Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Deep-Sea Research I 50 (2003) 119–130
DEEP-SEA RESEARCH PART I
www.elsevier.com/locate/dsr

Biochemical composition of deep-sea decapod crustaceans with two different benthic life strategies off the Portuguese south coast

R. Rosa*, M.L. Nunes

Departamento de Inovação Tecnológica e Valorização dos Produtos da Pesca, IPIMAR, Avenida de Brasília, Lisboa 1449-006, Portugal

Received 4 February 2002; received in revised form 23 August 2002; accepted 6 November 2002

Abstract

The objectives of the present study were to characterize the benthic life strategies of *Aristeus antennatus* (Crustacea: Penaeidea), *Parapenaeus longirostris* (Crustacea: Penaeidea) and *Nephrops norvegicus* (Crustacea: Astacidea) on the basis of biochemical composition (proximate chemical composition, total lipids, glycogen and cholesterol contents), and its response to biological and environmental factors (sex, maturation, reproduction, food availability and depth) into account. The specimens were collected at depths between 200 and 600 m off the Portuguese south coast (Algarve). The nektobenthic species (*A. antennatus* and *P. longirostris*) showed higher protein, lipid, cholesterol and glycogen contents, and lower moisture content in the muscle than the benthic–endobenthic species (*N. norvegicus*). Consequently, the energy content of the nektobenthic species was also higher. Principal component analyses were used to assess the relationship between the different biochemical contents and to relate them to the biotic and abiotic factors. Depth seems to have the most important role in the observed trends of the biochemical composition. The increase of the ovarian lipid levels occurs as a result of the maturation process. The highest values were obtained in mature *N. norvegicus* females. The differences can be due to maternal investment (lipid metabolism of the female is geared to the provision of egg lipid), since *N. norvegicus* produce large lecithotrophic eggs. The biochemical differences observed in the three species did not seem to be due to distinct trophic strategies, but instead were a consequence of depth, which may have a significant interspecific effect on food intake. It was also evident that reproductive cycle has profound effects upon the biochemistry of the three species. Gonadal maturation has large associated energy costs due to the increase in biosynthetic work. Moreover, the biochemical composition would be influenced by or synchronized with seasonal feeding activity or food availability.

Keywords: Biochemical composition; Crustaceans; Deep sea; Depth; Reproduction; Food availability

1. Introduction

In comparison with the extensive literature dealing with surface-living and mesopelagic (mid-water) species, fewer studies have considered biochemistry and metabolism of deep-sea species.

*Corresponding author. Tel.: +351-21-3027000; fax: +351-21-3015948.
E-mail address: rrosa@ipimar.pt (R. Rosa).

0967-0637/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0967-0637(02)00147-4

In fact, proximate chemical composition, energy content and metabolic rates of a large number of pelagic crustaceans and fishes have been studied in temperate and subtropical latitudes (Childress and Nygaard, 1973, 1974; Bailey and Robison, 1986; Ikeda, 1988; Childress et al., 1990b; Donnely et al., 1990; Cowles et al., 1991). Several of the cited authors revealed that mesopelagic species show variability in proximate composition as a function of depth of occurrence and as a function of regional productivity. Depth and productivity both affect food availability and thus influence chemical composition. In particular, lipid and protein content (% wet weight) both decline and as a result water concentration increases with increasing depth of occurrence (Childress et al., 1990b). Moreover, mesopelagic crustaceans and fishes living at greater depths have much lower metabolic rates than shallower-living pelagic species; the ammonia excretion and oxygen consumption rates decline with increasing depth (Donnely and Torres, 1988; Ikeda, 1988; Torres and Somero, 1988; Childress et al., 1990a). It is worth noting that these physiological and biochemical changes have been attributed to factors correlated to depth, aside from the possible influence of temperature (Childress, 1971; Torres et al., 1979) or hydrostatic pressure (Teal, 1971; Meek and Childress, 1973). Childress et al. (1990a) also found that the reduction in the metabolic rate with depth was also related to reduction in mobility. According to the visual interaction hypothesis of Childress et al. (1990a), the deep-sea species have a less active prey–predator relationships than shallow-water species.

Though benthic and benthopelagic life is defined by morphological, taxonomical, trophic and metabolic features, few studies have considered metabolism and energy content of benthic deep-sea crustaceans (Smith, 1978; Childress et al., 1990a). A characterization of two deep-sea benthic decapod life strategies on the basis of oxygen consumption and energy content was made by Company and Sardà (1998), whom the benthic–endobenthic species are poor swimmers and relatively large and heavy. They have high water contents and low organic matter contents, and they also have low oxygen consumption rates and energy contents. The nektobenthic strategy is characteristic of benthic species that are nonetheless quite good swimmers, and in comparison to benthic–endobenthic and mesopelagic species, they have intermediate energy values, water contents and oxygen consumption rates.

The study described in this paper was undertaken to characterize life strategies of *Aristeus antennatus* (Crustacea: Penaeidea), *Parapenaeus longirostris* (Crustacea: Penaeidea) and *Nephrops norvegicus* (Crustacea: Astacidea) on the basis of biochemical composition. In fact, given that the organisms tend towards an optimum biochemical composition (depending upon their adaptation strategy), the levels of nitrogen compounds, carbohydrate and lipids are an expression of an animal's adaptive characteristics (Company and Sardà, 1998). Since several biotic factors (e.g. maturation, reproduction, food availability) have an important effect on the biochemistry and physiology of the decapod crustaceans, manifested in changes in their physiological ecology and behaviour, the biochemical analyses (proximate chemical composition, total lipids, glycogen and cholesterol contents) were conducted taking into account temporal variations (during a period of one year), genders and different stages of gonadal development (only in females).

2. Material and methods

2.1. Samples

The study was performed over a period of 1 year, beginning in October 2000 and concluding in September 2001. Specimens were collected monthly off the Portuguese south coast (Algarve) by a commercial trawl vessel "Costa Sul". The fishery of *A. antennatus* was mainly between 400 and 600 m, *P. longirostris* between 200 and 300 m and *Norvegicus norvegicus* between 300 and 600 m. In each month, for the biochemical analyses in the muscle, the samples were pooled in triplicate according to sex. The biochemical analyses in the gonads and hepatopancreas were done only in females and the samples were pooled in triplicate according to the different stages of ovarian

development. The ovary maturation scale of Arculeo et al. (1995) was used for *A. antennatus*, Ribeiro-Cascalho's (1987) scale for *P. longirostris* and Farmer's (1974) for *N. norvegicus*.

2.2. Proximate chemical composition

Moisture, protein, fat and ash contents were determined according to AOAC procedures (1998). Moisture content was determined by constant-weight drying in oven at 100°C, Protein levels by a modified Kjeldahl method, Using the value 6.25 as a conversion factor of total nitrogen content to protein, lipid content by the soxhlet extraction method with ethyl ether, and ash determination was performed in a muffle furnace at 550°C to constant weight. The energy content was estimated according to FAO (1989) and calculated as: proteins—4.27 kcal/g wet wt.; lipids—9.02 kcal/g wet wt.; carbohydrates—4.11 kcal/g wet wt. (1 kcal = 4.184 kJ). In this study, the carbohydrate fraction was subestimated since it was quantified only by the glycogen content.

2.3. Total lipids and cholesterol analyses

Total lipids were extracted by the Bligh and Dyer (1959) method. The quantification of cholesterol content was based on the experimental procedure of Naemmi et al. (1995) modified by Oehlenschläger (1998). The cholesterol was analysed in a Hewlett Packard 5890 gas chromatograph. The separation was carried out with helium as carrier gas in a column HP5 (30 m × 0.5 mm id). The temperatures of the oven, injector and detector were 280°C, 285°C and 300°C, respectively. Cholesterol was identified and quantified by comparison with standards (Sigma) from which a standard curve was prepared.

2.4. Glycogen analysis

Glycogen concentrations were determined according to the method described by Viles and Silverman (1949). Tissue samples were boiled with 1 ml of 33% potassium hydroxide for 15 min. After cooling, 50 µl of a saturate sodium sulphate solution and 2 ml of 96% ethanol were added. Samples were placed in an ice bath for precipitation (~30 min). Following centrifugation, the precipitate was dissolved in 0.5 ml of distilled water, again precipitated with 1 ml of ethanol and redissolved in 0.4 ml of distilled water. Glycogen was then measured by the anthrone-reagent method (38 ml of sulphuric acid concentrated was added to 15 ml of distilled water and 0.075 g of anthrone; the mixture was heated at 90°C for 20 min) and the absorbance read at 620 nm. A calibration curve was prepared with a glycogen (Sigma) standard.

2.5. Statistical analysis

Seasonal changes in the biochemical data were analysed by ANOVA. Previously, normality and homogeneity of variances were verified by Kolmogorov–Smirnov and Bartlett tests, respectively. When data did not meet the assumptions of ANOVA, the non-parametric ANOVA equivalent (Kruskal–Wallis test) was performed. Having demonstrated a significant difference somewhere among the groups with the ANOVA and Kruskal–Wallis test, we applied the Tukey Test and the Dunn Test, respectively, to find out where those differences were. The relationships between biotic/abiotic factors and biochemical composition were first investigated by correlation analyses (non-parametric Spearman correlation coefficients). Principal component analysis (PCA) was performed on the correlation matrix of the biochemical variables. The resulting loadings provided a measure of association between each original variable and the resultant principal components (Zar, 1996).

3. Results

3.1. Muscle biochemical composition of males and females

The monthly variations of the proximate chemical composition of the muscle of *Nephrops norvegicus* (N.n.), *Parapenaeus longirostris* (P.l.) and *Aristeus antennatus* (A.a.) females and males

are shown in Figs. 1 and 2, respectively. The progression of protein content through the year revealed significant seasonal variations; the highest values were obtained in the spring months (N.n. females/males: $F_{11,24}=2.54$, Tukey Test $p<0.05$, $F_{11,24}=1.58$, $p\geqslant 0.05$; P.l.: $F_{11,24}=4.63/4.25$, Tukey Test $p<0.05$; A.a. $F_{11,24}=4.54/3.73$, Tukey Test $p<0.05$). The moisture revealed significant monthly fluctuations in both genders of the three species (N.n. females/males: $F_{11,24}=4.22/3.32$, Tukey Test $p<0.05$; P.l.: $F_{11,24}=5.35/4.43$, Tukey Test $p<0.05$; A.a.: $F_{11,24}=3.44/3.25$, Tukey Test $p<0.05$). The lipid content exhibited significant seasonal changes due to the considerable rise in spring (N.n. females/males: 5.70/4.07, Tukey Test $p<0.05$; P.l.: $F_{11,24}=5.02/4.74$, Tukey Test $p<0.05$; A.a.: $F_{11,24}=5.32/4.68$, Tukey Test $p<0.05$). The ash levels did not reveal any regular seasonal trend (N.n.: $F_{11,24}=2.33/1.58$, $p>0.05$; P.l.: $F_{11,24}=1.77/1.89$, $p>0.05$; A.a.: $F_{11,24}=2.11/1.59$, $p>0.05$). The cholesterol content varied significantly between winter and summer months, being the lowest in August (N.n. females/males: $F_{11,24}=4.68/4.17$, Tukey Test $p<0.05$; P.l.: $F_{11,24}=4.55/4.09$, Tukey Test $p<0.05$; A.a. $F_{11,24}=4.37/4.28$, Tukey Test $p<0.05$). The glycogen content varied significantly throughout the year, being the highest in spring, namely April (N.n. females/males: $F_{11,24}=4.18/3.76$, Tukey Test $p<0.05$; P.l.: $F_{11,24}=4.32/4.25$, Tukey Test

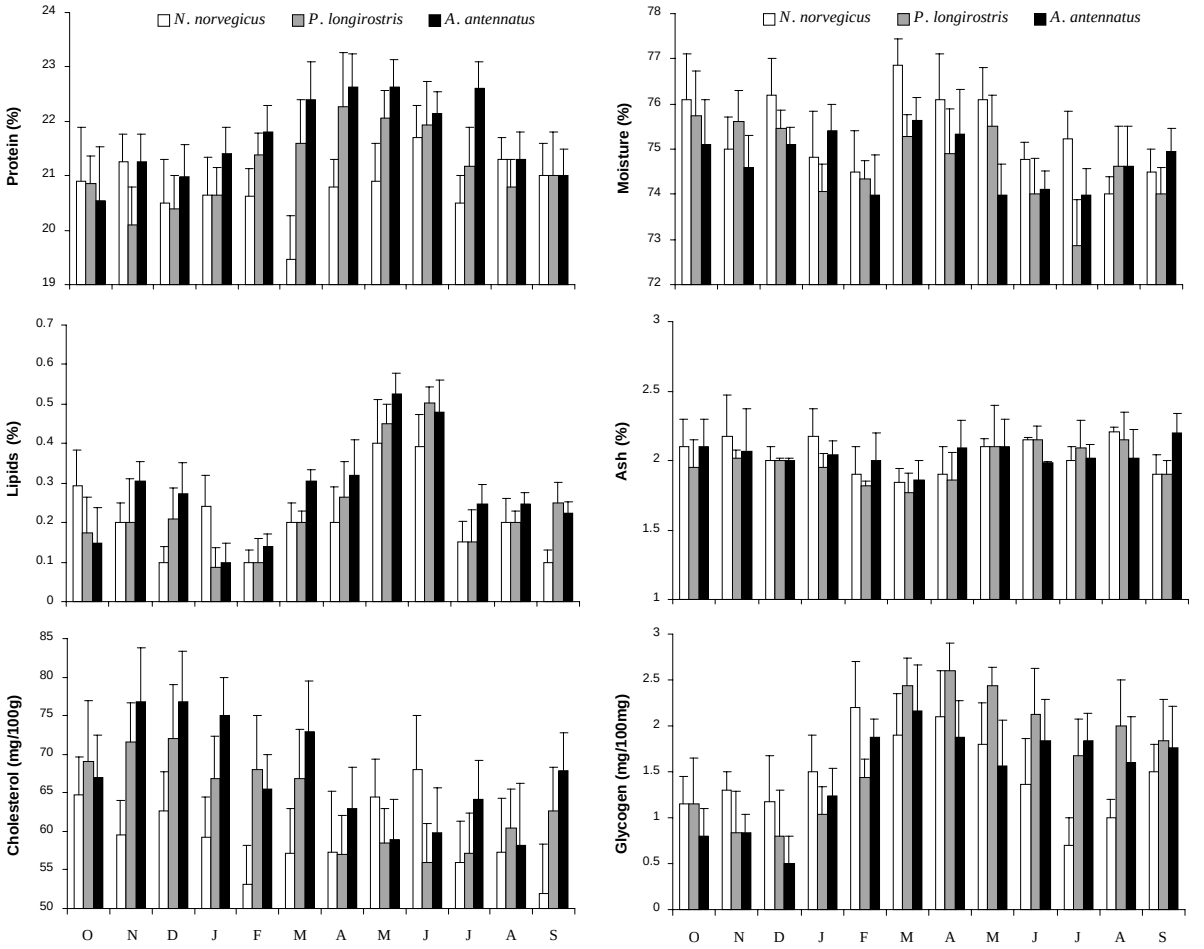

Fig. 1. Monthly fluctuations in proximate chemical composition (% wet wt.) and cholesterol (mg/100 g dry wt.) and glycogen (mg/100 mg wet wt.) contents in the muscle of *N. norvegicus*, *P. longirostris* and *A. antennatus* females.

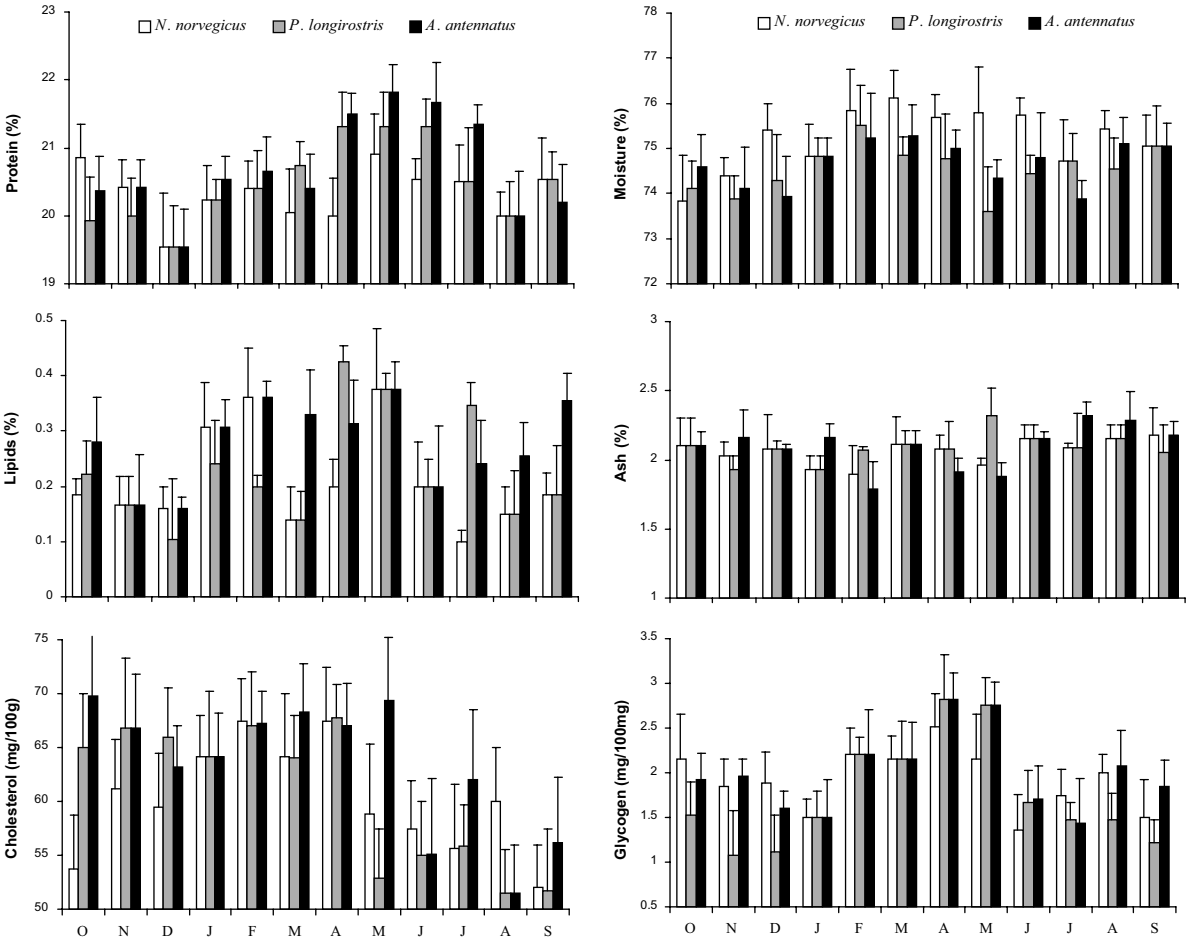

Fig. 2. Monthly fluctuations in proximate chemical composition (% wet wt.) and cholesterol (mg/100 g dry wt.) and glycogen (mg/100 mg wet wt.) contents in the muscle of *N. norvegicus*, *P. longirostris* and *A. antennatus* males.

$p<0.05$; A.a. $F_{11,24}=3.88/2.76$, Tukey Test $p<0.05$). In respect to the energy content, significant seasonal fluctuations were also attained (N.n. females/males: $F_{11,24}=3.54/2.68$, Tukey Test $p<0.05$; P.l.: $F_{11,24}=7.56/6.25$, Tukey Test $p<0.05$; A.a. $F_{11,24}=6.59/3.57$, Tukey Test $p<0.05$); in fact, the highest values were obtained in spring, namely in April/May (Fig. 3).

Biochemical variables interrelationships among the component loadings of the PCA are presented in Table 1. The first principal component (PC), explaining 29% of the variance, relates to the protein and lipid contents (as shown by the high factor loadings); the second PC, explaining 24% of the variance, relates to the cholesterol and glycogen contents. The third PC explains 17% of the variance and relates to the moisture content. As the other PCs had eigenvalues less than 1.0 and explained only a small proportion of the variance, they were not investigated any further. The three species studied could be well separated on the basis of the first 2 PCs (Fig. 4a). Most of the data belonging to *A. antennatus* had a positive value in the first PC, suggesting that this species is characterized by higher protein and lipid contents. Species distinction is not clear in the second PC. The influence of several biological (sex, maturation stage, life strategy) and environmental (season, depth) factors was also investigated and a clear pattern was obtained only with depth (Fig. 4b).

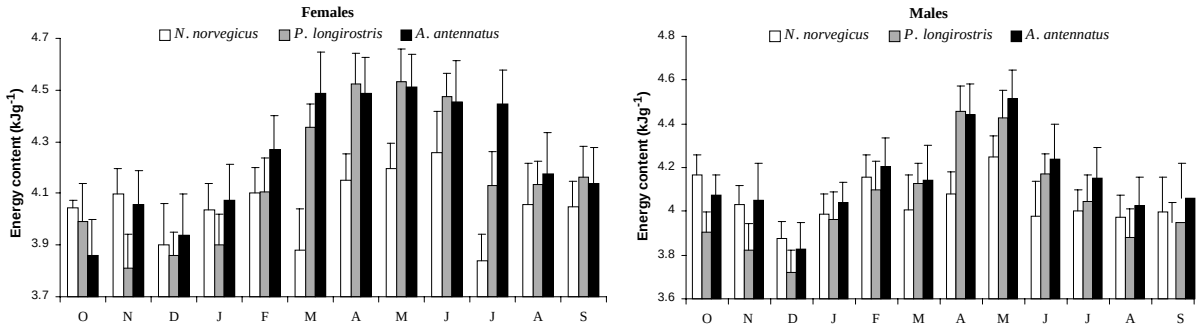

Fig. 3. Monthly fluctuations in the energy content (kJg^{-1} wet wt.) in the muscle of *N. norvegicus*, *P. longirostris* and *A. antennatus* females and males.

Table 1
Relationships between biochemical variables using principal component analysis

	Principal components		
	1	2	3
Eigenvalues	1.73	1.45	1.03
% total variance	28.80	24.20	17.23
Moisture	−0.27	−0.58	−0.58
Protein	0.78	−0.09	0.37
Lipid	0.79	−0.12	−0.04
Ash	0.63	−0.09	0.00
Glycogen	0.01	0.76	−0.48
Cholesterol	−0.09	−0.71	0.50

The highest depths were mainly observed in the positive axis of the first PC, suggesting that the increase of depth at the interval <300–500 m may positively affect the protein and lipid contents (Spearman coefficient of correlation between depth and first PC was 0.41).

3.2. Biochemical composition of female tissues in different stages of the maturation process

In order to elucidate how biochemical changes may be associated with the sexual maturation, the biochemical analyses were determined in the muscle, ovary and hepatopancreas of *N. norvegicus*, *P. longirostris* and *A. antennatus* females in different stages of the maturation process (Table 2).

The muscle protein content of immature, in maturation and mature females varied significantly between species (statistical analyses are summarized in the table with superscript letters). In fact, *N. norvegicus* females always showed lower values in relation to *P. longirostris* and *A. antennatus*. Moreover, these two nektobenthic species showed significant changes in this content during the maturation process. In respect to protein content in the ovaries, it varied significantly between species only in mature females, where the highest values were obtained for *N. norvegicus* females. In contrast, in the hepatopancreas of mature females the lowest values were also attained by this species. Moreover, in *N. norvegicus* significant variations of the protein content throughout the maturation process in this tissue was not detected.

The glycogen content did not vary significantly between species and during the gonad maturation in the three tissues. The cholesterol levels in the muscle decreased significantly during maturation and significant differences between species were observed in immature, in maturation and mature females. On the other hand, in the ovaries and hepatopancreas, the cholesterol content increased significantly during maturation. The highest values in the ovaries were obtained in *N. norvegicus* mature females. In the hepatopancreas, significant higher values were obtained in *A. antennatus* and the lowest were always attained by *N. norvegicus*.

The variations of muscle total lipids between species and during maturation were not significant. However, in the ovaries and hepatopancreas this content increased significantly during the maturation process. Significant differences were

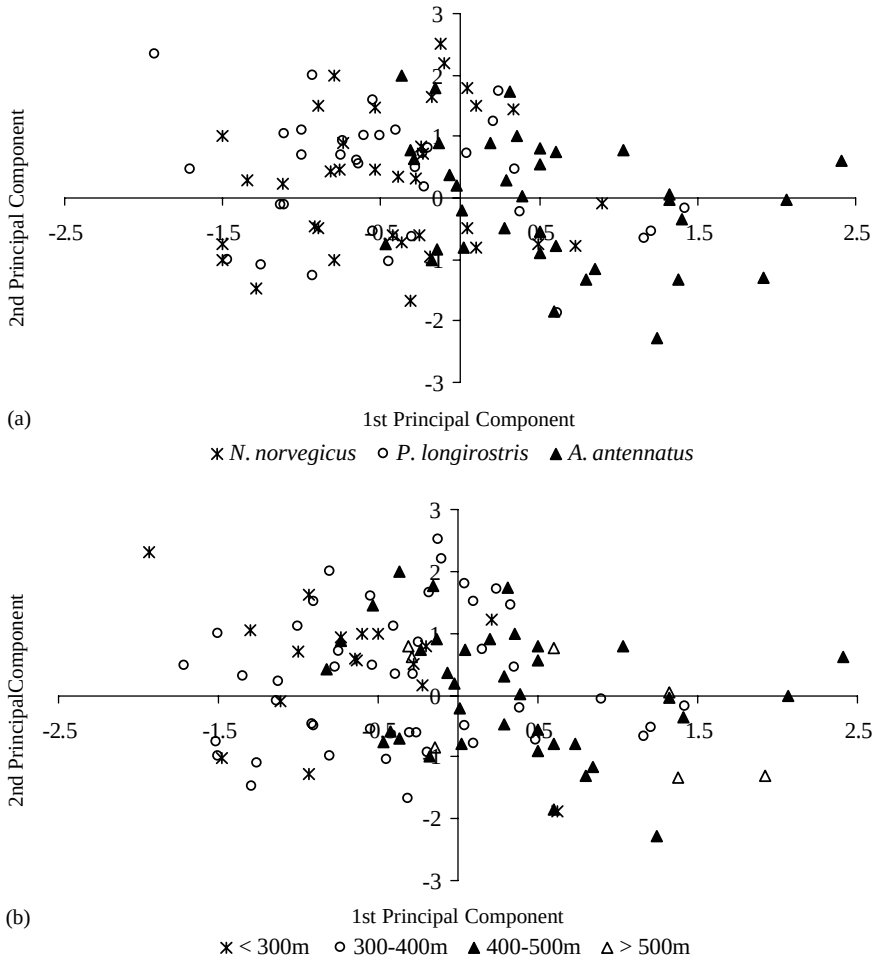

Fig. 4. Separation of (a) *N. norvegicus*, *P. longirostris* and *A. antennatus* and (b) species' occurrence depths, on the basis of the first 2 principal components.

detected between species and were similar to what was obtained with cholesterol content; in the ovaries, the highest values were obtained in *N. norvegicus* mature females and, in the hepatopancreas, significant higher values were obtained in *A. antennatus* mature females.

4. Discussion

Accumulation of energy reserves in species dependent upon unstable food resources has been reported by several authors (Slobodkin and Richman, 1961; Lee et al., 1971; Griffiths, 1977). In fact, the dependence of the deep-sea crustacean species on food resources that fluctuate in their availability (Cartes, 1994) could be an explanation for the species biochemical differences, namely in the hepatopancreas (the major storage centre of organic and inorganic reserves in decapods crustaceans) (Gibson and Barker, 1979). Both *A. antennatus* and *P. longirostris* have a highly diversified diet (as is generally characteristic of bathyal penaeoideans), probably related to a rather non-specialized hunting strategy (Cartes and Sardà, 1989) that enables them to adapt to

Table 2
Variations in protein (% wet wt.), glycogen (% wet wt.), cholesterol (mg/100 g dry wt.) and total lipids (% dry wt.) in the muscle of *Nephrops norvegicus* (N.n.), *Parapenaeus longirostris* (P.l.) and *Aristeus antennatus* (A.a.) females at different stages of the maturation process

Females		Immatures			In maturation			Matures		
		N.n.	P.l.	A.a.	N.n.	P.l.	A.a.	N.n.	P.l.	A.a.
Muscle	Protein	20.3 ± 0.3^{a}	$20.6\pm0.4^{a,b}$	21.3 ± 0.5^{b}	20.5 ± 0.5^{a}	21.2 ± 0.3^{b}	22.5 ± 0.4^{c}	$20.9\pm0.3^{a,b}$	22.3 ± 0.2^{c}	21.08 ± 0.5^{b}
	Glycogen	1.5 ± 0.4	1.9 ± 0.2	1.3 ± 0.5	1.8 ± 0.2	2.1 ± 0.2	1.8 ± 0.4	1.9 ± 0.4	1.4 ± 0.3	1.5 ± 0.2
	Cholesterol	64.5 ± 2.1^{a}	70.0 ± 2.1^{b}	76.8 ± 3.2^{c}	59.6 ± 2.8^{d}	68.8 ± 2.8^{b}	63.0 ± 2.1^{a}	59.2 ± 2.5^{d}	56.7 ± 3.2^{d}	64.2 ± 2.8^{a}
	Total lipids	2.7 ± 0.3	2.9 ± 0.3	2.8 ± 0.3	3.0 ± 0.6	3.1 ± 0.2	3.3 ± 0.5	2.6 ± 0.4	3.4 ± 0.4	3.2 ± 0.3
Ovary	Protein	$42.1\pm1.6^{a,b}$	40.3 ± 1.5^{a}	$41.5\pm1.2^{a,b}$	$43.9\pm1.4^{a,b}$	44.7 ± 1.6^{b}	45.5 ± 1.8^{b}	48.7 ± 1.2^{c}	45.7 ± 1.3^{b}	45.2 ± 1.6^{b}
	Glycogen	2.8 ± 0.3	2.7 ± 0.3	2.2 ± 0.2	2.5 ± 0.5	2.6 ± 0.5	2.4 ± 0.2	2.4 ± 0.2	2.5 ± 0.6	2.5 ± 0.3
	Cholesterol	65.3 ± 4.5^{a}	62.3 ± 4.5^{a}	62.7 ± 2.4^{a}	60.4 ± 3.7^{a}	64.4 ± 3.7^{a}	72.2 ± 3.7^{b}	80.1 ± 4.4^{c}	73.5 ± 3.4^{b}	69.9 ± 4.5^{b}
	Total lipids	21.9 ± 2.2^{a}	19.8 ± 1.9^{a}	22.3 ± 2.7^{a}	21.3 ± 1.7^{a}	25.2 ± 1.7^{b}	27.5 ± 1.7^{b}	36.5 ± 1.9^{c}	29.1 ± 2.2^{b}	$32.4\pm41.5^{b,c}$
Hepat.	Protein	8.9 ± 0.2^{a}	8.5 ± 0.4^{a}	8.8 ± 0.8^{a}	8.6 ± 0.5^{a}	8.4 ± 0.4^{a}	9.9 ± 0.6^{b}	8.7 ± 0.4^{a}	9.7 ± 0.5^{b}	10.6 ± 0.4^{c}
	Glycogen	2.9 ± 0.4	2.6 ± 0.4	2.4 ± 0.3	2.8 ± 0.3	2.7 ± 0.3	2.6 ± 0.3	3.0 ± 0.5	$2.5+0.3$	2.4 ± 0.4
	Cholesterol	112.5 ± 6.7^{a}	150.2 ± 6.7^{b}	145.6 ± 7.8^{b}	140.1 ± 5.8^{b}	173.3 ± 6.2^{c}	184.7 ± 5.8^{c}	180.2 ± 7.8^{c}	181.9 ± 5.3^{c}	191.5 ± 6.7^{d}
	Total lipids	29.9 ± 2.4^{a}	37.8 ± 2.1^{b}	41.2 ± 2.2^{b}	28.3 ± 2.1^{a}	44.6 ± 2.5^{b}	49.8 ± 3.1^{c}	45.8 ± 1.9^{b}	48.3 ± 2.6^{c}	51.5 ± 2.4^{c}

Values are the means $\pm$ SD of three pooled samples. Different superscript letters within rows represent significant differences ($p<0.05$).

possible changes in resource availability (Cartes, 1994, 1995). *N. norvegicus* feeds directly on benthic macrofauna, and detritus is abundant in its stomach contents (Cristo and Cartes, 1998).

In the present study, the two nektobenthic species (*P. longirostris* and *A. antennatus*) showed higher protein, lipid, cholesterol and glycogen contents, and lower moisture content in the muscle than the benthic–endobenthic species (*N. norvegicus*). Consequently, the energy content of the penaeideans species was also higher. A similar distinction between the two groups, with energy content (by calorimetry) and water content, were obtained by Company and Sardà (1998).

Though these species present two different trophic strategies, based on the PCA analysis, depth seems to have the most important role in the observed trends of the biochemical composition. In fact, depth influences the resource stability, i.e., it has a significant interspecific effect on food intake, which decreases with increasing depth (Labropoulou and Kostikas, 1999). The high proportion of empty stomachs in decapod species that inhabit deep slopes has been reported by several authors (Cartes and Abelló, 1992; Cartes, 1993), and it has been suggested as an adaptative strategy to the low food availability in the deep-sea environment.

The benthic marine invertebrates exhibit a seasonal cycle in activities such as feeding, growth and reproduction and this seasonality is typically correlated with coincident cycles of temperature and food availability (Brockington and Clarke, 2001). The biological response to this seasonal variability has profound effects on the biochemical composition of the organisms. For example, biochemical changes during maturation have been examined for a number of crustacean species (Pillay and Nair, 1973; Read and Caulton, 1980; Castille and Lawrence, 1989). Many of these studies were about the lipid dynamics, since the accumulation and mobilization of these organic reserves constitute one of the most significant metabolic processes in the physiology of crustaceans (Teshima et al., 1989).

In this study, the increase of lipid levels obtained in the ovaries occurred as a result of the maturation process. In fact, ovarian lipids provide fuel for the biosynthetic processes of oogenesis and vitellogenesis and are apparently taken up and accumulated by the developing oocytes (Harrison, 1990). Thus, it is normal to expect that lipid

requirements of maturing crustaceans are higher than those of juveniles and non-reproductive adults (Wouters et al., 2001). Comparing the ovarian lipid levels of the three species, the highest values were obtained in mature *N. norvegicus* females. This was expected because *N. norvegicus* produce large lecithotrophic eggs (Farmer, 1974) and the egg size has been correlated with maternal investment (lipid metabolism of the female is geared to the provision of egg lipid) (Wehrtmann and Kattner, 1998). The lipids are the main source of metabolic energy throughout embryonic development, and their amount is generally correlated with the size of the egg and with the time interval between spawning and hatching or larval first feeding (Rainuzzo et al., 1997).

The seasonal variation of protein content in the muscle of the species may be linked with changes in the feeding activity, because protein muscle loss during starvation has been observed in *N. norvegicus* (Dall, 1981). Since during that period of starvation, the abdominal muscle makes the largest contribution of protein to energy metabolism, small changes in this tissue are sufficient to make a substantial contribution to the overall animal maintenance (Barclay et al., 1983). The variations of the protein content during ovarian development can be the result of an increase in the biosynthesis of several proteins, including peptide hormones, enzymes and egg yolk proteins (namely high-density lipoproteins—HDLs), which are especially important in maturation (Yehezkel et al., 2000).

Since cholesterol is a precursor of steroid hormones (Kanazawa and Teshima, 1971), it was not surprising to find an increase of ovarian cholesterol with maturation in the three species analysed. A similar trend was observed in hepatopancreatic cholesterol, which differs from the results obtained by other authors (Adiyodi and Adiyodi, 1970; Lautier and Lagarrigue, 1988), where the decrease in hepatopancreatic cholesterol during vitellogenesis suggests that mobilization of these cholesterol stores may contribute to the build-up of ovarian cholesterol. However, the explanation of our findings could be given by the experiments of Teshima et al. (1988), which indicated that cholesterol is sequestered to the ovaries from the muscle stores. The seasonal changes in the muscle cholesterol content of the nektobenthic species seem to confirm this hypothesis: the lowest values were attained in summer, which seem to be concordant with the seasonal spawning pattern observed by Arrobas and Ribeiro-Cascalho (1987) in *A. antennatus* and by Ribeiro-Cascalho and Arrobas (1987) in *P. longirostris* from Portuguese waters. According to these authors, *A. antennatus* has one major peak of spawning in early summer (June–July) and *P. longirostris* has two peaks, one at the end of spring and another at the beginning of autumn in October. In fact, though the predominant reproductive pattern found in deep-sea organisms is continuous, reproduction throughout the year (Gage and Tyler, 1991; Tyler, 1986, 1988), these three species seem to have seasonal peaks of reproduction (Rosa and Nunes, in press). Similar findings were obtained in another deep-sea crustacean species (George and Menzies, 1967, 1968; Harrison, 1988). Moreover, Company and Sardà (1997) showed an increasing seasonality in the reproductive patterns of pandalid shrimps with depth, which can be a general trend of life-history adaptation with depth.

Among the different tissues analysed in this study, the glycogen is stored mainly in the hepatopancreas and to a lesser extent in the muscle, but according to Hagerman et al. (1990) and Baden et al. (1994) in *N. norvegicus*, the occurrence of glycogen depletion in the muscles, following hypoxia and starvation, suggests that the muscle contains a particularly important store of glycogen as it is more readily accessible when there is a shift to anaerobic metabolism or when there is a decrease in the feeding activity during winter. This can explain the seasonal variation of glycogen content in the muscles of the three species. In fact, the lowest values were obtained in the winter, which corresponds to the period of the year with the highest percentage of empty stomachs in *N. norvegicus* off the Portuguese south coast (Cristo and Cartes, 1998). In relation to the nektobenthic species, though there is no evidence of a decreasing feeding activity in this period of the year, the diet composition of *P. longirostris* and *A. antennatus* should vary significantly between seasons as in

other deep-sea crustaceans species (Cartes and Sardà, 1989; Cartes, 1993), since these changes correspond basically to the period of abundance of the different dietary groups in the deep-sea environment (Cartes, 1994).

The glycogen content did not show significant variations throughout the maturation process, contrary to what was stated by Kulkarni and Nagabhushanam (1979). Moreover, since carbohydrates have specific roles in the production of nucleic acids, as precursors of metabolic intermediates in the production of energy and non-essential amino acids, and as a component in ovarian pigments (Harrison, 1990), they have to be especially important for maturation and for embryogenesis.

Significant differences between the muscle biochemical composition of females and males were obtained. It has been hypothesized that males do not invest much energy for reproduction per se, but rather use most of it for somatic growth, i.e., males have lower energetic requirements than females to form a fully developed gonad (Kyomo, 1988; Jeckel et al., 1989).

In conclusion, the biochemical differences observed in the three species did not seem to be due to distinct trophic strategies, but instead were a consequence of depth, which must have a significant interspecific effect on food availability and food intake. It was also evident that the reproductive cycle has profound effects upon the biochemistry of the three species. Gonadal maturation has large associated energy costs due to the increase in biosynthetic work, which will support the lecithotrophic strategy (reliance on egg yolk nutrition) of the embryos and pre-feeding larval stages. Moreover, these processes seem to be influenced or synchronized with seasonal feeding activity or food availability. In addition, it is worth saying that marine invertebrate intra- and inter-population differences in biochemical composition have also been detected and related to differences in the environment, to food availability and to differential demands on resource allocation (Griffiths, 1977; Norrbin and Bamstedt, 1984). Therefore, it should be kept in mind that the present study has looked at single populations, and the biochemical differences should thus be interpreted with some caution.

Acknowledgements

The Foundation for Science and Technology (FCT) supported this study through a doctoral grant to the first author.

References

Adiyodi, K.G., Adiyodi, R.G., 1970. Endocrine control of reproduction in decapod crustacea. Biological Reviews 46, 121–165.

AOAC, 1998. Official Methods of Analysis. 16th Edition, 4th Revision. Association of Official Analytical Chemistry, Washington, DC.

Arrobas, I., Ribeiro-Cascalho, A., 1987. On the biology and fishery of *Aristeus antennatus* (Risso 1816) in the south Portuguese coast. Investigacion Pesquera 51, 233–243.

Arculeo, M., Payen, G., Cuttitta, A., Galioto, G., Riggio, S., 1995. A survey of ovarian maturation in a population of *Aristeus antennatus* (crustacea: Decapoda). Animal Biology 4, 13–18.

Baden, S.P., Depledge, M.H., Hagerman, L., 1994. Glycogen depletion and altered copper and manganese handling in *Nephrops norvegicus* following starvation and exposure to hypoxia. Marine Ecology Progress Series 103, 65–72.

Bailey, T.G., Robison, B.H., 1986. Food availability as a selective factor on the chemical composition of midwater fishes in the eastern North Pacific. Marine Biology 91, 131–141.

Barclay, M.C., Dall, W., Smith, D.M., 1983. Changes in lipid and protein during starvation and the moulting cycle in the tiger prawn, *Penaeus esculentus* Haswell. Journal of Experimental Marine Biology and Ecology 68, 229–244.

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

Brockington, S., Clarke, A., 2001. The relative influence of temperature and food on the metabolism of a marine invertebrate. Journal of Experimental Marine Biology and Ecology 258, 87–99.

Cartes, J.E., 1993. Diets of deep-water pandalid shrimps on the Western Mediterranean slope. Marine Ecology Progress Series 96, 49–61.

Cartes, J.E., 1994. Influence of depth and season on the diet of the deep-water aristeid *Aristeus antennatus* along the continental slope (400–2300 m) in the Catalan Sea (western Meditterranean). Marine Biology 120, 639–648.

Cartes, J.E., 1995. Diets of, and trophic resources exploited by, bathyal penaeoidean shrimps from the western Mediterranean. Marine Freshwater Research 46, 889–896.

Cartes, J.E., Abelló, P., 1992. Comparative feeding habits of polychelid lobsters in the Western Mediterranean deep-sea communities. Marine Ecology Progress Series 84, 139–150.

Cartes, J.E., Sardà, F., 1989. Feeding ecology of the deep-water aristeid crustacean *Aristeus antennatus*. Marine Ecology Progress Series 54, 229–238.

Castille, F.L., Lawrence, A.L., 1989. Relationship between maturation and biochemical composition of the gonads and digestive glands of the shrimps *Penaeus aztecus* and *Penaeus setiferus* (L.). Journal of Crustacean Biology 9, 202–211.

Childress, J.J., 1971. Respiratory rate and depth of occurrence of midwater animals. Limnology and Oceanography 16, 104–106.

Childress, J.J., Nygaard, M.H., 1973. The chemical composition of midwater fishes as a function of depth occurrence off Southern California. Deep-Sea Research 20, 1093–1109.

Childress, J.J., Nygaard, M.H., 1974. The chemical composition and relative buoyancy of midwater crustaceans as a function of depth occurrence off Southern California. Marine Biology 27, 225–238.

Childress, J.J., Cowles, D.L., Favuzzi, J., Mickel, T.J., 1990a. Metabolic rates of benthic deep-sea decapod crustaceans decline with increasing depth primarily due to the decline in temperature. Deep-sea Research 37 (6), 929–949.

Childress, J.J., Price, M.H., Favuzzi, J.A., Cowles, D.L., 1990b. Chemical composition of midwater fishes as a function of depth occurrence off the Hawaiian Islands: food availability as a selective factor. Marine Biology 105, 235–246.

Company, J.B., Sardà, F., 1997. Reproductive patterns and aspects of deep-water pandalid shrimp life-histories in the Western Mediterranean along a depth gradient (150–1100 m). Marine Ecology Progress Series 148, 49–58.

Company, J.B., Sardà, F., 1998. Metabolic rates and energy content of deep-sea benthic decapod crustaceans in the Western Mediterranean Sea. Deep-Sea Research I 45, 1861–1880.

Cowles, D.L., Childress, J.J., Wells, M.E., 1991. Metabolic rates of midwater crustaceans as a function of depth occurrence off the Hawaiian Islands: food availability as a selective factor? Marine Biology 110, 75–83.

Cristo, M., Cartes, J.E., 1998. A comparative study of the feeding ecology of *Nephrops norvegicus* (L.), (Decapoda: Nephropidae) in the bathyal Mediterranean and the adjacent atlantic. Sciencia Marina 62, 81–90.

Dall, W., 1981. Lipid absorption and utilization in the Norwegian lobster *Nephrops norvegicus* (L.). Journal of Experimental Marine Biology and Ecology 50, 33–45.

Donnely, J., Torres, J.J., 1988. Oxygen consumption of midwater fishes and crustaceans from the eastern Gulf of Mexico. Marine Biology 97, 483–494.

Donnely, J., Torres, J.J., Hopkins, T.L., Lancraft, T.M., 1990. Proximate composition of Antarctic mesopelagic fishes. Marine Biology 106, 13–23.

FAO, 1989. Yield and nutritional value of the commercially more important fish species. FAO Fisheries Technical Papers 309, pp. 1–187.

Farmer, A.S.D., 1974. Reproduction in *Nephrops norvegicus* (Decapoda: Nephropidae). Journal of Zoology 174, 161–183.

Gage, J.D., Tyler, P.A., 1991. Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge University Press, London.

George, R.Y., Menzies, R.J., 1967. Indication of cyclic reproduction activity in abyssal organisms. Nature 215, 878.

George, R.Y., Menzies, R.J., 1968. Further evidence for seasonal breeding cycles in the deep-sea. Nature 220, 80–87881.

Gibson, R., Barker, P.L., 1979. The decapod hepatopancreas. Oceanography and Marine Biology Annual Reviews 17, 285–346.

Griffiths, D., 1977. Caloric variation in Crustacea and other animals. Journal of Animal Ecology 46, 593–605.

Hagerman, L., Sondergaard, T., Weile, K., Hosie, D., Uglow, R.F., 1990. Aspects of blood physiology and ammonia excretion in *Nephrops norvegicus* under hypoxia. Comparative Biochemistry and Physiology 97A, 51–55.

Harrison, K., 1988. Seasonal reproduction in deep-sea Crustacea (Isopoda: Asellota). Journal of Natural History 22, 175–197.

Harrison, K.E., 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. Journal of Shellfish Research 9, 1–28.

Ikeda, T., 1988. Metabolism and chemical composition of crustaceans from the Antarctic mesopelagic zone. Deep-Sea Research 35 (12), 1991–2002.

Jeckel, W.H., Moreno, J.E., Moreno, V.J., 1989. Biochemical composition, lipid classes and fatty acids in the male reproductive system of the shrimp *Pleoticus muelleri* bate. Comparative Biochemistry and Physiology 93B, 807–811.

Kanazawa, A., Teshima, S.-I., 1971. In vivo conversation of cholesterol to steroid hormones in the spiny lobster, *Panulirus japonicus*. Bulletin of the Japanese Society of Scientific Fisheries 37, 891–897.

Kulkarni, G.K., Nagabhushanam, R., 1979. Mobilisation of organic reserves during ovarian development in a marine penaeid prawn, *Parapenaeopsis hardwickii* (Miers). Aquaculture 18, 373–377.

Kyomo, J., 1988. Analysis of the relationship between gonads and hepatopancreas in males and females of the crab *Sesarma intermedia*, with reference to resource use and reproduction. Marine Biology 97, 87–93.

Labropoulou, M., Kostikas, I., 1999. Patterns of resource use in deep-water decapods. Marine Ecology Progress Series 184, 171–182.

Lautier, J., Lagarrigue, J.-G., 1988. Lipid metabolism of the crab *Pachygrapsus marmoratus* during vitellogenesis. Biochemical Systematics and Ecology 16, 203–212.

Lee, R.F., Hirota, J., Barnett, A.M., 1971. Distribution and importance of wax esters in marine copepods and other zooplankton. Deep-Sea Research 18, 1147–1166.

Meek, R.P., Childress, J.J., 1973. Respiration and the effect of pressure in the mesopelagic fish Anoplogaster cornuta (Beryciformes). Deep-Sea Research 20, 1111–1118.

Naemmi, E.D., Ahmad, N., Al-sharrah, T.K., Behbahani, M., 1995. Rapid and simple method for determination of

cholesterol in processed food. Journal of AOAC International 78, 1522–1525.

Norrbin, F., Bamstedt, U., 1984. Energy contents in the benthic and planktonic invertebrates of Kosterfjorden, Sweden. A comparison of energetic strategies in marine organisms groups. Ophelia 23 (1), 47–64.

Oehlenschläger, J., 1998. Cholesterol content in edible part of marine fish species and crustacean shellfish. 28th Annual Meeting of WEFTA, Tromsø, Norway, October 4–7.

Pillay, K.K., Nair, N.B., 1973. Observations on the biochemical changes in gonads and other organs of *Uca annulipes*, *Portunus pelagicus*, and *Metapenaeus affinis* (Decapoda: Crustacea) during the reproductive cycle. Marine Biology 18, 167–198.

Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155, 103–116.

Read, G.H.L., Caulton, M.S., 1980. Changes in mass and chemical composition during the molt cycle and ovarian development in immature and mature *Penaeus indicus* Milne Edwards. Comparative Biochemistry and Physiology 66A, 431–437.

Ribeiro-Cascalho, A., 1987. Some biological data on *Parapenaeus longirostris* (Lucas, 1846) from the southwest coast of Portugal. ICES C.M.K. 44, 12.

Ribeiro-Cascalho, A., Arrobas, I., 1987. Observations on the biology of *Parapenaeus longirostris* (Lucas, 1846) from the south coast of Portugal. Investigacion Pesquera 51 (Suppl. 1), 201–212.

Rosa, R.A., Nunes, M.L. Changes in organ indices and lipid dynamics during the reproductive cycle of *Aristeus antennatus*, *Parapenaeus longirostris* and *Nephrops norvegicus* (Crustacea: Decapoda) females from the south Portuguese coast. Crustaceana, in press.

Slobodkin, L.B., Richman, S., 1961. Calories/gm in species of animals. Nature 191, 299.

Smith Jr., K.L., 1978. Benthic community respiration in the N.W. Atlantic Ocean: in situ measurements from 40 to 5200 m. Marine Biology 47, 337–347.

Teal, J., 1971. Pressure effects on the respiration of vertically migrating decapod crustacea. American Zoologist 11, 571–576.

Teshima, S.-I., Kanazawa, A., Koshio, S., Horinouchi, K., 1988. Lipid metabolism in destalked prawn *Penaeus japonicus*: induced maturation and transfer of lipids reserves to the ovaries. Nippon Suisan Gakkaishi 54, 1123–1129.

Teshima, S.-I., Kanazawa, A., Koshio, S., Horinouchi, K., 1989. Lipid metabolism of the prawn *Penaeus japonicus* during maturation: variation in lipid profiles of the ovary and hepatopancreas. Comparative Biochemistry and Physiology 92B, 45–49.

Torres, J.J., Somero, G.N., 1988. Metabolism, enzymatic activities and cold adaptation in Antarctic mesopelagic fishes. Marine Biology 98, 169–180.

Torres, J.J., Belman, B.W., Childress, J.J., 1979. Oxygen consumption rates of midwater fishes as a function of depth of occurrence. Deep-Sea Research 26, 185–197.

Tyler, P.A., 1986. Studies of a benthic time series: reproductive biology of benthic invertebrates in the Rockall Trough. Proceedings of the Royal Society of Edinburgh 88B, 175–190.

Tyler, P.A., 1988. Seasonality in the deep-sea. Oceanography and Marine Biology: Annual Reviews 26, 227–258.

Viles, P., Silverman, J., 1949. Determination of starch and cellulose with anthrone. Journal of Analytical Chemistry 21, 950–953.

Wehrtmann, I.S., Kattner, G., 1998. Changes in volume, biomass, and fatty acids of developing eggs in *Nauticaris magellanica* (Decapoda: Caridea): a latitudinal comparison. Journal of Crustacean Biology 18 (3), 413–422.

Wouters, R., Piguave, X., Bastidas, L., Claderón, J., Sorgeloos, P., 2001. Ovarian maturation and haemolymphatic vitellogenin concentration of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed increasing levels of total dietary lipids and HUFA. Aquaculture Research 32, 573–582.

Yehezkel, G., Chayoth, R., Abdu, U., Khalaila, I., Sagi, A., 2000. High-density lipoprotein associated with secondary vitellogenesis in the hemolymph of the crayfish *Cherax quadricarinatus*. Comparative Biochemistry and Physiology 127B, 411–421.

Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall, Upper Saddle River, NJ.