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Food Chemistry 77 (2002) 57–65

Food Chemistry

www.elsevier.com/locate/foodchem

Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites

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Received 5 June 2001; received in revised form 10 August 2001; accepted 10 August 2001

Abstract

Seasonal changes, occurring during a 1-year period, in mussels (*Mytilus galloprovincialis*), cultivated in long-line plants in two different Italian sites, one off the northern Adriatic coast and the other in a sea lake along the Central Tyrrhenian coast, were investigated. Biometric parameters, percent meat and intervalval fluid contents, Condition Index, proximate composition and lipid profile of mussels were studied. The low meat content and Condition Index values registered in December, in mussels from the Adriatic site (142.0±9.9), and in September, in mussels from the Tyrrhenian sea lake (126.0±2.3) were coincident with the depletion of glycogen and lipid reserves. Gas chromatographic analysis of total lipids showed the prevalence of polyunsaturated fatty acids (37–48% of total fatty acids) over the saturated (26–38%) and monounsaturated (16–29%) ones throughout the year. Seasonal fluctuations of vitamin A, vitamin E, cholesterol, phytosterols and carotenes levels, in mussels from either site, were detected by high performance liquid chromatography.

Keywords: Mussels; *Mytilus galloprovincialis*; Chemical composition; Nutritional quality

1. Introduction

Bivalve molluscs, fresh and processed, play an ever-increasing role in the Italian diet. In Italy the culture of bivalve molluscs, a well established activity that amounted to about 148,000 metric tons in the year 1998, is mostly represented by mussels, the main product of the national aquaculture. *Mytilus galloprovincialis,* the species present along the national coast, is appreciated by consumers for its organoleptic properties, retained also after processing, and for the competitive price if compared with other bivalves.

The quality requisites of bivalve molluscs are primarily dependent on the quality of the aquatic environment, assuring a healthy product and a safe consumption. However, from a nutritional standpoint, other characteristics may influence the product quality (Beninger & Lukas, 1984; Karakoltsidis, Zotos, & Constantinides, 1995). Water, protein, lipid, mineral and glycogen contents of the meat, together with minor components of a hydrophilic or lipophilic nature, contribute to the nutritional value and organoleptic characteristics of mussels. A parameter of ecophysiological and economic relevance, especially in view of the industrial processing, is represented by the Condition Index, a measure of the apparent health and commercial quality of bivalves.

It is known that water temperature, food availability and reproductive cycle of animals may influence the meat yield and biochemical composition of mussels (Fernandez-Reiriz, Labarta, & Babarro, 1996; Okumus & Stirling, 1998). However, poor information is available from the literature on the nutritional characteristics of *M. galloprovincialis* cultured in the Italian coasts and on their seasonal variations.

This study was aimed at evaluating the seasonal changes in meat and intervalval fluid contents, Condition Index, proximate composition and lipid profile of mussels (*M. galloprovincialis*), cultivated in long-line plants in two different Italian sites, one off the northern Adriatic coast and the other in a sea lake along the central Tyrrhenian coast.

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0308-8146/02/$ - see front matter
PII: S0308-8146(01)00322-3

2. Materials and methods

2.1. Sample collection and treatment

This study was carried out between June 1998 and June 1999. Mussels (*Mytilus galloprovincialis*) were cultivated in long-line plants in two different sites. One site was placed in the northern Adriatic sea, 1.5 miles off the Italian coast, near Cattolica (Rimini). The average water temperature in this site fluctuated between a minimum of 6.2 °C in February 1999 and a maximum of 26.5 °C in August 1998 and water salinity between 33 and 36 g L^{-1}.

The other site was a sea lake along the central Tyrrhenian coast, the lake of Sabaudia (4.16 miles length, 400 ha area, 4–4.5 m average depth), connected to the sea by two mouths. Water temperature in the sea lake fluctuates during the year between 6–8 °C in January–February and 28–30 °C in July–August and water salinity between 25 g L^{-1} and 35 g L^{-1}.

Mussels of commercial size were collected contemporaneously from the two sites at seasonal intervals in June, September, December 1998 and in February and June 1999.

Samples (about 5 kg) were immediately transported under refrigeration (+6 °C) to the National Institute for Food and Nutrition Research in Rome. Upon arrival, mussels were inspected and dead animals discarded. 20 individuals were randomly selected for biometric measurements and Condition Index determination. The remaining mussels were rapidly washed and manually shucked by cutting the adductor muscle with a knife. The mean percent weights of shells, intervalval fluid and edible meat in the mussel population were calculated.

Pools of about 30 individuals, together with their intervalval fluids, were constituted and stored in vacuum-sealed multilayer barrier bags at −75 °C for chemical analysis. On the day of analysis, the content of one bag, thawed rapidly under running cold water, was homogenised in a laboratory blender (model 8010E, Waring® Products Division, New Hartford, CT, USA) for 45 s, at a low speed, using a previously cooled stainless steel cup. A total of three pools of about 30 individuals, for each sampling period and geographical site, was analysed.

2.2. Reagents

α-Tocopherol (vitamin E), all-*trans* retinol (vitamin A), cholesterol, desmosterol, stigmasterol, campesterol, β-sitosterol, fucosterol, ergosterol, 7-dehydrocholesterol, α-carotene, β-carotene and pure fatty acid standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Brassicasterol was from Larodan Fine Chemicals (Malmö, Sweden). *tert*-Butylhydroquinone (TBHQ) was from Fluka Chemie AG (Buchs, Switzerland). Boron trifluoride in methanol was from Aldrich Chemical Co. (Milwaukee, WI, USA). All solvents (Carlo Erba, Milan, Italy) were of analytical or high performance liquid chromatography (HPLC) grade as required.

2.3. Biometric parameters and Condition Index

Thickness, length, width and height of the shells were measured using a 0.05 mm precision calliper as described by Fisher, Schneider, and Bauchot (1987). After the biometric measurements, meat and shells of the 20 specimens were grouped in four pools, weighed and dried at 105 °C for 24 h. Condition Index (CI) was calculated as follows:

$$CI = (MDW/SDW) \times 1000$$

where MDW is meat dry weight (g) and SDW is shell dry weight (g).

2.4. Chemical evaluations

2.4.1. Proximate composition

Moisture, crude protein and ash contents of mussels were determined by AOAC (1990) methods. Glycogen content was assayed enzymatically (Keppler & Decker, 1974) after cold perchloric acid extraction (Dalrymple & Hamm, 1973). Total lipids were extracted by the method of Bligh and Dyer (1959), slightly modified according to Kinsella, Shimp, Mai, and Weihrauch (1977).

2.4.2. Fatty acids

Fatty acid profiles were determined by gas chromatography using a 6890 Hewlett Packard gas chromatograph with flame ionization detector, equipped with a SPB™ PUFA fused silica capillary column, 30 m × 0.25 mm inner diameter, 0.20 μm film thickness (Supelco Inc., Bellefonte, PA, USA). Operating conditions were as previously described (Orban et al., 2000). Fatty acids were identified by comparison of retention times to authentic standards for area percent normalisation. Relative quantities were expressed as weight percent of total fatty acids in each sample.

2.4.3. Unsaponifiable lipids

Total lipids were saponified and α-tocopherol, cholesterol, desmosterol, stigmasterol + campesterol, β-sitosterol, fucosterol + brassicasterol, all-*trans* retinol, α-carotene and β-carotene quantified by HPLC. For α-tocopherol and sterols evaluations, the total lipid extract was saponified for 15 min at 70 °C with ethanolic potassium hydroxide (2 M) while, for all-*trans* retinol and carotenes determinations, the saponification was conducted overnight at room temperature. In both cases saponification occurred in screw-capped amber vials under a nitrogen atmosphere in the presence of TBHQ as described previously (Orban et al., 2000).

Following the saponification, thin layer chromatography of the recovered unsaponifiable lipids was accomplished to separate α-tocopherol from sterols. Fluoresceinated silica gel plates (size 20 × 20 cm, layer thickness 500 μm, porosity 60 Å, Whatman Inc., Clifton, NJ, USA) were run in the dark for 55 min using hexane: diethyl ether: acetic acid (70:30:1.5 v/v/v) as the developing solvent. The two spots, corresponding to sterols and α-tocopherol, were identified under UV light by comparison with standard compounds spotted alongside, scraped off and recovered with an adequate solvent. The purified sterols and α-tocopherol were injected separately into the HPLC for quantification. The HPLC system used in the analysis of α-tocopherol, sterols, all-*trans* retinol and carotenes was a Hewlett Packard (Waldbronn, Germany) 1100 Series liquid chromatograph equipped with an UV/visible photodiode array detector. The analytical separations were performed using a 25 cm × 4.6 mm inner diameter, 5 μm Ultrasphere C18 column (Beckman, Palo Alto, CA., USA). α-Tocopherol and sterols were eluted isocratically using acetonitrile/methanol (82:18, v/v) as mobile phase at a flow rate of 1.7 ml min^{-1} and at a constant temperature (25 °C). Runs were monitored at 215 nm and 272 nm. All-*trans* retinol, α-carotene and β-carotene were separated at a flow rate of 1 ml min^{-1} by gradient elution of solvent A (acetonitrile:tetrahydrofuran 3:1 v/v) and solvent B (methanol:1% w/v ammonium acetate 3:2 v/v). The gradient program was as follows: time 0–35 min: 20–100% A, time 35–50 min: 100% A. Runs were monitored at 325 and 450 nm. Analytes were identified by their retention times and UV/visible spectra. Peak areas were used to determine analyte concentrations in the samples by reference to standard curves obtained by chromatographing pure substances under identical conditions.

2.5. Statistics

Results are expressed as mean ± standard deviation. All chemical analyses at any seasonal sampling were carried out in duplicate on three pools of about 30 mussels. In mussels from the same site, the analysis of variance (One-Way ANOVA) was conducted to test significant seasonal fluctuations for each parameter considered (Statgraphics®, Statistical Graphic System by Statistical Graphic Corporation, Version 6.0, Manugistics™ Inc, Rockville, MD, USA).

3. Results and discussion

3.1. Biometric characteristics, percent meat and intervalval fluid contents, Condition Index

The biometric characteristics of the shells of mussels from the Adriatic site and from the Tyrrhenian sea lake, throughout the experimental period, are listed in Table 1. The seasonal variations of percent meat and intervalval fluid contents and of Condition Index of mussels from the two sites were statistically significant ($P \leqslant 0.01$, Fig 1a and b). In mussels of either origin, Condition Index and meat content showed very similar patterns during the year, with peak values occurring in the winter months, coinciding with low intervalval fluid contents. The differences in times of peak values observed between Adriatic and Tyrrhenian mussels may be related to the different environmental conditions of the two sites. Condition Index and meat content of mussels are affected by a variety of extrinsic and intrinsic factors, such as water temperature and salinity, food availability and gametogenic cycle of animals (Okumus & Stirling, 1998).

Table 1
Biometric characteristics of the shells of mussels (*Mytilus galloprovincialis*) from Cattolica (Adriatic sea) and from the lake of Sabaudia (Tyrrhenian sea)[a]

	1998			1999	
	June	September	December	February	June
Cattoliea (Adriatic sea)					
Thickness (mm)	0.67±0.09	0.59±0.12	0.71±0.14	0.51±0.19	0.67±0.09
Length (mm)	63.60±5.61	54.55±3.32	57.43±6.54	66.56±4.87	51.98±4.12
Height (mm)	32.38±2.56	27.84±1.35	28.80±4.11	33.46±3.03	27.99±4.45
Width (mm)	23.46±2.18	19.95±2.35	21.12±2.92	23.58±2.35	19.13±1.72
Lake of Sabaudia (Tyrrhenian sea)					
Thickness (mm)	0.65±0.09	0.69±0.15	0.62±0.19	0.68±0.11	0.68±0.12
Length (mm)	66.42±2.58	65.97±6.55	58.75±5.02	66.75±5.90	56.94±5.84
Height (mm)	31.84±1.39	32.33±4.32	29.52±3.43	33.00±2.22	29.03±3.11
Width (mm)	24.06±2.02	21.40±5.61	21.31±2.37	23.20±1.87	19.84±1.80

[a] Mean ± standard deviation ($n = 20$).

3.2. Proximate composition

The proximate compositions of mussels from Cattolica and from the lake of Sabaudia underwent a seasonal cycle characterised by phases of accumulation and depletion of reserves, reflecting the stage of gonadal development as well as the availability of food. The fluctuations of total lipid (8.1–13.4% in mussels from Cattolica vs 5.8–12.1% in mussels from the lake of Sabaudia), glycogen (14.7–24.8% vs 12.7–23.2%), protein (44.0–54.4% vs 42.4–56.2%) and ash (11.7–17.0% vs 11.0–21.0%) contents of mussels on a dry basis during the experimental period are shown in Fig. 2 a and b. Seasonal fluctuations were statistically significant ($P \leqslant 0.05$) with the exception of those regarding the protein content of Adriatic mussels. Glycogen and lipid contents followed an almost similar pattern and reached the lowest levels in December and in September, for Adriatic and Tyrrhenian mussels, respectively, coinciding with low meat contents (25.0% vs 25.5% on a wet basis) and Condition Index values (142.06 vs 126.03). The ash content of the meat reached peak values, coinciding with minimum glycogen and lipid reserves.

Stress conditions, environmental situations requiring major energy expenditure or gametes release, may be

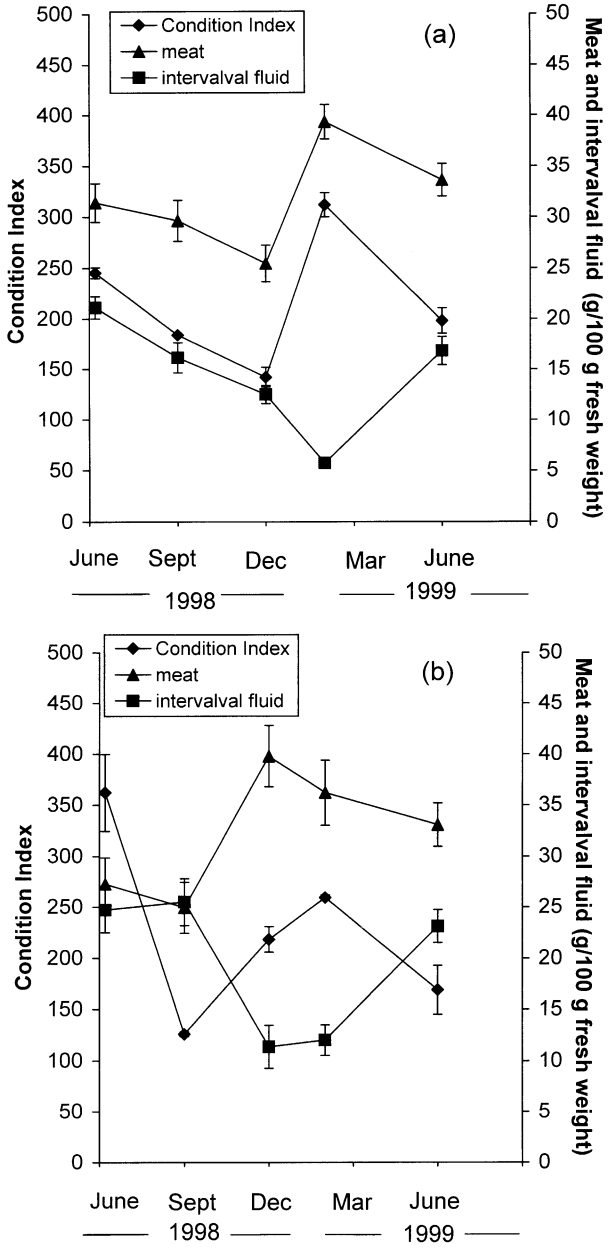

Fig. 1. Seasonal variations of Condition Index and of meat and intervalval fluid contents (g/100 g fresh weight) in mussels (*M. galloprovincialis*) from Cattolica, Adriatic sea (a) and from the lake of Sabaudia, Tyrrhenian sea (b).

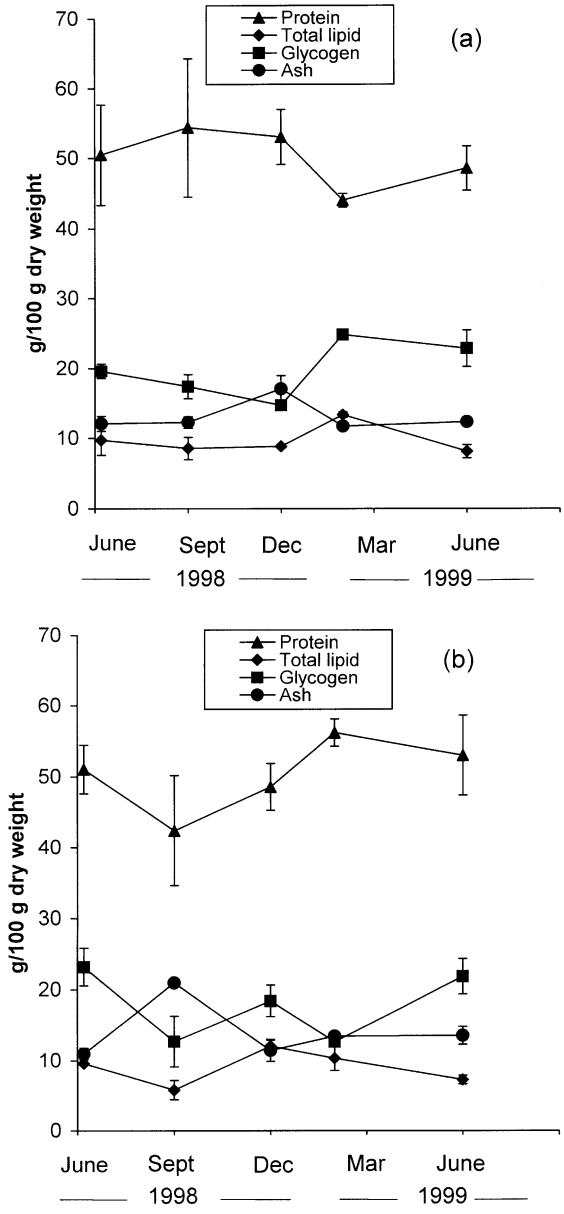

Fig. 2. Seasonal variations of protein, total lipid, glycogen and ash contents (g/100 g dry weight) in mussels (*M. galloprovincialis*) from Cattolica, Adriatic sea (a) and from the lake of Sabaudia, Tyrrhenian sea (b).

responsible of the low Condition Index, meat content and biochemical reserves observed in certain periods of the year. An accumulation of glycogen over the summer season, followed by a decline through the winter in coincidence with low meat yields and Condition Index values and with spawning, was reported by Okumus and Stirling (1998) in mussels (*M. edulis*) cultured in two Scottish sea lochs.

3.3. Fatty acids

The fatty acid profiles of mussels (*M. galloprovincialis)* from Cattolica and from the lake of Sabaudia during the period under study are shown in Figs. 3 and 4, respectively. Palmitic (16:0) and palmitoleic (16:1, n-7) acids were the predominant saturated and monounsaturated fatty acids, respectively, while, among polyunsaturated fatty acids, eicosapentaenoic (20:5, n-3) and docosahexaenoic (22:6, n-3) acids were prevalent. A similar fatty acid profile was observed in *M. galloprovincialis* from the Aegean Sea by Karakoltsidis et al. (1995). A high contribution of 20:5 n-3 and 22:6 n-3 to the total fatty acids was also reported by Fernandez-Reiriz et al. (1996) in a study on *M. galloprovincialis* grown in two different zones in Spain.

Most of the fatty acids underwent statistically significant changes during the period under study. In mussels from either site, the percent levels of 20:5 n-3 and 22:6 n-3 showed changes opposite to each other, as also pointed out by other authors working on different mollusc species (Chu, Webb, & Chen, 1990; Pollero, Ré, & Brenner, 1979). The seasonal fluctuations of 20:5 n-3 ($P \leqslant 0.01$) and 22:6 n-3 ($P \leqslant 0.05$), fatty acids known to be synthesised by diatoms and dinoflagellates, and the timing differences observed in the two fatty acid levels between mussels from Cattolica and from the lake of Sabaudia, may be related to the type of food ingested by the molluscs during the year and to their reproductive cycles. The conversion of 20:5 n-3 to 22:6 n-3 might also explain the inverse relationship observed between the two fatty acid levels. The total percentages of saturated, monounsaturated and polyunsaturated fatty acids in the lipids of mussels from Cattolica and from the lake of Sabaudia are shown in Fig. 5a and b. In mussels from both sites, the prevalence of polyunsaturated fatty acids, amounting to about 37–48% of total fatty acids, over the saturated (26–37%) and monounsaturated ones (16–29%) is evident. The seasonal changes observed in the percentage levels of total saturated and polyunsaturated fatty acids were not statistically significant ($P > 0.05$), while monounsaturated fatty acid levels varied significantly in Tyrrhenian ($P \leqslant 0.05$) and Adriatic ($P \leqslant 0.01$) mussels.

High levels of n-3 polyunsaturated fatty acids (32–42% of total fatty acids), low levels of n-6 polyunsaturated fatty acids (2–9%) and high n-3/n-6 ratio

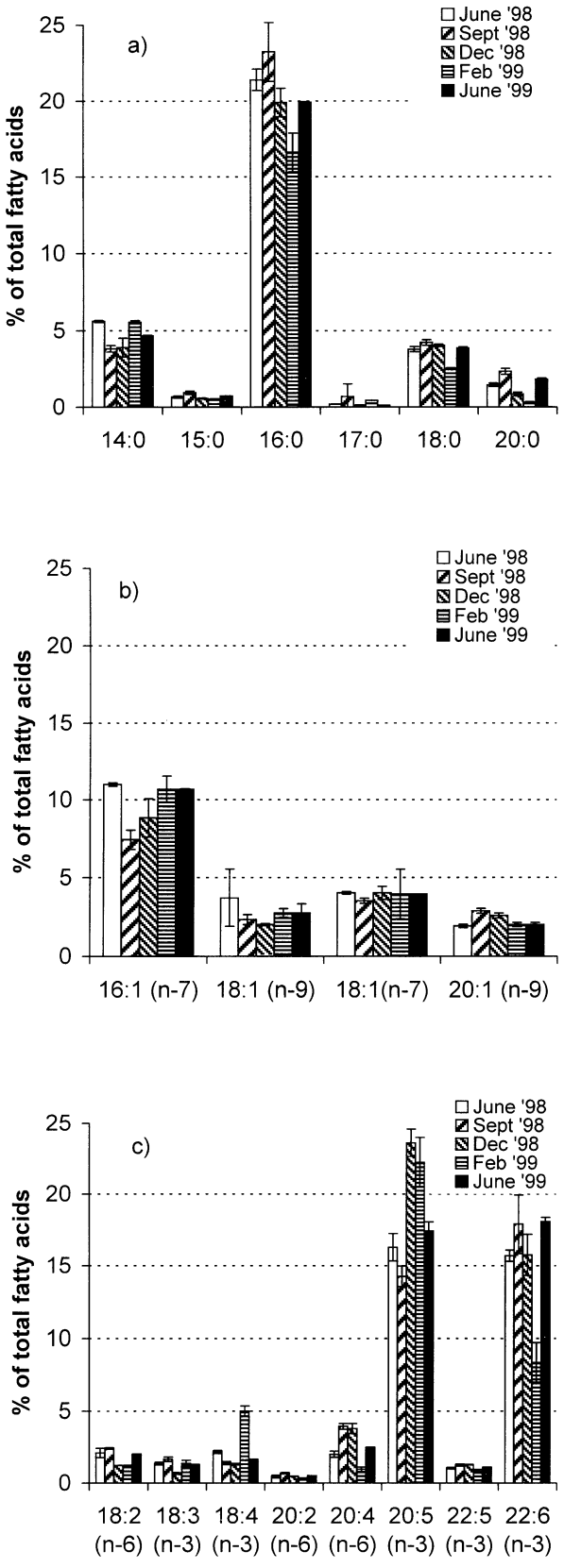

Fig. 3. Seasonal variations in the fatty acid composition of mussels (*M. galloprovincialis)* from Cattolica, Adriatic sea (% of total fatty acids): (a) saturated fatty acids, (b) monounsaturated fatty acids, (c) polyunsaturated fatty acids.

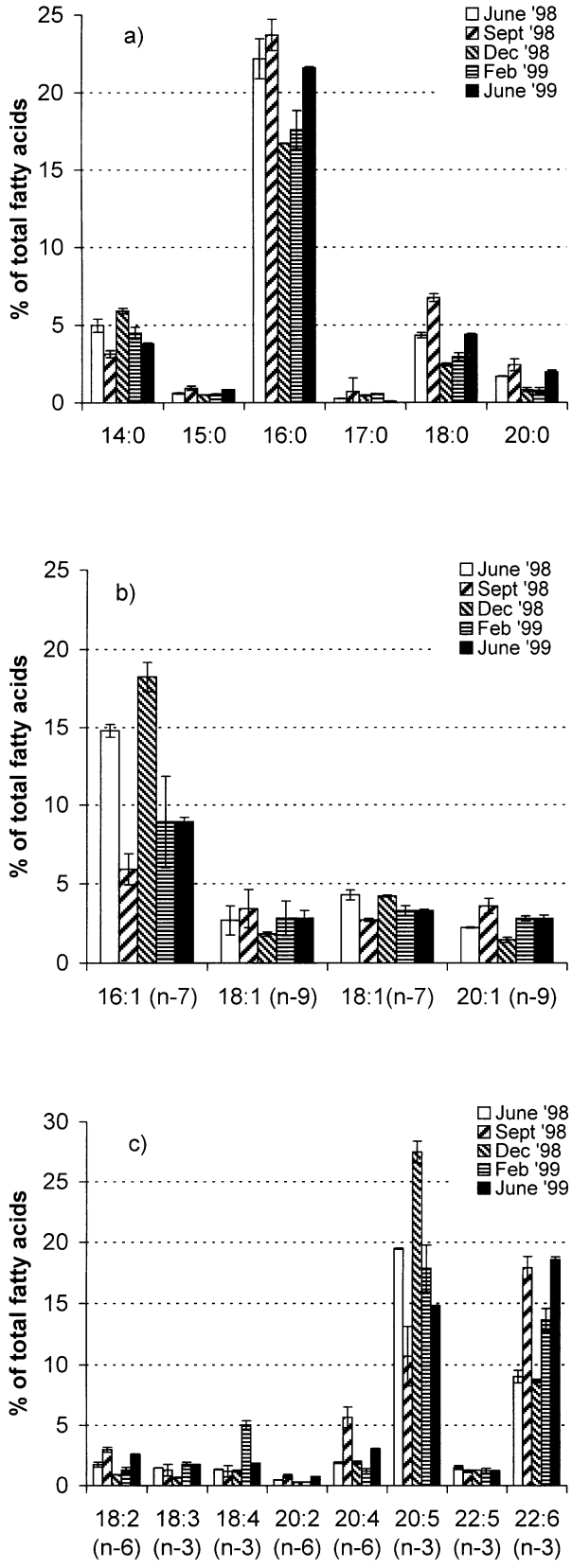

Fig. 4. Seasonal variations in the fatty acid composition of mussels (*M. galloprovincialis*) from the lake of Sabaudia, Tyrrhenian sea (% of total fatty acids): (a) saturated fatty acids, (b) monounsaturated fatty acids, (c) polyunsaturated fatty acids.

values (3.4–15.6), especially in the winter season, characterised mussels from Cattolica and from the lake of Sabaudia (Fig. 6a and b).

3.4. Unsaponifiable lipids

Total lipids of mussels from the two sites were assayed for cholesterol, plant sterols, α-tocopherol, all-*trans* retinol and carotenes contents.

Desmosterol, β-sitosterol, stigmasterol + campesterol and fucosterol + brassicasterol, originating from the phytoplankton-based diet of mussels, were the plant sterols quantified.

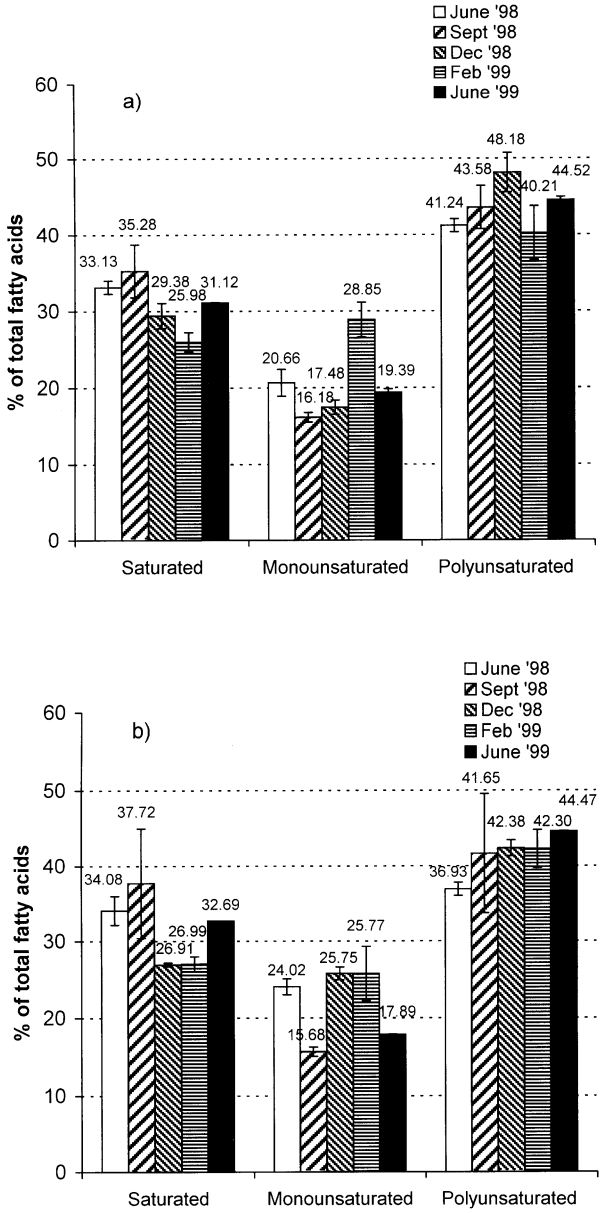

Fig. 5. Seasonal variations in the percent distribution of total saturated, monounsaturated and polyunsaturated fatty acids in mussels (*M. galloprovincialis*) from Cattolica, Adriatic sea (a) and from the lake of Sabaudia, Tyrrhenian sea (b) (% of total fatty acids).

A statistically significant seasonal variation of the single sterol levels was found in the lipid fraction of mussels from either zone of culture (Fig. 7a and b). Cholesterol, the dominant sterol, was present in mussels from Cattolica and from the lake of Sabaudia, at levels ranging from a minimum of 10 mg/lipid g in February to a maximum of about 30 mg/ lipid g in September, when plankton blooming was probably occurring. β-Sitosterol, stigmasterol + campesterol and fucosterol + brassicasterol showed seasonal patterns similar to those of cholesterol, while desmosterol, reaching peak values in December (4.0 mg/lipid g in mussels from Cattolica vs 3.8 mg/lipid g in mussels from the lake of Sabaudia) showed opposite changes. Desmosterol is known to be an intermediate of the metabolic transformation of 24-alkylsterols, present in marine vegetable organisms, into cholesterol. High desmosterol levels in coincidence with low amounts of cholesterol, have been observed in zooplancton samples from the Gulf of Trieste, northern Adriatic sea (Serrazanetti, Pagnucco, Conte, Artusi, Fonda-Umani, & Bergami, 1994).

The presence in *M. galloprovincialis* of phytosterols, poorly absorbed by the small intestine, assumes nutritional relevance in consideration of their capacity to compete with cholesterol and reduce its absorption and of their anticarcinogenic in vivo effects (Pelletier et al., 1995; Rao & Janezic, 1992).

A wide range of $\Delta^{5,7}$-sterols, vitamin D precursors known to constitute a large proportion of the unsaponifiable fraction of mollusc lipids, was detected by their

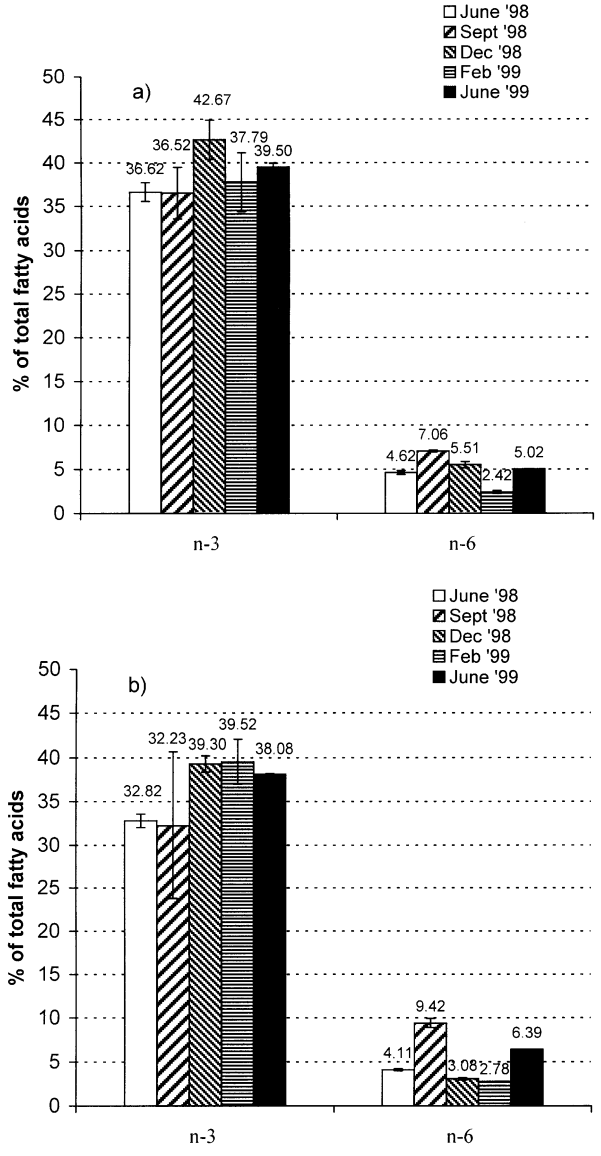

Fig. 6. Seasonal variations of total n-3 and n-6 polyunsaturated fatty acids in mussels (*M. galloprovincialis*) from Cattolica, Adriatic sea (a) and from the lake of Sabaudia, Tyrrhenian sea (b) (% of total fatty acids).

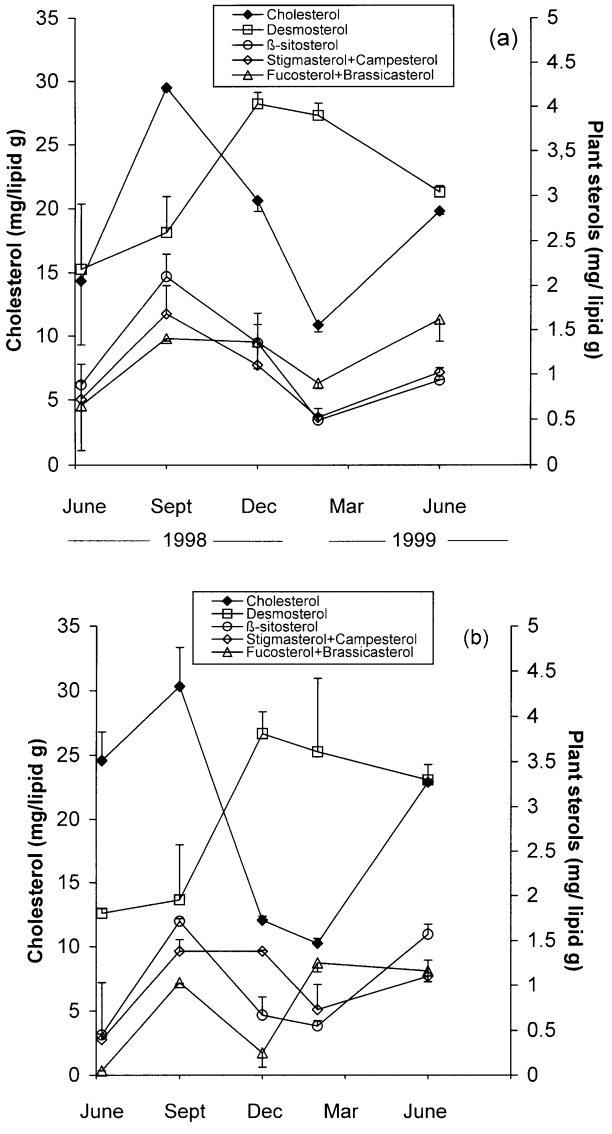

Fig. 7. Seasonal variations of cholesterol and plant sterols levels in the total lipids of mussels (*M. galloprovincialis*) from Cattolica, Adriatic sea (a) and from the lake of Sabaudia, Tyrrhenian sea (b) (mg/lipid g).

typical UV spectra in the mussels under study (results not shown). Among these compounds 7-dehydrocholesterol, a vitamin D_3 precursor, was identified in a highly impure form and therefore not quantified. On the other hand, ergosterol, the vitamin D_2 precursor reported to be present in mussels (Navia, 1971), was not found in our samples.

Since molluscs are known to have a limited capacity for sterol synthesis, the wide diversity of sterols detected in their tissues may be ascribed to their exogenous origin and is as an indication of the complex metabolic transformation undergone by exogenous sterols (Jarzebski, 1991).

Furthermore, as steroid hormones precursors, sterols may play specific roles in the gonadal development of marine molluscs (Jarzebski & Wenne, 1989).

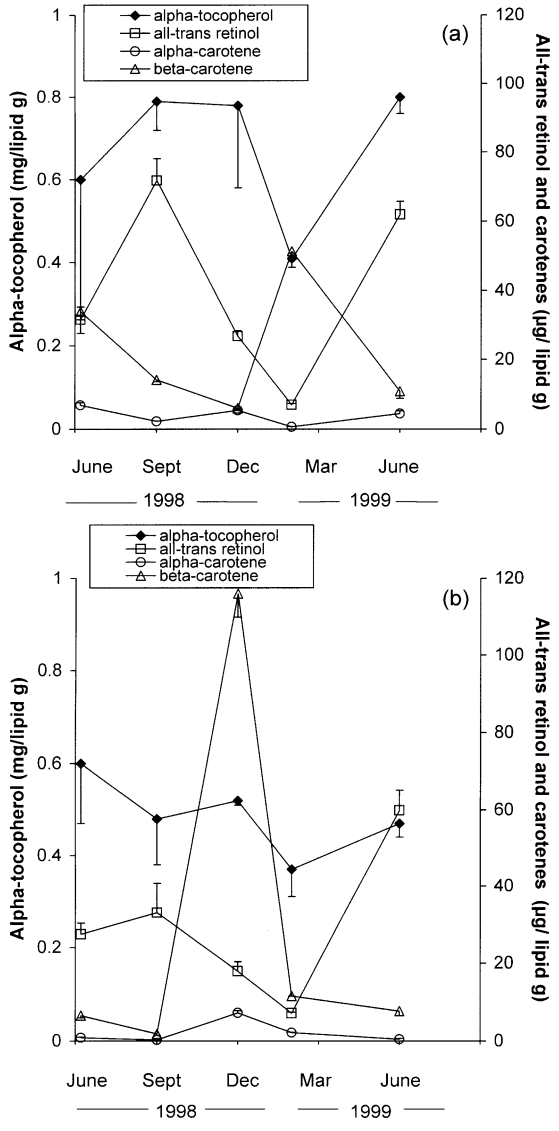

Fig. 8. Seasonal variations of α-tocopherol (mg/lipid g), all-*trans* retinol, α-carotene and β-carotene (μg/lipid g) levels in total lipids of mussels (*M. galloprovincialis)* from Cattolica, Adriatic sea (a) and from the lake of Sabaudia, Tyrrhenian sea (b) .

The α-tocopherol content of mussel lipids showed light, unsignificant fluctuations during the year ($P > 0.05$). All-*trans* retinol underwent highly insignificant fluctuations reaching lower levels in the winter months ($P \leqslant 0.001$) (Fig. 8a and b).

In all samples, HPLC of unsaponifiable lipids showed also that oxygenated carotenoids, characterised by typical absorption spectra, were prevalent over the less polar, late-eluting, α-carotene and β-carotene (results not shown). Due to the lack of suitable standard compounds, the early-eluting xanthophylls were not identified and the quantification of α-carotene and β-carotene only was possible. Their contents in mussel lipids showed significant seasonal changes during the year ($P \leqslant 0.001$). Peak values of α-carotene and β-carotene occurred in December in samples from the Tyrrhenian sea lake while in those from the Adriatic sea, high values were registered in different periods (Fig. 8a and b). Bivalve molluscs are known to be non-selective assimilators of carotenoids, compounds responsible of their meat pigmentation, and to store in the reproductive parts and in other organs, the oxygenated and hydrocarbon carotenoids ingested with phytoplankton (Simpson, Katayama, & Chichester, 1981). Besides their vitaminic and provitaminic activities, α-tocopherol and carotenoids retain interesting antioxidant properties and product ensure tissue protection from oxidative damages. Therefore, their presence constitutes a peculiar quality attribute in *M. galloprovincialis* mussels.

4. Conclusions

In conclusion, the quality characteristics of *M. galloprovincialis* mussels, harvested at seasonal intervals in two Italian sites, one off the northern Adriatic coast (Cattolica) and the other in a sea lake along the central Tyrrhenian coast (Lake of Sabaudia), reflect the different environmental conditions met by the animals during their growth. The sessile habit of mussels makes, in fact, their chemical composition strictly dependent on the phytoplankton resources available and therefore on the season of harvest. Furthermore, the seasonal variations of Condition Index and of the biometric and biochemical parameters, here described, apply only to the specific period considered, since a certain year-to-year variability of the climatic conditions, and consequently of the phytoplankton populating the sea water and of mussels' gametogenic cycle, may occur.

Apart from the observed seasonal variability of nutrient levels, mussels from both sites were characterised by low lipid contents. In particular, the lipid fraction contained elevated levels of n-3 polyunsaturated fatty acids, cholesterol, plant sterols and carotenoids and low levels of n-6 polyunsaturated fatty acids.

The data obtained from the present study may be useful to indicate the periods of the year more suitable for the marketing and consumption of mussels cultivated in the two geographical sites considered.

Acknowledgements

This study was supported by the Italian Ministry for Agricultural Policies. Authors gratefully acknowledge Dr. Giuliana Giulini and Dr. Nadia Mietti (Cooperativa M.A.R.E.—Consorzio Mediterraneo, Cattolica, Rimini) for their scientific collaboration and for providing the samples.

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