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Sex-related differences in the lipid and micronutrient profile, but not the proximate composition, of the Mediterranean mussel *Mytilus galloprovincialis*: a pilot study with implications for sex-targeted aquaculture

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Bivalves are a sustainable, low-carbon-footprint source of high-quality animal protein and polyunsaturated fatty acids (PUFA), particularly the long-chain (LC) n-3 forms eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The Mediterranean mussel (*Mytilus galloprovincialis*) is among the most farmed and consumed bivalves in Europe, yet the role of sex in its nutritional profile at marketed-product level is poorly documented. *M. galloprovincialis* shows skewed sex-biased progenies, possibly influencing product nutrient content. In this pilot study, pooled soft tissues of farmed adults purchased as human food were sex-sorted into a female (F) and a male (M) pool (60 animals each) and analyzed for proximate composition, fatty acid profile, minerals, and vitamin B₁₂. Data were summarized as absolute and relative differences and as log₂(F/M). Moisture, protein, ash, carbohydrates, and energy were nearly superimposable between sexes. In contrast, the female pool has 25.6% more total lipids and ~five-fold higher PUFA fraction, driven by an order-of-magnitude higher EPA and DHA; total n-3 fatty acids were nearly six-fold higher, the n-3/n-6 ratio was more than twice, and EPA+DHA was more than ten times higher than in the male pool. Vitamin B₁₂, iron, selenium, and iodine were higher in the female pool, whereas

sodium and zinc were higher in the male pool. These data suggest that sex-targeted aquaculture could be explored as a route to differentiated, n-3 LC-PUFA and vitamin B₁₂-enriched mussel products, providing a quantitative basis for properly replicated, season-resolved follow-up studies, and for breeding programs aimed at strengthening the nutritional and socio-economic value of mussel farming.

KEYWORDS

DHA, doubly uniparental inheritance (DUI) of mitochondria, EPA, *Mytilus galloprovincialis*, sex-related differences, vitamin B₁₂

1 Introduction

Bivalve mollusks are a high-protein, low-saturated-fat seafood and a rich source of polyunsaturated fatty acids (PUFA), especially the long-chain (LC) n-3 forms eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Wright et al., 2018; Tan et al., 2022). Most commercially important edible bivalves contain protein exceeding 50% of their dry weight, with a high-quality essential amino acid profile and a digestibility above 90% (Song et al., 2025; Zhang et al., 2025). Moreover, their filter-feeding biology delivers it with one of the lowest carbon and freshwater footprints among animal protein sources, requiring no external feed (Willer and Aldridge, 2020). Globally, mollusks accounted for 20.0% of total aquaculture production of aquatic animals in 2022, and for 52.9% of the marine and coastal component (FAO, 2024). Bivalve production grew by ~7% in wet weight between 2018 and 2022, with a 6.5% increase in EPA+DHA yield (Tan et al., 2025), and bivalves rank among the most sustainable and climate-efficient sources of high-quality protein (Zhang et al., 2025; Sun, 2026). Increasing the nutritional value of bivalve aquaculture is therefore a priority on the European Union (EU) and global food research agendas (European Commission, 2020; FAO, 2024).

Bivalve nutritional composition is, however, intrinsically variable. Lipid content and fatty acid profile respond to environment, season, geographical site, diet, life stage, and reproductive cycle (Martínez-Pita et al., 2012b, 2016; Mutić et al., 2021; Silva et al., 2021; Sun, 2026). Among these factors, sex has been shown to contribute to the lipid signature of adult bivalves, because female gonads accumulate large amounts of PUFA, particularly EPA, DHA, and arachidonic acid (AA), that are mobilized into oocytes during gametogenesis as both structural membrane components and energy reserves for embryonic and larval development (Palacios et al., 2007; Martínez-Pita et al., 2012b; Laudicella et al., 2023). Sex-related differences in proximate composition and lipid profile have been documented in scallops, specifically *Nodipecten subnodosus* (Palacios et al., 2007) and *Chlamys nobilis* (Zheng et al., 2012), as well as in three oyster species of the genus *Crassostrea* (Qin et al., 2023) and in other bivalves, including the wedge clam *Donax trunculus* (Martínez-Pita et al., 2012a) and the Manila clam *Ruditapes philippinarum* (Fernández-Reiriz et al., 2017). Yet, despite the central commercial role of *M. galloprovincialis* in Mediterranean countries, dedicated side-by-side comparisons of female and male soft tissues at the marketed-product level remain scarce.

A peculiar feature of *M. galloprovincialis* (and more than 100 other bivalve species) is doubly uniparental inheritance (DUI) of mitochondria, an exception to strictly maternal inheritance typical in animals (Zouros, 2013; Ghiselli et al., 2021), alongside a mother-dependent sex ratio. Pair-mating experiments in *M. galloprovincialis* and other *Mytilus* species showed that the proportion of male offspring is largely determined by the female parent, with individual females producing sex-biased, sometimes females-only, progenies independently from the fertilizing male (Saavedra et al., 1997; Kenchington et al., 2002). This outcome is mainly attributed to maternal nuclear factors that are deposited into eggs (Saavedra et al., 1997; Diz et al., 2013; Yusa et al., 2013; Iannello et al., 2025). If sex influences nutritional value, this heritable, mother-dependent bias might be tested as a route to obtain mussel lineages with differentiated, certifiable nutritional profiles without genetic manipulation, provided the underlying difference proves consistent, replicated, and seasonally stable.

While this gonadal lipid dimorphism is well documented across bivalves, two questions remain open: whether sex-related differences are large enough to matter in the marketed, ready-to-eat product, including nutrients beyond lipids, and whether they could be translated into production through the maternal control of sex ratio, which may offer a distinctive feature for *Mytilus* and other farmed DUI bivalves.

As a first step in this direction, we performed a pilot nutritional characterization of female and male *M. galloprovincialis* soft tissues from pooled specimens of marketed (farmed) animals, with the aim of (i) exploring whether sex-related differences in proximate composition, fatty acid and mineral profile are detectable in the commercial product, and (ii) assessing whether these differences are sufficient to justify a properly replicated follow-up study.

2 Materials and methods

2.1 Sampling and sex identification

Adult farmed Mediterranean mussels (*M. galloprovincialis*) were purchased as human food during the gametogenesis season (March 2025) at two retail outlets, one in Naples (farmed in Golfo di Oristano, Sardinia, Italy) and one in Bologna (farmed in Goro, Po River Delta, Ferrara, Italy). The farm provenance of each batch was tracked through the identification tags mandatory for live bivalves

in the market. The mussels were fresh, depurated, and of average size as for fresh trade (~7 cm) (Lee et al., 2008). Shells were opened, and individuals were preliminarily sex-sorted by visual inspection of gonad color (orange/coral in females and cream-white in males at the fully sexual maturity stage) (Figure 1), a criterion routinely used in *Mytilus* reproductive biology studies. Furthermore, in each specimen, a small portion of the mantle (site of gamete storage in *Mytilus*) was dissected with a blade, a small amount of stored gametes was removed, smeared on a slide, and promptly visualized using a standard optical microscope (Martínez-Pita et al., 2012b). The sex of individual mussels was confirmed by the presence in the smear of oocytes (~70 µm) or sperm (head ~3 µm) (Figure 2, inset).

To facilitate gametogenesis stage recognition from fresh gamete smears, we compared cells in the smears with cells in immunohistochemistry (IHC) staining on exemplary mature specimens using an antibody against the protein VASA (a germ cell marker). The IHC protocol was as described in Filanti et al. (2021) for the Manila clam (*Ruditapes philippinarum*). Briefly, the entire body was fixed, included in paraffin, sectioned, and sections were incubated with the primary antibody anti-VASA/VAS (Abcam ab209710; polyclonal, developed in rabbit), diluted 1:100, and the secondary antibody HRP anti-rabbit in goat (Santa Cruz Biotechnology Inc.) at the dilution of 1:100. Imaging was performed with an Olympus BH-2 microscope, equipped with BEL Photonics BlackL 5000 USB digital camera, through the acquisition software BEL Photonics Eurisko 2.9 (Figure 2). According to IHC, mature females were recognized by the presence of big cells, the oocytes, whose diameter is up to ~70 µm, with a high nucleus-to-cytoplasm ratio (Figure 2A). Males were recognized by the presence of spermatozoa (~3 µm head) (Figure 2B).

The soft tissues of sexed specimens in advanced gametogenesis (presence of mature gametes) for nutritional analyses were shelled and frozen at -20 °C within ~3 h of shucking. Samples from Naples were transported frozen to Bologna and pooled with the samples from Bologna by sex into two batches (sample F, females; sample M, males) for lipid analysis. Totally, each sample group was weighed at ~450 g each with approximately 60 individuals per pool. The individual mussel shell metrics and meat weight were not recorded. Both production areas were represented in comparable proportions in each pool. The sample pools were shipped frozen to the analytical laboratory for lipid extraction and quantification.

2.2 Proximate composition, fatty acid profile, and micronutrient analyses

Proximate composition, fatty acid profile, and micronutrient content were determined according to official methods and regulatory guidelines. Moisture was measured by oven-drying at 103 °C (Baldini et al., 1996). Crude protein was quantified using the Kjeldahl method from total nitrogen using a conversion factor of 6.25 (International Organization for Standardization, 2009), consistent with the factor prescribed for protein declaration under Regulation (EU) No 1169/2011. Total crude lipids were determined by acid hydrolysis followed by gravimetric extraction (European Commission, 2024). The fatty acid composition was analyzed as fatty acid methyl esters by gas chromatography (Baldini et al., 1996), screening a panel of 36 fatty acids (C4:0–C24:1), and expressed as percentages of total identified fatty acids (% TFA), with a limit of quantification (LQ) of 0.01% for individual fatty acids. Fatty acid percentages were converted to absolute contents (mg/100 g edible tissue) by multiplying total crude lipid by a conversion factor for low-lipid mollusks, $X_{FA} = 0.956 - 0.296/TL$, where TL is total crude lipid (g/100 g) (Weihrach et al., 1977; Charrondière et al., 2012; Nowak et al., 2014); each fatty acid percentage was then applied to the resulting total fatty acid mass. Two established lipid-quality indices were additionally derived from the fatty acid profile: the PUFA/SFA ratio and the unsaturation index (UI), the latter calculated as Σ (percentage of each fatty acid $\times$ its number of double bonds) (Chen and Liu, 2020). Total dietary fiber was assessed using an enzymatic–gravimetric method (AOAC, 2023); ash was quantified gravimetrically (European Commission, 2009); and total sugars were determined after inversion (European Commission, 2009), while total carbohydrates were calculated by difference. The energy value was calculated according to the European Union Regulation 1169 (European Union, 2011). Vitamin B₁₂ (cyanocobalamin) was quantified using a commercial ELISA kit (RIDASCREEN® FAST; R-Biopharm, Darmstadt, Germany), according to the manufacturer's instructions. Sodium was determined by flame photometry (International Organization for Standardization, 2001), and salt content was computed as sodium $\times$ 2.5. Zinc, iron, selenium, and iodine were quantified by inductively coupled plasma spectrometry by an ISO/IEC-accredited



FIGURE 1
Sex-sorting of *Mytilus galloprovincialis* by gonad color: orange/coral in females (left) and cream-white in males (right).

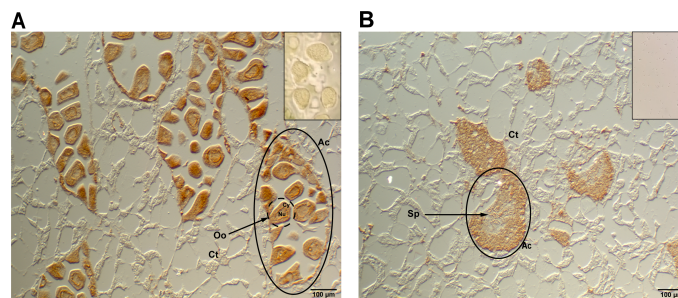


FIGURE 2

Mytilus galloprovincialis gamete morphology. (A) Oocytes labelled with anti-VASA antibody (brownish color) in an immunohistochemistry prepared female body section (some pedunculated still connected at the acinus wall, some free in the acinus lumen; an oocyte is circled with a dashed line), and fresh oocytes at the optical microscope ocular (upright inset). (B) Sperm labelled with anti-VASA antibody (brownish color) in an immunohistochemistry prepared male body section (early germ cells at the acinus border normally have a stronger labelling than mature male gametes in the acinus lumen), and fresh sperm at the optical microscope ocular (upright inset). The histological districts observed include germ cells inside acini (the gonadal units; one is circled with a solid line) and somatic tissues (connective tissue). Ac, acini; Oo, oocytes (big cells with a high nucleus-to-cytoplasm ratio); Sp, spermatozoa (free in the acinus lumen); Ct, connective tissue.

laboratory (Laboratorio Gamma, Cavriago, Italy), following the ISTISAN protocols (Baldini et al., 1996) for zinc, iron, and selenium, and the UNI EN standard (Ente Nazionale Italiano di Unificazione, 2007) for iodine.

2.3 Data analysis

Given the descriptive pilot nature of this study, no inferential statistics were performed (Hurlbert, 1984; Nakagawa and Cuthill, 2007). For each parameter, and to favor biological interpretation, the following summary descriptors were calculated: (i) the absolute difference ($F - M$); (ii) the relative difference, $100 \times (F - M)/M$ (%); and (iii) the $\log_2$ fold change, $\log_2(F/M)$, a dimensionless measure that is symmetric around zero, with a value of +1 indicating a two-fold higher level in females and -1 a two-fold higher level in males. Where relevant, nutrient contents calculated per standard edible portion were compared with dietary reference values (DRVs) for adults, established by the European Food Safety Authority (EFSA) (EFSA, 2017).

3 Results

The presence of mature gametes indicated advanced gametogenesis in mussels used in this study for nutritional analyses. All between-sex contrasts below are descriptive, reflecting the single-pool-per-sex design.

3.1 Proximate composition and energy

Moisture, crude protein, ash, and carbohydrate contents differed by less than 5% between the two pools, with $|\log_2(F/M)| < 0.1$ for all four parameters (Table 1). Energy content differed marginally (Table 1).

3.2 Total lipids and fatty acid profile

Total crude lipids were 25.6% higher in the pooled female sample than in the pooled male sample (Table 1). The fatty acid

profile also differed between pools (Table 2): SFA accounted for 41.9% and 56.6% of fatty acids in the female and male pools, respectively, and MUFA for 29.0% and 37.4%, whereas PUFA were ~five-fold higher in the female pool (29.2 vs 6.0% TFA). Both the PUFA/SFA ratio and the UI were correspondingly higher in the female pool, reflecting this PUFA enrichment. Within PUFA, EPA was about ten-fold and DHA more than ten-fold higher in the female pool; total n-3 was ~six-fold higher, total n-6 was also higher, and the n-3/n-6 ratio was 5.10 versus 2.35. AA was detected only in the female pool, being below the LQ in the male pool.

To translate these proportions in terms of dietary intake, percentage fatty acid profiles were converted to absolute amounts using conversion factors appropriate for low-lipid mollusks (as detailed in the Materials and Methods). On this basis, the female pool provided 382 mg of EPA+DHA per 100 g of edible tissue, roughly an order of magnitude higher than the 28 mg per 100 g calculated for the male pool (Supplementary Table 1). The Italian reference portion for fish, mollusks, and crustaceans is 150 g, equivalent to ~25 mussels (Società Italiana di Nutrizione Umana, 2024). A single serving of this size would therefore provide about 570 mg of EPA+DHA from the female pool, roughly 2.3 times the 250 mg/day adequate intake recommended for adults (EFSA, 2010).

3.3 Minerals and vitamin B₁₂

Iron, selenium, iodine, and vitamin B₁₂ were higher in the female pool (by 56, 24, 29, and 77%, respectively), whereas sodium and zinc were higher in the male pool (Table 1). Referred to a standard 150 g edible portion (Società Italiana di Nutrizione Umana, 2024), the female pool reached the adequate intake (AI) for vitamin B₁₂, whereas the male pool provided about 57% of it (4.1 and 2.3 μg vs 4 $\mu\text{g}/\text{day}$) (EFSA, 2015a). Both pools exceeded the AI for iodine (269 and 209 μg vs 150 $\mu\text{g}/\text{day}$) (EFSA, 2014a) and selenium (147 and 119 μg vs 70 $\mu\text{g}/\text{day}$) (EFSA, 2014b), whereas iron covered only a minor fraction of the population reference intake (PRI), approximately 17–38% depending on sex and reference value (4.2 and 2.7 mg vs 11–16 mg/day) (EFSA, 2015b). For the two minerals higher in the male pool, a 150 g portion provided 3.1 mg and 3.9 mg of zinc in the female and male pools, respectively,

TABLE 1 Proximate composition, energy value, mineral content, and vitamin B₁₂ of pooled female (F) and male (M) soft tissues of marketed *Mytilus galloprovincialis*.

Parameter	Unit	Females (F)	Males (M)	F – M	Rel. diff (%)	log ₂ (F/M)
Proximate composition						
Moisture	g/100 g	82.34	82.27	+0.07	+0.1	+0.001
Crude protein	g/100 g	9.13	9.08	+0.05	+0.6	+0.008
Total crude lipids	g/100 g	2.11	1.68	+0.43	+25.6	+0.33
Total dietary fiber	g/100 g	0.43	0.85	–0.42	–49.4	–0.98
Ash	g/100 g	1.83	1.76	+0.07	+4.0	+0.06
Carbohydrates	g/100 g	4.16	4.36	–0.20	–4.6	–0.07
of which sugars	g/100 g	2.36	2.54	–0.18	–7.1	–0.11
Energy	kJ/kcal	307/73	297/71	+10/+2	+3.4/+2.8	+0.05
Minerals and vitamin B₁₂						
Sodium	mg/100 g	314.5	372.9	–58.4	–15.7	–0.25
Salt	g/100 g	0.79	0.93	–0.14	–15.1	–0.24
Iron	mg/100 g	2.8	1.8	+1.0	+55.6	+0.64
Zinc	mg/100 g	2.08	2.58	–0.5	–19.4	–0.31
Selenium	µg/100 g	98	79	+19	+24.1	+0.31
Iodine	µg/100 g	179	139	+40	+28.8	+0.36
Vitamin B ₁₂	µg/100 g	2.71	1.53	+1.18	+77.1	+0.82

Values refer to a single pooled sample per sex (F, M). For each parameter, the absolute difference (F – M), the relative difference [$100 \times (F - M)/M$], and the log₂ fold change [$\log_2(F/M)$] are reported; log₂(F/M) is a unit-independent descriptor symmetric around zero, with positive values indicating higher levels in females and negative values indicating higher levels in males.

corresponding to approximately 24–41% of the adult sex-specific PRI, depending on dietary phytate intake (EFSA, 2014c), and 472 mg and 559 mg of sodium, respectively, remaining well below the 2 g/day safe and adequate intake for adults (EFSA, 2019).

4 Discussion

4.1 Sex-related differences in nutritional composition

4.1.1 Biological interpretation and comparison with previous studies

The main feature of these pilot data is a quantitative and qualitative remodeling of the lipid fraction in *M. galloprovincialis* females, whereas the proximate composition remained essentially unchanged between sexes. The shift in the female pool toward higher total lipids, lower MUFA, much higher PUFA, and a ~six-fold higher n-3 contribution is consistent with the gametogenic biology of bivalves: EPA, DHA, and AA are accumulated in female gonads as both phospholipid membrane components and energy reserves for oocytes, and are mobilized at gametogenesis (Palacios et al., 2007; Martínez-Pita et al., 2012b). Consistently, female bivalve gonads show a richer metabolite and lipid complement than males, reflecting the greater reproductive investment in oocytes (Lance et al., 2023). Because the commercial mussels analyzed here had been depurated before sale, residual gut microalgae are unlikely to explain these differences. AA, detected only in the female pool, is an

established precursor of prostaglandins and other eicosanoids involved in oocyte maturation and spawning in mollusks (Deridovich and Reunova, 1993). Comparably, female-biased PUFA enrichments have been reported in *Nodipecten subnodosus* (Palacios et al., 2007), in golden and brown morphs of the noble scallop *Chlamys nobilis* (Zheng et al., 2012), and in three *Crassostrea* species (Qin et al., 2023), which together support a generalized pattern across Bivalvia. A pronounced sex-related difference of the gonad lipidome has also been reported within the genus *Mytilus* (Laudicella et al., 2023), indicating that the lipid asymmetry observed here is not species-specific. Sex was confirmed on gamete smears in animals sampled during the gametogenic season, so the lipid differences are best read as reflecting active gametogenesis.

The parallel enrichment of vitamin B₁₂, iron, and selenium in the female pool, though less firmly characterized than the lipid signature, is consistent with the greater biosynthetic investment of maturing female gonads. Cobalamin is a cofactor of one-carbon metabolism, which sustains the nucleotide biosynthesis and methylation reactions of developing oocytes (EFSA, 2015a); the higher female B₁₂ may thus reflect the metabolic demands of oogenesis, in line with the sex- and maturation-related element partitioning reported in *M. galloprovincialis* gonads (Kapranov et al., 2021).

4.1.2 Nutritional significance

From a human nutrition standpoint, the EPA+DHA difference is substantial: the *M. galloprovincialis* female pool ranks among the most LC-PUFA-rich bivalves reported (e.g., *Pecten maximus*, *Chlamys nobilis*) (Tan et al., 2022). The male pool falls below

TABLE 2 Fatty acid profile of pooled female (F) and male (M) soft tissues of marketed *Mytilus galloprovincialis*, expressed as percentage of total identified fatty acids (% TFA).

Parameter	Unit	Females (F)	Males (M)	F – M	Rel. diff (%)	log ₂ (F/M)
Fatty acid summary (% of total fatty acids)						
SFA	% TFA	41.86	56.61	–14.75	–26.1	–0.44
MUFA	% TFA	28.98	37.44	–8.46	–22.6	–0.37
PUFA	% TFA	29.16	5.97	+23.19	+388	+2.29
PUFA/SFA ratio	—	0.70	0.11	+0.59	+536	+2.72
Unsaturation Index (UI)	—	165.5	58.5	+107	+183	+1.50
Total n-3 PUFA	% TFA	24.38	4.19	+20.19	+482	+2.54
Total n-6 PUFA (LA + AA)	% TFA	4.78	1.78	+3.00	+169	+1.43
n-3/n-6 ratio	—	5.10	2.35	+2.75	+117	+1.12
EPA + DHA	% TFA	22.17	2.12	+20.05	+946	+3.39
Selected individual fatty acids (% of total fatty acids)						
Lauric (C12:0)	% TFA	<LQ	1.09	—	—	—
Myristic (C14:0)	% TFA	8.37	13.02	–4.65	–35.7	–0.64
Pentadecanoic (C15:0)	% TFA	0.66	0.89	–0.23	–25.8	–0.43
Palmitic (C16:0)	% TFA	26.48	31.93	–5.45	–17.1	–0.27
Palmitoleic (C16:1 n-7)	% TFA	22.91	29.17	–6.26	–21.5	–0.35
Heptadecanoic (C17:0)	% TFA	0.71	0.85	–0.14	–16.5	–0.26
Stearic (C18:0)	% TFA	5.37	8.28	–2.91	–35.1	–0.62
Oleic (C18:1 n-9)	% TFA	4.70	6.49	–1.79	–27.6	–0.47
LA (C18:2 n-6)	% TFA	4.02	1.78	+2.24	+126	+1.18
ALA (C18:3 n-3)	% TFA	2.21	2.07	+0.14	+6.8	+0.09
Arachidic (C20:0)	% TFA	0.27	0.27	0.00	0.0	0.00
Eicosenoic (C20:1 n-9)	% TFA	1.37	1.78	–0.41	–23.0	–0.38
AA (C20:4 n-6)	% TFA	0.76	<LQ	—	—	—
EPA (C20:5 n-3)	% TFA	14.23	1.43	+12.80	+895	+3.31
Heneicosanoic (C21:0)	% TFA	<LQ	0.20	—	—	—
Behenic (C22:0)	% TFA	<LQ	0.08	—	—	—
DHA (C22:6 n-3)	% TFA	7.94	0.69	+7.25	+1051	+3.52

The upper section reports fatty acid class sums and derived indices; the lower section reports selected individual fatty acids. Difference descriptors are defined as in Table 1.

Values refer to a single pooled sample per sex (F, M). Total crude lipid content, from which absolute fatty acid amounts (mg/100 g, as detailed in the Materials and Methods) were derived, is reported in Table 1. All other fatty acids in the analytical panel (C4:0–C24:1) were below the LQ (<0.01% TFA) in both pools. AA, arachidonic acid; ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, total fatty acids.

whole-tissue values reported for Mediterranean *M. galloprovincialis* (Wright et al., 2018; Willer and Aldridge, 2020), whose non-sex-resolved profile instead matches our female pool, consistent with a sex-related difference. The higher female content of vitamin B₁₂, iron, selenium, and iodine further compounds this asymmetry, as these micronutrients are also among the public-health-relevant components for which bivalves are recommended dietary sources (Wright et al., 2018; Willer and Aldridge, 2020). Compositional values reported here refer to the raw product, the matrix required for labelling; however, the nutrients that ultimately reach the human body may differ (Walther et al., 2019). Published evidence nonetheless indicates that the female LC-PUFA advantage would largely persist: gentle cooking (steaming, boiling) retains, or on a wet-weight basis concentrates, the EPA+DHA of *M. galloprovincialis*, whereas only frying degrades them markedly (Biandolino et al., 2021; Psycheva et al., 2022); these LC-PUFA are stored predominantly as

phospholipids, a highly stable and bioaccessible form (Rincón-Cervera et al., 2020; Tan et al., 2022), and cooked-mussel consumption measurably raises the human omega-3 index (Carboni et al., 2019).

Cholesterol was not determined in this pilot study, and a sex effect on its content cannot be formally excluded. It is, however, unlikely to alter the picture reported here: bivalves are intrinsically low in cholesterol relative to terrestrial meats (Wright et al., 2018), and their sterol fraction largely mirrors dietary microalgae rather than sex, with free-sterol composition not differing between female and male gonads (Palacios et al., 2007). Moreover, no DRV has been established for dietary cholesterol, and current cardiovascular dietary guidance focuses primarily on limiting saturated fat rather than dietary cholesterol, which is no longer considered a primary target for cardiovascular risk reduction in most individuals (EFSA, 2010; Lichtenstein et al., 2026).

For iodine and selenium, the enrichment observed in the female pool does not change the overall classification of mussels as an excellent dietary source, because a single portion of either sex already exceeds the respective AI (EFSA, 2014a, b), consistent with the recognized value of molluscan shellfish as selenium-rich foods (Wright et al., 2018). For vitamin B₁₂, the female pool reached the adult AI, whereas the male pool provided more than half of it (EFSA, 2015a). This difference may therefore provide an additional nutritional margin for consumers with marginal B₁₂ status or limited intake of animal-source foods, including older adults and individuals following predominantly plant-based diets (EFSA, 2015a; Niklewicz et al., 2023).

4.2 Limitations

Two limitations should be noted. First, the single pooled sample per sex (450 g, 60 individuals) buffered individual variability but precluded within-sex variance estimation and formal statistical testing; because sex and batch are consequently confounded, the reported differences are exploratory and cannot be formally distinguished from batch effects. Second, individuals were pooled from two different basins (Sardinian and northern Adriatic). Although including material from two production areas broadened the commercial relevance of the pools, the design does not allow origin effects or sex × origin interactions to be evaluated. The contrasts reported here should therefore be read as hypothesis-generating, requiring confirmation in replicated, season-resolved studies.

4.3 Applied perspectives and translation

From an applied perspective, two tentative implications can be outlined. First, the apparent magnitude of the lipid and micronutrient asymmetry, if confirmed by a replicated study, could become relevant to product labeling, consumer information, and the design of sex-sorted, nutrient-declared mussel products. Second, because *Mytilus* spp. show, alongside DUI, the mother-dependent sex-ratio bias outlined above, the sex-related nutritional differences reported here could, in principle, be used (if confirmed across seasons and replicated families) to develop female-enriched lines with high-LC-PUFA, high-B₁₂ profiles, or sex-enriched lines tailored to specific processing or product-quality traits, without genetic manipulation, complementing rather than replacing climate-adaptive approaches such as selective breeding for thermal tolerance (Sun, 2026) and breeding that already enhances PUFA and essential-amino-acid content in farmed bivalves (Tan et al., 2024). On the feed side, the LC-PUFA available in farmed bivalves derives from their microalgal diet, whose lipid content can now be monitored in hatcheries (Kallau and Yang, 2025).

The feasibility of such an approach would depend on seed production. Because much mussel seed is still wild-collected, two non-exclusive routes could be explored if the present findings are confirmed. The first, independent of seed origin, could be applied immediately and is based on post-harvest sex sorting of shucked product into value-added, nutrient-declared lines. The second is propagation-based and may become feasible as hatchery output

expands; it would rely on the maternal control of sex ratio described in *Mytilus*, a reproductive feature that co-occurs with DUI, as a hypothetical non-transgenic lever to be further tested in dedicated experiments. Under this route, female-biased progeny could in principle be obtained by selecting the female broodstock alone, without genetically manipulating the parental population.

5 Conclusion

This pilot characterization indicates that female and male *M. galloprovincialis* differ markedly in lipid content, n-3 LC-PUFA enrichment (EPA and DHA), and vitamin B₁₂, despite similar proximate composition. The absence of biological replicates prevents formal testing, but the data support exploring sex-targeted aquaculture to develop nutritionally differentiated mussel products. Combined with the mother-controlled sex-ratio bias in *Mytilus*, the magnitude of these differences justifies a replicated, season-resolved follow-up integrated with food-safety endpoints before any application.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because mussels were purchased as human food from a retail supplier; no live experimental animals were used.

Author contributions

FD: Writing – original draft, Formal analysis. EDA: Investigation, Writing – review & editing. TC: Investigation, Writing – review & editing. MS: Investigation, Writing – review & editing. Data curation. HY: Writing – review & editing. SL: Writing – review & editing. LM: Writing – review & editing. Conceptualization, Funding acquisition, Methodology, Project administration, Writing – original draft.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author LM declared that they were an editorial board member of *Frontiers*, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2026.1917273/full#supplementary-material>

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