

# Effect of the farming site and harvest time on the nutritional, elemental and volatile profile of mussels: A comprehensive analysis of the PDO ‘Cozza di Scardovari’

Francesco Bordignon<sup>a,\*</sup>, Eugenio Aprea<sup>b</sup>, Emanuela Betta<sup>c</sup>, Gerolamo Xiccato<sup>a</sup>, Angela Trocino<sup>a,d</sup>

<sup>a</sup> Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, Legnaro, Padova, Italy

<sup>b</sup> Center of Agriculture Food Environment C3A, University of Trento, Via Edmund Mach 1, 38098 San Michele all'Adige, Trento, Italy

<sup>c</sup> Sensory Quality Unit, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, San Michele all'Adige, Trento, Italy

<sup>d</sup> Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Viale dell'Università 16, Legnaro, Padova, Italy

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## ABSTRACT

This study comprehensively characterised a protected designation of origin mussel ‘Cozza di Scardovari’ (*Mytilus galloprovincialis*) by examining how it is affected by the farming site (outer vs. inner area of the lagoon) and harvest time (21 April vs. 18 May vs. 16 June). Harvest time affected the marketable traits and fatty acid profile of mussels, whereas farming site scarcely affected marketable traits and mussel yields. Mussels from the inner area of the lagoon displayed a superior nutritional profile, including higher contents of proteins (7.8% vs. 7.4%;  $P < 0.05$ ), lipids (1.2% vs. 1.0%;  $P < 0.001$ ) and essential amino acids such as tryptophan (+24%;  $P < 0.05$ ) and valine (+8%;  $P < 0.05$ ), with a more favourable n-3/n-6 ratio (7.7 vs. 7.0;  $P < 0.001$ ) than those from the close-to-sea area. Volatile organic compounds, mainly octanoic acid, dimethyl sulphide and 1-penten-3-ol, differed between farming sites within the same lagoon.

## 1. Introduction

Fisheries and aquaculture products account for a small fraction (3.7%) of the foodstuffs protected under geographical indications (GIs), which is a promising tool for achieving sustainable development goals and rural development (Girard, 2022). Recently, there has been a growing interest in protecting seafood products through GI, with the primary focus on obtaining a protected designation of origin (PDO) certification for freshwater fish and bivalve molluscs, while labelling processed seafood products with protected geographical indication (Girard, 2022).

Only three PDO labels were granted to Mediterranean mussels (*Mytilus galloprovincialis*), namely ‘Mejillón de Galicia’ (EC, 2006a; Costas-Rodríguez, Lavilla, & Bendicho, 2010), ‘Cozza di Scardovari’ (EC, 2006b) and ‘Moules de bouchot de la Baie du Mont Saint-Michel’ (EC, 2011). The PDO certification might enhance the economic sustainability of mussel farming (Girard, 2022), particularly at a European level where mussel production is challenged by limited seed availability, climate change and low product prices (Avdelas et al., 2021).

Italy is one of the major mussel producers in Europe (Carvalho & Guillen, 2021). Farming sites are mainly located in the Northern Adriatic regions, including Po Delta lagoons such as Sacca degli Scardovari (44°51'N, 12°24'E). The unique geographical and morphological features of the lagoon and the expertise of local farmers guarantee the quality of ‘Cozza di Scardovari’ mussels, protected with the PDO label since 2013. To date, no studies have comprehensively investigated the nutritional, elemental and volatile profile of this PDO mussel. Among several characteristics reported in the product specification, these mussels have a high meat yield (>25%), peculiar sweetness (sodium content <210 mg/100 g edible part), tenderness and ‘melt-in-the-mouth’ properties in addition to a distinctive taste (EC, 2006b).

The environmental conditions of a specific farming site impart unique characteristics to food products, playing a crucial role in determining their authenticity and origin (Costas-Rodríguez et al., 2010), which is paramount in the case of PDO food. The effects of farming sites and harvest time on mussel proximate composition have been previously explored (Bongiorno et al., 2015; Lopez, Bellagamba, & Moretti, 2023; Orban et al., 2002). However, farming sites often derive from different

\* Corresponding author.

E-mail address: [francesco.bordignon@unipd.it](mailto:francesco.bordignon@unipd.it) (F. Bordignon).

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marine ecosystems and regions. In this study, we hypothesise that changes could also occur within the same lagoon embayment. Along with farming sites, harvest time (from April to September for 'Cozza di Scardovari') affects mussel size and quality (Bongiorno et al., 2015). Increasing temperatures due to climate change, currently observed in the Adriatic region, induce premature harvesting of 'Cozza di Scardovari' mussels (Avdelas et al., 2021). Early harvesting often leads to the non-labelling of the product, as mussels do not reach the minimum fresh meat yield (>25%) required by the consortium's rule to obtain the PDO certification (Avdelas et al., 2021). In addition, early harvesting may affect the organoleptic and nutritional properties of mussels, including their volatile fingerprint. To the best of our knowledge, this is the first study to investigate the effects of different harvest times and farming sites within the same lagoon on the volatile profile of mussels. Only one study investigated the effect of farming sites in different Spanish coastal environments on the volatile organic compounds (VOCs) of mussels (Fuentes, Fernández-Segovia, Escriche, & Serra, 2009).

This study provided the first comprehensive characterisation of the PDO 'Cozza di Scardovari' mussels and tested the hypothesis that farming sites within the same lagoon environment (outer vs. inner part of the lagoon) and harvest time (April, May and June) could influence not only mussel condition and marketable traits, but also nutritional, elemental and volatile fingerprints.

## 2. Materials and methods

### 2.1. Study area

The PDO mussels were farmed in Sacca degli Scardovari (44°51'N, 12°24'E), a large lagoon located on the southern side of the Po River delta, specifically between its terminal southern branches (Po di Donzella and Po di Tolle). The lagoon occupies an area of 32.0 km<sup>2</sup> with an average depth of 1.5 m. It features a broad southern entrance connecting it to the Northern Adriatic Sea, protected by partially submerged sand banks. The tidal ranges are relatively small, with a maximum range of approximately 1 m during spring tides, while the northern bottom of the bay is permanently submerged. This northern section of the lagoon receives nutrient-rich agricultural runoffs. The southern part is influenced by seawater exchanges and hosts several shellfish farms, primarily cultivating clams and mussels. Eutrophication contributes to seasonal blooms of opportunistic macroalgae in the more sheltered areas during late spring and summer (Andreoli et al., 1994).

The mussels sampled in this study were cultured in culture ropes suspended from raft systems. They were sampled three times, in April, May and June, in accordance with the typical harvesting period in the lagoon. At each sampling time, mussels were harvested from two farming sites: the Southeast site (44°51'N, 12°25'E), located in the outer area of the lagoon and characterised by both fluvial and tidal flows, great water hydrodynamics and influenced by marine load (Andreoli et al., 1994); the Northwest site (44°52'N, 12°23'E), an area located in the inner area of the lagoon, is characterised by lower water exchange and higher nutrient and phytoplanktonic densities (Andreoli et al., 1994) compared with the outer area of the lagoon.

### 2.2. Mussel sampling and biometric measurements

At each sampling time (one day per month on 21 April, 18 May and 16 June), 8.0 kg of mussels per farming site were collected directly from the rearing structures (raft system). Temperature and salinity during each month of harvesting were retrieved from probes installed by the Regional Agency for Environmental Protection and Prevention of the Veneto (ARPAV, 2021) near the sampling sites. In the Southeast site, the temperature was 14.1 °C ± 1.8 °C in April, 18.1 °C ± 1.6 °C in May and 21.5 °C ± 0.5 °C in June, while salinity was 27.3 ± 2.0 ppt, 29.8 ± 2.4 ppt and 29.0 ± 0.7 ppt, respectively. In the Northwest site, the temperature was 15.3 °C ± 2.5 °C in April, 19.9 °C ± 1.2 °C in May and

25.2 °C ± 1.1 °C in June, while salinity was 27.4 ± 0.5 ppt, 28.2 ± 0.7 ppt and 28.9 ± 2.4 ppt, respectively. At harvesting, collected mussels were immediately placed into refrigerated polystyrene boxes. The temperature inside the boxes was maintained at 4.0 °C ± 0.5 °C during the transport to the laboratory, which lasted 1:30 h. Mussels were brushed and washed soon after their arrival. Subsequently, 120 samples per sampling time and farming site were randomly selected, among which 30 mussels were destined for measuring the cooked meat yield. The remaining 90 were weighed and used to measure meat yield and for biometric measurements, i.e. shell length, width and height, along with thickness at the shell margin and the maximum curvature of the valves using a digital calliper (Maurer 1/100 mm, Padova, Italy), as described in Bongiorno et al. (2015). Subsequently, among these 90 mussels, 30 were destined for shell strength assessment, 30 for the condition index measurement and 30 for water holding capacity (WHC) measurement, as further detailed.

After the biometric measurements, the byssus was cut, and soft tissues were gently removed from the shell using a scalpel. Meat and shell weights were measured before and after oven-drying (105 °C). Condition index and meat yield were calculated as per Bongiorno et al. (2015), as follows:

$$\text{Condition index (\%)} = [\text{meat dry weight (g)} / \text{shell dry weight (g)}] \times 100$$

$$\text{Fresh meat yield (\%)} = [\text{meat wet weight (g)} / \text{total mussel weight (g)}] \times 100$$

Shell strength was evaluated using a texture analyser (TA.XTplus, Stable Micro Systems, Godalming, UK), with a compression platen of 3 cm × 4 cm and a test speed of 0.5 mm/s, and the collected data was treated using the TEXT EXPONENT 32 software (Stable Micro Systems).

Cooked meat yield was calculated after 7 min of boiling and 5 min of cooling, as follows (Bongiorno et al., 2015):

$$\text{Cooked meat yield (\%)} = [\text{meat weight after cooking (g)} / \text{total mussel weight (g)}] \times 100$$

### 2.3. Water holding capacity

At each sampling, six pools (20 g each) per farming site of whole soft tissues (approximately five randomly sampled mussels per site in addition to the previously described specimens) were obtained to assess the WHC of the edible part (Bongiorno et al., 2015). The samples were weighed and then centrifuged (210g for 15 min) at 5 °C, and the WHC was assessed as follows:

$$\text{WHC (\%)} = [\text{weight of liquid released (g)} / \text{total mussel wet weight (g)}] \times 100$$

### 2.4. Chemical analysis

In addition to the specimens described above, 100 mussels per farming site were randomly sampled at each sampling time. Four pools (160.0 ± 11.0 g) of mussel fresh meat were obtained, freeze-dried, ground and stored at -80 °C for subsequent analysis of proximate composition, amino acid profile, fatty acid (FA) profile and mineral contents. A further sample (20.0 ± 2.6 g) of mussel edible parts (approximately five randomly sampled mussels) was directly processed to measure the total volatile basic nitrogen (TVB-N) content (EC, 1995). All chemical analyses were performed in duplicate.

#### 2.4.1. Proximate composition

The freeze-dried mussels were analysed according to the AOAC (2000) methods to determine the contents of dry matter (934.01), crude protein (2001.11) and ash (967.05). Ether extract was determined after acid hydrolysis treatment (EC, 1998).

#### 2.4.2. Fatty acid profile

The lipid fraction was obtained from freeze-dried samples through accelerated solvent extraction (Application Note 334; ASE®, Dionex, Sunnyvale, CA, USA). This involved two extraction cycles, utilising petroleum ether (petroleum ether 32,247; puriss.; Sigma-Aldrich; St. Louis, MO, USA) as a solvent, a temperature of 125 °C and a pressure of 10.3 Mpa. The process consisted of a 6-min heating phase and a 2-min extraction phase. Subsequently, extracted lipids were added with 10 ml of Na<sub>2</sub>SO<sub>4</sub> (LiChropur™ 80,948; HPLC grade; Sigma-Aldrich) (0.47% in H<sub>2</sub>O). Subsequently, samples were kept at 4 °C for 30 min, and the supernatant (petroleum ether and lipids) was collected in another vial previously weighed. After dry evaporation in N<sub>2</sub> stream (Genevac EZ-2, SP Industries, Warminster, PA, USA), residual samples (extracted lipids in vials) were weighed and then added with 2 ml of 2% H<sub>2</sub>SO<sub>4</sub> (Sulphuric acid 339,741; HPLC grade; Sigma-Aldrich) in methanol (Methanol 24,860; HPLC grade; Sigma-Aldrich) as per Christie (1989). Vials were stored at 50 °C in a heater overnight. Afterwards, hexane (Hexane 293,253; HPLC grade; Sigma-Aldrich) (1 ml hexane/20 mg lipids) and potassium bicarbonate 2% (Potassium bicarbonate 237,205; puriss.; Sigma-Aldrich) (5 ml) were added. After centrifugation, the supernatant was subjected to gas chromatography (GC) using an Agilent 7890 A Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA). The gas chromatograph had a split flow rate of 40 ml/min and a rate set of 160:1. A capillary column (30 m × 0.25 mm internal diameter, 0.14 µm film thickness; Supelco SP 2560, Sigma-Aldrich) was used with hydrogen as a carrier at a flow rate of 1.4 ml/min. The oven temperature was initially set at 50 °C for 2 min, then increased to 220 °C at a rate of 4 °C/min, and held for 17.5 min. The injector and detector temperatures were both set at 250 °C. The identification of FAs was accomplished by comparing the retention time of 37 standard fatty acid methyl esters (FAMES) (37 Component FAME Mix, 47,885-U; and PUFA n-3 with Menhaden Oil, 47,085-U; Supelco; Sigma-Aldrich). The individual FAMES were expressed as the percentage of the total area of eluted FAMES.

#### 2.4.3. Amino acid profile

The freeze-dried samples underwent acid hydrolysis, and were later subjected to pre-column derivatisation using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AA218 Amino Acid Standard; Sigma-Aldrich). Afterwards, amino acids were separated through RP-HPLC and detected using UV, as per a method adapted from the European Pharmacopoeia (2003). Briefly, to determine alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine, the freeze-dried samples were hydrolysed with 6 M hydrochloric acid at 105 °C for 24 h. Cysteine was quantified after a preliminary reaction with dithiodipropionic acid (109,010; HPLC grade; Sigma-Aldrich), followed by hydrolysis. The hydrolysed samples were then neutralised with 8 M sodium hydroxide (LiChropur™ 69,413; HPLC grade; Supelco; Sigma-Aldrich), filtered and adjusted to volume. The derivatisation step followed the manufacturer's instructions (AccQTag Ultra Derivatization Kit, Waters Corporation, Singapore). Tryptophan analysis involved basic hydrolysis with barium hydroxide at 105 °C for 24 h, followed by neutralisation, filtration and direct RP-HPLC analysis (European Pharmacopoeia, 2003). The separation and quantification of amino acids were performed using an Agilent 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a C18 reversed-phase column (CORTECS C18, 2.7 µm, 2.1 × 150 mm; Agilent Technologies) maintained at 45 °C and a diode array detector (DAD VL+, Agilent Technologies).

#### 2.4.4. Mineral profile

Mineral analysis was conducted on ground freeze-dried mussel samples. Sample preparation followed the protocol described in the AOAC Official Method 2013.06 (AOAC, 2016a). In detail, each sample weighing between 0.30 and 0.35 g was placed in a vessel with 2 ml of 30% hydrogen peroxide (H1009; Merck Chemicals GmbH, Darmstadt,

Germany) and 7 ml of concentrated (65%) nitric acid (1.00441; Merck Chemicals GmbH, Darmstadt, Germany), both of suprapur quality. The prepared samples underwent microwave digestion (Ethos 1600 Milestone S.r.l., Sorisole, BG, Italy) following these steps: Step 1, sample heating from 25 °C to 200 °C in 15 min at 1200 W with a maximum pressure of 100 bar; Step 2, maintenance of a temperature of 200 °C for 18 min at 1200 W with a maximum pressure of 100 bar; Step 3, cooling from 200 °C to 35 °C in 15 min. After cooling to room temperature, the dissolved sample was diluted with ultrapure water (with a resistivity of 18.2 MΩ cm at 25 °C) to a final volume of 25 ml (AOAC, 2016a). The mineral concentrations were determined using Spectro Arcos EOP inductively coupled plasma–optical emission spectrometry (Spectro Analytical Instruments GmbH, Kleve, Germany), as per the AOAC Official Method 985.01 (AOAC, 2016b). Calibration standards were prepared by mixing multi- and single-element standard solutions (Inorganic Ventures Inc., Christiansburg, VA, USA) with 30% suprapur nitric acid (Merck Chemicals GmbH) to achieve similar matrices as the samples (AOAC, 2016b).

#### 2.5. Volatile organic compound analysis

To analyse VOCs, 10 fresh mussels were randomly sampled at each site at each harvesting time. Mussels were shucked and muscles were immediately placed in liquid nitrogen. Frozen muscles were powdered with a grinder (Analytical mill 11, IKA-Werke GmbH & Co, Staufen, Germany). The powder was placed in a falcon at –80 °C until VOC analysis.

Headspace solid-phase microextraction coupled with GC-mass spectrometry was employed to extract, separate and identify volatile compounds. GC analyses were carried out using a Clarus 500 GC system (PerkinElmer AutoSystem XL, Waltham, MA, USA) linked to a mass spectrometer (TurboMass Gold; PerkinElmer) and furnished with a PAL triaxis autosampler (CTC Analytics, Zwingen, Switzerland).

The method starting from a previously optimised procedure on milk samples was adapted to the analysis of mussels (Bottiroli, Aprea, Betta, Fogliano, & Gasperi, 2020). In details, a 1 g sample of thawed mussel powder was inserted in a 20-ml GC amber vial together with 1 ml of distilled water, 0.5 g of NaCl and a magnetic stir bar and spiked with 50 µl of 2-octanol (96% GC grade; Aldrich, Milan, Italy) water solution (0.9875 µg/l) used as internal standard. A 2-cm DVB/Car/PDMS fibre (50/30 µm thickness; Supelco; Sigma-Aldrich; St. Louis, MO, USA) was used. Before sampling, the vials underwent a 20-min equilibration at 40 °C with continuous stirring. Subsequently, the triphasic fibre was introduced to the vial headspace and exposed for 60 min at 40 °C while continuously stirred. The volatile compounds were thermally released from the fibre coating into the GC injector port, maintained at 250 °C, in splitless mode. Analytes separation was conducted using an HP-Innowax fused-silica capillary column (30 m length, 0.32 mm inner diameter, 0.5 µm film thickness) (J&W Scientific, Agilent Technologies, Santa Clara, CA, USA). Helium was used as carrier gas at a 1.5 ml/min flow rate. The oven temperature was programmed as follows: 40 °C held for 3 min, increased at a rate of 4 °C/min to 220 °C and held for 1 min; further increased at a rate of 10 °C/min to 250 °C and held for 1 min. The transfer line temperature was set at 220 °C. The mass spectrometer was operated under the electron ionisation mode (70 eV), scanning the range from 33 to 300 *m/z*. Samples were analysed in triplicates. The content of each compound was expressed as of 2-octanol equivalents (µg/l). Compound identification was based on mass spectra matching with the standard NIST/EPA/NIH (NIST 14) and Wiley 7th Mass Spectral Libraries and linear retention indices (LRI) compared with the literature. The LRI were calculated under the same chromatographic conditions after injection of a C7–C30 n-alkane series (Supelco; Sigma-Aldrich; St. Louis, MO, USA).

## 2.6. Statistical analysis

The data were submitted to analysis of variance (ANOVA) using PROC GLM of SAS (SAS, 2013), with harvest time, farming site and their interactions as fixed effects. Bonferroni's test was used to compare means, with means assumed to be statistically different at  $P \leq 0.05$  and to be highly statistically different at  $P \leq 0.001$ . Principal component analysis (PCA) was used to explore the data after a Log transformation and Pareto scaling of the variables. To reduce the dimensionality of the dataset, only the variables that were considerably different ( $P < 0.05$ ) among the harvest time (April, May and June) and/or between farming sites (Southeast and Northwest) after ANOVA analysis were used for the PCA. Multivariate analysis was performed using the software Simca v.17 (Sartorius Stedim Data Analytics AB, Göttingen, Germany).

## 3. Results and discussion

### 3.1. Effect of farming site

#### 3.1.1. Biometry and marketable traits

The biostatistical analysis of mussels could offer several insights about mussel growth and shell shape, which are important indicators to optimize farming sites and harvesting period.

The limited impact of the farming sites within the same lagoon on mussel biometry (weight 18.7 g and length 61.3 mm, average of the data related to the Southeast and Northwest sites reported in Table 1), condition index (20.5%) and marketable traits aligns with previous reports about the Northern Adriatic Sea (Bordignon et al., 2024; Moschino, Bressan, Cavaleri, & Da Ros, 2015). In contrast, the thicker shell margin of mussels collected from the outer-Southeast site compared with those harvested in the inner-northwest site (+20%;  $P < 0.001$ ) (Table 1) could be attributed to the higher water dynamics in the closer-to-sea environment, leading to stronger forces acting on mussel shells (Moschino et al., 2015; Steffani & Branch, 2003). Thicker shells could also result from the effect of increasing temperatures (Keskin & Ekci, 2020; Mele, McGill, Thompson, Fennell, & Fitzer, 2023) and salinity (Wang et al., 2012), but this was likely not the case in our study. The highest thickness measured in the Southeast site in June (Table S1), could not be associated with a higher temperature (21.5 °C vs. 25.0 °C in the Southeast compared with the Northwest) or salinity (approximately 29 ppt in both sites). Despite the increased thickness of the shell margin, there were no noticeable impacts on mussel fresh meat yield (37.7%), shell incidence (33.0%) or shell strength (261.0 N/cm<sup>2</sup>) (Table 1).

**Table 1**

Effect of farming site and harvest time on biometry, shell characteristics, and marketable traits of the PDO "Cozza di Scardovari" mussels.

	Farming site (S)		Harvest time (T)			P-value			RMSE
	Southeast	Northwest	April	May	June	S	T	S × T	
Mussels, n	270	270	180	180	180				
Weight, g	18.4	18.9	13.8 <sup>a</sup>	21.5 <sup>b</sup>	20.9 <sup>b</sup>	0.22	<0.001	0.99	4.51
Length, mm	61.3	61.3	57.7 <sup>a</sup>	63.7 <sup>b</sup>	62.6 <sup>b</sup>	0.97	<0.001	0.53	5.16
Width, mm	30.7	30.4	28.6 <sup>a</sup>	31.2 <sup>b</sup>	31.8 <sup>b</sup>	0.12	<0.001	0.58	2.45
Height, mm	23.3	23.1	21.9 <sup>a</sup>	24.1 <sup>b</sup>	23.5 <sup>b</sup>	0.35	<0.001	0.17	2.37
TM, mm	0.1	0.2	0.2 <sup>b</sup>	0.1 <sup>a</sup>	0.2 <sup>b</sup>	<0.001	<0.001	<0.001	0.06
TC, mm	0.7	0.7	0.6 <sup>a</sup>	0.7 <sup>b</sup>	0.8 <sup>c</sup>	0.83	<0.001	<0.001	0.11
Mussels, n	90	90	60	60	60				
Condition index, %	20.0	21.0	15.5 <sup>a</sup>	23.2 <sup>b</sup>	22.8 <sup>b</sup>	0.16	<0.001	0.11	4.77
Water holding capacity, %	8.4	9.8	20.0 <sup>c</sup>	5.6 <sup>b</sup>	1.7 <sup>a</sup>	0.15	<0.001	0.31	2.73
Fresh meat yield, %	37.7	36.9	38.2 <sup>b</sup>	40.0 <sup>b</sup>	33.8 <sup>a</sup>	0.21	<0.001	<0.001	6.03
Cooked meat yield, %	19.0	21.2	16.6 <sup>a</sup>	19.4 <sup>b</sup>	24.4 <sup>c</sup>	<0.001	<0.001	<0.05	4.50
Shell incidence, %	33.3	32.7	33.1 <sup>ab</sup>	31.4 <sup>a</sup>	34.5 <sup>b</sup>	0.41	<0.01	<0.001	4.60
Shell strength, N/cm <sup>2</sup>	266	256	186 <sup>a</sup>	271 <sup>b</sup>	326 <sup>c</sup>	0.39	<0.001	0.63	83.0
Mussels, n	15	15	10	10	10				
TVB-N, mg/100 g ww	17.9	19.0	16.0 <sup>a</sup>	19.2 <sup>ab</sup>	20.2 <sup>b</sup>	0.37	<0.05	0.55	2.87

TM: thickness at the shell margin; TC: thickness at the maximum curvature of the valve; TVB-N: total volatile basic nitrogen; ww: wet weight; RMSE: root mean square error. Values with different superscript letters in a column are significantly different ( $P \leq 0.05$ ).

### 3.1.2. Nutritional profile

Mussels from the inner area of the lagoon (Northern) exhibited a better nutritional profile, characterised by a higher content of proteins (7.8% vs. 7.4%;  $P < 0.05$ ), lipids (1.2% vs. 1.0%;  $P < 0.001$ ) (Table 2) and essential amino acids such as cysteine and cystine (+7%;  $P < 0.05$ ), phenylalanine (+7%;  $P < 0.05$ ), tryptophan (+24%;  $P < 0.05$ ) and valine (+8%;  $P < 0.05$ ) (Table 3) compared with those from the other close-to-sea area, likely owing to more favourable nutritional (i.e. high phytoplanktonic density) and environmental conditions of the inner area of the lagoon (Andreoli et al., 1994; Bongiorno et al., 2015). As for FAs (Table 2), the higher proportion of eicosapentaenoic acid (C20:5 n-3; EPA) (12.6% vs. 11.9%;  $P < 0.05$ ) and the more favourable n-3/n-6 ratio (7.7 vs. 7.0;  $P < 0.001$ ) in mussels from the inner-northeast site compared with the outer-southeast site might be associated with the prevalence of EPA-rich phytoplankton species, such as *Skeletonema* and *Chaetoceros* (Piveteau et al., 2000; Rodríguez-Núñez & Toledo-Agüero, 2017), which are known to colonise the inner northern area of Sacca degli Scardovari (Andreoli et al., 1994). Conversely, mussels from the outer region of the lagoon showed higher docosahexaenoic acid (C22:6 n-3; DHA) (6.80% vs. 6.09%;  $P < 0.001$ ), likely due to a greater abundance of *Thalassiospira* spp., which contains higher levels of DHA than *Skeletonema* spp. (Boelen, Van Mastrigt, Van De Bovenkamp, Heeres, & Buma, 2017). Overall, the FA profile of the mussels analysed in the present study reflected the typical profile of molluscs from the Northern Adriatic coastal environments (Bongiorno et al., 2015; Bordignon et al., 2024), where SFA account for 30%–40% of total FA (38.8% in our study; Table 2), followed by PUFA (34.9%) and MUFA (24.6%). This is because of the high abundance of SFA-rich diatoms in these waters (Bordignon et al., 2024). Peculiar FAs of marine molluscs, including *M. galloprovincialis*, were also found, such as dienoic C20:2 non-methylene-interrupted (NMI) (0.6%) and C22:2 NMI (5.8%), the proportions of which are influenced by differences in mussel diet and environmental conditions that occur in different farming sites (Barnathan, 2009).

The elemental profile has been recently used as a fingerprint tool in wild and farmed mussels derived from different locations and harvested at different times (Kapranov, Kozintsev, Bobko, & Ryabushko, 2023; Peycheva et al., 2023). Under our conditions, the influence of farming sites on mussel mineral profile was negligible when compared with the impact of the harvest time. This was also observed in other aquatic environments (Peycheva et al., 2023). Overall, the contents of heavy metals such as cadmium (0.1 mg/kg, on average), lead (0.2 mg/kg), arsenic (1.7 mg/kg) and nickel (0.7 mg/kg) in the 'Cozza di Scardovari' mussels were remarkably lower than those commonly found in mussels

**Table 2**

Effect of the farming site and harvest time on the proximate composition and fatty acid (% of total FAME) profile of PDO “Cozza di Scardovari” mussels.

	Farming site (S)		Harvest time (T)			P-value			RMSE
	Southeast	Northwest	April	May	June	S	T	S × T	
Pools <sup>1</sup> , n	12	12	8	8	8				
Proximate composition, %									
Water	85.7	85.2	86.5 <sup>b</sup>	85.2 <sup>a</sup>	84.6 <sup>a</sup>	0.06	<0.001	<0.05	0.63
Protein	7.36	7.81	7.37 <sup>a</sup>	7.93 <sup>b</sup>	7.40 <sup>a</sup>	<0.05	<0.05	<0.05	1.26
Lipids	1.01	1.16	0.89 <sup>a</sup>	1.11 <sup>b</sup>	1.25 <sup>c</sup>	<0.001	<0.001	<0.001	0.32
Ash	2.28	2.16	2.52 <sup>b</sup>	2.46 <sup>b</sup>	1.60 <sup>a</sup>	<0.01	<0.001	<0.05	1.15
Fatty acids, % total FAME									
C14:0	5.78	6.45	4.18 <sup>a</sup>	6.47 <sup>b</sup>	7.69 <sup>c</sup>	<0.001	<0.001	<0.001	0.24
C15:0	0.73	0.73	0.84 <sup>c</sup>	0.74 <sup>b</sup>	0.60 <sup>a</sup>	0.79	<0.001	<0.001	0.03
C16:0	24.4	24.8	25.4 <sup>b</sup>	24.4 <sup>a</sup>	24.1 <sup>a</sup>	0.09	<0.001	0.61	0.54
C17:0	2.21	2.01	2.45 <sup>c</sup>	2.10 <sup>b</sup>	1.78 <sup>a</sup>	<0.001	<0.001	<0.001	0.09
C18:0	4.95	4.72	5.74 <sup>c</sup>	4.71 <sup>b</sup>	4.04 <sup>a</sup>	<0.01	<0.001	0.06	0.18
Other SFA	0.37	0.31	0.39 <sup>b</sup>	0.26 <sup>a</sup>	0.38 <sup>b</sup>	<0.05	<0.001	0.09	0.05
C16:1 n-7	11.6	12.3	11.0 <sup>a</sup>	10.8 <sup>a</sup>	14.1 <sup>b</sup>	<0.01	<0.001	<0.001	0.55
C18:1 n-9	1.70	1.60	1.78 <sup>c</sup>	1.66 <sup>b</sup>	1.52 <sup>a</sup>	<0.001	<0.001	<0.05	0.07
C18:1 n-7	4.30	4.25	4.65 <sup>b</sup>	4.12 <sup>a</sup>	4.05 <sup>a</sup>	0.13	<0.001	0.61	0.09
C20:1 n-11	1.44	1.37	1.55 <sup>b</sup>	1.50 <sup>b</sup>	1.17 <sup>a</sup>	<0.01	<0.001	<0.001	0.06
C20:1 n-9	2.45	2.23	2.98 <sup>c</sup>	2.36 <sup>b</sup>	1.68 <sup>a</sup>	<0.001	<0.001	<0.001	0.11
C20:1 n-7	1.50	1.53	1.67 <sup>b</sup>	1.45 <sup>a</sup>	1.43 <sup>a</sup>	0.11	<0.001	<0.01	0.05
Other MUFA	1.49	1.39	1.50 <sup>b</sup>	1.49 <sup>b</sup>	1.33 <sup>a</sup>	<0.001	<0.001	<0.01	0.04
C16:2 n-4	0.68	0.79	0.66 <sup>a</sup>	0.72 <sup>b</sup>	0.83 <sup>c</sup>	<0.001	<0.001	<0.001	0.04
C18:2 n-6	1.67	1.52	1.16 <sup>a</sup>	1.63 <sup>b</sup>	1.98 <sup>c</sup>	<0.001	<0.001	<0.01	0.03
C18:3 n-3	1.75	1.78	1.39 <sup>a</sup>	2.07 <sup>c</sup>	1.84 <sup>b</sup>	0.30	<0.001	<0.001	0.05
C18:4 n-3	1.64	1.77	1.87 <sup>b</sup>	1.96 <sup>b</sup>	1.28 <sup>a</sup>	<0.01	<0.001	<0.001	0.09
C20:2 NMI	0.63	0.61	0.58 <sup>a</sup>	0.67 <sup>c</sup>	0.61 <sup>b</sup>	0.08	<0.001	<0.001	0.02
C20:4 n-6	1.48	1.36	1.12 <sup>a</sup>	1.23 <sup>b</sup>	1.91 <sup>c</sup>	<0.001	<0.001	<0.01	0.07
C20:5 n-3	11.9	12.6	10.6 <sup>a</sup>	12.5 <sup>b</sup>	13.8 <sup>c</sup>	<0.05	<0.001	0.85	0.59
C22:2 NMI	6.13	5.37	7.41 <sup>c</sup>	5.94 <sup>b</sup>	3.91 <sup>a</sup>	<0.001	<0.001	<0.001	0.23
C22:5 n-3	0.61	0.62	0.61	0.61	0.63	0.61	0.16	<0.01	0.04
C22:6 n-3	6.80	6.09	5.91 <sup>a</sup>	6.86 <sup>b</sup>	6.57 <sup>b</sup>	<0.001	<0.01	<0.001	0.43
Other PUFA	1.87	2.08	2.11 <sup>b</sup>	2.00 <sup>b</sup>	1.82 <sup>a</sup>	<0.001	<0.001	<0.001	0.11
4,8,12-TMTD	1.80	1.60	2.50 <sup>c</sup>	1.70 <sup>b</sup>	0.90 <sup>a</sup>	0.06	<0.001	<0.001	0.11
SFA	38.5	39.1	39.0	38.7	38.6	0.09	0.53	0.36	0.78
MUFA	24.5	24.7	25.1 <sup>b</sup>	23.4 <sup>a</sup>	25.3 <sup>b</sup>	0.53	<0.001	0.001	0.55
PUFA	35.2	34.6	33.4 <sup>a</sup>	36.2 <sup>b</sup>	35.2 <sup>b</sup>	0.20	<0.001	0.06	1.15
Isoprenoids	1.80	1.60	2.50 <sup>c</sup>	1.70 <sup>b</sup>	0.90 <sup>a</sup>	0.06	<0.001	<0.001	0.11
PUFA n-6	3.37	3.08	2.40 <sup>a</sup>	3.07 <sup>b</sup>	4.21 <sup>c</sup>	<0.001	<0.001	<0.001	0.09
PUFA n-3	22.8	23.0	20.4 <sup>a</sup>	24.1 <sup>b</sup>	24.2 <sup>b</sup>	0.73	<0.001	0.06	1.05
n-3/n-6	7.04	7.74	8.49 <sup>c</sup>	7.92 <sup>b</sup>	5.76 <sup>a</sup>	<0.001	<0.001	<0.001	0.24

<sup>1</sup> Each pool consisted of the meat of 25 randomly selected mussels. NMI: Non-methylene-interrupted fatty acid; 4,8,12-TMTD: Trimethyltridecanoic acid; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; RMSE: Root mean square error. Values with different superscript letters in a column are significantly different ( $P \leq 0.05$ ).

**Table 3**

Effect of the farming site and harvest time on the amino acid (mg/100 g wet weight) content of PDO “Cozza di Scardovari” mussels.

	Farming site (S)		Harvest time (T)			P-value			RMSE
	Southeast	Northwest	April	May	June	S	T	S × T	
Pools <sup>1</sup> , n	12	12	8	8	8				
Alanine	381	396	356 <sup>a</sup>	405 <sup>b</sup>	405 <sup>b</sup>	0.26	<0.01	0.08	32.9
Arginine	411	431	408 <sup>a</sup>	498 <sup>b</sup>	357 <sup>a</sup>	0.26	<0.001	0.10	43.5
Aspartic acid	812	844	693 <sup>a</sup>	797 <sup>b</sup>	993 <sup>c</sup>	0.23	<0.001	0.06	62.9
Cysteine	68	73	66 <sup>a</sup>	70 <sup>ab</sup>	76 <sup>b</sup>	<0.05	<0.001	0.10	4.5
Glutamic acid	1052	1108	959 <sup>a</sup>	1078 <sup>b</sup>	1203 <sup>c</sup>	0.13	<0.001	0.19	87.3
Glycine	665	682	713 <sup>b</sup>	804 <sup>c</sup>	504 <sup>a</sup>	0.55	<0.001	0.18	68.4
Histidine	225	236	221 <sup>a</sup>	251 <sup>b</sup>	218 <sup>a</sup>	0.25	<0.01	0.73	22.2
Isoleucine	196	207	207 <sup>b</sup>	241 <sup>c</sup>	157 <sup>a</sup>	0.16	<0.001	<0.05	18.3
Leucine	381	403	373 <sup>a</sup>	433 <sup>b</sup>	371 <sup>a</sup>	0.12	<0.001	0.07	32.9
Lysine	687	735	619 <sup>a</sup>	771 <sup>b</sup>	742 <sup>b</sup>	0.06	<0.001	0.07	59.0
Methionine	117	124	98.0 <sup>a</sup>	125 <sup>b</sup>	137 <sup>b</sup>	0.25	<0.001	0.32	14.5
Phenylalanine	237	254	236	261	240	<0.05	0.07	0.15	18.5
Proline	294	302	260 <sup>a</sup>	309 <sup>b</sup>	324 <sup>b</sup>	0.43	<0.001	0.07	24.1
Serine	341	353	233 <sup>a</sup>	403 <sup>b</sup>	405 <sup>b</sup>	0.23	<0.001	<0.05	25.0
Threonine	341	359	341	356	354	0.14	0.52	0.15	28.1
Tryptophan	79.7	98.8	75.6 <sup>a</sup>	77.8 <sup>a</sup>	114 <sup>b</sup>	<0.05	<0.001	0.07	18.2
Tyrosine	224	237	200 <sup>a</sup>	244 <sup>b</sup>	248 <sup>b</sup>	0.16	<0.001	0.25	21.1
Valine	216	234	219 <sup>b</sup>	272 <sup>c</sup>	183 <sup>a</sup>	<0.05	<0.001	0.12	20.0

<sup>1</sup> Each pool consisted of the meat of 25 randomly selected mussels. RMSE: Root mean square error. Values with different superscript letters in a column are significantly different ( $P \leq 0.05$ ).

from the Italian market (Chiesa et al., 2018). Moreover, cadmium levels were considerably below the maximum limit set by the EU Regulation 1881/2006 (1.0 mg/kg wet weight) and lower than those reported in studies on mussels harvested in the Black Sea (0.3–0.7 mg/kg wet weight), Marmara Sea (0.3–1.0 mg/kg wet weight) and Southeast Adriatic Sea (0.2–0.6 mg/kg wet weight) (Peycheva et al., 2023). Similarly, lead content was substantially lower (–87%) than the maximum content accepted in bivalve molluscs (1.5 mg/kg wet weight; EC, 2006) and not affected by the farming site, which is consistent with previous findings (Çevik et al., 2008).

### 3.1.3. Volatile organic compounds

A total of 70 volatile compounds were identified in the headspace of the analysed mussels (Table S5). Alcohols represented the major class (16 identified compounds), which however scarcely contributed to the overall flavour of the product due to their high threshold value (Fuentes et al., 2009). Instead, aldehydes (13 compounds), originating from the degradation of FAs (Aprea, Gasperi, Betta, Sani, & Cantini, 2018) and amino acids (Piveteau et al., 2000) play a major role in the flavour of many food products and are responsible for a wide range of oxidised flavours (Aprea et al., 2018; Le Guen, Prost, & Demaimay, 2000). In our mussels, octanoic acid was the most abundant volatile compound, conferring a characteristic fatty, oily and cheesy aroma (Ianni, Bennato, Martino, Grotta, & Martino, 2020; Kalua et al., 2007), followed in abundance by dimethyl sulphide and 1-penten-3-ol, imparting plant-like aromas (Cecchi, Sacchini, & Felici, 2018) and a characteristic sulphury odour, distinctive of wild mussels (Le Guen et al., 2000). Along with them, other VOCs observed in our fresh mussels such as benzaldehyde, 1-octen-3-ol and 3,5-octa-dien-2-one, could have a great impact on their typical flavour, contributing to a desirable plant-organic aroma and fishy smell, and their contents were shown to change according to mussel origin (Fuentes et al., 2009; Le Guen et al., 2000). Consistently, the concentrations of some key volatiles, including dimethyl sulphide, benzaldehyde, (E,E)-2,4-heptadienal, ethyl hexanoate and ethyl octanoate, as well as hexanal, heptanal and octanal, were different in mussels farmed in two sites of the same lagoon. Specifically, mussels from the inner area of the lagoon could have a more sweet, fruity-like aroma as a result of higher ethyl hexanoate (fruity, apple-like), ethyl octanoate (sweet, fruity, apple) and (E,E)-2,4-heptadienal (fatty, hay) and as result of the increase over time of benzaldehyde (fruity, nutty, almond-like aroma) (Mathatheeranan et al., 2023). In contrast, high levels of aldehydes obtained from PUFA oxidation, such as hexanal, heptanal and octanal (green, grassy and/or citrus fruits-like odours), which were higher in the mussels of the outer area of the lagoon, can be associated with the off-flavours of seafood products (Varlet, Prost, & Serot, 2007).

## 3.2. Effect of harvest time

### 3.2.1. Biometry and marketable traits

Seasonal fluctuations in Mediterranean mussel conditions, yields and quality have been already reported (Bongiorno et al., 2015; Lopez et al., 2023; Orban et al., 2002). However, changes in the marketable traits of our PDO mussel occurred also within the same season (spring). Overall, the optimal harvest period coincides with mussels displaying their highest fresh meat yield and condition index. The results of the present study agree with previous findings obtained on mussels collected from the Italian market (Lopez et al., 2023): mussels reached the highest fresh meat yield in May (+18%;  $P < 0.001$ ) compared with June and a better condition index in May–June (+48%;  $P < 0.001$ ) compared with April (Table 1). Under our conditions, early harvesting did not compromise PDO labelling, as all sampled mussels exhibited a high meat yield regardless of the harvest time, exceeding the consortium's PDO labelling requirements of >25%. Nevertheless, June emerged as the best harvest time to obtain PDO mussels, with the highest cooked meat yield (+26% compared with May and +47% compared with April) and shell strength (+20% compared with May and +75% compared with April) (Table 1).

Temporal fluctuations of WHC have substantial implications on mussel organoleptic properties, as WHC directly influences mussel texture, softness and juiciness. Noteworthy, the mussels collected in June exhibited a considerably ( $P < 0.001$ ) lower WHC compared with May (1.7% vs. 5.6%) and April (1.7% vs. 20.0%), and lower than that observed in mussels from another Northern Adriatic area (Gulf of Trieste) and collected in the same month (Bongiorno et al., 2015). The TVB-N content increased with the harvest period, but always remained below the limit indicated for seafood by the European Union (35.0 mg N/100 g wet weight; Commission Decision 95/149/ECC of 8 March, 1995) and the acceptability limit for mussels suggested by previous studies (22.0–25.0 mg N/100 g wet weight; Bongiorno et al., 2018).

### 3.2.2. Nutritional profile

The temporal variations observed in the chemical composition of 'Cozza di Scardovari' mussels aligned with previous observations (Bordignon et al., 2024; Grkovic et al., 2023; Irisarri, Fernández-Reiriz, & Labarta, 2015), with May and June representing a period of energy reserve accumulation. In addition, the lower water content and higher lipid levels recorded during the warmer months (–2% and +33% in May and June compared with April;  $P < 0.001$ ) (Table 2) could be correlated with the gonadal development stage of mussels (Lopez et al., 2023).

FAs are effective as the fingerprint for mussels derived from different farming sites; however, harvest time remains the main factor influencing the FA profile of mussels (Bongiorno et al., 2015; Bordignon et al., 2024). Under our conditions, the decrease from April to June in odd-numbered SFA (C15:0: –29%;  $P < 0.001$ ; C17:0: –27%;  $P < 0.001$ ), MUFA (C18:1 n-7: –13%;  $P < 0.001$ ) and the simultaneous increase in PUFA (n-3: +17%;  $P < 0.001$ ) (Table 2), suggest a transition from bacteria to phytoplankton in the primary food source (Bongiorno et al., 2015), consistent with food particle dynamics already observed in the lagoon (Andreoli et al., 1994).

As for amino acids, the harvest in May produced the best profile in terms of essential amino acids, particularly isoleucine (+54% compared with June and +16% compared with April;  $P < 0.001$ ), leucine (+16% compared with June and April;  $P < 0.001$ ) and valine (+48% compared with June and +24% compared with April;  $P < 0.001$ ), whereas the increased content of glutamic acid and aspartic acid from April to June (+25% and +43%, respectively;  $P < 0.001$ ) (Table 3) could result in an improvement in mussel taste (Fuentes et al., 2009).

As for the elemental profile, the decrease in the contents of various minerals, such as calcium, potassium, manganese, and sodium, during warmer months has already been observed in Mediterranean mussels (Grkovic et al., 2023; Peycheva et al., 2023) and could be linked to the stage of resource utilisation during gonadal development (Rouane-Hacene et al., 2015) to the size of the mussels (Regoli & Orlando, 1994), or to changes in water physio-chemical characteristics. For instance, the reduction in sodium content from April to June (–33%;  $P < 0.001$ ) (Table 4) may be linked to the decreased water salinity during spring as a result of increased rain and/or higher freshwater flows from agricultural runoffs (Sfriso, Facca, Bon, Giovannone, & Buosi, 2014). In June, mussels showed the lowest sodium concentration (158.0 mg/100 g wet weight), substantially below the consortium's limit for PDO labelling (<250.0 mg Na/100 g).

### 3.2.3. Volatile compounds

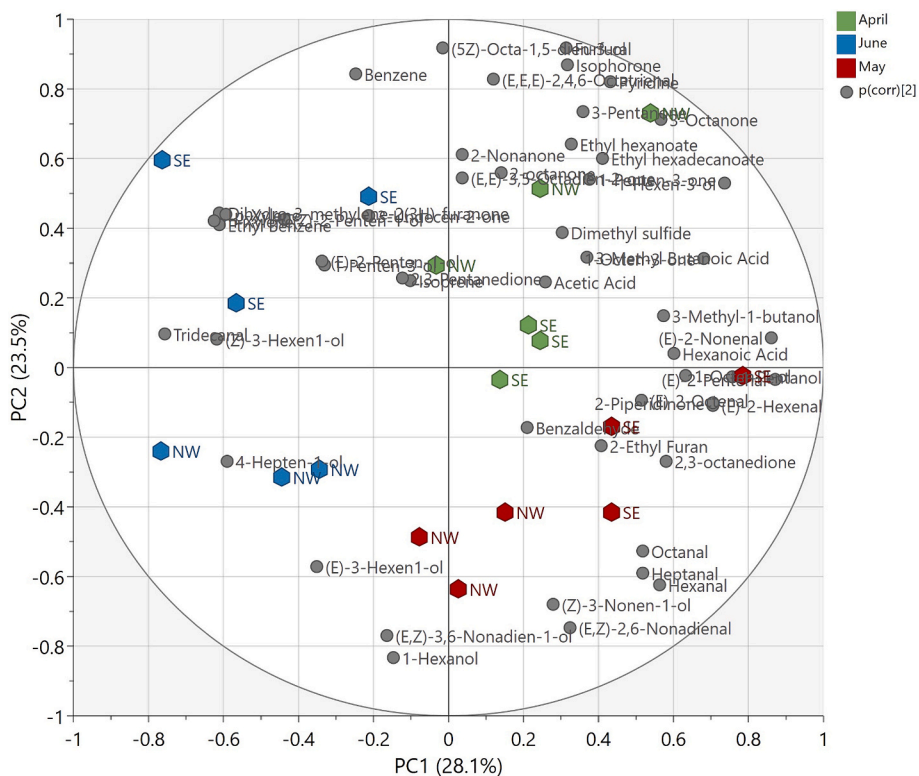
PCA was performed to have a general overview of the distribution of the VOCs among the mussels analysed. The first two components of the PCA biplot accounted for 51% of the variance of the data, showing a clear separation among the three harvest times and a distinction of the sampling sites within each harvest time (Fig. 1).

Mussels sampled in April were characterised by a higher amount of ketones and further discriminated from the other harvest times by acetic and 3-methylbutanoic acids. The compounds that contributed to the separation of samples collected in May were mainly aldehydes (C6–C8

**Table 4**  
Effect of the farming site and harvest time on the mineral (mg/kg wet weight) content of PDO “Cozza di Scardovari” mussels.

	Farming site (S)		Harvest time (T)			P-value			RMSE
	Southeast	Northwest	April	May	June	S	T	S × T	
Pools <sup>1</sup> , n	12	12	8	8	8				
Aluminium	154	172	266 <sup>c</sup>	158 <sup>b</sup>	64.9 <sup>a</sup>	0.10	<0.001	<0.001	24.0
Arsenic	1.59	1.81	1.57 <sup>a</sup>	2.01 <sup>b</sup>	1.53 <sup>a</sup>	<0.001	<0.001	<0.001	0.09
Boron	3.66	3.59	4.11 <sup>c</sup>	3.95 <sup>b</sup>	2.82 <sup>a</sup>	0.14	<0.001	<0.001	0.12
Barium	0.67	0.72	1.09 <sup>c</sup>	0.67 <sup>b</sup>	0.32 <sup>a</sup>	0.19	<0.001	<0.001	0.10
Calcium	589	660	768 <sup>b</sup>	580 <sup>ab</sup>	525 <sup>a</sup>	0.28	<0.05	0.13	145
Cadmium	0.12	0.11	0.12 <sup>b</sup>	0.11 <sup>ab</sup>	0.10 <sup>a</sup>	<0.05	<0.001	<0.01	0.01
Cobalt	0.15	0.15	0.24 <sup>c</sup>	0.14 <sup>b</sup>	0.08 <sup>a</sup>	0.34	<0.001	<0.001	0.01
Chromium	0.55	0.61	0.91 <sup>c</sup>	0.56 <sup>b</sup>	0.27 <sup>a</sup>	0.09	<0.001	<0.001	0.07
Copper	1.29	1.38	1.52 <sup>b</sup>	1.41 <sup>b</sup>	1.08 <sup>a</sup>	0.12	<0.001	0.20	0.14
Iron	114	125	191 <sup>c</sup>	117 <sup>b</sup>	50.6 <sup>a</sup>	0.11	<0.001	<0.001	16.3
Potassium	1758	1799	1968 <sup>b</sup>	1876 <sup>b</sup>	1493 <sup>a</sup>	0.30	<0.001	0.11	93.8
Lithium	0.27	0.29	0.42 <sup>c</sup>	0.28 <sup>b</sup>	0.14 <sup>a</sup>	<0.001	0.06	<0.001	0.02
Magnesium	699	677	771 <sup>b</sup>	758 <sup>b</sup>	536 <sup>a</sup>	0.08	<0.001	<0.001	29.4
Manganese	5.07	5.90	7.95 <sup>c</sup>	5.38 <sup>b</sup>	3.13 <sup>a</sup>	<0.001	<0.001	<0.001	0.55
Sodium	2167	2013	2364 <sup>b</sup>	2331 <sup>b</sup>	1577 <sup>a</sup>	<0.01	<0.001	<0.01	103.1
Nickel	0.65	0.71	1.11 <sup>c</sup>	0.65 <sup>b</sup>	0.28 <sup>a</sup>	0.06	<0.001	<0.001	0.08
Phosphorous	1474	1628	1518 <sup>a</sup>	1665 <sup>b</sup>	1469 <sup>a</sup>	<0.001	<0.01	<0.001	95.8
Lead	0.18	0.21	0.26 <sup>b</sup>	0.18 <sup>a</sup>	0.14 <sup>a</sup>	0.25	<0.001	<0.05	0.04
Sulphur	2271	2270	2257 <sup>b</sup>	2432 <sup>c</sup>	2124 <sup>a</sup>	0.97	<0.001	<0.05	95.2
Selenium	0.60	0.66	0.75 <sup>b</sup>	0.62 <sup>a</sup>	0.52 <sup>a</sup>	0.13	<0.001	<0.05	0.08
Silicon	57.0	65.8	43.6 <sup>a</sup>	67.7 <sup>b</sup>	73.0 <sup>b</sup>	<0.05	<0.001	<0.001	8.78
Strontium	5.42	5.42	6.43 <sup>b</sup>	5.20 <sup>a</sup>	4.63 <sup>a</sup>	0.99	<0.001	0.08	0.80
Titanium	3.14	3.41	4.63 <sup>c</sup>	3.57 <sup>b</sup>	1.62 <sup>a</sup>	0.20	<0.001	<0.05	0.51
Vanadium	0.34	0.37	0.56 <sup>c</sup>	0.37 <sup>b</sup>	0.13 <sup>a</sup>	0.14	<0.001	<0.001	0.05
Zinc	13.8	13.6	15.1 <sup>b</sup>	14.7 <sup>b</sup>	11.2 <sup>a</sup>	0.75	<0.001	0.10	1.20

<sup>1</sup> Each pool consisted of the meat of 25 randomly selected mussels. RMSE: Root mean square error. Values with different superscript letters in a column are significantly different (P ≤ 0.05).



**Fig. 1.** Biplot of the first 2 components of the principal component analysis. The explained variance of each component is reported in parentheses. Hexagons represent the mussels sampled in April (green), June (blue) and May (red) at the Northwest (NW) and Southeast (SE) sites of the Sacca degli Scardovari lagoon. Grey dots represent the loadings. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and (E,Z)-2,6-nonadienal), hexanol, 1-octen-3-ol and ethyl furan. In June, the samples were characterised by a prevalence of aromatic hydrocarbons (ethyl benzene, *m*-xylene and *p*-xylene) and tridecanal, 1-

penten-3-ol, (Z)-3-hexen-1-ol, 4-hepten-1-ol and dihydro-3-methylene-2 (3H)-furanone. For several compounds, a clear trend over harvest time was observed (Table S5). Two compounds, 4-hepten-1-ol and tridecanal,

increased with the harvest time from April to June in both farming sites, whereas only 1-octen-3-one decreased.

In the conditions of the present study, changes in the volatile fingerprints observed with harvest time suggest a transition from the sour-vinegary notes of April, attributed to higher levels of acetic acid and 3-methyl butanoic acid, to a fruitier and mushroom-like taste in May, as a result of increased levels of 1-octen-3-ol and (E,Z)-2,6-nonadienal (Zhou et al., 2020). Moving into June, the flavour profile shifted towards a more pronounced green, leafy and plant-like character due to increased concentrations of 1-penten-3-ol, (Z)-3-hexen-1-ol and 4-hepten-1-ol (Cecchi et al., 2018).

The correlations ( $P < 0.05$ ) between FAs and VOCs determined in this study are reported in Table 5. Substantial correlations were found for aldehydes and alcohols originating from the oxidation of FAs. The oxidation of specific FAs affects the volatile profile of a given matrix (Aprea et al., 2018). However, based on the available literature, the relationships between FAs and VOC profiles remain inconclusive, particularly in Mediterranean mussels. For instance, a strong correlation ( $r = 0.90\text{--}0.99$ ) was found between C20:2 NMI and (E,E)-2,4-heptadienal, 2,3-pentanedione and 2-nonanone (Fratini, Lois, Pazos, Parisi, & Medina, 2012), which was not confirmed by our results. Instead, a substantial correlation between C20:2 NMI and the aldehyde (E,Z)-2,6-nonadienal and the alcohols 1-hexen-3-ol and 1-octen-3-ol was observed in our trial. In addition, previous observations found a relationship between PUFA n-6 and (E)-2-pentenal, hexanal, (E)-2-hexenal and heptanal (Fratini et al., 2012), which is not confirmed by our results. In contrast, a correlation between C22:6 n-3 and the aldehyde (E,E)-2,4-eptadienal was found. Although correlation among variables does not necessarily indicate a causal effect, different findings compared with previous observations may be due to various reasons. For example, seasonal changes in lipid composition (Hagar & Dietz, 1986) or variations in lipoxygenase activity (Coffa & Hill, 2000) could contribute to these differences.

#### 4. Conclusions

Farming sites had a limited impact on the biometry and marketable traits of mussels. In contrast, mussels from the inner area of the lagoon compared with those from the outer close-to-sea site, showed a superior nutritional profile in terms of protein, lipid and essential amino acid contents and a more favourable n-3/n-6 ratio. They also showed a higher content of volatile compounds associated with sweet, fruity-like aromas. In contrast, farming sites did not affect the mineral content of the mussels, showing very low heavy metals such as cadmium, lead, arsenic and nickel.

As for harvest time, early harvesting in April did not compromise PDO labelling, as all sampled mussels exhibited a high meat yield, exceeding the consortium's PDO labelling requirements of >25%. June emerged as the optimal harvesting period for achieving high-quality PDO mussels with the highest yields and n-3 PUFA concentration and the lowest sodium concentration. In contrast, the harvest in May provided the best profile in terms of essential amino acids.

Changes in the volatile fingerprints observed with harvest time suggest a transition from the sour-vinegary notes to fruitier and plant-like flavours in June. Based on these findings, further investigation to assess the sensory characteristics of the studied mussel is envisaged.

#### CRediT authorship contribution statement

**Francesco Bordignon:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Eugenio Aprea:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Emanuela Betta:** Methodology, Investigation, Formal analysis. **Gerolamo Xiccato:** Writing – review & editing, Supervision, Conceptualization. **Angela Trocino:** Writing – review &

**Table 5**

Main correlation (Pearson correlation coefficient,  $r$ ) between fatty acids and volatile organic compounds.

Fatty acids	Volatile compounds	Pearson's $r$	P-value
C16:1 n-7	(Z)-3-Hexen1-ol	0.94	<0.01
C20:1 n-11	(E)-2-Nonenal	0.93	<0.01
C20:2 NMI	(E,Z)-2,6-Nonadienal	0.93	<0.01
C22:5 n-3	(5Z)-Octa-1,5-dien-3-ol	0.91	<0.05
C18:4 n-3	Nonanal	0.90	<0.05
C20:1 n-9	1-Hexen-3-ol	0.87	<0.05
C20:1 n-11	1-Hexen-3-ol	0.87	<0.05
C16:2 n-4	(E)-3-Hexen1-ol	0.86	<0.05
C22:2 NMI	1-Hexen-3-ol	0.84	<0.05
C16:2 n-4	(E,Z)-3,6-Nonadien-1-ol	0.83	<0.05
C16:2 n-4	(Z)-3-Hexen1-ol	0.82	<0.05
C20:2 NMI	1-Octen-3-ol	0.82	<0.05
C22:6 n-3	(E,E)-2,4-eptadienal	0.82	<0.05

editing, Writing – original draft, Supervision, Resources, Project administration, 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.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2024.140078>.

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