UNIVERSITÀ DEGLI STUDI DI PADOVA

Department of Comparative Biomedicine and Food Science
First Cycle Degree (B.Sc.) in Animal Care

Exploring Climate Change Effects on Clams: Behavioural Indicators and Priming-Based Mitigation

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1. Abstract

Climate change is altering marine ecosystems at an unprecedented pace. The rapid increase in sea-surface temperature, ocean acidification, and the intensification of marine heatwaves (MHWs) are disrupting metabolic and ecological balances in aquatic species. Bivalves, being sedentary filter feeders that directly interact with both the water column and sediments, are particularly vulnerable. This thesis explores how mild “priming” treatments such as thermal and chemical, may enhance clam tolerance to heat stress while offering new behavioural tools to monitor welfare under extreme climatic events.

Experiments were carried out at the University of Padova using commercially sourced clams (*Ruditapes philippinarum*). Individuals were subjected to controlled priming exposures: Thermal (30 °C) and hydrogen-peroxide (H_2O_2) at a concentration of 50 μM chemical priming, followed by a simulated heatwave challenge. A behavioural “burrowing test” was developed to evaluate vitality and welfare, and videos were analysed frame-by-frame to obtain quantitative measurements.

This project represents the first applications of chemical priming in live clams and demonstrates the feasibility of behavioural welfare metrics for invertebrates. The work contributes to the MANILA project (*Priming and Microbiota Manipulation to Mitigate Shellfish Vulnerability*), linking physiology, behaviour, and applied aquaculture management.

2. Introduction

2.1 Climate change and marine ecosystems

Since the start of the industrial era, atmospheric CO_2 concentrations have risen from about 280 ppm to over 420 ppm (IPCC, 2021). More than 90% of the resulting heat imbalance has been absorbed by the oceans, producing a steady increase in sea-surface temperature and a wave of secondary effects like acidification, deoxygenation, and changes in circulation patterns. Among the most visible symptoms are marine heatwaves (MHWs), defined as periods of abnormally high temperature persisting for at least five days above the seasonal mean (Garrabou et al., 2009).

The Mediterranean Sea has become a climate-change hotspot. In 2019 MHW affected approximately 95% of its surface, and the Northern Adriatic Sea recorded temperatures exceeding 30 °C for more than a month during summer (Galli et al., 2017). Model projections suggest that by 2050 the region may experience tenfold more heatwave days (IPCC, 2021). These events damage biodiversity, triggering mass mortalities of invertebrates and fish, and reducing ecosystem services such as water filtration and nutrient cycling (Froehlich et al., 2018).

In coastal and lagoon areas, where most bivalve aquaculture occurs, thermal anomalies interact with eutrophication, chemical pollution, and low water renewal. This combination creates “multiple stressors,” a term used to describe situations where several environmental pressures act simultaneously and synergistically (Sokolova et al., 2021). The ability of organisms to cope with such conditions depends on their physiological plasticity and the speed of adaptive responses.

2.2 Bivalves as sentinels of environmental change

Bivalves are key components of coastal ecosystems. Through filter feeding they contribute to water clarity and nutrient cycling and serve as habitat engineers for many other species. Their fixed lifestyle and constant interaction with sediments make them excellent bioindicators (Milan et al., 2018). However, these same traits increase their susceptibility to environmental disturbance. When heatwaves coincide with pollution peaks or hypoxia, bivalves often suffer mass mortality events (Munari et al., 2011).

Economically, clam farming is a pillar of Mediterranean aquaculture. Thousands of workers depend on it, and production supports both local consumption and export markets. Yet the industry has faced repeated crises linked to environmental stress. In the Venice Lagoon alone, annual harvest fell from over 40 000 tons in 2000 to less than 3 000 tons by 2019 (Veneto Agricoltura, 2020). Identifying methods to increase clam resilience is therefore a priority for both ecological and socio-economic reasons.

2.3 Physiological responses to thermal stress

Clams are ectothermic and cannot thermoregulate internally. Their metabolism, digestion, and immune activity all depend on ambient temperature. When exposed to rapid warming, oxygen solubility in water declines while metabolic demand increases, producing an oxygen mismatch and energy crisis (Sokolova et al., 2021). This imbalance causes the formation of reactive oxygen species (ROS), which can damage cell membranes, proteins, and DNA (Ahmad et al., 2020).

To counteract oxidative stress, bivalves activate antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Heat-shock proteins (HSPs) also play a vital role by refolding denatured proteins and preventing aggregation. While these responses offer short-term protection, they require energy and divert resources from growth and reproduction. Chronic activation leads to reduced fitness and increased susceptibility to disease (Benedetti et al., 2022).

Because bivalves cannot avoid stressful conditions by migration or behavioural thermoregulation, their survival depends on the efficiency of cellular defense mechanisms and the capacity to adjust metabolism quickly. This has stimulated interest in approaches aimed at enhancing or pre-conditioning these defense mechanisms before extreme events occur.

2.4 The concept of priming and stress memory

Priming is the process by which a sub-lethal stress exposure enhances tolerance to future stress of the same or different nature. In plants, this phenomenon has been extensively studied to improve resistance to heat, drought, and pathogens (Hilker et al., 2016; Liu et al., 2021). During priming, specific molecular pathways are activated, and epigenetic marks or metabolic changes persist after the initial stimulus, enabling a faster or stronger response during subsequent exposure to real stress, thereby increasing resistance to potentially lethal conditions. Evidence of priming in animals is emerging. Gurr et al. (2022) showed that clams pre-exposed to moderate pCO_2 developed a form of stress memory that improved survival during subsequent acidification events. Similarly, heat-hardening in insects and corals confers transient resistance to thermal stress (Hackerott et al., 2021).

In bivalves, thermal priming has been linked to a range of physiological responses, such as the induction of heat-shock proteins (HSPs) and antioxidant enzymes, which contribute to cellular protection during subsequent stress exposure. Chemical priming, in contrast, is a relatively new concept in animals and has been studied mainly in plants. In the present study, hydrogen peroxide (H_2O_2) was used as a mild oxidative signal because low concentrations of this molecule can act as a signaling cue that stimulates antioxidant defense pathways without inducing toxic effects, a mechanism widely described in plants. Accordingly, controlled oxidative cues may activate protective mechanisms that prepare organisms for future stress. In addition to individual treatments, a combined chemical and thermal priming was tested to evaluate whether activating multiple stress-response pathways could produce additive, synergistic, or antagonistic effects before exposure to heatwave conditions.

2.5 Behaviour as a welfare indicator in clams

Animal welfare is now a cornerstone of sustainable aquaculture policy within the European Union. Although traditionally centered on vertebrates, welfare principles are increasingly being applied to invertebrates, acknowledging their capacity to experience stress and impaired functioning. Assessing welfare in bivalves, however, is challenging because they lack overt behavioural repertoires.

Among the few measurable behaviours, **burrowing** provides particularly valuable information. It represents a complex motor sequence that depends on adequate muscle tone, energy reserves, and sensory responsiveness. Healthy clams typically re-bury rapidly when placed on sediment surfaces, whereas stressed individuals exhibit delays or fail to bury completely. Therefore, burrowing latency and completion rate serve as reliable proxies for physiological condition (Peruzza et al., 2024).

Behavioural testing also aligns with the ethical principle of “Refinement,” minimizing invasive sampling. By observing clams rather than dissecting them, researchers can assess welfare repeatedly in the same individuals. The development of automated or semi-automated systems further enhances objectivity and throughput.

2.6 Relevance of the study

Heatwaves are projected to become a dominant feature of Mediterranean summers. For aquaculture producers, this means increasing losses unless proactive measures are implemented. The concept of “conditioning” or “priming” animals before high-risk periods offers a practical and low-cost mitigation strategy. At the same time, reliable welfare indicators are essential to evaluate success and comply with ethical standards.

By combining priming as a preventive measure with burrowing behaviour as a diagnostic tool, this thesis aims to bridge clams’ physiology with applied aquaculture management. Burrowing performance is a key indicator of clam health, but it also reflects susceptibility to predation, since individuals that burrow quickly are less likely to be captured. This study further contributes to the understanding of stress memory in invertebrates, a topic that remains largely unexplored. The results will provide information valuable both for academic research and for hatchery and farm-level monitoring practices.

2.7 Multiple stressors and the MANILA framework

Marine organisms rarely experience a single, isolated stressor. In coastal environments, temperature fluctuations and marine heatwaves are among the most critical challenges for shellfish, as they can strongly affect physiological stability and survival. Recognizing the importance of understanding these thermal challenges, the Italian national project MANILA Priming and Microbiota Manipulation to Mitigate Shellfish Vulnerability was established through collaboration between the University of Padova and the Polytechnic University of Marche. Its central objective is to test whether priming and microbiota management can strengthen shellfish resilience during heat-wave events.

This bachelor thesis forms part of Work Package 1 (WP1) of MANILA, which focuses specifically on thermal and chemical priming strategies. While the broader project investigates molecular and epigenetic mechanisms through high-throughput techniques such as RNA-Seq and ATAC-Seq, this thesis contributes the behavioural perspective by developing and testing a quantifiable welfare indicator the burrowing test under controlled heat-wave scenarios.

3. Aims of Thesis

This project was designed to answer specific questions emerging from the MANILA framework and from broader concerns about climate-change impacts on aquaculture. The main aims are:

1. Evaluate thermal and chemical priming effects. Determine whether short-term exposure to controlled sub-lethal temperature (30°C) or mild hydrogen-peroxide concentration can enhance clam tolerance during simulated marine-heatwave events.

2. Develop a behavioural welfare test. Design and validate a reproducible burrowing-behaviour protocol that quantifies reactivity, vitality, and stress tolerance under experimental conditions.

3. Monitoring approaches. Assess the practicality manual frame-analysis (ImageJ) for objective measurement of behavioural endpoints.

4. Materials and Methods

4.1 Experimental site and facilities

The study was carried out in the Aquarium Room of the University of Padova, which provides controlled aquatic systems suitable for thermal and chemical exposure experiments.
Each experimental tank (capacity 60 L) was equipped with digital thermostats (accuracy ± 0.2 °C), continuous aeration, and an external filtration circuit using synthetic seawater.
Lighting was maintained on a 12 h light : 12 h dark photoperiod at 400–500 lux to simulate natural daylight conditions.
Temperature, salinity, pH, and dissolved oxygen were monitored daily using a multiparametric probe (YSI ProDSS).
Water parameters were kept at:

- Salinity = 30 ± 1 ppt
- pH = 8.1 ± 0.1
- Dissolved oxygen > 90 % saturation
- Ammonia < 0.1 mg L^{-1}

Acclimation and recovery tanks were cleaned every morning, and one-third of the water was replaced with freshly prepared artificial seawater.

4.2 Animals and acclimation

Commercially sourced clams were transported from a European hatchery in aerated containers at ~18°C and transferred immediately to holding tanks upon arrival.
Individuals showing damaged shells or prolonged valve closure were excluded.

Mean shell length was 18 ± 1 mm, representing post-juvenile, pre-reproductive stages commonly used in resilience studies.

During a seven-day acclimation period, clams were maintained at 25 °C and fed twice daily with a mixed algal diet (*Isochrysis galbana* and *Tetraselmis suecica*, 1:1 ratio, 5×10^4 cells mL^{-1}). Feeding was stopped 24 h before the start of the priming phase to normalise metabolic state and avoid waste accumulation.

4.3 Experimental design

Four treatment groups were established, each consisting of 60 clams divided into three replicate tanks (20 per tank):

Control (C): maintained at 20 °C, no H_2O_2 added.
Thermal Priming (HP): short exposure at 30 °C.
Chemical Priming (CP): exposure to H_2O_2 at 50 µM .
Combined Priming (CHP): sequential chemical (H_2O_2 at 50 µM) + thermal treatment (30 °C).

For behavioural testing and subsequent analyses, clams from each treatment were further subdivided into four experimental subgroups of 15 individuals each, identified as **TQ**, **H**, **M**, and **K**. These subgroup designations were used throughout laboratory data collection to facilitate tracking and comparison between replicates.

4.3.1 Thermal priming

Temperature was gradually increased from 25 °C to 30 °C, over 6 h to minimise shock. Continuous aeration prevented hypoxia. Mortality was recorded twice daily.

4.3.2 Chemical priming

Hydrogen peroxide (H_2O_2) was used as a redox-active compound to elicit mild oxidative signalling.
Solutions were renewed daily to maintain stability; residual H_2O_2 was checked spectrophotometrically (A_{405} nm).
No visible bleaching or gaping occurred at these concentrations, confirming sub-lethal exposure.

4.3.3 Combined priming

For the CHP treatment, clams were first exposed to the optimal H_2O_2 concentration (for 7 days) and then to the heat-priming regime (30 °C for 7 days).

This sequence was chosen to mimic potential field conditions where oxidative stress may precede heat stress.

4.3.4 Recovery phase

After each priming treatment, all clams were returned to 20 °C for 7 days to allow physiological recovery.

Feeding and maintenance followed the acclimation protocol. Mortality, shell condition, and filtration activity (valve opening) were monitored.

4.3.5 Heatwave challenge

Following recovery, the primed and control groups were exposed to a simulated heatwave of 33–35 °C for 14 days, based on temperature records from the Northern Adriatic (Garrabou et al., 2009). Half of the control group remained at 25 °C to serve as unstressed reference. Mortality was logged twice daily.

4.4 Behavioural assessment: the burrowing test

4.4.1 Burrowing Experimental setup

Each clam was individually placed on 5 cm of sand in a transparent tank containing filtered seawater (25 °C or heatwave temperature, depending on the trial).
A GoPro Hero 8 camera was fixed above the tank and set to record continuously for 2 h at 60 fps.
The procedure was performed under consistent lighting and without external vibrations.

4.4.2 Video analysis

Consequently, all footage was re-analysed using ImageJ 1.54.
Videos were converted to 1 frame s^{-1} sequences and reviewed manually. For each clam, the following times were recorded:

t_1 = first detectable movement (start of burrowing);
t_2 = partial burial (shell partially covered in the sand);
t_3 = complete burial (shell no longer visible).

Latency to burrow = $t_3 - t_1$.
Clams failing to complete burial within 2 h were assigned 120 min as censored value.

As first attempt, we used DeepLab, a machine-learning program that estimates movement from video frames.

A training dataset of ~500 manually annotated frames was used to identify shell margins, but model performance was inconsistent because of low contrast between shells and sand.

5. Results

5.1 Mortality

Low mortality was recorded during both the priming phase and the subsequent heatwave challenge. During the priming period, very low mortality events occurred across treatments, with the highest daily mortality observed in the M treatment (4 individuals on 13 April) and in the H treatment (3 individuals on 11 April). During the challenge phase, mortality was minimal, with only one individual recorded in treatments H, M, and K, corresponding to mortality rates below 1%, while no mortality occurred in the TQ treatment. Overall survival remained high across all treatments, indicating that mortality was not a major discriminating response variable in this experiment. Consequently, behavioural responses, particularly burrowing performance, were considered the primary indicators of treatment effects.

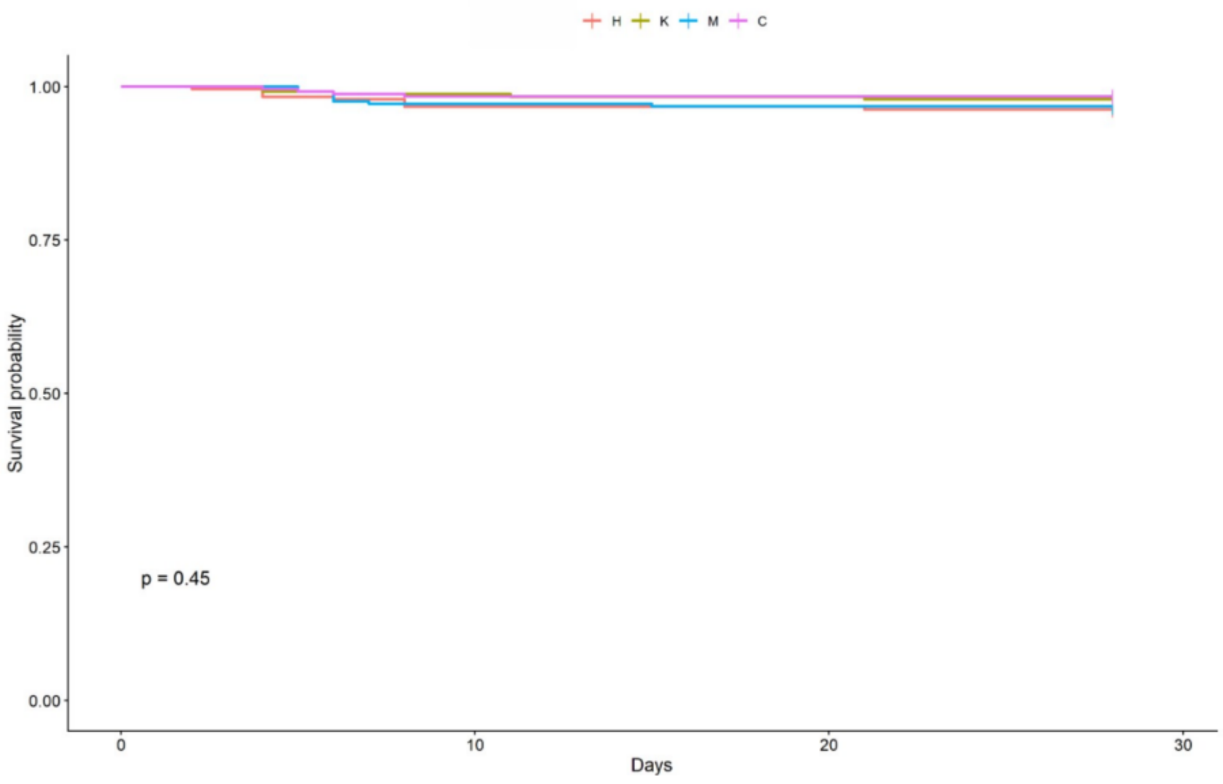

Figure S1: Kaplan-Meyer curves of mortality observed during the priming experiment among groups. Log-rank p-value is reported. H= heat priming, K= chemical priming, M= mixed priming, C= control.

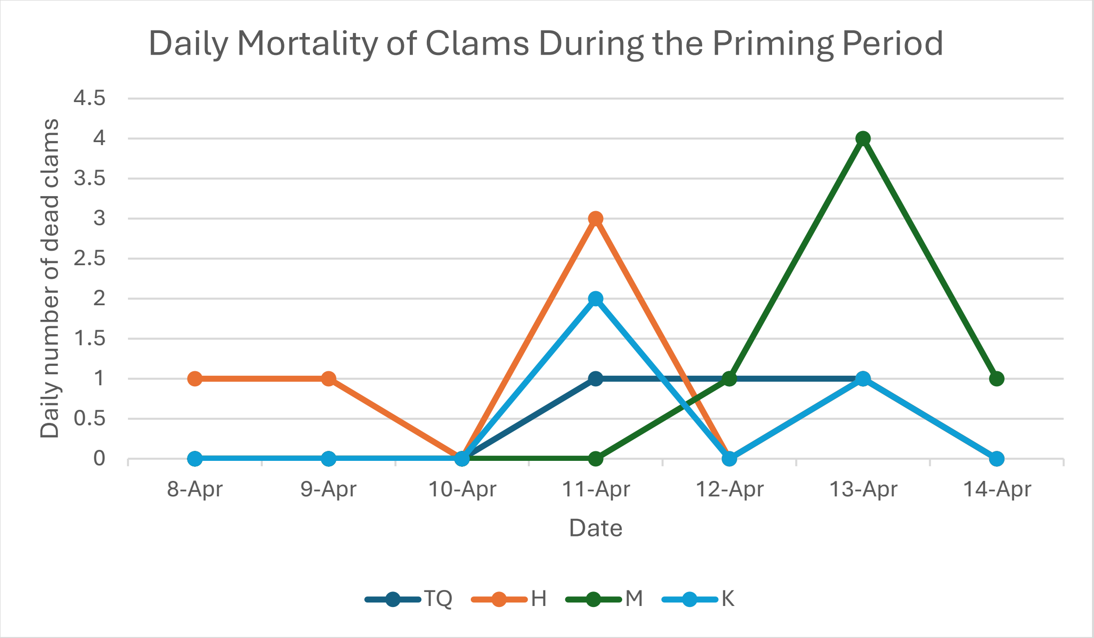

Figure S2. Daily mortality of clams during the priming period (8–14 April). Daily number of dead clams recorded across treatments: TQ (control), H (heat priming), M (mixed priming), and K (chemical priming)

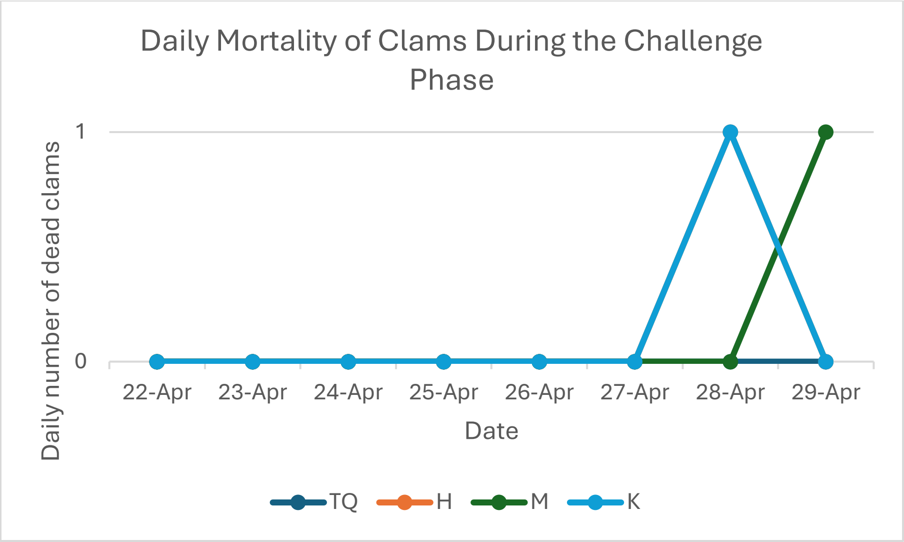

Figure S3. Daily mortality of clams during the heatwave challenge phase (22–29 April). Daily number of dead clams recorded across treatments: TQ (control), H (heat priming), M (mixed priming), and K (chemical priming). Mortality during the challenge phase was minimal, with only isolated mortality events observed.

5.2 Burrowing Behaviour

Visual Scoring

Visual observations showed a progressive increase in cumulative burial across all treatments during the observation period. After the priming phase, control and chemically primed clams showed relatively rapid burrowing behaviour, whereas heat-primed individuals exhibited slightly slower burial during the early time points.

Following the heatwave challenge, primed groups displayed improved burrowing performance compared with controls. At the final observation time (30 minutes), chemically primed clams showed the highest number of completely buried individuals, followed by heat-primed clams. These patterns were clearly visible in the plotted results and indicate enhanced behavioural performance in primed groups.

Manual scoring allowed direct visual confirmation of burrowing stages and enabled clear identification of complete burial.

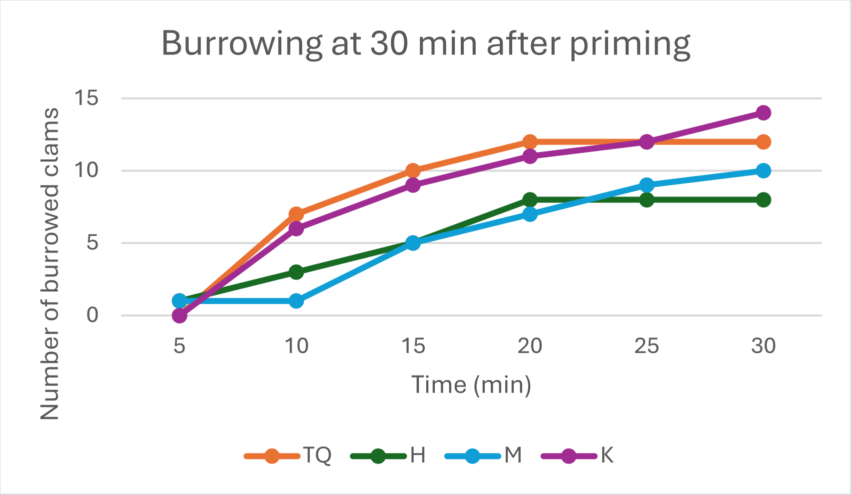

Figure S4. Burrowing performance of clams after priming. Number of clams fully burrowed over time during the 30-minute observation period following the priming phase. Burrowing was recorded at 5-minute intervals across treatments: TQ (control), H (heat priming), M (mixed priming), and K (chemical priming).

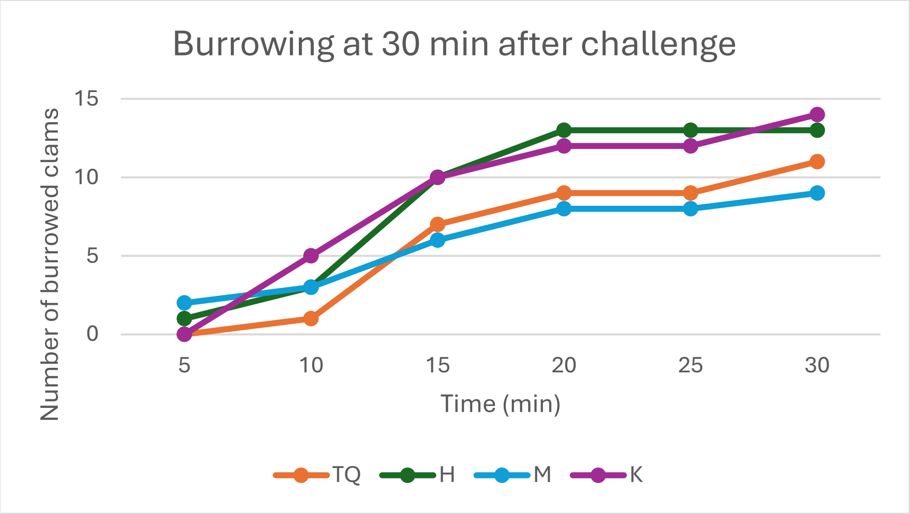

Figure S5. Burrowing performance of clams after the heatwave challenge. Number of clams fully burrowed over time during the 30-minute observation period following the heatwave challenge. Burrowing was recorded at 5-minute intervals across treatments: TQ (control), H (heat priming), M (mixed priming), and K (chemical priming).

ImageJ Analysis

Frame-by-frame analysis using ImageJ revealed behavioural trends consistent with those observed through visual observations. Burial increased progressively over time across all treatments, and the ranking of treatments at the final 30-minute time point matched the results obtained through direct observation.

The development of automated detection methods was one of the objectives of this study. Although a fully automated image-analysis pipeline was not achieved, the ImageJ-based approach represented an initial step toward quantifying burrowing behaviour from video recordings. The analysis required manual frame-by-frame evaluation to identify complete burial, making the procedure labor-intensive and time-consuming. Nevertheless, the burrowing test proved to be a useful and meaningful indicator of clam vitality and stress response. Future work could build on this approach by implementing automated image-analysis tools to streamline and accelerate the detection of burrowing events.

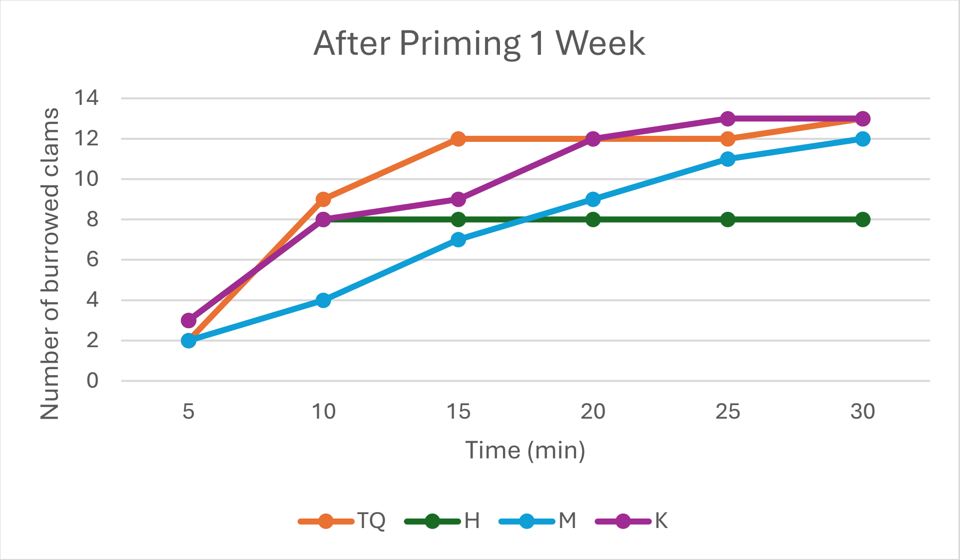

Figure S6. Burrowing performance of clams one week after the priming phase. Number of clams fully burrowed over time during the 30-minute observation period, recorded at 5-minute intervals. Treatments included TQ (control), H (heat priming), M (mixed priming), and K (chemical priming).

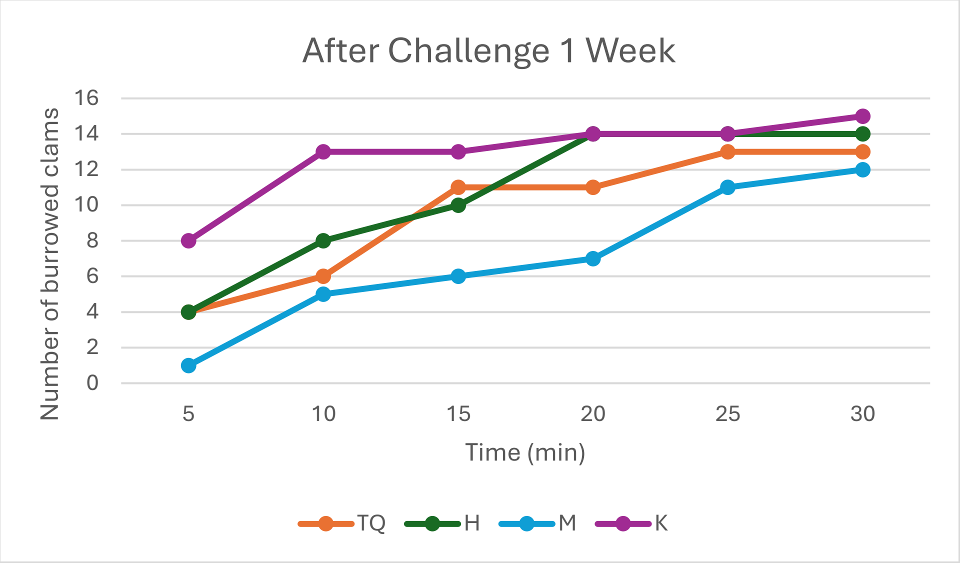

Figure S7. Burrowing performance of clams one week after the heatwave challenge. Number of clams fully burrowed over time during the 30-minute observation period, recorded at 5-minute intervals across treatments: TQ (control), H (heat priming), M (mixed priming), and K (chemical priming).

6. Discussion

The absence of mortality during both the priming phase and the heatwave challenge indicates that the experimental conditions were not severe enough to induce lethal stress. However, this does not imply the absence of physiological stress. Instead, the observed behavioural changes suggest that the treatments affected organismal performance at a sublethal level. This highlights burrowing behaviour as a sensitive indicator of physiological stress or resilience, capable of detecting effects that are not captured by survival alone.

Burrowing is a key behaviour in clams, providing protection from environmental stressors and predators. The progressive increase in burial observed across treatments over time likely reflects a normal behavioural response as individuals re-establish their position within the sediment. However, differences among treatments suggest that priming may influence behavioural performance during subsequent stress exposure. In particular, chemically primed individuals exhibited the highest level of burial at the final observation time, suggesting that prior exposure to chemical cues may enhance the capacity of clams to cope with environmental stress. Heat-primed individuals also showed improved performance compared with controls after the challenge phase, indicating a potential acclimation effect.

The comparison between manual scoring and ImageJ analysis demonstrated that both approaches produced consistent biological interpretations despite minor numerical differences at intermediate time points. These differences likely arise from methodological variation: manual scoring depends on the observer's judgement when determining the moment of complete burial, whereas ImageJ applies standardized digital criteria for detecting embedding. Although the two methods differed slightly in the exact timing of burial events, the overall patterns across treatments remained consistent, indicating that both approaches reliably captured the behavioural response of the clams to the experimental conditions.

Within the broader context of climate change and increasing marine heatwaves, early detection of sub-lethal stress responses is important for improving aquaculture management strategies. Behavioural monitoring methods such as burrowing analysis may therefore provide useful indicators of resilience and stress preparedness in shellfish populations, supporting the objectives of the MANILA project to enhance sustainability in shellfish farming.

6.1 Climate change, stress and clam welfare

The present study adds behavioural and practical evidence to the wide literature describing how climate change alters aquatic animal physiology. Ocean warming and the intensification of marine heatwaves now represent among the most acute threats to coastal biodiversity. For sessile or sedentary organisms such as clams, these events cause a rapid rise in metabolic rate coupled with reduced oxygen availability. The resulting metabolic stress manifests as accumulation of reactive oxygen species, impairment of immune function, and disruption of reproduction (Sokolova et al., 2021).

The Northern Adriatic and other shallow Mediterranean basins are particularly exposed. Summer heatwaves lasting more than 30 days have already produced episodes of large-scale mortality in wild and farmed stocks (Garrabou et al., 2009). Because clams cannot migrate to cooler waters, the only feasible strategy to sustain production is to enhance their physiological resilience. The current work contributes to this goal by testing controlled *priming* as a prophylactic intervention and by validating *burrowing behaviour* as a simple welfare indicator under thermal stress.

The behavioural perspective is valuable because it links cellular stress to whole-animal performance. Rapid and complete burrowing reflects adequate energy reserves, muscular coordination and neural control; delays signal fatigue or sub-lethal damage. In this experiment, primed clams buried faster and more consistently than unprimed controls, suggesting that mild pre-exposure prepared their physiology to cope with subsequent heat stress.

Thermal priming stimulates production of heat-shock proteins (HSP70, HSP90) that stabilize proteins and membranes. Chemical priming using hydrogen peroxide creates a controlled oxidative signal that up-regulates antioxidant enzymes such as superoxide dismutase and catalase (Anjum et al., 2022). These responses represent only part of a broader set of physiological adjustments that may occur during priming, which can also involve metabolic regulation, cellular repair processes, and other stress-response pathways. The combined treatment likely triggers multiple branches of the defence system. The improved burrowing observed in CHP animals in this study support the hypothesis of additive benefits.

Similar outcomes were described by Gurr et al. (2022), who found that clams pre-exposed to elevated pCO_2 maintained transcriptional “front-loading” of stress-response genes and subsequently tolerated acidification better. These converging lines of evidence suggest that moderate pre-stress, regardless of the exact nature of the stressor, can induce a generic preparedness state that crosses environmental modalities.

6.2 Behavioural indicators and non-invasive welfare monitoring

Animal welfare assessments in aquaculture often rely on mortality counts or biochemical assays and molecular analyses that require sacrificing animals. While informative, these approaches provide only endpoint data. Behavioural observation offers a dynamic, non-destructive alternative. For bivalves, behavioural repertoires are simple but ecologically meaningful valve closure, filtration rate, siphon extension, and burrowing.

The burrowing assay developed in this thesis proved both repeatable and sensitive. Because it integrates muscular performance and motivation, it can detect subtle impairment before mortality occurs. Moreover, it can be applied repeatedly to the same individuals, allowing longitudinal monitoring through different treatments.

Beyond manual behavioural scoring, the attempt to automate burrowing analysis using DeepLab highlighted both the potential and current limitations of machine-learning approaches for behavioural monitoring. Automated image-analysis methods offer important advantages, particularly in reducing analysis time and minimizing observer bias through standardized detection criteria. However, achieving sufficient sensitivity and accuracy to reliably detect subtle behavioural transitions remains challenging, especially in benthic species with limited movement and uniform backgrounds. In this study, the algorithm struggled under such conditions, emphasizing the need for improved image contrast and larger annotated datasets for training. With further optimization, automated methods could facilitate behavioural monitoring and allow a much larger number of individuals to be analysed within a single experiment, thereby increasing statistical power and experimental throughput.

6.3 Linking behaviour to molecular and biochemical responses

Behavioural changes are ultimately rooted in physiological processes. When a clam experiences heat stress, energy allocation shifts toward maintenance rather than growth. ATP consumption rises, anaerobic metabolism may occur, and antioxidant defenses are mobilized to control oxidative damage. Primed individuals, by contrast, already possess elevated baseline levels of these defenses and can respond more efficiently.

The integration of behavioural and molecular endpoints is a powerful approach: behaviour gives rapid, low-cost feedback, while molecular data validate the underlying mechanisms. Once correlated, behavioural metrics could serve as proxies for physiological state, simplifying welfare monitoring in field conditions.

6.4 Applications to aquaculture

From an applied perspective, priming could be integrated into hatchery protocols as a pre-conditioning stage. Juveniles could undergo mild temperature or H_2O_2 exposure before transfer to grow-out sites, reducing mortality during summer peaks. Such “training” would require minimal equipment: temperature-controlled tanks and standard aeration or H_2O_2. The cost is negligible compared with the economic losses from mass mortalities.

The behavioural assay developed here could also serve as a routine welfare check. Farmers could periodically test a subset of individuals: delayed or incomplete burrowing would warn of suboptimal conditions, prompting management actions such as water exchange or shading. Because the test is simple and non-invasive, it could be adopted widely with minimal training.

Moreover, incorporating welfare monitoring aligns aquaculture with EU ethical frameworks. Directive 2010/63/EU encourages refinement and reduction of suffering in all experimental animals, and similar principles are increasingly extended to farmed invertebrates. Demonstrating attention to welfare also enhances consumer confidence in sustainable seafood products.

6.5 Limitations and future directions

While the present findings are encouraging, several limitations must be acknowledged:

1. **Species-specific responses:** Different clam species can exhibit varying physiological tolerances to environmental stressors such as temperature increases and oxidative exposure. Consequently, the behavioural and stress responses observed in this study may not necessarily be representative of all clam species or bivalves in natural or aquaculture systems..
2. **Incomplete data set:** The present study explored DeepLab, an artificial-intelligence platform that uses convolutional neural networks to track body parts across video frames. Although the model did not yield stable detection due to low visual contrast between shells and sand, this attempt demonstrates the growing potential of machine learning in aquaculture welfare monitoring. Manual analysis with ImageJ was ultimately adopted, providing precise frame-by-frame timing of burrowing events.
3. **Automation limits.** DeepLab failed to track clams reliably. Future neural-network training with higher-contrast imagery should resolve this.
4. **Environmental scope.** Only thermal stress was examined; future experiments should integrate salinity, hypoxia, and contaminant exposure to reproduce realistic multi-stressor conditions.
5. **Temporal scale.** Priming effects were evaluated over short periods. Long-term persistence of stress memory across seasons or generations remains unknown.

Future research should also explore microbiota interactions, another key component of the MANILA project. Host-associated microbes influence detoxification and immune modulation, nutrient metabolism, and other physiological processes that contribute to host resilience and overall physiological regulation. Combining priming with microbiome transplantation may yield additive or synergistic benefits, enhancing survival and growth even further.

7. Conclusion

Climate change, and particularly the increasing frequency of marine heatwaves, represents a significant challenge for clam populations and shellfish aquaculture. This study explored whether thermal and chemical priming could enhance clam tolerance to heat stress and whether burrowing behaviour could serve as a practical indicator of animal welfare. The results suggest that primed clams displayed improved behavioural performance during heat exposure, with faster and more consistent burrowing compared with unprimed individuals. These findings support the idea that mild pre-exposure to controlled stressors can activate protective mechanisms and increase resilience to subsequent environmental challenges. Furthermore, the burrowing test proved to be a simple and non-invasive method for evaluating clam vitality, highlighting its potential use in both experimental research and aquaculture monitoring. In addition, this work explored the potential for automating the analysis of burrowing behaviour, which could facilitate behavioural monitoring and improve the efficiency of future experimental studies. Overall, integrating priming strategies with behavioural assessment may contribute to improving the sustainability and resilience of shellfish production under changing climatic conditions.

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La pesca in Veneto: flotta, imprese, produzione e commercio.

9. Acknowledgments

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