Some observations on the life history of the freshwater amphipod *Echinogammarus longisetosus* Pinkster, 1973 (Gammaridae) from Catalonia (Spain, N Iberian peninsula)

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Guerao, G., 2003. Some observations on the life history of the freshwater amphipod *Echinogammarus longisetosus* Pinkster, 1973 (Gammaridae) from Catalonia (Spain, N Iberian peninsula). *Animal Biodiversity and Conservation*, 26.1: 31–39.

Abstract

Some observations on the life history of the freshwater amphipod Echinogammarus longisetosus *Pinkster, 1973 (Gammaridae) from Catalonia (Spain, N Iberian peninsula).*— Aspects of the population structure and reproductive biology of the freshwater amphipod *Echinogammarus longisetosus* were studied in the north-eastern Iberian Peninsula (Catalonia, Spain). Amphipods were sampled at approximately monthly intervals from September 1999 to October 2000. Pairs in precopula and ovigerous females were present all year round. The sex ratio was not significantly different from 1:1. Juveniles were abundant in all samples (> 40%). The number of eggs carried by females (N) was related to the size of the females (L_{P3}) (range: 9–68, mean value: 28.2): $N = 0.594\ L_{P3}^{3.141}$ ($n = 80$, $r^2 = 0.7136$, L_{P3} were measured from the anterior part of the head to the posterior edge of the third pereional segment; total length was approximately 3.5 times greater than L_{P3}). The mean embryo diameter was 0.45 mm (mean of measurements of the long and short axes of recently laid eggs). The egg volume increased during development (2 fold by eggs close to hatching).

Key words: *Echinogammarus longisetosus*, Amphipoda, Life cycle, Population structure, Reproduction, Fecundity.

Resumen

Algunas observaciones sobre el ciclo biológico del anfípodo de agua dulce *Echinogammarus longisetosus* Pinkster, 1973 (Amphipoda: Gammaridae) en Cataluña (España, N de la península ibérica).— Se estudian varios aspectos relacionados con la estructura poblacional y la biología reproductiva del anfípodo *Echinogammarus longisetosus*, basándose en muestras obtenidas en el nordeste de la península ibérica (Cataluña, España). Los anfípodos se muestrearon a intervalos de aproximadamente un mes desde septiembre de 1999 hasta octubre de 2000. Se capturaron parejas en precópula y hembras ovígeras en todas las muestras obtenidas durante el período estudiado. La relación de sexos no fue significativamente diferente de 1:1. Los individuos jóvenes eran abundantes en todas las muestras (> 40%). El número de huevos transportados por las hembras ovígeras (N) estaba relacionado con el tamaño de éstas (L_{P3}) (rango: 9–68, valor medio: 28,2): $\log N = 3{,}142 L_{P3} - 0{,}226$ ($n = 80$, $r = 0{,}845$; L_{P3} = longitud del céfalon más los tres primeros pereionitos; la longitud total era aproximadamente 3,5 veces L_{P3}). El diámetro medio de los huevos era de 0,45 mm (media de la longitud del eje mayor y del eje menor de los huevos al principio del desarrollo embrionario). El volumen de los huevos se incrementaba durante el desarrollo embrionario (2 veces en los huevos a punto de eclosionar).

Palabras clave: *Echinogammarus longisetosus*, Amphipoda, Ciclo vital, Estructura poblacional, Reproducción, Fecundidad.

(Received: 9 IV 02; Conditional acceptance: 30 VII 02; Final acceptance: 31 XII 02)

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ISSN: 1578–665X

Introduction

The genus *Echinogammarus* Stebbing, 1899 is found in a wide area extending from Europe and Northern Africa to Asia Minor (Pinkster, 1993). *E. longisetosus* Pinkster 1973, is a freshwater amphipod endemic of the Iberian peninsula. It is known from Guipúzcoa, Vizcaya, Navarra, Álava, Burgos, Zaragoza, Valencia, Teruel, Guadalajara, Cuenca, Madrid, Lleida, Girona, Tarragona and Barcelona (Pinkster, 1993). Knowledge of *E. longisetosus* to date has been limited to its description and a few ecological and zoogeographical notes (Pinkster, 1993); its biological cycle and reproduction was unknown.

The aim of the present work was to study the life cycle and reproduction of *E. longisetosus* and to compare it with literature data concerning freshwater species of the genus *Echinogammarus* and *Gammarus*.

Material and methods

The population studied inhabits the Torrent de Vallbardina (near Font Freda) in the locality of Gelida (Córdoba, 1999), about 28 km southwest of Barcelona (Catalonia, Spain, 41.437° N, 1.839° E) (fig. 1).

Specimens for the present study were collected once a month from October 1999 to October 2000. Samples were taken with a fine–meshed hand–net along the detritus and vegetal debris. On each occasion the water temperature was measured and the presence of pairs in precopula was noted. *Echinogammarus longisetosus* individuals were counted and classified into four demographic categories: juveniles without distinctive characteristics; non–ovigerous females with oostegites; ovigerous females with fully developed setose oostegites and carrying embryos in their marsupium; males with ventral genital apophyses (penial papillae) in the 7th segment of the pereion.

Amphipods were sorted from the samples with the aid of a dissecting microscope and measured with an ocular micrometer to the nearest 0.1 mm. Individuals were measured from the anterior part of the head (front) to the posterior edge of the third pereional segment (L_{P3}). In addition, 80 amphipods were measured for total length (L_t) measured from the front of the head to the base of the telson.

In order to determine the relationship between female size and brood size, 80 females with recently laid eggs were examined and the number of eggs counted.

Diameter of three axes (length, width and height) of live eggs was measured with a binocular microscope incorporating an ocular micrometer. Embryo diameter (ED) was the mean of measurements of the length (long axis) and short axis of recently laid eggs (width = height in recently laid eggs). Egg volume was calculated assuming their shape to be ellipsoidal ($V = 4/3\pi r_1 r_2 r_3$).

Results

Water temperature in the sampling locality varied from 8°C in December 1999 to 16°C in September 2000 (fig. 2). The substrate consists mainly of sand and stones with detritus and vegetal debris. Depth ranges from 0.1–0.5 m.

The relationship between L_{P3} and total length (L_t) is expressed as:

$$L_t = 3.5303\ L_{P3}^{0.9978} \quad (r^2 = 0.9917,\ n = 80)$$

L_{P3} was used as individual size reference because it can be measured more accurately than total

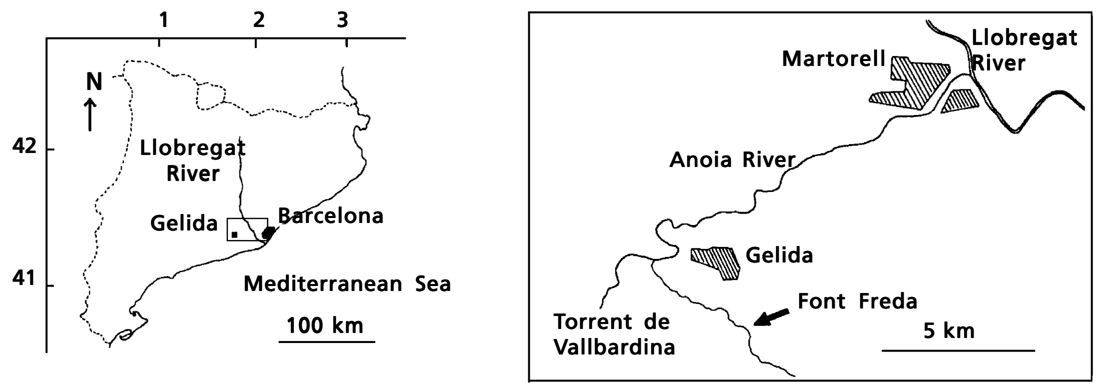


Fig. 1. Sampling area inhabited by a population of *Echinogammarus longisetosus*.

Fig. 1. Zona de muestreo habitada por una población de Echinogammarus longisetosus.

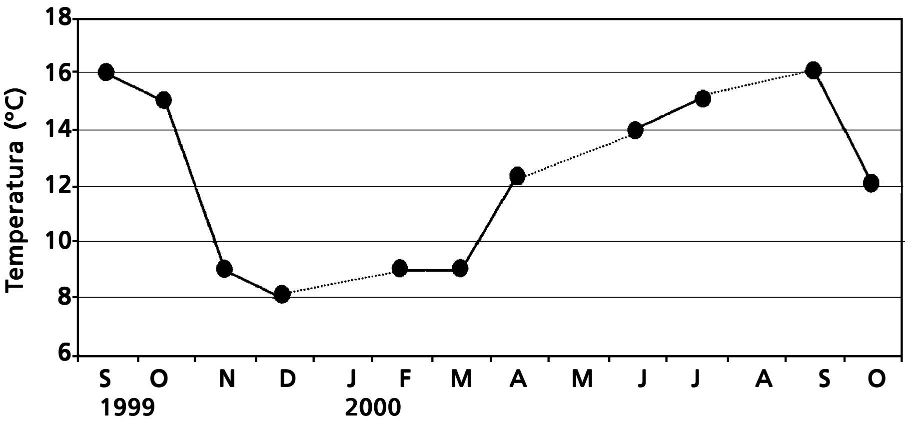


Fig. 2. Monthly water temperature of the investigated area: S. September; O. October; N. November; D. December; J. January; F. February; M. March; A. April; M. May; J. June; J. July; A. August.

Fig. 2. Temperatura mensual del agua de la zona muestreada: S. Septiembre; O. Octubre; N. Noviembre; D. Diciembre; J. Enero; F. Febrero; M. Marzo; A. Abril; M. Mayo; J. Junio; J. Julio; A. Agosto.

body length. Total length was approximately 3.5 times greater than L_{P3}.

Males reached larger sizes than females. Maximum L_{P3} was observed in specimens collected in April 2000 and December 1999: 5.1 mm (~17.7 mm L_t) and 4.05 mm (~14.1 mm L_t) for males and females, respectively. Males of ~1.5 to 1.8 mm L_{P3} were already recognisable by the presence of genital apophyses, while females of ~1.6 to 1.9 mm L_{P3} had just begun to develop oostegites. The minimum L_{P3} of ovigerous females was 2.6 mm (~9.1 mm L_t). The minimum L_{P3} of juveniles was 0.5 mm (~1.9 mm L_t). The size of juveniles hatched from eggs produced by females maintained in the laboratory was ~1.8 mm L_t.

The life cycle of *Echinogammarus longisetosus* is illustrated by data presented in figure 3, together with the water temperature. Pairs in precopula and ovigerous females were recorded in all samples; the percentage of ovigerous females reared a maximum in July 2000 (72.7%) and a minimum in October 1999 (12.5%). The structure of the *E. longisetosus* population was similar throughout the year and no discrete generations were evident because of the intensive periods of breeding. Juveniles were abundant in all samples (> 40%). The percentage of juveniles reached a maximum in February (74.6%) and spring (72.2% in April and 73.9% in June).

The sex ratio was not significantly different from 1:1 (χ^2 test, $P < 0.05$) for all months. However, males outnumbered females in all samples except in September 2000 (fig. 4).

The number of eggs (N) carried by the female of *E. longisetosus* was related to the size (L_{P3}) (fig. 5): $N = 0.594\ L_{P3}^{3.141}$ ($n = 80$, $r^2 = 0.7136$). The highest number of eggs was 68 from a female of 4 mm L_{P3} (~13.9 mm L_t). The lowest number of eggs was 9, from a female of 2.6 mm L_{P3} (~9.12 mm L_t). The mean number of eggs was 28.2 ($\sigma = 12.1$, $n = 80$).

The long axis length of recently laid eggs varied from 0.48 to 0.53 mm, with a mean value of 0.51 mm ($\sigma = 0.02$, $n = 100$); the small axis length ranged from 0.400 to 0.425 mm, with a mean value of 0.39 mm ($\sigma = 0.02$, $n = 100$); the mean embryo diameter was 0.45 mm, with a mean volume of 0.0420 mm^3. The eggs showed an increase in volume of the order of 100% during development: the mean volume of the eggs close to hatching was 0.0854 mm^3 (length = 0.675 mm; width = 0.537 mm; height = 0.450 mm). Seasonal differences in egg size were not observed.

Discussion

The life history types of amphipods were distributed according to latitudinal gradients: longevity, breeding periods, body size at maturity, brood size, and size of embryos in gammarid amphipods seem to be clearly related to temperature (Gable & Croker, 1977; Morino, 1978; Kolding & Fenchel, 1981; Wildish, 1982; Sainte–Marie, 1991). High–latitude gammaridean amphipods are characterized in general by univoltinism, delayed maturity, large embryos, and few broods in a lifetime. Iteroparous or semiannual population types, with high reproductive potentials, are more characteristic of low latitude habitats (Sainte–Marie, 1991).

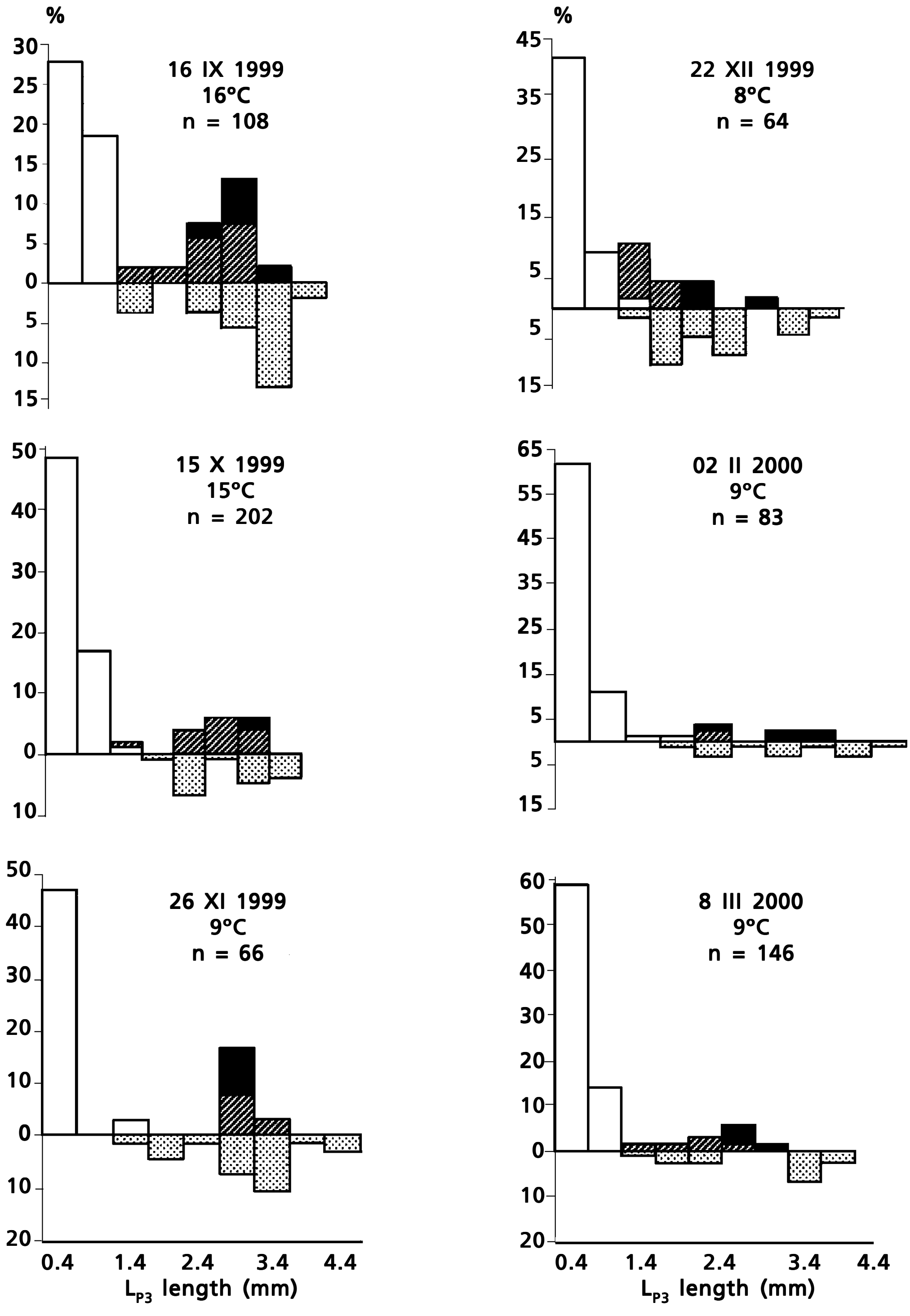
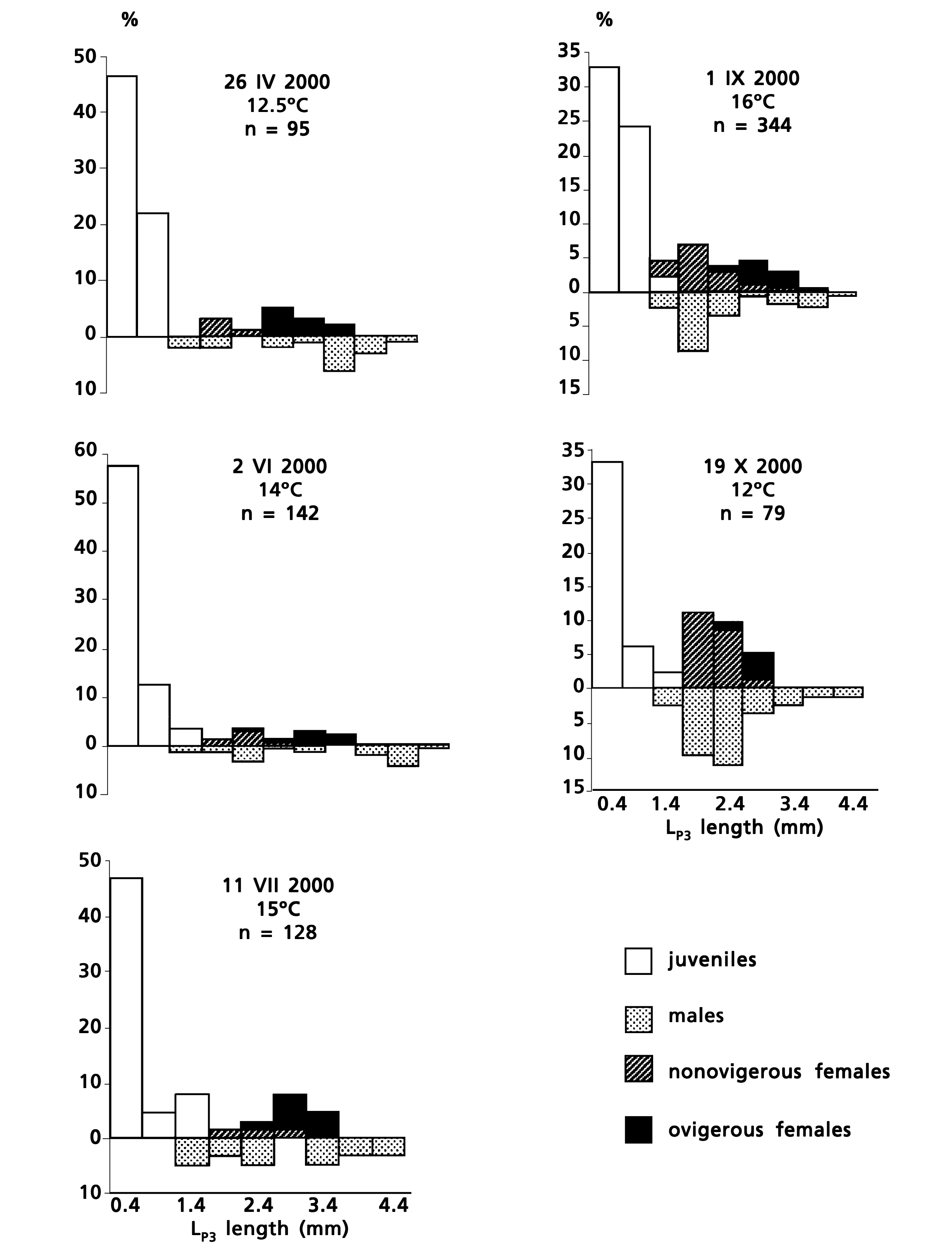


Fig. 3. Monthly distribution of L_{P3} length in juveniles, males and females of *Echinogammarus longisetosus.*

Fig. 3. Distribución mensual de clases de talla (L_{P3}) de individuos jóvenes, machos y hembras de Echinogammarus longisetosus.



There is a tendency towards an extended reproductive season with decreasing latitude in freshwater gammarid species (SAINTE–MARIE, 1991). Moreover, there is a relationship between the length of the breeding period and the altitude of the sampling locality (ZIELIŃSKI, 1995). In localities of constant water temperature amphipods may have an acyclic breeding without a winter pause (table 1). ZIELIŃSKI

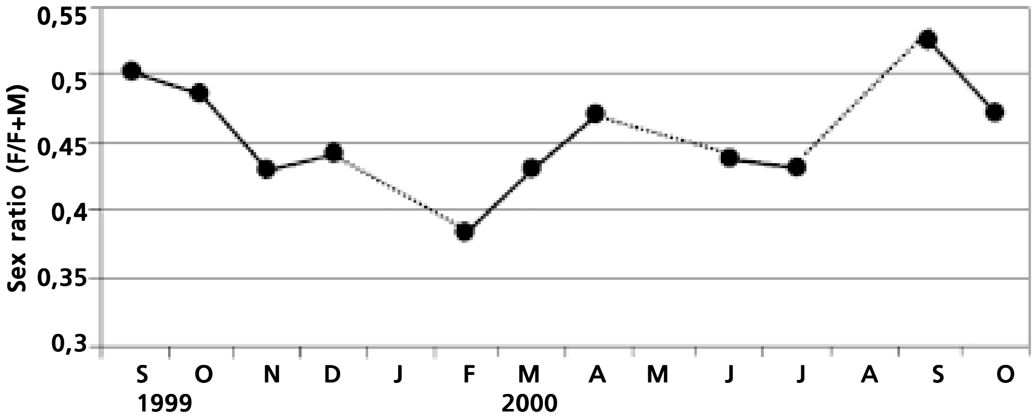


Fig. 4. Monthly sex ratio females / (females + males) of the *E. longisetosus* in the study area: S. September; O. October; N. November; D. December; J. January; F. February; M. March; A. April; M. May; J. June; J. July; A. August.

Fig. 4. Distribución mensual de la proporción de sexos hembras / (hembras + machos) de E. longisetosus *en la zona muestreada: S. Septiembre; O. Octubre; N. Noviembre; D. Diciembre; J. Enero; F. Febrero; M. Marzo; A. Abril; M. Mayo; J. Junio; J. Julio; A. Agosto.*

(1995) reported that low temperatures (below 4–7ºC) stop the breeding of *Gammarus balcanicus*. The population of *Echiogammarus longosetosum* from Torrent de Vallbardina (Catalonia, Spain) sampled on the present study showed continuous reproduction throughout the year and the maximum percentage of ovigerous females appeared in spring and summer. During the sampling period, the water temperatures in winter months were from 9ºC to 8ºC (fig. 2). In contrast, KONOPACKA & JESIONOWSKA (1995) noted the presence of ovigerous females of *E. ischnus* only from February to October in Licheńskie lake

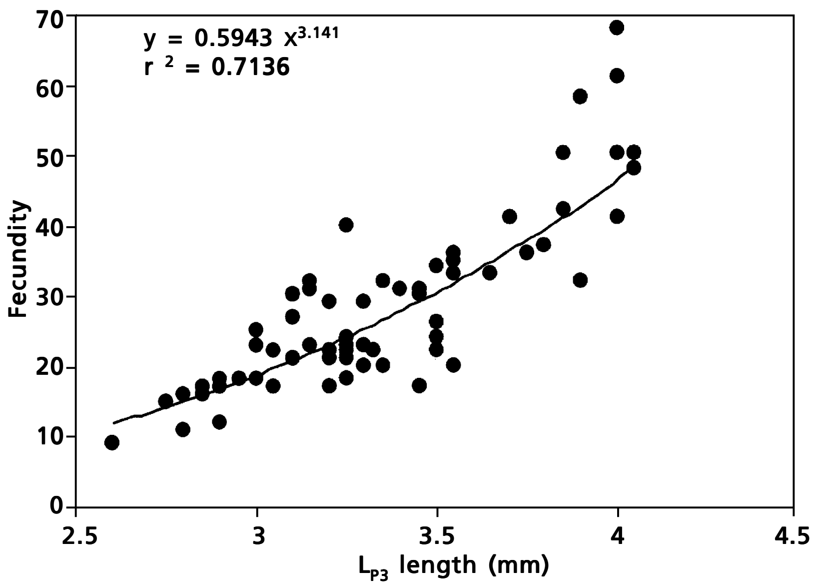


Fig. 5. Relationship between fecundity (number of eggs) and female size (L_{P3}) on *E. longisetosus.*

Fig. 5. Relación entre la fecundidad (número de huevos) y el tamaño de la hembra (L_{P3}) en E. longisetosus.

Table 1. Breeding period of several freshwater and brackish gammarid amphipods: WT. Range of water temperature (°C).

Tabla 1. Periodo de puesta en varias especies de amfípodos gammáridos de agua dulce y salobre: WT. Rango de temperaturas del agua (°C).

Species	WT	Breeding period	Reference
E. longisetosus	8–16	Continuous	Present study
E. ischus	10–12	Continuous	Konopacka & Jesionowska (1995)
E. ischus	5.2–29	February–October	Konopacka & Jesionowska (1995)
G. leopoliensis	0.2–15.3	February–October	Zieliński (1998)
G. balcanicus	2–7.4	April–October	Zieliński(1995)
G. fossarum	8–10	Continuous	Brzezińska–Blaszczyk & Jażdżewski (1980)
G. troglophilus	11–13	Continuous	Jenio (1980)
G. palustris	-2–20	April–November	Gable & Croker (1977)
G. lacustris	4.5–11	April–October	Hynes & Harper (1972)
G. lacustris	2–12	May–August	Hynes & Harper (1972)
G. pseudolimnaeus	0–19.5	February–October	Hynes & Harper (1972)

(Poland) (table 1). Similar life cycles were found in several freshwater species of the genus *Gammarus* from high latitudes (table 1).

The percentage of juveniles in the studied *Echinogammarus longisetosus* population may indicate a high mortality due to predation. In the sampled stream, there was a great density of benthic predators (Guerao, pers. obs.): mainly salamander larvae (*Salamandra salamandra*) and naiads of anisopter odonats. Jenio (1980) indicated that salamander larvae can be potential predators of gammarids.

In amphipods, it is usual that the sex ratio shows seasonal fluctuations and the females are more numerous than males (Fish & Preece, 1979; Hughes, 1978; Moore, 1981). Sexually biased pre-

Table 2. Fecundity and egg size of several freshwater and brackish gammarid amphipods: FL. Female total length (max.); MNE. Mean number of eggs (range); ED. Embryo diameter (in mm).

Tabla 2. Fecundidad y tamaño del huevo en varias especies de amfípodos gammáridos de agua dulce y salobre: FL. Longitud total de la hembra (max.); MNE. Número medio de huevos (rango); ED. Diámetro del embrión (en mm).

Species	FL	MNE	ED	Reference
E. longisetosus	14.1	28 (9–68)	0.45	Present study
E. ischnus	12.0	14 (3–27)	–	Konopacka & Jesionowska (1995)
G. fasciatus	15.0	29 (1–86)	0.46	Clemens (1950)
G. lacustris	16.0	22 (1–40)	–	Hynes & Harper (1972)
G. duebeni	18.0	25.4	0.56	Steele & Steele (1969)
G. minus	–	15 (5–27)	–	Jenio (1980)
G. pseudolimnaeus	15.0	39 (10–91)	–	Hynes & Harper (1972)
G. mucronatus	15.5	34.6	0.42	Fredette & Díaz (1986)
G. leopoliensis	12.3	16.7 (8–31)	–	Zieliński (1998)
G. balcanius	14.0	9.4 (2–20)	–	Zieliński (1995)

dation has been used as a hypothesis to explain the female–biased sex ratio. MOORE (1981) emphasized the impact of predation on the active sex (male). However, in *E. longisetosus* a sex ratio is not biased in favour of females and males are slightly more abundant than females. GABLE & CROKER (1977) also showed an unusual sex ratio for *Gammarus palustris*; males are more abundant than females throgouth the year.

The sex ratio is also influenced by physical environmental factors. KINNE (1961) suggested that sex determination in *Gammarus duebeni* is temperature–dependent. In the laboratory young *G. duebeni* became predominantly male under long–day conditions and predominantly female under short–day conditions (BULNHEIM, 1978; WATT, 1994). Future studies in the laboratory should be carried out to clarify the factors influencing *E. longisetosus* sex ratio.

The number of eggs produced per female increases with female size in *E. longisetosus*, as in many other gammarid amphipods (NELSON, 1980; KOLDING & FENCHEL, 1981; FREDETTE& DÍAZ, 1986; SAINTE–MARIE, 1991; KONOPACKA & JESIONOWSKA, 1995; ZIELIŃSKI, 1995, 1998). The mean number of eggs carried by *E. longisetosus* females are not markedly different from other freshwater and brackish gammarid species (SUTCLIFFE, 1993; ZIELIŃSKI, 1998) (table 2).

The mean embryo size (ED) obtained from *E. longisetosus* is similar to that reported for many other freshwater gammarid amphipods (SAINTE–MARIE, 1991) (table 2). The eggs of amphipods increase in size during development and the size increment varies according to the amphipod species (MOORE, 1981; SHEADER, 1983, 1996). Eggs of gammarids generally show an increase in volume of the order of 140–200% during development (SHEADER, 1996). In *E. longisetosus*, the eggs volume increment is lower than in other gammarid amphipods (about 100%).

Seasonal changes in egg volume are common in many species of amphipods (SHEADER, 1983, 1996). The length of the intermoult period is important in determining the size of oocytes; therefore, egg size is related to the temperature during oocyte development (SHEADER, 1996). SHEADER (1996) showed a seasonal cycle in egg size of *Gammarus insensibilis*, with small egs in the warmer summer months and large eggs in winter; the temperature values of the sampling area ranges from ~5°C in winter to ~25°C in summer. In the present study, seasonal differences in egg size were probably not been detected because of the relatively small yearly range of stream water temperature (fig. 2).

Acknowledgements

I wish to thank Dr. Joan Pretus and Mr. Joaquim Margarit for their valuable help in locating necessary references and documentation.

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