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Effects of Temperature and Salinity on Life History of the Marine Amphipod *Gammarus locusta*. Implications for Ecotoxicological Testing

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Abstract. The life history of *Gammarus locusta* was analysed in the laboratory under the following temperature and salinity combinations: 20 °C–33‰, 15 °C–20‰ and 15 °C–33‰ (reference condition). Life history analysis comprised survival, individual growth, reproductive traits and life table parameters. Compared to 15 °C, life history at 20 °C was characterised by at least a four-week reduction in the life-span, lower life expectancy, shorter generation time, faster individual growth, anticipation of age at maturity and higher population growth rate. These temperature effects constituted an acceleration and condensation of the life cycle, compared to the reference condition. Concerning salinity effects, with few exceptions, results show that overall this amphipod life history did not differ significantly between the salinity conditions tested. Regarding ecotoxicological testing implications, findings from this study indicate that the range of temperature and salinity conditions acceptable for testing was substantially expanded both for acute and chronic assays. A temperature of 20 °C or higher (for a salinity of 33‰) is suggested for routine chronic sediment toxicity testing with *G. locusta*, in order to reduce the life cycle and consequently improve cost-effectiveness and standardisation. Results also suggest that a multiple-response approach, including survival, growth and reproduction, should be applied in chronic toxicity tests.

Keywords: amphipod; ecotoxicology; life history; temperature; salinity

Introduction

Amphipods are often chosen for ecological and ecotoxicologial studies due to their ecological relevance, sensitivity to environmental disturbance and their amenability for culture and experimentation (ASTM, 1992; DeWitt et al., 1992; Reish, 1993; Conlan, 1994). Our interest has focused on the amphipod species *Gammarus locusta* since this species has a wide distribution along the European Atlantic coast, and it is abundant in many Portuguese coastal ecosystems. Previous studies on *G. locusta* included the study of its life history in the field (Costa and Costa, 1999) and the development of an acute sediment toxicity test (Costa et al., 1998). Current research efforts are focused on the development of chronic toxicity testing and application of sub-lethal endpoints to evaluate contamination-induced stress.

Temperature and salinity are key environmental variables that rule estuarine organisms' life history. Temperature effects on amphipods' reproductive bionomics were reviewed by Sainte-Marie (1991) which concluded that high-latitude amphipods are generally characterised by biannual or perennial life cycles, large body size, delayed maturity and single

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or few broods with many relatively large embryos, while low-latitude species are characterised by the opposite properties. Regarding salinity, in several amphipod species an optimum salinity, or range of salinities, in which there is no impairment of physiological capabilities, has been demonstrated (Steele and Steele, 1991; Bulnheim, 1979, 1984; Meadows and Ruagh, 1981).

Physiological adaptation to sub-optimal environmental conditions, such as osmoregulatory adaptation, requires energetic costs that may compromise other physiological needs, such as growth and reproduction. The energetic trade-off between these physiological needs may have consequences for each species' life history. Therefore, the characterisation of test organisms responses to these environmental factors is required for a correct interpretation of ecotoxicological tests and to define strategies for chronic and long-term toxicity testing.

Temperature and salinity effects on *G. locusta* were only partially addressed up to now, whereas a deeper description and quantification of its life-history traits is required to allow the conductance of ecotoxicological tests with this amphipod. Costa et al. (1998) attempted to establish *G. locusta* osmotic tolerance by testing this amphipod survival after acute exposure to a gradient of decreasing salinities at three distinct temperatures. They concluded that the amphipods were sensitive to sudden decreases of salinity and that this effect was more pronounced at higher temperatures. However, this experiment only considered acute responses to low salinity, and it is not known whether with an acclimation period, this species would show different tolerance limits. In addition, the sub-lethal effects of osmotic stress are still unknown.

Here we characterise and quantify effects of temperature and salinity on *G. locusta* life-history characteristics. This information will be used to define strategies for chronic toxicity testing such as the range of temperature and salinity conditions in which tests may be performed, their length and endpoints to be used.

Materials and methods

Amphipod collection and culturing

Gammarus locusta were collected from a clean reference site, located on the south margin of Sado estuary (38°27′N, 08°43′W), Portugal. This site is free of direct exposition to contaminated effluents, which are confined to the north margin, and contains a wealthy zoobenthic community (Mucha and Costa, 1999). Sediment from this location is not contaminated and is used as control sediment in ecotoxicological tests (Costa et al., 1998). Two culturing systems were developed at two different salinities, 33‰ and 20‰. The 20‰ solution was prepared by diluting the filtered seawater (33‰) with distilled water. Both culturing systems were established at 15 °C, in plastic aquariums with 0.45 μm filtered seawater and a sediment layer of about 1 cm. The sediment collected in the above-mentioned location, was a fine to medium sand with 2.4% fine fraction (fraction < 0.063 mm), 1–2% total volatile solids (TVS) and 20.7% of water. The culturing systems were semi-static with a 100% water replacement twice a week. Water was sieved through a battery of screens of decreasing mesh size (1500, 1000, 475 and 250 μm) to sort the animals in 4-size classes (adults, sub-adults, juveniles and newborns). Temperature, salinity and pH were monitored prior to every replacement of water. Food consisted of the macroalgae *Ulva lactuca* and/or *Ulva rigida*, depending on their abundance in the amphipod's sampling location.

Experimental design and procedure

Laboratory assays were performed in order to chronicle the life history of three cohorts of *G. locusta* under distinct environmental conditions. Two salinities and two temperatures were used, combined in three different treatments as follows: (A) 20 °C–33‰, (B) 15 °C–20‰ and (C) 15 °C–33‰. This way, life history traits of the test organism under reference conditions, that is 15 °C–33‰, which are used for culturing and acute sediment toxicity testing (Costa et al., 1998), were compared with the same traits at a higher temperature 20 °C on one hand, and at a lower salinity 20‰ on the other hand. These conditions were selected according to the range of salinities and temperatures annually registered in the locations of the Portuguese coast—17–35‰ and 11–24 °C where natural populations of *G. locusta* occur. (Marques and Bellan-Santini, 1990; Costa and Costa, 2000).

The general design of the three assays is summarised in (Table 1). Twenty-four hours before the beginning of the experiment, a sub-stock of newborns (up to 7-days-old) was isolated from the main

Table 1. Summary of general conditions of the assay and of specific assay procedures for each treatment

General assay conditions			
Photoperiod	12 h light: 12 h dark		
Sediment	Fine to medium sand, up to 1 cm layer		
Water	0.45 µm filtered sea water		
Aquaria dimension	10 l		
Aeration	Plastic tips, constant bubbling		
Sheltering structures	Small stones		
Feeding	*Ulva sp*., several times/week		
Starting amphipod's age	Under 7-days-old (newborns)		
Initial density	70 amphipods/aquarium		
Analysed parameters	Survival, growth, reproductive traits		
Specific assay conditions			
Treatments	A	B	C
Temperature and salinity	20 °C–33‰	15 °C–20‰	15 °C–33‰
Destructive sampling	3 aquaria/2 weeks	3 aquaria/2 weeks	2 aquaria/4 weeks
Water change	100%/week	100%/week	100%/2 weeks

culturing stock and kept at the assay temperature in freshly renewed water and unlimited food. The assays were conducted in 10 l plastic aquaria, each of them with exactly 70 newborns produced in laboratory. Aquaria were filled with a sediment layer up to 1 cm and 0.45 µm filtered seawater to a depth of 5 cm, and placed in a water bath with automatically controlled temperature. Aeration was provided with plastic tips placed at least 1 cm above the sediment surface and small stones (about 12 cm^2 of surface area) were furnished to provide shelter. During the assays, the organisms were fed with macroalgae *Ulva* spp. on an *ad libitum* basis. Aquaria were inspected daily for aeration and feeding needs and to remove dead animals. Temperature, salinity and pH were monitored and ranged between 14.5–15.5 °C (cohort B and C) and 19.5–20.5 °C (cohort A), 20–21% (cohort B) and 33–34% (cohorts A and C), and pH 7.6–8.7 (all cohorts), respectively.

Each cohort's life history parameters were determined biweekly for conditions A and B (in triplicate), or monthly for conditions C (in duplicate), using destructive sampling. Cohort C did not require a short sampling interval like it was applied for cohorts A and B, since some life history information was already available for the former. The cohort C conditions (reference situation) were used before for culturing and testing non-contaminant variables for assay periods equivalent to chronic tests (Costa et al., 1996).

At each sampling period, the aquaria to be sampled and removed from the experiment were randomly chosen, and their contents sieved through 1000 and 250 µm screens to collect the amphipods from the original cohort and newborns, respectively. In addition to this sampling, newborn production was also determined during water renewal operations, weekly for conditions A and B and biweekly for condition C. Contents were sieved and the newborns collected in the 250 µm sieve, after which they were fixed in 70% ethanol for later counting.

Life-history analysis

Life-history analysis comprised survival, individual growth, reproductive traits (age at maturity, offspring production and fecundity) and life-table parameters. Cohort's amphipods were counted, sexed and their length and weight measured, while newborns were only counted.

Individual length was measured to the nearest 0.1 mm using a binocular microscope for cohort A and B and as described in Costa and Costa (1999) for cohort C. Length was determined separately for males and females. It is difficult to determine the total length (TL) because of the resting position of these amphipods. Alternatively, the metasomatic length (ML) was used, which is defined as the distance between the anterior end of the rostrum and the posterior end of the last metasomatic segment. The relation between TL and ML was previously determined in a sample of 478 animals with an ML length range from 3 to 16 mm. This is described by the following linear regression equation

(Costa and Costa, 1999):

$$\mathrm{TL} = -0.153 + 1.218 * \mathrm{ML} (r = 0.9884) \quad (1)$$

Dry weight was determined only for males. Female's weight was not measured due to weight losses during manipulation to extract embryos and determine fecundity. Males from of each replicate were pooled and collectively weighed. The samples were dried at 65 °C until reaching constant weight.

Sexual dimorphism was identified by the size and shape of the gnathopods after Lincoln (1979). The animals were only sexed from 4th week onwards, since in younger and smaller animals this distinction is difficult to perform. For this reason the survival and growth curves data up to the age of 4 weeks was common for males and females. All females were recorded as ovigerous or non-ovigerous and the former were carefully manipulated at binocular microscope to determine fecundity. Brood size or fecundity was defined as the number of embryos present in the brood-pouch. For brood-size estimation the last stage of embryonic development—newly hatched young—was not considered, since they can freely leave and enter the brood-pouch (Sheader and Chia, 1970).

In order to record the first appearance of ovigerous females in each cohort, the aquaria were inspected daily. The week in which ovigerous females were observed for the first time was considered as a rough estimate of the age at maturity. Similarly the age of first reproduction is here defined as the week in which newborns were collected from the aquaria for the first time. Our personal observations of this species in laboratorial cultures and experimentation support the validity of these estimates. Offspring production of each replicate was quantified as the number of newborns produced per female per week.

Cohort life tables were constructed for each of the three experimental treatments according to Skadsheim (1990) and Pianka (1994). Life-table parameters were estimated for every two-week intervals throughout the life-span of each cohort using only female data. Life-table indexes estimated were:

l_x: age-specific survivorship: probability to survive to age or week x, that is the number of females alive at the beginning of age x as a fraction of initial female cohort size.

m_x: age-specific fertility: number of female neonates produced per female during age x, that is half the number of newborns produced during age x divided by the number of living females at age x.

$l_x m_x$: expected number of female progeny at week x expressed for newborn females rather than just for females who live to age x.

e_x: age-specific life expectancy: the expectation of future life, which was estimated as follows:

$$e_x = \frac{\sum_{y=x}^{\infty} l_y}{l_x}$$

GRR: gross reproductive rate: expected total number of female offspring produced by a female which lives through all age groups, that is the sum of m_x over all ages.

R_0: net reproductive rate: expected number of female progeny produced by each female which enters the population, an average expectation if a large number of females is considered, that is the sum of $m_x l_x$ over all ages.

r: intrinsic rate of natural increase, which was estimated as follows: $\sum e^{-rx} l_x m_x = 1$

T: generation time: average parental age at which all offspring are born, which was estimated as follows: $T = (\ln R_0)/r$.

The following assumptions were considered for life-table shaping:

—since animals were not sexed until 4th week, a sex ratio of 1 : 1 was assumed up to that age. Preliminary assays and laboratory observations confirmed the validity of this assumption.

—at the age intervals where survival was not determined, the average between previous and posterior age survivorship was used.

Data analyses

For each of the three cohorts mortality rates were estimated using linear regression fitted for survival data, and the regression coefficients were compared using analysis of covariance (ANCOVA).

The full lifetime growth of amphipods, has been shown to follow a sigmoid curve, although in the earliest developmental stages it is generally accepted that growth is linear (Mills, 1967; Highsmith and Coyle, 1991). Therefore, linear growth models were fitted separately for males and females using only data from the linear sections of the growth curves.

The individual growth rates (regression coefficients) of the three cohorts together were compared

by ANCOVA and *post-hoc* comparisons performed using Student's *t*-test (Zar, 1984). Cumulative number of offspring produced per female per week was used for estimating the offspring production rate. Regression lines were fitted to this data and the resultant slopes compared by ANCOVA. The relationship between brood size and female body length was determined by linear regression and the resultant coefficients compared as above described for individual growth.

According to Levin et al. (1996), no specific statistical analysis for life-table data has been developed, therefore, the bootstrap resampling method suggested by these authors was applied to compare the parameters GRR, R_0, r and T among treatments. These indices were compared against the null hypothesis that the treatment received by an individual had no effect on its response. For instance, for treatment 20 °C–33‰ and the index r, under the null hypotheses, $\theta = 0$, the statistics used were:

$$\theta = |r^{20\,^\circ\mathrm{C}-33‰} - r^{15\,^\circ\mathrm{C}-20‰}| \quad \text{and}$$
$$\theta = |r^{20\,^\circ\mathrm{C}-33‰} - r^{15\,^\circ\mathrm{C}-23‰}|$$

The distribution of θ under the null hypotheses was calculated by permuting individuals among treatments, maintaining the sample size for each treatment. From each data permutation the test statistic θ was determined. Because the number of possible permutations is enormous, a random sample of 5,000 permutations was used. For each permutation, the whole series of calculations of life-tables indexes (GRR, R_0, r or T) was repeated and then θ estimated. If the observed value of the test statistic is greater than $(1-\alpha)\%$ of the value in the permutation distribution, then the observed value of θ is significant at the $\alpha\%$ level. The confidence limits were also determined after Levin et al. (1996), using 3,000 permutations.

Life expectancy (e_x) computed for all age classes was compared among the three cohorts using Tukey Honest Significant Difference (HSD test) (Statistica 4.1/Mac^{TM}, 1991), after checking the homogeneity of variances with Barlett's test.

Results

The designations cohort A, B and C will be used in the following sections to refer to the 20 °C–33‰, 15 °C–20‰ and 15 °C–33‰ treatments, respectively.

Survival

Survival curves obtained for each cohort and separately for males and females were plotted in Fig. 1. Mortality was low and approximately the same for the three cohorts in the first four weeks of life and comparatively higher during most of the remaining life-span. Besides the occurrence of mortality oscillations during life-span, there was a linear tendency in the survival percentage overtime for the three cohorts ($p < 0.01$). Considering the total (males plus females) survival results, the highest mortality rate recorded was $5.71 \pm 0.58\%$ per week for cohort A, whereas cohort B and C showed close mortality rates ($4.18 \pm 0.40\%$ and $4.85 \pm 0.87\%$ per week, respectively). The same findings were observed for males and females separately: cohort A showed higher mortality rate ($5.85 \pm 0.59\%$ and $5.56 \pm 0.81\%$ per week for males and females respectively) comparatively with cohort B ($4.25 \pm 0.56\%$ and $4.12 \pm 0.46\%$ per week for males and females respectively) and cohort C ($4.35 \pm 1.35\%$ and $5.35 \pm 0.87\%$ per week for males and females respectively). Comparison of the regression slopes of the three cohorts showed no significant differences (ANCOVA, $p > 0.05$).

Since the cohorts were not followed until all individuals died, life-span was defined as the number of weeks till cohort survival is below 10%. According to this criterion the lowest life-span was recorded for cohort A—16 weeks, compared with 22 and 20 for cohorts B and C. The last sampling of cohort C occurred at 20th week and survival was still 15.7%. It is likely that the life-span of this cohort would be longer (>20 weeks) and probably closer to cohort B.

Individual growth

Both male length and weight showed the same growth pattern in each cohort. Therefore, and since females' weight was not measured (manipulation to extract embryos and determine fecundity damages the female body) only length data is present here.

The growth curves were characterised by high growth rates for the first weeks of life and subsequent gradual reduction until the end of life-span, tending asymptotically for the maximum length (see Fig. 2). ANOVA detected significant differences on growth rates between cohorts, for both males and females ($p < 0.01$). *Post-hoc* comparisons revealed a

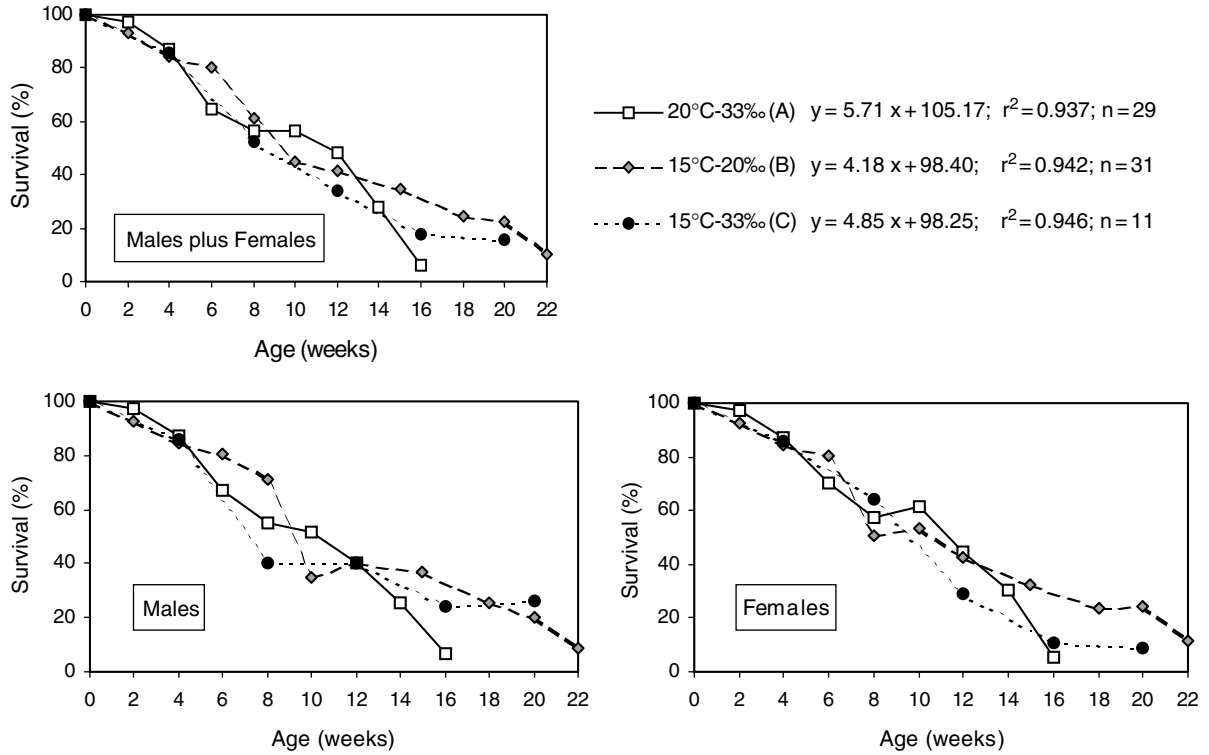

Figure 1. Survival curves of cohorts A, B and C, considering both sexes together and males and females separately. Regression equations, coefficient of determination (r^2) and number of observations (n) are indicated for each cohort.

significantly higher growth rate in cohort A than in cohort B and C (t-test, $p < 0.01$), and no differences between cohort B and C (t-test, $p > 0.05$). Individual growth rate results are outlined in (Table 2). Cohort A growth rates were 1.66 and 1.31 mm/week for males and females respectively, and cohorts B and C growth rates were respectively 1.27 and 1.23 mm/week (males) and 0.94 and 0.94 mm/week (females).

Reproductive traits

Age at maturity was estimated as 5 weeks for cohort A and as 7 weeks for cohorts B and C. The curves of weekly offspring production per female are shown in Fig. 3. The females of cohort A began the reproduction period two weeks earlier than cohort B and C. Offspring production oscillation was very similar along cohort's B and C life-span. Both began to breed at 8th week and the curves follow approximately the same pattern until the end of their lives. The offspring production rate was 35.44, 30.38, 33.16 offspring/female/week for cohorts A, B and C respectively. This parameter did not differ among the three cohorts (ANCOVA, $p > 0.05$). As shown in Fig. 4, the cumulative offspring production curves exhibit very close slopes and the only evident difference is that cohort A curve starts two weeks earlier.

For all treatments, the number of embryos varied directly with female body size, larger females producing larger broods in general. Both linear and curvilinear regressions have been used to describe the relationship between embryo number and female body length (Fish and Mills, 1975; Nelson, 1980; Skadsheim, 1989). In this study, we applied a linear regression since the obtained coefficients of determination were higher. A positive correlation between the number of embryos produced by female and the body length of ovigerous females was found for all treatments ($p < 0.01$) (see Fig. 5). Significant differences in fecundity–female body length relationships were found when the three cohorts were compared (ANCOVA, $p < 0.01$) and significant differences were detected between

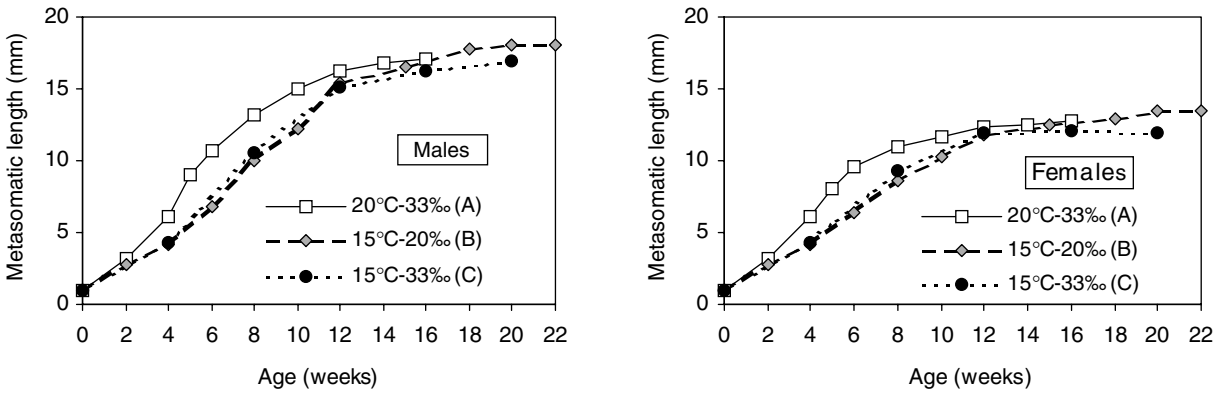

Figure 2. Length growth curves (metassomatic length–ML) for males and females of cohorts A, B and C.

Table 2. Summary of the survival, growth and reproductive traits and life-table parameters and probability levels for statistical comparisons between the cohorts A–C and B–C.[a,b,c]

	Treatments			Statistical analysis	
	Cohort A (20 °C–33‰)	Cohort B (15 °C–20‰)	Cohort C (15 °C–33‰)	Cohorts A–C	Cohorts B–C
Survival, growth and reproductive traits					
Survival					
Total mortality rate	b = 5.71	b = 4.18	b = 4.85	n.s.	n.s.
(Males + Females)	a = 105.17	a = 98.40	a = 98.25		
Males mortality rate	b = 5.85	b = 4.25	b = 4.35	n.s.	n.s.
	a = 105.90	a = 99.06	a = 94.26		
Females mortality rate	b = 5.56	b = 4.12	b = 5.35	n.s.	n.s.
	a = 105.08	a = 97.72	b = 102.11		
Longevity (weeks)	16	22	>20	—	—
Individual growth rate					
Males	b = 1.66	b = 1.27	b = 1.23	$P<0.01$	n.s.
	a = 0.06	a = 0.37	a = 0.16		
Females	b = 1.31	b = 0.94	b = 0.94	$P<0.01$	n.s.
	a = 0.84	a = 0.71	a = 0.97		
Reproductive traits					
Age at maturity (week)	5	7	7	—	—
Age of 1st reproduction (week)	6	8	8	—	—
Offspring production rate	b = 35.44	b = 30.38	b = 33.16	n.s.	n.s.
	a = 236.7	a = 277.5	a = 289.8		
Fecundity-female body length	b = 13.18	b = 19.57	b = 17.36	n.s.	$P<0.01$
	a = 98.29	a = 161.46	a = 138.64		
Life-table parameters					
e_x		*		$P<0.01$**	n.s.
GRR	195.33	234.20	187.29	n.s.	$P<0.01$
R_0	64.88	69.08	37.64	$P<0.001$	$P<0.001$
r	0.41	0.30	0.27	$P<0.001$	n.s.
T (weeks)	10.27	14.27	13.30	$P<0.001$	n.s.

[a] "n.s."—non significant: $p > 0.05$; "b" and "a"—the correlation coefficient and intercept coefficient of the linear regression analysis ($y = bx + a$) respectively; e_x—age specific life expectancy; GRR—gross reproductive rate; R_0—net reproductive rate; r— intrinsic rate of natural increase and T—generation time.

[b] Mortality rates units in percentage/week, growth rates units in mm/week, offspring production rate units in offspring/female/week.

[c] *Results from this parameter are not displayed in the table since they are age specific; **Significant differences only from 10th week onwards.

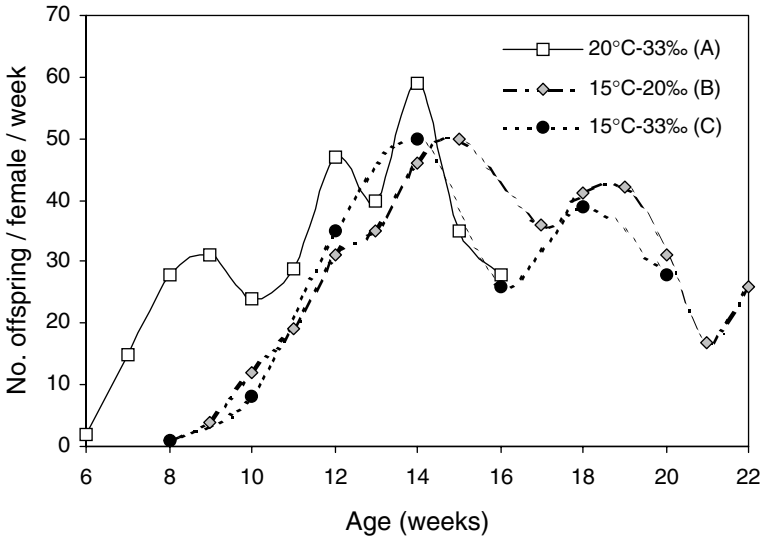

Figure 3. Weekly offspring production during the life-span of cohorts A, B and C.

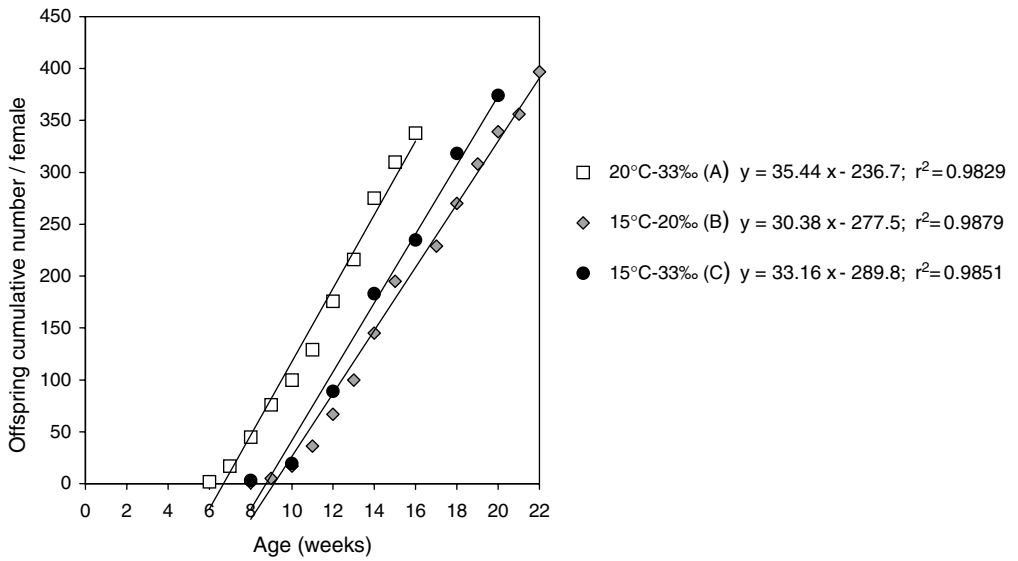

Figure 4. Curves fitted to cumulative number of weekly produced offspring per female in cohorts A, B and C. Regression equations and coefficients of determination (r^2) are indicated for each cohort.

cohorts A–B and between cohorts B–C (*t*-test, $p < 0.01$), but not between cohorts A–C (*t*-test, $p > 0.05$).

Life-table parameters

Age-specific life expectancy (e_x) was always lower in cohort A (20 °C–33‰) and the HSD statistics indicated that it was globally different from cohorts B (15 °C–20‰) and C (15 °C–33‰) ($p < 0.01$), although age-specific comparisons detected significant differences only from 10th week onwards for all age classes ($p < 0.01$). No differences were found between cohorts B and C ($p > 0.05$).

Table 2 summarises the results obtained for the population parameters derived from the life tables (GRR, R_0, r and T). Gross reproductive rate (GRR)

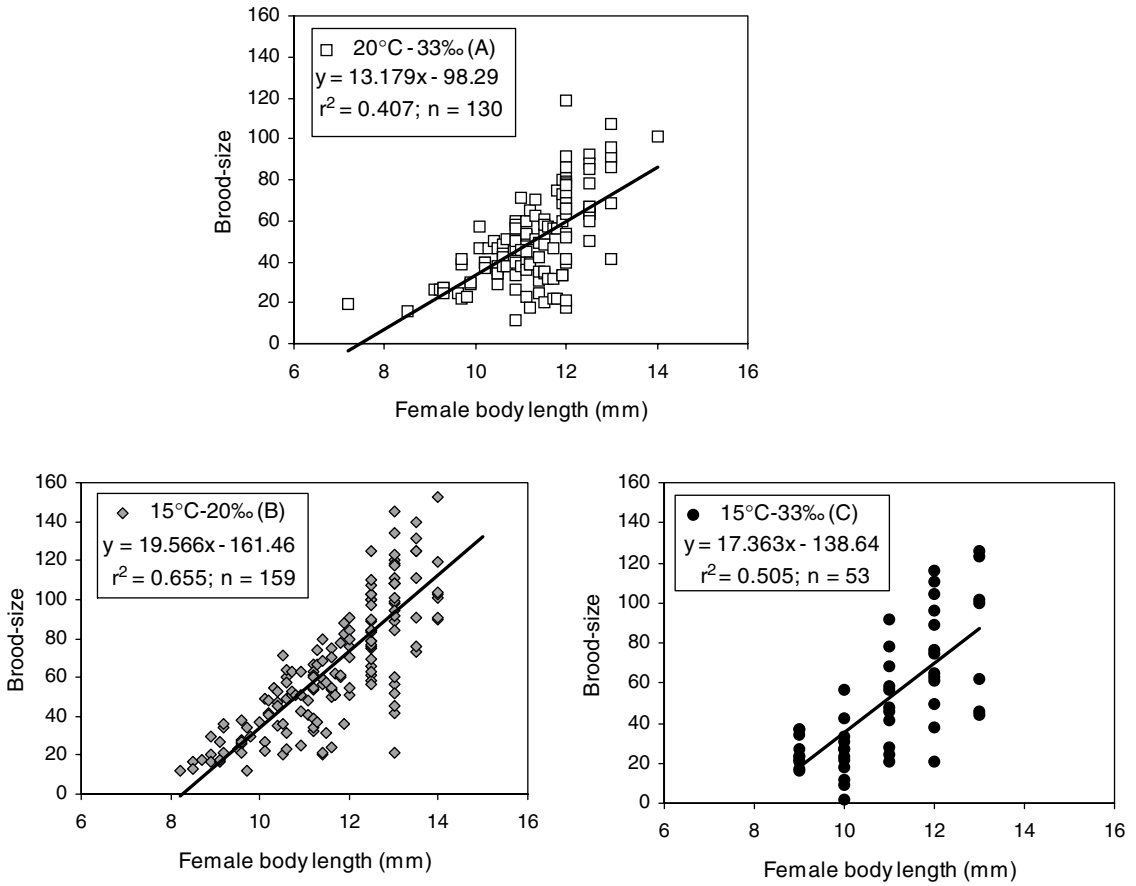

Figure 5. Brood size–female body length (ML) relationships determined for cohorts A, B, and C. Regression equations, coefficients of determination (r^2) and number of observations (n) is indicated in the respective graphs.

values were close for all treatments and only statistical differences were detected between treatments B and C ($p < 0.01$). The net reproductive rate (R_0) was significantly higher for treatments A and B comparing to treatment C ($p < 0.001$). The intrinsic rate of natural increase or population growth rate (r), was significantly higher for treatment A compared to treatments B and C ($p < 0.01$). The generation time (T index) was significantly shorter for treatment A ($p < 0.001$), and no differences were found between treatment B and C ($p > 0.05$).

Discussion

The present study shows how temperature plays an important role in *G. locusta* life history. Compared to 15 °C, life history at 20 °C was characterised by at least a 4-week reduction in the life-span, lower life expectancy, shorter generation time, faster individual growth, anticipated age at maturity, and higher population growth rate (r). All together these results describe an acceleration and condensation of the life cycle at 20 °C.

To be consistent with this findings the mortality rate should be significantly higher at 20 °C, but no differences were found. Yet, mortality rate values were higher at 20 °C than 15 °C, and other mortality-related parameters were affected by temperature, namely longevity and life expectancy, this last one being significantly higher at 20 °C from week 10 onwards. Therefore, the inconsistent results on mortality rates were probably a result of low discriminatory ability of the survival curves' statistics.

The temperature effects described above were not depicted and quantified before for *G. locusta*, and despite similar effects have been reported for other

amphipod species (Sheader, 1981; Sinervo and Doyle, 1990; Highsmith and Coyle, 1991; Sainte-Marie, 1991), there are still some aspects requiring clarification (Costa and Costa, 2000). The implications of these findings for ecotoxicological testing with *G. locusta* will be discussed later on.

Cohort A (20 °C–33‰) showed the highest individual growth rates, but the maximum length achieved, for both males and females, was not the highest in this cohort. This may be explained by the acceleration of the life cycle and consequent life-span reduction, which was also found in other amphipods (Sheader, 1981; Highsmith and Coyle, 1991). The acceleration and condensation of cohort A life cycle is very evident in Fig. 3. Cohort A shows three reproductive peaks (number of offspring/female/week) anticipated in time and closer to which other than cohorts B and C peaks, which appear later and more spaced in time. Since *Gammarus* spp. females are iteroparous, including *G. locusta* (Nelson, 1980; Costa and Costa, 1999), these reproductive peaks may represent a series of reproductive cycles that each cohort's females experienced. The closeness of cohort A's own reproductive peaks probably reflects the reduction of embryonic development period. Several authors have reported that the duration of embryonic development decreases with increasing temperature, since the temperature elevation accelerates the embryos cleavage rate and diminishes their incubation period (Sheader, 1981; Sinervo and Doyle, 1990; Highsmith and Coyle, 1991; Sheader, 1996).

Despite the observed acceleration of growth and life cycle at 20 °C, the offspring production rate did not differ from cohorts at 15 °C. The same is true for brood size–female body length relation, which did not differ between cohorts A and C. No consensus exists on how temperature may affect this reproductive feature in amphipods. In a major revision article (Sainte-Marie, 1991) concludes that this relation stays constant along a latitudinal gradient and thus over a temperature gradient.

By definition, population responses are more ecologically relevant than the individual-level traits, because the former integrates potentially complex interactions among life history traits and provides a more reliable measure of ecological impact (Forbes and Calow, 1999). However, we have shown that, except for some reproductive traits (offspring production rate compared with net reproductive rate (R_0)), the individual and populational responses to the environmental conditions tested point towards similar conclusions and therefore, we consider that both were able to evaluate the effects of temperature on *G. locusta* life history.

Net reproductive rate (R_0) is sometimes assumed to be a measure of population fitness but this assumption is risky and comparatively population growth rate (r) is a much more secure index to population fitness (Levin et al., 1996). The rationale behind this is that R_0 measures only expected reproductive output ($\sum m_x l_x$), and ignores its timing within the life cycle. Given this limitation, the current study's conclusions shall be supported preferentially on the parameter r, which shows that *G. locusta* populations at a temperature of 20 °C will present a higher growth rate comparatively to 15 °C. Generation time (T) results are in agreement with which was discussed earlier for the individual traits of growth and age at maturity; a population with a faster growth rate (treatment A) exhibits a shorter generation time.

The results indicate that, under a salinity of 33‰ or 20‰, *G. locusta* life-history traits remained very similar, except for brood size, which was significantly higher at 20‰ (for the same female body length), and for GRR and R_0. The fact that fecundity differs between cohorts B and C, under different salinity conditions, and offspring production did not, may indicate that there is a higher embryo loss or higher newborn mortality at 20‰. Reduced viability of embryos exposed to low salinities has been reported in *Echinogammarus marinus* by Vlasblom and Bolier (1971). These authors suggest that the egg membrane offered only weak protection to the embryos so that the extra-embryonic fluid of the eggs becomes isotonic in a short time. Thus, at low salinities the eggs swell by taking up water due to osmosis (Steele and Steele, 1991). Our unpublished results on the embryo developmental stages for cohorts A and B showed a markedly higher embryo loss for the latter (20.0% and 40.1% respectively). Differences found in R_0 between cohorts B and C must be interpreted with caution, given the above-mentioned uncertainties involving R_0. Conclusions based on this parameter did not match with the ones from r values, which indicate that there were no differences between cohorts B and C.

Natural history observations indicate that *G. locusta* is found most frequently in fully marine conditions (without freshwater influence) but it also shows that it is able to penetrate estuarine brackish environments to

some extent and live permanently in these conditions (Costa and Costa, 2000). Without doubt, the best evidence for this are the Baltic Sea populations of *G. locusta*, which live permanently at salinities between 5‰, and 7‰, (Bulnheim, 1979, 1984; Jazdzewski, 1973; Fenchel and Kolding, 1979). There are several laboratory culture records of this species' potential to adapt to low salinities. Fenchel and Kolding (1979) cultured five *Gammarus* species, including *G. locusta*, through several generations and during two years at salinities oscillating between 23‰ and 27‰. Bulnheim (1979, 1984) kept *G. locusta*, originally from the Wadden sea (Germany), for several weeks at salinities ranging from 10‰ to 15‰, and carried out physiological adaptation experiments with this amphipods under a salinity of 10‰.

This work shows that, given that a proper acclimation period is provided, the majority of *G. locusta* life-history traits at 20‰ do not differ from 33‰. It also shows that in laboratory controlled conditions with unlimited food, predation and other environmental constraints absent, *G. locusta* physiological adaptation potential is high, and overall its performance is not affected under the tested salinities. Considering this species affinity for marine salinities, it is reasonable to assume that the same is valid for the whole salinity range from 33‰ down to 20‰ (for a temperature of 15 °C). Yet, more conclusions regarding salinity tolerance limits can only be drawn after chronicle *G. locusta* life history under the same conditions but with a broader salinity range, down to the surviving limit.

Findings from the current study will have implications for future developments in ecotoxicological testing with *G. locusta*. The previously proposed acute toxicity test (Costa et al., 1998) only considered the reference temperature and salinity conditions that were used for culturing (15 °C–33‰). Compared to this reference situation, which corresponds to cohort C, the remaining temperature and salinity combinations did not lower the amphipod's performance and, therefore, can be considered within the tolerance limits of the species. The observed effects of temperature constituted only an acceleration of the life cycle, phenomenon that is known to occur in natural populations of amphipods distributed along temperature gradients (Sinervo and Doyle, 1990; Highsmith and Coyle, 1991; Sainte-Marie, 1991). Accordingly, with the present study the range of temperature and salinity conditions acceptable for testing was substantially expanded—from 15 °C to 20 °C at 33‰, and from 33‰ to 20‰ at 15 °C—both for acute (10 days) and chronic (28 days) or other long-term assays (several months). But still, the combined effect of the "extreme" conditions, that is 20 °C and 20‰, needs to be assessed before fully expanding the test range to all new potential temperature and salinity combinations.

Duration of the ecotoxicological tests, particularly chronic tests, may be a major technical problem. Ideally, a chronic test should comprise the entire organism life cycle, i.e. from birth to progeny production, or at least the most sensitive stages (DeWitt et al., 1992; Hill et al., 1994). Since life cycle length is usually temperature-dependent, unless higher temperatures are applied, the chronic tests would eventually extend for excessive periods of time (e.g. Borgmann, 1994; Conradi and Depledge, 1998). Although for scientific purposes long-term tests may be of great utility, routine application of such tests would not be practical and cost-effective. A compromise of 4 weeks (28 days) has been used namely for chronic sediment toxicity tests with amphipods and other invertebrates (DeWitt et al., 1992; Nipper and Roper, 1995; Moore et al., 1997; Ingersoll et al., 1998; Green et al., 1999; Martinez-Madrid et al., 1999; Stronkhorst et al., 1999). In some of these higher temperatures were used to achieve full life cycles in 4 weeks (e.g. DeWitt et al., 1992 Ingersoll et al., 1998), without compromising the tests' scientific validity.

At 20 °C, a concise version of *G. locusta* life cycle was obtained. Under this condition we were able to shorten *G. locusta* life cycle to about 5–6 weeks against 7–8 weeks at 15 °C. First ovigerous females were recorded at week 5 and first progeny at week 6. For standardisation and cost-effectiveness purposes, it would be useful to meet the other amphipod's tests length of 4 weeks. Though that is not possible under a temperature of 20 °C, in a recent chronic toxicity test (our unpublished results) conducted at 22 °C and 33‰ addressing sub-lethal effects of copper-spiked sediments in *G. locusta*, we obtained progeny within the 4-week period. The general experimental conditions of this assay were the same as described in (Table 1), except for the starting amphipod's age that was juveniles (2–3-week-old) instead of newborns (up to 1-week-old).

Therefore, we suggest to use juveniles and a temperature of 20 °C or higher (combined with a

salinity of 33‰) for routine chronic sediment toxicity testing with *G. locusta*. Regarding salinity, results from this study indicated that a set of salinities down to 20% may also be used in sediment toxicity tests with *G. locusta* (combined with a temperature of 15 °C), providing that a proper and gradual acclimation period to that salinity is granted.

Once established the life history in a non-contamination scenario for a set of temperature and salinity combinations, a new range of possibilities in ecotoxicological research with *G. locusta* is open. The range of environmental samples that can be tested was broaden, allowing now the inclusion of contaminated sediments from brackish sections of estuaries, down to 20‰. Simulations of summer (higher temperatures and salinities) versus winter conditions (lower temperature and salinites) can be performed, this way testing seasonal variations in toxicity that natural populations may experience. Finally, interactive effects of temperature and salinity and sediment toxicity can be addressed. Both temperature and salinity are recognized to affect toxicants bioavailability and to interfere in organisms' sensitivity (Bryant et al., 1985; Sze and Lee, 2000), therefore those implications must be considered in advance.

Findings from this study suggest that a multiple response criteria approach including survival, growth and reproductive traits shall be applied in chronic toxicity tests with *G. locusta*, in order to consubstantiate interpretations or eventually unravel underlying biological responses, which may not be found using sole or few criteria. In several cases of this study, responses were not homogenous among the criteria analysed leading to contradictory results, as for instance was observed for mortality rates versus longevity and life expectancy, when comparing cohorts A and C. The fact that the remaining growth and reproduction endpoints were consistent and pointed to the same direction, i.e. an acceleration of the life cycle at 20 °C, allowed to resolve this contradiction.

With the continuous development of ecotoxicological tests towards higher ecological relevance, life-table analysis has started to be used (Levin et al., 1996; Conradi and Depledge, 1998, 1999; Forbes and Calow, 1999). Life tables are a method that integrates individual-level responses to environmental factors (contaminants or others), at the population level, combining approaches that have traditionally been assessed independently (Levin et al., 1996; Forbes and Calow, 1999). The experimental approach used in this study may serve as a basis for implementation of life-table analysis in long-term tests with *G. locusta*, aiming to assess truly population-level responses to toxicants.

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References

ASTM (1992). Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. In: Annual Book of ASTM Standards, *Water and Environmental Technology*, Vol. 11.04, E1367-90. American Society for Testing and Materials, Philadelphia.

Borgmann, U. (1994). Chonic toxicity of ammonia to the amphipod *Hyalella azteca*; importance of ammonium ion and water hardness. *Envir. Pollut.* **86**, 329–35.

Bryant, V., Newberry, D.M., McLusky, D.S. and Campbell, R. (1985). Effect of temperature and salinity on the toxicity of nickel and zinc to two estuarine invertebrates (*Corophium volutator*, *Macoma balthica*). *Mar. Ecol. Prog. Ser.* **24**, 129–37.

Bulnheim, H.P. (1979). Comparative studies on the physiological ecology of five euryhaline *Gammarus* species. *Oecologia* **44**, 80–6.

Bulnheim, H.P. (1984). Physiological responses of various *Gammarus* species to environmental stress. *Limnologica* **15**, 461–67.

Conradi, M. and Depledge, M.H. (1998). Population responses of the marine amphipod *Corophium volutator* (Pallas, 1766) to copper. *Aquat. Toxicol.* **44**, 31–45.

Conradi, M. and Depledge, M.H. (1999). Effects of zinc on the life-history, growth and reproduction of the marine amphipod *Corophium volutator*. *Mar. Ecol. Prog. Ser.* **176**, 131–8.

Conlan, K.E. (1994). Amphipod crustaceans and environmental disturbance: a review. *J. Nat. Hist., Lond.* **28**, 519–54.

Costa, F.O., Correia, A.D. and Costa, M.H. (1996). Sensitivity of a marine amphipod to non-contaminant variables and to copper in the sediment. *Écologie* **27**, 269–76.

Costa, F.O., Correia, A.D. and Costa, M.H. (1998). Acute marine sediment toxicity: A potential new test with the amphipod *Gammarus locusta*. *Ecotox. Environ. Safety*. **40**, 81–7.

Costa, F.O. and Costa, M.H. (1999). Life history of the amphipod *Gammarus locusta* in the Sado estuary (Portugal). *Acta Oecologica* **20**, 327–32.

Costa, F.O. and Costa, M.H. (2000). Review of the ecology of *Gammarus locusta* (L.). *Pol. Arch. Hydrobiol.* **47**, 351–559.

DeWitt, T.H., Redmond, M.S., Sewall, J.E. and Swartz, R.C. (1992). Development of a chronic sediment toxicity test for marine benthic amphipods. U.S. Environmental Protection Agency for the Chesapeake Bay Program, Newport, 99 pp.

Fenchel, T. and Kolding, S. (1979). Habitat selection and distribution patterns of five species of the amphipod genus *Gammarus Oikos* **33**, 316–22.

Fish, J.D. and Mills, A. (1979). The reproductive biology of *Corophium volutator* and *C. Arenarium. J. Mar. Biol. Ass. U.K.* **59**, 355–68.

Forbes, V.E. and Calow, P. (1999). Is the per capita rate of increase a good measure of population-level effects in ecotoxicology?. *Environ. Toxicol. Chem.* **18**, 1544–56.

Green, A., Moore, D. and Farrar, D. (1999). Chronic toxicity of 2,4,6-trinitrotoluene to a marine polychaete and an estuarine amphipod. *Environ. Toxicol. Chem.* **18**, 1783–90.

Highsmith, R.C. and Coyle, K.O. (1991). Amphipod life histories: community structure, impact of temperature on decoupled growth and maturation rates, productivity and P : B ratios. *Amer. Zool.* **31**, 861–73.

Hill, I.R., Matthiessen, P. and Heimbach, F. (1994). Guidance document on sediment toxicity tests and bioassays for freshwater and marine environments, 105 pp Society of Environmental Toxicology and Chemistry, SETAC-Europe.

Ingersoll, C.G., Brunson, E.L., Dwyer, F.J., Hardesty, D.K. and Kemble, N.E. (1998). Use of sublethal endpoints in sediment toxicity tests with the amphipod *Hyalella azteca. Environ. Toxicol. Chem.* **17**, 1508–23.

Jazdzewski, K. (1973). Ecology of gammarids in the Bay of Puck. *Oikos Suppl.* **15**, 121–6.

Levin, L., Caswell, H., Bridges, T., Dibacco, C., Cabrera, D. and Plaia, G. (1996). Demographic responses of estuarine polychaetes to pollutants: life tables responses experiments. *Ecol. Appl.* **6**, 1295–313.

Lincoln, R.J. (1979). British Marine Amphipoda: Gammaridea. British Museum (Natural History), 658 pp.

Marques, J.C. and Bellan-Santini, D. (1990). Faune d'amphipodes des milieux lagunaires du Portugal: actualisation des données et comparaison avec d'autres systémes Atlantiques et Méditerranéens. *Mésogée* **50**, 53–61.

Martinez-Madrid, M., Rodriguez, P., Perez-Iglesias, J.I. and Navarro, E. (1999). Sediment toxicity bioassays for assessment of contaminated sites in the Nervion River (Northern Spain). 2. *Tubifex tubifex* reproduction sediment bioassay. *Ecotoxicology* **8**, 111–24.

Meadows, P.S. and Ruagh, A.A. (1981). Multifactorial analysis of behavioural responses of the amphipod *Corophium volutator* to temperature-salinity combinations. *Mar. Ecol. Prog. Ser.* **6**, 183–90.

Mills, E.L. (1967). The biology of an ampeliscid amphipod crustacean sibling species pair. *J. Fish. Res. Bd. Canada.* **24**, 305–55.

Moore, D.W., Bridges, T.S., Gray, B.R. and Duke, M. (1997). Risk of ammonia toxicity during sediment bioassays with the estuarine amphipod *Leptocheirus plumulosus. Environ. Toxicol. Chem.* **16**, 1020–7.

Mucha, A.P. and Costa, M.H. (1999). Macrozoobenthic community structure in two Portuguese estuaries: Relationship with organic enrichment and nutrient gradients. *Acta Oecologica* **20**, 363–73.

Nelson, W.G. (1980). Reproductive patterns of gammaridean amphipods. *Sarsia* **65**, 61–71.

Nipper, M.G. and Roper, D.S. (1995). Growth of an amphipod and a bivalve in uncontaminated sediments: Implications for chronic toxicity assessments. *Mar. Pollut. Bull.* **31**, 424–30.

Pianka, E.R. (1994). *Evolutionary Ecology*. Harper-Collins College publication. 486 pp.

Reish, D.J. (1993). Effects of metals and organic compounds on survival and bioacumulation in two species of marine gammaridean amphipod, together with a summary of toxicological research on this group. *J. Nat. Hist., Lond.* **27**, 781–94.

Sainte-Marie, B. (1991). A review of the reproductive bionomics of aquatic gammaridean amphipods: variation of life history traits with latitude, depth, salinity and superfamily. *Hydrobiologia* **223**, 189–27.

Sheader, M. (1981). Development and growth in laboratory-maintained and field populations of *Parathemisto gaudichaudi* (Hyperiidea: Amphipoda). *J. Mar. Biol. Ass. U.K.* **63**, 769–87.

Sheader, M. (1996). Factors influencing egg size in the gammarid amphipod *Gammarus insensibilis. Mar. Biol.* **124**, 519–26.

Sheader, M. and Chia, F.S. (1970). Development, fecundity and brooding behaviour of the amphipod, *marinoGammarus obtusatus. J. mar. Biol. Ass. U.K.* **50**, 1079–99.

Sinervo, B. and Doyle, R.W. (1990). Life-history analysis in "physiological" compared with "sidereal" time: an exemple with an amphipod (*Gammarus lawrencianus*) in a varying environment. *Mar. Biol.* **107**, 129–39.

Skadsheim, A. (1989). Regional variation in amphipod life history: effects of temperature and salinity on breeding. *J. Exp. Mar. Biol. Ecol.* **127**, 25–42.

Skadsheim, A. (1990). A cohort life table for *Gammarus salinus* (Amphipoda). *Oikos* **57**, 207–14.

Steele, D.H. and Steele, V.J. (1991). Effects of salinity on the survival, growth rate and reproductive output of *Gammarus lawrencianus* (Crustacea, Amphipoda). *Mar. Ecol. Prog. Ser.* **78**, 49–56.

Statistica/Mac™, (1991). User's Guide. StatSoft, Inc. Tulsa U.S.A. 410 pp.

Stronkhorst, J., Hattum, B.V. and Bowmer, T. (1999). Bioaccumulation and toxicity of tributyltin to a burrowing heart urchin and an amphipod in spiked, silty marine sediments. *Environ. Toxicol. Chem.* **18**, 2343–51.

Sze, P.W.C. and Lee, S.Y. (2000). Effects of chronic copper exposure on green mussel *Perna viridis Mar. Biol.* **137**, 379–92.

Vlasblom, A.G. and Bolier, G. (1971). Tolerance of embryos of *MarinoGammarus marinus* and *Orchestia gammarella* (Amphipoda) to lowered salinities. *Neth. J. Sea Res.* **5**, 334–41.

Zar, J.H. (1984). *Biostatistical Analysis*. Prentice-Hall, Inc. Englewood Cliffs, 718 pp.