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Biometry, estimates of production and seasonal variation in the biochemical composition of *Mesopodopsis slabberi* (Van Beneden, 1861) (Crustacea: Mysidacea)

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ABSTRACT

Morphometric relationships in *Mesopodopsis slabberi* (Van Beneden, 1861) were determined using freshly caught specimens from the Mondego estuary (western Portugal). Significant positive correlations between total length (TL) and cephalic length (CL) (TL = 2.5 CL + 0.012) and between dry weight (DW) and total length (ln DW = 3.0298 ln TL – 6.0229) were found. The annual production was 13.17 mg · m^{-3} · $year^{-1}$, and the annual P/B ratio was 9.32. This turnover rate supports the hypothesis that *M. slabberi* plays an important role in the Mondego estuary's food web. Contents of proteins, carbohydrates, chitin, lipids, phospholipids, and cholesterol were determined in freshly caught juveniles, males, and females throughout the year. Statistical analysis (ANOVA) revealed significant seasonal differences in biochemical composition, as well as differences among juveniles, females, and males. Environmental conditions (e.g., trophic conditions) and reproduction appeared to be the main processes influencing the seasonal patterns of variation in biochemical composition.

Key words: Biometry, production, biochemical composition, *Mesopodopsis slabberi*, Mysidacea.

RESUMEN

Biometría, estimaciones de producción y variación estacional en la composición bioquímica de* Mesopodopsis slabberi *(Van Beneden, 1861) (Crustacea: Mysidacea)

Se ha realizado un estudio morfométrico en la especie Mesopodopsis slabberi *(Van Beneden, 1861) a partir de ejemplares frescos capturados en el estuario de Mondego. Se han encontrado correlaciones significativamente positivas entre la longitud total (TL) y la longitud cefálica (CL) (TL = 2,5 CL + 0,012) y entre el peso seco (DW) y la longitud total (ln DW = 3,0298 ln TL – 6,0229). La producción anual fue de 13,17 mg · m^{-3} · $año^{-1}$, y la relación P/B anual fue de 9,32. Esta tasa de renovación consolida la hipótesis que* M. slabberi *desempeña un papel importante en la cadena trófica del estuario de Mondego. Los contenidos en proteínas, carbohidratos, quitina, lípidos, fosfolípidos y colesterol fueron determinados en ejemplares frescos, juveniles machos y hembras, capturados a lo largo del año.*

El análisis estadístico (ANOVA) muestra diferencias estacionales significativas en la composición bioquímica y entre juveniles, machos y hembras. Las condiciones ambientales (tróficas) y la reproducción parecen ser los procesos que más influyen en los modelos estacionales de variación en la composición bioquímica.

***Palabras clave**: Biometría, producción, composición bioquímica,* Mesopodopsis slabberi*, Mysidacea.*

INTRODUCTION

Mesopodopsis slabberi (Van Beneden, 1861) is a highly abundant species in the Mondego estuary (western Portugal), and it has an important ecological function in the pelagic communities, making a substantial contribution to the pelagic standing stock (Azeiteiro, 1999; Azeiteiro and Marques, 1999; Azeiteiro, Jesus and Marques, 1999). As in other estuaries, due to its abundance, *M. slabberi* plays a key role in the energy flows from benthic to plankton communities (Azeiteiro, Jesus and Marques, 1999).

The *Mesopodopsis slabberi* population reproduces continuously in the Mondego estuary, exhibiting clear spatial and temporal (tidal and seasonal) migration patterns (Azeiteiro, Jesus and Marques, 1999). This type of migration has been described in other estuaries (Collins and Williams, 1982; Webb and Wooldridge, 1990) for *M. slabberi*, and may have underlying salinity-related reproductive significance (Greenwood, Jones and Greenwood, 1989). Although reproduction and recruitment is continuous throughout the year, the main peaks have been observed in late summer/autumn and late spring/early summer (Azeiteiro, Jesus and Marques, 1999). Also, a smaller peak was recorded in early winter (Azeiteiro, Jesus and Marques, 1999). Such a recruitment pattern suggests the occurrence of two (bivoltinism) or three (trivoltinism) generations per year (Azeiteiro, Jesus and Marques, 1999). Spring females and males die after the late spring/early summer recruitment period (Azeiteiro, Jesus and Marques, 1999). In fact, large mysids disappear from the population in June as their progeny, the first summer generation, matures (Azeiteiro, Jesus and Marques, 1999). A similar pattern has been reported for other mysids (Azeiteiro, Jesus and Marques, 1999).

Seasonal variations in body composition yield information which may be useful in the understanding of a population's ecophysiology (Lehtonen, 1996). Seasonal changes in the biochemical composition of crustaceans over their life cycles may reflect metabolic processes related to the nutritional cycle and synthesis of reproductive products.

Bearing this in mind, the main objectives of the present study were:

(1) To determine the morphometric relationships of *M. slabberi* and to estimate its production.

(2) To identify the seasonal patterns of variation in *M. slabberi* biochemical composition.

(3) To verify to what extent the seasonal changes in biochemical composition depend on environmental or sexual conditions.

MATERIALS AND METHODS

Study site and environmental conditions

In the Mondego estuary (40º 08' N, 8º 50' W) the *M. slabberi* population is basically confined to the southern arm (Azeiteiro and Marques, 1999; Azeiteiro, Jesus and Marques, 1999).

Estuarine seasonal cycles of temperature and salinity can be seen in many temperate regions of other coastal areas (Valiela, 1995). Seasonal variations in river flow decrease salinity during the winter months, and light determines the highest temperatures in the summer months (Azeiteiro, 1999). The seasonal dynamics of phytoplankton are the result of such factors as light, depth of vertical mixing, nutrient supply and grazing pressure (Azeiteiro, 1999), generally resulting in a bimodal cycle (Azeiteiro, 1999).

Sampling programme

Suprabenthic (Azeiteiro and Marques, 1999) and crepuscular plankton samples (Azeiteiro, Jesus and Marques, 1999) were collected monthly, from June 1996 to July 1997, at a station where specimens of *M. slabberi* were known to be most abundant, according to a previous population dynamics study (Azeiteiro, Jesus and Marques, 1999). Always following the same sequence, quantitative samples of mysids were taken during spring tides, from subsurface waters (60 cm diameter and 335 µm mesh net) (sub-superficial tows) at high tide and sunset, and suprabenthic tows (50 cm diameter and 500 µm mesh net) were used for low-tide diurnal samples (Azeiteiro and Marques, 1999; Azeiteiro, Jesus and Marques, 1999).

Laboratory procedures

All samples were transported back to the laboratory in good condition, both in terms of temperature and oxygen, within two hours of collection. Freshly caught animals were kept on ice at ± 4 ºC

before they were classified and separated into juveniles, females, and males (Azeiteiro, Jesus and Marques, 1999). Samples from each group were lyophilised, weighed, and stored at –30 ºC; smaller portions of this material were later weighed and used for each analysis (analytical replicates).

Morphometric and production procedures

Specimens were measured (to the nearest 0.02 mm) from the anterior tip of the carapace to the posterior tip of the telson, as well as between the anterior and posterior tips of the carapace (Wooldridge, 1986; Azeiteiro, Jesus and Marques, 1999).

Individuals were classified into the following categories (Mauchline, 1980; Sorbe, 1984; Azeiteiro, Jesus and Marques, 1999): (1) juveniles: secondary sexual characteristics absent; (2) males, and (3) females (immature females: secondary sexual characteristics in process of development; mature incubant females: marsupium fully developed and carrying eggs or embryos; and mature empty females: with empty expanded marsupium from which young have recently emerged).

To estimate mysid standing stock (mg of dry weight · m^{-3}) it was necessary to determine the relationships between cephalic length and dry weight and total length and dry weight. For this purpose, a set of specimens were measured and then dried for 24 h at 60 ºC (Wooldridge, 1986; Azeiteiro, Jesus and Marques, 1999), and individually weighed (to the nearest 0.01 mg) with a Mettler microbalance. Following Matthews (1973) and Jørgensen and Matthews (1975), we pooled the entire set of specimens, belonging to the different categories, in order to estimate morphometric relationships considered valid for the whole population (Azeiteiro, Jesus and Marques, 1999).

Production was estimated using the Hynes, 1961 (in Sorbe, 1984) average cohort method, modified by Benke (1979), with particular significance in multivoltine populations, and called the size-frequency method by Waters and Hokenstrom (1980). The Hynes method for estimating aquatic invertebrate production involves, firstly, estimating the total number of individuals that developed into each size class, and then the calculation of losses in numbers between size classes. Production is then estimated as the sum of biomass losses between successive size classes. The Hynes method does not require the recognition and tracking of individual cohorts. It is therefore suitable for populations with continuous reproduction and no synchronization of larval release and egg extrusion (Sorbe, 1984; Azeiteiro, Jesus and Marques, 1999; Cunha, 2000).

Biochemical analyses

Proteins

Lyophilised material was homogenised using the proportion of 0.5 mg to 3 ml of pure water into different 10 ml test tubes. The water-soluble protein content was analysed using the method of Lowry *et al.* (1951), as modified by Fernandes *et al.* (1994).

Carbohydrates

We prepared the samples for analysis following essentially the same procedure as for proteins, except for the fact that taller test tubes were used. The homogenates were analysed using the method of Raymont *et al.*, as described in Båmstedt (1976) and Omori and Ikeda (1984), using 1 ml of 5 % phenol solution and 5 ml of concentrated sulphuric acid.

Chitin

The analysis was performed using the Båmstedt (1976) method for dried homogenised material. However, instead of being incinerated, the chitin final product was analysed for its content in carbohydrate using the method described above.

Total lipids

The analysis was performed following the method described by Lehtonen (1996). Approximately 15 mg of lyophilised material was weighed and homogenised in 0.5 ml of a chloroform/methanol (2:1) solution, and then centrifuged during 30 seconds. The precipitate was washed a second time with 0.5 ml of chloroform/methanol (2:1) and centrifuged again for 30 seconds. We added 20 % volume of 0.9 % NaCl solution to the chloroform/methanol (2:1) solution from both washes, and centrifuged again. The chloroform

phase containing the lipids in solvent solution was placed into tared cups and the solvents were evaporated. Following the evaporation of the solvent solution, the cups were weighed and the weight of the lipids calculated.

Lipid extracts were analysed for their phospholipid content by phosphate quantification. For this purpose we used the Bartlett (1958) phosphate determination with a Fiske and Subbarow reducing agent, according to the method described in Lowestein (1969). All volumes were reduced to one half of those indicated in the original description, and spectrophotometer readings were conducted at 830 nm.

Lipid extracts were also analysed for their cholesterol content using the method described by Fernandes *et al.* (1994). The lipid chloroform extraction was evaporated to complete dryness. Lipids were then dissolved with 20 ml of acetic acid and allowed to react with 1 ml of Liebermann-Burcherd reagent as described by Huang *et al.* (1961), adapted to tissue by Fernandes (pers. comm.). We did not consider it necessary to separate cholesterol from other lipids (Fernandes *et al.*, 1994), as there was no pigment interfering with spectrophotometer readings.

Data analysis

We performed an Analysis of Variance (ANOVA) to test differences between sexes and between months for all the components considered. A two-factor ANOVA or factorial analysis of variance was carried out in the first instance to test for interaction among factors (Zar, 1996). Since interaction was found, the means of levels were not compared (Zar, 1996). It was therefore necessary to perform a one-way ANOVA for each factor, in order to reveal significant differences among the levels of a factor.

RESULTS

The population density changed throughout the year, peaking in autumn (35 indiv · m^{-3} in October) and during spring (17 indiv · m^{-3} in the second half of May). No individuals were collected in August 1996, and a very sharp decrease was observed during the winter (January and February) (Azeiteiro, Jesus and Marques, 1999). In June, July and September 1996 and April 1997, the biochemical analyses could not be carried out because of the small biomass collected.

The following body size/weight relationships were estimated for freshly caught specimens of *M. slabberi* (figure 1): TL = 2.5 × CL + 0.012, ln DW = 3.0298 × ln TL - 6.0229 (TL: Total Length; CL: Cephalic Length; DW: Dry Weight).

Production estimates are summarised in table I. The annual net production was calculated at 13.17 mg · m^{-3} · $year^{-1}$, and the P/B ratio was estimated at 9.32.

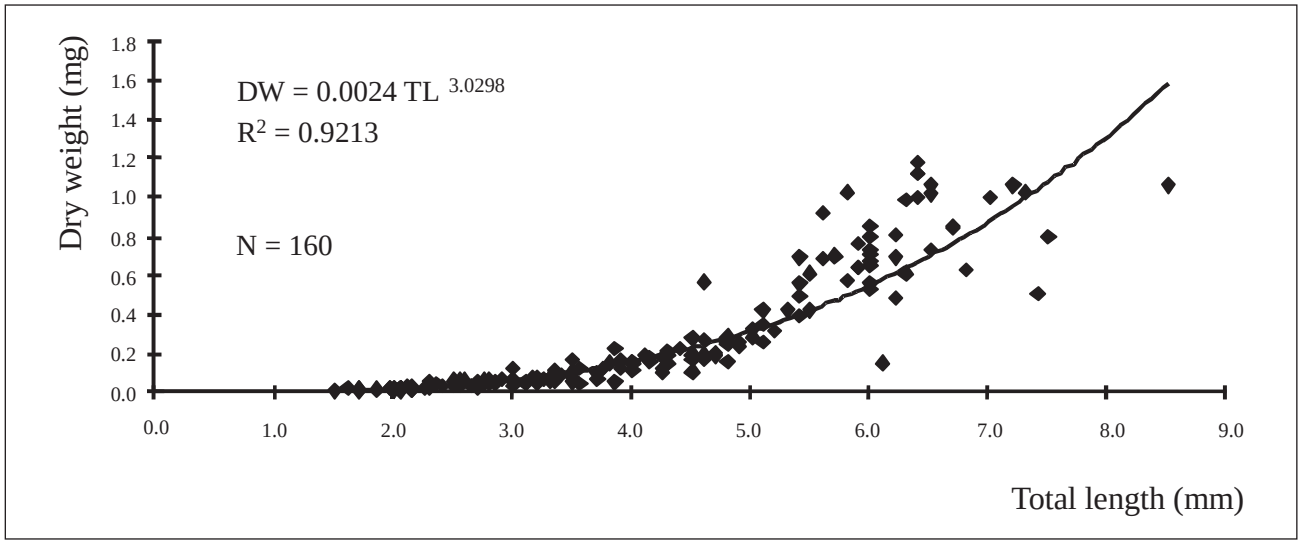

Figure 1. Regression model for weight-length relationships in *Mesopodopsis slabberi* from the Mondego estuary. Coefficient of determination (R^2) and sample size (N) are indicated

Table I. Production estimates for *M. slabberi* in the Mondego estuary. The Hynes average cohort method involves an estimation of the total number of individuals that developed into each size class, and then the calculation of loss numbers between size classes ($dm_j - dm_{j+1}$). Production is then estimated as the sum of biomass losses between successive size classes $P = \{ i \Sigma (dm_j - dm_{j+1}) (W_j \cdot W_{j+1})^{1/2} \} 365/CPI$ (annual) where CPI is the cohort production interval (the cohort longevity). The annual average biomass of the population ($mg \cdot m^{-3}$) is given by the expression ($\Sigma dm_j \cdot W_j$)

Total length (mm)	Year density	dm	$dm_j - dm_{j+1}$	W (mg)	($mg \cdot m^{-3}$)	$(W_j \cdot W_{j+1})^{1/2}$	Lost biomass	Production
1.450	0.67	5.5E-02		7.390E-03	4.098E-04			
1.825	4.41	3.7E-01	-3.1E-01	1.484E-02	5.458E-03	1.047E-02	-0.0033	-5.888E-02
2.200	12.30	1.0E+00	-6.6E-01	2.614E-02	2.680E-02	1.970E-02	-0.0129	-2.330E-01
2.575	19.41	1.6E+00	-5.9E-01	4.212E-02	6.815E-02	3.319E-02	-0.0197	-3.541E-01
2.950	20.15	1.7E+00	-6.2E-02	6.360E-02	1.068E-01	5.176E-02	-0.0032	-5.750E-02
3.325	14.47	1.2E+00	4.7E-01	9.140E-02	1.102E-01	7.624E-02	0.0361	6.504E-01
3.700	13.13	1.1E+00	1.1E-01	1.263E-01	1.383E-01	1.075E-01	0.0119	2.150E-01
4.075	10.04	8.4E-01	2.6E-01	1.693E-01	1.416E-01	1.462E-01	0.0377	6.788E-01
4.450	7.54	6.3E-01	2.1E-01	2.210E-01	1.388E-01	1.934E-01	0.0404	7.266E-01
4.825	3.62	3.0E-01	3.3E-01	2.824E-01	8.513E-02	2.499E-01	0.0816	1.469E+00
5.200	4.27	3.6E-01	-5.5E-02	3.543E-01	1.261E-01	3.164E-01	-0.0173	-3.106E-01
5.575	4.00	3.3E-01	2.3E-02	4.376E-01	1.458E-01	3.938E-01	0.0090	1.615E-01
5.950	2.17	1.8E-01	1.5E-01	5.330E-01	9.621E-02	4.829E-01	0.0737	1.327E+00
6.325	1.30	1.1E-01	7.2E-02	6.414E-01	6.937E-02	5.847E-01	0.0423	7.615E-01
6.700	1.19	9.9E-02	9.2E-03	7.638E-01	7.555E-02	6.999E-01	0.0065	1.162E-01
7.075	0.49	4.0E-02	5.8E-02	9.008E-01	3.648E-02	8.294E-01	0.0485	8.723E-01
7.450	0.35	2.9E-02	1.1E-02	1.053E+00	3.073E-02	9.741E-01	0.0110	1.986E-01
7.825	0.10	8.6E-03	2.1E-02	1.222E+00	1.047E-02	1.135E+00	0.0234	4.209E-01
			8.6E-03			5.882E-06	0.0000	9.068E-07
				Sum (biom.) = 1.412E+00				Sum (p) = 6.583E+00
				P = 13.167 mg · m-3 · year-1 P/B = 9.32				

Proteins

This was the primary body component throughout the year, constituting on average more then half of the dry weight (figure 2). Protein contents (% of dry weight) varied between 58.2 and 74.8 % for juveniles, 61.7 and 83.8 % for females, and 58.1 and 78.7 % for males. In general, we observed that, with the exception of the winter season, the protein contents in juveniles were lower than in females and males. Juveniles showed a small decrease in protein proportion in November, followed by a slow increase until the beginning of May. By the end of May, a new and more accentuated decrease was observed, followed again by a slow increase. Female protein contents exhibited a permanent variation throughout the year, with the lowest and highest values being observed in May, at the beginning and the end of the month, respectively. A small decrease was also observed in November. Males never showed a great deal of variation in protein proportion, except for a clear decrease observed in December and a smaller one observed in June.

Carbohydrates

Juveniles consistently presented a higher carbohydrate proportion than females or males, which showed a similar variation (figure 2). In fact, carbohydrate proportion (% of dry weight) varied between 6.24 and 16.12 %, for juveniles, between 4.86 and 28.99 % for females, and between 5.2 and 30.89 % for males. Juveniles showed the lowest value in November and the maximum at the end of May. With regard to males, we also observed the lowest values during winter, with a minimum in November, and the highest ones during spring, with a maximum in October and the beginning of May.

Chitin

The variation of chitin proportion was basically similar in juveniles, females, and males, with the average varying between 0.48 and 7 %. The highest values were observed in December and the lowest during spring.

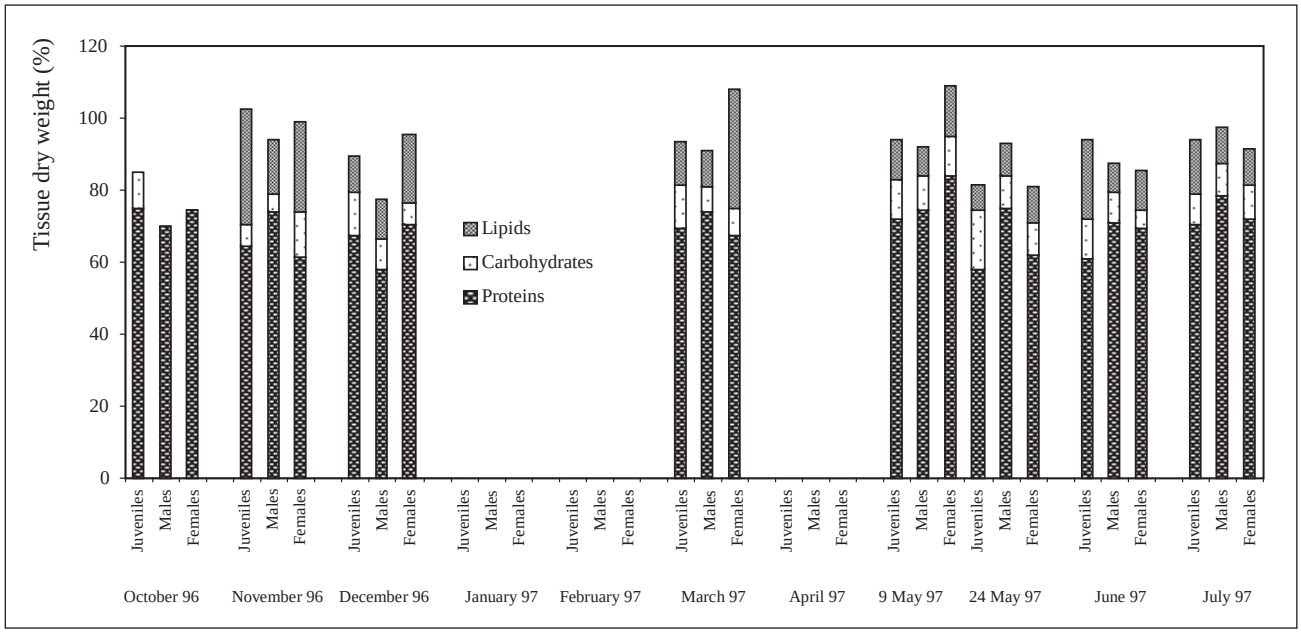

Figure 2. Seasonal changes in the relative amounts of several organic compounds (proteins, carbohydrates and total lipids, expressed as a % of tissue dry weight) in *Mesopodopsis slabberi* from the Mondego estuary

Lipids

The variation in lipid proportion was significantly different between juveniles and females, on the one hand, and males on the other. In fact, lipid contents (% of dry weight) varied from 7 to 42 % in juveniles, between 10 and 43 % in females, and from 8 to 15 % in males (figure 2). Juveniles showed the highest values in November, then a clear decrease, with low values being kept during the winter and early spring, followed by a new increase in June. Female lipid proportion exhibited a peak in March. In males, there was almost no variation throughout the year, although slightly higher values could be found during the winter.

Phospholipids

There was little variation in phospholipids proportion over the course of the year in all of the population groups. Phospholipids proportion (% of dry weight) ranged between 1.1 and 2.12 % in juveniles, from 1.12 to 2.17 % in females, and between 1.1 and 1.63 % in males (figure 3). Juveniles exhibited higher values in November and in June, while the lowest ones were recorded in May. Female phospholipids proportion showed slightly higher values in November and again in March and July. Finally, in males, there was no noticeable variation.

Cholesterol

Yearly variation was clearly more evident in juveniles than in the adults. In fact, cholesterol proportion (% of dry weight) ranged between 0.39 and 1.68 % in juveniles, while in females and males it ranged, respectively, from 0.41 to 0.61 % and from 0.57 to 0.73 % (figure 3). Therefore, while adults almost always presented the same cholesterol proportion, juveniles exhibited a strong variation throughout the year, with the highest values in December, early May, and June, and the lowest ones in March, the end of May, and July.

Triglycerides constituted the main fraction of lipid proportion (figure 3).

The proportion of ash weight in the total dry weight changed over the year, reaching a minimum in October (4.6 %) and a maximum in July (7.4 %) (Azeiteiro, Jesus and Marques, 1999)

The estimation of variation coefficients (VC) (VC = SD × 100/average) (Båmstedt, 1978) provided an index of the relative variability for each biochemical component. The largest variations occurred in carbohydrates and lipids, while proteins were the most stable components (table II).

ANOVA results are summarised in table III. The factors tested were sex (considering juveniles, females, and males) and months (with 8 levels,

Table II. Estimation of variation coefficients (VC) (VC = SD × 100/average) (Båmstedt, 1978) provided an index of the relative variability for each biochemical component

	Juveniles	Males	Females
Proteins	5.7	5	6
Carbohydrates	23.4	19.6	23.7
Lipids	18.4	13.45	14.7

coinciding with sampling dates: October, November, December, March, 9 May, 24 May, June and July).

DISCUSSION

Body size/weight relationships were consistent with values previously reported for other mysid species (Ladurantaye and Lacroix, 1980; Allen, 1984; Sorbe, 1984; Chigbu and Sibley, 1996).

The yearly cycle, as with other estuarine/neritic temperate species, appears to adjust to seasonal changes in each particular environment (Johnston and Northcote, 1989). *M. slabberi* feeds on phytoplankton (Webb, Perissinotto and Wooldridge, 1987), and the determining factors affecting its population dynamics and production in the Mondego estuary are temperature, salinity, oxygen, and chlorophyll *a* biomass (Azeiteiro, Jesus and Marques, 1999).

Marques *et al.* (1994) claimed that the prevailing conditions in the Mondego estuary, namely eutrophication, could result in the development of opportunistic adaptive strategies among invertebrate species. This may be related to the fact that the production of *M. slabberi* was found to be relatively low compared to other species in other systems. The P/B of the available measurements for zooplankton present a modal turnover rate of approximately 10-20 times a year (Valiela, 1995). The few available P/B ratios of meiofauna indicate an annual turnover of about 10 times, considerably higher than those of larger macrofauna (whose modal macrobenthic turnover rate is 1-2 times annually) (Valiela, 1995) (table IV provides a comparison between our results and data on the annual P/B ratios of different mysids). The high specific production –what we termed the P/B– of the *M. slabberi* population makes it an important producer. The faster turnover of smaller organisms means that although the biomass of small-sized species may be lower than that of larger species, the P/B of smaller species makes them proportionately more important producers than larger species (Valiela, 1995). The *M. slabberi* production and turnover rates support the hypothesis that this species plays a relevant role in the food web's energy flow (Azeiteiro, Jesus and Marques, 1999).

The marine crustaceans show a seasonal variation in biochemical composition. The protein,

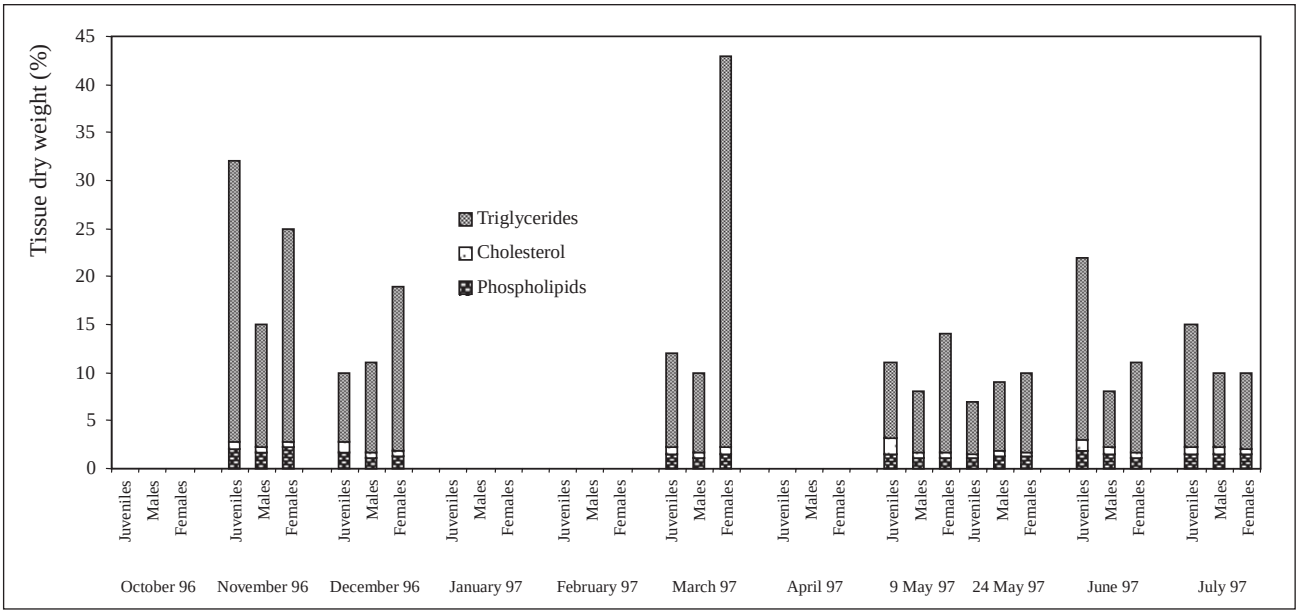

Figure 3. Seasonal changes in the relative amounts of the lipid component (phospholipids, cholesterol and tryglicerides, expressed as a % of tissue dry weight) in *Mesopodopsis slabberi* from the Mondego estuary

Table III. Analysis of variance: The variables under consideration are proteins, carbohydrates, chitin, phospholipids and cholesterol proportions (expressed as % of total dry weight), in juveniles, females, and males of *Mesopodopsis slabberi* over a thirteen months period. The factors tested were sex (considering juveniles, females, and males) and month (with 8 levels coincident with sampling dates*: October, November, December, March, 9 May, 24 May, June and July). (*): Specimens were collected only in these sampling dates (df): degrees of freedom; (F calc): calculated F-value; (P-value): probability value; (MS): mean square; (F crit): critical F-value ($p \leq 0.05$)

ANOVA: Two-factor with replication for testing interaction between factors: Sex groups _ months groups				ANOVA: Single factor for testing interaction between all groups for each biochemical component					
Biochemical component	df	F calc	P-value	Source of variation	df	MS	F calc	P-value	F crit
Proteins	14	5.5085	4.4655E-08	Between groups	23	0.0340	7.6636	1.391E-14	1.6197
				Within groups	120	4.439E-3			
Carbohydrates	14	4.3935	2.7809E-06	Between groups	23	0.0235	4.1492	1.411E-07	1.6197
				Within groups	120	5.675E-3			
Chitin	10	8.0821	3.5119E-09	Between groups	16	0.0242	7.8510	4.974E-11	1.7639
				Within groups	85	3.088E-3			
Phospholipids	12	2.9989	1.2150E-03	Between groups	20	0.0019	4.0721	1.115E-06	1.6714
				Within groups	105	4.712E-4			
Cholesterol	12	21.3605	1.1673E-20	Between groups	20	0.0010	34.2887	4.318E-32	1.6968
				Within groups	84	3.001E-3			

lipid and carbohydrate accumulation cycles show their maxima in spring, apparently related to variations in nutritional availability, the breeding cycle (Lethonen, 1996) and temperature (Clarke, 1977). Carbohydrates, lipids and proteins reached their maxima in late autumn (November) and spring. The species appeared to accumulate lipids coinciding with the beginning of the phytoplankton blooms in February (Azeiteiro, 1999). Females showed their lipid accumulation peak in March, and the juveniles in November, after a massive recruitment period occurring in late summer/autumn and spring/early summer, and the third minor phytoplankton bloom in September and October (Azeiteiro, 1999). Females seem to restore their body mass after the main recruitment periods when phospholipids peak. Gender influence is evident in a certain lack of synchronisation in reaching spring maxima.

Our results were basically consistent with the literature on the biochemical composition of crustaceans (Raymont, Austin and Linford, 1964, 1968; Båmstedt, 1975, 1976, 1978; Lethonen, 1996), particularly regarding other mysid species (e.g., *Boreomysis arctica* Kroyer and *Neomysis integer* Leach). Although there are few studies on mysids comparable to our results, these were consistent with data obtained for a number of benthic and suprabenthic peracarid species (Raymont, Austin and Linford, 1964, 1968; Båmstedt, 1975, 1976, 1978; Johnson and Hopkins, 1978; Omori and Ikeda, 1984; Lethonen, 1996).

Proteins were, proportionally, the main body component throughout the year, and also the most stable. In other groups (Moss and Lawrence, 1972; Ortega, López de Pariza and Navarro, 1984) the slight fluctuations in protein content were related to the water content of tissue, which suggests that protein variation reflects seasonal fluctuations in the tissues' hydration level (Ortega, López de Pariza and Navarro, 1984). The minimum values obtained in November for juveniles and females, and in December for males, both months of adverse environmental conditions, suggest that weight loss during winter months (Azeiteiro, Jesus and Marques, 1999) may also be sustained by proteins. Carbohydrates, and particularly lipids, displayed apparent seasonal variations. The carbohydrates showed a high temporal variability, which indicates both rapid accumulation and depletion (easily accessible reserve). Although a high accumulation occurred in autumn (October), it decreased towards winter (starvation period), particularly in the case of females. Juveniles showed the lowest value in November and the highest at the end of May. With regard to adults, we also observed the lowest values during winter, with minima in November and December for males and females

Table IV. Annual P/B ratios for other mysid species

	Reference	Annual P/B ratios
Neomysis americana	Richards and Riley, 1967	3.66
Gastrosaccus spinifer	Arntz, 1971	2.00
Mysis relicta	Hakala, 1978	3.0-3.8
Mysis relicta	Sell, 1982	2.2-3.3
Neomysis integer	Bremer and Vijverberg, 1982	4.00
Rhopalophthalmus terranatalis	Wooldridge, 1986	8.66
Mesopodopsis wooldridgei	Wooldridge, 1986	8.00
Rhopalophthalmus terranatalis	Wooldridge, 1986	7.85
Anchialina agilis	Sorbe, 1984	4.29
Schistomysis ornata	Sorbe, 1984	6.09
Schistomysis kervillei	San Vicente and Sorbe, 1990	9.38
Schistomysis parkeri	San Vicente and Sorbe, 1993	9.73
Schistomysis spiritus	San Vicente and Sorbe, 1995	6.77
Mesopodopsis slabberi	Azeiteiro, Jesus and Marques, 2001 (This paper)	9.32

respectively, and the highest ones during spring, with a maximum in May, following the higher chlorophyll *a* concentration months (Azeiteiro, 1999). The variation of chitin proportion was essentially similar in juveniles, females, and males. The highest values were observed in December and the lowest during spring; however, this might not reflect an absolute variation in chitin, but rather a changing relationship between the surface and volume of the individuals as they pass from a winter starvation period to a well-nourished spring (Azeiteiro, Jesus and Marques, 1999). Although these data are unavailable, we believe, based on our protein variation results, that they would confirm this hypothesis. Protein, as the main body component, is the most important component for weight variations, and has its lowest values in winter months. Lipid variation is a function of metabolism and reproductive strategy, depending therefore on the species's yearly cycle. In fact, many life-span traits of aquatic invertebrates depend on investments in depot lipids (Lehtonen, 1996; Ohman, 1997). In the present study, we observed seasonal variations in cholesterol and phospholipids. Phospholipids showed a continuous raise from December to July in males, June to July in females, and May to June in juveniles, which may indicate their structural role. Yearly variation in cholesterol was clearly more evident in juveniles than in adults. Therefore, while adults always presented essentially the same cholesterol contents (except for a slight decrease in June in females), juveniles exhibited a strong variation throughout the year, with the highest values in November, December, March, and June, which are also peak recruitment months (Azeiteiro, Jesus and Marques, 1999). This may indicate cholesterol's role as a precursor of growth hormones. Seasonal changes in lipids (especially triglycerides) and carbohydrates, i.e., the major cellular components in which energy is stored, appear to be mainly a function of the nutritional cycle (since they peak after favourable environmental trophic conditions) (Azeiteiro, 1999), while changes in phospholipids and cholesterol appear to be directly related to the reproductive cycle. In fact, as structural components of cell membranes, phospholipids forcibly change depending on cell proliferation and degeneration. On the other hand, cholesterol has a dual role as a structural component of cell membranes and as a precursor of sexual hormones involved in the reproductive control of crustaceans (Sastry, 1983).

In *M. slabberi*, as in other crustaceans, biochemical changes apparently resulted from metabolic needs in relation to the nutritional cycle and synthesis of reproductive products. Therefore, both environmental and trophic conditions appear to play an important role in determining seasonal changes in biochemical composition.

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