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ORIGINAL ARTICLE

Temporal dynamics of amino and fatty acid composition in the razor clam *Ensis siliqua* (Mollusca: Bivalvia)

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Abstract Few studies have been conducted on the temporal dynamics of both amino acid (AA) and fatty acid (FA) profiles in marine bivalves. We investigated the seasonal variation of these compounds in the pod razor clam *Ensis siliqua* in relation to food availability, salinity, water temperature and reproductive cycle. AA content varied between 46.94 and 54.67 % dry weight (DW), and the AAs found in greater quantity were glutamic acid, glycine and aspartic acid. FA content varied between 34.02 and 87.94 mg g^{-1} DW and the FAs found in greater quantity were 16:0 and 22:6*n*-3. Seasonal trends were observed for AAs and FAs. FAs increased with gametogenesis and decreased with spawning while AA content increased throughout spawning. The effect of increasing temperature and high food availability during the spawning season masked the loss of AAs resulting from gamete release. Still, a comparatively greater increase in the contents of glutamic acid and leucine with spawning indicate their possible involvement in a post-spawning gonad recovery mechanism. A post-spawning decrease in 14:0, 16:0, 16:1*n*-7, 18:1*n*-7 and 18:1*n*-9 is indicative of the importance of these FAs in bivalve eggs. An increase in 18:3*n*-3, 18:4*n*-3, 20:1*n*-9 and 20:2*n*-6 during gametogenesis suggests their involvement in oocyte maturation. The FA 22:4*n*-6, while increasing with spawning, appears to play a role in post-spawning gonad recovery. Salinity did not have an effect on the AA composition. None of the environmental parameters measured had an effect on FA composition.

Keywords Amino acids · Fatty acids · Biochemical profile · Seasonality · Razor clam · *Ensis siliqua*

Miguel Baptista and Tiago Repolho have contributed equally to this work.

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Introduction

Bivalves are primary consumers that constitute an important link in the aquatic food chain (Hamdani and Soltani-Mazouni 2011). Their significant ecological role and economical relevance have long contributed to a generous amount of academic focus. As a result, information on the biochemistry of a wide range of bivalve species became available (e.g., Bayne et al. 1978; Lewin et al. 1979; Jarzebski et al. 1986; Wenne and Styczyńska-Jurewicz 1987 and references therein). Studies of seasonal changes in biochemical content of organisms allow understanding how environment, ecology and physiology can affect their

composition. As such, seasonal changes in the biochemistry of bivalves are known to be linked to the complex interaction of both biotic factors (e.g., growth, reproduction and food availability) and abiotic factors (e.g., temperature and salinity) (Gabbott 1983). Levels of proteins, lipids and carbohydrates (glycogen) have been shown to fluctuate with food availability and reproductive cycle. Food abundance allows for the accumulation of proteins and lipids in bivalve tissues (Taylor and Venn 1979; Bressan and Marin 1985; Wenne and Styczyńska-Jurewicz 1987). Lipids generally increase during the course of gametogenesis (Barber and Blake 1981; Tarnowska et al. 2009; Yang et al. 2011) and decrease upon release of gametes (i.e., spawning; Pieters et al. 1980; Bressan and Marin 1985; Tarnowska et al. 2009). For proteins, diverging trends have been observed throughout gametogenesis and spawning. During gametogenesis, protein content was found to increase (Joaquim et al. 2008; Tarnowska et al. 2009; Yang et al. 2011), decrease (Taylor and Venn 1979; Davis and Wilson 1983) and even remain stable (Pieters et al. 1980). During spawning, levels of protein were found to increase (Davis and Wilson 1983; Yang et al. 2011) and decrease (Pieters et al. 1980; Bressan and Marin 1985). Differences in food availability and water temperature conditions may partially explain the observed discrepancies since these factors are known to influence protein accumulation (Taylor and Venn 1979). Focusing on proteins and lipids, compounds involved in most biochemical and physiological processes of any organism is therefore useful for the recognition of ecological (e.g., Bressan and Marin 1985; Darriba et al. 2005a) and physiological changes (e.g., Beukema and De Bruin 1977; Davis and Wilson 1983; Tarnowska et al. 2009). Greater detail in the measurement of biochemical changes is achieved, however, when analyzing amino acids (AAs) and fatty acids (FAs). Indeed, differences in seasonal trend have been observed among both AAs and FAs (e.g., Beninger and Stephan 1985; Fatima 1996; Ojea et al. 2004). More commonly reported, is the different behavior exhibited among free AAs in relation to salinity (Sokolowski et al. 2003) and that exhibited among FAs in relation to temperature (Holland 1978). Studies conducted so far provided a general view of the seasonal variations in bivalve biochemistry, but few assess the temporal variations occurring among AAs and FAs. Most studies focusing on AAs in bivalves investigate the effects of salinity change on the levels of free AAs (Allen 1961; Ellis 1985; Sokolowski et al. 2003; Kube et al. 2007) or the AA composition of shells (Dussart 1983; Goodfriend et al. 1997; Barbour Wood et al. 2006) or specific soft body parts (i.e., gills and mantle; Trytek and Allen 1980). Few studies report whole-body AA composition of bivalves (Sidwell et al. 1979; Fatima 1996; Özden and Erkan 2011). Whole-body FA composition has received greater attention (e.g., Lewin et al. 1979; Beninger and Stephan 1985; Teshima et al. 1990; Pazos et al. 1996; Taylor and Savage 2006). Still, among these studies, only four evaluated seasonality: one on the AA composition (Fatima 1996) and three on the FA composition (Beninger and Stephan 1985; Pazos et al. 1996; Taylor and Savage 2006).

The pod razor clam *Ensis siliqua* (Linnaeus 1758) is found over a wide latitudinal range, from the Norwegian Sea along the Eastern Atlantic and west coast of Africa, and also the Baltic and Mediterranean seas (Tebble 1966; von Cosel and Gofas 2011). It inhabits fine sand, silt or sandy-mud bottoms of coastal zones where they can form extensive beds commonly found between 3 and 7 m although occurring until 12 m (Tebble 1966; Monteiro and Gaspar 1993; Gaspar et al. 1999). They form deep burrows (Hauton et al. 2003; Wootton et al. 2003) with longitudinal axis of the shell in a vertical orientation (da Costa et al. 2010) and when feeding both siphons protrude above the sediment (Tuck et al. 2000; Breen et al. 2011). This species has a single spawning period (Lebour 1938; Gaspar and Monteiro 1998; Darriba et al. 2005b) with males and females exhibiting synchronism in the gametogenic development and spawning (Gaspar and Monteiro 1998). Studies on coastal waters of the Iberian Peninsula show that gametogenesis starts in November with spawning occurring mainly during April and May (Gaspar and Monteiro 1998; Darriba et al. 2005b). The pod razor clam is a high-value commercial species that has been heavily fished along the Portuguese coast since 1969 (Gaspar and Monteiro 1998; Gaspar et al. 1999). Though attention has been paid to the population genetics of this species (Fernández-Tajes et al. 2007, 2012) and limited studies conducted on its gametogenic cycle (Gaspar and Monteiro 1998; Darriba et al. 2005b), the AA and FA composition of this razor clam has not been assessed.

Thus, this study aims to evaluate the seasonal variation in AA and FA composition of *E. siliqua* over a 1-year period in relation to food availability, salinity, water temperature and reproductive cycle. FA levels are expected to increase with greater food availability and throughout gametogenesis and decrease with spawning. AA levels are expected to increase with food availability while no prediction is possible on reproduction-related fluctuations. Salinity and water temperature may also affect AA and FA composition.

Materials and methods

Biological sampling

Ensis siliqua individuals were hand collected in Caldeira de Tróia, a shallow water habitat near the mouth of Sado

estuary, Portugal. Sampling was performed at low tide, in the intertidal zone, once every 2 months, from April 2010 to February 2011.

For the biochemical analysis, three pooled samples (n = 20) were taken from 60 individuals selected for shell size (≥60 mm) indicative of reproductive capability (Gaspar and Monteiro 1998). The soft tissue was removed and then homogenized with a grinder (Retsch Grindomix GM200, Düsseldorf, Germany; 5,000 rpm; material: PP cup and stainless steel knifes), vacuum packed and frozen at −80 °C. The frozen samples were freeze-dried for 48 h at −50 °C under low pressure (approximately 10^{-1} atm), powdered and stored at −80 °C. Moisture content was determined according to the AOAC (2005) methodologies, by drying the sample overnight at 105 °C (laboratory heater, P-Selecta 207).

Amino acid analysis

To extract total amino acids (protein bound + free), 14.8–15.8 mg of sample were placed in 10-ml ampoules with 3 ml of 6 M HCl (Merck) containing 0.1 % phenol (Merck), according to the method described by AOAC (2005). Following the establishment of inert and anaerobic conditions, to prevent oxidative degradation of amino acids, ampules were sealed and samples hydrolyzed at 110 °C for 24 h; hydrolysates were filtered (0.45 μm pore size) and dissolved with Milli-Q distilled water to 20 ml. Samples were then frozen at −80 °C and freeze-dried for 48 h at −50 °C under low pressure (approximately 10^{-1} atm), after which they were dissolved in 5 ml of 0.1 M HCl (Merck) and stored at −80 °C until amino acid separation. Finally, thawed samples were filtered (0.2 μm pore size) and separation was performed with high-performance liquid chromatography (Agilent 1100 HPLC) using precolumn o-phthalaldehyde and 3-mercaptopropionic acid in borate buffer (OPA, Agilent Technologies) and 9-fluorenylmethylchloroformate in acetonitrile (FMOC; Agilent Technologies) derivatization, a Phenomenex Gemini ODS C18 guard column (4 mm × 3 mm) and a Phenomenex Gemini ODS C18 110A column (4.6 mm × 150 mm, 5 μm). The solvents and gradient conditions were those described by Henderson et al. (2000). Detection wavelengths were set at UV 338 and 262 nm and fluorescence 340/450 and 266/305 nm. The identity and quantity of the amino acids were assessed by comparison with the retention times and peak areas of standard amino acids (Sigma–Aldrich) using norvaline and sarcosine as internal standards. Trypthophan and cysteine were quantified in *E. siliqua*; however, because these are partially lost during the acidic hydrolysis, they were not considered for analysis.

Fatty acid analysis

Fatty acid methyl esters (FAME) profile from nonpolar and polar lipids was determined in triplicate for each sample and was based on the experimental procedure of Cohen et al. (1988); 300–330 mg of sample were dissolved in 5 mL of acetyl chloride/methanol (1:19 v/v; Merck), shaken and heated (80 °C; 1 h). After cooling, 1 mL of Milli-Q distilled water and 2 mL of *n*-heptane pro analysis (Merck) were added, and samples were shaken and centrifuged (2,300*g*, 5 min; 3K30 Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) until phase separation. The moisture content of the upper phase was removed using anhydrous sodium sulfate (Panreac). A 2 μL aliquot from the upper phase was then injected onto a gas chromatograph (Varian Star 3800 Cp, Walnut Creek, CA, USA) equipped with an autosampler and fitted with a flame ionization detector at 250 °C for FAME analysis. Fatty acid separation was carried out with helium as carrier gas at a flow rate of 1 mL min^{-1}, in a capillary column DB-WAX (30 m length × 0.32 mm internal diameter; 0.25 μm film thickness; Hewlett–Packard, Albertville, MN) programmed at 180 °C for 5 min, raised to 220 at 4 °C min^{-1}, and maintained at 220 °C for 25 min, with the injector at 250 °C. FAME identification was accomplished through comparison of retention times with those of Sigma standards. Quantitative data were obtained with Varian software using C21:0 FA (Sigma) as internal standard.

Environmental parameters

To ascertain the existence of a relationship between AA and FA levels in *E. siliqua* and environmental conditions in Caldeira de Tróia, environmental data were obtained from the Annual Reports of Tróia Monitoring Program in IMAR (2008–2011). Data on turbidity (here as proxy of food availability for these suspension feeders), salinity and water temperature acquired between September 2007 and September 2010 (during high tide) were used. The compiled data set was not continuous for the shellfish sampling period. Consequently, data were pooled into four seasonal periods: Spring (1 March–31 May), Summer (1 June–31 August), Autumn (1 September–30 November) and Winter (1 December–28 February).

Statistical analysis

Seasonal differences in the AA and FA composition and environmental conditions were tested with analysis of variance (ANOVA) followed by multiple comparisons test (Tukey's test). Where necessary, data were transformed (Log_{10}) to correct for homogeneity of variance. When data transformation still did not meet the assumptions of

ANOVA, differences were analyzed with the nonparametric ANOVA equivalent (Kruskall–Wallis). Pearson's correlation coefficients were used to identify the relationships among the potential explanatory environmental variables for biochemical composition patterns (Table 1). Simple and multiple regression analyses were used to test for associations among variables. The combined effect of the three environmental parameters (turbidity, salinity and water temperature) yielded akin or, mostly, lower R^2 values than those obtained with the more explanatory pair of parameters for each AA and FA. All statistical analyses were tested at 0.05 level of probability with the software STATISTICATM 6.1 (Statsoft, Inc., Tulsa, OK 74104, USA).

Table 1 Pearson's correlation coefficients between turbidity, salinity and water temperature in Caldeira de Tróia between September 2007 and September 2010

	Turbidity	Salinity
Turbidity	1	
Salinity	−0.37	1
Water temperature	−0.26	0.10

Correlation coefficients are non-significant ($P > 0.05$)

Results

Seasonal variations in amino acid composition

The general trend among AAs exhibited two peaks (June and February) and two minimums (April and December). A few different trends were, however, observed (Table 2). The main essential amino acids (EAAs) were arginine (Fig. 1C), leucine and lysine. The content of leucine was greater in June when compared to April and December (Fig. 1B, Tukey's test, $F = 6.11$, df = 5, $P < 0.01$). Methionine values did not vary significantly throughout the year (Fig. 1A, Tukey's test, $F = 2.18$, df = 5, $P > 0.05$). Total EAA ($\sum$EAA) content was significantly greater in June and August when compared to April and December (Fig. 1D, Tukey's test, $F = 7.27$, df = 5, $P < 0.01$).

The main non-essential amino acids (NEAAs) were glutamic acid, glycine (Fig. 2B) and aspartic acid.

Table 2 Amino acid composition (% dry weight) of *Ensis siliqua*, between April 2010 and February 2011

Amino acids	Spring	Summer		Autumn	Winter	
	April	June	August	October	December	February
Essential (EAA)						
Threonine	2.54 ± 0.03^{a}	2.83 ± 0.19^{a}	2.79 ± 0.05^{a}	2.66 ± 0.08^{a}	2.64 ± 0.05^{a}	2.84 ± 0.10^{a}
Valine	2.15 ± 0.02^{a}	2.38 ± 0.08^{b}	2.30 ± 0.05^{ab}	2.21 ± 0.11^{ab}	2.13 ± 0.02^{a}	2.36 ± 0.05^{b}
Methionine	0.90 ± 0.02^{a}	0.97 ± 0.19^{a}	1.10 ± 0.06^{a}	1.13 ± 0.15^{a}	0.89 ± 0.07^{a}	1.04 ± 0.05^{a}
Isoleucine	1.94 ± 0.02^{a}	2.20 ± 0.09^{b}	2.17 ± 0.05^{bc}	2.08 ± 0.09^{abc}	1.99 ± 0.03^{ac}	2.20 ± 0.07^{b}
Leucine	3.49 ± 0.03^{a}	4.16 ± 0.22^{b}	4.08 ± 0.17^{bc}	3.87 ± 0.24^{abc}	3.64 ± 0.04^{ac}	4.05 ± 0.18^{bc}
Phenylalanine	1.85 ± 0.02^{a}	2.06 ± 0.08^{bc}	2.05 ± 0.05^{abc}	1.90 ± 0.11^{abc}	1.86 ± 0.01^{ab}	2.10 ± 0.07^{c}
Histidine	0.96 ± 0.01^{abc}	1.02 ± 0.03^{bc}	1.00 ± 0.04^{abc}	0.93 ± 0.04^{ab}	0.92 ± 0.02^{a}	1.04 ± 0.03^{c}
Lysine	3.64 ± 0.19^{ab}	4.21 ± 0.08^{a}	3.83 ± 0.36^{ab}	3.59 ± 0.10^{b}	3.46 ± 0.06^{b}	3.98 ± 0.26^{ab}
Arginine	3.71 ± 0.03^{ab}	4.25 ± 0.05^{ab}	4.54 ± 0.13^{a}	4.01 ± 0.32^{ab}	3.20 ± 0.04^{b}	4.09 ± 0.56^{ab}
$\sum$EAA	21.19 ± 0.24^{ab}	24.08 ± 0.68^{c}	23.86 ± 1.04^{c}	22.39 ± 1.28^{abc}	20.73 ± 0.21^{a}	23.70 ± 1.38^{bc}
Non-essential (NEAA)						
Aspartic acid	4.78 ± 0.06^{a}	5.58 ± 0.29^{ab}	5.78 ± 0.16^{b}	5.34 ± 0.32^{ab}	4.96 ± 0.03^{ab}	5.56 ± 0.27^{ab}
Tyrosine	1.70 ± 0.01^{a}	1.94 ± 0.08^{b}	1.94 ± 0.06^{b}	1.82 ± 0.11^{ab}	1.73 ± 0.01^{a}	1.96 ± 0.05^{b}
Serine	2.41 ± 0.01^{ab}	2.59 ± 0.05^{a}	2.50 ± 0.09^{ab}	2.35 ± 0.15^{ab}	2.31 ± 0.01^{b}	2.50 ± 0.08^{ab}
Glutamic acid	7.12 ± 0.09^{a}	8.58 ± 0.43^{bc}	8.67 ± 0.30^{c}	8.17 ± 0.48^{abc}	7.46 ± 0.09^{ab}	8.21 ± 0.54^{abc}
Glycine	5.17 ± 0.10^{ab}	5.87 ± 0.30^{bc}	6.11 ± 0.22^{c}	5.83 ± 0.38^{bc}	4.86 ± 0.03^{bd}	4.23 ± 0.19^{d}
Alanine	3.71 ± 0.06^{ab}	4.01 ± 0.17^{b}	3.57 ± 0.11^{a}	3.42 ± 0.13^{ac}	3.05 ± 0.04^{c}	3.34 ± 0.23^{ac}
Proline	1.82 ± 0.04^{ab}	2.02 ± 0.07^{ab}	1.98 ± 0.07^{ab}	1.77 ± 0.14^{a}	1.83 ± 0.05^{ab}	2.04 ± 0.14^{b}
$\sum$NEAA	26.72 ± 0.31^{a}	30.59 ± 1.26^{b}	30.56 ± 1.00^{b}	28.73 ± 1.83^{ab}	26.21 ± 0.12^{a}	27.84 ± 1.63^{ab}
$\sum$AA	47.91 ± 0.18^{a}	54.67 ± 1.83^{b}	54.42 ± 2.04^{b}	51.11 ± 3.08^{ab}	46.94 ± 0.31^{a}	51.53 ± 2.99^{ab}

Different superscript letters within rows represent significant differences ($P < 0.05$) and should be interpreted in the following exemplificative manner: **a** not $\neq$ **a**, **a** $\neq$ **b**, **a** $\neq$ **c**; **ab** $\neq$ **cd**, **a** not $\neq$ **ab**, **ab** not $\neq$ **bc**

Values are means of triplicate samples (+SD). Amino acid essentiality was defined based on Knauer and Southgate (1999)

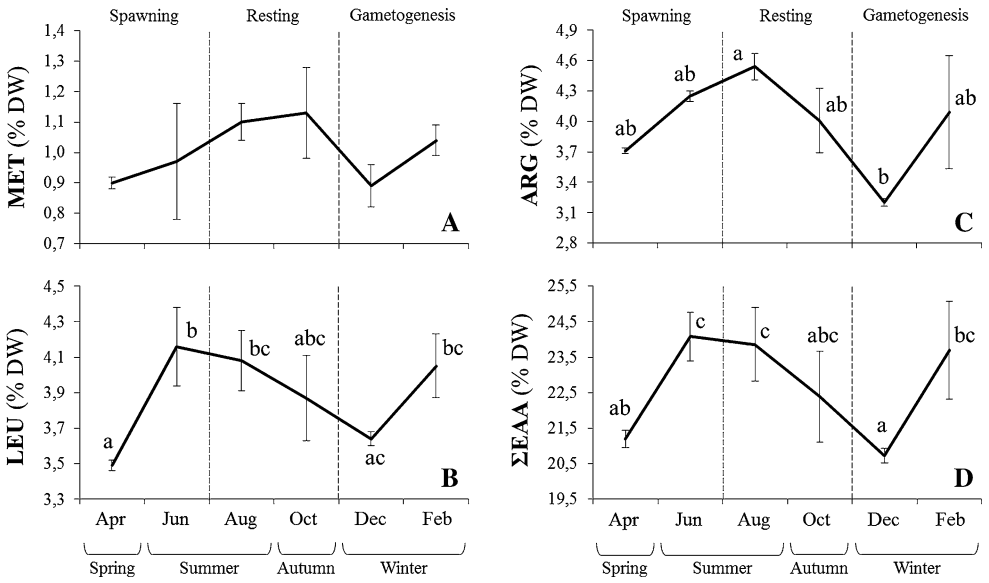

Fig. 1 Main essential amino acids content and total essential amino acid content, between April 2010 and February 2011. **A** Methionine, **B** leucine, **C** arginine, **D** total essential amino acid content. Values are means of triplicate samples (±SD). *Different superscript letters* represent significant differences between months ($P < 0.05$) and should be interpreted in the following exemplificative manner: **a** not ≠ **a**, **a** ≠ **b**, **a** ≠ **c**; **ab** ≠ **cd**, **a** not ≠ **ab**, **ab** not ≠ **bc**. Temporal evolution in gonad development stages as determined by Gaspar and Monteiro (1998) is presented. *MET* methionine, *LEU* leucine, *ARG* arginine, *ΣEAA* total essential amino acid content

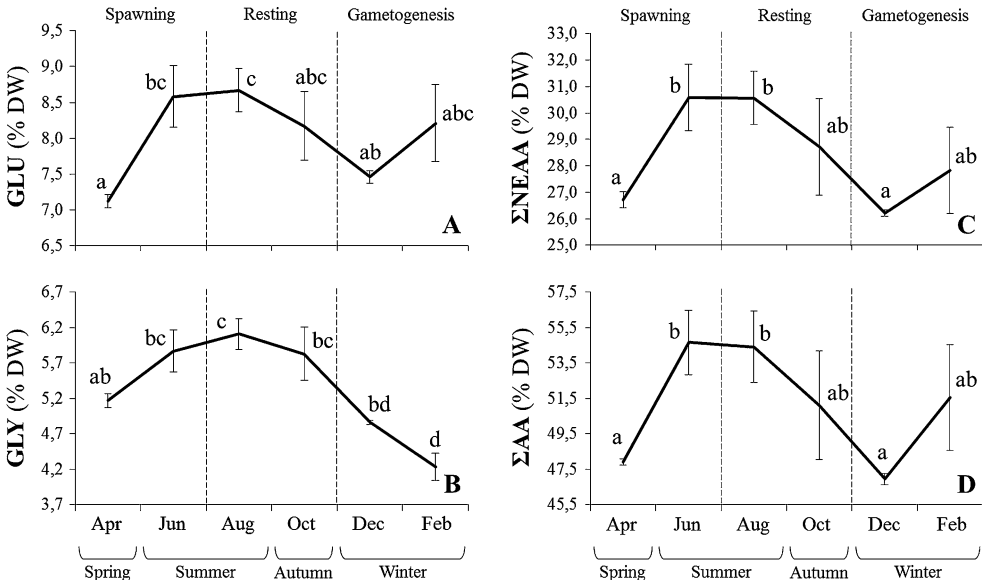

Fig. 2 Main non-essential amino acids content, total non-essential amino acid content and total amino acid content, between April 2010 and February 2011. **A** Glutamic acid, **B** glycine, **C** total non-essential amino acid content, **D** total amino acid content. Values are means of triplicate samples (±SD). *Different superscript letters* represent significant differences between months ($P < 0.05$) and should be interpreted as exemplified in Fig. 1 caption. Temporal evolution in gonad development stages as determined by Gaspar and Monteiro (1998) is presented. *GLU* glutamic acid, *GLY* glycine, *ΣNEAA* total non-essential amino acid content, *ΣAA* total amino acid content

Glutamic acid content was greater in August in relation to April and December (Fig. 2A, Tukey's test, $F = 6.91$, df $= 5$, $P < 0.01$). Differently, values for proline were higher in February than in October (Table 2, Tukey's test, $F = 4.74$, df $= 5$, $P < 0.05$). Similarly to $\sum$EAA, total NEAA ($\sum$NEAA) content was significantly greater in June and August when compared to April and December (Fig. 2C, Tukey's test, $F = 7.24$, df $= 5$, $P < 0.01$). Concomitantly, total amino acid ($\sum$AA) content was also significantly greater in June and August when compared to

Table 3 Fatty acid composition (mg g^{-1} dry weight) of *Ensis siliqua* between April 2010 and February 2011

Fatty acids	Spring	Summer		Autumn	Winter	
	April	June	August	October	December	February
Saturated (SFA)						
14:0	4.57 ± 1.48^{a}	2.65 ± 0.05^{b}	0.83 ± 0.15^{c}	0.72 ± 0.26^{c}	0.78 ± 0.02^{c}	0.79 ± 0.08^{c}
15:0	0.41 ± 0.11^{a}	0.30 ± 0.01^{ab}	0.16 ± 0.03^{c}	0.16 ± 0.05^{c}	0.19 ± 0.00^{bc}	0.22 ± 0.02^{bc}
16:0	19.01 ± 4.16^{a}	11.17 ± 0.73^{b}	5.31 ± 0.79^{c}	4.84 ± 1.34^{c}	4.99 ± 0.12^{c}	5.43 ± 0.55^{c}
17:0	0.88 ± 0.14^{a}	0.77 ± 0.07^{ab}	0.51 ± 0.08^{c}	0.45 ± 0.12^{c}	0.50 ± 0.01^{c}	0.58 ± 0.05^{bc}
18:0	6.04 ± 0.66^{a}	5.27 ± 0.23^{a}	3.48 ± 0.39^{b}	2.95 ± 0.69^{b}	2.87 ± 0.05^{b}	3.16 ± 0.19^{b}
19:0	0.20 ± 0.03^{a}	0.19 ± 0.01^{a}	0.12 ± 0.02^{b}	0.10 ± 0.03^{b}	0.10 ± 0.00^{b}	0.11 ± 0.00^{b}
20:0	0.43 ± 0.27^{a}	0.56 ± 0.11^{a}	0.39 ± 0.18^{a}	0.34 ± 0.11^{a}	0.35 ± 0.01^{a}	0.38 ± 0.05^{a}
22:0	0.12 ± 0.00^{ab}	0.16 ± 0.05^{a}	0.00 ± 0.00^{b}	0.05 ± 0.00^{ab}	0.07 ± 0.01^{ab}	0.07 ± 0.01^{ab}
Branched						
Iso 15:0	0.07 ± 0.00^{a}	0.03 ± 0.00^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}	0.03 ± 0.00^{a}
Anteiso 16:0	0.53 ± 0.08^{a}	0.42 ± 0.05^{a}	0.43 ± 0.08^{a}	0.39 ± 0.11^{a}	0.44 ± 0.01^{a}	0.56 ± 0.04^{a}
Iso 17:0	0.80 ± 0.15^{a}	0.55 ± 0.04^{b}	0.37 ± 0.07^{b}	0.33 ± 0.09^{b}	0.36 ± 0.01^{b}	0.49 ± 0.04^{b}
∑Branched	1.38 ± 0.28^{a}	1.00 ± 0.03^{ab}	0.82 ± 0.16^{b}	0.73 ± 0.20^{b}	0.81 ± 0.02^{b}	1.07 ± 0.07^{ab}
∑SFA	33.04 ± 7.13^{a}	22.07 ± 1.22^{b}	11.61 ± 1.78^{c}	10.35 ± 2.79^{c}	10.64 ± 0.22^{c}	11.81 ± 1.02^{c}
Monounsaturated (MUFA)						
16:1*n*-9	0.84 ± 0.07^{ab}	0.72 ± 0.08^{a}	0.73 ± 0.11^{a}	0.64 ± 0.16^{a}	0.69 ± 0.01^{a}	1.06 ± 0.06^{b}
16:1*n*-7	3.73 ± 0.72^{a}	2.82 ± 0.12^{b}	0.87 ± 0.13^{c}	0.76 ± 0.23^{c}	0.65 ± 0.08^{c}	0.78 ± 0.08^{c}
16:1*n*-5	0.16 ± 0.03^{a}	0.12 ± 0.01^{ab}	0.08 ± 0.01^{bc}	0.06 ± 0.01^{c}	0.06 ± 0.00^{c}	0.08 ± 0.01^{bc}
17:1	0.96 ± 0.15^{a}	0.81 ± 0.16^{ab}	0.76 ± 0.13^{ab}	0.50 ± 0.11^{b}	0.70 ± 0.01^{ab}	0.88 ± 0.05^{a}
18:1*n*-9	5.12 ± 0.99^{a}	2.81 ± 0.06^{b}	1.50 ± 0.09^{c}	1.30 ± 0.35^{c}	1.20 ± 0.04^{c}	1.24 ± 0.06^{c}
18:1*n*-7	2.36 ± 0.42^{a}	1.51 ± 0.02^{b}	0.90 ± 0.11^{c}	0.84 ± 0.22^{c}	1.03 ± 0.05^{bc}	1.13 ± 0.04^{bc}
18:1*n*-5	0.00 ± 0.00^{a}	0.03 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.02 ± 0.00^{a}	0.00 ± 0.00^{a}
20:1*n*-11	1.17 ± 0.11^{a}	1.25 ± 0.04^{a}	1.13 ± 0.16^{ab}	0.83 ± 0.21^{bc}	0.77 ± 0.02^{c}	0.81 ± 0.01^{c}
20:1*n*-9	2.12 ± 0.21^{a}	1.19 ± 0.06^{b}	0.80 ± 0.11^{c}	0.68 ± 0.17^{c}	0.67 ± 0.02^{c}	0.68 ± 0.02^{c}
20:1*n*-7	0.84 ± 0.08^{a}	0.76 ± 0.02^{ab}	0.57 ± 0.09^{ab}	0.46 ± 0.11^{b}	0.57 ± 0.01^{ab}	0.77 ± 0.01^{ab}
22:1*n*-9	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.03 ± 0.00^{a}	0.00 ± 0.00^{a}	0.06 ± 0.00^{a}
∑MUFA	17.29 ± 2.79^{a}	12.01 ± 0.28^{b}	7.35 ± 0.84^{c}	6.08 ± 1.59^{c}	6.32 ± 0.23^{c}	7.46 ± 0.33^{c}
Polyunsaturated (PUFA)						
16:2*n*-4	0.24 ± 0.01^{a}	0.27 ± 0.02^{a}	0.25 ± 0.04^{a}	0.25 ± 0.08^{a}	0.27 ± 0.00^{a}	0.33 ± 0.02^{a}
16:3*n*-4	0.17 ± 0.03^{ab}	0.20 ± 0.06^{a}	0.16 ± 0.02^{ab}	0.14 ± 0.03^{abc}	0.10 ± 0.00^{c}	0.12 ± 0.01^{bc}
16:3*n*-3	5.10 ± 0.38^{a}	4.54 ± 0.47^{ab}	4.45 ± 0.62^{ab}	3.46 ± 0.78^{b}	3.66 ± 0.07^{b}	4.17 ± 0.21^{ab}
16:4*n*-3	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}
18:2*n*-6	0.85 ± 0.20^{a}	0.40 ± 0.01^{ab}	0.50 ± 0.30^{ab}	0.31 ± 0.07^{ab}	0.25 ± 0.01^{b}	0.29 ± 0.01^{ab}
18:3*n*-6	0.21 ± 0.05^{a}	0.10 ± 0.01^{b}	0.00 ± 0.00^{b}	0.07 ± 0.01^{b}	0.05 ± 0.00^{b}	0.07 ± 0.00^{b}
18:3*n*-4	0.12 ± 0.01^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.03 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
18:3*n*-3	1.60 ± 0.50^{a}	0.38 ± 0.01^{b}	0.22 ± 0.05^{b}	0.35 ± 0.09^{b}	0.23 ± 0.02^{b}	0.44 ± 0.01^{b}
18:4*n*-3	2.14 ± 0.73^{a}	0.77 ± 0.01^{b}	0.34 ± 0.07^{b}	0.54 ± 0.20^{b}	0.40 ± 0.02^{b}	0.65 ± 0.06^{b}
20:2*n*-6	1.44 ± 0.20^{a}	0.78 ± 0.04^{b}	0.65 ± 0.11^{bc}	0.52 ± 0.14^{bc}	0.41 ± 0.01^{c}	0.44 ± 0.02^{c}
20:4*n*-6	1.86 ± 0.43^{ab}	2.77 ± 0.03^{a}	1.90 ± 0.46^{ab}	1.77 ± 0.56^{b}	1.53 ± 0.10^{b}	2.05 ± 0.05^{ab}
20:3*n*-3	0.18 ± 0.04^{a}	0.04 ± 0.00^{b}	0.00 ± 0.00^{b}	0.03 ± 0.02^{b}	0.02 ± 0.00^{b}	0.00 ± 0.00^{b}
20:4*n*-3	0.69 ± 0.18^{a}	0.32 ± 0.00^{ab}	0.17 ± 0.02^{ab}	0.15 ± 0.05^{ab}	0.13 ± 0.00^{b}	0.19 ± 0.01^{ab}
20:5*n*-3	8.38 ± 2.48^{a}	5.35 ± 0.16^{ab}	2.93 ± 0.69^{bc}	2.55 ± 0.86^{bc}	2.19 ± 0.06^{c}	3.17 ± 0.11^{bc}
21:5*n*-3	1.62 ± 0.30^{a}	1.32 ± 0.28^{ab}	1.03 ± 0.12^{b}	0.92 ± 0.27^{b}	0.94 ± 0.02^{b}	1.37 ± 0.02^{ab}
22:4*n*-6	1.08 ± 0.13^{a}	1.57 ± 0.06^{b}	1.03 ± 0.23^{ac}	0.95 ± 0.31^{ac}	0.93 ± 0.01^{ac}	1.16 ± 0.02^{abc}
22:5*n*-6	0.51 ± 0.10^{a}	0.43 ± 0.02^{a}	0.43 ± 0.10^{a}	0.36 ± 0.12^{a}	0.34 ± 0.00^{a}	0.60 ± 0.01^{a}
22:5*n*-3	2.20 ± 0.42^{a}	1.94 ± 0.06^{ab}	1.26 ± 0.34^{bc}	1.08 ± 0.38^{c}	1.14 ± 0.02^{c}	1.76 ± 0.02^{abc}

Table 3 continued

Fatty acids	Spring	Summer		Autumn	Winter	
	April	June	August	October	December	February
22:6*n*-3	9.27 ± 2.10^{a}	7.54 ± 0.15^{ab}	5.48 ± 1.35^{b}	4.67 ± 1.65^{b}	4.49 ± 0.06^{b}	6.03 ± 0.05^{ab}
∑PUFA	37.61 ± 8.24^{a}	28.41 ± 1.43^{a}	20.80 ± 3.87^{b}	18.11 ± 5.59^{b}	17.06 ± 0.36^{b}	22.82 ± 0.32^{b}
∑ *n*-3	31.18 ± 7.11^{a}	21.91 ± 1.32^{ab}	15.88 ± 3.23^{ab}	13.76 ± 4.28^{ab}	13.21 ± 0.23^{b}	17.80 ± 0.34^{ab}
∑ *n*-6	5.94 ± 1.10^{a}	6.02 ± 0.12^{a}	4.51 ± 0.61^{ab}	3.95 ± 1.23^{b}	3.49 ± 0.13^{b}	4.56 ± 0.07^{ab}
n-3/*n*-6	5.21 ± 0.25^{a}	3.64 ± 0.20^{b}	3.50 ± 0.28^{b}	3.49 ± 0.03^{b}	3.79 ± 0.09^{b}	3.90 ± 0.13^{b}
DHA/EPA	1.13 ± 0.10^{a}	1.41 ± 0.04^{b}	1.87 ± 0.04^{c}	1.82 ± 0.04^{c}	2.05 ± 0.03^{d}	1.90 ± 0.08 ^{cd}
∑FA	87.94 ± 18.15^{a}	62.49 ± 2.2^{b}	39.76 ± 6.46^{bc}	34.53 ± 9.97^{c}	34.02 ± 0.78^{c}	42.09 ± 1.65^{bc}

Different superscript letters within rows represent significant differences ($P < 0.05$) and should be interpreted in the following exemplificative manner: **a** not ≠ **a**, **a** ≠ **b**, **a** ≠ **c**; **ab** ≠ **cd**, **a** not ≠ **ab**, **ab** not ≠ **bc**

Values are means of triplicate samples (±SD)

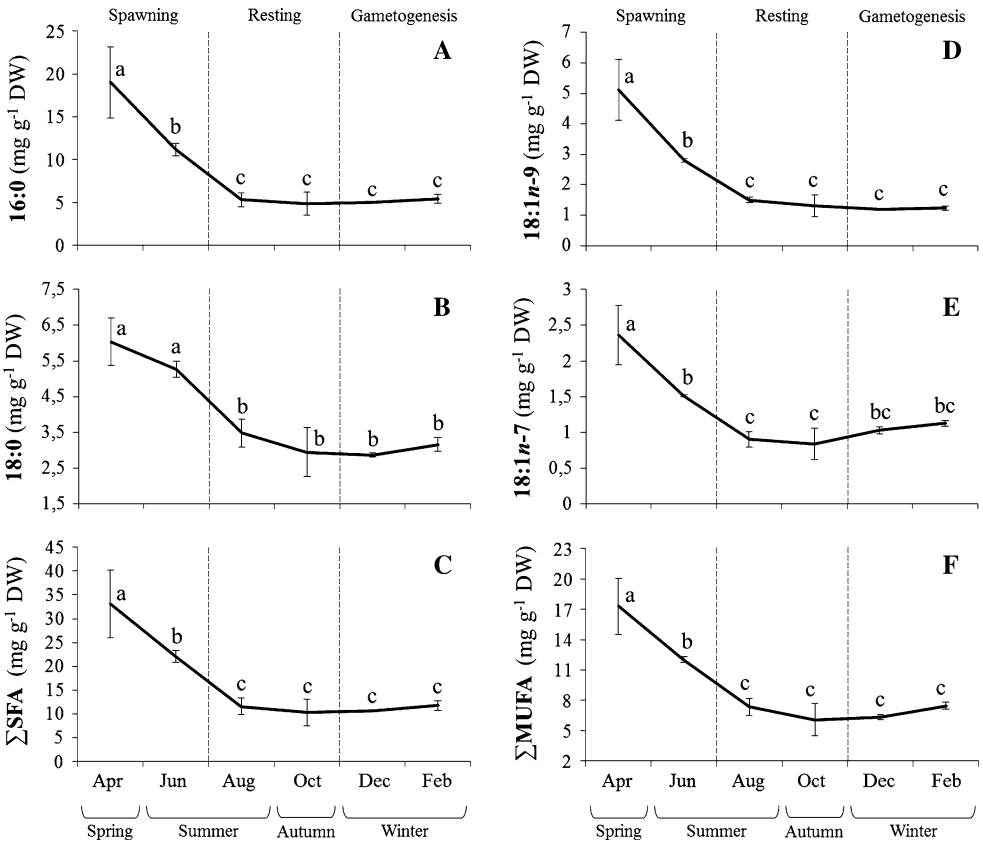

Fig. 3 Main saturated fatty acid, main monounsaturated fatty acid and respective fractions (SFA, MUFA) contents between April 2010 and February 2011. **A** Palmitic acid, **B** stearic acid, **C** saturated fatty acid fraction, **D** oleic acid, **E** vaccenic acid, **F** monounsaturated fatty acid fraction. Values are means of triplicate samples (±SD). *Different superscript letters* represent significant differences between months ($P < 0.05$) and should be interpreted as exemplified in Fig. 1 caption. Temporal evolution in gonad development stages as determined by Gaspar and Monteiro (1998) is presented

April and December (Fig. 2D, Tukey's test, $F = 7.10$, df = 5, $P < 0.01$). The complete AA profile of *E. siliqua* is shown in Table 2.

Seasonal variations in fatty acid composition

The general trend among FAs exhibited one peak in April and a minimum either in October or December. A few different trends were, however, observed (Table 3).

The main saturated fatty acids (SFAs) were palmitic (16:0) and stearic (18:0; Fig. 3B) acids. Content of 16:0 was significantly greater in April when compared to all other months (Fig. 3A, Tukey's test, $F = 30.56$, df = 5, $P < 0.01$). SFA fraction (∑SFA) levels were significantly higher in April when compared to all other months (Fig. 3C, Tukey's test, $F = 24.76$, df = 5, $P < 0.01$).

The main monounsaturated fatty acids (MUFAs) were oleic (18:1*n*-9), vaccenic (18:1*n*-7; Fig. 3E) and

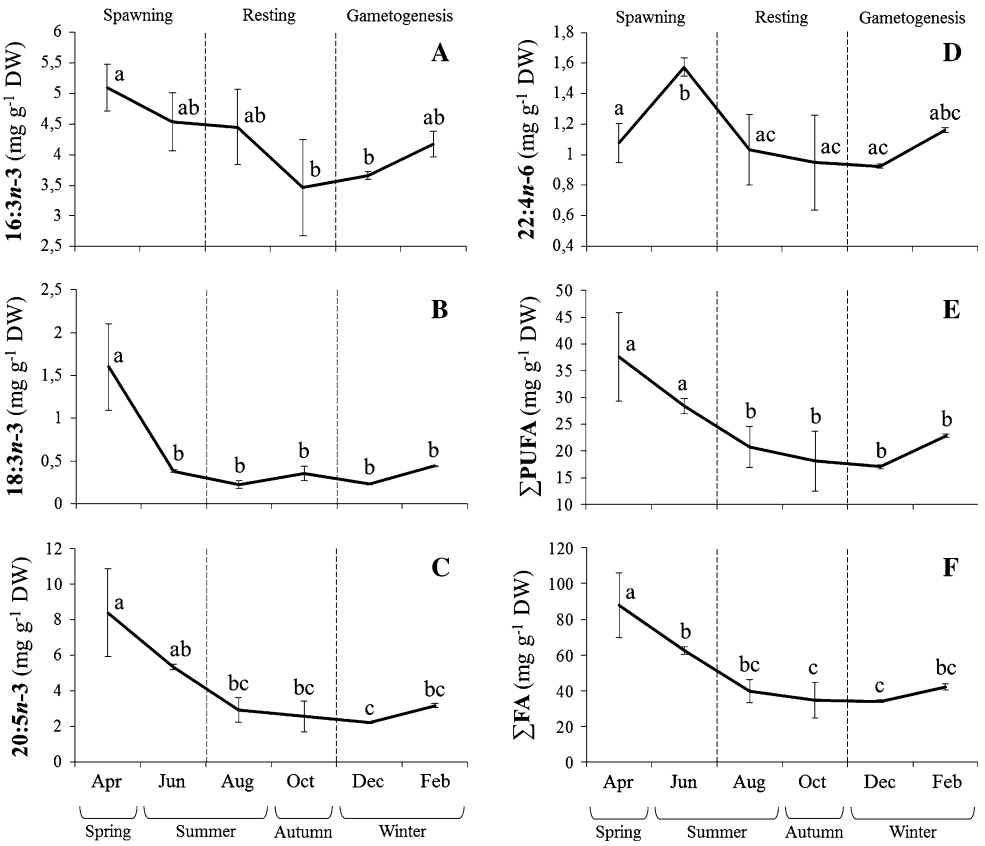

Fig. 4 Main polyunsaturated fatty acid, respective fraction (PUFA) and total fatty acid contents between April 2010 and February 2011. **A** Hexadecatrienoic acid, **B** α-linolenic acid, **C** eicosapentaenoic acid, **D** adrenic acid, **E** polyunsaturated fatty acid fraction, **F** total fatty acid content. Values are means of triplicate samples (±SD). *Different superscript letters* represent significant differences between months ($P < 0.05$) and should be interpreted as exemplified in Fig. 1 caption. Temporal evolution in gonad development stages as determined by Gaspar and Monteiro (1998) is presented

palmitoleic (16:1*n*-7) acids. 18:1*n*-9 content was significantly greater in April when compared to all other months (Fig. 3D, Tukey's test, $F = 38.65$, df = 5, $P < 0.01$). The content of 16:1*n*-7 surpassed that of 18:1*n*-7 in April and June. Levels of 16:1*n*-7 were also significantly higher in April when compared to all other months (Table 3, Tukey's test, $F = 51.16$, df = 5, $P < 0.01$). Concomitantly, MUFA fraction (∑MUFA) content was significantly greater in April when compared to all other months (Fig. 3F, Tukey's test, $F = 31.22$, df = 5, $P < 0.01$).

Among polyunsaturated fatty acids (PUFAs), the seasonal trends varied considerably. The *n*-3 family dominated this fraction. The main PUFAs were docosahexaenoic (DHA, 22:6*n*-3), hexadecatrienoic (16:3*n*-3; Fig. 4A) and eicosapentaenoic (EPA, 20:5*n*-3) acids. 16:3*n*-3 was the second main PUFA except in April and June, when it was surpassed by 20:5*n*-3. 20:5*n*-3 exhibited significantly higher values in April when compared to those obtained between August and December (Fig. 4C, Tukey's test, $F = 13.73$, df = 5, $P < 0.01$). Regarding α-linolenic acid (18:3*n*-3), a significantly higher value was observed in April when compared to all other months (Fig. 4B, Tukey's test, $F = 18.35$, df = 5, $P < 0.01$). On the other hand, adrenic acid (22:4*n*-6) content was significantly greater in June when compared to all other months (Fig. 4D, Tukey's test, $F = 5.96$, df = 5, $P < 0.01$). Differently from ∑SFA and ∑MUFA, PUFA fraction (∑PUFA) levels were significantly higher in April and June when compared to all other months (Fig. 4E, Kruskal–Wallis, $H = 9.26$, df = 5, $P < 0.01$). Finally, total FA (∑FA) content was significantly greater in April when compared to all other months (Fig. 4F, Tukey's test, $F = 16.98$, df = 5, $P < 0.01$). The complete FA profile of *E. siliqua* is shown in Table 3.

Environmental parameters and biochemical composition

Mean turbidity (here as a proxy of food availability) peaked in spring (Kruskal–Wallis, $H = 8.90$, df = 3, $P < 0.05$; Fig. 5A). Mean salinity did not vary significantly throughout seasons (Kruskal–Wallis, $H = 3.36$, df = 3, $P > 0.05$; Fig. 5B). Mean water temperature in Caldeira de Tróia varied from 15 °C in winter to 20 °C in summer (Kruskal–Wallis, $H = 9.34$, df = 3, $P < 0.05$; Fig. 5C). There was no significant relationship between environmental parameters (Table 1). Moreover, none of the three environmental parameters measured (i.e., turbidity, salinity and water temperature), nor a combination between parameters, had a significant effect on amino acid or fatty acid composition of *E. siliqua* (see the low R^2 values irrespective of significance levels in Tables 2 and 3).

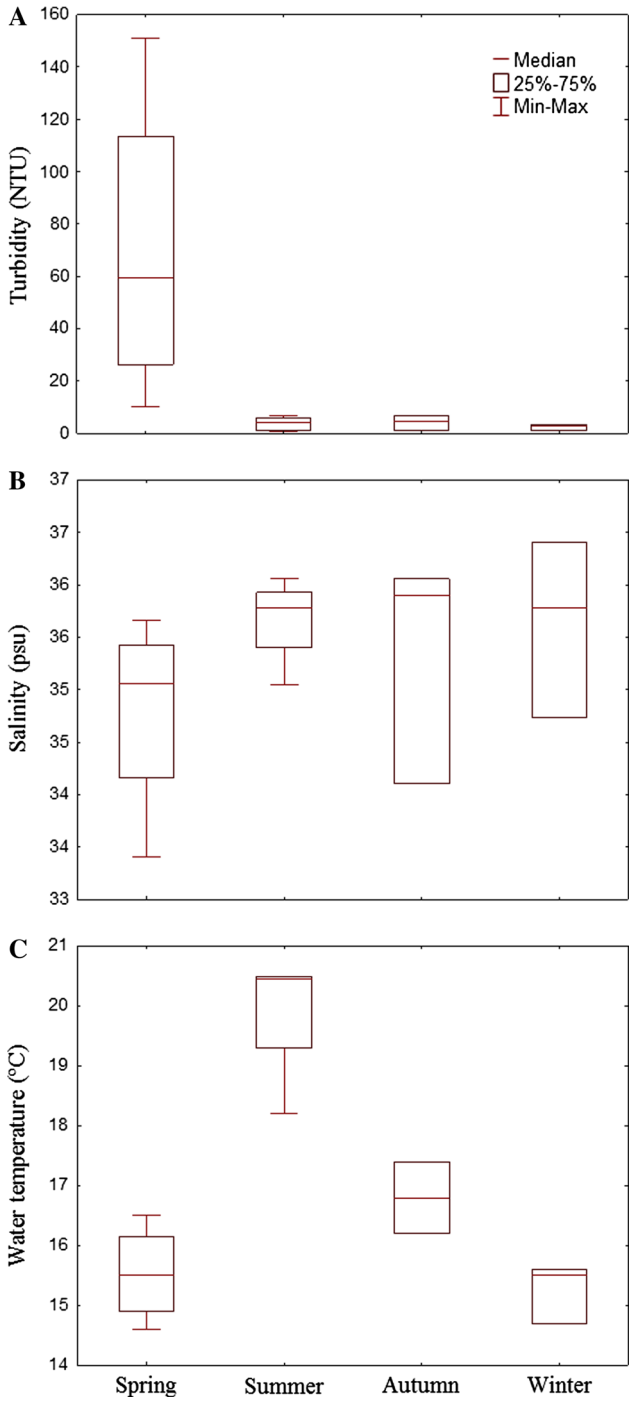

Fig. 5 Seasonal changes of biotic and abiotic factors in Caldeira de Tróia obtained from September 2007 to September 2010. **A** Turbidity (NTU, as a proxy of food availability), **B** salinity (psu), **C** temperature (°C). Spring: 1 March–31 May; Summer: 1 June–31 August; Autumn: 1 September–30 November; Winter: 1 December–28 February

Discussion

The biochemical composition of an organism is determined by endogenous processes (e.g., gametogenesis, maturation, spawning) and exogenous factors (e.g., food availability, salinity, temperature) (Rosa et al. 2002; Rosa and Nunes 2003; Ojea et al. 2004; Baptista et al. 2012). A temporal analysis of the biochemical compounds permits intercrossing with chronological data of other variables allowing the researcher to gather knowledge on the ecology and physiology of an organism and also to understand how the surrounding environment may affect it.

Studies dealing with both biochemical composition and gametogenesis of bivalves do not usually examine the extent of the differential loss of biochemical content by males and females (through release of gametes) in gonochoric species, or analogously, by the male and female reproductive tissues in hermaphroditic species (e.g., Barber and Blake 1981; Ojea et al. 2004; Tarnowska et al. 2009; Yang et al. 2011). Hayward and Gillooly (2011) studied the cost of gamete production for a broad variety of invertebrate and vertebrate organisms and found that although males and females (of the same species) invest similarly in gonad biomass, gamete biomass production rate is approximately two to four orders of magnitude higher in females. While studying *E. siliqua*, a sex ratio of 1:1 was observed in populations from the south of Portugal (Gaspar and Monteiro 1998) and northwest Spain (Darriba et al. 2005b; da Costa et al. 2010); thus, it is reasonable to assume that a similar ratio should occur among the population of Caldeira de Tróia. Still, even if such ratio is not verified, the immensely greater accumulation (during gametogenesis) and subsequent loss (through spawning) of AA and FA reserves (in eggs) by females should be regarded as essentially responsible for the AA and FA fluctuations observed in the present study.

Amino acid profile and seasonality

The EAAs found in greater quantity were arginine, leucine and lysine while the main NEAAs, and overall main AAs, were glutamic acid, glycine and aspartic acid. Upon comparison of these results with those obtained in other studies, it appears that bivalve AA composition varies considerably between species. Sidwell et al. (1979) found that the main AAs in *Crassostrea virginica* were glutamic acid, aspartic acid and lysine. On the other hand, Özden and Erkan (2011) studied *Flexopecten glaber* and found the main AAs to be proline, glutamic acid, phenylalanine and aspartic acid. Still, considering those two studies and the present one, it would appear that glutamic acid and aspartic acid are always found among the main AAs. In the study of Fatima (1996), however, the main AAs of *Perna viridis* were found to be arginine, leucine and lysine while glutamic acid and aspartic acid were the AAs found in smaller concentration. In terms of $\sum$AA content, values between 46.94 and 54.67 % dry weight (DW) were obtained for *E.*

Table 4 Models assessing the importance of environmental parameters in predicting amino acid composition of *E. siliqua* in Caldeira de Tróia

Amino acids	Model	Turbidity (*t* ratio)	Salinity (*t* ratio)	SST (*t* ratio)	Total R^2
Essential (EAA)					
THR	1	−1.92			0.19
	2		2.48*		0.28
	3			1.55	0.13
	4		2.41*	1.51	0.37
VAL	1	−1.29			0.09
	2		2.44*		0.27
	3			1.88	0.18
	4		2.42*	1.89	0.41
MET	1	−1.25			0.09
	2		0.90		0.05
	3			1.20	0.08
	4	−0.97		0.91	0.14
ILE	1	−2.14*			0.22
	2		2.79*		0.33
	3			1.95	0.19
	4		2.83*	2.04	0.47
LEU	1	−2.24*			0.24
	2		2.62*		0.30
	3			2.25*	0.24
	4		2.71*	2.36*	0.49
PHE	1	−1.49			0.12
	2		2.39*		0.26
	3			1.82	0.17
	4		2.35*	1.81	0.40
HIS	1	−0.26			0.00
	2		1.26		0.09
	3			1.29	0.09
	4		1.15	1.18	0.17
LYS	1	−0.70			0.03
	2		0.99		0.06
	3			1.62	0.14
	4		0.86	1.51	0.18
ARG	1	−0.76			0.03
	2		1.26		0.09
	3			2.90*	0.34
	4		1.21	2.80*	0.40
∑EAA	1	−1.46			0.12
	2		2.02		0.20
	3			2.46*	0.27
	4		2.05	2.47*	0.43
Non-essential (NEAA)					
ASP	1	−2.37*			0.26
	2		2.77*		0.32
	3			2.82*	0.33
	4		3.12**	3.16**	0.59
TYR	1	−1.98			0.20
	2		2.34*		0.25
	3			2.16*	0.23
	4		2.36*	2.19*	0.43
SER	1	−0.33			0.01
	2		1.81		0.17
	3			2.06	0.21
	4		1.75	1.99	0.34
GLU	1	−2.42*			0.27
	2		2.78*		0.33
	3			2.77*	0.32
	4		3.11**	3.10**	0.59
GLY	1	−0.29			0.01
	2		0.60		0.02
	3			4.05**	0.51
	4	0.64		4.01**	0.52
ALA	1	1.08			0.07
	2		0.51		0.02
	3			2.21*	0.23
	4	1.98		2.83*	0.39
PRO	1	−1.06			0.07
	2		2.48*		0.28
	3			1.54	0.13
	4		2.40*	1.49	0.37
∑NEAA	1	−1.30			0.10
	2		2.14*		0.22
	3			4.15**	0.52
	4		2.72*	4.60**	0.68
∑AA	1	−1.42			0.11
	2		2.17*		0.23
	3			3.47**	0.43
	4		2.51*	3.72**	0.60

For each variable, the *t* ratios are included. Models 1–4 evaluated the following: 1: turbidity; 2: salinity; 3: water temperature; 4: more explanatory pair of environmental parameters. * $P < 0.05$, ** $P < 0.01$

siliqua. These values are in agreement with total protein content found for other marine bivalves, namely, *Siliqua patula*, a species belonging to the same family as *E. siliqua* (i.e., Pharidae) (46.0–48.0 % DW; Lewin et al. 1979), *Venerupis decussata* (44.7–50.8 DW; Ojea et al. 2004), *P. viridis* (57.8–66.5 % DW; Fatima 1996), *C. virginica* (32.5–70.1 % DW; Sidwell et al. 1979) and *Cerastoderma glaucum* (37.5–77.4 % DW; Tarnowska et al. 2009).

Table 5 Models assessing the importance of environmental parameters in predicting fatty acid composition of *E. siliqua* in Caldeira de Tróia

Fatty acids	Model	Turbidity (*t* ratio)	Salinity (*t* ratio)	SST (*t* ratio)	Total R^2
Saturated (SFA)					
14:0	1	4.62**			0.57
	2		−1.02		0.06
	3			−0.21	0.00
	4	4.66**		0.88	0.59
15:0	1	3.86**			0.48
	2		−0.82		0.04
	3			−0.63	0.02
	4	3.60**	0.31		0.48
16:0	1	5.04**			0.61
	2		−1.28		0.09
	3			−0.32	0.01
	4	5.03**		0.80	0.63
17:0	1	3.20**			0.39
	2		−0.88		0.05
	3			−0.07	0.00
	4	3.24**		0.74	0.41
18:0	1	3.42**			0.42
	2		−1.07		0.07
	3			0.62	0.02
	4	4.03**		1.87	0.53
19:0	1	2.77*			0.32
	2		−0.85		0.04
	3			0.65	0.03
	4	3.22**		1.62	0.42
20:0	1	0.41			0.01
	2		0.85		0.04
	3			0.54	0.02
	4	0.78	1.07		0.08
22:0	1	1.33			0.10
	2		−0.55		0.02
	3			−0.45	0.01
	4	1.21		−0.13	0.10
Iso 15:0	1	2.57*			0.29
	2		−0.14		0.00
	3			−0.23	0.00
	4	2.67*	0.82		0.32
Anteiso 16:0	1	1.67			0.15
	2		−0.65		0.03
	3			−1.45	0.12
	4	1.35		−1.09	0.21
Iso 17:0	1	4.29**			0.54
	2		−1.19		0.08
	3			−0.73	0.03
	4	3.83**	−0.10		0.54
∑SFA	1	4.59**			0.57
	2		−1.17		0.08
	3			−0.14	0.00
	4	4.68**		0.99	0.59

Table 5 continued

Fatty acids	Model	Turbidity (*t* ratio)	Salinity (*t* ratio)	SST (*t* ratio)	Total R^2
Monounsaturated (MUFA)					
16:1*n*-9	1	0.64			0.02
	2		−0.45		0.01
	3			−1.20	0.08
	4	0.35		−1.03	0.09
16:1*n*-7	1	3.78**			0.47
	2		−1.05		0.06
	3			0.26	0.00
	4	4.12**		1.40	0.53
16:1*n*-5	1	3.63**			0.45
	2		−1.22		0.09
	3			0.51	0.02
	4	4.20**		1.78	0.55
17:1	1	1.94			0.19
	2		−0.34		0.01
	3			−0.13	0.00
	4	1.92		0.36	0.20
18:1*n*-9	1	5.27**			0.63
	2		−1.43		0.11
	3			−0.16	0.00
	4	5.42**		1.12	0.66
18:1*n*-7	1	4.99**			0.61
	2		−1.34		0.10
	3			−0.71	0.03
	4	4.43**	−0.22		0.61
18:1*n*-5	1	−0.76			0.03
	2		0.92		0.05
	3			1.08	0.07
	4		0.82	0.98	0.11
20:1*n*-11	1	1.43			0.11
	2		−0.54		0.02
	3			2.84*	0.33
	4	3.02**		4.14**	0.59
20:1*n*-9	1	5.50**			0.65
	2		−1.72		0.16
	3			−0.19	0.00
	4	5.65**		1.13	0.68
20:1*n*-7	1	2.16*			0.23
	2		−0.56		0.02
	3			−0.56	0.02
	4	2.01	0.16		0.23
22:1*n*-9	1	−0.68			0.03
	2		1.61		0.14
	3			−1.31	0.10
	4		1.83	−1.58	0.26
∑MUFA	1	4.55**			0.56
	2		−1.28		0.09
	3			−0.04	0.00
	4	4.74**		1.15	0.60

Table 5 continued

Fatty acids	Model	Turbidity (*t* ratio)	Salinity (*t* ratio)	SST (*t* ratio)	Total R^2
Polyunsaturated (PUFA)					
16:2*n*-4	1	−1.04			0.06
	2		−0.10		0.00
	3			−0.74	0.03
	4	−1.27		−1.06	0.13
16:3*n*-4	1	0.74			0.03
	2		−0.05		0.00
	3			2.81*	0.33
	4	1.77		3.34**	0.45
16:3*n*-3	1	2.57*			0.29
	2		−1.16		0.08
	3			1.02	0.06
	4	3.27**		2.08	0.45
16:4*n*-3	1	−0.84			0.04
	2		1.00		0.06
	3			−1.89	0.18
	4	−1.50		−2.28*	0.29
18:2*n*-6	1	3.78**			0.47
	2		−1.43		0.11
	3			0.41	0.01
	4	4.28**		1.66	0.55
18:3*n*-6	1	4.61**			0.57
	2		−1.48		0.12
	3			−0.93	0.05
	4	4.02**	−0.45		0.58
18:3*n*-4	1	3.96**			0.49
	2		−1.02		0.06
	3			−1.19	0.08
	4	3.59**		−0.60	0.51
18:3*n*-3	1	5.97**			0.69
	2		−1.54		0.13
	3			−1.34	0.10
	4	5.50**		−0.76	0.70
18:4*n*-3	1	5.49**			0.65
	2		−1.37		0.11
	3			−1.28	0.09
	4	5.04**		−0.68	0.66
20:2*n*-6	1	5.25**			0.63
	2		−1.71		0.16
	3			0.12	0.00
	4	5.78**		1.67	0.69
20:4*n*-6	1	−0.24			0.00
	2		0.82		0.04
	3			1.65	0.15
	4		0.69	1.54	0.17
20:3*n*-3	1	7.01**			0.75
	2		−2.11[a]		0.22
	3			−1.06	0.07
	4	6.19**	−1.33		0.78
20:4*n*-3	1	5.38**			0.64
	2		−1.38		0.11
	3			−0.52	0.02
	4	5.21**		0.51	0.65

Table 5 continued

Fatty acids	Model	Turbidity (*t* ratio)	Salinity (*t* ratio)	SST (*t* ratio)	Total R^2
20:5*n*-3	1	4.35**			0.54
	2		−0.99		0.06
	3			−0.15	0.00
	4	4.42**		0.92	0.57
21:5*n*-3	1	2.49*			0.28
	2		−0.81		0.04
	3			−0.74	0.03
	4	2.28*		−0.22	0.28
22:4*n*-6	1	−0.08			0.00
	2		0.70		0.03
	3			1.39	0.11
	4		0.57	1.29	0.13
22:5*n*-6	1	1.12			0.07
	2		−0.16		0.00
	3			−0.77	0.04
	4	0.92		−0.51	0.09
22:5*n*-3	1	2.57*			0.29
	2		−0.48		0.01
	3			−0.30	0.01
	4	2.48*	0.39		0.30
22:6*n*-3	1	3.28**			0.40
	2		−0.70		0.03
	3			0.06	0.00
	4	3.40**		0.95	0.44
∑PUFA	1	3.84**			0.48
	2		−0.95		0.05
	3			−0.06	0.00
	4	3.93**		0.92	0.51
∑FA	1	4.36**			0.54
	2		−1.12		0.07
	3			−0.11	0.00
	4	4.47**		0.99	0.57

For each variable, the *t* ratios are included. Models 1–4 evaluated the following: 1: turbidity; 2: salinity; 3: water temperature; 4: more explanatory pair of environmental parameters. * $P < 0.05$, ** $P < 0.01$, [a]marginally significant ($P = 0.05$)

Generally, the seasonal trend in the content of individual AA in *E. siliqua* exhibited two peaks (June and February) and two minimums (April and December) (Table 2). Though not statistically significant for all AAs, an increase in AA content is observable between April and June (∑EAA, ∑NEAA, ∑AA; Figs. 2D, 3C, D), which is coincident with the spawning period of *E. siliqua* (Gaspar and Monteiro 1998; Darriba et al. 2005b). Yet, given the release of gametes and therefore the loss of protein-bound amino acids contained in eggs and sperm (Bressan and Marin 1985; Tarnowska et al. 2009; Hamdani and Soltani-Mazouni 2011), a decrease in ∑AA content would be expected to occur. In fact, marine bivalve eggs are mainly composed of proteins, with these compounds accounting for 32.9–74.0 % of their dry weight (Bayne et al. 1978; Pieters et al. 1980; Lee and Heffernan 1991; Massapina et al. 1999; Park et al. 2003). Moreover, pod razor clam eggs are relatively large (i.e., 99.3 ± 3.2 μm in diameter; da Costa 2009) and the egg mass in adult bivalve females was shown to reach 26.8–38.9 % of the individuals total dry weight (Park et al. 2003; Park and Choi 2004). Nonetheless, similar trends in protein content were recorded for other bivalve species. In the study of Davis and Wilson (1983), an increase in protein content of *Nucula turgida* was observed immediately after the main spawning period. In *Spisula solida*, an increase in the protein content was witnessed in the period immediately after the onset of spawning (Joaquim et al. 2008). Last, in the study of Yang et al. (2011), protein content of *Fulvia mutica* increased during the first spawning period. Neither study attempted to explain such results. An increase in the protein content in adductor muscles was shown to be associated with increasing water temperature and food availability (Taylor and Venn 1979). In fact, in the three mentioned studies and

presumably in the present one, spawning occurred during a period of increasing or high water temperature (Fig. 5C). Additionally, a high amount of food was available at Caldeira de Tróia in the Spring of 2010 (Fig. 5A). Interestingly, in the present study, turbidity and water temperature were found not responsible for the measurable AA content fluctuations in *E. siliqua* (Fig. 5; Table 4). On the other hand, as mentioned above, spawning is known to be responsible for AA loss and as such is bound to have impacted AA content. It is possible that the simultaneous influence of these three factors has obscured their individual roles in AA dynamics. It then appears that the combined effect of increasing temperature and high food availability masked the loss of AAs resulting from gamete release. Still, a comparatively greater increase was registered for glutamic acid and leucine (April–June, Table 2). Such result may be indicative of an important role of these AAs at this stage of the reproductive cycle. Evidence of AAs involvement in reproduction exists for a variety of terrestrial and marine organisms (e.g., Kasschau and McCommas 1982; O'Brien et al. 2005; Wu et al. 2008) hence opening the possibility of such compounds holding a similar role in bivalve mollusks. Given the coincidence between gamete release and the increase in the contents of glutamic acid and leucine, it appears that these two AAs are involved in gonad maintenance, possibly as part of a post-spawning recovery mechanism. Free AAs (FAAs) are known osmolytes playing an important role in the osmoregulation of bivalves. Under hypoosmotic stress, the intracellular concentration of FAAs decreases as a result of release, while under hyperosmotic stress, the intracellular concentration of FAAs increases as a result of uptake (Zurburg and De Zwaan 1981; Matsushima and Hayashi 1988). The roles of alanine and glycine in osmoregulation, for example, have been studied considerably (Baginski and Pierce 1977; Ellis et al. 1985). Yet, none of the observed variations in the seasonal content of AAs appears to have occurred as a result of salinity changes (Fig. 5B).

Fatty acid profile and seasonality

The main SFAs were 16:0 and 18:0, while the main MUFAs were 18:1*n*-9 and 18:1*n*-7. PUFA fraction was dominated by *n*-3 FAs, mainly 22:6*n*-3, 16:3*n*-3 and 20:5*n*-3 surpassing 16:3*n*-3 in April and June. Overall, the main FAs were 16:0 and 22:6*n*-3 during the entire studied period. Similar results were obtained for several other marine bivalves, including *S. patula* (Lewin et al. 1979; Beninger and Stephan 1985; Teshima et al. 1990; Wenne and Polak 1992; Pazos et al. 1996). ∑FA content ranged between 34.02 and 87.94 mg g^{-1} DW in *E. siliqua*. In their study, Wenne and Styczyńska-Jurewicz (1987) reviewed the lipid content of 31 bivalve species. Values varied between approx. 20.00–345.00 mg g^{-1} DW, therefore placing *E. siliqua* in the lower range of lipid content among bivalves. Interestingly, lipid content found for *S. patula* (i.e., 400.00–430.00 mg g^{-1} DW; Lewin et al. 1979) far exceeds that of *E. siliqua* while also exceeding the values reviewed by Wenne and Styczyńska-Jurewicz (1987).

Generally, the seasonal trend in the content of individual FAs in *E. siliqua* exhibited one peak in April and a minimum either in October or December (Table 3). Differently from what occurs with ∑AA content, the seasonal variation observed in ∑FA content follows the trend described in most studies with FA levels increasing with gametogenesis and subsequently dropping with spawning (Fig. 4F; Pieters et al. 1980; Bressan and Marin 1985; Tarnowska et al. 2009). ∑FA content is highest in April, when sea water temperature is relatively low (Fig. 5C). A decrease in ∑FA content is observed between April and June, when greater food availability, resulting from Spring blooms (Fig. 5A), should have allowed for the accumulation of FAs (Bressan and Marin 1985; Jarzebski et al. 1986). This decrease takes place during the spawning season (Gaspar and Monteiro 1998; Darriba et al. 2005b) and should therefore be related to the loss of FAs contained in gametes (Taylor and Venn 1979; Ojea et al. 2004; Hamdani and Soltani-Mazouni 2011). In fact, lipids are the second main component of marine bivalve eggs, accounting for 11.4–38.0 % of their dry weight (Bayne et al. 1978; Pieters et al. 1980; Lee and Heffernan 1991; Massapina et al. 1999; Park et al. 2003) or even 47.5 % DW (Davis and Wilson 1983). In the present study, biochemical analysis was performed on whole-body tissue. Gonad-specific biochemical fluctuations, however, become diluted in whole-body dynamics (Ojea et al. 2004). Consequently, loss of gonadal FAs may have been masked by FA fluctuations related to other tissues (e.g., adductor muscle, gills). Between August and October, period comprising the reproductively inactive period (Gaspar and Monteiro 1998; Darriba et al. 2005b), ∑FA levels remained low and relatively stable. During the first stages of gametogenesis, lipids accumulate in the digestive gland. Later, as gonads grow, lipids are accumulated in gametes (Barber and Blake 1981). Through comparison of ∑FA values in April and December, it is plausible that an increase in FA content occurs throughout gametogenesis (Gaspar and Monteiro 1998; Darriba et al. 2005b). Thus, the main seasonal changes in ∑FA content of *E. siliqua* appear strongly connected with the reproductive cycle.

The content of some FAs did not exhibit the general seasonal trend followed by the rest. Between April and June (spawning period) a comparatively greater decrease in 14:0, 16:0, 16:1*n*-7, 18:1*n*-7, 18:1*n*-9, 18:3*n*-3, 18:4*n*-3, 20:1*n*-9 and 20:2*n*-6 was observed. Similar results were obtained in the work of Beninger and Stephan (1985) while

analyzing the seasonal variations in whole-body FA composition of *V. decussata* and *Venerupis philippinarum* and in the works of Besnard et al. (1989) and Ojea et al. (2004) while analyzing the FA composition of gonads from *Pecten maximus* and *V. decussata*, respectively. Moreover, the FAs 14:0, 16:0, 16:1*n*-7, 18:1*n*-7 and 18:1*n*-9 were found to be among the major FAs in unfertilized eggs of *V. decussata* (Ojea et al. 2004). Such result is indicative of the significance of these FAs in bivalve eggs, possibly possessing a crucial role in embryonic development. During gametogenesis (i.e., between December and April) the contents of 14:0, 16:0, 16:1*n*-7, 18:1*n*-9, 18:3*n*-3, 18:4*n*-3, 20:1*n*-9, 20:2*n*-6 and 20:5*n*-3 increase considerably more than other FAs. Similar results were obtained for *Mytilus edulis* females in the months preceding spawning (Kluytmans et al. 1985). Also, Besnard et al. (1989) found maximal concentrations of 18:3*n*-3, 18:4*n*-3 and 20:5*n*-3 to be related to oocyte maturation in *P. maximus*. These results suggest that such FAs are involved in oocyte maturation, especially 18:3*n*-3, 18:4*n*-3, 20:1*n*-9 and 20:2*n*-6 which are not actively accumulated in unfertilized eggs (Ojea et al. 2004). The FA 22:4*n*-6, on the other hand, increases with spawning when practically all other FAs decrease, and thus, it is plausible to assume that this FA possesses a chief role in gonad maintenance, possibly as part of a post-spawning recovery mechanism (see discussion about AA involvement in gonad maintenance in the previous sub-section). It is also noteworthy that none of the observed variations in the seasonal content of FAs occurred as a result of turbidity, salinity or temperature changes (Fig. 5; Table 5).

The results on the AA and FA composition of *E. siliqua* presented herein provide a baseline for future research. Also, the one-year period of study provides insight on the seasonal fluctuations of these compounds. By performing similar studies in subsequent years, for example, it will be possible for ecologists to register the interannual differences in biochemical composition and how environmental change will affect it. On a larger scale, comparison of the presented data with that of populations from other regions may allow for the direct analysis of the impact of environmental forcing. On a more economical point of view, the presented data on biochemical condition may be used by *E. siliqua*-directed fisheries on the selection of the harvesting period in order to maximize the nutritional quality of the catch. Lastly, aquaculture systems may benefit from the presented data. In their work, da Costa et al. (2010) pointed out that improvement of postlarval survival could lead to a large-scale development of *E. siliqua* aquaculture. Condition of bivalve adults has been shown to influence nutrient levels of eggs (Bayne et al. 1978), number of larvae produced and larvae growth rate (Helm et al. 1973). Complementarily, larvae viability was shown to be significantly correlated with their lipid level (Helm et al. 1973). It is possible that through the usage of a feed supplement enriched with the above highlighted AAs and FAs (potentially involved in different stages of the reproductive process), both condition of adults and survival of spats could be improved in *E. siliqua* and other bivalve species.

Conclusions

Here, we have shown that in *E. siliqua*, the AA and FA seasonal fluctuations did not follow the same general trend. FAs increased with gametogenesis and decreased with spawning while AA content increased throughout spawning. In AA dynamics, the combined effect of increasing temperature and high food availability seem to have masked the loss of AAs resulting from gamete release. Still, involvement of AAs in post-spawning gonad recovery may occur. Salinity did not have an effect on the AA composition. On the other hand, certain FAs appear to be deeply involved in different stages of the reproductive process (i.e., embryonic development, gametogenesis, post-spawning gonad recovery). None of the three environmental parameters measured: turbidity, salinity and water temperature had an effect on FA composition.

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Appendix

See Tables 2, 3, 4, 5

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