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Refrigerated transport modulates the health status and nutritional quality of Mediterranean mussels (*Mytilus galloprovincialis*): A case study from the western Adriatic Sea

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ABSTRACT

Mediterranean mussels (*Mytilus galloprovincialis*) are a cornerstone of Italian aquaculture, yet their quality and survival can be affected by post-harvest handling and long-distance refrigerated transport. This study investigated the effects of a 17-hour-simulated transport at 7 ± 1°C on mussel health status and quality, considering the position of mussels within stacked packaging layers (top, intermediate, and bottom). Results evidenced that the simulated transport conditions or the position within the pallet did not affect mussels' marketable indexes (condition index, edible fraction yield, shell incidence) but were able to affect the mussels' nutritional profile. In fact, mussels after transport exhibited reduced dry matter, crude protein, and crude lipid contents, coupled with fatty acid remodeling (mainly a decrease in palmitic acid and a parallel increase in n3-polyunsaturated fatty acids), regardless of their position within the pallet. These variations reflected the physiological mobilization of endogenous reserves to maintain basal metabolic functions during transport in emersion conditions as well as an active homeoviscous adaptation to counteract the reduction in membrane fluidity induced by the lower temperature. Interestingly, that fatty acid remodulation resulted in improved lipid quality indices (lower atherogenicity and thrombogenicity indexes, higher h/H ratio) leading to a more favorable nutritional profile. Finally, the simulated transport conditions majorly affected the health status of the mussels located at the bottom layers, possibly interested by the synergic effect of cold stress and hypoxia. In fact, mussels collected from these layers were characterized, at gills level, by an upregulation of markers involved in cellular (*hsp70*) and oxidative (*sod1* and *cat*) stress together with a higher incidence of histopathological indexes compared to those from the top and intermediate layers. These findings suggest that short-term refrigerated transport induces physiological adjustments to extend their period of survival in adverse conditions, that were exacerbated in mussels located at the lowest levels of the pile.

1. Introduction

Aquaculture is a fundamental component of the global seafood sector, supplying over half of the world's seafood demand, with production expected to reach 205 million tons by 2032 (FAO, 2024). Within this rapidly expanding sector, mollusks farming constitutes approximately 25% of global animal aquaculture (annual average growing rate of 3.46% since 2000), encompassing over 65 bivalve species (Montagnani et al., 2024; Theodorou and Tzovenis, 2023). European mussels (*Mytilus* spp.) represents around 22.38% of global mussel

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production, with Italy ranking second after Spain (Soliño and Figueras, 2025; Vuoso et al., 2025). Particularly, the western Adriatic Sea offers highly favorable conditions for Mediterranean mussel (*Mytilus galloprovincialis*) farming. In fact, its shallow depths and significant riverine inputs strongly increase organic matter availability and thus mussels' growth (della Malva et al., 2024; Neri et al., 2023; Žurga et al., 2024). This shoreline area serves as a nursery and an important mussel production site for both local coastal regions, like Emilia-Romagna and Marche, and also for other Italian regions characterized by less favorable sea areas (Brigolin et al., 2017; Orban et al., 2002). The predominant farming technique in this area is the longline system, where mussels colonize vertically suspended ropes hung from horizontal backbones (della Malva et al., 2024; Ramšak et al., 2024). This method ensures operational efficiency, low environmental footprint, and capacity to provide ecosystem services, including biofiltration, biodiversity enhancement, and coastal erosion mitigation, while producing nutrient-rich seafood and valuable by-products (Pacifico et al., 2024; Petersen et al., 2014; Suplicy, 2020; Tamburini et al., 2020). In fact, Italian Mediterranean mussels are valued for their exceptional nutritional profile, rich in high-quality proteins, essential vitamins and minerals, and long-chain polyunsaturated fatty acids (PUFA), making them a major seafood for the local market (Bongiorno et al., 2015; della Malva et al., 2024; Lopez et al., 2023). However, mussels' growth and biochemical composition can be highly variable, influenced by seasonal changes, climatic conditions, and seawater features (Acarli et al., 2024; Grkovic et al., 2023; Noor et al., 2021), which are notably dynamic in the western Adriatic Sea (Azpeitia et al., 2016; Gren, 2019). In addition, mussels' farms yields can also be affected by stress caused by human intervention during processes of handling and harvesting (Ramšak et al., 2024; Theodorou et al., 2020). In fact, following harvest, Mediterranean mussels undergo different post-production pathways depending on their market destination. A substantial portion of them is cleaned and processed directly at the farming site and then distributed to local markets, involving minimal transport and limited handling. Differently, a significant fraction of the western Adriatic Sea reared mussels is harvested prior to market size, shipped and then relocated to other farming areas to complete the production cycle in different marine environmental conditions (della Malva et al., 2024; Peruzzo et al., 2026). This relocation can modify the organoleptic properties, including taste and texture, and nutritional attributes, supporting the production of local varieties of high market value and regional economies (*i.e.* Sicily: cozze di Messina, cozze di Catania; Sardinia: cozze di Olbia). However, these mussels are often subjected to extended handling and long-distance refrigerated transport. This logistical phase imposes additional stressors, including possible temperature fluctuations, prolonged cold storage duration, and possible supply chain delays, which have been shown to negatively impact survival, metabolic condition, and overall product integrity (Nguyen et al., 2020). In fact, due to their significant water content, neutral pH, and endogenous enzymes activity, mussels are highly susceptible to rapid microbial spoilage when cold chain management is suboptimal. This may lead to the breakdown of nutrients like proteins and unsaturated fatty acids by microorganisms, causing a significant loss of nutritional quality and commercial value (Zhelyazkov et al., 2024; Zhou et al., 2019). For these reasons, the cold chain maintenance during transport plays a crucial role in ensuring the shelf life and nutritional quality of harvested mussels and other bivalve mollusks during distribution (Alfaro et al., 2019; Teng et al., 2022; Zhou et al., 2019). In particular, the reference legislation at European level states that fishery products transported alive must be kept at a temperature and under conditions which do not affect food safety or their viability (Regulation No 853/2004, 2004). Delivering live mussels over long distances, while maintaining their quality and safety is challenging. In fact, temperature variability during transport, packaging methods, and shipment duration markedly influence mussel physiological welfare and biochemical composition. Additionally, the position of mussels within stacked packaging can affect oxygen availability and temperature exposure, further modulating stress and quality outcomes (Barrento et al., 2013; Nguyen et al., 2020; Tuckey et al., 2023; Zamora et al., 2019).

Given these challenges, this study aimed to systematically assess the effects of refrigerated transport conditions (duration, temperature range, and packaging configuration) on the welfare and nutritional quality of Mediterranean mussels farmed in the mid-western Adriatic Sea. Particularly, harvesting, packaging, and transport procedures from a local aquaculture facility (Senigallia, Marche Region, IT) to Siracusa (Sicily, IT) were monitored and replicated under controlled laboratory conditions. The investigation evaluated changes in biometric parameters, physiological stress markers, and nutritional composition pre- and post-transport simulation, with particular emphasis on the influence of mussels positioning within packaging stacks.

2. Materials and methods

2.1. Ethics

In accordance with current legislation on animal experimentation, ethical approval was not required for this study. The Mediterranean mussel (*Mytilus galloprovincialis*) is an invertebrate species and therefore falls outside the regulatory framework that governs the use of vertebrate animals in scientific research. No specific permits were needed, and all handling procedures complied with general good practice for invertebrate research.

2.2. Case study

The complete production cycle of Mediterranean mussel was monitored at a commercial farm (Sena Gallica Soc. Cooperativa, Senigallia, AN, Italy; 43°43′N, 13°13′E), located along the mid-western Adriatic coast, from June 2024 and May 2025. The farming season started in early summer (June 2024) with seed collection, derived either from natural settlement on farm infrastructures (ropes, cables, and buoys) or from residual adult mussels recovered during routine cleaning operations. The mussels' seed was placed into tubular mesh nets made of cotton (mussels' socks) which are then tied and suspended vertically from the horizontal long-lines, usually hanging down to a depth of approximately 4–7 m. Up to 3 times during the on-growing phase, the farmers collect the socks and re-pack the mussels into new, wider-meshed plastic (polypropylene) ones to maintain optimal density and avoid biomass overload. The grow-out phase spans up to 11 months (May 2025), when mussels achieve their commercial size (typically 5 cm in maximum length) and are ready to be harvested and cleaned through specific automatic machine that allows specimens separation. After this step, cleaned mussels are packaged and prepared for the local distribution. The present study was focused on an intermediate phase of the production cycle, during which mussels under market size (around 3.5 cm in maximum length) were harvested and shipped (still on socks) to other Italian regions, through refrigerated trucks, to complete the productive cycle in different seawater environment. Particularly, in February 2025, mussels' socks were severed from the long-lines and then stacked on pallets (Fig. 1). Each pallet was wrapped with cling film to ensure containment and to reduce desiccation during transport.

Following harvest, mussels were transported to Siracusa (Sicily, IT) for the final relocation to a local farming area. This transfer was of great interest to monitor since it is the longest transport of mussels within Italy under refrigerated conditions. Mussels were transported in refrigerated trucks for approximately 17 h (duration of the shipment from Senigallia to Siracusa) under controlled conditions, with average temperature of 7 °C (ranging in an interval of ± 1 °C during the shipment) to preserve viability and minimize post-harvest stress.

2.3. Experimental design

Six mussels' socks (6 m each) destined to shipment, with mussels of

Fig. 1. (**a**) Stacking procedures of mussels' socks planned for shipment; (**b**) example of a pallet ready to be shipped with a monitoring device (temperature), Elitech RC−5 +.

3.47 ± 0.23 cm in maximum length, were randomly collected during the harvesting procedures at the farm and were divided into 36 one-meter samples that were transported in natural seawater at constant temperature to the laboratory (the one-meter subsamples of socks are named "socks" in the remaining part of the manuscript). A significant number of specimens were immediately sampled from 9 randomly selected socks (as described in the following sections) to determine the chemical properties and the overall health status prior to transport (t_0). Then, to replicate the transport conditions, the remaining 27 socks were stacked on three pallets (9 randomly selected socks each), composing three layers (3 socks each) defined as follows: bottom (BOT), intermediate (INT), and top (TOP). The stacked pallets (wrapped with the same cling film used by the farmers) were then maintained in a climatic chamber set at 7 ± 1 °C for 17 h, simulating the duration and temperature of actual refrigerated transport. Each layer was equipped with a temperature monitoring device (Elitech RC−5 +) to exclude eventual temperature fluctuations during the simulation. At the end of the 17-hour period (t_1), mussels were sampled from the socks located in each layer (TOP, INT, and BOT) to evaluate eventual changes in marketable indexes, chemical properties, and overall health status resulting from the transport process. The experimental design is resumed in Fig. 2.

2.4. *Marketable indexes*

To calculate the mussels' marketable indexes, 180 specimens from the 9 socks at t_0 (30 specimens per sock) and 180 specimens at t_1 for

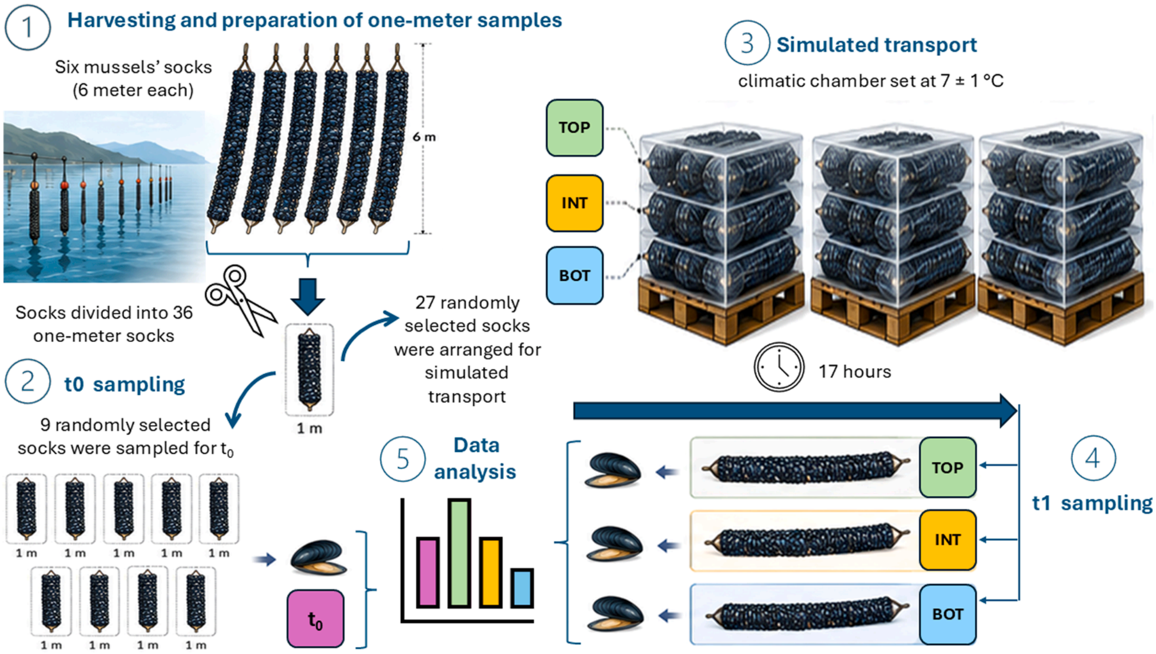

Fig. 2. Scheme of experimental design used in the present study.

TOP, INT, and BOT, respectively (9 socks for each layer, 30 specimens per sock) were randomly selected and individually weighted. Then, for each specimen, the *Adductor* muscle was severed to open the valves and separate the edible fraction. Both edible fractions and valves were individually weighted. Condition Index (CI), Edible Fraction Yield (EFY), and Shell Incidence (SI) were calculated as follows:

$$CI\ (\%) = (\text{meat dry weight} \ / \ \text{shell dry weight}) \times 100$$

$$EFY\ (\%) = (\text{wet weight of edible fraction} \ / \ \text{total mussel weight}) \times 100$$

$$SI\ (\%) = [\text{wet weight of valves} \ / \ (\text{wet weight of valves} + \text{wet weight of edible fraction})] \times 100$$

2.5. Chemical analysis

For the proximate composition analyses, the edible fraction of 90 randomly selected specimens from the 9 socks at t_0 (10 specimens per sock) and 90 randomly selected specimens at t_1 for TOP, INT, and BOT, respectively (9 socks for each layer, 10 specimens per sock) was collected, rinsed with distilled water, lyophilized, ground, and stored at −20 °C. Samples of mussels deriving from the same sock (10 mussels in total) were pooled together, obtaining 9 samples at t_0 and 9 samples at t_1 for TOP, INT, and BOT, respectively. Samples were then analyzed following the AOAC (AOAC, 2023) procedures to determine the content of dry matter, crude protein, crude lipid, and ash contents. Moisture content was obtained by calculating weight loss after samples' lyophilization. The crude protein and crude lipid contents were determined using the Kjeldahl method (after samples' acid digestion and using nitrogen-to-protein conversion factor of 6.25; AOAC 981.10) and the Soxhlet ether method (AOAC 991.36), respectively. Finally, ash content was determined through incineration in a muffle furnace by combustion at 550°C for three hours (AOAC 920.153).

For fatty acid profile determination, the edible fraction of 90 randomly selected specimens from the 9 socks at t_0 (10 specimens per sock) and 90 randomly selected specimens at t_1 for TOP, INT, and BOT, respectively (9 socks for each layer, 10 specimens per sock) was collected, lyophilized, ground, and stored at −20 °C. Samples of mussels deriving from the same sock (10 mussels in total) were pooled together, obtaining 9 samples at t_0 and 9 samples at t_1 for TOP, INT, and BOT, respectively. The total lipid fraction was obtained from lyophilized samples, according to Folch et al. (1957). The fatty acids in the lipid extract were trans-esterified to fatty acids methyl esters (FAME) using a base-catalyzed trans-esterification (Christie, 1982). A Varian GC 430 gas chromatograph (Varian Inc., Palo Alto, CA, USA), equipped with a flame ionization detector and a Supelco Omegawax™ 320 m capillary column (Supelco, Bellefonte, PA, USA), was used to determine the fatty acid composition. The resulting chromatograms were recorded with the Galaxie Chromatography Data System 1.9.302.952 (Varian Inc., Palo Alto, CA, USA). Fatty acids were identified by comparing their FAME retention times to those of the Supelco 37 component FAME mix standard (Supelco, Bellefonte, PA, USA) and then quantified using calibration curves, with tricosanoic acid (C23:0) (Supelco, Bellefonte, PA, USA) as internal standard.

Finally, the Atherogenicity Index (AI), the Thrombogenicity Index (TI), and the hypocholesterolemic / Hypercholesterolemic ratio (h/H ratio), as nutritional markers used to evaluate the quality of fatty acid profile in products intended for human consumption, were calculated as follows:

$$AI = [C12{:}0 + (4 \times C14{:}0) + C16{:}0] \ / \ (\Sigma\ MUFA + \Sigma\ n6\text{-}PUFA + \Sigma\ n3\text{-}PUFA)$$

$$TI = (C14{:}0 + C16{:}0 + C18{:}0) \ / \ [(0.5 \times \Sigma\ MUFA) + (0.5 \times \Sigma\ n6\text{-}PUFA) + (3 \times \Sigma\ n3\text{-}PUFA) + (n3\text{-}PUFA \ / \ n6\text{-}PUFA)]$$

$$h/H = (C18{:}1n9 + \Sigma\ PUFA) \ / \ (C12{:}0 + C14{:}0 + C16{:}0)$$

where MUFA and PUFA correspond to monounsaturated and polyunsaturated fatty acids, respectively.

2.6. Histological analyses

Samples of gills and digestive gland from 45 randomly selected specimens from the 9 socks at t_0 (5 specimens per sock) and 45 randomly selected specimens at t_1 for TOP, INT, and BOT, respectively (9 socks for each layer, 5 specimens per sock) were dissected and immediately fixed in Bouin's solution (Merck KGaA, Darmstadt, Germany) for 24 h at 4 °C. Samples were then included in paraffin (Bio Optica, Milan, Italy) to obtain 5 μm sections following the procedure described in Zarantoniello et al. (2023). For each sample, three transversal sections were collected (at 100 μm intervals from each other) and stained with Mayer's hematoxylin and eosin Y (Merck KGaA, Darmstadt, Germany). Sections were examined using a Zeiss Axio Imager.A2 microscope equipped with an Axiocam 105 combined-color digital camera (Zeiss, Oberkochen, Germany).

For each specimen, an individual semi-quantitative histopathological condition index (HCI) was calculated, according to Costa et al. (2013), using the following *formula*:

$$HCI_y = (\Sigma\ W_x \times S_{xy}) \ / \ \Sigma\ M_x$$

where W_x is the weight associated with the x^{th} alteration, S_{xy} is the score associated with the x^{th} alteration in the y^{th} specimen, and M_x is the maximum attributable value for the x^{th} alteration (weight of the alteration × maximum score of the alteration). Particularly, the weight value (W) attributed to each alteration ranged from 1 (minimal significance for individual health status) to 3 (health status severely affected), whilst the scores (S) were assigned as follows: 0, alteration not observed; 1, alteration scarcely observed; 2, alteration moderately diffused; 3, alteration highly diffused.

The HCI was calculated per organ (digestive gland and gills) on each specimen analyzed, considering the alterations reported in Table 1.

2.7. Gene expression analysis

Gills and digestive glands tissues were collected from 27 randomly selected specimens from the 9 socks at t_0 (3 specimens per sock) and 27 randomly selected specimens at t_1 for TOP, INT, and BOT, respectively (9 socks for each layer, 3 specimens per sock). Samples were pooled

Table 1
Histopathological alterations investigated in digestive gland and gills of Mediterranean mussels collected from each one-meter sample and the weight value attributed.

Organ	Alterations	Weight (W)
Digestive gland	Epithelial thinning and lumen enlargement	1
	Intracellular inclusions of lipofuscin-like substances	1
	Inter-tubular adipocytes infiltration	1
	Hemocyte infiltration	2
	Epithelial cell hyperplasia	2
	Epithelial atrophy	3
Gills	Intracellular inclusions of lipofuscin-like substances	1
	Epithelial detachment or vacuolization	1
	Lamellar deformations or fusion	1
	Hemocyte infiltration	2
	Epithelial cell hyperplasia	2
	Necrosis	3

For each alteration, scores were assigned as follows: 0, alteration not observed; 1, alteration scarcely observed; 2, alteration moderately diffused; 3, alteration highly diffused.

(each sample constituted of gills or digestive gland of mussels deriving from the same sock; 3 mussels in total) and total RNA was then isolated, using the RNAzol solution, following the manufacturers' instructions. The RNA extracted was quantified by a spectrophotometer, and its integrity was checked by gel electrophoresis. The IScript cDNA Synthesis Kit (BioRad, Milano, Italy) was used to synthesize the cDNA that was stored at −20 °C until use. Real-time qPCRs were performed in an iQ5 iCycler thermal cycler (Bio-Rad, Hercules, CA, USA) using sample set up, number of cycles, and thermal profile described in Cattaneo et al. (2023). Two no template-controls were added in each run to guarantee the absence of contamination (no peaks detected in each reaction). Using beta-actin (*β-actin*) and 18S ribosomal protein (*18 s*) as housekeeping genes, the relative quantification of heat-shock protein 70 (*hsp70*), superoxide dismutase 1 (*sod1*), and catalase (*cat*) was performed on both gills and digestive gland cDNA samples. Primer sequences used in the present study are shown in Table 2. For each pair of primers, the annealing temperature was preliminary optimized with a temperature gradient assay, whilst their efficiency was verified with a mix of cDNA (efficiency around 90% for all the primers) tested at different concentrations (1:1, 1:10, 1:100, 1:1000). Melting curve analysis demonstrated the specificity of the qPCR products by producing a single peak for each reaction. In addition, the amplification products were sequenced, and homology was verified.

For each gene considered, the relative expression was calculated using the geometric mean of the two housekeeping genes, after checking their stability of expression, using the Bio-Rad CFX Manager 3.1. software (Bio-Rad). Gene transcript expression variations are reported as relative mRNA abundance (arbitrary units).

2.8. Statistical analysis

The socks were used as experimental unit for all the analyses and data were checked for normality and homoscedasticity through Shapiro–Wilk and Levene's tests, respectively (Prism−8, GraphPad Software, version 8.0.2; San Diego, CA, USA). Data obtained from the different analysis were then independently analyzed using a one-way ANOVA followed by Tukey's test (Prism−8), comparing: (i) TOP, INT, and BOT *vs* t_0; (ii) TOP *vs* INT *vs* BOT. Significance was set at $p < 0.05$ and results were expressed as mean ± standard deviation (SD). Principal Component Analysis (PCA) was conducted with R Studio (version 4.6.0) on data related to fatty acid analysis, using the "prcomp" function from the "stats" package (R Studio Team, 2023). Variables were centered but not scaled prior to PCA to preserve the relative contribution of dominant fatty acids to the overall lipid profile. Visualization was carried out using the "factoextra" package (Kassambara and Mundt, 2020). In addition, data regarding fatty acid analysis were represented as relative variations of TOP, INT, and BOT profiles against t_0 in a two-dimensional heatmap conducted with R Studio (version 4.6.0), using the "dplyr" and "tidyr" packages. Statistically significant differences derived from the one-way ANOVA were directly mapped onto the matrix. The heatmap was generated using the "ggplot2" package within the "tidyverse" environment (Wickam et al., 2019).

3. Results

3.1. Marketable indexes

Considering all the marketable indexes analyzed (Table 3), no significant differences were detected between mussels collected at t_0 and t_1 as well as among TOP, INT, and BOT layers at t_1.

3.2. Proximate composition and fatty acid profile

Considering the proximate composition (Table 4), mussels collected from all layers at t_1 were characterized by a significantly lower content in dry matter ($p < 0.0001$), crude protein ($p < 0.0001$), and crude lipid ($p < 0.0001$), and by a significantly higher moisture ($p < 0.0001$) compared to those collected at t_0. Additionally, as regards the comparison among layers at t_1, mussels from TOP layer showed a significantly lower content in dry matter ($p < 0.0004$) and crude protein ($p < 0.0001$), and a significantly higher moisture ($p < 0.0004$) compared to those from INT and BOT layers. Crude lipid content was significantly higher ($p < 0.0001$) in mussels from BOT layer compared to those from TOP and INT ones. Finally, no significant differences were evident in terms of ash content.

Considering the fatty acid profile (summarized in Fig. 3 and reported in details in Supplementary Table 1), myristic (C14:0) and palmitic (C16:0) acids were the most represented saturated fatty acids (SFA), with the latter showing significantly lower values in mussels collected from all the layers at t_1 compared to those collected at t_0 ($p = 0.0411$; Fig. 3a). Among the monounsaturated fatty acids (MUFA) vaccenic acid (C18:1n7) was significantly higher in mussels collected from the TOP layer compared to those collected at t_0 ($p = 0.0067$) as well as those from BOT layer at t_1 ($p = 0.0468$) (Fig. 3a). The most representative polyunsaturated fatty acid (PUFA) was the eicosapentaenoic acid (C20:5n3, EPA), which was significantly higher in mussels collected from all layers at t_1 compared to those collected at t_0 ($p = 0.0202$; Fig. 3a). The PCA biplot based on the single fatty acids (Fig. 3b; 89.3% of the total cumulative variance) showed that the long vector of palmitic acid pointed toward the negative quadrant of Dim2 (18.7% of explained variance), acting as a major driver for the vertical segregation of the samples. In addition, the main axis of the single fatty acid PCA (Dim1, 70.6%, Fig. 3b) illustrated a horizontal shift from t_0 (samples clustering on the left, associated with vaccenic acid and other minor MUFA) toward t_1 conditions (samples shifting to the right, heavily driven by the long vectors of EPA and DHA).

Table 3

Marketable indexes of Mediterranean mussels sampled at t_0 and after the 17-hour simulated transport (t_1).

Index	t_0	t_1		
		TOP	INT	BOT
CI (%)	19.81 ± 0.81	20.97 ± 2.62	19.44 ± 0.72	20.98 ± 1.23
EFY (%)	28.84 ± 3.54	31.36 ± 2.17	30.30 ± 4.20	31.80 ± 3.39
SI (%)	45.60 ± 1.02	46.67 ± 1.70	46.09 ± 2.96	44.20 ± 1.26

Values are shown as mean ± SD ($n = 9$). Abbreviations: CI, condition index; *EFY*, edible fraction yield; *SI*, shell index.

Table 2

Sequences and NCBI ID of the primers used in the present study.

Gene	Forward primer (5'−3')	Reverse primer (5'−3')	NCBI ID
hsp70	ATAACTACTGAGATATGGCAGGAA	TGGTCGTTGGCTATGATGT	AJ783713.1
sod1	GTGACAGTGACAGGAGAGTTA	TCCAAATGGGTTGAAATGTGAT	FM177867.1
cat	CTGTTCTCTGACCGTGGAA	CCTTGTTGACCGTCTTAAATGT	AY743716.2
18 s (hk)	TCGATGGTACGTGATATGCC	CGTTTCTCATGCTCCCTCTC	L33451
β-actin (hk)	GCCCGATGGACAGGTTAT	CAAGAAGGATGGTTGGAATAATGA	AF157491.1

Abbreviations: hk, housekeeping genes

Table 4
Proximate composition of Mediterranean mussels sampled at t_0 and after the 17-hour simulated transport (t_1).

Proximate composition, %	t_0	t_1		
		TOP	INT	BOT
Dry matter	21.76 ± 0.95	$18.08 \pm 0.87^{a\,*}$	$19.26 \pm 1.24^{b\,*}$	$20.16 \pm 0.64^{b\,*}$
Moisture	78.24 ± 0.95	$81.92 \pm 0.87^{b\,*}$	$80.74 \pm 1.24^{a\,*}$	$79.84 \pm 0.64^{a\,*}$
Crude protein	9.66 ± 0.06	$8.08 \pm 0.24^{a\,*}$	$8.50 \pm 0.32^{b\,*}$	$8.82 \pm 0.17^{c\,*}$
Crude lipid	2.42 ± 0.20	$1.31 \pm 0.12^{a\,*}$	$1.26 \pm 0.11^{a\,*}$	$1.54 \pm 0.12^{b\,*}$
Ash	2.12 ± 0.12	2.58 ± 0.47	2.44 ± 0.80	2.30 ± 0.10

Values are shown as mean ± SD ($n = 9$; each sample consisting of a pool of 10 specimens). Within each row, means followed by different letters differ significantly; asterisk denotes significant difference from t_0; ($p < 0.05$).

Finally, as reported in Fig. 3a, the total amounts of SFA, MUFA, and PUFA were not significantly different between mussels collected at t_0 and t_1 as well as among TOP, INT, and BOT layers at t_1. However, both n3-PUFA and n6-PUFA contents were significantly higher in mussels collected from all the layers at t_1 compared to those collected at t_0 ($p = 0.0390$ and $p = 0.0030$ for n3-PUFA and n6-PUFA, respectively), whilst no significant differences were detected between sampling times and among layers in terms of n3/n6 ratio. The PCA biplot based on fatty acids categories (Fig. 3c; 95.1% of the total cumulative variance) captured a massive variance on Dim1 (85.5%), demonstrating a distinct transition from samples at t_0 characterized by SFA and MUFA vectors (negative side of Dim1) toward a profile more represented by PUFA classes at t_1 (positive side of Dim1).

Considering the lipid quality indexes (Table 5), mussels collected from all the layers at t_1 were characterized by significantly lower values of atherogenicity ($p = 0.0235$) and thrombogenicity ($p = 0.0186$) indexes, and by a significantly higher hypocholesterolemic/Hypercholesterolemic ratio ($p < 0.0382$) compared to those collected at t_0. No significant differences were evident among TOP, INT, and BOT layers at t_1.

3.3. Histological analysis

Mussels from both t_0 and t_1 (all the layers) showed a normal structure of the digestive gland, formed of clusters of blind-ending alveolo-tubular units characterized by a simple columnar epithelium of digestive cells (the most abundant cell type) with interspersed basophilic pyramidal cells (Fig. 4a-d). Overall, all the specimens analyzed were characterized by a comparable degree of adipocyte infiltration in the inter-tubular

Table 5
Lipid quality indexes of Mediterranean mussels sampled at t_0 and after the 17-hour simulated transport (t_1).

Index	t_0	t_1		
		TOP	INT	BOT
AI	0.98 ± 0.10	$0.83 \pm 0.09^*$	$0.88 \pm 0.07^*$	$0.87 \pm 0.05^*$
TI	0.25 ± 0.02	$0.21 \pm 0.02^*$	$0.22 \pm 0.02^*$	$0.22 \pm 0.02^*$
h/H	1.34 ± 0.12	$1.58 \pm 0.18^*$	$1.50 \pm 0.13^*$	$1.52 \pm 0.10^*$

Values are shown as mean ± SD ($n = 9$). Within each row, means followed by different letters differ significantly; asterisk denotes significant difference from t_0; ($p < 0.05$). Abbreviations: *AI*, atherogenicity index; *TI*, thrombogenicity index; *h/H*, hypocholesterolemic/Hypercholesterolemic ratio.

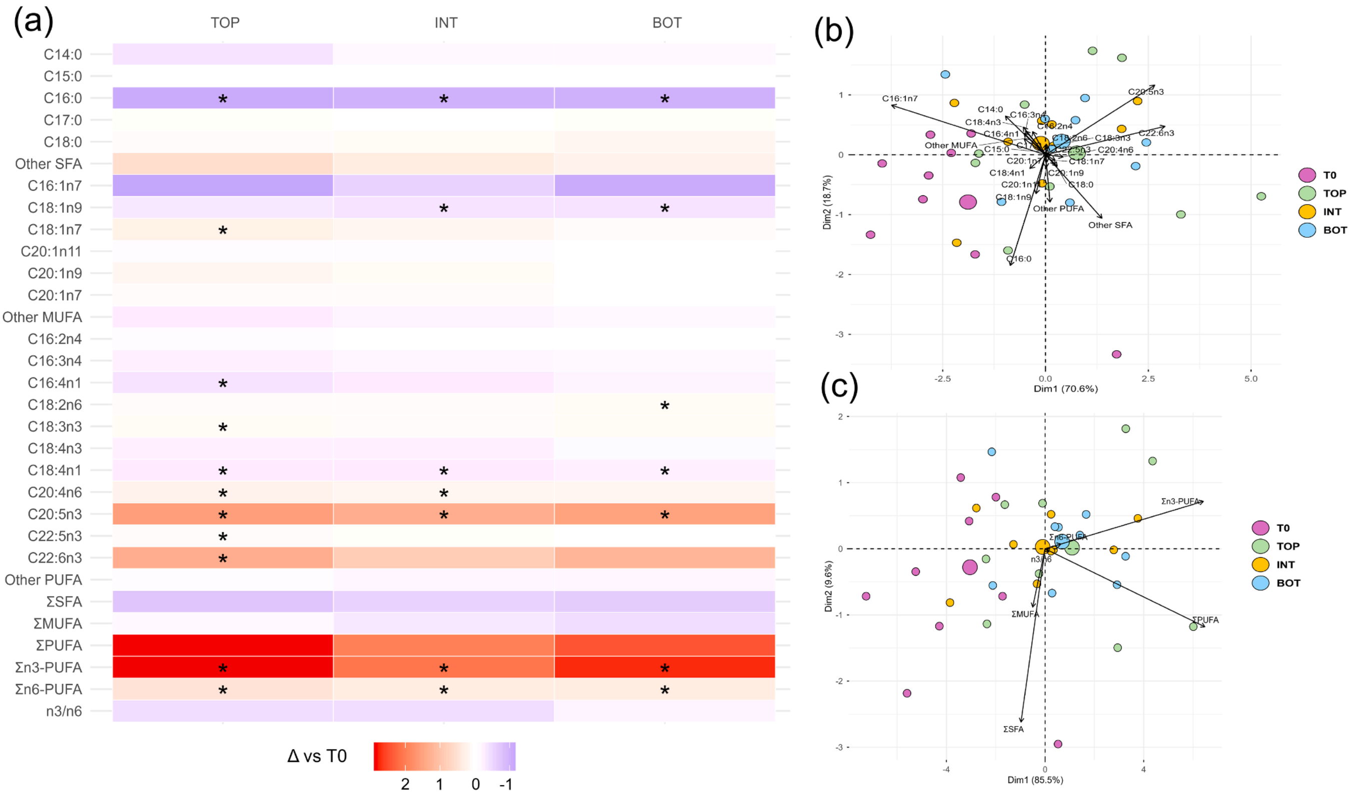

Fig. 3. Integrated overview of the fatty acid profile of Mediterranean mussels sampled at t_0 and after the 17-hour simulated transport (t_1). (**a**) Two-dimensional heatmap displaying the relative variations of TOP, INT, and BOT profiles against t_0. The chromatic scale ranges from purple (relative decrease compared to t_0) to intense red (relative increase compared to t_0). Asterisks denote significant difference from t_0; ($p < 0.05$). (**b**) PCA biplot based on single fatty acid percentages. (**c**) PCA biplot based on the main fatty acid categories and n3/n6 ratio. In both PCA biplots, large circles represent group centroids, smaller circles represent individual biological replicates, and vectors indicate variable loadings.

tissue (Fig. 4) and a moderate diffusion of tubules with reduced epithelium width and consequent lumen enlargement (Fig. 4e). Other episodes of tubular alterations, mainly consisting in inclusion of lipofuscin-like substances in digestive cells, epithelial cells hyperplasia or epithelium atrophy, as well as *foci* of hemocyte infiltration within the inter-tubular tissue, were scarcely recorded in mussels from both t_0 and t_1 (all the layers; Fig. 4f-h). Consequently, no significant differences were detected between mussels collected at t_0 and t_1 as well as among mussels from the different layers at t_1 in terms of HCI calculated in the digestive gland (Table 6).

Considering gills, mussels from t_0 and the TOP and INT layers at t_1 (Fig. 5a-c) showed the typical structure of the *ctenidium*, with well-defined lamellae characterized by a single layer epithelium attached to a basal lamina. A moderate presence of hemocytes was observed within the hemolymph sinuses of the intact gill filaments (Fig. 5e). Differently, mussels collected from the BOT layer, despite maintaining an overall functional gill morphology (Fig. 5d), were characterized by a high diffusion of intracellular inclusions of lipofuscin-like substances, epithelial vacuolization, and lamellar deformation and by a high incidence of hemocyte infiltration (Fig. 5f-h). Consequently, as reported in Table 6, the HCI calculated in mussels from the BOT layer was significantly higher compared to those collected at t_0 ($p < 0.0001$) and to those from the TOP and INT layers at t_1 ($p < 0.0001$).

3.4. Gene expression analysis

Considering the *cat*, *sod1*, and *hsp70* relative expression in digestive gland, no significant differences were detected between mussels collected at t_0 and t_1 as well as among mussels from the different layers at t_1 (Fig. 6a-c). Differently, as reported in Fig. 6d-f, mussels from BOT layer were characterized by a significantly higher *cat*, *sod1*, and *hsp70* relative expression in the gills compared to those collected at t_0 ($p = 0.0211$, 0.0395, and 0.0213 for *cat*, *sod1*, and *hsp70*, respectively) and to those from the TOP and INT layers at t_1 ($p = 0.0157$, 0.0275, and 0.0099 for *cat*, *sod1*, and *hsp70*, respectively).

4. Discussion

In the farm monitored in this case study, a substantial portion of production was harvested in February under the market size. Mussel socks were then transferred to Siracusa (Italy) where they were subsequently re-immersed to complete the production cycle. The present study investigated the effects of this 17-hour transport conducted at a constant temperature of 7 ± 1 °C, commonly adopted for short-term handling (Gökoglu, 2021; Huang et al., 2025), using a stacked arrangement. Results evidenced that these conditions were able to affect: (i) the mussels' nutritional profile, independently on the position of mussels within the layers of the piles; (ii) the overall health status of mussels located at BOT layer, possibly interested by the synergic effect of cold stress and hypoxia. In particular, the 17-hour simulated transport under refrigerated conditions did not affect the marketable indexes, regardless of the position of mussels within the stacked layers. Rather than short-term storage, these indexes appear to be more strongly related to the features of farming environment (temperature, salinity, pH, and nutrients' concentration) and mussels' growth and reproductive phase, identifying optimal harvesting windows during the productive cycle, specific for each specific coastal area (Acarli et al., 2024, 2023; Bordignon et al., 2024a; Yildiz et al., 2011). Accordingly, the values observed in the present study are consistent with those reported for mussels farmed in the western Adriatic Sea, which is strongly influenced by Po River inputs, and differ from those documented in the eastern Adriatic Sea and the Tyrrhenian Sea (Grkovic et al., 2023; Orban et al., 2002; Vernocchi et al., 2007). However, mussels were able to modulate their physiology and metabolism in response to environmental stressors, including thermal stress and hypoxia, as they are naturally adapted to the highly variable conditions of the intertidal environment, where they have evolved a high degree of physiological plasticity (Boroda et al., 2020). Accordingly, the simulated transport conditions of the present

Table 6
Histopathological condition index (HCI) calculated on digestive gland and gills of Mediterranean mussels sampled at t_0 and after the 17-hour simulated transport (t_1).

HCI	t_0	t_1		
		TOP	INT	BOT
Digestive gland	0.144 ± 0.055	0.141 ± 0.036	0.178 ± 0.052	0.185 ± 0.067
Gills	0.159 ± 0.057	0.174 ± 0.081^a	0.200 ± 0.085^a	0.418 ± $0.055^{b\ *}$

Values are shown as mean ± SD ($n = 9$). Within each row, means followed by different letters differ significantly; asterisk denotes significant difference from t_0; ($p < 0.05$).

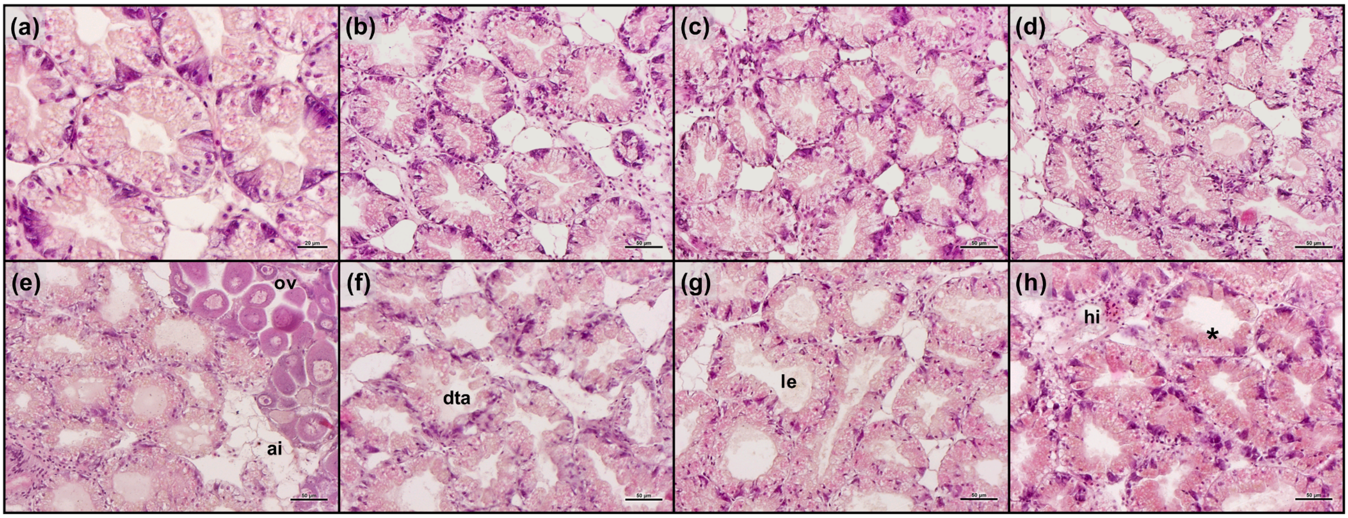

Fig. 4. Digestive gland histomorphology of Mediterranean mussels. (**a**) details of digestive tubules at t_0; representative pictures of digestive gland from mussels collected at (**b**) TOP, (**c**) INT, and (**d**) BOT layers after the 17-hour simulated transport (t_1); (**e-h**) details of alterations considered for the histopathological condition index calculation. Abbreviations: *AI*, adipocytes infiltration; *LE*, lumen enlargement; *ov*, ovary; *EA*, epithelial atrophy; *HI*, hemocyte infiltration. Symbols: arrow, example of epithelial cell hyperplasia; asterisk, example of intracellular inclusion of lipofuscin-like substances. Scale bars: (a) 20 μm, (b-h) 50 μm.

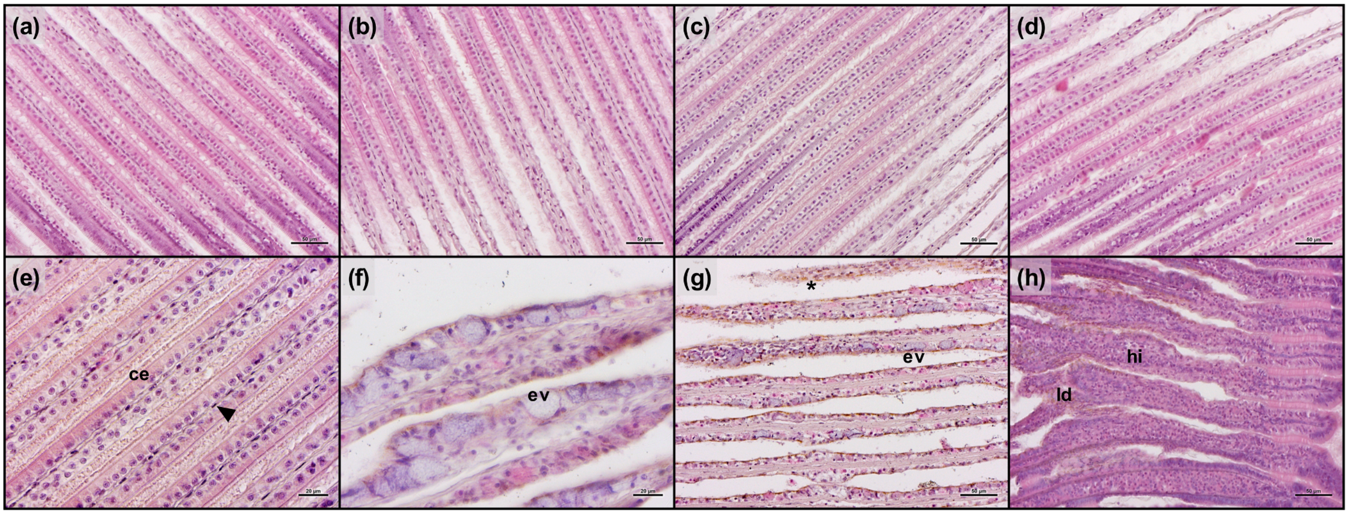

Fig. 5. Gills histomorphology of Mediterranean mussels. (**a**) details of gills filaments at t_0; representative pictures of gill filaments from mussels collected at (**b**) TOP, (**c**) INT, and (**d**) BOT layers after the 17-hour simulated transport (t_1); (**e**) details of gills filaments' ciliated epithelium; (**f-h**) details of alterations considered for the histopathological condition index calculation. Abbreviations: *CE*, ciliated epithelium; *EV*, epithelial vacuolization, *LD*, lamellar deformation, *HI*, hemocyte infiltration. Symbols: arrow, hemocyte; asterisk, example of intracellular inclusions of lipofuscin-like substances. Scale bars: (a-d and g, h) 50 µm, (e, f) 20 µm.

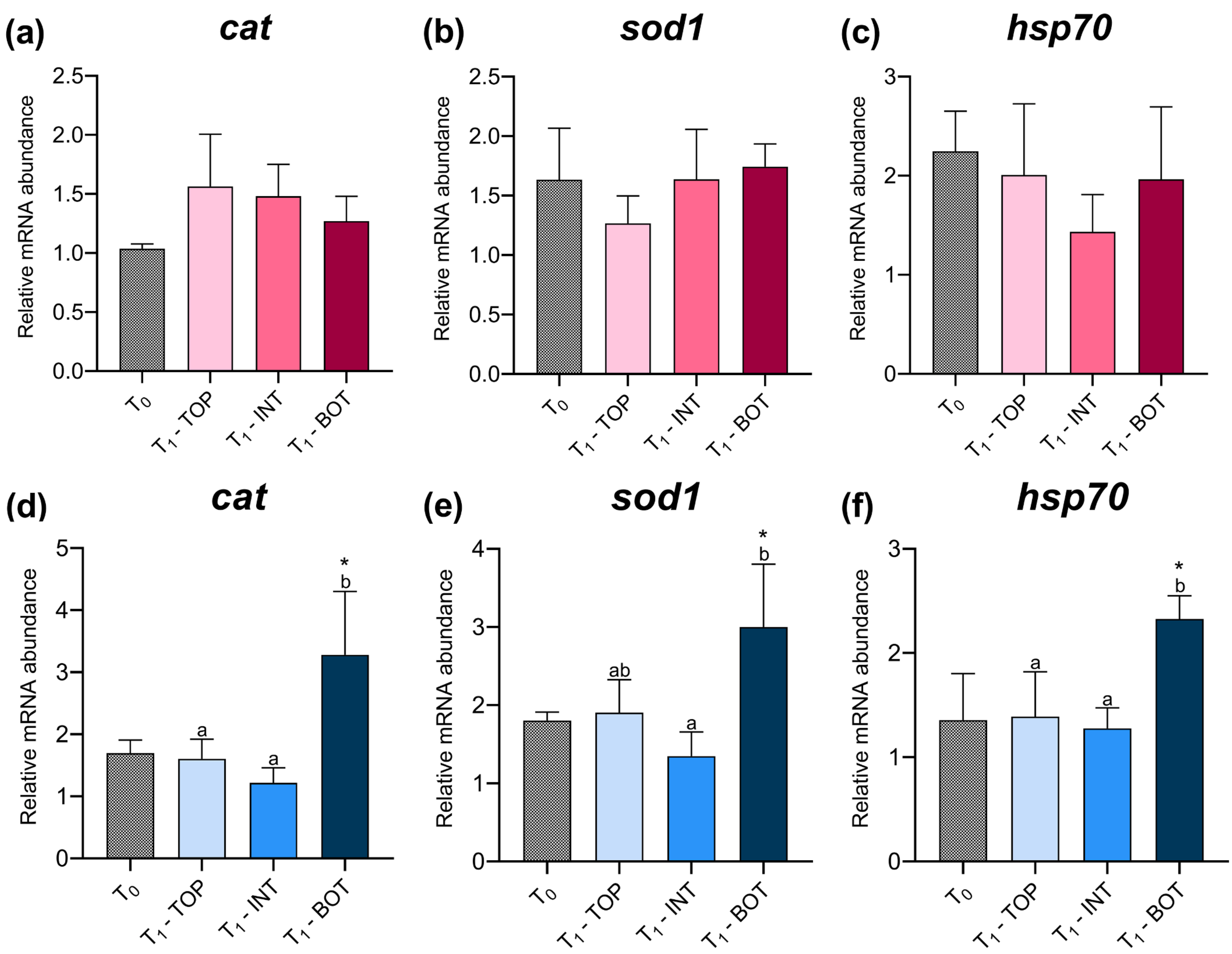

Fig. 6. Relative mRNA abundance of catalase (*cat*), superoxide dismutase 1 (*sod1*), and heat-shock protein 70 (*hsp70*) analyzed in (**a-c**) digestive glad and in (**d-f**) gills of Mediterranean mussels sampled at t_0 and after the 17-hour simulated transport (t_1). Values are shown as mean ± SD ($n = 9$). Different letters indicate significant differences among the layers within t_1, whilst asterisk denotes a significant difference from t_0; ($p < 0.05$).

study were able to affect the chemical profile of mussels. In fact, after 17-hour transport, a reduction in dry matter, protein, and lipid contents was observed, together with a remodeling of the fatty acid profile, regardless of the position of mussels within the pallet. Previous studies have shown that inadequate refrigeration or freezing storage can negatively affect the proximate composition and fatty acid profile of bivalves (Zhelyazkov et al., 2024; Zhou et al., 2019). Particularly, both long-term (up to 15 days) refrigerating (4 °C) and freezing storage (- 20 °C) have been reported to cause protein denaturation and lipid oxidation with a significant loss of PUFA (especially DHA and EPA) in Mediterranean mussels (Bejaoui et al., 2021). Nevertheless, in the present study, the variation in proximate composition highlighted at t_1 could reflect the physiological mobilization of endogenous reserves to maintain basal metabolic functions during the emersion phase rather than spoiling due to inadequate transport conditions (Albentosa et al., 2007; Bi et al., 2022). In fact, bivalves can use multiple metabolic pathways to ensure energy supply and maintain vital functions during transport in emersion conditions, including the mobilization of glycogen and protein reserves and the modulation of fatty acid metabolism (Calderwood et al., 2014; Venter et al., 2025, 2018). The rapid adaptation to the transport conditions was also evidenced by the specific fatty acid remodeling observed in all the mussels analyzed at t_1 compared to those analyzed at t_0. The observed decrease in palmitic acid reflected its prioritized use through beta-oxidation to meet the energy demands required for cellular maintenance and stress-response mechanisms in bivalves (Masanja et al., 2023; Zhukova, 2019). Conversely, the parallel increase in n3-PUFA (mainly driven by the EPA) reflects the activation of desaturase enzymes and the incorporation of long-chain PUFA, primarily EPA, into membrane phospholipids to counteract the reduction in membrane fluidity caused by the lower temperature during simulated transport (Fokina et al., 2014; Pernet et al., 2007; Vernocchi et al., 2007). The observed remodeling of fatty acid profile at t_1 suggested an active homeoviscous adaptation, a typical mechanism of poikilotherms organisms to modulate lipid composition of cells' membrane to maintain its fluidity and functionality under cold exposure (Biederman et al., 2021; Liu et al., 2025). In this regard, the increase in n3-PUFA indicated that even relatively short-term cold stress during transport triggers a rapid biochemical reorganization to protect cellular integrity (Pecl et al., 2025; Pernet et al., 2007). Interestingly, this physiological defense mechanism to cold stress resulted in improved lipid quality indices after transport, as evidenced by a decrease in both atherogenicity and thrombogenicity indexes and a parallel increase in the h/H ratio in mussels at t_1. This more favorable nutritional profile, considering human nutrition, could be particularly relevant for the small fraction of mussels that, upon reaching their destination, are sold directly in local markets rather than being relocated to the sea. Mediterranean mussels farmed in the Adriatic Sea typically show optimal lipid health indices regardless of harvest season (Bordignon et al., 2024b). This species, together with other bivalves, including scallops (*Flexopecten glaber*; Prato et al., 2019), Pacific oysters (*Crassostrea gigas*; Bordignon et al., 2020), and Manila clams (*Ruditapes philippinarum;* Biandolino et al., 2019), typically display a higher proportion of SFA during winter (especially in February) compared to warmer months (Lopez et al., 2023; Prato et al., 2010), as also observed in the present study. This feature can be related to the greater availability, during winter, of organic detritus in the water column and of SFA-rich phytoplankton species, like *Chaetoceros* spp. and *Skeletonema* spp. diatoms which bloom in this season (Bordignon et al., 2024b; Neri et al., 2023). Conversely, in spring and summer, at the end of the mussels' productive cycle when the final harvesting occurs, zooplankton and dinoflagellates filtered by the mussels are richer in MUFA and n−3 PUFA like DHA (Freites et al., 2002; Ventrella et al., 2008), rather than SFA (Ezgeta-Balić et al., 2012).

Finally, the simulated transport conditions had a major impact on the health status of the mussels located in the lowest layer of the pallet (BOT) which were characterized by more frequent spatial occlusion (possibly exacerbated by the cling-film) and reduced gaping activity. This condition may add hypoxia as synergistic stressor to the cold-stress condition in mussels located in the BOT layers. In fact, the reduced temperature experienced by mussels during the common refrigerated transports induce a reduction of the metabolic rate, extending survival time during emersion conditions and slows nutrient loss (Nie et al., 2024; Wu et al., 2025). Similarly, a rapid decrease in aerobic metabolism has been observed in bivalves exposed to short-term hypoxia (Nogueira et al., 2017; Sussarellu et al., 2013, 2010). Accordingly, in the present study, reserve mobilization was less pronounced in mussels located in the lower layers compared to those at the TOP one. The more stressful condition induced by the synergic effect of cold stress and hypoxia possibly further reduced the energy turnover rates of mussels located in INT and, especially, BOT layers, in order to extend their period of survival in adverse conditions (Liu et al., 2018; Sokolova et al., 2012; Zhang et al., 2020). In this regard, it has been recently demonstrated that immersion in magnesium chloride (40 g/L) was an effective pre-transport treatment for green-lipped mussel (*Perna canaliculus*), being able to reduce anaerobic stress biomarkers during transport and support faster recovery (Chen et al., 2026). In addition, the adverse effects of the synergic action of thermal stress and hypoxia experienced by mussels located at BOT level were further demonstrated by the upregulation of markers related to cellular (*hsp70*) and oxidative stress response (*sod1* and *cat*) at gills level. In fact, when bivalves are challenged by environmental stressors, including temperature variations and hypoxia, the mRNA expression levels of HSP isoforms are upregulated to facilitate the refolding of stress-damaged proteins, thus contributing to cellular homeostasis and survival under environmental stress (Nie et al., 2018). Particularly, since HSP70 maintains the tertiary structure of proteins during oxygen metabolic stress, a stimulus sufficiently strong to damage proteins may trigger transcription of its mRNA (Ramaglia and Buck, 2004). In addition, in Mediterranean mussels, cold stress is also responsible for increasing the production of reactive oxygen species (ROS) due to the disruption of oxygen-metabolic homoeostasis, with consequent activation of antioxidant enzymes in gills to prevent lipids peroxidation and enzyme inhibition (Wang et al., 2018). In the present study, this physiological response was possibly exacerbated in mussels located at BOT level due to the reduced airflow and spatial occlusion which facilitated hypoxia condition. The upregulation of both *sod1* and *cat* in BOT mussels is in line with results obtained in previous study which indicate that early hypoxia (within hours) triggers rapid increases in antioxidant enzyme expression to prepare intertidal mussels for future oxygen reintroduction which often causes a ROS burst, leading to oxidative damage (Nogueira et al., 2017). Finally, the more stressful condition for mussels located at BOT layer was also confirmed by the higher HCI in the gills compared to that obtained in mussels analyzed at t_0 and those from the TOP and INT layers at t_1. Particularly, the most frequent alterations observed in mussels from BOT layer were the diffuse infiltration of hemocytes (responsible for cell-mediated immunity mainly via phagocytosis or production of reactive oxygen species; Rocha et al., 2016b, 2016a) and the relatively high accumulation of lipofuscin-like pigments, a relevant marker of oxidative stress (Rocchetta et al., 2014). Conversely, the health status of the digestive gland was not altered by the transport conditions. The greater incidence of alterations in gills compared to the digestive gland may reflect acute, rather than chronic, exposure to adverse conditions, since the gills are more delicate structures that are primarily affected by environmental stressors and are more prone to oxidative damage (Almeida et al., 2005; Gostyukhina et al., 2022; Metian et al., 2009). These findings suggest that short-term refrigerated transport induces physiological adjustments to extend the period of survival in stressful conditions, particularly evident in mussels located at BOT level.

5. Conclusion

This study demonstrates that short-term refrigerated transport of Mediterranean mussels (*Mytilus galloprovincialis*) at 7 ± 1 °C for 17 h did not compromise marketable indexes, regardless of mussel position within stacked pallets. The exposure to low temperatures and the period of emersion induced the physiological adaptive mobilization of endogenous reserves and membrane remodeling. However, the arrangement of socks during transport affected the gills' health status of mussels located in the BOT layer. For these reasons, this study demonstrated the proper transport conditions used by the farm monitored, especially in terms of temperature management and shipment duration. Further considerations should be directed at the configuration of the socks (*i.e.* reducing weight on the lower layers or modifications in the wrapping method), as their arrangement could be a key factor in maintaining the functional integrity of sensitive organs such as the gills, especially in view of the relocation in the sea. The present study only represents a simulation of real transport but provides practical insights for optimizing mussel post-harvest handling and cold chain-based distribution of live mussels.

CRediT authorship contribution statement

Matteo Zarantoniello: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Adriano Mancini:** Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Antonio Bonelli:** Investigation, Formal analysis. **Marina Pasquini:** Writing – review & editing, Validation, Methodology. **Federico Conti:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Anna Laura Eusebi:** Validation, Project administration, Methodology. **Giuliana Parisi:** Writing – review & editing, Validation, Methodology, Data curation. **Alessia Arteconi:** Writing – review & editing, Validation, Methodology. **Giorgia Gioacchini:** Validation, Methodology. **Francesco Fatone:** Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Ike Olivotto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ike Olivotto reports financial support was provided by EU FOLOU project, European Union’s Horizon Europe. If there are other authors, they 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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2026.103720.

Data availability

Data will be made available on request.

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