Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 101 (2006) 1063–1070
Food Chemistry
www.elsevier.com/locate/foodchem

Nutritional and commercial quality of the striped venus clam, *Chamelea gallina*, from the Adriatic sea

Elena Orban [a,*], Gabriella Di Lena [a], Teresina Nevigato [a], Irene Casini [a], Roberto Caproni [a], Generoso Santaroni [a], Giuliana Giulini [b]

[a] *National Research Institute for Food and Nutrition, Seafood Study Unit, Via Ardeatina 546, 00178 Rome, Italy*
[b] *Cooperativa M.A.R.E. S.c.r.l., Via Enrico Toti 2, 47841 Cattolica, Rimini, Italy*

Received 23 December 2005; received in revised form 16 February 2006; accepted 6 March 2006

Abstract

Nutritional quality parameters (proximate and mineral composition, contents of glycogen, fatty acids, cholesterol, plant sterols, fat-soluble vitamins, carotenes) and ecophysiological and commercial quality indicators (Condition Index, percent content of meat and intervalvar fluid) of the striped venus clam, *Chamelea gallina,* from the central Adriatic coast of Italy were studied at seasonal intervals over a 1-year period. Contents of protein (8.55–10.7 g/100 g), total lipid (0.73–1.59 g/100 g), glycogen (2.25–4.96 g/100 g) and non-protein nitrogen (0.54–0.78 g/100 g) varied significantly during the year, reaching the highest values in winter, in coincidence with a peak of Condition Index. Gas chromatography of total lipids showed high percentages of $n-3$ polyunsaturated fatty acids (33.7–41.9% of total fatty acids), in particular eicosapentaenoic (8.16–20.0% of total fatty acids) and docosahexaenoic acids (12.5–20.3% of total fatty acids) and low levels of total $n-6$ polyunsaturated fatty acids (3.61–7.87% of total fatty acids). HPLC analysis of the unsaponifiable lipids showed low levels of cholesterol, the dominant sterol (28.3–34.2 mg/ 100 g), and variable amounts of plant sterols (stigmasterol + campesterol, β-sitosterol, fucosterol + brassicasterol), α-tocopherol and carotenes.

Keywords: Bivalve molluscs; Clam; *Chamelea gallina*; Nutritional quality; Commercial quality

1. Introduction

The consumption of bivalve molluscs in Italy has increased in the recent years in response to the higher availability, on the market, of products originating from aquaculture. The different bivalve molluscs consumed in our country may, in fact, originate from fishery or from aquaculture.

The striped venus clam, *Chamelea gallina*, belonging to the Veneridae family, is particularly present in the wild, mainly on the central and northern Adriatic coasts of Italy, where the seawater is rich in mineral salts and organic matter, contributed in part by the river Po and by other Appennine and Alpine rivers. This clam inhabits sea bottoms of clean and muddy sand from the lower shore to depths of approximately 15 m.

By the end of the first year of life, at a length of 12–17 mm, *C. gallina* in the Adriatic sea is already capable of reproducing. The commercial size, corresponding to a minimum length of 25 mm, as established by the Council of the European Union (1994), is reached during the second–third year of age. Gamete emission starts in early spring and continues until the end of summer, reaching a peak at its beginning. Therefore, in this area, autumn and winter coincide with the gametogenesis of *C. gallina* (Poggiani, Piccinetti, & Piccinetti-Manfrin, 1973; Salvatorelli, 1967).

It is known that the quality requisites of bivalve molluscs are primarily dependent on the water quality, assuring a healthy product and a safe consumption, and by a

* Corresponding author. Tel.: +39 06 51494452; fax: +39 06 51494550.
E-mail address: orban@inran.it (E. Orban).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.03.005

variety of factors, such as food availability, gametogenic cycle, water temperature and salinity (Beninger & Lucas, 1984; Karakoltsidis, Zotos, & Constantinides, 1995; Orban et al., 2004; Orban et al., 2002).

A parameter of commercial importance for bivalves, especially in view of their industrial processing, is represented by the Condition Index, commonly used to evaluate the healthy state of animals and adopted in foreign trade as a criterion to select the best products. This parameter, correlated with the meat yield, is reported to reflect the seasonal fluctuations, the gametogenic cycle and the environmental conditions (Lucas & Beninger, 1985; Okumus & Stirling, 1998).

Proteins, lipids, minerals and glycogen, together with minor components of hydrophilic and lipophilic nature, contribute to the nutritional value and organoleptic characteristics of clams. The presence of traces of sand affects the palatability of clams and is highly dependent on the method of harvesting which, in the case of *C. gallina,* is accomplished by hydraulic dredges.

The aim of this work was the study of the nutritional and commercial quality of *C. gallina* clam harvested on the central Adriatic coast of Italy in different seasonal periods.

2. Materials and methods

2.1. Sample collection and environmental parameters

This study was carried out between June 2002 and July 2003 in collaboration with cooperatives of fishermen. Clams (*C. gallina*) of commercial size were collected in June, September, December, 2002, and in February and July, 2003, by hydraulic dredges along the Italian coast of the central Adriatic sea, in the area between Rimini (Emilia-Romagna) and Pesaro (Marche). After collection, the clams were selected by a vibrating sieve and sampled randomly for this study.

The sea bottom on the Adriatic coast of Emilia Romagna and Marche is made of sand up to a depth of −2 and −4 m; at greater depths the silt content increases. The geological parameters of this coastal area are affected by many factors, such as the presence of the Po river and of other minor freshwater streams. During this study the average surface water temperature in the area of clam collection fluctuated between a minimum of 5–6 °C in February and a maximum of 26–28 °C in August. Under the influence of freshwater, the salinity of the seawater varied during the year between 24–25 g l^{-1} and 36–37 g l^{-1}.

2.2. Sample treatment

Soon after collection, samples (about 5 kg) were transported under refrigeration (+6 °C) to the laboratories of the National Research Institute for Food and Nutrition in Rome. Upon arrival, clams were inspected and dead animals, if any, discarded. Shells were rapidly washed under running cold water and rinsed on a paper towel. Twenty individuals were randomly selected for biometric measurements and Condition Index determination. The remaining clams were divided into at least three groups of 50–60 specimens for chemical evaluations. Each group was processed separately. Clams were manually shucked by cutting the adductor muscle with a knife. For each group, the mean percent weights of intervalvar fluid and edible meat were calculated. Animals and intervalvar fluid, from each group of 50–60 clams, were frozen in vacuum-sealed multilayer barrier bags at −75 °C for chemical analyses.

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 2 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

Within 2 weeks from collection and freezing, clam samples were rapidly thawed under running cold water, when still in the sealed bag, and 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. Three pools, of about 50–60 individuals for each sampling period, were analysed.

The pH was measured at 20 °C on a water:clam homogenate (2:1 w/w). Moisture, crude protein ($N \times 6.25$) and ash contents of clams were determined by the AOAC (1990) methods 950.46, 955.04 and 938.08, respectively. Total lipids were extracted by the method of Bligh and Dyer (1959) slightly modified according to Kinsella, Shimp, Mai, and Weihrauch (1977). The glycogen content was assayed enzymatically (Keppler & Decker, 1974) after cold perchloric acid extraction (Dalrymple & Hamm, 1973). Non-protein nitrogen was determined by the Kjeldahl method 955.04 (AOAC, 1990) after protein precipitation with 10% (w/v) trichloroacetic acid.

Sodium, potassium, calcium and magnesium were analysed by Ion Exchange Liquid Chromatography on clam homogenates after lyophilization. A weighed amount of lyophilised sample was ashed in a muffle furnace at 500 °C for 24 h. Ashes were dissolved with the smallest amount of concentrated nitric acid and the resulting solution brought up to 50 ml with bidistilled water.

A Dionex-Biolc Ion system equipped with a Dionex CS12 Ion Pack column and a suppressed conductivity detector (Camberley, UK) was used to analyse the inorganic elements. Cation separation was accomplished by

an isocratic 20 mM methane sulfonic mobile phase at a flow rate of 1 ml min^{-1}. Chromatographic runs lasted 12 min and a baseline separation of all components was achieved (Rey & Pohl, 1993).

Phosphorus was determined spectrophotometrically according to the AOAC method 965.17 (AOAC, 1990).

Zinc, iron, selenium, chromium, nickel and mercury were analysed by Instrumental Activation Analysis. Lyophilised clam homogenates, in pure quartz vials, were irradiated, without any pre-treatment, in a 1 MW Triga Reactor for about 14 h in a thermal neutron flux of approximately 2.6×10^{-12} n cm^{-2} s^{-1}. The continuous rotation of the irradiation facility ensured a uniform neutron flux to all samples. After cooling, samples were transferred to polyethylene containers and measured by gamma spectrometry using a high purity germanium detector with a relative efficiency of about 20% and a resolution of 1.9 keV at the 1332 keV peak. Gamma spectra analysis was performed using the Omnigam EG & G Ortec computer programme.

Fatty acid profiles were determined by Gas Chromatography, using a 6890 Hewlett Packard Gas Chromatograph with flame ionisation 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 with authentic standards for area percent normalisation. Relative quantities were expressed as weight percent of total fatty acids in each sample.

Total lipids were saponified and cholesterol, plant sterols (desmosterol, stigmasterol + campesterol, β-sitosterol, fucosterol + brassicasterol, ergosterol, 7-dehydrocholesterol), α-tocopherol, all-*trans* retinol and α-and β-carotene were assayed by High Performance Liquid Chromatography (HPLC). For α-tocopherol and sterol 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 carotene determinations, the saponification occurred overnight at room temperature. In both cases saponification occurred in screw-capped amber vials under a nitrogen atmosphere in the presence of *tert*-butylhydroquinone, 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 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, as previously described (Orban et al., 2002). Runs were monitored at 325 and 450 nm. Analytes were identified by their retention times and UV–visible spectra. Quantification was done by external standardisation. 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. Analytical quality control

The repeatability of the gas chromatographic response in the fatty acids analysis was evaluated for both samples and standards. Clam samples were prepared for the instrumental analysis and injected 3 times. The areas of peaks studied ($n = 25$) showed a mean coefficient of variation (CV) of 0.4%, while the CV for retention times showed a mean value of 0.03%. All analytical standards ($n = 25$) were set at four different concentrations in triplicate. An appropriate internal standard (15:1 $n - 5$) was used. Subsequently, 300 injections from the 300 prepared vials were performed and, in this way 25 calibration curves were obtained. The mean correlation coefficient observed (R^2, linear regression) was 0.9877, 0.9606 and 0.9991 being the minimum and maximum values, respectively. As regards recoveries, a standard oil, with known amounts of selected fatty acids (C14:0 = 33%, C16:0 = 33% and C18:1 $n - 9 = 33\%$), was analysed in triplicate. Results were as follows: C14:0 = 29.9%, C16:0 = 34% and C18:1 $n - 9 = 36.1\%$.

Finally, in order to test the robustness of the method of fatty acid determination, two different derivatization procedures were applied on the same sample (the first one was the derivatization step routinely used in our laboratory). The mean CV observed for the two sets of results was 4.2%.

The recovery of α-tocopherol and plant sterols after the saponification and TLC-purification steps was nearly 70%. A set of standards was routinely assayed in the silica gel plates, together with samples and, to compensate for losses, data were corrected on the basis of the recoveries daily obtained. In the HPLC separation of unsaponifiables, linear calibration curves were obtained for α-tocopherol (27–140 μg/ml, $R^2 = 0.9999$), cholesterol (800–4800 μg/ml, $R^2 = 0.9999$), all-*trans* retinol (60–350 ng/ml, $R^2 = 0.9972$), α-carotene (100–550 ng/ml, $R^2 = 0.9998$) and β-carotene (100–600 ng/ml, $R^2 = 0.9981$). To compensate for any day-to-day variations, each day of analysis a set

of standards was routinely saponified and run under the same conditions as samples.

Repeatability of the HPLC method was estimated by calculating the CV of analyte concentrations after repeated runs of a standard solution containing each compound at the level found in samples. The mean CVs for pure α-tocopherol, cholesterol, all-*trans* retinol and α- and β-carotene were 0.51%, 0.51%, 0.16%, 0.30% and 0.29%, respectively. The recoveries of standard compounds after saponification were: 96% for α-tocopherol, 98% for cholesterol and 86–95% for all-*trans* retinol and carotenes. After HPLC runs, the purity of analytes was checked by matching the UV/Vis spectra of each peak with those of the standards.

2.6. Statistics

Results are reported as means ± standard deviations. Chemical evaluations at any seasonal sampling were performed at least in duplicate on three pools of 50–60 clams. Analysis of variance (one-way ANOVA) and multiple-range LSD test were applied to find any significant ($P \leqslant 0.05$) seasonal fluctuations during the year (Statgraphics®, Statistical Graphic System by Statistical Graphic Corporation, Version 5 Plus, Manugistics™ Inc, Rockville, MD, USA).

3. Results and discussion

3.1. Biometric characteristics and Condition Index

The biometric characteristics of *C. gallina* shells harvested during the experimental period along the central Adriatic coast of Italy are listed in Table 1. These clams were harvested by hydraulic dredging, a collection technique often resulting in a certain amount of damaged specimens which were carefully discarded in our study.

The commercial quality and physiological state of bivalve molluscs are adequately described by the Condition Index, a parameter of economic relevance reflecting the ecophysiological conditions and the health of animals. The seasonal pattern of Condition Index in *C. gallina* clams was characterised by a peak value by the end of winter (February), just before spawning (Fig. 1). At the same time, a net depletion of the amount of intervalvar fluid was observed, with a consequent increase in the meat content. The low CI values registered in September, 2002, and July, 2003, periods of gamete emission, may reflect the major biological effort expended in the production and release of gametes.

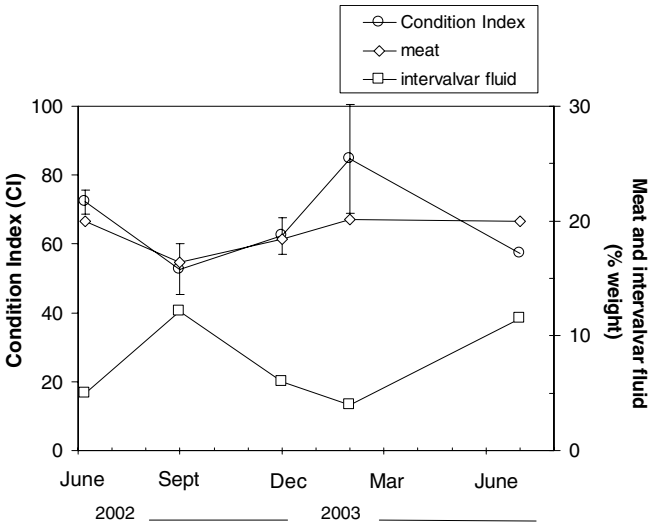

Fig. 1. Seasonal variations of the Condition Index and of meat and intervalvar fluid contents (g/100 g wet weight) in clams (*C. gallina*) from the central Adriatic sea.

3.2. Proximate composition

The biochemical composition of clams was characterised by phases of accumulation and utilisation of reserves (Table 2). Protein (8.55–10.75 g/100 g), total lipid (0.73–1.59 g/100 g), glycogen (2.25–4.96 g/100 g) and non-protein nitrogen (0.54–0.78 g/100 g) varied significantly during the year, following a similar pattern. Their fluctuations were also coincident with those of the Condition Index. A correlation between Condition Index and biochemical constituents of bivalves has also been reported in different mollusc species (Beninger & Lucas, 1984; Orban et al., 2004; Whyte & Englar, 1982).

By reporting values on a dry weight basis, these patterns are confirmed, notwithstanding the significant moisture fluctuations of clams during the year. This observation is important since the true nutrient content of clams seems not to be masked by the moisture fluctuations occurring during the year.

In particular, the increments of the contents of lipids, glycogen and proteins, biochemical reserves of bivalves

Table 1
Biometric characteristics of the shells of clams (*C. gallina*) harvested during the experimental period from the central Adriatic sea[A]

	2002			2003	
	June	September	December	February	July
Thickness (mm)	1.27 ± 0.14	1.45 ± 0.31	1.34 ± 0.23	1.08 ± 0.09	0.97 ± 0.22
Length (mm)	27.80 ± 2.00	30.60 ± 2.80	26.20 ± 4.40	24.18 ± 2.31	26.60 ± 1.14
Height (mm)	24.20 ± 1.90	25.80 ± 1.60	23.00 ± 2.70	20.89 ± 1.52	23.05 ± 0.63
Width (mm)	12.70 ± 1.00	14.29 ± 0.70	12.80 ± 1.50	10.90 ± 0.70	12.77 ± 0.58

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

Table 2
Chemical composition of clams (*C. gallina*) from the central Adriatic sea: seasonal variation (g 100 g^{-1} wet weight)[A]

	2002			2003	
	June	September	December	February	July
pH	6.46 ± 0.06^{a}	6.44 ± 0.05^{a}	6.48 ± 0.05^{a}	6.36 ± 0.01^{a}	6.48 ± 0.08^{a}
Moisture	79.8 ± 0.71^{b}	82.9 ± 0.06^{c}	79.7 ± 0.37^{b}	77.4 ± 0.71^{a}	83.1 ± 0.06^{c}
Protein	10.4 ± 0.57^{b}	8.55 ± 0.02^{a}	10.1 ± 0.11^{b}	10.8 ± 0.12^{b}	9.04 ± 0.25^{a}
Total lipid	1.55 ± 0.10^{c}	0.73 ± 0.08^{a}	1.16 ± 0.04^{b}	1.59 ± 0.25^{c}	0.84 ± 0.05^{ab}
Glycogen	3.51 ± 0.20^{c}	2.71 ± 0.03^{b}	4.33 ± 0.09^{d}	4.96 ± 0.05^{e}	2.25 ± 0.07^{a}
Nonprotein N	0.63 ± 0.03^{a}	0.54 ± 0.01^{a}	0.61 ± 0.02^{a}	0.78 ± 0.09^{b}	0.57 ± 0.05^{a}
Ash	3.37 ± 0.40^{a}	3.55 ± 0.23^{a}	3.08 ± 0.20^{a}	2.73 ± 0.13^{a}	3.37 ± 0.06^{a}

[A] Means ± standard deviations ($n = 3$). Within a row, values not sharing the same superscript are significantly different ($p \leqslant 0.05$).

important during gamete formation and food shortage, observed in winter, were coincident with the intense energetic demand of gametogenesis. The accumulation and mobilisation of energetic reserves, according to the stage of the reproductive cycle, is reported in different marine bivalve species (Berthelin, Kellner, & Mathieu, 2000; Okumus & Stirling, 1998).

3.3. Mineral elements

C. gallina clams, like other bivalve molluscs, may be considered a good source of nutritionally important minerals. In addition, as filter-feeders, clams may concentrate, in their tissues, trace elements present in the diet and in the aquatic environment, dissolved or as particulate inorganic matter. Therefore, they may be considered good sentinel organisms of environmental pollution (Usero, Morillo, & Gracia, 2005).

Table 3 shows the mineral content of clams *C. gallina* during the period of the experiment. Seasonal fluctuations are evident for some elements, mainly potassium, phosphorus and chromium. Changes in food availability and particulate metal run-off due to high precipitation, together with influences of the reproductive cycle, have been proposed to explain the seasonal fluctuations of minerals in bivalve molluscs (Fowler & Oregioni, 1976; Pàez-Osuna, Frias-Espericueta, & Osuna-Lopez, 1995). In our study we often observed a certain difficulty in the determination of minerals due to the presence of sand traces within the clam valves, probably resulting from the collection system utilised. This may have been a crucial factor in determining the variability of mineral contents in clam samples.

3.4. Fatty acids

The fatty acid profiles of total lipids extracted from clams (*C. gallina*) harvested in different seasons are listed in Table 4. A prevalence of total polyunsaturated fatty acids (PUFA, 41.6–48.1% of total fatty acids) over the saturated (SFA, 29.1–39.3% of total fatty acids) and monounsaturated ones (MUFA, 14.2–23.4% of total fatty acids) was evident throughout the year, as was also observed in mussels and oysters from different Italian sites (Orban et al., 2004, 2002). No significant seasonal changes of total PUFA were observed during the year, while total SFA, MUFA, $n-3$ PUFA and $n-6$ PUFA varied significantly ($P < 0.05$). Palmitic acid (C16:0, 19.1–22.5% of total fatty acids) and eicosapentaenoic (C20:5 $n-3$, 8.16–20.0% of total fatty acids) and docosahexaenoic (C22:6 $n-3$, 12.6–20.3% of total fatty acids) acids were the prevalent saturated and polyunsaturated fatty acids throughout the year. In consideration of the very low lipid content of clams, it may be assumed that the high percentages of C20:5 $n-3$ and C22:6 $n-3$ in their total lipid fraction

Table 3
Mineral contents of clams (*C. gallina*) from the central Adriatic sea: seasonal variation[A]

	2002			2003	
	June	September	December	February	July
Wet weight (mg 100 g^{-1})					
Na	528 ± 24.5^{a}	630 ± 55.3^{a}	525 ± 154^{a}	564 ± 26.7^{a}	710 ± 0.25^{a}
K	182 ± 21.6^{a}	190 ± 12.8^{a}	221 ± 26.9^{ab}	257 ± 32.5^{a}	181 ± 4.33^{a}
Mg	78.8 ± 6.64^{a}	87.5 ± 0.47^{a}	74.3 ± 5.66^{a}	89.4 ± 5.51^{a}	85.6 ± 7.57^{a}
Ca	245 ± 7.32^{a}	150 ± 123^{a}	327 ± 364^{a}	67.5 ± 8.56^{a}	304 ± 133^{a}
P	145 ± 14.1^{b}	108 ± 11.6^{a}	153 ± 11.6^{b}	177 ± 22.1^{b}	150 ± 7.28^{b}
Zn	1.23 ± 0.11^{a}	0.91 ± 0.14^{a}	1.38 ± 0.52^{a}	1.48 ± 0.25^{a}	1.28 ± 0.23^{a}
Fe	6.31 ± 1.29^{a}	6.73 ± 0.89^{a}	6.44 ± 0.79^{a}	10.7 ± 2.90^{a}	8.20 ± 1.70^{a}
Wet weight (μg 100 g^{-1})					
Se	57.5 ± 3.54^{a}	72.8 ± 11.5^{a}	59.7 ± 5.93^{a}	63.3 ± 11.2^{a}	69.6 ± 9.44^{a}
Cr	128 ± 17.7^{a}	139 ± 18.4^{a}	77.8 ± 7.58^{a}	116 ± 21.2^{a}	90.2 ± 12.8^{a}
Hg	1.10 ± 0.14^{a}	1.22 ± 0.15^{a}	1.02 ± 0.11^{a}	1.26 ± 0.06^{a}	1.05 ± 0.07^{a}
Ni	73.0 ± 7.85^{a}	57.2 ± 8.89^{a}	76.5 ± 6.03^{a}	75.5 ± 7.57^{a}	56.5 ± 4.95^{a}

[A] Means ± standard deviations ($n = 3$). Within a row, values not sharing the same superscript are significantly different ($p \leqslant 0.05$).

Table 4
Fatty acid composition of total lipid of clams (*C. gallina*) from the central Adriatic sea: seasonal variation (% of total fatty acids)[A]

Fatty acids	2002			2003	
	June	September	December	February	July
C14:0	4.67 ± 0.07^{c}	3.05 ± 0.22^{b}	3.13 ± 0.37^{b}	3.71 ± 0.59^{b}	1.88 ± 0.01^{a}
C15:0	0.49 ± 0.02^{a}	0.85 ± 0.04^{b}	0.45 ± 0.01^{a}	0.49 ± 0.01^{a}	0.47 ± 0.00^{a}
C16:0	19.7 ± 0.46^{a}	22.5 ± 1.48^{a}	20.8 ± 1.10^{a}	19.1 ± 0.65^{a}	20.7 ± 0.05^{a}
C17:0	0.30 ± 0.02^{a}	0.15 ± 0.02^{a}	0.13 ± 0.01^{a}	1.45 ± 0.23^{a}	0.09 ± 0.01^{a}
C18:0	5.06 ± 0.14^{b}	6.33 ± 0.33^{c}	4.38 ± 0.22^{b}	3.53 ± 0.60^{a}	6.40 ± 0.04^{c}
C20:0	1.94 ± 0.02^{b}	6.36 ± 0.40^{d}	2.22 ± 0.21^{b}	0.84 ± 0.04^{a}	4.24 ± 0.01^{c}
Total saturated	32.1 ± 0.55^{b}	39.3 ± 0.56^{d}	31.1 ± 0.76^{b}	29.1 ± 0.47^{a}	33.8 ± 0.01^{c}
C16:1 $n-7$	9.19 ± 0.06^{c}	4.54 ± 0.78^{a}	6.55 ± 0.67^{b}	15.1 ± 0.81^{d}	3.52 ± 0.12^{a}
C18:1 $n-9$	4.10 ± 0.33^{b}	4.95 ± 0.45^{c}	4.20 ± 0.06^{b}	2.54 ± 0.24^{a}	5.32 ± 0.04^{c}
C18:1 $n-7$	6.10 ± 0.30^{c}	2.78 ± 0.13^{a}	3.97 ± 0.08^{b}	4.82 ± 0.76^{b}	4.33 ± 0.39^{b}
C20:1 $n-9$	1.00 ± 0.07^{a}	1.89 ± 0.08^{c}	1.04 ± 0.01^{a}	0.93 ± 0.12^{a}	1.68 ± 0.03^{b}
Total monounsaturated	20.4 ± 0.16^{c}	14.2 ± 0.12^{a}	15.8 ± 0.81^{b}	23.4 ± 0.70^{d}	14.9 ± 0.27^{ab}
C18:2 $n-6$	1.59 ± 0.04^{b}	1.37 ± 0.16^{b}	1.30 ± 0.14^{b}	0.91 ± 0.16^{a}	1.30 ± 0.04^{b}
C18:3 $n-3$	1.07 ± 0.11^{b}	1.18 ± 0.10^{b}	0.38 ± 0.10^{a}	0.62 ± 0.11^{a}	2.62 ± 0.04^{c}
C18:4 $n-3$	1.93 ± 0.11^{c}	1.23 ± 0.11^{ab}	0.74 ± 0.10^{a}	3.68 ± 0.48^{d}	1.77 ± 0.04^{bc}
C20:2 $n-6$	0.78 ± 0.05^{b}	1.35 ± 0.14^{d}	0.77 ± 0.05^{b}	0.54 ± 0.04^{a}	1.12 ± 0.02^{c}
C20:4 $n-6$	1.86 ± 0.04^{a}	5.15 ± 0.24^{c}	4.12 ± 0.21^{b}	2.16 ± 0.43^{a}	3.54 ± 0.08^{b}
C20:5 $n-3$	18.9 ± 1.02^{c}	8.16 ± 0.43^{a}	20.0 ± 1.50^{c}	19.5 ± 0.91^{c}	11.8 ± 0.07^{b}
C22:5 $n-3$	2.44 ± 0.04^{a}	3.31 ± 0.08^{a}	3.27 ± 0.52^{a}	2.65 ± 0.78^{a}	3.92 ± 0.15^{b}
C22:6 $n-3$	14.0 ± 0.46^{ab}	19.8 ± 0.07^{c}	17.6 ± 1.21^{bc}	12.6 ± 3.39^{a}	20.3 ± 0.02^{c}
Total polyunsaturated	42.5 ± 0.72^{a}	41.6 ± 0.68^{a}	48.1 ± 0.06^{a}	42.6 ± 2.92^{a}	46.4 ± 0.25^{a}
Total $n-3$ polyunsaturated	38.3 ± 0.74^{b}	33.7 ± 0.62^{a}	41.9 ± 0.03^{c}	39.0 ± 2.67^{bc}	40.4 ± 0.28^{bc}
Total $n-6$ polyunsaturated	4.22 ± 0.02^{b}	7.87 ± 0.05^{d}	6.19 ± 0.02^{c}	3.61 ± 0.23^{a}	5.96 ± 0.01^{c}
Ratio $n-3/n-6$	9.07 ± 0.03^{c}	4.28 ± 0.05^{a}	6.77 ± 0.01^{b}	10.8 ± 0.04^{d}	6.78 ± 0.06^{b}

[A] Means ± standard deviations ($n = 3$). Within a row, values not sharing the same superscript are significantly different ($p \leqslant 0.05$).

reflect the preferential association of these fatty acids with phospholipids in cell membranes.

As regards MUFA, palmitoleic acid (C16:1 $n-7$, 3.52–15.1% of total fatty acids) reached high percentages in two samplings (June, 2002 and February, 2003, respectively 9.19% and 15.1% of total fatty acids) while, in the other periods, its percentages were lower (3.5–6.5% of total fatty acids) and equivalent to those of the other MUFA, mainly C18:1 $n-9$ and C18:1 $n-7$.

A correspondence between the fatty acid profile of aquatic filter-feeders and that of their diet is a known fact (Albentosa, Labarta, Fernandez-Reiriz, & Perez-Camacho, 1996; Beninger & Stephan, 1985). In addition, statistically significant seasonal fluctuations of single fatty acid percentages, as observed in *C. gallina* clams, are not uncommon among bivalve molluscs. It is generally regarded that, besides gametogenic cycle influences, the blooming of different planktonic populations during the year may affect the lipid profile of those organisms, like bivalves, relying on plankton as the main food source (Pollero, Ré, & Brenner, 1979).

High levels of $n-3$ PUFA (33.7–41.9% of total fatty acids), important in the human diet for their platelet anti-aggregating and blood pressure-reducing properties, low levels of $n-6$ PUFA (3.61–7.87% of total fatty acids) and relatively high $n-3/n-6$ PUFA ratio values (4.28–10.81) characterised clams during the period under study. These aspects contribute to a positive evaluation of the lipid quality of *C. gallina* from the central Adriatic sea. Seafood products are, in fact, the only significant source in the human diet of $n-3$ PUFA, a class of nutrients fundamental in the prevention of human chronic inflammatory and cardiovascular diseases. In consideration of the increased consumption of food rich in $n-6$ PUFA in industrialised countries and of the important re-establishment of a healthy balance between $n-3$ and $n-6$ PUFA, an increment of the consumption of seafood is recommended by the current dietary guidelines (Simopoulos, 2003).

3.5. Unsaponifiable lipids

The composition of the unsaponifiable lipid fraction extracted from total lipids of clams *C. gallina* is shown in Table 5. Cholesterol was the prevalent sterol in all seasons; its levels, varying insignificantly during the year, were always low in clams (28.3–34.2 mg/100 g) if compared to other protein sources. Along with cholesterol, several plant sterols deriving from the phytoplankton-based diet of clams were identified in the unsaponifiable lipid fraction of *C. gallina*. Stigmasterol+campesterol (2.70–5.24 mg/100 g), β-sitosterol (1.10–2.39 mg/100 g) and fucosterol + brassicasterol (1.25–8.30 mg/100 g) were the phytosterols identified and quantified. 7-Dehydrocholesterol, a $\Delta^{5,7}$-sterol precursor of vitamin D_3, was detected in a

Table 5
Unsaponifiable lipid components in clams (*C. gallina*) from the central Adriatic sea: seasonal variation[A]

	2002			2003	
	June	September	December	February	July
Wet weight (mg 100 g^{-1})					
Cholesterol	34.2 ± 1.84^{a}	28.3 ± 3.68^{a}	32.5 ± 0.86^{a}	32.8 ± 4.16^{a}	31.2 ± 1.31^{a}
Stigmasterol + Campesterol	3.80 ± 1.74^{a}	3.04 ± 0.21^{a}	5.24 ± 1.86^{a}	2.70 ± 0.30^{a}	4.91 ± 0.50^{a}
α-Tocopherol	0.92 ± 0.45^{a}	0.48 ± 0.03^{a}	1.16 ± 0.21^{a}	1.07 ± 0.20^{a}	0.88 ± 0.40^{a}
β-Sitosterol	2.19 ± 0.16^{b}	1.10 ± 0.27^{a}	2.39 ± 0.49^{b}	2.27 ± 0.44^{b}	1.76 ± 0.30^{ab}
Fucosterol + Brassicasterol	4.04 ± 0.82^{bc}	1.25 ± 0.43^{a}	5.25 ± 0.27^{c}	8.30 ± 0.42^{d}	3.42 ± 0.28^{b}
Desmosterol	–	–	–	–	–
Ergosterol	–	–	–	–	–
7-Dehydrocholesterol	i	i	i	i	i
Wet weight (μg 100 g^{-1})					
All-*trans* retinol	tr	tr	tr	tr	tr
α-Carotene	4.96 ± 0.04^{c}	1.28 ± 0.07^{b}	0.21 ± 0.02^{a}	0.42 ± 0.10^{ab}	6.15 ± 0.67^{d}
β-Carotene	26.8 ± 0.11^{c}	6.61 ± 0.18^{b}	1.64 ± 0.15^{a}	31.1 ± 1.76^{d}	3.92 ± 0.55^{a}

(–) not detectable; (i) present but not quantified because detected as an impure peak; (tr) trace.
[A] Means ± standard deviations ($n = 3$). Within a row, values not sharing the same superscript are significantly different ($p \leqslant 0.05$).

highly impure peak; therefore its quantification was not possible. Two plant sterols detected in other bivalves (Orban et al., 2004, 2002), desmosterol, an intermediate of cholesterol biosynthesis, and ergosterol, a vitamin D_{2-} precursor, were not identified in *C. gallina* clams. Since it is known that molluscs have a limited capacity for sterol biosynthesis (Goad, 1981), the presence of different phytosterols in bivalve molluscs, described by several authors (Jarzebski, 1991; Jarzebski & Wenne, 1989; Krzynowek, Wiggin, & Donahue, 1983; Murphy, Mooney, Mann, Nichols, & Sinclair, 2002; Polak, Jarzebsky, Wenne, & Falkowski, 1987), reflect the variety of phytoplankton food sources and may also be regarded as an indication of the complex metabolic transformations undergone by exogenous sterols after ingestion. From a nutritional point of view, plant sterols able to compete with cholesterol for intestinal absorption and to affect the metabolism of cholesterol and bile acids, are molecules of interest in the human diet (Pelletier et al., 1995; Rao & Janezic, 1992). Their presence in a balanced diet is known to reduce blood serum cholesterol levels in humans and to prevent colon cancer development (Ling & Jones, 1995; Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000).

The levels of α-tocopherol, a vitamin with anti-oxidant properties, in clams (*C. gallina*) were low and stable; all-*trans* retinol was present in trace amounts. Among minor unsaponifiable components, the liquid chromatographic analysis of clam oil detected the presence of xanthophylls and carotenes. These substances, characterised by a provitamin and antioxidant action, are nutrients absorbed from the diet and stored mainly in reproductive organs (Simpson, Katayama, & Chichester, 1981). Xanthophyll peaks, recognisable by their retention times and characteristic absorption spectra, could not be identified singularly, due to the lack of suitable standard compounds. As regards α-and β-carotene, their levels were highly variable during the year, as has also been observed in mussels and oysters (Orban et al., 2004, 2002).

4. Conclusions

Clams, *C. gallina*, from the central Adriatic coast of Italy may be regarded as a food item with interesting dietetic properties due to their low lipid and cholesterol contents, to the presence of phytosterols and to the high percentages of healthy polyunsaturated lipids.

The high seasonal variability observed in the chemical composition of this species is a feature typical of bivalve molluscs. In fact, apart from influences of the reproductive cycle, clams, as bottom-dwelling organisms, are highly dependent on the dietary resources available in their immediate habitat and therefore on the climatic conditions.

The fluctuations of nutrient contents observed in clams during the year have an impact on the commercial and nutritional qualities of the product. The period just preceding gamete release that, compatibly with the inter-annual variability, generally coincides with the end of winter, may be considered a period of the year when *C. gallina* from the central Adriatic area reaches its highest commercial and nutritional values.

Acknowledgement

This study was supported by the Italian Ministry for Agricultural and Forestry Policies (4th Three-Year Plan of Fishery and Aquaculture, Project no. 4C127).

References

Albentosa, M., Labarta, U., Fernandez-Reiriz, M. J., & Perez-Camacho, A. (1996). Fatty acid composition of *Ruditapes decussatus* spat fed on different microalgae diets. *Comparative Biochemistry and Physiology, 113A*, 113–119.

AOAC (1990). Official methods of analysis (15th ed.). Arlington, VA: Association of Official Analytical Chemists.

Beninger, P. G., & Lucas, A. (1984). Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L.

(Jeffreys) and *Tapes philippinarum* (Adams & Reeve). *Journal of Experimental Marine Biology and Ecology, 79*, 19–37.

Beninger, P. G., & Stephan, G. (1985). Seasonal variations in the fatty acids of the triacylglycerols and phospholipids of two populations of adult clam (*Tapes decussatus* L. and *T. philippinarum*) reared in a common habitat. *Comparative Biochemistry and Physiology, 81B*, 591–601.

Berthelin, C., Kellner, K., & Mathieu, M. (2000). Storage metabolism in the Pacific oyster (*Crassostrea gigas*) in relation to summer mortalities and reproductive cycle (West Coast of France). *Comparative Biochemistry and Physiology, 125B*, 359–369.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Council of the European Union (1994). Council Regulation (EC) No. 1626/94 of 27 June 1994 laying down certain technical measures for the conservation of fishery resources in the Mediterranean. *Official Journal of the European Communities L171*, 6/7/1994.

Dalrymple, R. H., & Hamm, R. (1973). A method for the extraction of glycogen and metabolites from a single muscle sample. *Journal of Food Technology, 8*, 439–444.

Fisher, W., Schneider, M., & Bauchot, M. L. (1987). *Fiches FAO d'identification des especes pour les besoins de la peche: Mediterranee et Mer Noire. Végétaux et invertebrés* (Vol. I). Rome: FAO.

Fowler, S. W., & Oregioni, B. (1976). Trace metal in mussels from the NW Mediterranean. *Marine Pollution Bulletin, 7*, 26–29.

Goad, L. J. (1981). Sterol biosynthesis and metabolism in marine invertebrates. *Pure and Applied Chemistry, 53*, 837–852.

Jarzebski, A. (1991). 4-Desmethylsterols from the marine bivalve *Macoma balthica*. *Lipids, 26*, 561–563.

Jarzebski, A., & Wenne, R. (1989). Seasonal changes in content and composition of sterols in the tissues of the bivalve *Macoma balthica*. *Comparative Biochemistry and Physiology, 93B*, 711–713.

Karakoltsidis, P. A., Zotos, A., & Constantinides, S. M. (1995). Composition of the commercially important Mediterranean finfish, crustaceans and molluscs. *Journal of Food Composition and Analysis, 8*, 258–273.

Keppler, D., & Decker, K. (1974). Glycogen. In H. U. Bergmeyer (Ed.). *Methods of Enzymatic Analysis* (Vol. 3, pp. 1127–1131). Weinheim: Verlag Chemie.

Kinsella, J. E., Shimp, J. L., Mai, J., & Weihrauch, J. (1977). Fatty acid content and composition of freshwater finfish. *Journal of the American Oil Chemists' Society, 54*, 424–429.

Krzynowek, J., Wiggin, K., & Donahue, P. (1983). Sterol and fatty acid content on three groups of surf clams (*Spisula solidissima*): wild clams (60 and 120 mm size) and cultured clams (60 mm size). *Comparative Biochemistry and Physiology, 74B*, 289–293.

Ling, W. H., & Jones, P. J. H. (1995). Dietary phytosterols: a review of metabolism, benefits and side effects. *Life Sciences, 57*, 195–206.

Lucas, A., & Beninger, P. G. (1985). The use of physiological condition indices in marine bivalve aquaculture. *Aquaculture, 44*, 187–200.

Murphy, K. J., Mooney, B. D., Mann, N. J., Nichols, P. D., & Sinclair, A. J. (2002). Lipid, fatty acid and sterol composition of New Zeeland green lipped mussel (*Perna canaliculus*) and Tasmanian blue mussel (*Mytilus edulis*). *Lipids, 37*, 587–595.

Okumus, I., & Stirling, H. P. (1998). Seasonal variations in the meat weight, Condition Index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. *Aquaculture, 159*, 249–261.

Orban, E., Di Lena, G., Masci, M., Nevigato, T., Casini, I., Caproni, R., et al. (2004). Growth, nutritional quality and safety of oysters (*Crassostrea gigas*) cultured in the lagoon of Venice (Italy). *Journal of the Science of Food and Agriculture, 84*, 1929–1938.

Orban, E., Di Lena, G., Nevigato, T., Casini, I., Marzetti, A., & Caproni, R. (2002). Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites. *Food Chemistry, 77*, 57–65.

Orban, E., Di Lena, G., Ricelli, A., Paoletti, F., Casini, I., Gambelli, L., et al. (2000). Quality characteristics of sharpsnout sea bream (*Diplodus puntazzo*) from different intensive rearing systems. *Food Chemistry, 70*, 27–32.

Pàez-Osuna, F., Frias-Espericueta, M. G., & Osuna-Lopez, J. I. (1995). Trace metal and concentrations in relation to season and gonadal maturation in the oyster *Crassostrea iridiscens*. *Marine Environmental Research, 40*, 19–31.

Pelletier, X., Belbraouet, S., Mirabel, D., Mordret, F., Perrin, J. L., Pages, X., et al. (1995). A diet moderately enriched in phytosterols lowers plasma cholesterol concentrations in normo-cholesterolemic humans. *Annals of Nutrition and Metabolism, 39*, 291–295.

Piironen, V., Lindsay, D. G., Miettinen, T. A., Toivo, J., & Lampi, A. M. (2000). Plant sterols: biosynthesis, biological function and their importance to human nutrition. *Journal of the Science of Food and Agriculture, 80*, 939–966.

Poggiani, L., Piccinetti, C., Piccinetti-Manfrin, G. (1973). Osservazioni sulla biologia dei molluschi bivalvi *Venus gallina* L. e *Tapes aureus* nell'Alto Adriatico. In: *Note del Laboratorio di Biologia Marina e Pesca 4, Fano, Italy* (pp. 189–212).

Polak, L., Jarzebsky, A., Wenne, R., & Falkowski, L. (1987). Seasonal changes in condition and lipids composition of the bivalve *Macoma balthica* L. from the gulf of Gdansk (Southern baltic). *Comparative Biochemistry and Physiology, 88B*, 881–885.

Pollero, R. J., Ré, M. E., & Brenner, R. R. (1979). Seasonal changes of the lipids of the mollusc *Chlamys tehuelcha*. *Comparative Biochemistry and Physiology, 64A*, 257–263.

Rao, V. A., & Janezic, S. A. (1992). The role of dietary phytosterols in colon carcinogenesis. *Nutrition and Cancer, 18*, 43–52.

Rey, M. A., & Pohl, C. A. (1993). Novel weak acid cation exchange column. *Journal of Chromatography, 640*, 65–71.

Salvatorelli, G. (1967). Osservazioni sul ciclo riproduttivo annuo di *Venus gallina* (molluschi lamellibranchi). *Annali Università di Ferrara, 27*, 15–22.

Simopoulos, A. P. (2003). Importance of the ratio omega-6/omega-3 essential fatty acids: evolutionary aspects. In *Omega-6/omega-3 essential fatty acid ratio: the scientific evidence*. In A. P. Simopoulos & L. G. Cleland (Eds.). *World review of nutrition and dietetics* (Vol. 92, pp. 1–22). Basel: Karger.

Simpson, K. L., Katayama, T., & Chichester, C. O. (1981). Carotenoids in fish feed. In *Carotenoids as colorants and vitamin A precursors*. In J. C. Bauernfeind (Ed.). *Technological and nutritional application* (pp. 463–538). New York: Academic Press.

Usero, J., Morillo, J., & Gracia, I. (2005). Heavy metal concentrations in molluscs from the Atlantic coast of Southern Spain. *Chemosphere, 59*, 1175–1181.

Whyte, J. N. C., & Englar, J. R. (1982). Seasonal variation in the chemical composition and condition indices of Pacific oyster *Crassostrea gigas* grown in trays or on the sea bed. *Canadian Journal of Fisheries and Aquatic Sciences, 39*, 1084–1094.