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Physiological plasticity and life history traits affect *Chamelea gallina* acclimatory responses during a marine heatwave

Letizia Iuffrida [a], Rachele Spezzano [b], Giulia Trapella [c], Nicolo Cinti [c], Luca Parma [d], Antonina De Marco [d], Giorgia Palladino [c,e], Alessio Bonaldo [d,e], Marco Candela [c,e], Silvia Franzellitti [a,e,*]

[a] Animal and Environmental Physiology Laboratory, Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, 48123, Ravenna, Italy
[b] Ocean EcoSystems Biology Unit, Marine Biological Laboratory, Woods Hole, 02543, Massachussets, United States
[c] Unit of Microbiome Science and Biotechnology, Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, 40126, Bologna, Italy
[d] Department of Veterinary Medical Sciences, University of Bologna, 40064, Ozzano Emilia, Italy
[e] Fano Marine Center, The Inter-Institute Center for Research on Marine Biodiversity, Resources and Biotechnologies, 61032, Fano, Italy

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ABSTRACT

The striped venus clam (*Chamelea gallina*) is a relevant economic resource in the Adriatic Sea. This study explored the physiological status of *C. gallina* at four sites selected along a gradient from high to low incidence of recorded historical mortality events and low to high productivity in the Northwestern Adriatic Sea. Investigations were performed during the marine heatwave in 2022 (from July to November). The optimal temperature range for *C. gallina* was exceeded in July and September, exacerbating stress conditions and a poor nutritional status, particularly at the low productivity sites. Transcriptional profiles assessed in digestive glands showed that clams from the low productivity sites up-regulated transcripts related to feeding/digestive functions as a possible compensatory mechanism to withstand adverse environmental conditions. Clams from the high productivity sites, that in a previous study showed enrichment of health-promoting microbiome components, displayed a healthier metabolic makeup (*IDH* up-regulation) and induction of protective antioxidant and immune responses. These features are hallmarks of putative enhanced resilience of the species towards environmental stress. Despite the well-known high sensitivity of *C. gallina* to environmental variations and its narrow window of acclimatory potential, results highlight that local conditions may influence physiological plasticity of this clam species and shape either positively or negatively its response capabilities to environmental changes. The identification of health-promoting endogenous mechanisms both from the animal (this study) and from its associated microbiome may provide the foundation for developing novel tools and strategies to improve clam health and production in low productivity areas or under adverse environmental conditions.

1. Introduction

The striped Venus clam, *Chamelea gallina* (Linnaeus, 1758), is a bivalve belonging to the Veneridae family. It is widely distributed along the Eastern Atlantic coast, from Norway and the British Isles to Morocco, including Madeira and the Canary Islands. It is also found in the Black Sea and the Mediterranean Sea, and it is particularly abundant in the Adriatic Sea (Carducci et al., 2020; Rufino et al., 2006), where it occupies a well-defined ecological niche determined by limited ranges of chemical and physical parameters of water column and sediments (Grazioli et al., 2022; Milan et al., 2016).

C. gallina is a high economic-valuable bivalve in Italy, contributing to 11% of the country's total fishery production by weight, and 6% in terms of revenue in 2018 and 2019 (DGPEMAC, 2019). The primary area for harvesting this clam species is the Northcentral Adriatic Sea, which accounts for over 98% of clam production at the national level (Bargione et al., 2023). Nevertheless, the high fishing pressure in this area led to a strong decline in stock abundance (Scarcella and Cabalenas, 2016),

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* Corresponding author. Animal and Environmental Physiology Laboratory, Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, 48123, Ravenna, Italy.
E-mail address: silvia.franzellitti@unibo.it (S. Franzellitti).

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further exacerbated by several mortality events that occurred in the past years (Grazioli et al., 2022). The precise causes of *C. gallina* mass mortalities remain unclear, and multiple factors, operating synergistically, should be considered, including wide and sudden fluctuations in water temperature, salinity, pH, dissolved oxygen, particulate organic matter, nutrients, and quality of sediments, that routinely occur in coastal areas of the Northern Adriatic Sea (Matozzo et al., 2012; Monari et al., 2011; Sordo et al., 2021; Vasconcellos and Ünal, 2022). Additionally, phytoplankton blooms (Romanelli et al., 2009), heightened incidences of infectious diseases (Matozzo et al., 2012; Milan et al., 2019; Torresi et al., 2011), and pollution (Guglielmi et al., 2023; Monari et al., 2011; Visciano et al., 2015) may be further potential detrimental factors for clam health status. However, the absence of a comprehensive monitoring plan over the past decades has made quite challenging to elucidate the possible connections between fluctuations of the resource and environmental variability (Carlucci et al., 2024). *C. gallina* shows relatively low stress tolerance compared to other bivalve species (Moschino and Marin, 2006), and impairment of defense mechanisms have been observed in clams from wild stocks affected by periodic mass mortality events (Milan et al., 2016). Furthermore, due to its peculiar ecological niche, *C. gallina* can only be harvested directly from natural beds. These findings highlight the importance of implementing measures to protect wild clam stocks and ensure their sustainable management.

This study focused on clam natural stocks of the Northwestern (NW) Adriatic Sea (Italy). In this area, the general circulation pattern, dominated by the North Adriatic Current originating from density gradients off the Po River delta, determines latitudinal gradients, particularly of salinity, chlorophyll, and nutrients (Cozzi and Giani, 2011; Grilli et al., 2020; Kraus et al., 2019). Despites these features sustain the large bivalve productivity of these coastal zones (Brigolin et al., 2017; Minarelli et al., 2018), they are also responsible for the remarkable environmental variability and natural stress pressure on marine fauna, which is further exacerbated by high anthropogenic pressures, and by strong climate hazards under ongoing climate changes (Hala and Bakiu, 2024). Among these, extreme events including long-lasting marine heatwaves and extreme rainfalls are projected to increase in frequency and intensity (Tojčić et al., 2024). While stress responses of *C. gallina* has been widely investigated under laboratory experiments, as triggered by temperature alone or in combination with further stressors (Matozzo et al., 2012, 2013; Monari et al., 2011; Soms-Molina et al., 2024), to our knowledge no field investigations reported the impacts of environmental stress during a heatwave on clam natural populations showing a different degree of physiological plasticity.

In this study, the physiological status of *C. gallina* was explored at four sites selected along a gradient from high to low incidence of recorded historical mortality events and low to high productivity. Investigations were performed during the exceptional heatwave that hit the Mediterranean Sea in 2022, with recorded average sea surface temperature (SST) anomalies for the North Adriatic as high as +2 °C (Marullo et al., 2023). The aim is to assess the impact of a heatwave event on striped Venus clams already facing site-specific physiological impairments. The parameters assessed in clams included biometric parameters, condition indices, and transcriptional profiles of genes related to metabolic, antioxidant, and cytoprotective/detoxification responses (which are the main processes previously explored in wild stock clams) (Carducci et al., 2020; Milan et al., 2016). Physiological data were integrated with a dataset of environmental parameters to unravel putative signatures of ongoing physiological impairments related either to the local or the global environmental threats.

2. Materials and methods

2.1. Study area and clam sampling

Clam samplings were performed at four sites within the FLAG (Fishery Local Action Group) Area Costa Emilia Romagna of the Northwestern (NW) Adriatic Sea (Italy), which covers a stretch of 130 km of coastal waters off the Emilia Romagna Region (Fig. 1) (EU Regulation No 1303, 2013). The Sites (i.e., Ravenna, Savio, Cesenatico, Rimini) were selected considering the gradient of productivity and historical trends of mortality events in the area (Carducci et al., 2020; DGPEMAC, 2019; Romanelli et al., 2009), and with technical advice of fishermen from the Co.ge.mo Ravenna Consortium that provided the technical support for sampling procedures. Ravenna (44.48333 N, 12.28633 E) and Savio (44.30277 N, 12.34763 E) fall within a low productivity district (low productivity sites), while Cesenatico (44.206367 N, 12.40463 E) and Rimini (44. 07388 N, 12.58054 E) are within a high productivity district (high productivity sites). At each Site, specimen of *C. gallina* were collected by commercial fishermen in July, September, and November 2022. For each sampling site and time point, 65 clams were collected for microbiological (Trapella et al., 2023) and physiological analyses. The clams were immediately stored in coolers at +4 °C and transferred to the laboratory, where shell length, total tissue weight, soft tissue weight, and shell weight were recorded ($N = 20$ per condition). Meat yield (soft tissue weight/total weight) and condition index (dried soft tissue weight/dried shell weight) ($N = 20$ per condition) were calculated as reported previously (Kurtay and Lök, 2023). Digestive glands (DG) were dissected from each individual clam ($N = 10$ per condition), snap-frozen in liquid nitrogen and stored at −80 °C until further analyses.

2.2. Assessment of environmental parameters

Timeseries of temperature, salinity, dissolved oxygen, and chlorophyll concentration were retrieved from E.U. Copernicus Marine Service Information (https://marine.copernicus.eu, last accessed February 2024). The temperature and salinity data were obtained from the Mediterranean Sea Physics Analysis and Forecast dataset (https://doi.org/10.25423/cmcc/medsea_analysisforecast_phy_006_013_eas8). The time series data for chlorophyll and dissolved oxygen were obtained from the Mediterranean Sea Biogeochemistry Analysis and Forecast dataset (https://doi.org/10.25423/cmcc/medsea_analysisforecast_bgc_006_014_medbfm4).

Extracted timeseries span the sampling period and extend back to January 2022. For each sampling site, data were extracted at the sea-bottom level (about 8 m, the approximate depth of clam collection) and provided as monthly means.

2.3. RNA extraction and cDNA preparation

For each animal, the whole digestive gland (about 40 mg fresh tissue) was homogenized in a suitable volume of the TripleXtractor reagent (Grisp, Porto, Portugal), and total RNA extraction was carried out using the DirectZol™ RNA MiniPrep kit (Zymo Research, Freiburg, Germany) following the manufacturer's instructions. RNA concentration and quality were confirmed using the Qubit system (Thermo Scientific, Milan, Italy) and electrophoresis with a 1.2% agarose gel under denaturing conditions. The iScript™ Reverse Transcription Supermix (Bio-Rad Laboratories, Milan, Italy) was used to synthesize cDNA from 1 μg of total RNA, following the manufacturer's instructions.

2.4. Quantitative real-time PCR (qPCR) analyses

A *de novo* transcriptome for *C. gallina* was assembled through the Trinity pipeline (Haas et al., 2013) using RNAseq experiments available at the NCBI Gene Bank database (GeneBank Ac. Numb.: SRR7957478 to SRR7957492). The *de novo* transcriptome was used together with the Chameleabase database (Coppe et al., 2012) to retrieve nucleotide sequences for the selected gene transcripts to be used for qPCR primer design using the PrimerQuest Tool (https://eu.idtdna.com/pages/tools/primerquest, last accessed March 2023). Primer pair specificities were checked both in silico and empirically by BLAST analysis and

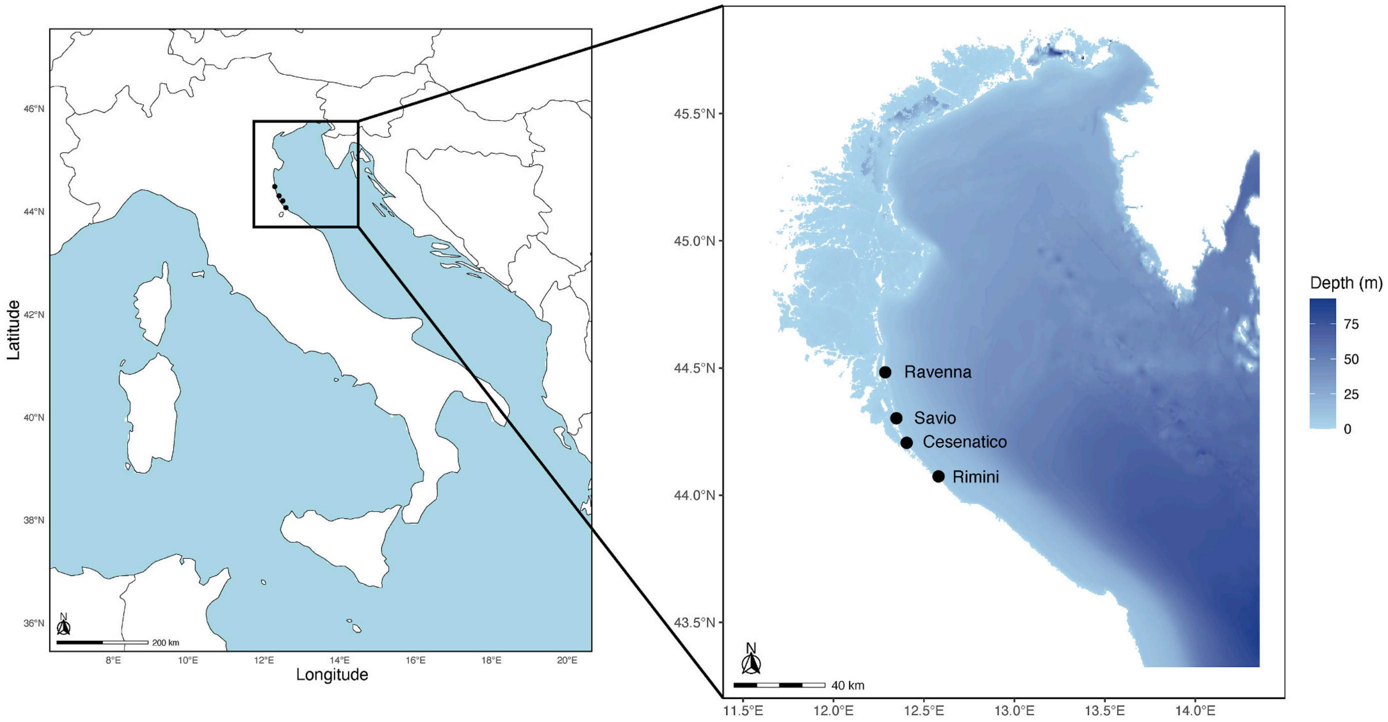

Fig. 1. Study area and location of the sampling sites in the NW Adriatic Sea (Italy). Maps were generated using the *ggplot2* and *ggmaps* packages in R (R Development Core Team, 2021). ***Colored figure is intended only for the online and PDF version.***

using melting profiles, electrophoresis, and sequencing of PCR products. The amplification efficiency of each primer pair was calculated through a cDNA dilution series (Table S1).

qPCR reactions were performed in duplicate for each sample in a final volume of 10 μL. The reaction mixture contained 5 μL iTaq Universal Master mix with ROX (BioRad Laboratories, Milan, Italy), 1 μL diluted cDNA, and 0.2 μM specific primers. To assess specificity, both a no-template control and a minus-reverse transcriptase (no-RT) control were incorporated into the qPCR analysis. Amplifications were performed in a StepOne real-time PCR system apparatus (Thermo Fisher, Milan, Italy) using a standard “fast mode” thermal protocol. Melting curves and agarose gel electrophoresis were used to verify the product for each target mRNA, ensuring specificity and confirming the absence of artifacts. *EF1* and *TUB* were selected as reference genes to normalize qPCR data following the procedure previously described (Balbi et al., 2016). Relative expression values of target mRNAs were determined using the comparative delta C_T method (Schmittgen and Livak, 2008) and analyzed with the StepOne and the DataAssist softwares (Thermo Fisher, Milan, Italy). Data are reported as relative expression (fold change) with respect to a reference sample (Ravenna - July).

2.5. Statistical analyses

Data visualization and graphics were performed using the *ggplot2* package in R (R Development Core Team, 2021). Environmental parameters, biometric parameters of clams, and qPCR data were analyzed by a 2-way permutation multivariate analysis of variance (PERMANOVA) using PRIMER v6 (Anderson et al., 2008) to test for variations among Sites and sampling Months. Log-transformed variations of variables were used to calculate similarity matrices based on the Euclidean distance (999 permutations). When the PERMANOVA main tests revealed statistical differences ($P < 0.05$), PERMANOVA pairwise comparisons were carried out. Principal-component analysis (PCA) of normalized environmental parameters was performed with the *stats* package in R (R Development Core Team, 2021) to examine possible gradients across the study sites. Distance-based redundancy linear modeling (DistLM) with a test of marginality in PRIMER was also performed to account for the contribution of environmental parameters in explaining the observed variances in the biometric and gene transcription datasets. DistLM used the BEST selection procedure and Akaike information criterion adjusted for small sample sizes (AICc) selection criterion. In all cases, statistical differences were considered significant when $P < 0.05$.

3. Results

3.1. Environmental parameters

The extraction of time series was made for 11 months, from January to November 2022. The timeseries of the monthly means and violin plots showing variations of temperature, salinity, dissolved oxygen, and chlorophyll at the sampling months are shown in Fig. 2. The onset of the heatwave was set in May 2022 (Marullo et al., 2023), where monthly trends showed a sudden increase of sea temperatures (Fig. 2A), paralleled by remarkable increase of salinity levels (Fig. 2B) and decrease of dissolved oxygen (Fig. 2C) and chlorophyll concentrations (Fig. 2D) at all sampling Sites.

The variability of the environmental parameters at the sampling timepoints was analyzed through PERMANOVA, which demonstrated that the individual factors "Site" and "Month" exerted a significant effect on all parameters, except for the effect of "Site" on temperature ($P < 0.05$; Table S2). For all the assessed parameters, a significant interaction between the factors ($P < 0.05$; Table S2), except for chlorophyll, is reported. In July, water temperature was significantly lower in Rimini compared to the other three study sites (Fig. 2A). In November, significantly lower temperatures were observed in Savio (Fig. 2A). Salinity showed complex patterns across the sampling months. In July, average values recorded in Ravenna were significantly lower compared to the other sites (Fig. 2B). In September, a salinity gradient was observed from north to south, with all sites exhibiting significant differences from one another (Fig. 2B). In November, the low productivity sites (Ravenna and Savio) were characterized by significantly lower salinity values

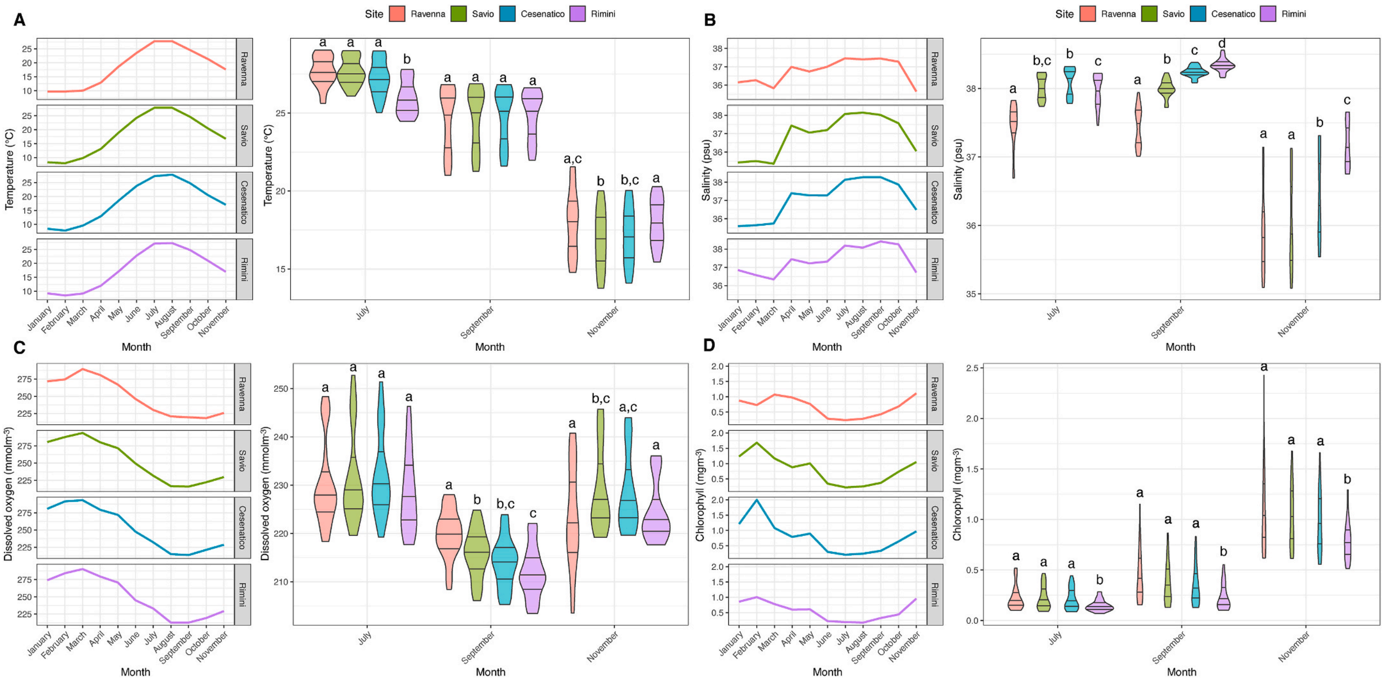

Fig. 2. Timeseries of seawater parameters recorded at the sampling sites. (A) temperature; (B) salinity; (C) dissolved oxygen; (D) chlorophyll. For each parameter, left-side graphs display trends of monthly mean between January and November 2022. Violin plots show variations of the parameters at the sampling time months (lines within violins indicate median, 25th and 75th percentiles). Different letters indicate statistical differences between Sites within each sampling time Months ($P < 0.05$). ***Colored figure is intended only for the online and PDF version.***

compared to the high productivity sites (Cesenatico and Rimini) (Fig. 2B). Dissolved oxygen measured in September showed a decrease from north to south, with Ravenna and Savio showing significantly higher values than Rimini (Fig. 2C). In November, Savio showed higher dissolved oxygen concentrations compared to Ravenna and Rimini (Fig. 2C). Finally, chlorophyll values in Rimini were significantly lower compared to the other sampling sites in all sampling months (Fig. 2D).

For all parameters, further pairwise comparisons between different sampling months within the sampling sites are reported in Table S3.

The Principal Component Analysis (PCA) showed clustering along the primary (PC1) and secondary (PC2) axes, which accounted for 97% of the total observed variance (Fig. S1). The permutation test showed that both PCA components were significant ($P < 0.05$). A clear clustering by sampling month was observed, mainly correlated with temperature and chlorophyll variations. Within each cluster, slight Site-related differences related to dissolved oxygen and salinity were observed (Fig. S1).

3.2. *C. gallina* biometric parameters and calculated physiological indices

Fig. 3 shows variations of *C. gallina* biometric parameters and calculated meat yield and condition index at the different sampling sites and months. All parameters showed significantly lower values in low productivity *vs* high productivity sites (Fig. 3A–E).

Results from PERMANOVA analyses demonstrated that the single factors “Site” and “Month” had a significant effect on all parameters except for “Month” on meat yield and condition index ($P < 0.05$; Table S4). Furthermore, PERMANOVA analyses showed a significant interaction between the factors ($P < 0.05$; Table S4). Pairwise comparisons within each sampling month showed that in July and September values of shell length, shell weight, and soft tissue weight of clams collected in Rimini were significantly higher compared to other sampling sites (Fig. 3A–C). In November, values of the same biometric parameters detected in both high productivity sites (Cesenatico and Rimini) were significantly higher than those assessed in Ravenna and Savio ($P < 0.05$) and not significantly different between each other ($P > 0.05$) (Fig. 3A–C). Calculated meat yield and condition index of clams collected in Ravenna in July and September were significantly lower than those assessed in the other sites, while values recorded in clams from Rimini in September were significantly higher than those assessed in clams from the other sites (Fig. 3D–E). Finally, calculated meat yield and condition index values of clams collected in Rimini in November were significantly lower than those assessed in the other sites (Fig. 3D–E).

For all parameters further pairwise comparisons between different sampling time months within the sampling sites are reported in Table S5.

Distance-based linear model (DistLM) analysis (Fig. 3F) revealed that temperature and dissolved oxygen mainly account for differences between sampling months, that explained about 40% total variation, while salinity and chlorophyll accounted for about 11% total variation explaining the observed changes in biometric parameters across sampling sites. The tests of marginality from DistLM analyses, performed on separate low productivity and high productivity sites datasets by considering environmental parameters, showed that in low productivity sites dissolved oxygen explained most of the variation (about 68%) of the observed biometric parameters and physiological indices, with contribution of the other environmental parameters being not statistically significant ($P > 0.05$; Fig. 3G). Chlorophyll, salinity, and water temperatures cumulatively explained about 90% of the variation of biometric parameters and physiological indices in high productivity sites ($P > 0.05$; Fig. 3G).

3.3. *C. gallina* transcriptional profiles in digestive glands

Variations of *C. gallina* gene transcription profiles at the different Sites and sampling Months are reported in detail in Fig. 4. *IDH*, *PK*, and *GST* showed significantly lower values in low productivity vs high productivity sites (Fig. 4B,C,E).

Results from PERMANOVA analyses demonstrated that at least one amongst the single factors “Site” and “Month” had a significant effect on all gene transcriptional profiles except *AMYL* ($P < 0.05$; Table S6).

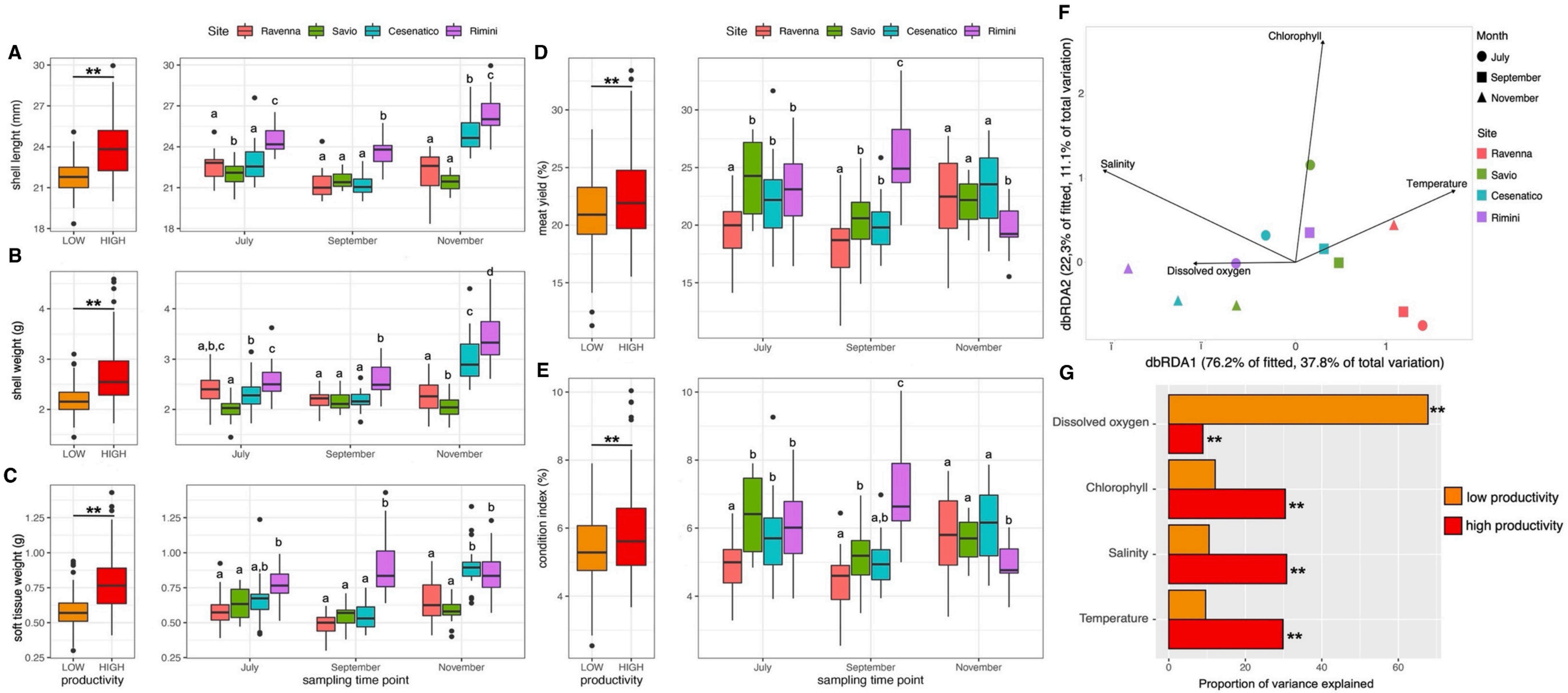

Fig. 3. Biometric parameters and physiological indices assessed in *C. gallina* from low and high productivity sites in the NW Adriatic Sea during the 2022 heatwave. (A) Shell length, (B) shell weight, (C) soft tissue weight, and calculated (D) meat yield (D) and (E) condition index. For each parameter, left-side box plots display the overall observed values in low productivity and high productivity sites (*$P < 0.05$). Right-side box plots show the variability of the parameters across sampling Sites and Months. Different letters indicate statistical differences between Sites within sampling time Months ($P < 0.05$). (F) Redundancy Analysis (RDA) plot of the Distance-based Linear Model (DistLM) analysis. Symbols indicate the mean value for each sampling month and site. (G) Results from the tests of marginality related to the DistLM analysis showing contribution of each environmental parameter to the total variance observed in low productivity and high productivity site datasets of biometric parameters and meat yield and condition index. DistLM used the BEST selection procedure and Akaike information criterion adjusted for small sample sizes (AICc) selection criterion. ***Colored figure is intended only for the online and PDF version.***

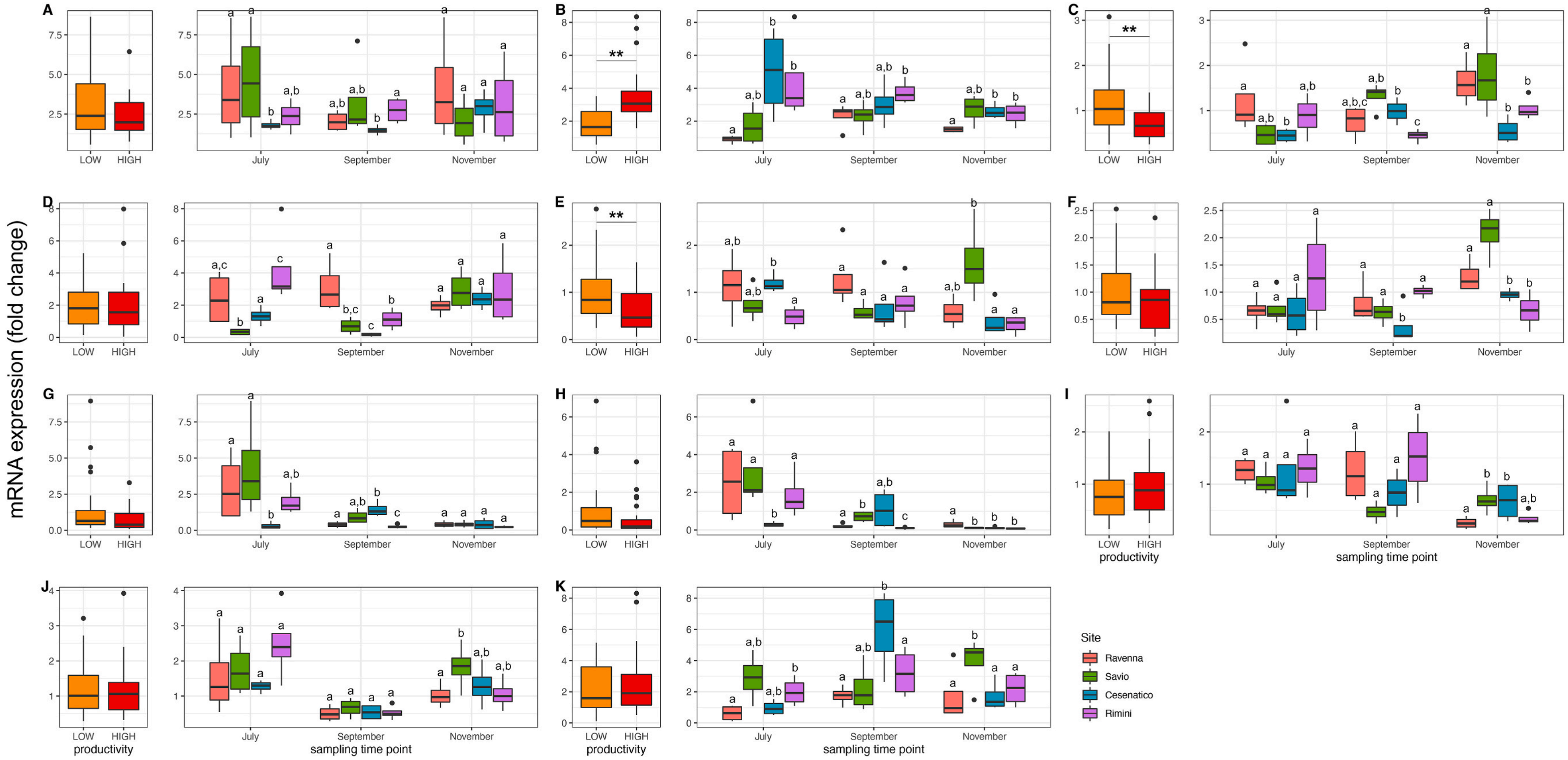

Fig. 4. Transcriptional profiles of metabolic (*AMYL*, A; *IDH*, B; *PK,* C), antioxidant (*CAT,* D; *GST*, E; *SOD*, F) and cytoprotective/detoxification (*ABCC*, G)*; LYS*, H*; MT10*, I; *HSP90*, J*; CSTL*, K) gene products assessed in digestive glands of *C. gallina* from low and high productivity sites in the NW Adriatic Sea during the 2022 heatwave. For each transcript,box plots on the left side display the overall expression levels in samples from low productivity and high productivity sites (*P < 0.05). Box plots on the right side show the expression levels across sampling timepoints in the different sites. Different letters indicate statistical differences between Sites within sampling Months (P < 0.05). Full gene transcripts names are reported in Table S1. ***Colored figure is intended only for the online and PDF version.***

Furthermore, PERMANOVA analyses showed a significant interaction between the factors for all gene transcriptional profiles except *AMYL and HSP90* ($P < 0.05$; Table S6).

All gene products showed complex transcriptional patterns across sites and sampling months (Fig. 4A–K). Pairwise comparisons within each sampling months showed that low productivity sites (Ravenna and Savio) displayed significant over expression for *AMYL*, *ABCC*, *LYS* in September, and for *PK, GST* (Savio), *SOD, MT10* (Savio), and *CTSL* (Savio) in November (Fig. 4). High productivity sites (Cesenatico and Rimini) displayed significant over-expression for *IDH* and *SOD (*Rimini) in July (Fig. 4). For all gene transcripts further pairwise comparisons between different sampling time months within the sampling Sites are reported in Table S7, highlighting significant variations for some gene transcripts according to sampling month.

Distance-based linear model (DistLM) analysis (Fig. 5A) performed on the whole dataset revealed that temperature and chlorophyll mainly account for differences between sampling Months, that explained about 22% total variation, while salinity and dissolved oxygen accounted for about 11% total variation explaining the observed changes in transcriptional profiles across sampling Sites. Fig. 5B reports the tests of marginality from DistLM analysis performed on separate low productivity and high productivity sites gene transcription datasets by considering environmental parameters. Results showed that for transcriptional profiles all the environmental parameters almost equally contribute explaining the total variance observed either in the low productivity or in the high productivity site dataset ($P < 0.05$; Fig. 5B).

4. Discussion

During 2022, the Mediterranean Sea experienced an unprecedented persistent and intense marine heatwave event. The basin average SST anomaly was 0.8 ± 0.3 °C in 2022, well above the value of 0.5 ± 0.2 °C recorded in 2021 (Marullo et al., 2023). The Mediterranean SST warming started in May 2022, when the mean anomaly increased abruptly from 0.01 °C (April) to 0.76 °C (May), reaching the highest values between June and July (about 1.7 °C) (Marullo et al., 2023). Results of this study showed that in the NW Adriatic Sea, the 2022 heatwave generated peak temperatures in July as high as about 29 °C, a high salinity (about 38 psu), and chlorophyll concentrations as low as 0.1 mg/m^3. These values are the result of the prolonged drought and extremely low riverine inputs of freshwater and nutrients related to a concomitant major atmospheric heatwave over Europe (Faranda et al., 2023). Within this general scenario, a differentiation of environmental conditions between the *C. gallina* low productivity and high productivity sites of the NW Adriatic Sea assessed in this study can be observed. Indeed, the PCA analysis showed that high productivity sites, Cesenatico and Rimini, cluster together in all sampling timepoints and are clearly differentiated by the low productivity site of Ravenna. The Savio site had a distinct pattern, clustering with Cesenatico and Rimini in July and adopting an intermediate position in the remaining two months. These

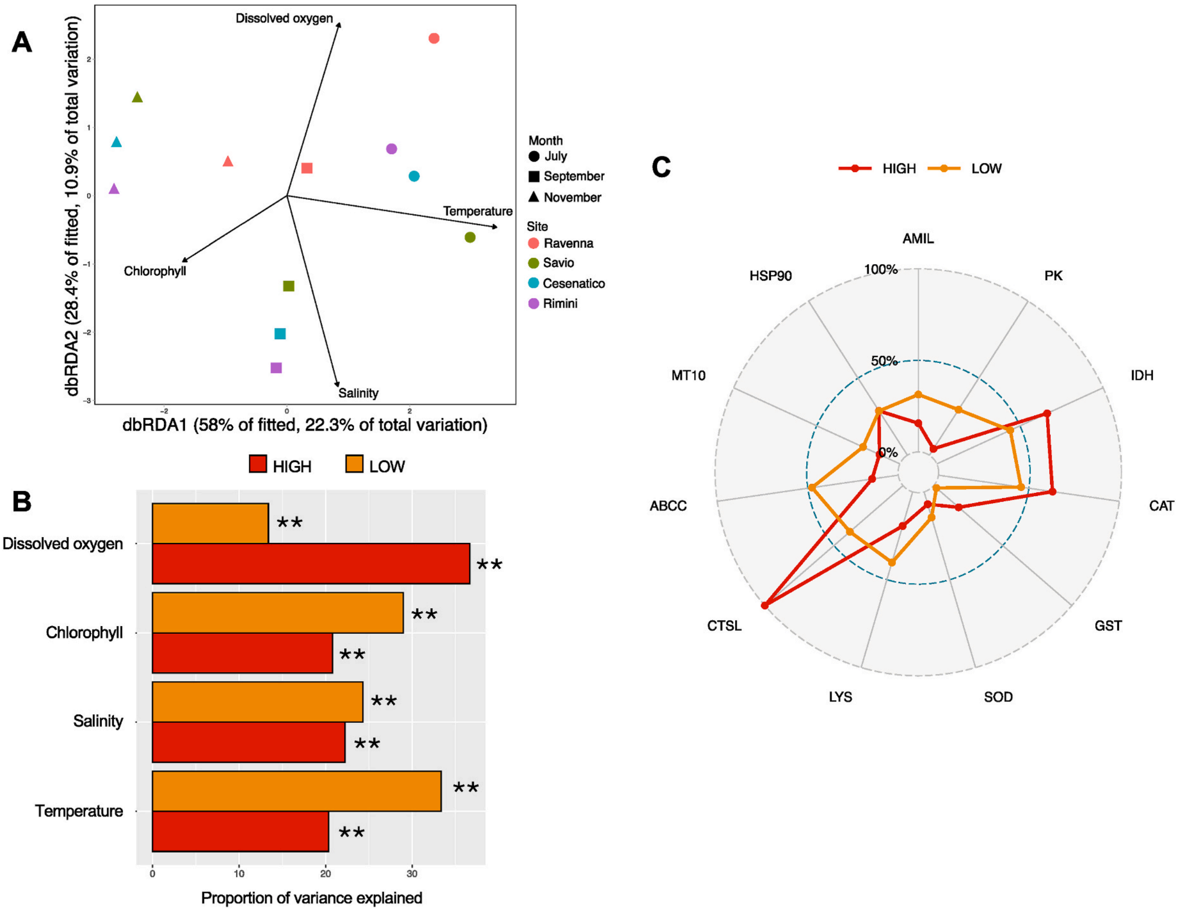

Fig. 5. Multivariate analyses of gene transcription data. (A) Redundancy Analysis (RDA) plot of the Distance-based Linear Model (DistLM) analysis**.** Symbols indicates means for each sampling site and month. (B) Results from the tests of marginality related to the DistLM analysis showing contribution of each environmental parameter to the total variance observed low productivity and high productivity site datasets of gene transcription profiles. DistLM used the BEST selection procedure and Akaike information criterion adjusted for small sample sizes (AICc) selection criterion. Asterisks indicate level of statistical significance related to the result (**$P < 0.01$). (C) Radar plots that summarize Month-related transcriptional changes in digestive glands of *C. gallina* from low productivity and high productivity sites (geometric means). For each endpoint, month-related variation is expressed by the normalized Area Under the Curve (AUC) (Wathsala et al., 2018). ***Colored figure is intended only for the online and PDF version.***

Site-related variations of environmental parameters seem mainly correlated with salinity and dissolved oxygen latitudinal patterns, whereas patterns of water temperatures and chlorophyll dominate within the Month-related data clustering.

It is known that *C. gallina* has a very narrow window of optimal environmental conditions, in particular concerning temperature, dissolved oxygen, and salinity (Moschino and Marin, 2006). For example, the optimum of water temperature for its growth ranges between 10 °C and 27 °C (Bargione et al., 2021; Moschino and Marin, 2006). Temperatures only slightly out of this range are known to reduce clam growth and impair reproduction (Bargione et al., 2021; Moschino and Marin, 2006). Such a narrow acclimatory capability may explain the observed differences in the overall biometric parameters and physiological indices. Clams from the low productivity sites have significantly lower overall values of biometric parameters as well as meat yield and condition index, thus suggesting that environmental conditions at the low productivity sites are generally less favorable for clam somatic growth and health status compared to those at the high productivity sites. Furthermore, natural stocks of *C. gallina* in the study area have already experienced several severe mortality events. For example, the present-day stocks are recovering from the 2018 mass mortality that generated a differential reduction of clam production between the Ravenna compartment and the Rimini compartment (DGPEMAC, 2019). Signatures of these peculiar life history traits may contribute to the different clam physiological status between these compartments. Time course variations of the biometric parameters across the sampling period pointed out that environmental conditions assessed at all sampling sites in July and September (when the influence of the heatwave was at its maximum) may have contributed to reduce the Site/productivity -related variations of biometric parameters, as no significant differences were observed among the sampling sites at the July sampling time point, except for shell length and soft tissue weight from clams collected in Rimini. It must be observed that seawater temperatures recorded in June and July overrode the upper thermal limit (about 27 °C) afterwhich clam growth is reduced and reproduction is inhibited (Moschino and Marin, 2006), although in Rimini water temperatures were on average about 1 °C lower than values assessed at Cesenatico, Savio, and Ravenna. This may combine with the very low water concentrations of chlorophyll, a proxy for food availability, to produce a situation of generalized stress and poor nutrional status for the clams. In November, when water temperature decreased back to about 18 °C and chlorophyll concentration increased at all sites, biometric parameters accounted for the signifcant physiological differences between low productivity and high productivity sites.

The physiological indices meat yield and condition index are widely used as metrics to evaluate bivalve fitness and physiological status (Andral et al., 2004; Kanduč et al., 2018). Although it is known that conditions experienced by bivalves during heatwaves may cause a reduction in the condition index and in the energy reserves (De Marco et al., 2023; Lattos et al., 2023), in this study, clams collected at the different sampling sites showed different Month-related patterns for these parameters. In general, Ravenna showed a significant increase in these parameters in November compared to July/September, indicating an improvement of clam physiology as the environmental conditions became more favorable (in particular, temperature). Rimini showed an opposite trend compared to Ravenna, while Cesenatico and Savio showed more stable meat yield/condition index values. Although in this study no reproduction related parameters have been assessed, we can hypothesize the influence of the reproductive cycle progression, at least for clams collected in Rimini. In the Adriatic Sea, *C. gallina* spawning commonly occurs from April to September, with some additional events in early autumn (Bargione et al., 2021; Grazioli et al., 2022). Furthermore, *C. gallina* showed an opportunistic reproductive strategy, as spawning takes place only when conditions are suitable for the development of planktotrophic larvae, i.e. when phytoplankton is abundant (García-Fernández et al., 2024), and the water temperature ranges from 18 °C to 27 °C (Bargione et al., 2021). It must be hypothesized that in Rimini the July/September environmental conditions were not so extreme as in Ravenna or in the other sites; therefore, gonad development was not impaired and spawning could take place in November sampling time-point at this Site, as suggested by the significant decrease of meat yield and condition index. This finding is in agreement with a prevailing effect of spawning on utilization of energy reserves, which are depleted from growth (Hong et al., 2020; Mladineo et al., 2007; Moschino and Marin, 2006).

A previous investigation showed the relevant metabolic and stress response pathways related to health status of *C. gallina* collected from sites affected by events of reduced productivity (Milan et al., 2016). Specifically, clams sampled across a 3-months period showed permanent immune response activation in DGs, increased protein turnover (via up-regulation of several ribosomal proteins and the lysosomal proteolytic enzyme cathepsin L), and reduced energy metabolism (mainly the glycolytic pathway). It has been proposed that individuals may need to allocate part of their energy reserves to cope with the long-lasting environmental stress condition or to sustain immune responses causing the degradation of the energy balance. Furthermore, local variation in food quality or even declined feeding activity induced by deterioration of animal health may be considered as possible physiological mechanisms related to the observed molecular outcomes (Milan et al., 2016). Accordingly, results reported in this study showed variations of some gene transcripts related to metabolism, antioxidant responses, and lysosomal functions that may be consistent with the above findings. The radar plots (Fig. 5C) that summarize month-related transcriptional changes in DGs, showed that transcriptional profiles in clams from the low productivity sites are characterized by differential expression of the metabolism related gene transcripts *AMYL* and *PK*, of the lysozyme *LYS*, and of the ABC transporter *ABCC*. Alpha-Amylase (*AMYL*) and pyruvate kinase (*PK*) are key enzymes in glycolytic metabolism (Canesi et al., 2007; Liu et al., 2017), and acknowledged biomarkers of mussel digestive functions (Connor et al., 2016). Their over-expression in clams from Ravenna and Savio in July may sign the induction of glycolysis and digestive activity as a requirement from increased energy budget for acclimation to the challenging environmental conditions. This finding is further corroborated by modulation of *LYS* and *ABCC* in these same clams. Although the primary role attributed to lysozymes in bivalves is the defense of the host in the circulating hemolymph, a digestive function for some *LYS* isoforms in the digestive gland has been suggested (Xue et al., 2010, and reference therein). *ABCC* encodes a Multidrug Resistance-Related Proteins (MRP), an ABC transporter pivotal in marine bivalve Multixenobiotic resistance system (Franzellitti et al., 2016). Its expression and transport activity can be triggered as a part of a generalized response to food ingestion and stimulation of digestive and immune functions, as previously observed in marine mussels (Franzellitti et al., 2019).

Transcriptional profiles assessed in DGs of clams from the high productivity sites across the heatwave are dominated by over-expression of transcripts encoding a NADP-dependent isocitrate dehydrogenase (*IDH*), catalase (*CAT*) and cathepsin-L (*CTSL*). *IDH* catalyzes oxidative decarboxylation of isocitrate to α-ketoglutarate in the Krebs' cycle. A primary function of *IDH* is to supply reduced NADP (NADPH) for the antioxidant systems (Yang et al., 2002), and the biosynthetic pathways leading to the production of nucleic acids and lipids (Al-Khallaf, 2017). The concomitant *CAT* over-expression corroborates the potential stimulation of the antioxidant system. The lysosomal enzyme Cathepsin-L (*CTSL*) is involved in intracellular protein catabolism, and it is a component of the autophagic pathway (Xue et al., 2010). Therefore, its over-expression may indicate stimulation of the clam innate immune system through a *CTSL*-dependent lysosomal proteolytic pathway, as observed in mussels (Romero et al., 2022). A previous study revealed that DG associated microbiome in clams from the high productivity site of Rimini was enriched of microorganisms capable of biosynthesizing health-promoting cofactors and vitamins, such as pyridoxine, folate, riboflavin, and the antioxidants and steroid-precursors terpenoids (Trapella et al., 2023). The healthier physiological performances of the Rimini clams under the heatwave would have been supported by this health promoting components of the DG microbiome, that may boost antioxidant, immune, and health-promoting metabolic processes in clam hosts, while reducing the need for digestive functions. The influence of endogenous factors on modulating gene transcriptions is further supported by the DistLM analysis, which indicated that only the 34% of total variation in the gene transcription dataset might be explained by environmental parameters profiles, suggesting the involvement of additional factors in regulating these transcripts. These factors may further include the widespread pollutants distributed in coastal habitats of the NW Adriatic Sea. In particular, sediments are reported to be a source of metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides and pharmaceuticals for benthic organisms (Barra et al., 2020; Combi, 2020; Combi et al., 2016; Maggi et al., 2022). Indeed, different types of pollutants have been reported in soft tissues of mussels collected in farms located in the same areas of clams sampled in this study (Gomiero et al., 2019; Interino et al., 2023; Palladino et al., 2023). Both sediment and mussel investigations show north to south trends of pollutant concentrations, but also local enrichments, likely reflecting the significant riverine inputs from the Italian border, the coastal-based human activities, as well as the hydrological features of the Adriatic Sea. Although the focus of the project presented in this study was not on pollutant survey and effects, previous microbiome data (Trapella et al., 2023) suggested that pollutants in sediments from the low productivity sites (e.g., Ravenna) may negatively interact with clam DG-associated microbial components causing the onset of dysbiosis, a condition increasingly associated with pathogenesis and impaired clam health status (Iannello et al., 2021; Peruzza et al., 2023).

5. Conclusions

This study provides a snapshot of the physiological status and acclimatory potential of *C. gallina* from some high and low productivity sites of the NW Adriatic Sea across the 2022 heatwave event. As such, the results of this study may not be universally applicable to all populations of *C. gallina*, since physiological responses may vary significantly between different geographical regions and environmental contexts. However, this study may provide a valuable example of how this clam species, which is well-known for its sensitivity to environmental variability and its narrow range of acclimation, may indeed be affected either positively or negatively by local conditions that shape its resilience to environment changes. Clams from low productivity sites showed enhanced transcripts related to feeding/digestive functions as a possible compensatory response to withstand the physiologically challenging environmental conditions at the peak of the heatwave. Clams from high productivity sites are endowed with health promoting DG microbiome components (Trapella et al., 2023), that may have supported a healthier metabolic makeup (i.e. synthesis of NADPH metabolic intermediate through *IDH* activity) and the effective induction of protective mechanisms (antioxidant and immune responses) at the peak of the heatwave. These features may be addressed as a hallmark of enhanced resilience of the species towards environmental stress, that allowed clams at these sites to maintain their fitness despite the adverse environmental conditions (as suggested by trends of meat yield and condition index).

The oceanographic models project that the Adriatic Sea will run towards an increase of average SSTs and incidence of extreme events faster than the whole Mediterranean Sea (Tojčić et al., 2024). Furthermore, local anthropogenic threats make coastal habitats in this area remarkable models to study the impacts of global and local environmental changes on Mediterranean marine commercial fauna (Capolupo et al., 2017; Iuffrida et al., 2025; Palladino et al., 2023). In this scenario, the identification of health-promoting endogenous mechanisms either from the animal side (this study) or from its associated microbiome (Trapella et al., 2023) would aid in developing novel tools and strategies to improve the physiological conditions of *C. gallina* at the low productivity sites and/or during the onset of adverse environmental conditions, thereby sustaining animal well-being and clam production. Future research should build on this study by incorporating long-term monitoring and broader geographic coverage to address the chronic biological impacts of (repeated) extreme events and climate changes on *C. gallina* physiological resilience and, tentatively, forecast mitigation solutions.

CRediT authorship contribution statement

Letizia Iuffrida: Writing – original draft, Formal analysis, Data curation. **Rachele Spezzano:** Writing – original draft, Formal analysis, Data curation. **Giulia Trapella:** Writing – original draft, Methodology, Formal analysis, Data curation. **Nicolo Cinti:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Luca Parma:** Writing – original draft, Investigation, Funding acquisition, Data curation, Conceptualization. **Antonina De Marco:** Writing – original draft, Formal analysis, Data curation. **Giorgia Palladino:** Writing – original draft, Project administration, Formal analysis, Data curation. **Alessio Bonaldo:** Writing – original draft, Investigation, Formal analysis, Data curation. **Marco Candela:** Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Silvia Franzellitti:** Writing – original draft, Supervision, Funding acquisition, Formal analysis, Data curation, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.120287.

Data availability

Data will be made available on request.

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