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PROLONGED HEAT WAVES REDUCE THE CONDITION INDEX AND ALTER THE MOLECULAR PARAMETERS IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS*

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Abstract

The entire shellfish farming sector is negatively affected by heat waves. Predictive models show that while heat waves are not predicted to exceed 28° C in the northern Adriatic Sea over the coming decades, their duration will increase to periods of up to 30 days. Knowledge regarding the effects of heat waves on bivalves at physiological and molecular level is still limited. This study attempted to simulate what will happen in the future in Pacific oysters exposed to prolonged heat waves, assessing morphometric and physiological indices, and investigating the expression level of a number of genes, including the chaperone heat shock proteins *HSP70*, *HSP72* and *HSP90,* and the factor *P53*. A state of stress in the heat wave-exposed animals was found, with loss of body weight and energy resources: despite showing a higher clearance rate, these animals were unable to absorb the nutrients required to maintain homeostasis, as well as demonstrating an alteration in hemolymphatic AST activity, total calcium and magnesium concentration. mRNA levels of all examined genes increased in response to thermal stress, with long-term overexpression, activating cell stress defense mechanisms and modulating the cycle cell. The results of this study indicate that heat waves affect oyster welfare, with consequences for the productivity of the sector due to the lack of salable products.

Keywords

Heat wave; Thermal stress; Concave oyster; Condition index; Overexpression; Heat shock protein

1. Introduction

The aquaculture sector is growing in importance, contributing an increasing percentage of animal protein to the per capita requirement of the global population, with shellfish products accounting for 15% of total production [1]. The entire sector, however, is negatively affected by climate change, and by ocean warming in particular, with a serious decline in bivalve mollusk production expected over the coming decades [2]. In Italy, one of the world's top bivalve mollusk-producing countries, 65% of all national aquaculture production consists of bivalves, but climate change could cause these numbers to collapse in the near future. The most worrying component of climate change is the heat

wave - an extreme climatic event with a minimum duration of three days [3,4], whose frequency, duration and severity are predicted to increase.

According to the predictive models developed in the study conducted by Galli et al., 2017 [5] on heat waves in the Mediterranean Sea, heat waves above 30° C will be recorded in the southern Mediterranean in the years 2041-2050, whereas they will not exceed 28° C in the northern Adriatic Sea, where the situation will instead be aggravated by an increase in heat wave duration of up to 30 days, with devastating consequences for ecosystems in general, and widespread death of bivalve mollusks due to their sensitivity to thermal stress.

In this study, we used the heat wave to simulate the expected conditions of the northern Adriatic Sea. The experimental animal was the concave oyster, *Crassostrea gigas*, as in recent years attempts have been made to introduce this species to the area, best known for the production of mussels and clams [6]. Over time, the concave oyster has functionally adapted to sessile life in intertidal areas affected by sudden environmental changes, and to daily and seasonal fluctuations in temperature. Ideal temperatures for the growth of this animal range from 11-34° C [7].

In the Pacific oyster (*C. gigas*), several studies have evaluated the effects of heat stress on phenotypic and genetic response. Meistertzheim et al., 2007 [8], in oysters exposed to long-term thermal stress of 25°C for 24 days, found a tissue specific *HSPs* synthesis, with *HSP70* and *HSP23* upregulated more and faster in the gill than in the mantle and *HSP12A* was induced in gill tissue only. Moreover, the expression of these transcripts showed a fluctuating trend with a significant increase at 3–7 days of exposure, a decrease at 14 days, and a lower second increase at 17–24 days. Even in Clegg et al., 1998 [9], gill tissue was analyzed to evaluate heat shock proteins in oyster exposed to a thermal stress (an acute exposure of 37°C for 1 h with an otherwise lethal heat treatment). This study found that the increases in *HSP72*, *HSP77* and *HSP69* synthesis occurred in the first 2 days of exposure. Then, their levels remained higher than in the control group for 2 weeks following the stress, recognized as the period of induced thermotolerance. Recently, the effects of acute heat stress on genetic and phenotypic response of thermotolerant selected and control oysters' populations were evaluated by Ding et al., 2020 [10]. The selected population showed no different growth, but a higher survival rate, increased oxygen consumption at higher temperatures and higher enzyme activity than the control one. The authors also, demonstrated a different genome structure and gene expression in candidate genes, at gills level, associated with thermal tolerance (*IF4A2, IF6, EIF3A, MANBA, DDX43, RECS, CAT2,* and *BAG4*).

To the best of our knowledge, the effects of such a chronic and intense thermal treatment on oysters, 30 days at 28°C, have not been evaluated yet. Moreover, the expression of the genes

involved in the physiological stress and repair response, was often performed in gills but not in adductor muscle, of which few information is available. Thus, in this study, the Pacific oyster was exposed to a water temperature of 28°C for 30 days, simulating predicted future conditions in the north Adriatic Sea. The morphometric, physiological and hemolymphatic variables and the expression levels of the *HSP70*, *HSP72*, *HSP90* and *P53* were then evaluated by mRNA quantification at different times of exposure (3, 5, 10 and 30 days) as welfare indicators of commercial product quality.

2. Materials and methods

2.1. Animals and experimental design

Farmed Pacific oysters (*Crassostrea gigas)* were purchased from the *NaturEdulis* facility (Goro, Ferrara, Italy) and immediately transferred to the laboratory of Aquaculture at the Department of Veterinary Medical Sciences of the University of Bologna (Cesenatico, in the Forlì-Cesena province of Italy). The animals had an average body weight of 38.22 ± 8.16 g and a shell length of 70 ± 6.2 mm. 120 specimens were randomly distributed into twelve 70-liter aquaria filled with recirculating natural Adriatic seawater at a salinity of 28-30 ppt and a temperature of 20 ± 0.5 °C, equal to that recorded in the field during animal harvesting, and acclimatized for one week. The oysters were fed daily with a maintenance ration of live microalgae *Isochrysis galbana,* consisting of 3% of the mean dry-meat weight of the animals in dry weight of algae feed per day, in accordance with FAO hatchery protocol for the culture of bivalves [11].

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) and oyster mortality were checked daily.

2.2. Biometric and physiological parameters

Three oysters from the control and treatment groups were sampled at different time points (3, 5, 10 and 30 days after heat wave exposure) to determine the most significant biometric indices. The animals were individually weighed, then the soft body was separated from the shell to weigh the valves and the wet meat to calculate the condition index (CI). Developed by Hughes-Games (1977) [12], this tool indicates pulp yield (percentage of edible portion), calculated as follows: CI = wet soft tissue weight (g)/ whole specimen weight (g)* 100 [13,14]. At the final sampling time point (30 days exposure), the soft bodies were dried in an oven at 60°C for 24h, then weighed to calculate the shell-free dry weights (SFDW). At the same time point, the hepatopancreas of a further three specimens per group was dissected and weighed to calculate the hepatosomatic index (HSI), which expresses the ratio of liver weight to total body weight, and which is calculated as follows: HSI = hepatopancreatic weight (g)/ whole specimen weight (g) * 100 [15].

Three oysters per group were sacrificed at each sampling time point to collect 0.5 - 1.0 ml of hemolymph from the pericardial cavity using a 1 mL syringe with 25 gauge needle [16]. The hemolymph was then centrifuged for 3 min 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 total protein, aspartate transaminase (AST) activity, and concentrations of magnesium, total calcium, phosphate and glucose. Ammonia concentration was measured using a point of care analyzer (FUJI DRI-CHEM NX10N).

Three oysters per group were randomly chosen at the final sampling time point to evaluate the physiological index of clearance rate (CR) according to Romano et al., 2011 [17]. In brief, the oysters were individually placed in beakers containing 1-l of filtered seawater at the experimental temperatures. After a period of 20 minutes to allow the oyster to open and resume pumping, algal cells (*Isochrysis galbana*) at a mean initial concentration of $8 \cdot 10^5$ cell ml^{-1} were added to each beaker. At 30 min intervals over a two-hour period, 10-15 ml aliquots were sampled from each beaker to assess the exponential decrease of cell numbers as a result of filtering by the oysters. The decline in cell concentration was monitored using a cell counting chamber by inverted microscope. Two beakers without bivalves were used as controls, which showed no significant decline throughout the experimental period. The CR ($l\ h^{-1}\ g^{-1}$) was then calculated using the following equation: $CR = (\ln C_0 - \ln C_1)\ V / t / SFDW$, where C_0 and C_1 were the algal cell concentrations at the beginning and end of

each time increment (t = 0.5 h), and mean SFDW values per group at 30 days were used to standardize clearance rates [18]. The CR of one specimen was based on the average rate over the two-hour period [19,20].

2.3. Gene expression analysis

Adductor muscle and gill tissues (≤ 50mg) were collected from six oysters per group at 3, 5, 10 and 30 days after exposure, immersed in RNAlater (10 µL/mg) to preserve the integrity of the nucleic acids, and stored at -80°C.

The total RNA was extracted by RNeasy® Plus Universal Mini Kit (Qiagen) according to the manufacturer's instructions. In brief, the tissues immersed in RNAlater were removed and placed in clean tubes containing 900µL QIAzol, in which the tissues were mechanically homogenized. To remove the genomic DNA and to separate the RNA, 100 µL of gDNA Eliminator solution and 180 µL of chloroform were added to the lysate, shaken for 15 seconds and then centrifuged at 12000 x g at 4°C for 15 minutes. Following the centrifugation, 600 µL of the separated aqueous phases containing the RNA were loaded into an automatic RNA extraction *QIAcube* (Qiagen). The extracted RNA was quantified using a *Nanodrop 2000* spectrophotometer and then used for the reverse transcription reaction, using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions.

Finally, semiquantitative analysis of the gene expression was performed using real-time qPCR (CFX 96 machine; Bio-Rad), loading 10 ng of cDNA per each analysis, using Sso Advanced Universal SYBR Green Supermix (Bio-Rad). The sequences of the forward and reverse primers used in real-time PCR were specific for *C. gigas,* synthesized by Integrated DNA Technologies, and are listed in Table 1. Amplification conditions were: a denaturation step of 3 min at 98°C, followed by 40 cycles for 10 s at 95°C and 1 min at 60°C, followed by a melting curve analysis consisting of 80 cycles of 10 s, with an increase of 0.5°C between each cycle from 65°C to 95°C. A single peak was observed on the melting curve, confirming the specificity of the reaction.

GAPDH and *18S* genes were selected as housekeeping genes [21,22].

Gene	Forward primer	Reverse primer
HSP70	5'-AGCAAGCCAGCACAGCA-3'	5'-GCGATGATTTCCACCTTC-3'
HSP72	5'-GAGGATCGCAGCCAAGAA-3'	5'-TATCGCCCTCGCTGATCT-3'
HSP90	5'-GGAGAGCAAAACCCTCACC-3'	5'-TGGCAATGGTTCCAAGGT-3'
P53	5'-ACCCAGCTCCGACTCATTT-3'	5'-TCATGGGGGATGATGACAC-3'
GAPDH	5'-TTCTCTTGCCCCTCTTGC-3'	5'-CGCCCAATCCTTGTTGCTT-3'

18S	5'-CGGGGAGGTAGTGACGAA-3'	5'-ACCAGACTTGCCCTCCAA-3'

Table 1. Sequences from *C. gigas* used to design the primers [22] (Farcy et al., 2009)

2.4. Statistical analyses

The data from the biometric variables 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 ($P < 0.05$). An unpaired t-test was used to evaluate the hepatopancreatic weight, hepatosomatic index and clearance rate differences between the groups at final sampling.

The mRNA expression data for *HSPs* and *P53* were analyzed using Student's t-test, and the data from the exposed group were normalized to controls for each time point to assess the effect of temperature during exposure.

All statistical analyses were conducted using GraphPad Prism (8 and 9 version).

3. Results

3.1. Heat waves alter biometric and physiological parameters

The biometric values of the whole oyster weight (Figure 1A) and shell weight (Figure 1B) showed no significant differences between the experimental groups over time. However, there was a significant different in the wet soft-body weight of the exposed group between the 5th day (the day with the highest value) and the 30th day (Tukey's post-hoc test, $p = 0.007$) (Figure 1C). The CI of the exposed group was affected by time, showing lower values after 30 days of exposure between the groups ($p = 0.0009$), and in the exposed group itself ($p < 0.0001$) (Figure 1D). The HSI at the end of the trial was affected by temperature, with lower values in the exposed group compared to the control group ($p = 0.008$) (Figure 1E), while there was no difference between the groups in terms of CR at the end of the trial (Figure 1F).

The heat wave did not affect the glucose (Figure 2A), phosphorus (Figure 2B), ammonia (Figure 2C) and total protein (Figure 2D) hemolymphatic results. Instead, AST activity showed a significant difference between interaction time and temperature, with a higher value at 5 days in the exposed

group compared to the control group at the same time point (p = 0.029), and at 30 days in the same group (p = 0.036) (Figure 2E). With regard to total calcium, there were differences between the time points, with the 30-day time point significantly different to all the others, and in the interactions between temperature and time, with a significant increase in the exposed group values over time, which also differed from the entire control group (p < 0.0001) (Figure 2F). Considering the magnesium concentrations, the results showed significant differences among the exposed group at 30 days, where there were the higher concentrations, and the same group at 3 days (p = 0,017) and the control group at 3 (p = 0,024) and 30 days (p = 0,014) (Figure 2G).

3.2. Heat waves generate a long-term overexpression of HSPs in muscles and gills

For the gene expression analysis, two representative tissues were chosen to detect responses to the heat waves. Muscles were chosen due to their sensitivity to stress, while gills were chosen due to their role as the initial sensor of the external environment.

In the muscles, *HSP70* showed an increased expression compared to non-exposed controls at 5 (Student's t-test, p = 0.0034) and 30 (p = 0.0139) days (Figure 3A). *HSP72* showed a similar pattern, with overexpression at 3 (p = 0.0322), 5 (p = 0.0015) and 30 (p = 0.0018) days (Figure 3B), while *HSP90* expression increased at late time points only, namely at 10 (p = 0.0003) and 30 (p = 0.0013) days (Figure 3C).

HSP70 and *HSP72* were also temporally regulated in the exposed group in a similar way to the non-exposed animals, while *HSP90* showed a drastic increase in expression over time in the heat wave-exposed animals only (Supplementary Figure S1).

In the gills, the *HSP70* and *HSP72* genes were affected by the heat wave in a temporally different manner: *HSP70* was overexpressed at early and intermediate time points only (3 days, p = 0.0189; 10 days, p = 0.0148; Figure 4A), as was *HSP72* (3 days, p = 0.0060; 10 days, p = 0.0018; Figure 4B). *HSP90* genes, however, were regulated in the same way as the muscles, showing an overexpression at 10 (p = 0.0100) and 30 (p = 0.0392) days (Figure 4C). All three genes tended to be similar to control expression levels at late time point.

Expression of the *P53* gene, a downstream responder controlled by *HSPs* expression, was also analyzed. Results showed this gene to be undetectable in the muscles, while it was overexpressed at 10 days in the gills (p = 0.0479; Figure 4D).

P53 and all three *HSP* genes showed a time pattern of expression along the four time points similar to the non-exposed group (Supplementary Figure S2).

Further the gene expression of the three *HSPs* between the two considered tissues was compared.

HSP70 resulted more expressed in the muscle at 10 and 30 days in control groups (Student t-test, 10 days, $p = 0.0010$; 30 days, $p = 0.0001$), which was amplified in exposed group with a huge overexpression at 30 days (Student's t-test, $p < 0.0001$) (Figure 5A).

The same gene expression dynamic was described for *HSP72*, with a higher expression at 10 and 30 days in control groups (Student t-test, 10 days, $p = 0.0393$; 30 days, $p = 0.0113$), amplified at 30 days when animals are exposed to the heat wave (Student's t-test, $p = 0.0003$) (Figure 5B).

The *HSP90* expression in control groups resulted even less expressed in the muscle at early time points with a significant difference at 5 days (Student's t-test, $p = 0.0070$) but, again, resulting more expressed at 30 days compared to gills ($p = 0.0006$). Also in exposed groups, in the muscle the *HSP90* gene was less expressed compared to gills at early time points (Student's t-test, 3 days, $p = 0.0012$; 5 days, $p = 0.0227$) but resulting upregulated of almost 10 times at 30 days ($p = 0.0001$) (Figure 5C).

4. Discussion

4.1. Heat waves reduce the assimilation of food filtered in oysters

Oysters are ectothermic organisms, meaning that external temperatures far outside their optimum range for homeostasis lead to higher energy requirements due to an increase in both feeding and metabolic rates [23,24].

This study showed a reduction in pulp yield by almost a third following 30 days of thermal stress. One possible explanation for this effect is that the exposure to a lengthy heat wave negatively impacted the weight of soft tissue and consequently the condition index, thus reducing pulp yield: the decrease in body mass is due to metabolization of the flesh, converted into energy to combat the thermal stress. This hypothesis is confirmed by the metabolic rate, which was higher in the exposed animals than the control group compared to body weight, and by clearance rate, as will be discussed further on.

Previous studies have shown that metabolic reserves stored in the digestive gland are also mobilized for maintenance during physiological stress such as seasonal changes [25,26]; in fact the

exposed group in our study showed significantly poor welfare with an elevated mobilization of energy, causing a halving of hepatopancreatic weight compared to the control group.

A physiological problem arises at higher temperatures, when metabolic demands may outstrip feeding ability leading to reduced growth [27,28] or reduced body size. One possible explanation is a reduction in the efficiency of microalgal nutrient absorption by the animals: while the clearance rate on day 30 shows that statistically both groups filtered at the same rate [29], the exposed group had a lower average soft tissue weight and a higher clearance rate than the control group at the end of the test [27], therefore a higher metabolic rate. It would therefore appear that none of the filtered microalgae were assimilated for tissue metabolism maintenance or stored as an energy reserve, instead being exploited in full together with part of the flesh to respond to the prolonged stress. One of these response mechanisms is *HSP* activation: the synthesis of stress proteins such as heat shock proteins is energetically costly, accounting for 20–25% of the total energy budget in mollusks [30,31].

In addition to altering the internal parameters of the oyster, increased temperatures also affect the ultrastructure of the valves, made of calcium carbonate (CaCO3) for 95.994% and magnesium oxide (MgO) for 0.649% in addition to other minerals of trivial amounts. [32]. This alteration leads the animals to sequester calcium and magnesium from the environment and transport it through the hemolymph to the mantle cells used for shell formation [33,34]. In the present study, the heat wave may have caused an increased sequestration of hemolymphatic calcium and magnesium in the exposed group, mostly at the end of exposure, intended for protection against possible gradual alteration of the shell structure.

Aspartate transaminase plays an essential role in the regulation of amino acid metabolism, and its activity in oysters undergoes a seasonal increase during early summer [35,36]. In the present study, AST was activated early on, presumably because the period of five days at 28°C triggered a metabolic behavior in the oysters similar to that which occurs naturally in the early summer: the high temperature increased the AST biosynthesis activity of amino acids, in correlation with the higher metabolic rate described above. This effect was also shown by the increase in total protein and decrease in ammonia concentrations in hemolymph, not significantly but in absolute terms.

4.2. Heat shock proteins mediate the condition index deficit

The Pacific oyster genome contains a broad spectrum of genes involved in the cellular defense mechanisms against stressful stimuli. These genes encode for *HSPs*, with 88 genes encoding for *HSP70* alone [37,38,9]. The action of the *HSPs* allows the oyster to acquire resistance to a broad range of stressors, including thermal stress, but at great cost: high energy expenditure [39,40,22].

While Clegg et al., 1998 [9] found that an acute thermal stress causes high levels of *HSPs* synthesis in oysters' gills, in the present study of chronic stress, the data showed that the *HSPs,* in the gills and also in the muscle tissue, were activated just three days after the start of exposure and the values continued to remain above the control threshold for the subsequent 27 days. This prolonged effect is a clear sign of thermal stress, requiring the animals to mobilize energy from their body mass and hepatopancreatic stores.

In this context, only *HSP90* was detected in the muscle, with significant overexpression after 10 days. The differential expression response to high temperatures may be due to the fact that the energy expenditure required to synthesize certain *HSPs* overrides the energy expenditure for the synthesis of other functional proteins, causing a deficiency of the latter [41,42], a mechanism which may explain why the *HSP90* isoform was not detected at 3 days. The requirement for high levels of *HSP70* and *HSP72* to combat temperature stress may inhibit the production of *HSP90.*

Another correlation between *HSPs* genes expression and physiological parameters is shown by the AST hemolymphatic concentrations. *HSP70* and *HSP72* overexpression at 5 days followed the same trend of the AST levels, suggesting a direct correlation: high temperature is a well-known cause of misfolded protein formation (AST synthesis of amino acids), inducing an overexpression of *HSP70* and *HSP72* which act as molecular chaperons to the folding of nascent and altered proteins [43,44].

However, sustained thermal stress inevitably causes an activation of pro-apoptotic factors, such as the *P53* gene, directly induced by *HSPs* [22]; in fact its expression followed the same trend as the *HSPs*, with a peak at 10 days of heat exposure.

To give an all-encompassing picture: in the first days after the beginning of the exposure, the genetic response with the overexpression of the *HSPs* at 5 days in muscle and 10 days in the gills, did not affect the biometric parameters as a tolerance response [7]. However, with the prolongation of exposure, at 30 days, significant differences in phenotype and functional characteristics of exposed organisms occurred. Although the genetic expression of *HSPs* in muscle remained above the control threshold (maybe due to the presence of constitutive forms [8]), in the gills the *HSPs* expression tend to be similar to the control values. A such reduction was also observed in the wet soft body weight,

condition index, hepatosomatic index and AST values of the exposed group, indicating a general alteration of the animal homeostasis where oysters were not able to endure chronic temperature stress.

Moreover, from the comparative analysis of the gene expression levels in two tissues, muscle and gill, an interesting point emerged: the muscle was physiologically expressing higher levels of *HSPs* in late time points (after 30 days of trial). This higher expression was amplified by the heat wave exposure. Meistertzheim et al., 2007 [8] found a tissue specific *HSPs* synthesis in the gill and in the mantle of oysters exposed to long-term thermal stress. The present study confirms that there is a tissue-specific expression, also for the muscle that is more sensitive and more responsive compared to gills to the heat waves. In general, the thermal stress alters the sarcoplasmic reticulum Ca^{2+}-ATP_{ase} (SERCA) pump present in the muscle. During this condition, the *HSPs*, especially *HSP70*, bind with the SERCA preventing its inactivation, stabilizing muscle structure and function [45]. So, the thermal stress leads the muscle to have a high complement of *HSPs*, making it less susceptible to thermal damage. At 30 days, the data of the present study showed an *HSPs* overexpression higher in muscle than in the gills (where the expression tended to decrease, in line with the phenotypic damage of the animal). From these gene responses, it could be deduced that the muscle is a tissue even more resistant than the gills, it better supports thermal stress. Consequently, *HSPs* gene expression in adductor muscle could be used in resistance and thermal tolerance studies on oysters.

Farcy et al., 2009 [22] proposed that the quantification of some cell stress genes expression, such as *HSPs*, may be used to obtain sensitive indicators of oyster environmental stress, at least for acute heat stress (37°C for 1h). The present study confirmed that expression of *HSPs* genes family may also be used as a bioindicator of health status in oyster under a prolonged thermal exposure condition.

The predicted increase in the frequency and intensity of heat waves poses an undeniable threat to oyster farming. To help farmers in mitigating the consequences of these acute climate events, it could be necessary to perform two different actions: I) investigate the potential molecular and physiological mechanisms underlying acclimation/adaptation of the pacific oyster to heat waves, in order to select the most thermal tolerance broodstock and to create a more resistant progeny. In fact, as proved in Ding et al., 2020 [10], a thermotolerant oyster population, exposed to an acute heat stress, shows high survival rate, high enzyme activity and particular expression of the genes associated with thermal tolerance; II) increase oyster thermal tolerance by performing thermal pretreatment, since, the expression of *HSPs* by thermal shock ensures greater tolerance to a second more intense thermal shock [9,39].

5. Conclusion

The results of our study show that heat waves alter the homeostasis and cellular metabolism of concave oysters: If the heat wave has a short duration (up to 10 days), oysters can be able to activate genetic and metabolic responses against stress, without compromising the weight of the soft body and the pulp yield that define the quality of the product. However, if the heat wave prolongs for up to 30 days, a reduction in the quality of the product and a possible extension of the production cycle times with negative economic consequences for the sector could be expected.

In conclusion, in order to support this sector, it is crucial to develop predictive systems capable of warning stakeholders about the arrival of heat waves Further analysis on the effects of heat waves, should be performed directly on farms to precisely define the percentage of pulp loss and the overall product quality reduction.

Declaration of competing interest

The authors confirm they have no known conflicts of interest.

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Key to figure

Figure 1. Oyster weight (A), valve weight (B), wet soft body weight (C), CI (D), HIS (E), CR (F).
Statistical analysis. 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) stand for significant differences between times for the same group. The symbols (*, †) stand for significant differences between groups at the same time.

Figure 2. Glucose (A), phosphorus (B), ammonia (C), total protein (D), aspartate transaminase (E), calcium (F), magnesium (G) hemolymphatic concentrations.
Statistical analysis. Data are reported as mean value ± S.D. Lowercase letters stand for significant differences between times for the same group (ab, group Ctrl; a'b'c', group Exp). The symbols (*, †) stand for significant differences between the groups at the same time.

Figure 3. Effect of temperature on *HSP70* (A)*, HSP72* (B)*,* and *HSP90* (C). mRNA expression in muscle tissues of the exposed group were normalized on controls for each time point.
Statistical analysis. Data are reported as mean value + SEM (calculated on ΔCq). Asterisks represent the differences between the heat wave-exposed and non-exposed group at the same time point (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure 4. The effect of temperature on *HSP70* (A)*, HSP72* (B)*, HSP90* (C), *P53* (D) mRNA expressions of the exposed group normalized on controls for each time point in gill tissues.
Statistical analysis. Data are reported as mean value + SEM (calculated on ΔCq). Asterisks represent the differences between the heat wave-exposed and non-exposed group at the same time point (* $p < 0.05$; ** $p < 0.01$).

Figure 5. Expression of *HSP70* (A), *HSP72* (B), and *HSP90* (C) in muscles normalized on gills (horizontal dotted line) in control (white columns) and heat wave exposed (black columns) groups.
Statistical analysis. Data are reported as mean value + SEM (calculated on ΔCq). Asterisks represent the differences between the muscles and the gills at the same time point (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

Figure 1

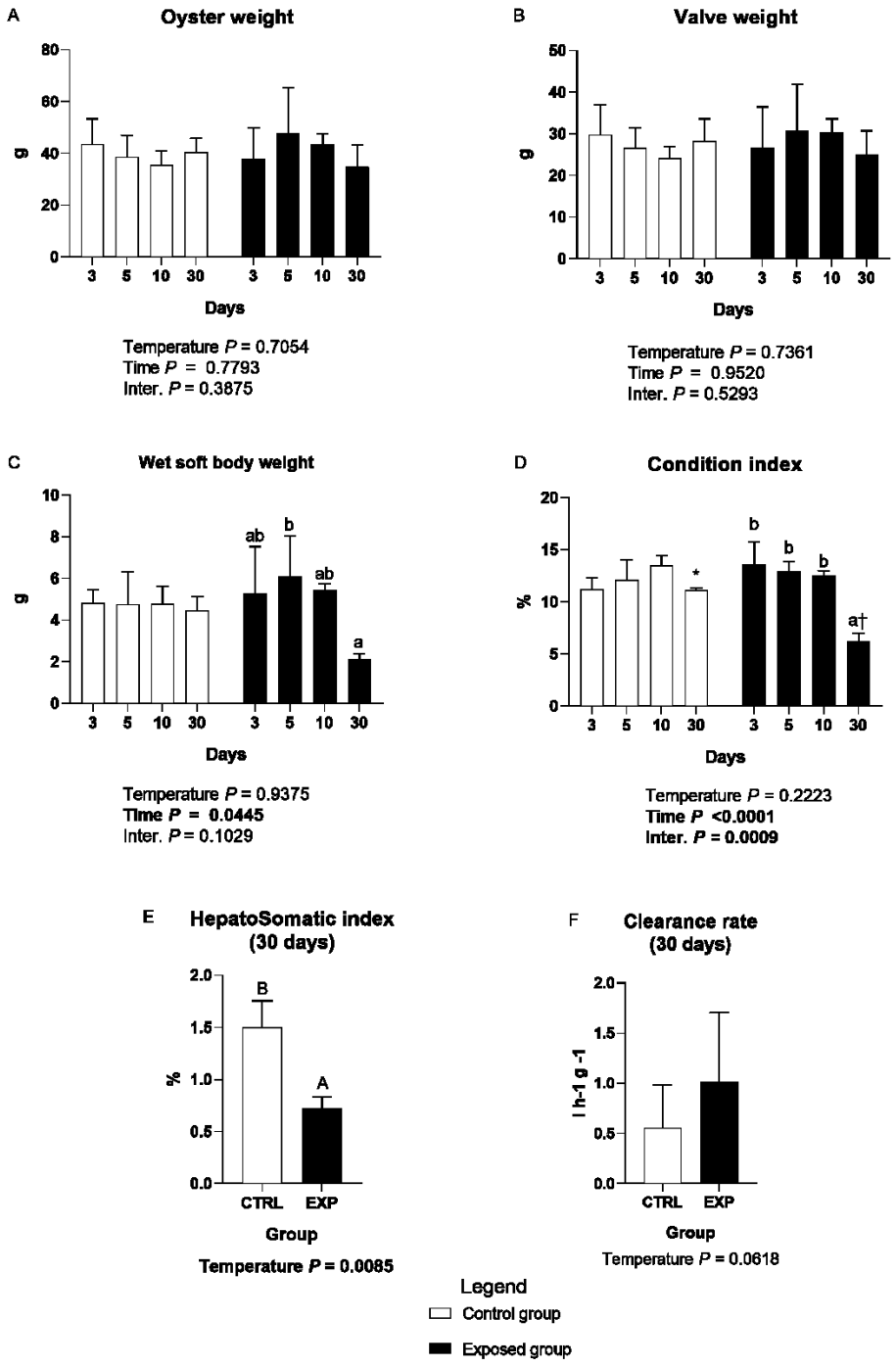

A
Oyster weight
g
Days
Temperature P = 0.7054
Time P = 0.7793
Inter. P = 0.3875
B
Valve weight
g
Days
Temperature P = 0.7361
Time P = 0.9520
Inter. P = 0.5293
C
Wet soft body weight
g
Days
Temperature P = 0.9375
Time P = 0.0445
Inter. P = 0.1029
D
Condition index
%
Days
Temperature P = 0.2223
Time P <0.0001
Inter. P = 0.0009
E
HepatoSomatic index
(30 days)
%
CTRL EXP
Group
Temperature P = 0.0085
F
Clearance rate
(30 days)
l h-1 g -1
CTRL EXP
Group
Temperature P = 0.0618
Legend
Control group
Exposed group

Figure 2

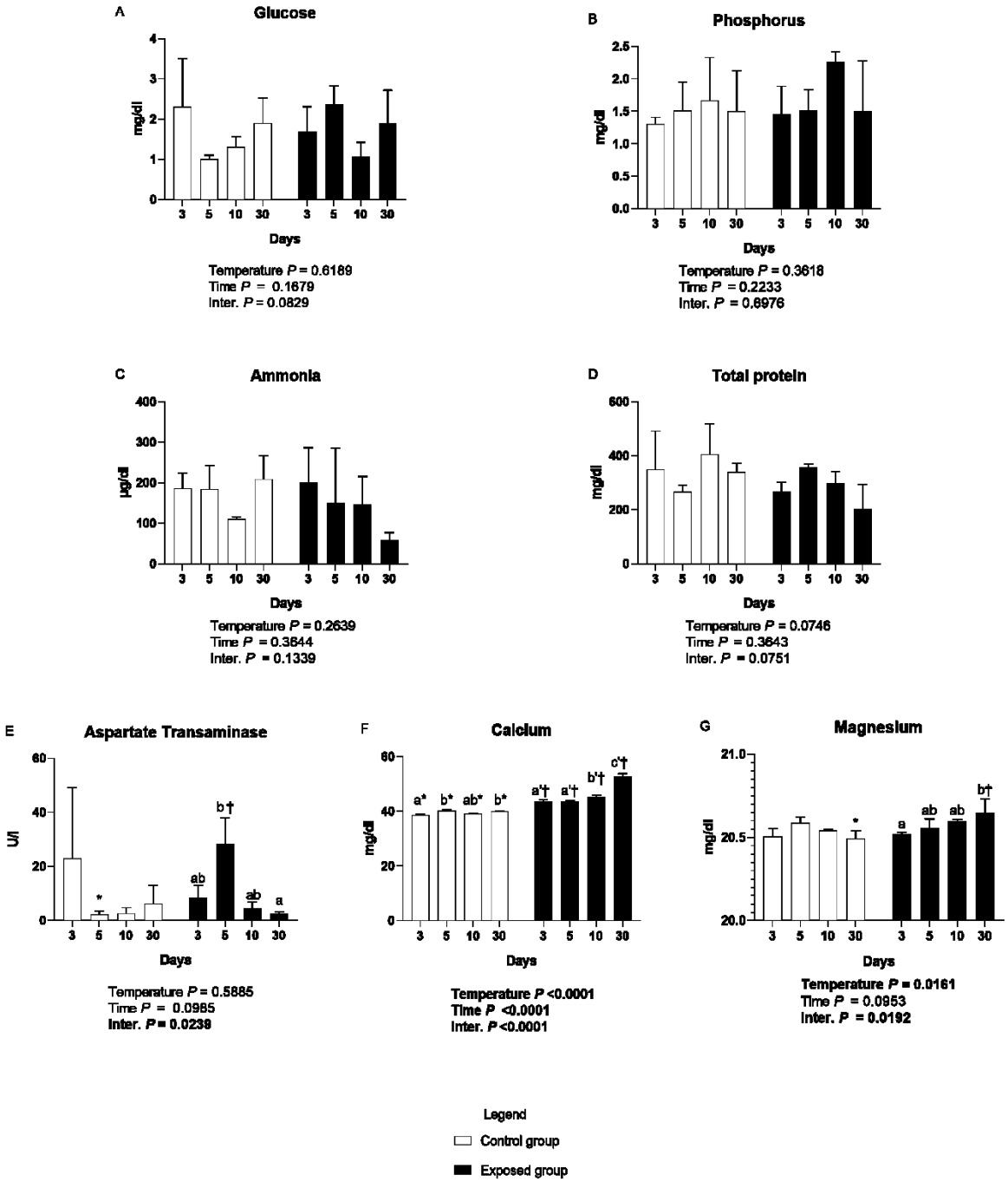

A
Glucose
mg/dl
Days
Temperature P = 0.6189
Time P = 0.1679
Inter. P = 0.0829
B
Phosphorus
mg/dl
Days
Temperature P = 0.3618
Time P = 0.2233
Inter. P = 0.6976
C
Ammonia
µg/dl
Days
Temperature P = 0.2639
Time P = 0.3644
Inter. P = 0.1339
D
Total protein
mg/dl
Days
Temperature P = 0.0746
Time P = 0.3643
Inter. P = 0.0751
E
Aspartate Transaminase
U/l
Days
Temperature P = 0.5885
Time P = 0.0985
Inter. P = 0.0238
F
Calcium
mg/dl
Days
Temperature P <0.0001
Time P <0.0001
Inter. P <0.0001
G
Magnesium
mg/dl
Days
Temperature P = 0.0161
Time P = 0.0953
Inter. P = 0.0192
Legend
Control group
Exposed group

Figure 3

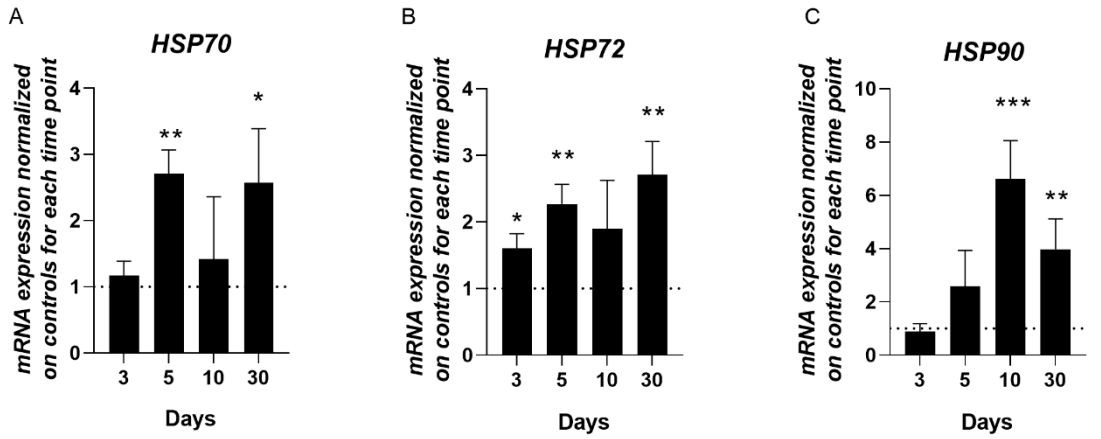


Figure 4

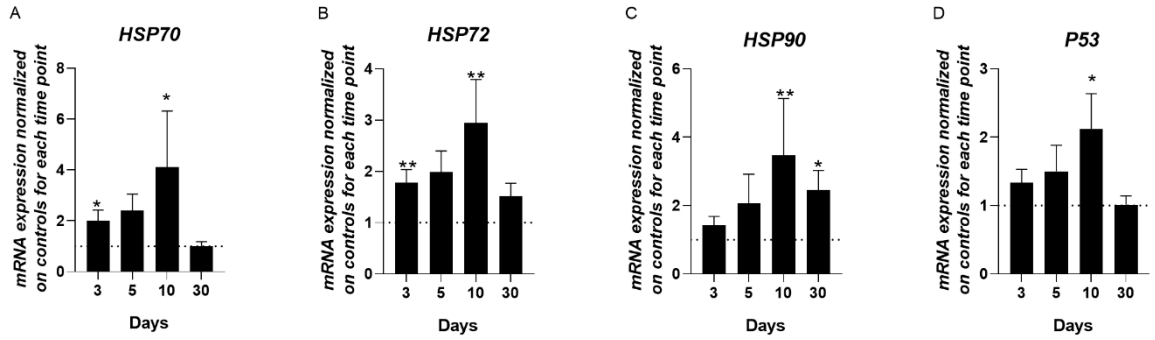
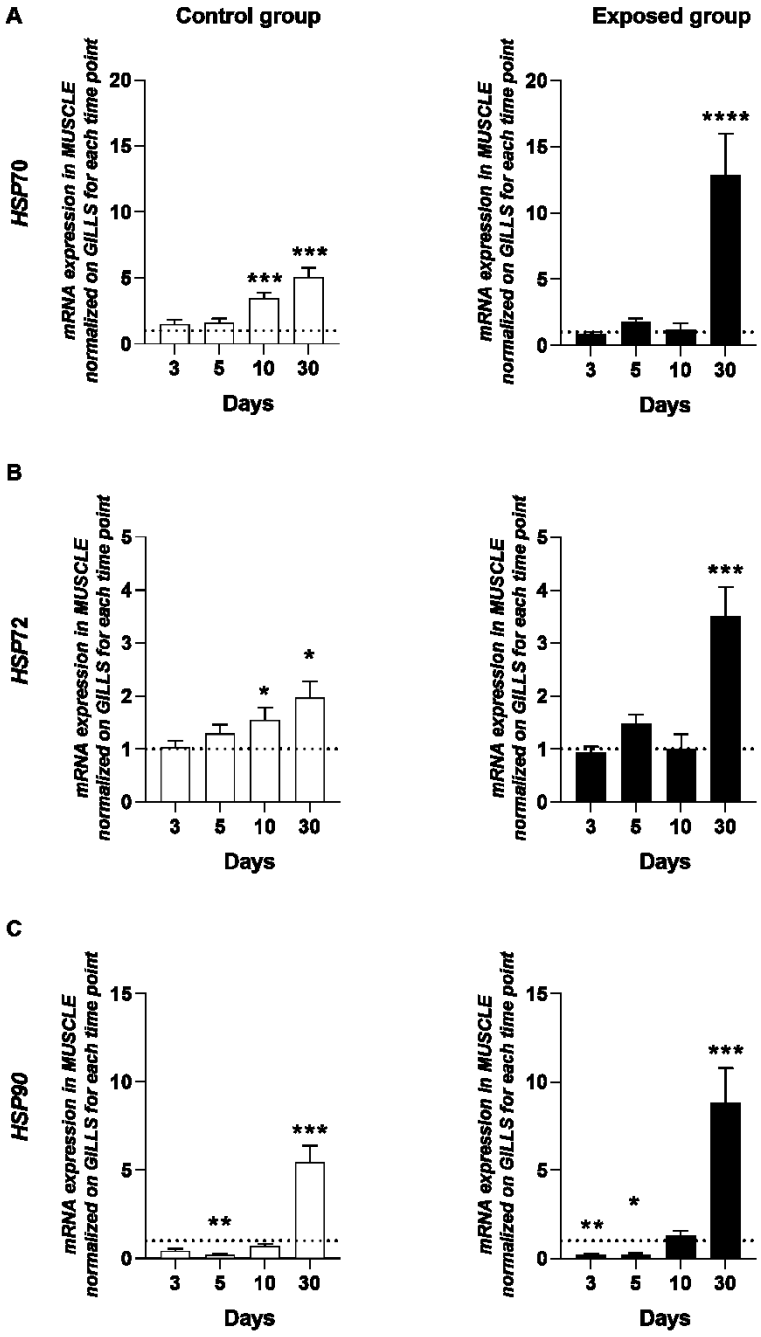
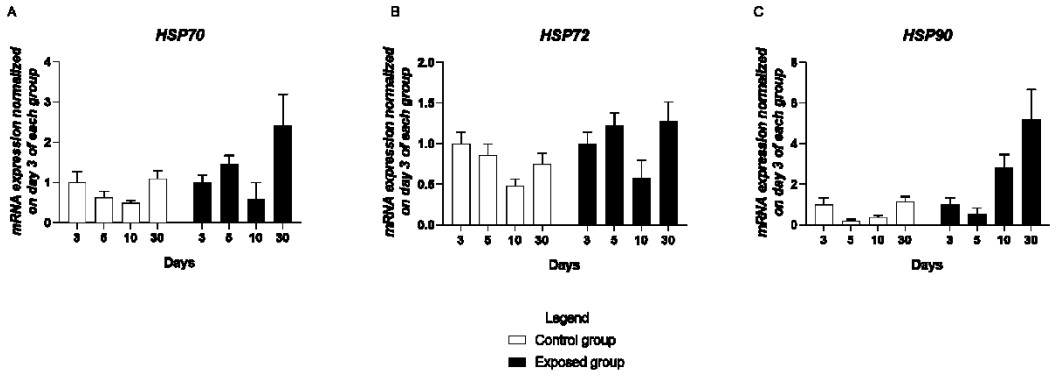


Figure 5


A
Control group
Exposed group
HSP70
mRNA expression in MUSCLE normalized on GILLS for each time point
Days
B
HSP72
C
HSP90

Supplementary Figure S1



Supplementary Figure S2