

# The influence of environmental factors on the population dynamics, reproductive biology and productivity of *Echinogammarus marinus* Leach (Amphipoda, Gammaridae) in the Mondego estuary (Portugal)

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**Abstract** – The population density of *Echinogammarus marinus* in the Mondego estuary changed throughout the year, with a maximum during spring. The lowest densities were found in the north arm of the estuary, and the highest ones in the inner areas of the south arm. Higher densities appeared associated with the presence of muddy deposits under *Fucus vesiculosus* (Phaeophyta) and also with the presence of green macroalgae biomass over the sediments. Females were morphologically recognisable at smaller sizes than males, but males became larger than females. Fecundity increases with the size of females and is influenced by temperature and salinity. Sexual activity and recruitment take place continuously throughout the year, although it almost ceases by the end of winter. Present results are in opposition to the hypothesis of discontinuous recruitment presented in a previous study. Productivity (ash free dry weight- AFDW) was estimated at 1.74 to 2.45 g·m<sup>-2</sup>·year<sup>-1</sup> in the north arm of the estuary corresponding to an annual turnover ratio ( $P/\bar{B}$ ) of 4.14 to 6.18. In the south arm, productivity was estimated at 1.96 to 2.74 g AFDW·m<sup>-2</sup>·year<sup>-1</sup> in the middle section ( $P/\bar{B}$  of 4.68 to 6.56), and at 3.85 to 5.38 g AFDW·m<sup>-2</sup>·year<sup>-1</sup> in the innermost sampling area ( $P/\bar{B}$  of 4.54 to 6.36). Differences in productivity appeared to depend only on population density, while annual  $P/\bar{B}$  ratios were similar over the estuary. Evidence was found that several features of *E. marinus* population dynamics were dependent on environmental factors resulting from the particular estuary hydraulic regime. © 2001 Éditions scientifiques et médicales Elsevier SAS

*Echinogammarus marinus* / environmental factors / population dynamics / productivity / reproductive biology

## 1. INTRODUCTION

*Echinogammarus marinus*, a strongly euryhaline amphipod, able to support long emersion periods [43], is extremely well adapted to life in estuaries. The species distribution extends from Norway to Portugal, having the Mondego estuary as the southern most limit [25, 26, 27].

In the Mondego estuary, *E. marinus* is the most abundant species in hard substrate intertidal communities [28]. It constitutes an important prey for upper trophic levels, namely for birds [38, 39], therefore playing an important role in the trophic web energy transfers. *E. marinus* occurs in close association with *Fucus vesiculosus*, a brown macroalgae, which constitutes an eulittoral belt on hard substrates. Rocky

margins make up about 60% of the perimeter of the Mondego estuary, representing therefore a significant part of the intertidal area, and are essentially the result of the installation of human activities (harbour facilities, aquaculture farms, salt works). *E. marinus* is typically found underneath *Fucus*, in association with muddy deposits, being less common on clean sands [26].

During the last decade seasonal intertidal macroalgae blooms (mainly of *Enteromorpha* spp.) have been reported in the south arm of the Mondego estuary due to nutrient enrichment of estuarine waters [14, 24, 31, 32, 40, 41]. This benthic eutrophication has been giving rise to qualitative changes in the Mondego estuary benthic community [24].

In Europe some studies were already carried out on different aspects of *E. marinus* biology and ecology [25, 26, 43, 47, 48]. A previous study on the biology,

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population dynamics, and productivity of *E. marinus* in the Mondego estuary was carried out in 1986/87 [27]. The major results of this study were: seasonal change of *E. marinus* population density, with maximum during spring and summer, continuous sexual activity throughout the year, being the recruitment described as discontinuous. Based on recognition of cohorts the authors performed the estimation of *E. marinus* lifespan (ten to twelve months), growth production (6.36 to 8.81 g AFDW·m<sup>-2</sup>·year<sup>-1</sup>) and other life cycle traits (iteroparous females; semi-annual species). However, monthly sampling periodicity then used was not fully adequate [15] and consequently some assumptions could be incorrect.

Uncertainties about the biological cycle of *E. marinus*, and the possible effect of the ongoing eutrophication process lead to a new field study carried out in 1994/95. The purpose was to enlighten the response of *E. marinus* population features to environmental factors, namely comparing the two arms of the estuary, and to produce more reliable information on the contribution of this abundant species to the estuarine food web.

## 2. MATERIALS AND METHODS

### 2.1. Study area

The Mondego estuary consists of two arms, north and south, that become separated by an island at the estuarine upstream area, at about 7 km from the sea,

joining again near the mouth (figure 1). The two arms of the estuary present very different hydrographical characteristics. The north arm is deeper (5 to 10 m during high tide, tidal range about 2 to 3 m), while the south arm (2 to 4 m deep, during high tide) is almost silted up in the upstream areas, which causes the freshwater of the river to flow essentially by the north arm. The water circulation in the south arm is mostly due to tides and to the relatively small freshwater input of a tributary, the Pranto River, which is artificially controlled by a sluice, located at 3 Km from the confluence with the south arm of the estuary (figure 1). In addition, due to differences in depth, the penetration of the tide is faster in the north arm, causing daily changes in salinity to be much stronger, whereas daily temperature changes are higher in the south arm [28, 29].

### 2.2. Field programme

The *E. marinus* population was followed for 13 months at three sampling stations, one in the north arm and two in the south arm (figure 1). Results from previous studies were taken into account in defining the sampling strategy. Sampling stations were located in order to represent different conditions where *E. marinus* occurs in the estuary. Sampling was performed during low tide, which facilitated the access to the sampling sites. Samples were taken fortnightly from November 1994 to December 1995. Five replicates were taken randomly each time at each station. Each replicate was obtained by scraping the rocky surface where the algae were attached. In addition, the superficial 1 cm sediment layer around the rocks,

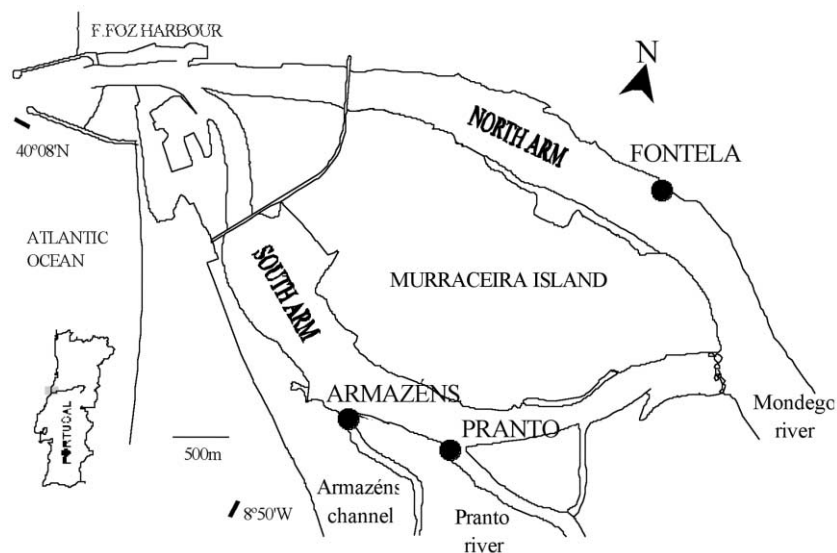


Figure 1. The Mondego estuary: location of the sampling stations in both arms.

under the fronds of algae, was also taken to ensure that all amphipods on the sampled area were collected. Since the sampled surface was irregular the area was estimated by projecting its shape onto a sheet of polyethylene, which was later weighed. Samples were preserved in 4% buffered formalin, and later sieved in the laboratory through a 0.5 mm mesh size bag. Given the size of newly hatched juveniles, this mesh size retains all individuals [27]. After separation, amphipods were kept in 70% ethanol for posterior analysis. At each sampling date and at each site, salinity, temperature, dissolved oxygen, pH, nitrites, nitrates, and phosphates in the water column were determined. Salinity, temperature, dissolved oxygen and pH were measured *in situ*, while water samples were taken from puddles around the algae for nutrient analysis. Sediment samples were also collected to estimate the organic matter content.

### 2.3. Laboratory procedures

Amphipods were counted, sexed, and the cephalic length ( $C_L$ ) was measured to the nearest 0.02 mm.

The determination of sex was based on the presence or absence of oostegites and/or broods (females), and of genital papillae (males). Animals without these features were considered to be juveniles. Non-brooding females were examined for the presence or absence of setae on the oostegites and classified as mature or resting females. If broods were present, eggs were counted, to estimate fecundity, measured and examined to determine the development stage. Five stages were considered, taking into account the criteria described in Skadsheim [47], adapted by Marques and Nogueira [27], respectively: A, newly laid eggs, spherical grouped and resembling a gelatinous mass; B, eggs well separated, oblong internally homogeneous; C, embryo comma-shape with vestigial pereopods already visible; D, constriction of the comma clearly visible, appendages segmented and looking slender, eyes visible, cephalotorax orange-red; E, hatched and free juveniles (these may stay some days in the brood pouch). Due to some difficulties in distinguishing stages A and B, data regarding these two stages were pooled in an AB stage. Since eggs become oval in shape during development, an average of their length and width was taken as measurement of their size.

The biomasses of *F. vesiculosus*, green macroalgae (mainly *Enteromorpha* spp.), and red macroalgae (mainly *Gracillaria* spp.), were estimated. Algae species were separated, dried at 60°C for 48 h, and their dry weight determined. The same procedure was followed to determine sediment dry weight. Afterwards the ash free dry weight of plants and the organic matter content of sediments were calculated after muffled for 8h at 450°C.

### 2.4. Data analysis

Principal component analysis (PCA) was used to identify the major sources of variation in the physicochemical and biotic data [23]. Data were organised into a matrix of 11 variables (temperature, salinity, dissolved oxygen, pH, nutrients [nitrates, nitrites, and phosphates], percentage of organic matter in sediment, *F. vesiculosus* biomass, green macroalgae biomass, and red macroalgae biomass)  $\times$  81 samples. This matrix was standardised to make the variances independent of the means [23]. Correlation matrices were calculated using the Pearson's coefficient and a cophenetic correlation coefficient is determined [23]. Data analysis was performed using the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis) version 1.80 software package [45].

Stepwise, least squares multiple regression models for *E. marinus* were developed to examine the relationship between some *E. marinus* population features (density, biomass, sex-ratio, percentage of ovigerous females and fecundity) and environmental factors: physicochemical factors and macroalgae biomass (*F. vesiculosus*, green macroalgae and red macroalgae). Normality of the distributions and the equality of variances were analysed using respectively the Kolmogorov-Smirnov and Bartlett's tests. When necessary a  $\ln(1+x)$  transformation was used prior to analysis. Regressions were estimated using the Stepwise Forward Analysis method performed with the STATGRAPHICS 4.0 statistical package.

Annual production ( $P$ ) was estimated using the Hynes average cohort method modified by Benke [2] essentially to address multivoltine populations, and called the size-frequency method by Waters and Hokenstrom [34]:

$$P = \left[ i \sum_1^i (\bar{d}_j - \bar{d}_{j+1}) \times \sqrt{(\bar{w}_j \times \bar{w}_{j+1})} \right] \frac{12}{CPI}$$

where:  $i$ , number of size classes;  $\bar{d}_j$ , mean density in size class  $j$  (ind·m<sup>-2</sup>);  $\bar{w}_j$ , mean individual weight in size class  $j$  (mg<sub>AFDW</sub>);  $CPI$ , cohort production interval (months).

The mean individual ash free dry weight ( $W$ ) in each size class ( $\bar{w}_j$ ) is obtained from the allometric equation,  $W$  (mg) = 1.592924  $\times$   $C_L^{3.984435}$ , determined by Marques and Nogueira [27].

The Hynes method does not require the recognition and tracking of individual cohorts. Therefore, it is suitable for populations with continuous reproduction and no synchronisation of larval release and egg extrusion [33]. The Hynes method for estimating production of aquatic invertebrates involves first an

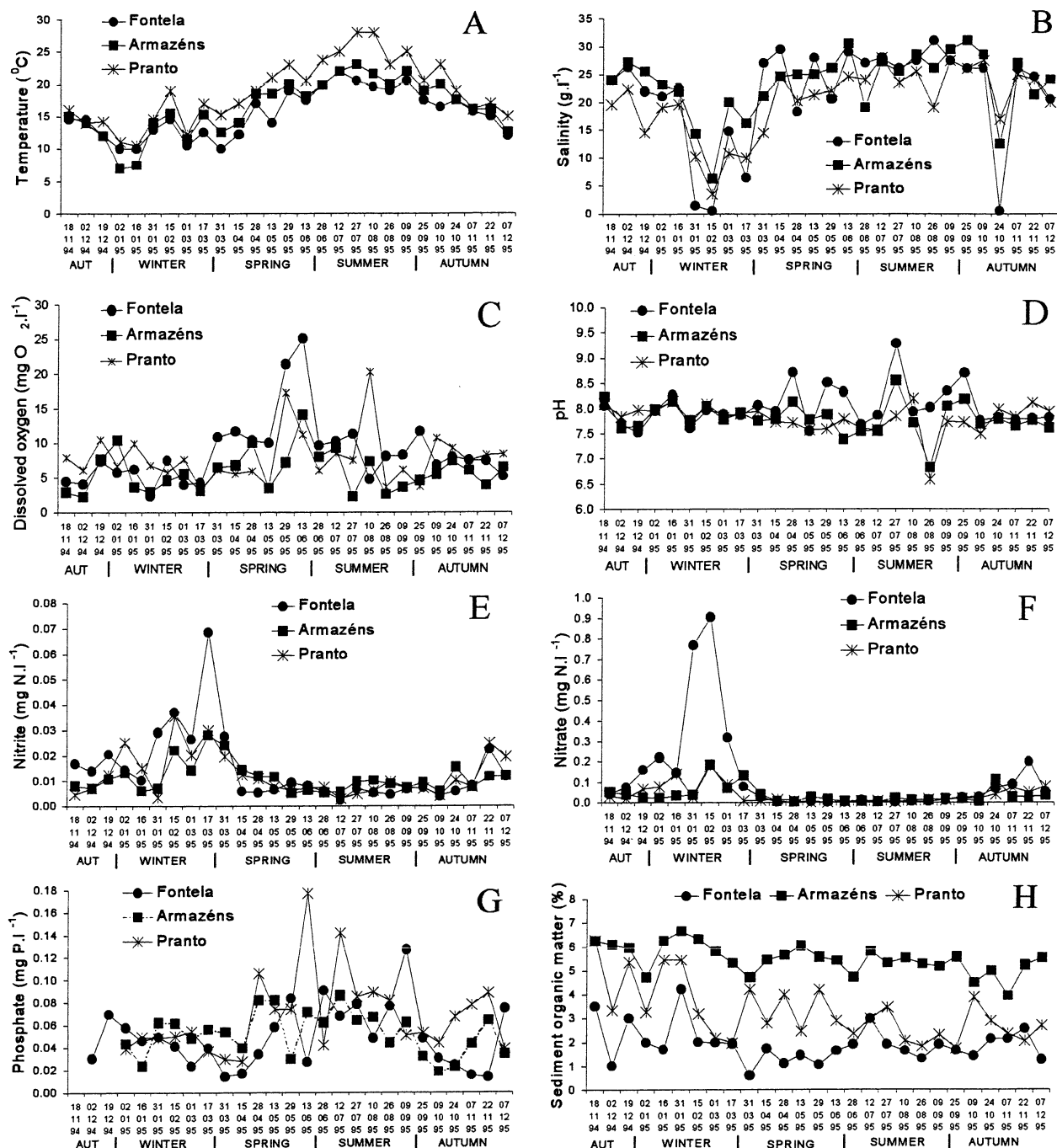


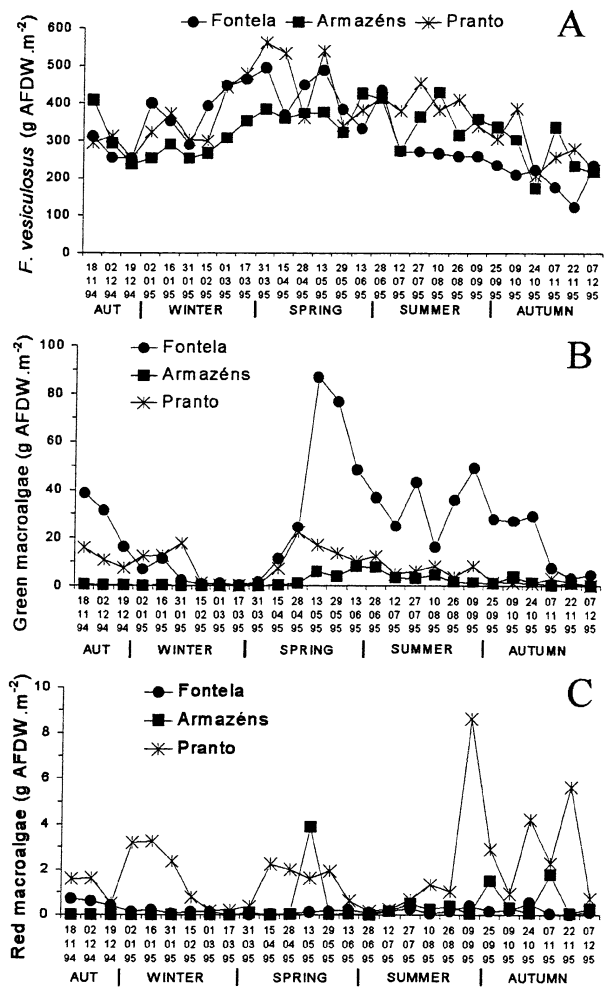
Figure 2. Variation of physicochemical conditions throughout the study period, at the three sampling places. A. Temperature, B. Salinity, C. Dissolved oxygen, D. pH, E. Nitrite concentration, F. Nitrate concentration, G. Phosphate concentration, H. sediment organic matter.

estimation of 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 total production value obtained was multiplied by a cohort production interval (*CPI*) correction factor [2], where the *CPI* is the time interval from hatching to the attainment of the largest size class. Based on previous studies (Vlasblom in Saint-Marie [27], [46], [47]), a *CPI* of 10–14 months was assumed.

### 3. RESULTS

#### 3.1. Environmental factors

Large seasonal and spatial variations in physico-chemical and biological conditions were observed in the study area (figure 2, 3). Water temperature and salinity (figure 2A, B) were strongly influenced by season changing from maxima in summer to minima during winter, in both arms of the estuary. Higher water temperatures recorded in the south arm during the study period were most certainly a consequence of the sampling procedures. In fact, despite all sampling was carried out during the morning, we always sampled first at Fontela and afterwards, 2 h later, at the Pranto station. Dissolved oxygen (figure 2C) reached the highest values in spring and summer, in both arms. Such values apparently resulted from the occurrence of large quantities of green macroalgae biomass, recorded in this period, in both arms (figure 3B). During the day, due to photosynthetic activity, green macroalgae contribute to increasing dissolved oxygen and the fact that all measures were taken during the morning is most probably the reason of these observations. The pH values (figure 2D) presented low variation during autumn and winter, but during spring and summer we could observe considerable oscillations. Nitrites and nitrates concentration in the water column (figure 2E, F) exhibited a seasonal variation, reaching higher values during autumn and winter, in both arms. Due to an increase in precipitation, during winter, great amounts of leaching waters (enriched with nutrients) from rice fields located upstream from the estuary were discharged, joining the natural freshwater runoff of the Mondego River. Phosphate concentration in water (figure 2G) reached higher values during the warmer period. This is normally observed in temperate estuaries like the Mondego [40] and is due to phosphorus release from the sediments to the water column in warmer periods [49]. The muddy sediment of Armazéns presented the higher percentage of organic matter content, while coarse sediments of Fontela presented the lowest values (figure 2H). *F. vesiculosus* biomass (figure 3A) showed an increase



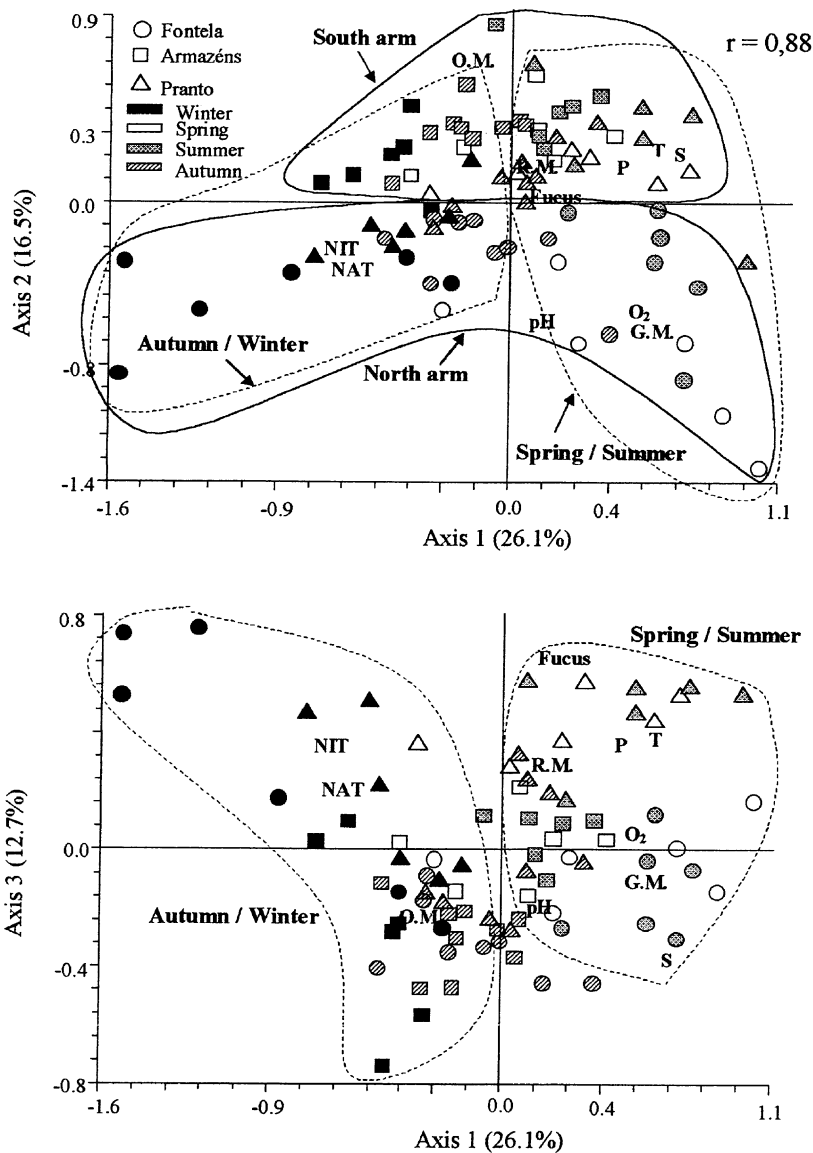
**Figure 3.** Biomass variation of *F. vesiculosus* (A), green macroalgae (B) and red macroalgae (C) throughout the study period, at the three sampling places.

during winter, reaching the highest values in spring, and a decrease along the rest of the year, in both arms. The occurrence of green macroalgae along the sampling period presented clear seasonal variation. The biomass of green macroalgae (figure 3B) was higher during spring and summer, and practically absent in winter. In fact, during the winter, the decrease of salinity, due to precipitation and an increase of current velocity do not allow the normal fixation and development of these algae. Red macroalgae (figure 3C) were practically absent from the north arm, reaching the highest values at the Pranto sampling station, but its biomass was always quite low.

Principal component analysis of the environmental factors x sampling stations dates matrix reveals a

consistent pattern (figure 4). This figure represents the projection of environmental factors (descriptors) and sampling stations (objects) in the same diagram (bi-plot). The projection of a descriptor on a principal axis shows its positive or negative contribution to the position of the objects along the axis [23]. From the projection against the first three axes of variability, it could be observed the existence of clear spatial and seasonal differences. Along the first axis of variability, samples collected during spring and summer were clearly separated from samples collected during autumn and winter. Spring and summer samples were

mainly characterised by higher green macroalgae biomass and by higher values of temperature, salinity, dissolved oxygen, and phosphorus concentration in water column. Autumn and winter samples were mainly characterised by higher concentrations of nitrites and nitrates in the water column. The second axis of variability allowed us to separate north arm sampling dates from samples collected in the south arm stations. North arm samples are mainly characterised by higher values of green macroalgae biomass, and by higher concentrations of dissolved oxygen, nitrites, and nitrates in the water column. The higher values of



**Figure 4.** Principal component analysis (PCA) projection of the descriptors and sampling sites of the Mondego estuary in the space defined by the first three axes of variability. NIT: nitrites; NAT: nitrates; O.M.: percentage of organic matter in sediment; O<sub>2</sub>: dissolved oxygen; G.M.: biomass of green macroalgae; R.M.: biomass of red macroalgae; P: phosphate; T: temperature; S: salinity. The percentage of variability associated with each axis is indicated in parentheses.  $r$  = cophenetic correlation coefficient.

green macroalgae biomass observed in the north arm were, to a certain extent, unexpectedly high when compared with those quantified in the south arm. Indeed, in the north arm, due to strong river current, green macroalgae can only find suitable places for fixation and development near the rocky margins, while in the south arm, due to low hydrodynamics, green macroalgae normally occur on the muddybanks, where they can reach massive amounts of biomass ( $\approx 400 \text{ g-afdw.m}^{-2}$ ) [24, 31, 40, 41]. In our case, samples were taken close to the rocky margins, which explains the higher than expected amounts of algae found in the north arm station. The south arm samples

are mainly characterised by higher values of temperature, salinity, organic matter in the sediments, phosphorus concentration in the water column, and by large red macroalgae biomass.

### 3.2. Abundance

Population density and biomass changed seasonally, with a winter peak in the Pranto station, and another during spring at all sampling stations (figure 5). Density was lower in the north arm, reaching the highest values in the upstream areas of the south arm. In addition, in the south arm, abundance was clearly higher in the innermost sampling station.

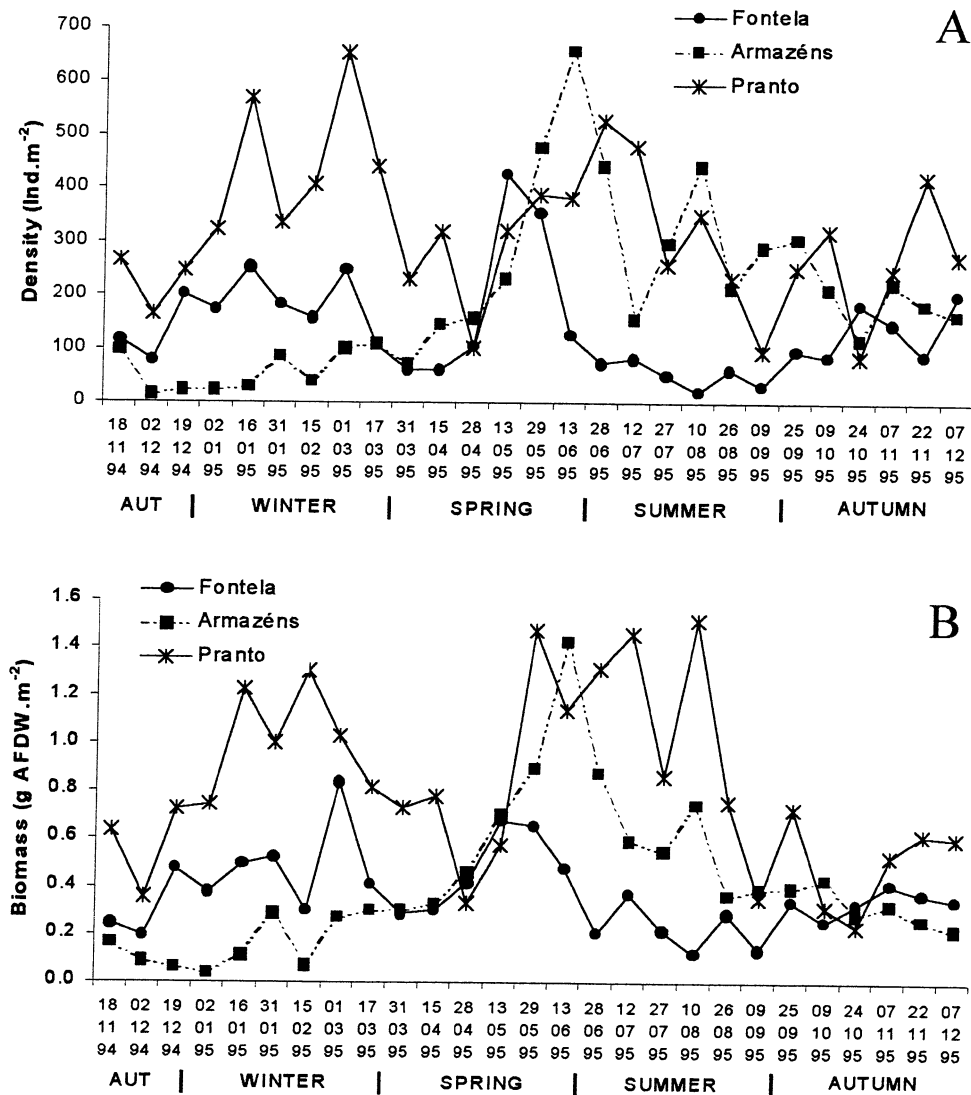


Figure 5. *Echinogammarus marinus*. Density (A) and biomass (B) at the three sampling places.

Stepwise multiple regression analysis showed that density and biomass of *E. marinus* were correlated with environmental factors. At the Fontela station, density ( $D$ ) was negatively correlated with water temperature ( $TEMP$ ) and salinity ( $SAL$ ) and positively correlated with green macroalgae biomass ( $GMB$ ) ( $r = 0.74$ ;  $P < 0.0001$ ), and biomass ( $B$ ) was also negatively correlated with temperature and positively with green macroalgae biomass ( $r = 0.48$ ;  $P < 0.05$ ):

$$\ln(1 + D) = 7.13 - 0.14 TEMP - 0.04 SAL + 0.02 GMB$$

$$B = 0.74 - 0.03 TEMP + 0.003 GMB$$

At the Armazéns station *E. marinus* density ( $r = 0.82$ ;  $P < 0.0001$ ) and biomass ( $r = 0.85$ ;  $P < 0.0001$ ) were positively correlated with green macroalgae biomass:

$$D = 92.38 + 55.43 GMB$$

$$B = 0.21 + 0.15 GMB$$

### 3.3. Population structure

Size-frequency distributions were analysed to interpret the population structure. The same pattern was observed in the three sampling places. Small individuals mainly constitute the population, with juveniles representing almost always more than 50 % of the samples (figure 6). The percentage of mature and ovigerous females was always less than 16%. Females were morphologically recognisable at smaller sizes than males, but males became larger than females. Thus, males predominantly constituted higher size classes. The largest individuals collected presented 2.22 mm cephalic length ( $CL$ ) (male) and 2.00 mm  $CL$  (female) at the Fontela station, 2.18 mm  $CL$  (male) and 1.90 mm  $CL$  (female) at the Armazéns station, and 2.20 mm  $CL$  (male) and 1.88 mm  $CL$  (female) at the Pranto station. By the end of winter, no small individuals were found at the Fontela (north arm) and Pranto (inner area of the south arm) stations, but the same was not observed at Armazéns station. The absence of juveniles in the population appeared clearly related with a decrease in the percentage of ovigerous females (figures 6).

### 3.4. Reproduction, sex-ratio, and fecundity

The *E. marinus* population was sexually active throughout the year in the Mondego estuary. However, taking into account the percentage of ovigerous females over the total female population (figures 6), sexual activity decreased at the end of winter, almost ceasing, in both arms. A new increase was observed in spring. A second period during which sexual activity decreased was observed at Fontela during summer.

The percentual variation of eggs in different development stages confirms that sexual activity takes place

throughout the year. In fact, eggs in all stages of development were found along the study period. Stepwise multiple regression analysis showed a positive correlation between the percentage of ovigerous females (%  $OF$ ) and salinity at the Pranto station ( $r = 0.39$ ;  $p < 0.05$ ):

$$\ln(1 + \% OF) = 2.76 + 0.038 SAL$$

To confirm in a sounder way that recruitment was continuous, additional sets of samples were collected at the Pranto station with a three days periodicity during a fifteen days period, in winter, and again during a thirty days period, in spring. The methodology used was the same already described. It was possible to confirm that emission of new recruits occurred every day, without any relation with tidal amplitude.

Sex-ratio variation presented a different pattern in the two arms of the estuary. At Fontela females were more abundant than males in autumn and winter while the contrary was observed in spring and summer, namely during the hottest period. In south arm males were almost always more abundant than females, namely during winter, although females outnumbered males during part of the summer and autumn 1995. Stepwise multiple regression analysis showed that the sex ratio (males/females) was positively correlated with water temperature at Fontela sampling station ( $r = 0.42$ ;  $p < 0.05$ ):

$$\ln(1 + \text{Sex - ratio}) = 0.45 + 0.02 TEMP$$

and with green macroalgae biomass at Armazéns station ( $r = 0.51$ ,  $p < 0.01$ ):

$$\ln(1 + \text{Sex - ratio}) = 0.61 + 0.03 GMB$$

Despite the fact that individual fecundity was extremely variable, a significant correlation was found between the number of eggs in stage AB within brood pouches and the size of females collected each season. Higher values of fecundity were observed in spring and autumn and lower values in winter and summer (figure 7A). The mean volume of eggs AB was higher during colder months and lower during the summer period (figure 7B). Stepwise multiple regression analysis indicated that fecundity ( $F$ ) was positively correlated with salinity ( $r = 0.45$ ;  $p < 0.05$ ):

$$F = 6.30 + 0.25 SAL$$

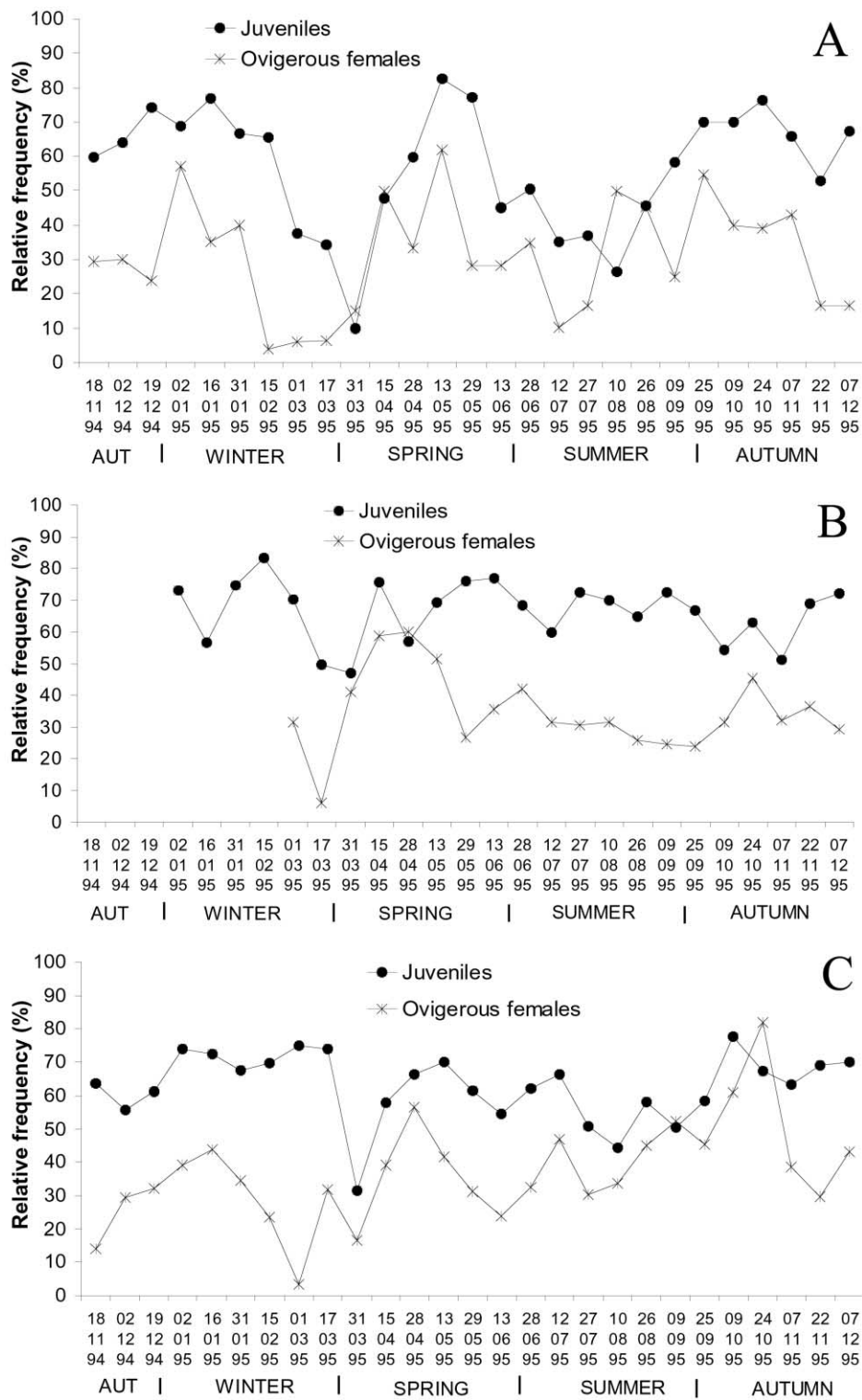
and that AB egg volume ( $ABvol$ ) was negatively correlated with temperature and salinity ( $r = 0.78$ ;  $p < 0.0001$ ):

$$\ln(1 + ABvol) = 0.95 - 0.009 TEMP - 0.01 SAL$$

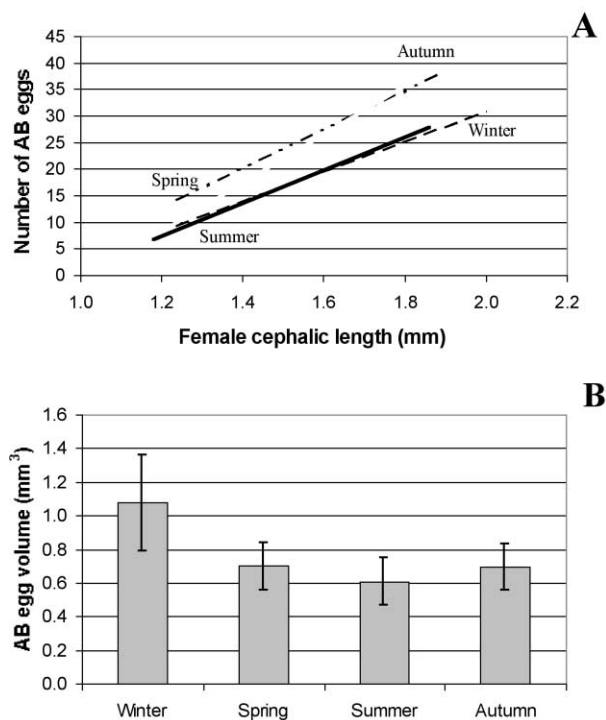
### 3.5. Production estimates

Length-weight relationships previously established by Marques and Nogueira [27] were used in produc





**Figure 6.** *Echinogammarus marinus*. Variation of ovigerous females in the total female population and of juveniles in the population. **A.** Fontela, **B.** Armazéns, **C.** Pranto.



**Figure 7.** *Echinogammarus marinus*. Seasonal regression lines of AB eggs number (Y) on cephalic length (X) of ovigerous females (A) and AB egg volume (mean  $\pm$  s. d.) (B) during the study period.

**Table I.** *Echinogammarus marinus*. Production estimates at the three sampling places.

	P (g AFDW·m <sup>-2</sup> ·year <sup>-1</sup> )	$\bar{B}$ (g·m <sup>-2</sup> )	P/ $\bar{B}$
Fontela	1.74 – 2.44	0.39	4.14 – 6.18
Armazéns	1.96 – 2.74	0.42	4.68 – 6.56
Pranto	3.85 – 5.38	0.86	4.54 – 6.36

tion estimates (table I). Production and annual average biomass were higher at the Pranto station, located in south arm, while the lowest values were found at Fontela, in the north arm. Despite these differences, annual P/ $\bar{B}$  ratios were similar in all sampling stations.

## 4. DISCUSSION

### 4.1. Abundance

Population density showed clear seasonal and spatial variation. At the Pranto station, *E. marinus* population was stable all over the study period, which

agrees with results obtained by Marques and Nogueira [27]. This is probably favoured by the fact that muddy sediments with high organic content surround hard substrates at this station. It is well known that *E. marinus* is typically found underneath algae (fucoids) associated with muddy deposits, but less common on clean sands [26, 27, 43]. The Fontela station in north arm is characterised by more coarse sediments, with low content of organic matter, which may explain lower abundance. Lower densities recorded in the beginning of spring at the three sampling stations were due to a drop of recruitment by the end of winter, probably as a response to more harsh conditions. On the contrary, an increase in recruitment was responsible for the significant increase in density observed during spring.

The positive correlation found between *E. marinus* density and green macroalgae biomass at Fontela and Armazéns stations suggests a dependence on the yearly dynamics of that macroalgae. This is consistent with observations on some other amphipod species [5, 12, 15, 22, 41]. Such dependence is probably related with the variation of food resources, since *E. marinus* can feed directly on algae [13], although it does not ingest fresh *Fucus*, regarded as unpalatable due to their content of phenolic compounds [42]. The negative correlation between *E. marinus* density and temperature and salinity at Fontela station may express the fact that higher temperature increases desiccation rates during emersion, intensifying the environmental stress upon organisms in intertidal areas. Salinity does not play a role in this process but higher salinities were also recorded in the warmest period of the year. Juveniles inhabiting the intertidal zone, when emerged, can lose water much faster than adults, since small individuals have greater surface-to-volume ratios [16]. Juveniles are consequently much more vulnerable to desiccation stress than adults. It was observed that, during summer and part of autumn, *E. marinus* density reached the lowest values in the north arm. This was probably due to the different hydrographic characteristics of the two arms that caused organisms living in the intertidal area of the north arm to spend more time in emersion.

In the south arm, between the autumn of 1994 and the winter of 1995, *E. marinus* density was very different at the two sampling stations. During this period, the Pranto station exhibited higher densities while the Armazéns station attained its lowest values. The Armazéns sampling station is located in the confluence of the Armazéns channel with the south arm (figure 1). There is no freshwater discharge in this channel but the tidal wave washes out the channel, where wastewaters from several industries are discharged [14]. Although the available data are not consistent enough to demon

strate it, a possible explanation for *E. marinus* density variations is that individuals moved upstream, from the Armazéns to the Pranto area, to avoid extreme conditions during this period.

*E. marinus* densities recorded during this study were lower than those found by Marques and Nogueira [27] (500 to 4000 ind·m<sup>-2</sup>). This may signify a decline of the *E. marinus* population in the Mondego estuary, which could be explained by an eventual decrease of the area occupied by *F. vesiculosus* during the last years. Although there are no previous data on *F. vesiculosus* biomass in the Mondego estuary, considerable changes in environmental conditions, like periodic dredging, namely in the north arm, discharge of domestic effluents, discharge of waters contaminated with pesticides (never quantified) proceeding from rice fields located upstream, and eutrophication could be reasons for the decrease of both *F. vesiculosus* and *E. marinus* populations.

#### 4.2. Reproduction, sex-ratio, and fecundity

*E. marinus* ovigerous females were found all over the year in the Mondego estuary, as referred by Marques and Nogueira [27], and the recruitment was continuous. Continuous sexual activity during the year is a common feature in amphipods [5, 6, 12, 17, 36, 41]. In a previous work, Marques and Nogueira [27] tried to recognise and track cohorts, justifying their approach based on the assumption that, although sexual activity was continuous, juveniles release was synchronised with spring tides, when conditions for juveniles survival were optimised. It appeared to us now that this assumption was not correct, and therefore that cohort recognition would not be a suitable approach. Therefore, we were not able to estimate growth rates and life span from field data. In the Mondego estuary, the southern limit of the known distribution of *E. marinus*, this species reproduce throughout the year, having probably a multivoltine life cycle. In fact, an experimental study showed that ovigerous females appeared in the population after 8 to 12 weeks of development, and each female could produce at least three broods consecutively (unpublished data). Other *E. marinus* populations also show different life cycles, varying from univoltine, in a cold-temperate to sub-polar climate (Denmark), with a reproduction period from April to May/June [47], to bivoltine in a maritime temperate climate (Normandy), with a reproduction period from May to July [43]. Actually, this is a common phenomenon among amphipods, which may exhibit large intraspecific variations of life cycles as a function of latitude [11, 18, 46, 53], probably because a lack of extreme temperatures, especially cold temperatures, might allow prolonged reproduction periods [19]. A period where sexual activity almost ceased was ob-

served in the Mondego estuary population, by the end of the winter, although this was almost imperceptible at the Armazéns station. Low water salinity tends to induce lower viability of *E. marinus* eggs [51]. This may explain the decrease of *E. marinus* ovigerous females observed by the end of winter at the Fontela and Pranto stations actually following a period of low salinity. On the other hand, higher salinities values recorded at Armazéns, during the same period, probably allowed the completion of egg embryonic development and the release of juveniles. Therefore, the occurrence of higher salinities allowed in general the occurrence of higher percentage of ovigerous females. Marques and Nogueira [27] also related a period of low sexual activity during the first year of their study, but not in the second year. A possible explanation is the fact that temperature and salinity were lower during that period in the first year. Therefore, a decrease in the reproduction effort might have been a response of *E. marinus* population to more harsh conditions.

Males outnumbered females, notably during spring and summer in the north arm, and during the winter in the south arm. This is not a common feature with other amphipod species populations [1, 6–10, 18, 41, 42], neither with other *E. marinus* populations [47]. The previous study by Marques and Nogueira [27] also related a sex ratio favourable to females in *E. marinus*. Mechanisms that generate male-biased sex ratios in *E. marinus* are not known. Stepwise multiple regression analysis suggested that higher temperatures and bigger biomass of green macroalgae could influence positively a predominance of males, but does not explain what could be the causal relation. On the other hand, differential rates of growth, maturation, longevity or predation can all result in skewed sex ratios [1]. Watt [52] showed that photoperiod influence the sex ratio of *Gammarus duebeni*, with preponderance of males when exposed to long days (> 13–14 h light), and preponderance of females on exposure to short days (< 13–14 h light) at 15°C. Kneib [20] suggest that temporal variations in the sex ratio in *Uholorchestia spartinophila* populations could be influenced by differential mortality caused by seasonal changes in the quality or availability of food. Moore [35] related a substantial swing in sex ratio from season to season in *Lembos websteri*, involving a winter period of male domination, giving way in the spring and summertime either to female domination or to a ratio close to unity. Parasitism by microsporidians can also alter sex ratios of *Gammarus duebeni* populations by causing sex transformation (Bulnheim in Conlan [4]). Finally, male sexual maturity is relatively difficult to determine and since immature females are included in sex ratio estimations, it is acknowledged that the ratios quoted do not represent the real operational sex ratio of reproductively competent individuals [1].

Fecundity of females of similar size was quite variable which could be due to constraints during sampling and sorting in the laboratory. In fact, Drake & Arias [12] reported the tendency of preserved females to lose their broods. However, significant correlation was found between number of eggs in stage AB within brood pouches and size of females. A positive relationship between fecundity and body size is indeed common in amphipods [1, 5, 7, 8, 9, 10, 15, 17, 22, 27, 42, 50]. Northern Gammaridea species usually produce smaller clutches during the colder months, associated with an increase in egg size, giving the offspring a better chance of surviving harsh conditions [21, 41, 48, 50]. This was also observed in *E. marinus*. However, in our case, the number of eggs produced per female was similar in winter and summer, although we observed, in an experimental study (unpublished data), that *E. marinus* females reared at 20 °C presented smaller broods and shorter periods of embryonic development when compared with females cultivated at lower temperatures (10 °C). A possible interpretation is that *E. marinus* females had smaller broods during colder months because egg volume was higher, while in summer broods were smaller probably as a consequence of shorter periods of embryonic development, which allowed production of more broods in the same period of time. Stepwise multiple regression analysis showed a positive correlation between fecundity and salinity. As said above, Vlasblom & Bolier [51] found that lower viability of the eggs in *E. marinus* was related to egg swelling at low salinities due to water intake by osmosis. Early developmental

stages are especially vulnerable at low salinities, suggesting that any osmoregulatory ability, whether due to active transport or reduced membrane permeabilities, do not appear until later on [37]. A negative correlation between AB egg volume and temperature and salinity was observed by us. In colder months (normally associated to lower salinities, essentially due to rainfall), developing embryos presented bigger volumes, which may optimise chances of survivorship because they contain more reserves. With higher temperatures and salinities embryos may probably survive easier, and therefore such conditions favour the production of smaller eggs by *E. marinus* females.

### 4.3. Production estimates

Production values estimated presented spatial variations. Production and annual average biomass were higher in the Pranto station (south arm), where density was also higher, while the lowest values were recorded at Fontela (north arm), also associated with lower abundances. Differences in annual production of *E. marinus* in the Mondego estuary were essentially due to differences in population density, but annual  $P/\bar{B}$  ratios were similar at all sampling places. Annual  $P/\bar{B}$  ratios estimated can be considered relatively high when compared with values obtained to other amphipod species from temperate zones [3, 7, 8–10, 18, 44], and also to *Cyathura carinata*, an isopod species considered important to the structure and functioning of the Mondego estuary biological community [30] (table II). However, the fact that different methods were used to estimate secondary production in each

**Table II.** Production estimates of some amphipod and one isopod species.

Species, Location	Production (g·m <sup>-2</sup> ·yr <sup>-1</sup> )	$P/\bar{B}$	Source
<i>Ampelisca araucana</i>			
Chile	8.03–12.43 (DW)	3.65–4.45	[3]
<i>A. tenuicornis</i>			
English Channel	0.70–1.68 (DW)	3.12–4.20	[7]
<i>A. typica</i>			
English Channel	0.07–0.16 (DW)	4.06–4.36	[9]
<i>A. armoricana</i>			
English Channel	0.12 (DW)	1.76–2.74	[10]
<i>A. brevicornis</i>			
English Channel	0.04–0.49 (DW)	2.15–2.80	[8]
England	1.31–1.68 (DW)	2.49–3.21	[20]
<i>Corophium curvispinum</i>			
The Netherlands	5.85–11.26 (AFDW)	2.7–3.5	[44]
<i>Cyathura carinata</i> (Isopoda)			
Portugal	5.7–9.9 (AFDW)	1.65–2.03	[30]

DW – dry weight; AFDW – ash-free dry weight.

case makes a sound comparison difficult. But the ample intertidal distribution of *E. marinus*, its accessibility to aquatic predators and birds, and high turnover rates suggest that this amphipod may play an important role in the trophic dynamics of the Mondego estuary community.

## 5. CONCLUSION

Evidence is shown that *E. marinus* population distribution, dynamics and annual production in the Mondego estuary were affected by the hydraulic regime. Temperature, salinity, and green macroalgae biomass also influenced the *E. marinus* population. In the north arm, where dredging takes place regularly and environmental conditions are characterised by coarse sediments and stronger daily changes in salinity [28, 29], *E. marinus* shows the lowest values of density and annual production. On the contrary, in the south arm, characterised by a weaker hydraulic regime, muddy sediments and smaller daily salinity changes [28, 29] *E. marinus* presents higher densities and annual production, namely in the inner areas. The present results showed that in the southern limit of the species the recruitment is continuous through the year and, as a consequence, no cohorts are distinguished on the population. This information clearly reports new insight of the species, in opposition to the previous study, which assumed a discontinuous recruitment occurring only in spring tides. The generalised notion that estuaries are highly productive systems is reinforced by these results and, in the case of the Mondego, *E. marinus* may definitively be seen as an important element of this energy flow.

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