

## Lipid and Fatty Acid Compositions of *Mytilus galloprovincialis* Cultured in the Mar Grande of Taranto (Southern Italy): Feeding Strategies and Trophic Relationships

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**Ermelinda Prato, Antonio Danieli, Michele Maffia, and Francesca Biandolino (2010)** Lipid and fatty acid compositions of *Mytilus galloprovincialis* cultured in the Mar Grande of Taranto (southern Italy): feeding strategies and trophic relationships. *Zoological Studies* 49(2): 211-219. Lipid and fatty acid (FA) compositions were determined in the mussel *Mytilus galloprovincialis* collected from June 2006 to May 2007 in the Mar Grande of Taranto, southern Italy. Total lipids significantly differed throughout the study period (ANOVA,  $p < 0.05$ ), with higher values in summer (24.7% dry weight (DW)) and the lowest values in winter (3.5% DW). Triacylglycerols (TAGs) were the dominant lipid class in spring and summer accounting for 55.28% and 60.3% of total lipids, respectively, while in the autumn and winter phospholipids (PLs) were considerably greater than TAGs, comprising 55.16% and 47.5% of total lipids, respectively. Cholesterol did not show large variations over the seasons. Predominant FAs were saturated FAs (SAFAs) followed by monounsaturated FAs (MUFAs). The amount of polyunsaturated FAs (PUFAs) was low. The 14:0, 16:0, 18:0, and 22:0 SAFAs, together with 14:1, 16:1 $\omega$ 7, 18:1 $\omega$ 9, 18:1 $\omega$ 7, 20:1 $\omega$ 9, and 24:1 $\omega$ 9 MUFAs, and the PUFA, non-methylene interrupted dienoic (NMID), were the most abundant FAs. FA biomarkers are frequently used to identify trophic relationships among marine invertebrates. In order to obtain indications on food sources of *M. galloprovincialis*, a variety of FA ratios and the sum of some FAs were determined. The sum of 18:1 $\omega$ 7 + odd-branched FAs indicated a moderate bacterial contribution to the mussel diet. A high 18:1 $\omega$ 9/18:1 $\omega$ 7 ratio together with a high level of 20:1 $\omega$ 9 indicated an animal dietary input. Trophic markers suggested low contribution of diatoms and dinoflagellates to the diet of *M. galloprovincialis*. <http://zoolstud.sinica.edu.tw/Journals/49.2/211.pdf>

**Key words:** Lipid composition, Mussels, Seasonal variations, Trophic markers.

*Mytilus galloprovincialis* is an important commercial species in several zones of Mediterranean coasts. In Italy, it represents the main product of the national bivalve culture and is cultured on suspended ropes situated in lagoons and the open sea along the shore of the Mediterranean Sea.

Despite the large amount of research devoted to characterizing the biology and ecology of *M. galloprovincialis* in the Mar Grande in Taranto (Pastore et al. 1976, Tursi et al. 1991, Matarrese et

al. 1993), information about its biochemistry is very scarce. In marine bivalves, some polyunsaturated FAs (PUFAs) and sterols are important biochemical constituents, taking part in numerous biochemical processes and representing an energetic supply under critical nutritional conditions.

It is well known that seasonal variations in lipid contents and fatty acid (FA) compositions of adult bivalves are closely linked to the reproductive cycle and climate changes and are affected by the availability and composition of the natural diet

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(Fernandez-Reiriz et al. 1996, Caers et al. 2000). Previous studies reported that changes occurring in lipid content are mainly determined by fluctuations in triacylglycerols (TAGs), not in phospholipids (PLs). In fact PLs mainly have a structural function as components of all biological membranes, and their content is nearly constant throughout the year (Pazos et al. 1997); instead TAGs, with mainly an energetic function, show variable contents, and therefore they are considered to be indicators of the nutritional state (Swift et al. 1980).

Generally, marine organisms reflect, to varying degrees, exogenous sources in their FA components. There are PUFAs that are typical of certain primary producers (algae and micro-organisms), and there are certain PUFAs known to be essential FAs for marine invertebrates. Indeed, bivalves have a very limited or no capability to synthesize PUFAs (Waldock and Holland 1984, Chu and Graeves 1991) but still require them for their development (Langdon and Waldock 1981, Chu and Webb 1984, Knauer and Southgate 1997). These PUFAs are used as biomarkers to assess relationships between primary producers and invertebrate consumers (Pollero et al. 1981, Kharlamenko et al. 1995, Müller-Navarra et al. 2000).

An organism's FA composition is determined by its position in the food chain and is affected by trophic relationships among organisms in the marine environment; thus they can serve as a suitable trophic marker. The trophic biomarker concept is based on observations that marine organisms have specific FA patterns, and some of these compounds are incorporated into the consumer's unmodified lipids, providing trophic information (Sargent and Whittle 1981, Drazen et al. 2008).

This approach was used in several studies on specific FA markers available for different groups of primary producers and consumers (Perry et al. 1979, Volkman et al. 1989), and FAs were successfully used as trophic markers to identify food sources, habitat preferences, feeding strategies, and trophic links (Kharlamenko et al. 1995 2001). PUFAs indicate organic matter derived from fresh phytoplankton (Volkman et al. 1989), while detritus is rich in saturated FAs (SAFAs) (Fahl and Kattner 1993).

The 16:1 $\omega$ 7/16:0 ratio is used to discriminate between diatom versus dinoflagellate feeding. This is due to the fact that 16:1 $\omega$ 7 is abundant in diatoms, while 16:0 is common in dinoflagellates (Graeve et al. 1994a b, St. John and Lund 1996,

Auel et al. 2002, Maazouzi et al. 2007).

Another ratio with the potential to differentiate between these 2 food sources is 20:5 $\omega$ 3/22:6 $\omega$ 3 (Nelson et al. 2001, Phleger et al. 2002). The sum of 16:1 $\omega$ 7+18:1 $\omega$ 7 is considered to be an indicator of a diatom-based diet (Graeve et al. 2001, Nelson 2001, Auel et al. 2002). Literature data indicate that 18:4 $\omega$ 3 is a dinoflagellate marker (Mansour et al. 1999).

The 18:1 $\omega$ 7/18:1 $\omega$ 9 ratio was proposed as a biomarker for an herbivorous vs. a carnivorous diet (Graeve et al. 1997, Falk-Petersen et al. 2000, Auel et al. 2002). The sum of branched and odd FAs (15:0-17:0 + 18:1 $\omega$ 7) is used to estimate bacterial dietary input, and 20:1 $\omega$ 9 is indicative of a carnivorous diet (Virtue et al. 2000).

The lipid and FA compositions of *M. galloprovincialis* from the Mar Grande of Taranto have not been investigated to date. The aim of this study was to examine seasonal variations in lipid classes (TAGs, PLs, and cholesterol) and the FA composition. In addition, this work is an attempt to identify major food sources of *M. galloprovincialis* from the Mar Grande in Taranto, using FA markers.

## MATERIALS AND METHODS

### Lipid extraction

Adult stocks of *M. galloprovincialis* were collected monthly from June-Aug. 2006 (summer), Sept.-Nov. 2006 (autumn), Dec. 2006-Feb. 2007 (winter) and Mar.-May 2007 (spring), from a commercial mussel culture farm in Mar Grande at Taranto, Italy (the Ionian Sea). The shell length of mussels used for the lipid analysis ranged 3.7-5.7 cm. Prior to lipid extraction, the shells were opened to collect the tissues. Tissues were thoroughly cleaned with filtered seawater and finely chopped, and around 0.5 g of tissue was homogenized in 1.1% NaCl. Tissues from 5 different individuals (with 3 replicates) were prepared for lipid extraction. Lipids were extracted using chloroform: methanol (2: 1, v/v) following the method of Folch et al. (1957). The total lipid content was determined gravimetrically.

TAGs and total cholesterol were measured by the colorimetric enzymatic Trinder (1969) method (with a commercial kit from SGM, Rome, Italy). PLs were quantified by a colorimetric enzymatic method (Takayama et al. 1977) with a commercial kit (SGM).

## FA analysis

After evaporation to dryness, the lipid extract fraction was transesterified to methyl esters in boron trifluoride catalyzed by a methanol: benzene solution (1: 2 v/v) at 90°C for 20 min (Allinger 1986). When the methylation was completed, 2 ml of distilled water was added to the mixture. FA methyl esters were analyzed by gas-liquid chromatography using an HP 6890 series gas chromatograph (Hewlett Packard, USA) equipped with an Omegawax 250 capillary column (Supelco, USA). The column temperature program was as follows: from 150 to 250°C at 4°C/min, then maintained at 250°C. Helium was used as the carrier gas at a flow of 1 ml/min.

Methyl esters of FAs were identified by the FAME mix (Supelco) as the standard, and the results are reported as seasonal average percentages of total identified methyl ester FAs.

Lipid analyses were carried out in triplicate, and results are expressed as seasonal average percentages of the dry weight (DW) of the animals. TAG, PL, and cholesterol levels are also expressed as average seasonal percentages of total lipids.

## Data analysis

Data are presented as the seasonal average  $\pm$  the standard deviation (SD). Statistical analyses were performed using the SPSS statistical package (SPSS, version 16.0, IBM Company, Chicago, USA). Differences in lipid compositions among seasons were analyzed by analysis of variance (ANOVA) at the 5% confidence level.

Multiple mean comparisons were made using Fisher's least significant difference (LSD) test to estimate differences in FA markers among specific sampling seasons (statgraphics plus vers. 2.1, Statistical Graphics Corp., Princeton, NJ, USA).

## RESULTS

### Lipid class analysis

The seasonal average trend of total lipids in *M. galloprovincialis* is shown in figure 1. In summer, total lipids were higher at 24.7% DW, while the lowest value was encountered in winter (3.5% DW).

Results of the statistical analysis indicated that total lipids of *M. galloprovincialis*, determined in each season, significantly differed from each

other (ANOVA,  $p < 0.05$ ).

The amount of TAGs significantly changed over the seasons (ANOVA,  $p < 0.05$ ). TAGs were the major lipid class in spring and summer accounting for 55.28% and 60.3% of total lipids, respectively, while in autumn and winter, they reached the lowest levels (32.58% and 38.63% of total lipids, respectively). In contrast, PLs showed maximum values in autumn and winter (56.16% and 47.5% of total lipids, respectively) and lower values in spring and summer (31.1%-29.7% of total lipids, respectively). For this lipid class, no significant difference was found between spring and summer (ANOVA,  $p > 0.05$ ). The cholesterol content did not exhibit large variations over the seasons (ANOVA,  $p > 0.05$ ) (Fig. 2).

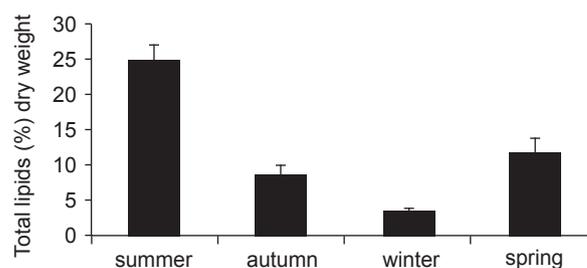
### FA composition

In total, 31 FAs were identified, and their seasonal variations are shown in table 1. The FA profile of *M. galloprovincialis* was characterized by a predominance of SAFAs in all seasons with percentages of 49.29%-57.22% of total FAs, followed by MUFAs which ranged 33.19%-39.63% of total FAs and PUFAs at 7.55%-11.16%.

The major SAFA was palmitic acid (16:0), accounting for up to 33.46% of total FAs in summer, followed by stearic acid (18:0), myristic acid (14:0), and behenic acid (22:0).

The great majority of MUFAs consisted of myristoleic acid (14:1: 3.01%-9.3% of total FAs), palmitoleic acid (16:1 $\omega$ 7: 4%-7.14% of total FAs), oleic acid (18:1 $\omega$ 9: 2.31%-8.4% of total FAs), nervonic acid (24:1 $\omega$ 9: 4.4%-8.5% of total FAs), and eicosenoic acid (20:1 $\omega$ 9: 4.05%-5.80% of total FAs). Other MUFAs, such as 18:1 $\omega$ 7 and 20:1 $\omega$ 7, appeared in lower proportions.

The predominant PUFAs were 22:2 (2.01%-2.87% of total FAs) and also 22:6 $\omega$ 3 (1.2%-2.17 of total FAs), followed by 18:2 $\omega$ 6 (0.83%-2.27%). PUFAs with 18 carbon



**Fig. 1.** Total lipids (% dry weight)  $\pm$  standard deviation (SD) of *Mytilus galloprovincialis* during the study period.

atoms and 3 or 4 double bounds and those with 20 carbon atoms were present in lower concentrations. No significant differences were found among seasons for myristic acid (14:0) (ANOVA,  $p > 0.05$ ); the percentage of 16:0 significantly differed only between autumn and summer (ANOVA,  $p < 0.05$ ), and for 18:0, a significant difference was found between winter and spring (ANOVA,  $p < 0.05$ ). Most MUFAs showed significant differences between winter and summer seasons (ANOVA,  $p < 0.05$ ), except for 16:1 $\omega$ 7 and 20:1 $\omega$ 9 (ANOVA,  $p > 0.05$ ).

During the entire study period, 18:2 $\omega$ 6, 18:4 $\omega$ 3, 20:4 $\omega$ 6, and 22:2 PUFAs showed no

significant differences among seasons. Linolenic acid (18:3 $\omega$ 3) did not significantly change between spring and summer or between autumn and winter (ANOVA,  $p > 0.05$ ). There were significant differences in eicosapentaenoic (EPA, 20:5 $\omega$ 3) among seasons (ANOVA,  $p < 0.05$ ), except between autumn and summer (ANOVA,  $p > 0.05$ ).

### Trophic markers

Table 2 shows the summary statistics from the multiple mean comparisons, comparing average contents of selected FAs which are used as trophic markers. SAFAs in mussels collected

**Table 1.** Fatty acid composition (% of total fatty acids (FAs)) of *Mytilus galloprovincialis*

Fatty acid	Summer	± SD	Autumn	± SD	Winter	± SD	Spring	± SD
C11:0	1.37	0.36	3.85	0.7	3.44	0.84	3.23	1.04
C12:0	0.09	0.02	0.5	0.02	0.2	0.08	0.1	0.04
C14:0	7.32	0.56	7.58	0.9	5.68	1.15	6.58	0.63
C15:0	0.09	0.03	1.03	0.2	1.02	0.56	1.01	0.27
C16:0	33.46	2.69	26.9	2.4	31.95	2.06	28.88	2.17
C17:0	1.38	0.42	1.7	0.8	1.61	0.32	1.45	0.23
C18:0	6.83	1.21	7.92	0.9	8.72	1.05	5.58	1.08
C20:0	0.4	0.06	0.7	0.09	0.3	0.09	-	
C22:0	2.77	0.63	7.04	1.1	4.2	0.96	2.46	1.1
<b>Σ SAFAs</b>	<b>53.71</b>		<b>57.22</b>		<b>57.12</b>		<b>49.29</b>	
C14:1	3.01	0.54	9.3	1	7.6	1.12	8.87	0.55
C15:1	0.32	0.05	0.31	0.02	0.3	0.07	0.3	0.06
C16:1 $\omega$ 7	4.26	0.52	4.5	0.82	4.00	1.11	7.14	1.04
C16:1 $\omega$ 5	-		0.9	0.08	-		-	
C17:1	0.72	0.03	1.1	0.1	0.75	0.12	0.70	0.09
C18:1 $\omega$ 9	7.53	0.98	2.31	0.98	4.44	0.85	8.40	1.10
C18:1 $\omega$ 7	4.42	0.62	2.5	0.75	2.52	0.66	3.02	0.78
C20:1 $\omega$ 9	5.25	0.74	4.05	0.62	5.00	1.04	5.80	0.87
C20:1 $\omega$ 7	-		2.07	0.15	2.47	0.84	3.70	1.02
C22:1 $\omega$ 9	1.77	0.45	1.5	0.09	1.71	0.36	1.70	0.58
C24:1 $\omega$ 9	8.5	0.94	6.71	0.85	4.4	1.03	-	
<b>Σ MUFAs</b>	<b>35.78</b>		<b>35.25</b>		<b>33.19</b>		<b>39.63</b>	
C18:2 $\omega$ 6	2.27	0.76	0.83	0.34	1.07	0.21	1.10	0.16
C18:3 $\omega$ 3	2.19	0.43	0.51	0.18	0.83	0.11	1.65	0.21
C18:3 $\omega$ 6	0.38	0.06	0.82	0.28	0.33	0.03	0.70	0.08
C18:4 $\omega$ 3	-		-		1.42	0.16	1.59	0.58
C20:2	0.81	0.02	-		0.72	0.15	0.56	0.05
C20:3 $\omega$ 3	0.07	0.01	-		-		-	
C20:3 $\omega$ 6	0.31	0.08	-		-		-	
C20:4 $\omega$ 6	0.63	0.06	1.1	0.31	-		0.90	0.03
C20:5 $\omega$ 3	0.15	0.03	0.22	0.05	1.09	0.08	0.36	0.04
C22:2	2.01	0.12	2.87	0.78	2.05	0.51	2.70	0.98
C22:6 $\omega$ 3	1.70	0.18	1.2	0.55	2.17	0.10	1.60	0.09
<b>Σ PUFAs</b>	<b>10.52</b>		<b>7.55</b>		<b>9.68</b>		<b>11.16</b>	

SAFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

in autumn and winter significantly differed from those found in spring (Fisher's test,  $p < 0.05$ ). In spring and summer, PUFAs significantly differed from those in autumn (Fisher's test,  $p < 0.05$ ). MUFAs and the PUFAs/MUFAs and PUFAs/SAFAs ratios did not significantly differ among seasons (Fisher's test,  $p > 0.05$ ). In spring, the 16:1 $\omega$ 7/16:0 ratio significantly differed from those in summer and winter. The sum of 16:1 $\omega$ 7+18:1 $\omega$ 7 in spring significantly differed from all other seasons.

The 20:5 $\omega$ 3/22:6 $\omega$ 3 ratio showed significant differences between summer-winter and autumn-spring samples (Fisher's test,  $p < 0.05$ ). Furthermore, the 18:1 $\omega$ 7/18:1 $\omega$ 9 ratio, the sum of 15:0+17:0+18:1 $\omega$ 7, and the trophic markers, 18:4 $\omega$ 3 and 20:1 $\omega$ 9, did not significantly differ during the study period (ANOVA,  $p > 0.05$ ).

## DISCUSSION

The population of *Mytilus galloprovincialis* in the Mar Grande at Taranto showed significant variations in total lipids throughout the year. Lower lipid contents were observed in winter, with higher ones in summer.

Several patterns of temporal variability of lipids in bivalve molluscs, reported in previous studies (Besnard et al. 1989, Pazos et al. 1996 2003), are the result of several environmental factors acting simultaneously, such as temperature, food availability, plankton composition, and physiological factors. In many temperate bivalves, the lipid content steadily increases during summer months until spawning occurs (Gabbott 1983). In this study, the lipid contents of *M. galloprovincialis* were relatively high compared to values reported for most other temperate bivalves: values for the lipid contents of *M. edulis* by Zandee et al. (1980)

were 10%-11%; Wenne and Polak (1989) reported 18% for *Macoma baltica*; and a value of 12% was reported for *Mercenaria mercenaria* (Klingensmith and Stillway 1982).

Qualitatively, the lipid class composition of *M. galloprovincialis* being dominated by TAGs and PLs was characteristic of many other marine bivalves. TAGs are known as major reserve lipids in many marine organisms (Sargent 1976). In adult bivalves, TAG deposits in gonads indicate a role as a major energy reserve for reproduction, while TAG deposits in other tissues serve to maintain energy during the food-limited winter period.

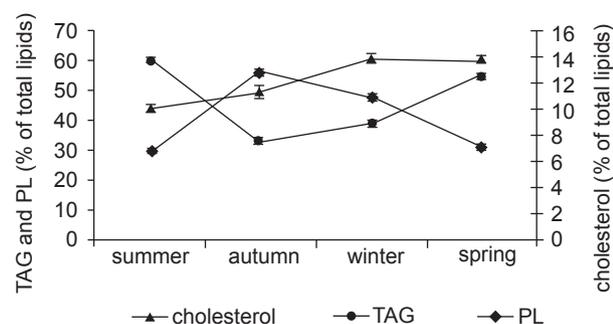
Mussels, like other marine bivalves in temperate latitudes, exhibit cyclic changes in reproductive stages as a consequence of the seasonality of environmental conditions. For *M. galloprovincialis* in the Mar Grande, Matarrese et al. (1993) reported 1 phase of reproductive activity (autumn-winter), one in which a decrease in gametogenic activity begins (spring), and one of quiescence (summer), when the water temperature rises to  $> 20^{\circ}\text{C}$ . During periods of minimal gametogenic activity, TAGs were the main class of lipids according to our results; this occurred during the spring and summer. Although TAGs are described as the main lipid form in different bivalves (Lubet et al. 1985, Pazos et al. 2003), the results of this study show that in autumn and winter, PLs were considerably greater than TAGs, as occurs in *Pecten magellanicus* (Napolitano et al. 1992) and *Crassostrea gigas* (Allen and Conley 1982).

The increase in PLs during autumn and winter may be related to their role as structural membrane lipids and as part of the lipovitellines that accumulate in oocytes, as a reserve for the cellular division process, following fertilization (Napolitano et al. 1992, Li et al. 1998).

Sterols showed a similar seasonal cycle to that of PLs, but at lower levels. This may have been due to the fact that the major function of sterols is to maintain the structural integrity of cell membranes (Nes 1974).

Even though the influence of the diet on the lipid composition of the next trophic level becomes more blurred with each step up the food chain, data from the literature indicate that dietary quality affects the FA composition of different bivalves (Pazos et al. 1996, Knauer and Southgate 1997, Linehan et al. 1999).

The potential of FAs to improve interpretation of trophic interactions has proven to be a useful tool



**Fig. 2.** Seasonal changes in lipid classes (expressed as a percentage of the dry weight of tissue) in *Mytilus galloprovincialis*.

for this kind of investigation (Graeve et al. 1994a b, St. John and Lund 1996). In order to obtain indications of feeding behavior and food sources of *Mytilus galloprovincialis*, a variety of FA ratios and the sum of some FAs were examined as trophic markers. The FA profile of *M. galloprovincialis* showed a considerable contribution of SAFAs in tissues during all seasons, while PUFAs were less abundant. This contrasts with previously published data in other species of bivalves, such as *Chlamys islandica* (Bell et al. 1985) and *Pecten maximus* (Besnard 1988), in which PUFAs were the major FAs measured, indicating that phytoplankton is the main feeding source in those species. In this study, the major SAFA proportion, throughout the year, was primarily due to the high percentage of short-chain, palmitic 16:0 and stearic 14:0 FAs, indicating omnivorous feeding habits (Graeve et al. 1994a b). This may have been due to the seston food quality collected in the oligotrophic Mar Grande having high concentrations of SAFAs and low concentrations of PUFAs as reported by Fahl and Kattner (1993) for oligotrophic Antarctic waters. Rossi et al. (2008) reported that SAFA contents of seston accounted for 73%-93% of total lipids, with 12:0, 14:0, 16:0, 18:0, and 22:0 SAFAs isolated in the highest concentrations for seston from Georges Basin and Oceanographer Canyon (in the NW Atlantic). These data obtained for seston in the NW Atlantic are similar to those obtained in this study for *M. galloprovincialis* and may indicate that 14:0 and 16:0 SAFAs were

conservatively transferred between seston and *M. galloprovincialis*.

In addition, FAs become saturated as particulate organic matter is oxidized in the water column, especially during conditions of low nutrient availability, high levels of detritus, and limited phytoplankton growth (Goutx and Saliot 1980, Mayzaud et al. 1989, Fahl and Kattner 1993, Baldi et al. 1997, Parrish et al. 2005).

In addition, Brett et al. (2006) reported a pattern of FAs in cyanophytes similar to that observed in this study for *M. galloprovincialis*, which suggests that *M. galloprovincialis* also feeds on cyanophytes.

The MUFA proportion was also high (33.19%-39.63% of total FAs) compared to PUFAs (7.55%-11.16% of total FAs) and did not significantly vary during the period investigated in contrast to SAFAs and PUFAs.

The bacterial contribution to the diet of bivalves can be evaluated by the presence of bacterial acids. Parkes and Taylor (1983) reported that odd-numbered branched FAs, 15:0 and 17:0, and some MUFAs, such as 18:1 $\omega$ 7, are predominantly synthesized by bacteria, and these FAs constitute a significant proportion in marine invertebrates, indicating potential bacterial sources in the bivalve diet (Perry et al. 1979, Gillan and Johns 1986, Kharlamenko et al. 1995). In this study, the sum of 18:1 $\omega$ 7 and odd-branched FAs indicated a moderate and constant bacterial contribution to the mussel diet. Although in a

**Table 2.** Multiple mean comparisons of the main fatty acids trophic markers among different seasons

	Summer	Autumn	Winter	Spring
$\Sigma$ SAFAs	53.71 <sup>ab</sup>	57.22 <sup>a</sup>	57.12 <sup>a</sup>	49.29 <sup>b</sup>
$\Sigma$ MUFAs	35.78 <sup>a</sup>	35.25 <sup>a</sup>	33.19 <sup>a</sup>	39.63 <sup>a</sup>
$\Sigma$ PUFAs	10.52 <sup>b</sup>	7.55 <sup>a</sup>	9.68 <sup>ab</sup>	11.16 <sup>b</sup>
PUFAs/SAFAs	0.19 <sup>a</sup>	0.13 <sup>a</sup>	0.17 <sup>a</sup>	0.23 <sup>a</sup>
PUFAs/MUFAs	0.29 <sup>a</sup>	0.21 <sup>a</sup>	0.29 <sup>a</sup>	0.28 <sup>a</sup>
16:1 $\omega$ 7/16:0	0.13 <sup>a</sup>	0.17 <sup>ab</sup>	0.12 <sup>a</sup>	0.25 <sup>b</sup>
16:1 $\omega$ 7+18:1 $\omega$ 7	8.68 <sup>a</sup>	7 <sup>a</sup>	6.52 <sup>a</sup>	10.16 <sup>b</sup>
20:5 $\omega$ 3/22:6 $\omega$ 3	0.09 <sup>a</sup>	0.18 <sup>b</sup>	0.05 <sup>a</sup>	0.22 <sup>b</sup>
18:1 $\omega$ 7/18:1 $\omega$ 9	0.59 <sup>a</sup>	1.08 <sup>a</sup>	0.57 <sup>a</sup>	0.34 <sup>a</sup>
15:0+17:0+18:1 $\omega$ 7	5.89 <sup>a</sup>	5.23 <sup>a</sup>	5.15 <sup>a</sup>	5.48 <sup>a</sup>
18:4 $\omega$ 3	-	-	1.42 <sup>a</sup>	1.52 <sup>a</sup>
20:1 $\omega$ 9	5.25 <sup>a</sup>	4.05 <sup>a</sup>	5 <sup>a</sup>	5.80 <sup>a</sup>

SAFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids. Significant differences among the means are indicated by different superscript letters on the same line (at the 5% confidence level).

previous study carried out in the Mar Grande at Taranto (Stabili and Cavallo 2001), it was reported that bacteria reached the highest density during summer months, *Mytilus galloprovincialis* did not show significant differences in bacterial acid inputs among seasons.

According to Falk-Petersen et al. (2000), 18:1 $\omega$ 9 is a major FA of most marine animal lipids. The 18:1 $\omega$ 7 FA is also frequently present in great quantities, being derived from chain elongation of the phytoplankton 16:1 $\omega$ 7 FA (e.g., Graeve et al. 1997, Falk-Petersen et al. 2000, Kharlamenko et al. 2001). Therefore, 16:1 $\omega$ 7 and 18:1 $\omega$ 7 in animal lipids tend to reflect phytoplanktonic dietary inputs, while 18:1 $\omega$ 9 reflects carnivorous dietary inputs. In this study, *M. galloprovincialis* showed a high 18:1 $\omega$ 9/18:1 $\omega$ 7 ratio indicating an animal dietary input. On the other hand, the relatively high concentration of 20:1 $\omega$ 9 (4.05%-5.8%) suggested once more that *M. galloprovincialis* feeds on zooplankton. The use of zooplankton as food was also reported in the oyster *Ostrea edulis* (Knox 1986). Falk-Petersen et al. (2002) showed that copepods usually contain large amounts of 20:1 $\omega$ 9 and 20:1 $\omega$ 11, which together constituted 60% of total fatty acids. These trophic markers did not significantly vary during the entire study period, even though zooplankton abundance in the Mar Grande was highest during summer months (Belmonte et al. 2001).

Regarding the contribution of phytoplankton to the diet of *M. galloprovincialis*, the 16:1 $\omega$ 7/16:0 and 20:5 $\omega$ 3/22:6 $\omega$ 3 ratios, which allow differentiation between diatom- and dinoflagellate-based diets, were very low. Trophic markers for diatoms (16:1 $\omega$ 7 and 20:5 $\omega$ 3) (Graeve et al. 1994a b) and those for dinoflagellates (18:4 $\omega$ 3 and especially 22:6 $\omega$ 3), suggest that diatoms and dinoflagellates contribute less to the diets of *M. galloprovincialis* than do other phytoplankton groups. Caroppo et al. (2006), in a study of phytoplankton and cyanobacteria in the Taranto Seas, reported for Lido Azzurro (Mar Grande) that the phytoplankton communities consisted mainly of nanosized phytoflagellates and diatoms. Their contributions to the composition of the community in terms of abundances differed: diatoms had a higher average abundance percentage (30.4%), while coccolithophorids never dominated the algal community, and among picophytoplankton species, *Synechococcus* was the most abundant.

It is known that bivalves have no or only a very limited capability to synthesize PUFAs (Waldock and Holland 1984, Chu and Graeves

1991), but seem to have the capability of de novo synthesis of some peculiar FAs called non-methylene interrupted (NMI) FAs (20:2 and 22:2; Zhukova 1991), even if they are often not considered in nutrition studies.

In this study, among PUFAs, NMID FAs constituted the dominant part. It was suggested that NMI FAs have functional and structural roles in membranes (Kraffe et al. 2004) and also act as a substitute for essential FAs such as 20:4 $\omega$ 3, 20:5 $\omega$ 3, and 22:6 $\omega$ 3 (Klingensmith 1982, Pond et al. 1998, Zhukova et al. 1991). Indeed, these FAs may be related to biosynthetic pathways based on chain elongation and desaturation of  $\omega$ 7 MUFAs. Whyte (1988) reported for *Crassostrea gigas* that the increase in 22:2 coincided with low levels of 20:5 $\omega$ 3, and Klingensmith (1982) reported an inverse relationship between  $\omega$ 3 PUFAs and NMI FA levels.

Our results provide useful information on lipid contents and fatty acid compositions in an attempt to delineate the diet composition of *M. galloprovincialis* from the Mar Grande at Taranto (southern Italy). These preliminary data shed some light on the food sources utilized by *M. galloprovincialis* and on their potential nutritional value for animal and human consumers. In this context, FAs were employed as biomarkers to examine the ability of *M. galloprovincialis* to use food sources available in its environment and develop different feeding strategies, which can be key factors in ecosystem functioning. Furthermore, the results are a valid addition to the literature on lipid physiology in marine invertebrates and might be of interest to local farmers, in order to optimize aquaculture conditions.

## REFERENCES

- Allen WV, H Conley. 1982. Transport of lipid in the blood of the Pacific oyster, *Crassostrea gigas* (Thunberg). *Comp. Biochem. Phys. B* **71**: 201-207.
- Allinger NL, MP Cava, DC De Jough, CR Johnson, NA Lebel, CL Stevens. 1986. *Chimica organica*. Bologna, Italy: Zanichelli.
- Auel H, M Harjes, R da Rocha, D Stübing, W Hagen. 2002. Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellula*. *Polar Biol.* **25**: 374-383.
- Baldi F, A Minacci, A Saliot, L Mejanelle, P Mozetic, V Turk et al. 1997. Cell lysis and release of particulate polysaccharides in extensive marine mucilage assessed by lipid biomarkers and molecular probes. *Mar. Ecol.-Prog. Ser.* **153**: 45-57.

- Bell MV, JR Sargent. 1985. Fatty acid analysis of phosphoglycerides from tissues of the clam *Chlamys islandica* (Muller) and the starfish *Ctenodiscus crispatus* (Retzius) from Balsfjorden, northern Norway. *J. Exp. Mar. Biol. Assoc. UK* **58**: 825-841.
- Belmonte G, G Fanelli, C Gravili, F Rubino. 2001. Composition, distribution and seasonality of zooplankton in Taranto seas (Ionian Sea, Italy). *Biol. Mar. Mediterr.* **8**: 352-362.
- Besnard JY. 1988. Etude des constituents lipidiques dans la gonade femelle et les larves de *Pecten maximus* L. (Mollusque Lamellibranche). PhD dissertation, Univ. de Caen, Caen France, 154 pp.
- Besnard JY, P Lubet, A Nouvelot. 1989. Seasonal variations in the fatty acids content of the neutral lipids and phospholipids in the female gonade of *Pecten maximus* L. *Comp. Biochem. Phys. B* **93**: 21-26.
- Brett MT, CD Müller-Navarra, AP Ballantyne, JL Ravet, CR Goldman. 2006. Daphnia fatty acid composition reflects that of their diet. *Limnol. Oceanogr.* **51**: 2428-2437.
- Caers M, P Coutteau, P Sorgeloos. 2000. Impact of starvation and of feeding algal and artificial diets on the lipid content and composition of juvenile oysters (*Crassostrea gigas*) and clams (*Tapes philippinarum*). *Mar. Biol.* **136**: 891-899.
- Caroppo C, S Turicchia, MC Margheri. 2006. Phytoplankton assemblages in coastal waters of the northern Ionian Sea (eastern Mediterranean), with special reference to cyanobacteria. *J. Mar. Biol. Assoc. UK* **86**: 927-937.
- Chu FLE, J Graeves. 1991. Metabolism of palmitic, linoleic, and linolenic acids in adult oysters, *Crassostrea virginica*. *Mar. Biol.* **110**: 229-236.
- Chu FLE, KI Webb. 1984. Polyunsaturated fatty acids and neutral lipids in developing larvae of the oyster *Crassostrea virginica*. *Lipids* **19**: 815-820.
- Drazen JC, CF Phleger, MA Guest, PD Nichols. 2008. Lipid, sterols and fatty acid composition of abyssal holothurians and ophiuroids from the North-East Pacific Ocean: food web implications. *Comp. Biochem. Phys. B* **151**: 79-87.
- Fahl K, G Kattner. 1993. Lipid content and fatty acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica). *Polar Biol.* **13**: 405-409.
- Falk-Petersen S, TM Dahl, CL Scott, JR Sargent, B Gulliksen, S Kwasniewski et al. 2002. Lipid biomarkers and trophic linkages between ctenophores and copepods in Svalbard waters. *Mar. Ecol.-Prog. Ser.* **227**: 187-194.
- Falk-Petersen S, W Hagen, G Kattner, A Clarke, J Sargent. 2000. Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. *Can. J. Fish. Aquat. Sci.* **57**: 178-191.
- Fernandez-Reiriz MJ, U Labarta, JMF Babarro. 1996. Comparative allometries in growth and chemical composition of mussel (*Mytilus galloprovincialis* Lmk) cultured in two zones in the Ria Sada (Galicia, NW Spain). *J. Shell. Res.* **15**: 349-353.
- Folch J, M Lees, GH Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497-509.
- Gabbott PA. 1983. Developmental and seasonal metabolic activities in marine molluscs. In PW Hochachka, KM Wilbur, eds. *The Mollusca*, vol. 2. Environmental biochemistry and physiology. New York: Academic Press, pp. 165-217.
- Gillan FT, RB Johns. 1986. Chemical markers for marine bacteria: fatty acids and pigments. In RB Johns, ed. *Biological markers in the sedimentary environment*. Amsterdam: Elsevier Science Publisher, pp. 291-309.
- Goutx M, A Saliot. 1980. Relationship between dissolved and particulate fatty acids and hydrocarbons, chlorophyll a and zooplankton biomass in Villefranche Bay, Mediterranean Sea. *Mar. Chem.* **8**: 299-318.
- Graeve M, P Dauby, Y Scailteur. 2001. Combined lipid, fatty acid and digestive tract content analyses: a penetrating approach to estimate feeding modes of Antarctic amphipods. *Polar Biol.* **24**: 853-862.
- Graeve M, W Hagen, G Kattner. 1994a. Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. *Deep Sea Res.* **41**: 915-924.
- Graeve M, G Kattner, W Hagen. 1994b. Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. *J. Exp. Mar. Biol. Ecol.* **182**: 97-110.
- Graeve M, G Kattner, D Piepenburg. 1997. Lipids in Arctic benthos: Does the fatty acid and alcohol composition reflect feeding and trophic interactions? *Polar Biol.* **18**: 53-61.
- Kharlamenko VI, SI Kiyashko, AB Imbs, DI Vyshkvartzev. 2001. Identification of food source of invertebrates from the seagrass *Zostera marina* community using carbon and sulphur stable isotope ratio and fatty acid analyses. *Mar. Ecol.-Prog. Ser.* **220**: 103-117.
- Kharlamenko VI, NV Zhukova, SV Khotimchenko, VI Svetashev, GM Kamenev. 1995. Fatty acids as markers of food sources in a shallow-water hydrothermal ecosystems (Kraternaya Bight, Yankich Island, Kurile Islands). *Mar. Ecol.-Prog. Ser.* **120**: 231-241.
- Klingensmith JS. 1982. Distribution of methylene and non methylene-interrupted dienoic fatty acids in polar lipids and triacylglycerols of selected tissue of the hard-shell clam (*Mercenaria mercenaria*). *Lipids* **17**: 976-981.
- Klingensmith JS, LW Stillway. 1982. Lipid composition of selected tissues of the hardshell clam *Mercenaria mercenaria*. *Comp. Biochem. Phys. B* **71**: 111-112.
- Knauer J, PC Southgate. 1997. Growth and fatty acid composition of Pacific oysters (*Crassostrea gigas*) spat fed a spray-dried freshwater microalga (*Spongiococcum excentricum*) and microencapsulated lipids. *Aquaculture* **154**: 293-303.
- Knox GA. 1986. *Estuarine ecosystems: a systems approach*. Vol. 1. Boca Raton, FL: CRC Press, 198 pp.
- Kraffe F, P Soudant, Y Marty. 2004. Fatty acids of serine, ethanolamine, and choline plasmalogens in some marine bivalves. *Lipids* **39**: 59-66.
- Langdon CJ, MJ Waldock. 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. *J. Mar. Biol. Assoc. UK* **61**: 431-448.
- Li G, M Osada, T Suzuki, M Sato, K Mori. 1998. Degradation of vitellin during embryonic and larval development in the Pacific oyster *Crassostrea gigas*. *Invertebr. Reprod. Develop.* **33**: 1-9.
- Linehan LG, TP O'Connor, G Burnell. 1999. Seasonal variation in the chemical composition and fatty acid profile of Pacific oysters (*Crassostrea gigas*). *Food Chem.* **64**: 211-214.
- Lubet P, G Bichon, JY Besnard, G Zwingelstein. 1985.

- Composition and metabolism of lipids in some tissues of the mussel *Mytilus galloprovincialis* L. (Moll. Bivalvia) *in vivo* and *in vitro* incorporation of 1(3)-[<sup>3</sup>H]-glycerol. *Comp. Biochem. Phys. B* **82**: 425-431.
- Maazouzi C, G Masson, MS Izquierdo, JC Pihan. 2007. Fatty acid composition of the amphipod *Dikerogammarus villosus*: feeding strategies and trophic links. *Comp. Biochem. Physiol.* **147**: 868-875.
- Mansour MP, JK Volkman, AE Jackson, SI Blackburn. 1999. The fatty acid and sterol composition of five marine dinoflagellates. *J. Phycol.* **35**: 710-720.
- Matarrese A, A Tursi, G Costantino, R Pollicoro. 1993. The reproductive cycle of *Mytilus galloprovincialis* Lamark in the Mar Piccolo and in the Mar Grande of Taranto (Ionian sea). *Oealia* **19**: 1-11.
- Mayzaud P, JP Chanut, RG Ackman. 1989. Seasonal changes of biochemical composition of marine particulate matter with special reference to fatty acids and sterols. *Mar. Ecol.-Prog. Ser.* **56**: 189-204.
- Müller-Navarra DC, MT Brett, AM Liston, CR Goldman. 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature* **403**: 74-77.
- Napolitano GE, RG Ackman. 1992. Anatomical distributions and temporal variations of lipid classes in sea scallop *Placopecten magellanicus* (Gmelin) from Georges Bank (Nova Scotia). *Comp. Biochem. Phys.* **103**: 645-650.
- Nelson MM, BD Mooney, PD Nichols, CF Phleger. 2001. Lipids of Antarctic amphipods: food chain interactions and the occurrence of novel biomarkers. *Mar. Chem.* **73**: 53-64.
- Nes WR. 1974. Role of sterols in membranes. *Lipids* **9**: 596-612.
- Parkes RJ, J Taylor. 1983. The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. *Estuar. Coastal Shelf. Sci.* **16**: 173-189.
- Parrish CC, RJ Thompson, D Deibel. 2005. Lipid classes and fatty acids in plankton and settling matter during the spring bloom in a cold ocean coastal environment. *Mar. Ecol.-Prog. Ser.* **286**: 57-68.
- Pastore M, P Panetta, C Andreoli, B Dell'Angelo. 1976. Accrescimento di *Mytilus galloprovincialis* (Lam.) nei mari di Taranto. *Oealia* **2**: 20-61.
- Pazos JA, G Román, CP Acosta, JL Sánchez, M Abad. 1997. Lipid classes and fatty acid composition in the female gonad of *Pecten maximus* in relation to reproductive cycle and environmental variables. *Comp. Biochem. Phys. B* **117**: 393-402.
- Pazos JA, C Ruiz, O García-Martín, M Abad, JL Sánchez. 1996. Seasonal variations of the lipid content and fatty acid composition of *Crassostrea gigas* cultured in El Grove, Galicia, NW Spain. *Comp. Biochem. Phys. B* **114**: 171-179.
- Pazos JA, JL Sanchez, R Guillermo, ML Péres-Parallé, M Abad. 2003. Seasonal changes in lipid classes and fatty acid composition in the digestive gland of *Pecten maximus*. *Comp. Biochem. Phys. B* **134**: 367-380.
- Perry GJ, JK Volkman Jr, RB Johns. 1979. Fatty acids of bacterial origin in contemporary marine sediments. *Geochim. Cosmochim. Acta* **43**: 1715-1725.
- Phleger CF, MM Nelson, BD Mooney, PD Nichols. 2002. Interannual and between species comparison of the lipid, fatty acids and sterols of Antarctic krill from the US AMLR Elephant Island survey area. *Comp. Biochem. Phys.* **131**: 733-747.
- Pollero RJ, RR Brenner. 1981. Effects of the environment and fasting on the lipid and fatty acid composition of *Diplodom patagonicus*. *Lipids* **16**: 685-690.
- Pond DP, MV Bell, DR Dixon, AE Fallick, M Segonzac, J Sargent. 1998. Stable-carbon-isotope composition of fatty acids in hydrothermal vent mussels containing methano-trophic and thiotrophic bacterial endosymbionts. *Appl. Env. Microbiol.* **64**: 370-375.
- Rossi S, MJ Youngbluth, CA Jacoby, F Pagès, X Garrofé. 2008. Fatty acid trophic markers and trophic links among seston, crustacean zooplankton and the siphonophore *Nanomia cara* in Georges Basin and Oceanographer Canyon (NW Atlantic). *Sci. Mar.* **72**: 403-416.
- Sargent JR. 1976. The structure, metabolism and function of lipids in marine organisms. *Biochem. Biophys. Perspect. Mar. Biol.* **3**: 149-212.
- Sargent JR, KJ Whittle. 1981. Lipids and hydrocarbons in the marine food web. In A Longhurst, ed. *Analysis of marine ecosystems*. London: Academic Press, pp. 491-533.
- Stabili L, RA Cavallo. 2001. Confronto tra la flora batterica eterotrofa dell'Adriatico meridionale e dello Ionio settentrionale. *Atti Assoc. Ital. Oceanol. Limnol.* **14**: 369-378.
- St. John MAC, T Lund. 1996. Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea. *Mar. Ecol.-Prog. Ser.* **131**: 75-85.
- Swift ML, D White, MB Ghassemieh. 1980. Distribution of the neutral lipids in the tissues of the oyster *Crassostrea virginica*. *Lipids* **15**: 129-132.
- Takayama M, S Itoh, T Nagasaki, I Tanimizu. 1977. A new enzymatic method for determination of serum choline-containing phospholipids. *Clin. Chim. Acta* **79**: 93-98.
- Trinder P. 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J. Clin. Pathol.* **22**: 158-161.
- Tursi A, D Posa. 1991. Growth model of *Mytilus galloprovincialis* Lam. on the Mar Grande and on the Mar Piccolo of Taranto (southern, Italy). *Stat. Appl.* **3**: 23-31.
- Virtue P, P Mayzaud, E Albessard, P Nichols. 2000. Use of fatty acids as dietary indicators in northern krill, *Meganyctiphanes norvegica*, from northeastern Atlantic, Kattegat, and Mediterranean waters. *Can. J. Fish. Aquat. Sci.* **57**: 104-114.
- Volkman JK, SW Jeffrey, PD Nichols, GI Rogers, CD Garland. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* **128**: 219-240.
- Waldock MJ, DL Holland. 1984. Fatty acid metabolism in young oysters, *Crassostrea gigas*: polyunsaturated fatty acids. *Lipids* **19**: 332-336.
- Wenne R, L Polak. 1989. Lipid composition and storage in the tissues of the bivalve, *Macoma balthica*. *Biochem. Syst. Ecol.* **17**: 583-587.
- Whyte JNC. 1988. Fatty acid profiles from direct methanolysis of lipids in tissue of cultured species. *Aquaculture* **75**: 193-203.
- Zandee DI, JH Kluytmans, W Zurburg, H Pieters. 1980. Seasonal variations in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis. *Neth. J. Sea Res.* **14**: 1-29.
- Zhukova NV. 1991. The pathway of the biosynthesis of non-methylene-interrupted dienoic fatty acids in molluscs. *Comp. Biochem. Phys. B* **100**: 801-804.