

Original article

The influence of temperature and salinity on the duration of embryonic development, fecundity and growth of the amphipod *Echinogammarus marinus* Leach (Gammaridae)

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Abstract

The effects of salinity and temperature on the duration of embryonic development, fecundity and growth of the amphipod *Echinogammarus marinus* Leach from the Mondego estuary (Portugal) were studied in laboratory experiments. Combinations of three temperatures (10, 15 and 20 °C) and four salinities (10, 15, 20 and 25 ‰) were used. The duration of embryonic development was 33 ± 0.7 d (mean \pm S.E.) at 10 °C, 32 ± 0.5 d at 15 °C, and 17 ± 0.3 d at 20 °C. Analysis of variance demonstrated that the duration of *E. marinus* embryonic development, reared under different combinations of salinity and temperature, was significantly affected only by temperature ($P < 0.001$). A positive correlation between the number of newborn juveniles and the size of *E. marinus* females (as head length) was observed. The number of juveniles released per female was higher at 10 °C and lower at 20 °C. Analysis of variance showed that only temperature significantly affected the number of juveniles released per female ($P < 0.001$). Experimental data were used to calibrate the von Bertalanffy growth model. Results showed that growth was continuous throughout life under all laboratory conditions. Intrinsic growth rates were higher at 20 °C and lower at 10 °C. Analysis of covariance applied over the initial 90 d after hatching showed significant differences between growth rates of *E. marinus* under different salinity and temperature conditions. Extrapolation of laboratory data to the field scenario suggests that *E. marinus* in the Mondego estuary have a multivoltine life cycle.

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Keywords: *Echinogammarus marinus*; Embryonic development; Fecundity; Growth rates; Salinity; Temperature

1. Introduction

Estuaries are characterised by fluctuating conditions of salinity and temperature to the extent that both are considered dominant “ecological master factors”, which may act either singly or in concert to modify the structure, function, and distribution of estuarine organisms (McKenney, 1996). Organisms in the tidal zone experience major short-term and long-term or seasonal variations in environmental temperature and seawater osmolality. Estuarine amphipods must be able to adapt functionally to these dynamic environmental variables. Temperature, through its control of metabolic rates and linked effects on reproductive effort, and salinity, through its effect on moulting and reproductive success, may

be important physiological constraints to reproduction and determinants of life-history patterns of amphipods in estuaries (Cunha et al., 2000a).

Echinogammarus marinus Leach is very well adapted to life in estuaries, since it is a strongly euryhaline amphipod, able to support long emersion periods (Dorgelo, 1973; Pinkster and Broodbakker, 1980). The Mondego estuary is the southernmost distribution limit of this species, which extends from Norway to Portugal (Maren, 1975a, b; Marques and Bellan-Santini, 1993). In the Mondego estuary, *E. marinus* is the most abundant species in intertidal communities on hard substrata (Marques et al., 1993). This species is an important prey for birds (Múrias et al., 1996, 1997) feeding among eulittoral *Fucus vesiculosus* (Phaeophyta) on rocky shores that make up about 60% of the perimeter of the Mondego estuary. Previous studies on the biology, population dynamics, and productivity of *E. marinus* there (Marques and

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Nogueira, 1991; Maranhão et al., 2001) showed that density, percentage of ovigerous females, sex ratio, fecundity, and egg volume were affected by temperature and salinity. The salinity and temperature ranges of this species in Mondego estuary were 4–31‰ and 7–29 °C (Marques and Nogueira, 1991; Maranhão et al., 2001).

The objectives of the present study were to determine the ecophysiological response of *E. marinus* to a range of salinity–temperature combinations, with emphasis on the duration of embryonic development, fecundity (number of juveniles released), and growth. The results obtained will assist in making extrapolations from field data previously obtained, and in developing a population dynamics model for this species (Martins et al., 2002) that will allow in simulating changes resultant from variations of environmental factors like temperature and salinity (forcing functions). Due to continuous recruitment, many traits of this amphipod's life cycle, such as age at maturity, lifespan, number of broods per female, and number of generations per year remain unknown.

2. Materials and methods

2.1. Collection and acclimation of amphipods

E. marinus individuals were collected among *F. vesiculosus* in the Mondego estuary and transported to the laboratory with its algae biotope. The amphipods used in laboratory experiments, performed at 10, 15 and 20 °C (and all salinities, see below) were collected, respectively, under the following temperature and salinity conditions: 9 °C–19‰, 13 °C–22‰ and 19 °C–27‰. Once in the laboratory, the algae were washed and the amphipods were separated and placed in a 37 × 22 cm plastic tank filled with 3 l of continuously aerated test water, for 1 month, to acclimate the individuals to experimental conditions. One tank with a large group of amphipods was used to acclimate the organisms to each pair of temperature–salinity conditions. Three room temperatures (10, 15, and 20 °C) and four salinities (10, 15, 20, and 25‰) were chosen. Therefore, 12 tanks were used for acclimation, each one corresponding to one possible combination of temperature and salinity. The salinity ranges used were prepared by mixing estuarine filtered water with deionised water or by adding commercial iodine-free seawater salt to the estuarine water. A light regime of 12 h light: 12 h dark was employed. Twice a week, the water was changed and the amphipods were fed with dry and chopped *F. vesiculosus* supplied ad libitum. At least once per week, Tetramin® fish food was supplied. To provide shelter and mimic the natural environment, small rectangles of black polyethylene sheet were placed in the tanks.

2.2. Duration of embryonic development

A mature female amphipod is available for fertilisation for only a short time immediately after moulting when the cu-

ticle is sufficiently flexible to allow release of the eggs into the brood pouch through the genital pores. A mature male will have only this short time between moulting and ovulation to deposit sperm into the brood pouch. Therefore, the reproductive behaviour of *E. marinus* involves a precopulatory guarding phase in which the male holds and carries the female. This ensures that insemination can occur as soon as the female moults and is ready to release eggs into the brood pouch. After 1 month acclimation, 10 pairs of amphipods in precopula were removed from the initial tank (for each temperature–salinity condition); each pair was placed in a 10 × 10 cm aquarium with 0.25 l of water and observed daily until the female separated from the male and was found to be ovigerous. The male was then removed from the container. The conditions in the small aquarium were basically the same as for the stock acclimated population; only the small rectangles of black polyethylene sheet were not used since the pieces of *F. vesiculosus* supplied as food provided enough shelter to individuals. The embryonic development time was recorded as the period from oviposition to release of juveniles from the brood pouch. A second set of three tanks, each one with a pair in precopula, was used to determine how many broods each female could produce in every experimental condition. In this case, the male was not removed when the separation occurred. When embryonic development was completed, the juveniles released by the female were removed from the aquarium with a plastic pipette, leaving the male and the female in the tank. The number of broods produced by each female was recorded.

2.3. Fecundity

The fecundity of *E. marinus* females was estimated as the number of juveniles released by a female in each replicate. Newborn juveniles were removed to avoid cannibalism by the female. After releasing all juveniles from the brood pouch, the female head length was measured to the nearest 0.02 mm. All measurements were taken with a calibrated ocular micrometer under a dissecting microscope. When possible, females were measured alive and returned to the respective acclimation tank. When necessary, they were killed by immersion in diluted ethanol. The allometric relation between total length (TL; mm) and head length (HL; mm) previously established by Marques and Nogueira (1991) ($TL = -1.211995 + 10.668509HL$) was used to determine the total length of amphipods.

2.4. Growth

The juveniles released in the fecundity experiments were placed in a tank with the same conditions as for the mother. Some of the water from the female's aquarium was also transferred since Sexton (1928) concluded that the young did better in a mixture of "parent" water and "clean" water. These juveniles were then used to estimate the growth under laboratory conditions. Ten replicates for each salinity–temperature pair were used. Groups of individuals (5–10) re-

moved from different replicates for each salinity–temperature condition were measured for the head length to the nearest 0.02 mm, after 1, 5, 10, 15, 20, 25, 30, 40, 50, etc., days following their release from the brood pouch, until the last one died. When possible, individuals were measured alive and returned to the respective aquarium. When necessary, individuals were killed by immersion in diluted ethanol.

2.5. Data analysis

Experimental data on the duration of embryonic development and fecundity were analysed using two-way analysis of variance (ANOVA), with the head length of females being a covariate in the fecundity test. Data were tested for conformity to normality (Kolmogorov–Smirnov’s test) and variance homogeneity (Cochran’s test) (Underwood, 1997). When necessary, an $\ln(1+x)$ transformation was used prior to the analysis. Multiple mean comparisons were made using Tukey’s test to estimate differences between laboratory conditions (Zar, 1996). Regressions were estimated using the Stepwise Forward Analysis method performed with the STATGRAPHICS 4.0 statistical package.

The overall growth of many animals has been shown to follow a sigmoid curve while during the earliest stages it is generally accepted that growth is exponential (Welton and Clark, 1980). Kinne (1953 in Savage, 1982) indicated that growth was exponential in *Gammarus duebeni*, another amphipod found in the Mondego estuary (Lopes, 2000), for at least 90 d after hatching when maintained at 10‰ and 19 °C, with adults reaching the same length as *E. marinus*. For this reason, growth rates of *E. marinus* under different experimental conditions were also compared, based on an estimation for 90 d after hatching, using the exponential growth model:

$$HL_t = HL_0 \times e^{\mu t} \quad (1)$$

where HL_t is the head length of amphipod at time t , HL_0 the initial head length, and μ the specific growth rate (d^{-1}) (Brown and Rothery, 1994). The exponential growth model was then fitted to linear regressions using \ln transformed data (Sokal and Rohlf, 1995). The estimated slopes of linear regressions corresponded to *E. marinus* growth rates in each aquarium. Covariance analysis (ANCOVA), with time as the covariate, was used to compare slopes between aquaria and significant differences in growth rates were determined using Tukey’s test (Zar, 1996).

To express laboratory growth rates, the von Bertalanffy growth model was used:

$$L_t = L_\infty [1 - e^{-k(t-t_0)}]^D \quad (2)$$

where L_t is the length of the organism at a given moment t ; L_∞ the maximum possible length of the organism; t_0 the instant when the organism would have a length = 0; k the intrinsic growth rate and D is the parameter that expresses metabolic deviations from the von Bertalanffy 2/3 rule. In this case, D was assumed to be 1.

Table 1

Echinogammarus marinus. Two-factor ANOVA of effects of salinity and temperature on embryonic development

Source	d.f.	MS	F	P
Salinity	3	0.023	2.1	0.1097
Temperature	2	5.514	487.2	0.0000
Salinity × temperature	6	0.014	1.3	0.2818
Residual	108	0.011		

3. Results

3.1. Duration of embryonic development

ANOVA demonstrated that the duration of embryonic development of *E. marinus*, reared under a range of salinities and temperatures, was significantly ($P < 0.001$) affected by temperature, but not by either salinity or a salinity–temperature interaction (Table 1). The mean duration of embryonic development was 33 ± 0.7 d (mean \pm S.E.) at 10 °C, 32 ± 0.5 d at 15 °C, and 17 ± 0.3 d at 20 °C (Fig. 1). Embryos of amphipods reared at 10 and 15 °C required about 15 d more than those reared at 20 °C to complete development. The Tukey test carried out a posteriori revealed significant differences between results obtained at 20 °C and results obtained at 10 and 15 °C.

All pairs of males and females that remained together after the release of juveniles produced at least three broods consecutively. The maximum possible number of broods produced by *E. marinus* females was not determined in the present study.

3.2. Fecundity

A positive correlation was observed between the number of newborn juveniles and the size (head length) of *E. marinus* females (Table 2). Since the number of juveniles released varied as a function of size of the females, a standard female head length of 1.60 mm (15.86 mm of total length) was used to solve the regression equations obtained under the various experimental laboratory conditions. For standard length females, the number of juveniles released per female was

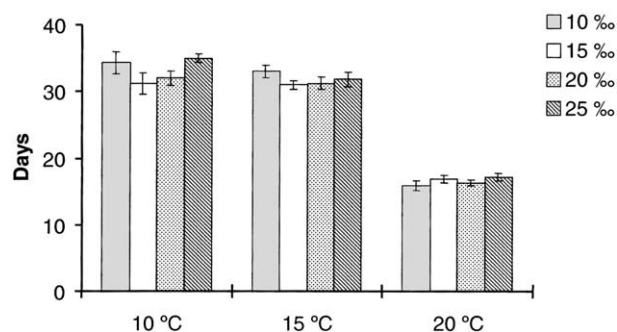


Fig. 1. *Echinogammarus marinus*. Duration of embryonic development at different combinations of salinity and temperature in laboratory conditions (mean \pm standard error).

Table 2

Echinogammarus marinus. Linear regression equations linking the number of juveniles released (NJ) and head length (HL) of females, and also the female head length range and fecundity mean values at all experimental conditions. *r*, correlation coefficient; *n*, number of females; S.E., standard error

Experimental conditions	Equations	<i>r</i>	<i>P</i>	<i>n</i>	Female HL range (mm)	Fecundity mean ± S.E.
10 °C–10‰	NJ = –38.763 + 37.035 HL	0.62	<0.05	10	1.32–1.62	15 ± 2
10 °C–15‰	NJ = –70.305 + 62.8 HL	0.88	<0.001	10	1.32–1.78	24 ± 4
10 °C–20‰	NJ = –162.36 + 120.98 HL	0.96	<0.001	10	1.36–1.84	33 ± 7
10 °C–25‰	NJ = –85.203 + 68.644 HL	0.83	<0.01	10	1.36–1.62	18 ± 2
15 °C–10‰	NJ = –120.37 + 85.627 HL	0.91	<0.001	10	1.52–1.82	20 ± 3
15 °C–15‰	NJ = –17.881 + 22.154 HL	0.31	0.39	10	1.44–1.80	16 ± 2
15 °C–20‰	NJ = –50.358 + 44.773 HL	0.65	<0.05	10	1.44–1.84	23 ± 4
15 °C–25‰	NJ = –71.661 + 53.869 HL	0.72	<0.05	10	1.52–1.76	17 ± 2
20 °C–10‰	NJ = –51.141 + 38.998 HL	0.82	<0.01	10	1.40–1.76	11 ± 2
20 °C–15‰	NJ = –11.695 + 12.159 HL	0.81	<0.01	10	1.40–1.72	7 ± 1
20 °C–20‰	NJ = –10.494 + 12.374 HL	0.22	0.54	10	1.42–1.70	10 ± 2
20 °C–25‰	NJ = –81.706 + 56.478 HL	0.92	<0.001	10	1.58–1.76	13 ± 1

higher at 10 °C and lower at 20 °C (Fig. 2). A female with a head length of 1.84 mm (corresponding to 18.42 mm of the total length), reared at 10 °C and at a salinity of 20‰ released 67 juveniles, the largest number observed in our experiments. On the other hand, the minimum number of recruits produced by a female was 3, recorded for two females reared at 20 °C, one with a head length of 1.40 mm (13.72 mm total length) and reared at 10‰ salinity, and the other with a head length of 1.52 mm (15.00 mm total length), and reared at 20‰ salinity. ANOVA showed that temperature significantly affected the number of recruits released by a female ($P < 0.001$), but not salinity or salinity–temperature interaction (Table 3). The Tukey test performed a posteriori revealed significant differences between the results obtained at 20 °C and those obtained at 10 and 15 °C.

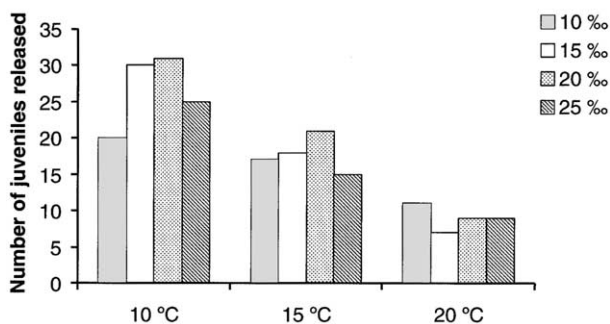


Fig. 2. *Echinogammarus marinus*. Number of juveniles released by a standard female of 1.60 mm head length, at all experimental conditions.

Table 3

Echinogammarus marinus. Two-factor ANOVA of effects of salinity and temperature on fecundity, with female head length as covariate

Source	d.f.	MS	F	P
Covariate	1	9.119	91.5	0.0000
Salinity	3	0.117	1.2	0.3217
Temperature	2	7.947	79.7	0.0000
Salinity × temperature	6	0.190	1.9	0.0859
Residual	107	0.099		

3.3. Growth

Specific growth rates obtained using the exponential growth model for *E. marinus* reared for 90 d after hatching under different laboratory conditions are presented in Table 4. Organisms reared at 10 °C showed the lowest growth rates while individuals maintained at 20 °C had the highest growth rates.

Significant differences were found between growth rates of *E. marinus* at different salinities and temperatures (ANCOVA, $P < 0.05$). Tukey's test (Table 5) showed that growth at 10 °C was significantly different from growth at 20 °C ($P < 0.05$) at all experimental salinity conditions, while growth at 15 °C is not significantly different from growth at 20 °C ($P > 0.05$) at any salinity. Specific growth rates of individuals reared at 15 °C at salinities of 10 and 25‰ were not significantly different from growth rates of amphipods maintained at 10 °C in all salinity conditions (Table 5). On the other hand, specific growth rates at 15 °C for salinities ranging between 15 and 20‰ were significantly different from growth rates at 10 °C for salinities ranging from 15 to 25‰. Finally, the specific growth rate of amphipods maintained at 10 °C and 10‰ salinity was not different from growth rates of organisms reared at 15 °C at all salinities tested.

The von Bertalanffy growth model calibrated with our experimental data showed that the growth of *E. marinus* was continuous throughout life (Fig. 3) under all laboratory conditions. Nevertheless, *E. marinus* growth rates were higher at 20 °C and lower at 10 °C (Fig. 3).

The largest individuals under laboratory conditions were observed at 10 °C, reaching a maximum head length of

Table 4

Echinogammarus marinus. Specific growth rate (d^{-1}) of individuals reared under laboratory conditions

Conditions (‰)	10 °C	15 °C	20 °C
10	0.0075	0.0094	0.0135
15	0.0066	0.0114	0.0123
20	0.0066	0.0108	0.0122
25	0.0065	0.0099	0.0125

Table 5

Echinogammarus marinus. Results from the multiple comparison test (Tukey's test) between growth rates (slopes) obtained in laboratory experiments. *, Significant differences between slopes ($P < 0.05$); NS, non-significant differences between slopes ($P > 0.05$)

Slopes	10 °C/10‰	10 °C/15‰	10 °C/20‰	10 °C/25‰	15 °C/10‰	15 °C/15‰	15 °C/20‰	15 °C/25‰	20 °C/10‰	20 °C/15‰	20 °C/20‰	20 °C/25‰
10 °C/10‰ 0.00749	–											
10 °C/15‰ 0.00655	NS	–										
10 °C/20‰ 0.00656	NS	NS	–									
10 °C/25‰ 0.00647	NS	NS	NS	–								
15 °C/10‰ 0.00943	NS	NS	NS	NS	–							
15 °C/15‰ 0.01138	NS	*	*	*	NS	–						
15 °C/20‰ 0.01079	NS	*	*	*	NS	NS	–					
15 °C/25‰ 0.00998	NS	NS	NS	NS	NS	NS	NS	–				
20 °C/10‰ 0.01355	*	*	*	*	NS	NS	NS	NS	–			
20 °C/15‰ 0.01232	*	*	*	*	NS	NS	NS	NS	NS	–		
20 °C/20‰ 0.01226	*	*	*	*	NS	NS	NS	NS	NS	NS	–	
20 °C/25‰ 0.01248	*	*	*	*	NS	NS	NS	NS	NS	NS	NS	–

1.85 mm (corresponding to 18.56 mm total length). In field studies (Maranhão et al., 2001), the largest individuals collected ranged between 2.00 and 2.22 mm head length, a female and a male respectively, of 20.16 to 22.47 mm total length. Moreover, it was observed that individuals kept at higher temperatures (20 °C) had a shorter lifespan (ranging from 190 to 230 d), while individuals kept at 10 °C exhibited not only a longer lifespan (ranging from 380 to 440 d) but also reached a larger body size.

4. Discussion

The duration of embryonic development in amphipods is related to temperature (Steele and Steele, 1973; Borowsky, 1980; Welton and Clarke, 1980; Fredette and Diaz, 1986; Steele and Steele, 1986; Takeuchi and Hirano, 1991). This relationship is clearly consistent with our laboratory results where a decrease in the duration of embryonic development was observed with a temperature rise from 10–15 to 20 °C. The same relationship was observed in experiments carried out by Vlasblom (1969) with *E. marinus* from the Oosterschelde (the Netherlands). In his study, a rapid increase in the rate of development from egg to hatching stage occurred at temperatures from 5 to 10 °C, reaching an optimum at ca. 20 °C. Nevertheless, the duration of embryonic development observed by Vlasblom (1969) for *E. marinus* was shorter compared with our data. He recorded 44, 18 and 11 d for the duration of embryonic development at 5, 9–12 and 15 °C, respectively. Differences in environmental conditions (mainly temperature) between the Mondego and the Oosterschelde estuaries may help account for this intraspecific variation.

Significant correlation was found between the number of juveniles released and the size of the females. Brood size in gammarideans is often reported as being proportional to body length of females (e.g. Beare and Moore, 1996; Costa and Costa, 1999; Persson, 1999; Cunha et al., 2000b). Moreover, the highest production of juveniles by *E. marinus* females was observed at 10 °C and the lowest at 20 °C.

Nevertheless, northern Gammaridean species usually produce smaller clutches during the colder months, in association with an increase in egg size. This strategy might provide the offspring with a better chance of surviving harsh conditions (Van Dolah and Bird, 1980; Kolding and Fenchel, 1981; Skadsheim, 1984; Pardal et al., 2000). In the Mondego estuary, *E. marinus* females produced smaller broods of larger eggs during winter (colder months) (Maranhão et al., 2001). However, under the present experimental conditions *E. marinus* females produced larger clutches in colder water. Bell and Fish (1996) stated that stable laboratory conditions, with food supplied ad libitum might well favour the production of larger broods. In our case, it is possible that favourable conditions in the laboratory allowed the reduction of egg size at lower temperatures, making a larger production of juveniles possible. Our results were consistent with the findings of Vlasblom (1969) that the greatest yield of juveniles by *E. marinus* females was at about 15 °C. Moreover, *E. marinus* females reared at 20 °C exhibited smaller broods and shorter periods of embryonic development (by about a half) when compared with females cultured at 10 °C. Skadsheim (1989) also observed that *Gammarus oceanicus* produced smaller broods of small eggs during acclimation to 20 °C. This might have been due to metabolic constraints. Brood and embryo size are limited by the amount of energy devoted by the female to reproduction, which depends on growth and maintenance costs (Clark, 1987 in Sainte-Marie, 1991). Reproductive success of an organism is known to require the allocation of a certain amount of the assimilated energy to the gonads, in competition with the interrelated processes of maintenance and somatic growth. Moreover, higher water temperatures may result in increased metabolic maintenance costs that only allow a decreased allocation of energy for reproduction (Cunha et al., 2000a). Dorgelo (1973) reported an increase in oxygen consumption rates in *E. marinus* with increased temperature, suggesting the occurrence of a greater energetic demand from metabolic processes of maintenance and, as a consequence, the production of smaller broods might be induced to reduce the energy expended in reproduc-

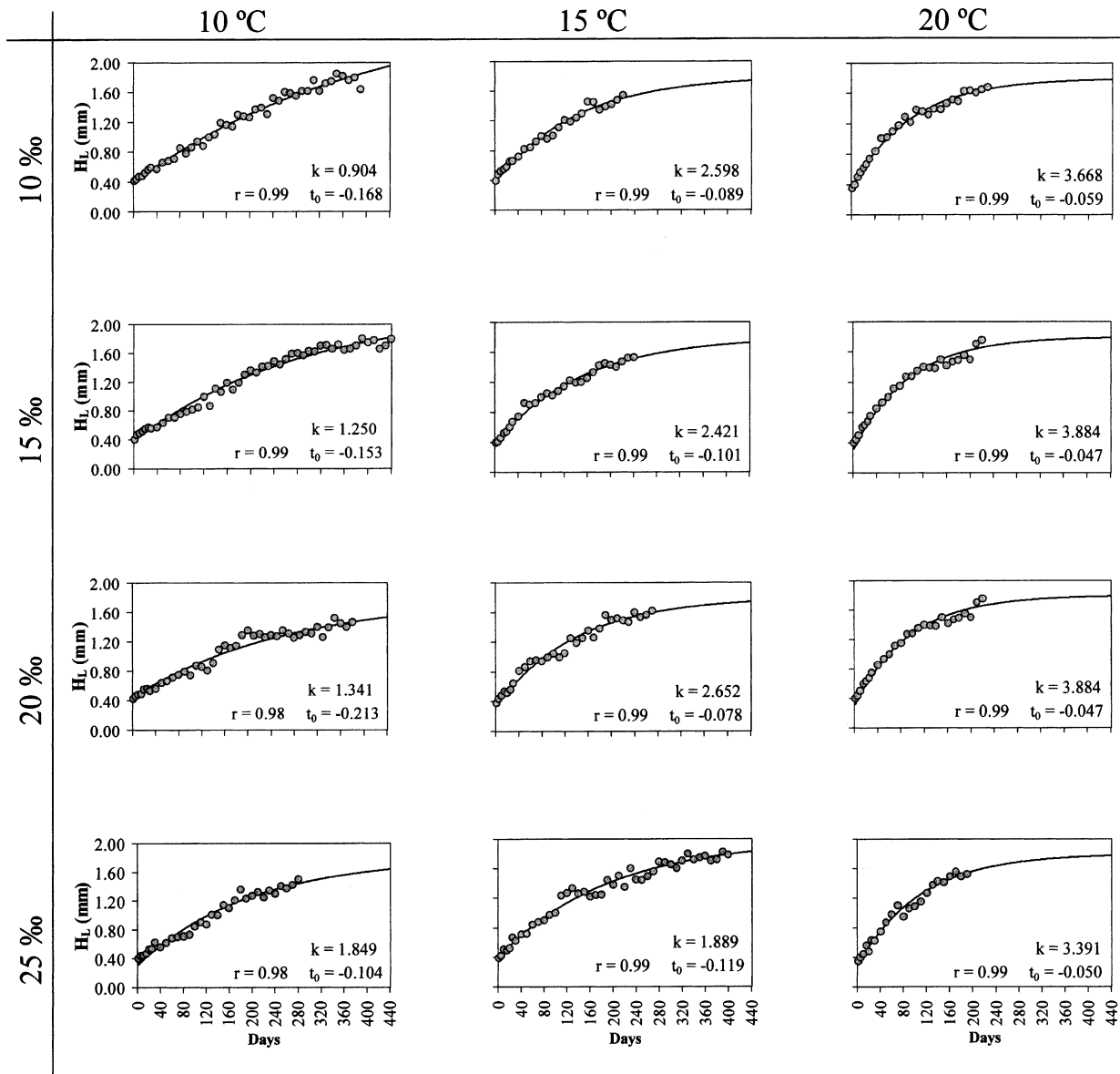


Fig. 3. *Echinogammarus marinus*. Growth models calibrated with laboratory data (plotted points). H_L , head length; k , intrinsic growth rate; t_0 , instant when the organism would have a length = 0; r , correlation between predicted and observed values.

tion. Variation of the percentage of intramarsupial loss with fluctuations of temperature and/or salinity may explain some observed differences. Vlasblom (1969) in his experiments with *E. marinus* found that at 15 °C about 75% of the eggs really hatched whereas at 9 °C this percentage was as low as 17%. Marques and Nogueira (1991) reported a 28% intramarsupial loss for *E. marinus* in the Mondego estuary, nonetheless without establishing any relation with the environmental factors. Nevertheless, percentages of brood mortality were not estimated during the present study. Ultimately, Nelson (1980) points out that a decrease of the brood size (fecundity) in summer vs. winter populations is general among amphipods, although by no means universal.

It was observed that a female could produce at least three broods consecutively. These results were similar to those obtained by Vlasblom (1969), who found a maximum of four

broods, and Skadsheim (1982), who referred to two or three broods for *E. marinus* populations from Oosterschelde (The Netherlands) and Oslofjorden (Norway), respectively.

Salinity changes had no visible effects on the duration of embryonic development and fecundity in *E. marinus*. This was probably because this species is strongly euryhaline (Dorgelo, 1973; Pinkster and Broodbakker, 1980). Although salinity is often considered of less significance for reproductive activities, some studies suggested the influence of salinity on amphipods' brood size. Vlasblom and Bolier (1971) stated that although embryos of *E. marinus* could develop in salinities ranging between 4 and 7‰, the number of emergent juveniles was then reduced. Pinkster and Broodbakker (1980) observed a reduction in brood size with decreasing salinity in laboratory experiments with *Eulimnogammarus obtusatus*. Cunha et al. (2000a) also reported the significance

of salinity on the brood size in wild populations of *Corophium multisetosum*.

The growth of *E. marinus* individuals was found to be continuous throughout life for all laboratory conditions. Nevertheless, growth rates were higher in the early phases, when it is commonly accepted that individuals grow exponentially (Welton and Clarke, 1980). Growth rates under different laboratory conditions were influenced by temperature, with higher rates occurring at higher temperatures. It is well known that individual growth depends on physiological processes influenced by temperature. The observed effect of temperature on growth rates of *E. marinus* and the continuous growth throughout life of this species have been widely found for other amphipod populations in temperate areas (Hastings, 1981; Savage, 1982; Dauvin 1988a, b; Uitto and Sarvala, 1991; Covi and Kneib, 1995; Drake and Arias, 1995; Cunha et al., 2000b; Pardal et al., 2000). Our results suggest that, in the Mondego estuary, *E. marinus* growth rates should be higher during spring and summer and lower during winter, as observed for other local amphipod species, e.g. *Amphitoe valida* (Pardal et al., 2000).

Apparently, salinity also influenced growth rates at temperatures of 10 and 15 °C, although not at higher temperatures. Salinity–temperature interactions have been shown to modify growth rates of a number of estuarine crustaceans (McKenney and Celestial, 1995). In *E. marinus*, this was probably because different combinations of temperature and salinity produce variations in respiration rates, as demonstrated by Dorgelo (1973). Due to difficulties in manipulating individuals without damaging the amphipods, these were not sexed during the experiments. It is possible, therefore, that the amphipods measured in the experiments at 15 °C at salinities of 10 and 25‰ were predominantly females. Since *E. marinus* males and females reach different sizes at the same age (the males being larger), the fact that a greater percentage of females may have been measured in those experiments, might have biased the head length of the experimental population. In each instance, the determined head length might then have been smaller, and the estimated growth rates at different temperatures been similar. Some growth variations could also be associated with poor food quality. *E. marinus* individuals exhibited cannibalistic behaviour in the laboratory, eating dead conspecifics and ingesting moulted exoskeletons, which supports the idea that this species requires a high nitrogen food source at times (Agnew and Moore, 1986).

The maximum body size attained by individuals under laboratory conditions was smaller than the body size attained by organisms in the field (see Marques and Nogueira, 1991; Maranhão et al., 2001). In addition to the influence that the quality of the food supplied could have had on these results, the lower body size reached by individuals under laboratory conditions could also be attributable to a decrease in the scope for growth in response to stress. For example, Chen et al. (1990) recorded higher mortalities and lower growth rates of penaeid shrimps in culture due to deterioration of water

Table 6

Echinogammarus marinus. Days needed to reach sexual differentiation and maturation, obtained by extrapolation of laboratory data to the field data

	Experimental conditions (°C)		
	10	15	20
Male sexual differentiation (days)	76–87	56–64	42–48
Female sexual differentiation (days)	76–83	56–61	45–46
Female maturation (days)	152–186	106–142	77–102

quality caused by ammonia and nitrite. Ammonia, excreted by cultured animals and derived from ammonification of unconsumed food or organic detritus, is the most common toxicant. Nitrite, formed from ammonia by *Nitrosomonas* spp., is rather more toxic than ammonia to crustaceans (Chen et al., 1990). In our case, it is possible that water quality problems were caused by ammonia and nitrite since the food was supplied ad libitum and the water was changed only twice per week. The size of the aquaria used could also have had some effect on individual growth rates. Clancy (1997) noted that enclosure effects might have biased growth measures of *Jassa marmorata* during an experimental study. That said, however, in the laboratory, *E. marinus* attained the largest dimensions and lived longer at 10 °C, which is consistent with common observations in field and laboratory studies, where maximum body size, lifespan and development time of ectothermic animals are negatively correlated with environmental temperature (Strong and Daborn, 1980; Panov and McQueen, 1998).

Using the growth models calibrated with data from the experiments at 10, 15 and 20 °C (Fig. 3) some extrapolations of data obtained in a fieldwork recently carried out by Maranhão et al. (2001) in the Mondego estuary were attempted. In the field, males attained sexual differentiation at 0.86 ± 0.03 mm head length (mean \pm S.D.) (corresponding to 7.92 mm total length) and females at 0.85 ± 0.02 mm head length (mean \pm S.D.) (7.82 mm total length). *E. marinus* females reach maturity at 1.19 ± 0.08 mm head length (mean \pm S.D.) (11.48 mm total length). The days that males and females need to attain these sizes when living at 10, 15 and 20 °C in the field are presented in Table 6. Sexual differentiation and maturity of *E. marinus* individuals were attained earlier at higher temperatures and later at lower temperatures (Table 6). A female's lifespan was estimated to be 369–484 d at 10 °C, at least 344 at 15 °C and no less than 261 d at 20 °C. The models did not allow any estimation of a male's lifespan, essentially due to different sizes attained under field and laboratory conditions.

In the Mondego estuary, the southern limit of its known distribution, *E. marinus* reproduce throughout the year showing continuous recruitment (Maranhão et al., 2001). Since *E. marinus* females could produce broods consecutively (iteroparity) under laboratory conditions and taking into account the results obtained in the present study for the duration of embryonic development, and the extrapolated values for the time required for female maturation and the estimated female's life span, one should expect three or four generations per year to occur in the Mondego estuary (multivoltine

life cycle). This is clearly consistent with previous field results that described this species as having an *r* adaptative strategy (Marques and Nogueira, 1991), which is the most common pattern in epifaunal species (van Dolah and Bird, 1980) from physically controlled communities.

5. Conclusion

The duration of *E. marinus* embryonic development was significantly affected by temperature. Embryos of amphipods reared at 10 and 15 °C required about 15 d more than those reared at 20 °C to complete development. A positive correlation was observed between the number of newborn juveniles and the size of *E. marinus* females (as head length). Temperature significantly affected the number of juveniles released per female which was higher at 10 °C and lower at 20 °C. The growth of *E. marinus* was continuous throughout life under all laboratory conditions and significant differences were found between growth rates of *E. marinus* at different salinities and temperatures. Intrinsic growth rates were higher at 20 °C and lower at 10 °C.

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