low molecular weight). In both cases, expression was evaluated at the same time point as a percentage change from unexposed controls in the same gel.

Assuming that the high molecular weight form represents the dimer of *HSP70* and that a dimerization of the protein has been observed to underlie its activation (Takakuwa et al., 2019), we also assessed the relative abundance of the heavy isoform of about 140 kDa. The abundance was expressed as a percentage of the densitometric volume of the dimer, relative to the total densitometric volume of both isoforms.

2.4. Statistical analyses

The data from the biometric variables, clearance rate, resistance to air exposure and biochemical hemolymph indices were analyzed by a two-way analysis of variance (ANOVA) and Tukey's post-hoc test performed in the event of significance ($P < 0.05$). The Western blot data for *HSP70* quantification were analyzed using different tests: *t*-test in HSP70 monomeric and *HSP70* isoforms abundance to assess differences between exposed and unexposed; one way Anova in *HSP70* monomeric and *HSP70* isoforms abundance to assess differences between different time-points and two way Anova in relative abundance of the heavy isoform to assess differences due to both heat exposure and timepoint. All statistical analyses were conducted using GraphPad Prism (Version 10.3.1).

3. Results

3.1. Biometric and physiological parameters

The biometric values of the whole clam weight (Fig. 1A), valve weight (Fig. 1B), wet soft body weight (Fig. 1C) and condition index (Fig. 1D) showed no significant differences between the experimental groups over time. There were however significant differences in the hepatosomatic index values (Fig. 1E), which decreased at the end of exposure in the exposed group, showing the presence of a time effect ($p = 0.0041$). HSI was also affected by temperature, with a significant difference between the groups on days 10 and 30 ($p < 0.0001$). The survival rate was 100 % for both groups during the whole trial.

While the heat wave did not affect the physiological clearance rate parameter (Fig. 2A), it did influence resistance to air exposure, a parameter affected by temperature ($p = 0.0028$), time of exposure ($p < 0.0001$) and the time-temperature interaction ($p = 0.0022$) (Fig. 2B). The exposed group showed higher LT_{50} values at days three and five than the control group at the same time points ($p = 0.0028$). The control group also showed an increase over the experimental time, with higher value at the end of exposure, while the LT_{50} value increased in the exposed group from the fifth day of exposure onwards ($p < 0.0001$).

The heat wave did not affect the glucose (Fig. 3A), AST activity (Fig. 3B), calcium (Fig. 3C), magnesium (Fig. 3D) or potassium (Fig. 3E) hemolymphatic results. Sodium concentration in the hemolymph showed several significant changes. Sodium levels significantly decreased over time in the control group, also dropping in the exposed group by the end of the exposure period, indicating a clear effect of time ($p < 0.0001$). A comparison of the groups shows sodium concentrations to be consistently higher in the exposed than in the control group ($p < 0.0001$), suggesting both a temperature and a time-temperature interaction effect ($p = 0.0003$) (Fig. 3F)

3.2. HSP70 protein quantification

Fig. 4 shows the images of all muscle samples analyzed by Western blot. The presence of the chemiluminescent marker allowed us to identify the band corresponding to *HSP70*, just below the band of the marker corresponding to 75 kDa. The protein is expressed at all times in both heatwave-exposed and non-heatwave-exposed samples. A second band, with a molecular weight just under 150 kDa, is also present in all the samples analyzed.

Fig. 5 shows the results of both assessments. In Fig. 5A, the control group was used as a normalization group and is therefore not shown. No significant differences in *HSP70* (Fig. 5A) abundance were observed either between treated and controls (*t*-test 70 kDa *HSP*: 3 days $p =$

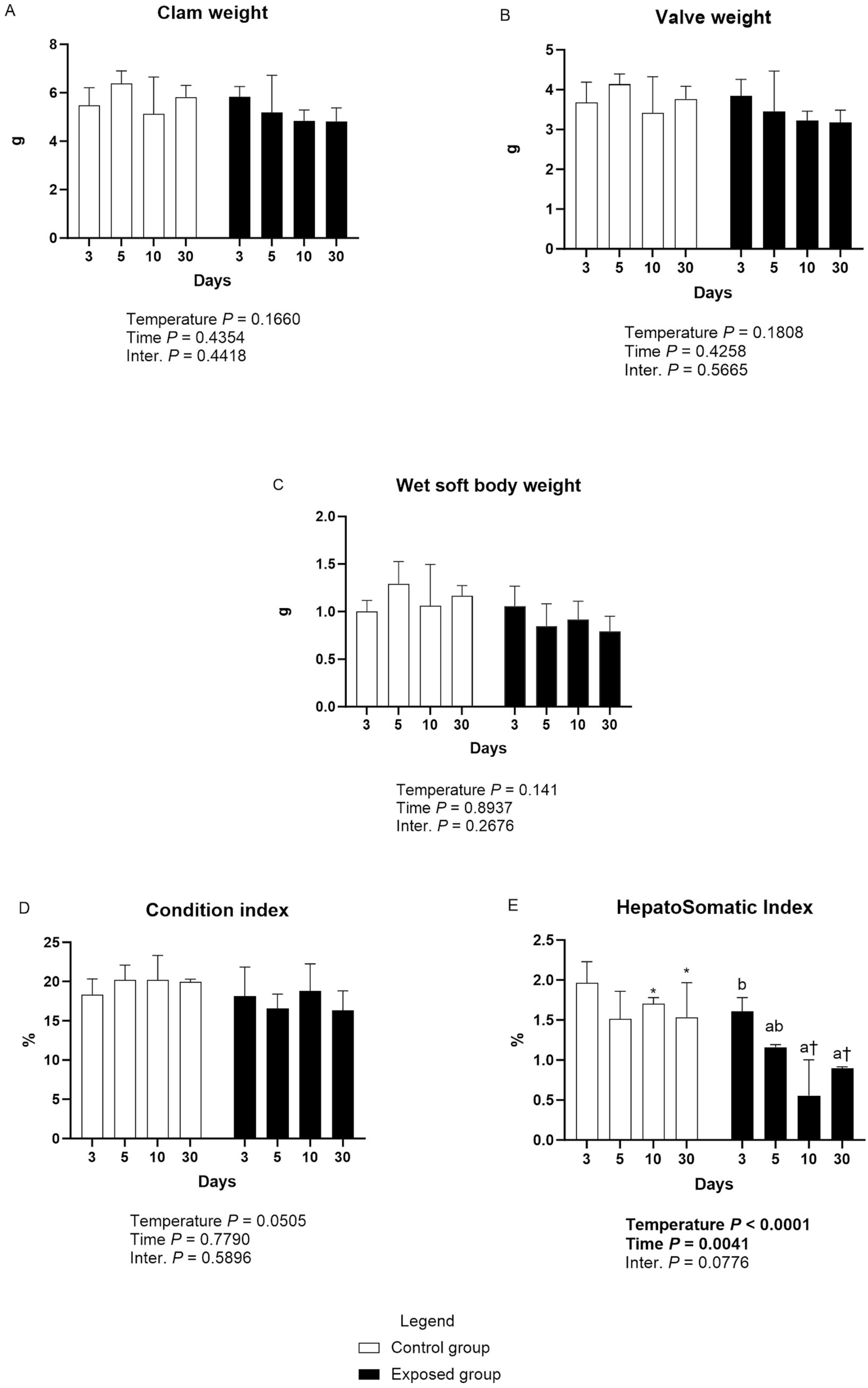

Fig. 1. Clam weight (A), valve weight (B), wet soft body weight (C), CI (D), HIS (E).
Data are shown as average value ± S.D. Lowercase letters (a,b) stand for significant differences in two-way ANOVA ($P < 0.05$) between times for the same group. The symbols (*, †) stand for significant differences between groups at the same time point.

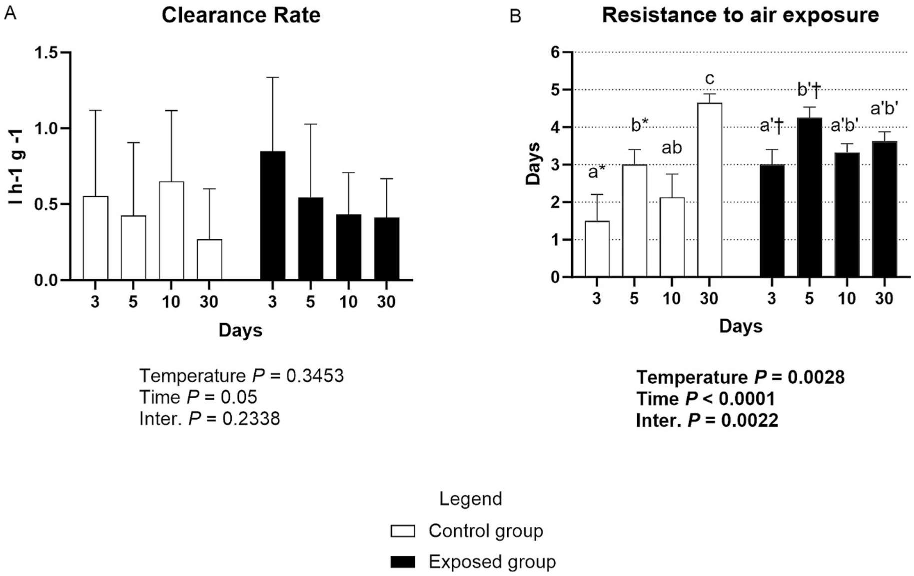

Fig. 2. Clearance rate (A), resistance to air exposure (B).
Data are shown as average value ± S.D. The uppercase letters (A, B) represent significant differences in two-way ANOVA ($P < 0.05$) between the groups. Lowercase letters (a,b for control; a',b' for exposed) stand for significant differences between times for the same group. The symbols (*, †) stand for significant differences between groups at the same time point.

0.8182; 5 days $p = 0.3517$; 10 days $p = 0.5701$; 30 days $p = 0.3939$; tot *HSP*: 3 days $p = 0.8182$; 5 days $p = 0.3532$; 10 days $p = 0.6803$; 30 days $p = 0.4848$) or at different timepoints (one way Anova 70 kDa *HSP* $p = 0.3745$; tot *HSP* $p = 0.3647$). In contrast, the level of the high molecular weight (MW) isoform was influenced by time ($p = 0.0025$), it changed over time in exposed sample (Fig. 5B). A peak in abundance is observed after five days. However, no significant differences were observed between control and exposed samples at the same exposure time.

4. Discussion

4.1. Heat waves have a slight influence on biometric parameters

In general, the thermal stress induced in this study was not high enough to affect clam body weight or pulp yield (represented by the CI), which showed no differences. The high temperature may have caused the animals' metabolic rate to rise, with a partial use of hepatopancreatic resources. The clams exposed to the prolonged thermal stress showed no change in hepatopancreatic weight until the fifth day of exposure, but as the heat wave persisted, their HIS values fell significantly more than those of the control group from the tenth day of exposure onwards. While the clams initially appeared able deal with the stress via their normal metabolic activity without resorting to an additional energy source, the continuation of the heat wave required them to mobilize the contents of the digestive gland on the tenth day of exposure, so as to provide them with sufficient energy to maintain their physiological state. However, their response then stabilized, with no increase in this mobilization over the final 20 days of exposure: indeed the HSI values in the exposed group did not change from the tenth day of exposure onwards, appearing to indicate that the continued high temperatures did not require greater energy. The 2016 study by Beaudry et al. on *M. edulis* exposed to an increase in temperature for 70 days, for example, shows that HSI levels decreased after 42 days of exposure, before remaining stable for the subsequent 28 days (Beaudry et al., 2016).

In nature, bivalve filtration rate increases as temperatures rise, due to an increase in metabolic rate, with seasonal variations (Moschino and Marin, 2006). In this study however, the CR of the two groups showed no differences, possibly because the clams exposed to stress had adapted to the temperature of 28 °C, with filtration comparable to that of the control conditions.

In nature, clams are regularly exposed to the air due to the movement of the tides, requiring them to survive out of water and exposing them to stress. This occurs not only at low tide, but also during harvest and transport, and especially at the time of sale (Yin et al., 2017; Zhou et al., 2021). Clams are classified as edible animals, with a legal obligation to be sold while still alive (European Regulation 1379/2013). Stress that alters their physiological state may however cause them to die more rapidly when they are harvested and are required to spend time out of water prior to sale, resulting in a poor quality product. Animals exposed to air, in fact, have less access to oxygen and suffer severe hypoxic stress, and many studies demonstrate the adverse effects of such exposure, including oxidative stress damage, cell apoptosis, destruction of histological structure, alteration of energy homeostasis, and even death (Arlinghaaus and Hallermann, 2007; Gu et al., 2017; Lu et al., 2021). In this study, however, we found that during the first five days of heat wave, stressed animals survived longer than control animals, possibly due to the activation of multiple adaptive mechanisms to cope with drying stress and hypoxia. Yin et al., 2017 demonstrated that during air exposure, Manila clam (*R. philippinarum*) changed its metabolism from aerobic to anaerobic with high activities of antioxidant enzymes. This anaerobic respiration requires energy produced through gluconeogenesis - an important metabolic response activated during aerial exposure (Nie et al., 2020; Nie et al., 2020a). In the study of Zhang et al., 2012, on *M. gigas*, another mechanism to resist air exposure emerged, the upregulation of the inhibitors of apoptosis. Such strategies would permit clams under stress to survive longer than unexposed animals and suggest that thermic stress may stimulate greater resistance to additional stress inputs. Matozzo et al. (2009) also discovered that the resistance to air exposure of the clams with a prolonged exposure to B(*a*)P (12 days) did not significantly differ from the resistance of the control animals, probably due to the capability of clams to metabolize the contaminant (stress input), thus reducing its negative effects on the physiological performance of bivalves. A similar event occurred in this study: after five days of exposure, the survival rate had stabilized in the exposed group, not differing significantly from the other group or time points. Clams subjected to prolonged stress have probably retained the mechanisms which allow them to respond to other stressors. In biometric terms, the

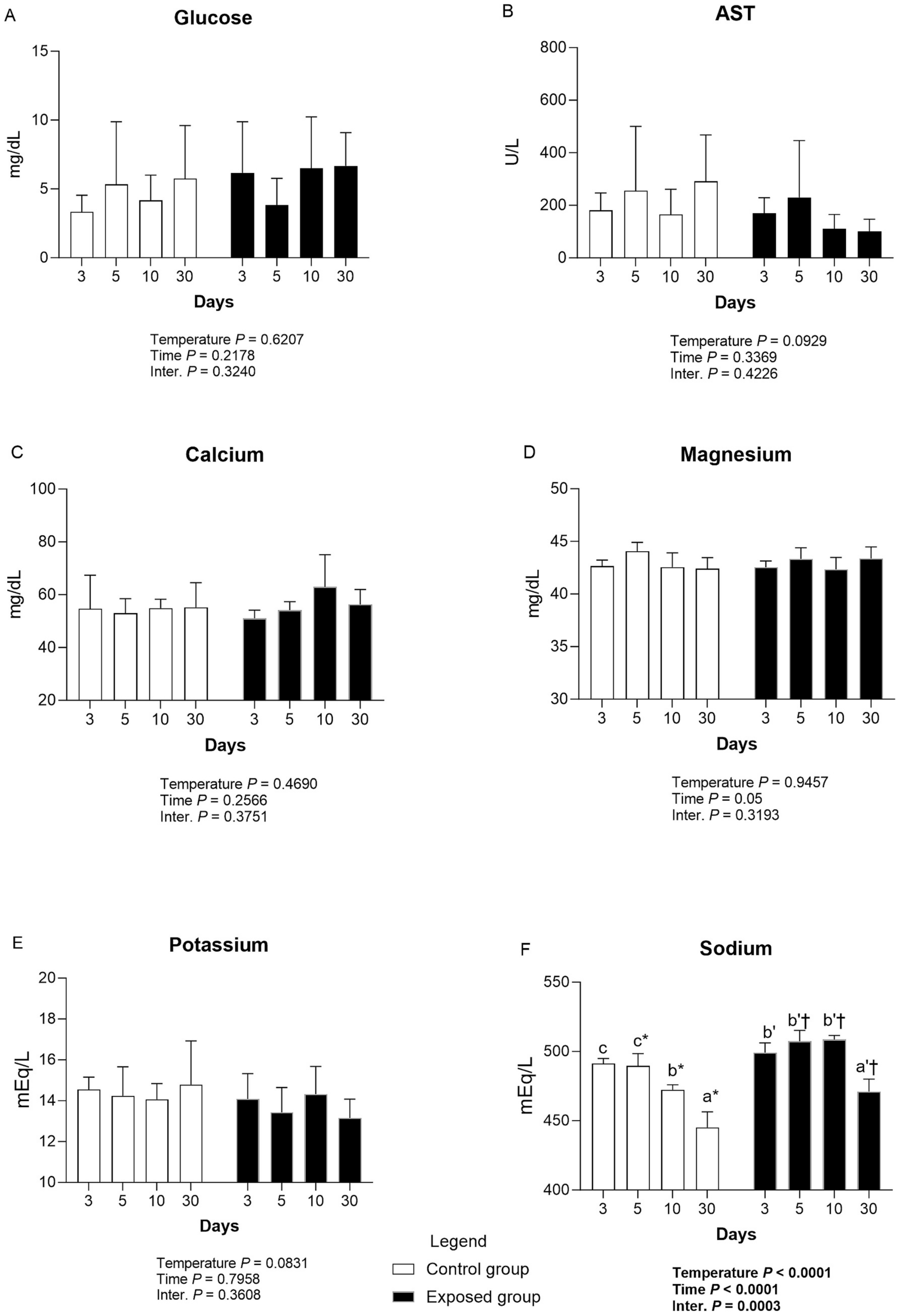

Fig. 3. Glucose concentration (A), aspartate transaminase activity (B), calcium (C), magnesium (D), potassium (E), and sodium (F) hemolymphatic concentrations. Data are shown as average value ± S.D. Lowercase letters (abc: Ctrl group; a'b': Exp group) stand for significant differences in two-way ANOVA ($P < 0.05$) between times for the same group. The symbols (*, †) stand for significant differences between groups at the same time point.

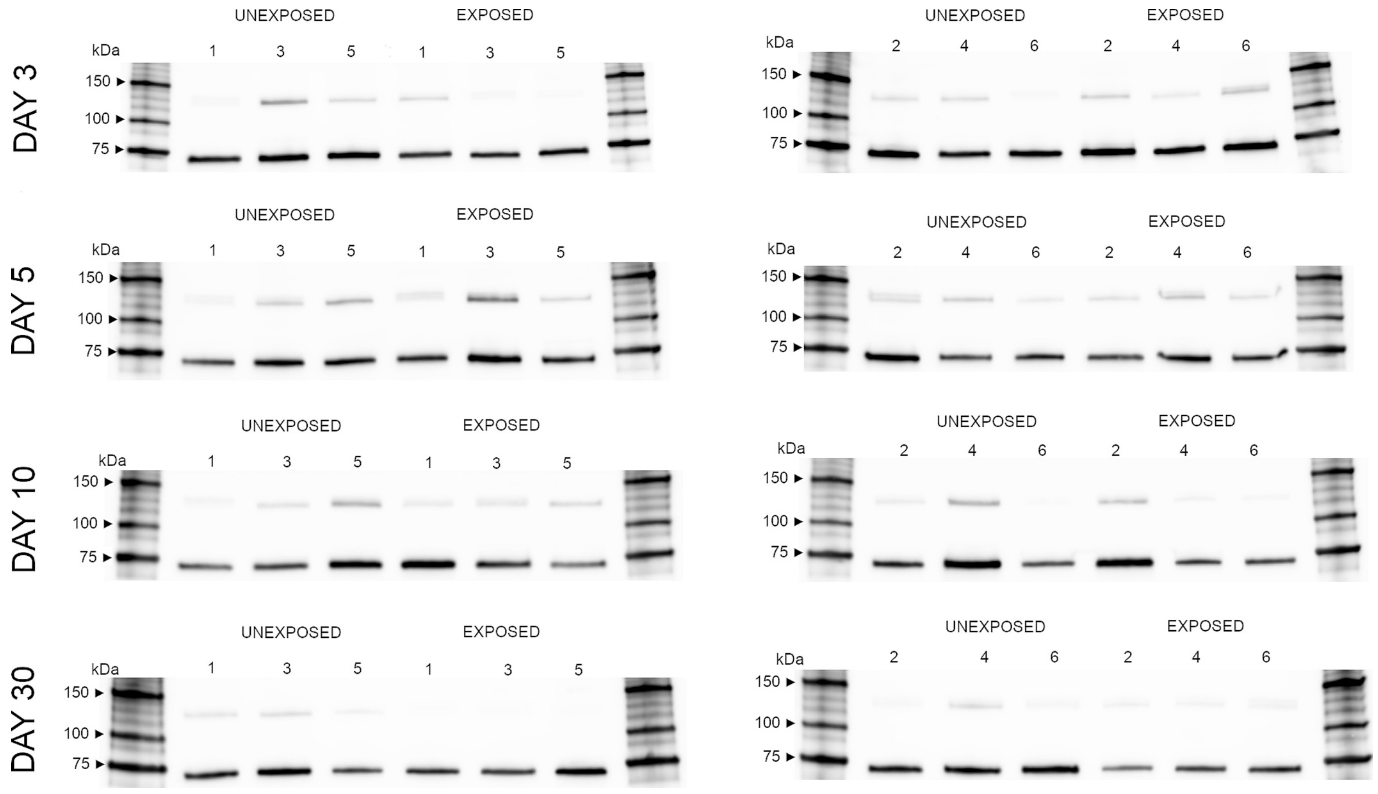

Fig. 4. Western Blot in clam adductor muscle. The numbers above the lanes refer to the sample IDs. Each gel contains three samples from subjects not exposed to HWs (unexposed, on the left of the gel) and three samples from subjects exposed to HWs (exposed, on the right of the gel). The comparison between exposed and unexposed animals was conducted on samples loaded in the same gel.

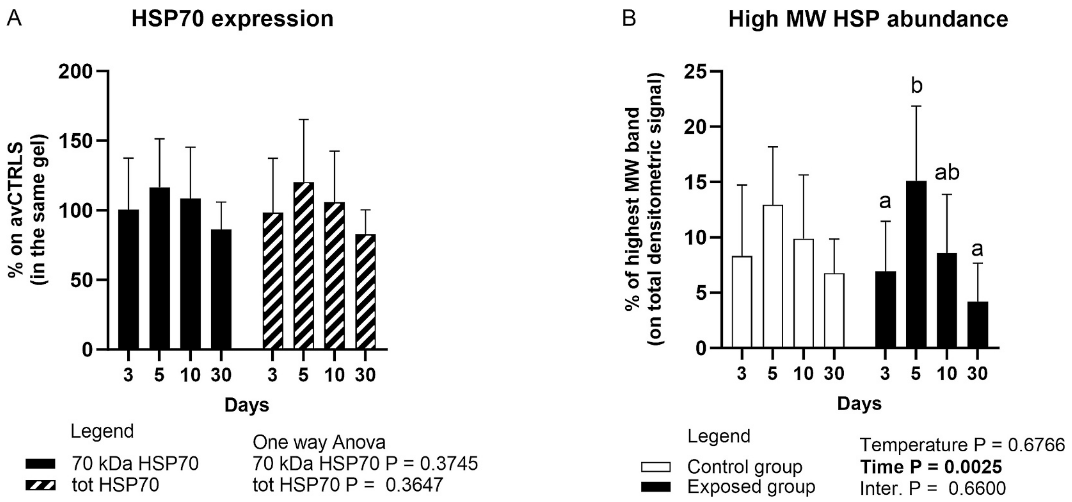

Fig. 5. Expression level of *HSP70* isoforms (A), and relative abundance of the high molecular weight *HSP70* isoform (B). The control group was used as a normalization group and is therefore not shown. Data are shown as average value ± S.D. Lowercase letters (a,b) stand for significant differences in two-way ANOVA ($P < 0.05$) between times for the same group.

clams may have mobilized energy from the hepatopancreas for the first five days, a strategy which may also have increased their resistance, allowing them to maintain a stable constant state without further increasing their metabolism and causing its degeneration.

To the best of our knowledge, the osmoregulatory response of bivalve mollusks to high temperature conditions has not yet been studied. However, from research on marine ectothermic animals, it is known that the gilthead sea bream *Sparus auratus* shows active osmoregulation during the summer season with high temperatures. It increases plasma sodium levels due to increased Na^+,K^+-ATPase activity with a change in cell membrane fluidity in the gills (Metz et al., 2003; Vargas-Chacoff et al., 2009). This could explain the results obtained in our test where there was a difference between the groups, with exposed clams showing significantly higher sodium levels than the control animals, especially from the tenth day of high temperature exposure onwards. Moreover, the use of hepatopancreatic energy resources may have slightly increased the animals' metabolic rate, causing a fall in pH. In the event of internal fluid acidosis, these species mainly react by increasing in the activity of the Na^+/H^+ exchanger. The greater the number of H^+ ions excreted, the more Na^+ ions enter the body, with an increase in hemolymph Na^+ level (Lannig et al., 2010). In our study, however, the prolonged heat wave did not cause acute damage to the clams' metabolism, as shown by the absence of changes in glucose and AST activity in the exposed group, two parameters used as biomarkers of general stress in a variety of aquatic invertebrates (Fritts et al., 2015; Hannan et al., 2016).

4.2. Heat waves do not alter HSP70 synthesis in muscle

The Western blot tests in this study revealed two bands, with the 70 kDa band corresponding to the molecular weight of the monomeric form of *HSP70*. Based on that described in the literature in other species, it could be assumed that the highest molecular weight band is the dimer of *HSP70*, which should migrate at a height corresponding to approximately 140 kDa. Other research groups, in fact, although working in very different species (murine and human models), have obtained comparable results in terms of molecular weight, and have classified the major band as homodimer (Grunwald et al., 2014; Lornage et al., 2019). *HSPs* are molecular chaperones which play a central role in proteostasis, with *HSP70* constituting a molecular chaperone capable of refolding proteins which lose their conformation under stress conditions, restoring their functionality (Rosenzweig et al., 2019). It exists in different forms, and is equipped with a mechanism of self-association (Takakuwa et al., 2019). Thompson et al. (2012) have shown that high temperatures alter the chaperone code, and that the ability of *HSP70* to oligomerize also increases, leading to the formation of more dimer forms (Thompson et al., 2012). This is reflected in the dimer presence data of this study, shown by the relative abundance of the high molecular weight *HSP70* isoform. The dimer percentage below 5 % in almost all samples may be a sign of the presence of the constitutive form of the protein which provides an essential housekeeping function, while the dimeric form of *HSP70* becomes noticeably more present in exposed samples at five days of heat wave. The increase may be due to the thermal stress which induced a greater degree of oligomerization, since the dimeric form probably has a more efficient stress response. Looking at the quantity of total *HSP70*, however, shown by the level of *HSP70* isoforms, the lack of over-activation of this *heat shock protein* deviates from the mechanisms used by other bivalve mollusks. The Pacific oyster, for example, exposed to the same heat wave simulation, generates a long overexpression of *HSPs* to fold any altered proteins (De Marco et al., 2023). The markedly different response of the striped venus clam may be linked to intrinsic characteristics of the species, associated with its original habitat (Lassoued et al., 2021). A native of the Mediterranean Sea, *C. gallina* has always been present in the northern Adriatic (Vincenzetti et al., 2017), an area which has experienced significant warming over the few last decades, with the most extreme and highest number of HWs ever recorded (von Schuckmann et al., 2019). This may have caused the clam to develop mechanisms over time which have allowed it to adapt or become more resilient to the increased water temperatures, becoming more tolerant to high temperature changes. The 2011 study by Monari et al. showed a consistent *HSP70* expression in the hepatopancreas at 20 °C and 25 °C, signs of a probable adaptative mechanism to these temperatures. But the expression was insignificant compared to the overexpression which emerged after seven days of exposure at 30 °C, a stress which probably exceeded the 28 °C simulation chosen for this study (Monari et al., 2011). Chen et al. (2018) conducted a high temperature test on clams (*Meretrix meretrix*) from different geographical populations, discovering a possible link between habitat of origin and heat tolerance, observing that clams from a certain site possessed better heat resistance. Iuffrida et al. (2024) demonstrated that the physiological status of a natural *C. gallina* population responded differently to the heat wave that hit the Mediterranean Sea in the summer of 2022, a difference based on geographical region. The same study revealed an interaction between sampling sites and the time of year, when the animals were harvested, for all analyzed transcriptional profiles, including metabolic, antioxidant and cytoprotective genes, except for alpha-amylase and *HSP90*. This finding is consistent with the absence of differences in *HSP70* expression observed in this study.

Many studies, especially those on other bivalves such as the Pacific oyster, have also shown that thermal treatment boosts tolerance to subsequent thermal shock (Hamdoun et al., 2003; Ding et al., 2020). It is therefore possible that clams in the Adriatic Sea have already experienced acute thermal stress which has made them more tolerant to stress in the future, a possible explanation for the failure to activate *HSP70*, as well as for the slight changes in biometric and physiological parameters. Of these, the mobilization of hepatopancreatic resources may have been intended solely to maintain metabolic balance, and not for *HSP* synthesis.

This study aimed to define a time limit beyond which prolonged high temperatures may weaken clam metabolism. Our results appear to show an ability of the clam to withstand the 28 consecutive days of thermal stress, and that there was no specific critical day beyond which the animal goes into extreme distress.

It should however be noted that this study was conducted considering thermal stress only. While this variable alone may not damage the clam fishing industry, the predicted decline in production may be due to the interaction of different variables (Froehlich et al., 2018). The marine environment is governed by a synergy of multiple factors which alter its ecosystems and animal physiology in different ways, therefore future research should combine the insights into temperature which have emerged this study, combining them with other factors such as dissolved oxygen concentration, salinity and pH to give a picture which reflects the true situation offshore as accurately as possible (Bertolini and Pastres, 2021).

5. Conclusion

This study showed the striped venus clam to have exceptional resistance to prolonged thermal stress compared to other bivalves. The heat wave did not affect the animal's general homeostasis, and was not so extreme as to trigger the molecular cascade that activates *HSP70*; indeed we recorded no heat limit time beyond which the animal underwent significant metabolic changes. There was no loss of pulp yield or reduction in resistance to air exposure, characteristics of essential importance for their sale quality. Despite this species offers great potential for the shellfish farming sector due to its excellent performance in such conditions, our temperature findings should integrate with other environmental variables to better represent future impact on clam farming.

CRediT authorship contribution statement

Antonina De Marco: Writing – original draft, Methodology, Investigation, Formal analysis. **Vito Antonio Baldassarro:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Maura Cescatti:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Marzia Moretti:** Writing – original draft, Methodology, Formal analysis. **Laura Calzà:** Writing – review & editing, Validation, Project administration, Conceptualization. **Luciana Giardino:** Writing – review & editing, Validation, Supervision, Project administration, Conceptualization. **Francesco Dondi:** Writing – review & editing, Validation, Methodology, Formal analysis, Conceptualization. **Maria Giulia Ferrari:** Writing – review & editing, Methodology, Formal analysis. **Pier Paolo Gatta:** Writing – review & editing, Validation, Supervision. **Luca Parma:** Writing – original draft, Validation, Data curation, Conceptualization. **Alessio Bonaldo:** Writing – original draft, Validation, Supervision, Project administration, Investigation, Conceptualization.

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.

Acknowledgments

The authors would like to thank Elisa Pignoni for her assistance in Western blot analysis during her Master's degree thesis.

Data availability

Data will be made available on request.

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