

Postembryonic Growth, Development and Reproduction of *Gammarus aequicauda* (Martynov, 1931) (Gammaridae) in Laboratory Culture

Ermelinda Prato*, Francesca Biandolino, and Christian Scardicchio

CNR - Institute for Coastal Marine Environment, Section of Taranto, Via Roma 3, 74100 Taranto, Italy

(Accepted December 7, 2005)

Ermelinda Prato, Francesca Biandolino, Christian Scardicchio (2006) Postembryonic growth, development, and reproduction of *Gammarus aequicauda* (Martynov, 1931) (Gammaridae) in laboratory culture. *Zoological Studies* 45(4): 503-509. *Gammarus aequicauda* (Martynov, 1931) (Gammaridae) is an epibenthic amphipod very well adapted to life in estuaries and shallow coastal waters. In Mar Piccolo in Taranto, Italy, it is the most abundant macrofaunal species of soft-bottom communities. The life history of *G. aequicauda* was analyzed in laboratory culture under controlled conditions of 18°C and a salinity of 36‰ in order to assess postembryonic growth, development, and reproduction. Pairs of amphipods in precopula were observed daily until the female was found to be ovigerous. The fecundity was estimated as the number of juveniles released by each female. Juveniles produced in the laboratory were transferred to new aquaria, and at an interval time of 7 d, the head length was measured, the antennal segments were counted, and the sexual maturation stage was assessed. The mean time spent in precopula ranged 1-3 d and embryonic development ranged 5-8 d. The head length of newly born *G. aequicauda* was approximately 0.23 mm (0.1 mg dry weight), and the head reached a maximum length of about 1.8 mm (11.85 mg) after 140 d. There was a significant correlation between head length and the number of articles on the 1st antennae. A positive correlation between the number of juveniles and the size of *G. aequicauda* females (in terms of head length) was also observed. Females produced at least 3 consecutive broods with a mean offspring number of 19.3 ± 13.3 . Males were on average larger than females. The sex of the males could be determined at an age of 30-37 d, and the 1st precopula was observed after 40 d. The increase in egg volume during development was not significant ($p > 0.05$). Growth continued throughout life under laboratory conditions. One important feature of this study was to provide the essential basis for the development of laboratory toxicity tests with cultured animals. There are several advantages of using laboratory cultured populations: the individuals are close to a normal physiological state, they are capable of growing and reproducing in captivity; and the animals are of known ages and are available throughout the year. <http://zoolstud.sinica.edu.tw/Journals/45.4/503.pdf>

Key words: Gammaridean amphipod, Life cycle, Laboratory culture.

Gammarus aequicauda (Martynov, 1931) (Gammaridae), is an epibenthic amphipod that lives in lagoon systems and shallow coastal waters, down to a depth of ca. 20 m, usually in localities with freshwater influence (lagoons and river mouths) under stones or among algae. In Mar Piccolo in Taranto, Italy, *G. aequicauda* is the most abundant macrofaunal species of soft-bottom communities. This species is an important prey for birds and fishes (Kevrekidis and Koukouras 1989) which feeding among the green

macroalgae, *Chaetomorpha linum* and *Ulva* sp. (Prato and Biandolino, 2003).

Previous studies on the biology, population dynamics, and productivity of this species were conducted by Brun (1975), Chassany de Casabianca (1979), Diviacco (1983), and Porcu and Tagliasacchi Masala (1983). The natural life-cycle of *G. aequicauda* was previously studied in the Mar Piccolo estuary (Prato and Biandolino, 2003). This study showed a clear seasonal variation in density with a maximum in spring and sum-

*To whom correspondence and reprint requests should be addressed. E-mail:linda.prato@iamc.cnr.it

mer and a minimum in autumn and winter. Ovigerous females were present year-round in the population and produced 2 generations each year: juveniles born in the spring, grew, matured, and became reproductively active during the summer months, giving rise to the next input of recruits in autumn. The sex ratio varied considerably over the year, exhibiting a preponderance of females during the winter months. The size of the brood was directly correlated to the cephalic length of the female.

The short life cycle of *G. aequicauda*, its amenability to experimental investigation, and its sensitivity to pollutants make the species an ideal laboratory organism for the assessment of toxicity (Cesar et al. 2002, Prato and Biandolino, in press). In order to fully understand the effects of pollutants in acute and chronic toxicity investigations, it is necessary to have reliable data on the biology of the organism under laboratory conditions, in the absence of pollutant stresses.

The objective of this study was to provide fundamental information on the life history traits of *G. aequicauda*, obtained from culture and maintained under optimal conditions in the laboratory. To achieve this, the duration of embryonic development, fecundity, growth, and development of juveniles produced from precopula pairs were determined. These data can subsequently be used as a basis for evaluating the effects of toxicants on the life history traits of laboratory-cultured *G. aequicauda*.

MATERIALS AND METHODS

Collection and acclimation of amphipods

Stock populations of *G. aequicauda* were collected in Jan. 2005, from the Mar Piccolo estuary (Ionian Sea, Italy; 40° 29' 17" N; 17° 14' 23" E) among the green macroalga *Chaetomorpha linum*. In the laboratory, the amphipods were isolated from the alga and transferred to 30 L plastic aquaria with their native seawater and sediment. The aquaria were continuously aerated. Table 1 shows the physicochemical characteristics of the sampling site.

The culture aquaria were maintained at a temperature of 18°C and a salinity of 36‰ under a 12: 12-h light: dark regime in a climate-controlled room for 10 d to acclimate the individuals to the experimental conditions. These conditions were selected according to the annual ranges of the

water variables registered in the Mar Piccolo. The culturing systems were semistatic, with 100% water replacement twice a week. Food consisted of the macroalga *C. linum* fed ad libitum and Tetramin fish feed. To provide shelter and in order to mimic the natural environment, small stones (about 10 cm² in surface area) were also furnished.

Precopula time

After 10 d of acclimation, 10 pairs of amphipods in precopula, in which the male holds and carries the female, were removed from the initial aquarium, and each pair was placed inside a 0.50 L glass beaker with 0.25 L of seawater. The conditions in these small aquaria were the same as for the acclimated population. They were observed daily until the female separated from the male and was determined to be ovigerous. When separation occurred, the male was removed from the beaker.

Fecundity

In order to determine how many broods each female could produce under these experimental conditions, another set (30 pairs) of amphipods in precopula was used. The male was removed when the separation occurred and was added when embryonic development was completed. The number of broods produced by each female was recorded.

Fecundity was estimated as the number of juveniles released by each female. After releasing juveniles, the female head length, was measured, and when possible, the female was returned live to the acclimation aquarium. All juveniles were counted, and the head length (of sub-stocks of 50 juveniles) was measured to the nearest 0.1 mm

Table 1. Physical and chemical data of the sampling site

| | |
|------------------------------------|-----------------|
| Native Sediment characteristics | |
| Organic matter (%) | 0.8 |
| Sand > 63 mm (%) | 85.8 |
| Silt : 3- 63 mm (%) | 12.9 |
| Clay :< 3 mm (%) | 1.2 |
| Overlying water-quality parameters | |
| Temperature | 16°C |
| Salinity | 36 - 37‰ |
| pH | 8.3 (7.9 - 8.6) |
| Dissolved oxygen (mg/L) | 8.3 (8.0 - 8.5) |

using a binocular microscope with a graduated eyepiece.

Growth

Juveniles released by the females were removed from the beaker with a glass pipette and transferred to new aquaria. In this way, it was possible to obtain animals of the same age for studying growth and development under controlled conditions. Three aquaria, with 70 juveniles each produced in the laboratory, were filled with about 2 cm of native sediment, and filtered (0.45 µm) seawater was added to a depth of 10 cm.

At 7 d intervals, pools of 10 animals were removed, their head length was measured, the antennal segments were counted, and the sexual maturation stage was assessed. Head length (HL) was used instead of the total length (TL), in view of the difficulty of measuring an amphipod's recurved body. Head length was measured from the anterior end of the rostrum to the posterior margin of the cephalon. The relation between TL and HL was previously determined in a sample of 370 animals, and it can be described by the following linear regression equation:

$$TL = -0.3605 + 9.0353HL; R = 0.91.$$

The segments of the 1st pair of antennae were counted on the primary flagellae with a binocular microscope. After measurement, living individuals were returned to the respective aquarium. The aquaria were inspected daily for mortality, aeration, feeding needs, and reproduction.

Sexual dimorphism was determined by the size and shape of the gnathopods. Male sexual maturation was determined by the appearance of an enlarged propodus on the 2nd gnathopod; female sexual maturation was determined by the appearance, presence, and condition of the oostegites. Oostegites of immature females lacked setae; mature females had fully developed, setose oostegites, and ovigerous females had eggs in the brood pouch. Animals without these characteristics were considered to be juveniles.

Eggs were then removed from the marsupium, counted to estimate fecundity, measured, and classified in to 4 developmental stages (A, B, C, and D) as described by Janssen et al. (1979) for the genus *Gammarus*. Egg size was determined using a binocular microscope with a graduated eyepiece; the longest axis was measured and this was referred to as the length, with perpendicular axis referred to as the width. Depth was not measured separately, but was assumed to be equivalent

to the width. These measurements were then used to determine the volume of each egg using the equation:

$$V = 4/3 \pi r_1 r_2 r_3;$$

where V is the volume of the egg (mm³), r_1 is the length/2, and r_2 and r_3 are the width/2.

Every 7 d, 10 randomly selected amphipods were removed for determination of weight and growth. Growth was calculated as a rate, using the following equation:

$$G = (WT_{t_2} - WT_{t_1}) / (t_2 - t_1);$$

where G is the growth rate (mg/d), WT_{t_2} is the estimated individual dry weight (mg) of *G. aequicauda* at time t_2 ; WT_{t_1} is the estimated individual dry weight (mg) at time t_1 ; and $t_2 - t_1$ is the length of the time interval (d). The experiment was terminated after 140 d, when the last animal had died.

Data analysis

All data were tested for normality (using the Shapiro-Wilk test) and homogeneity of variance (using Bartlett's test). The statistical software package, SPSS (Chicago, IL, USA), was used to compare egg volumes of different stages with analysis of variance (ANOVA). Correlation analysis was performed to identify and compare the relationships between head length and number of antennular articles, between head length and dry weight, and between male and female head length, during the precopulatory guarding phase. Regression analysis was performed to test the relationship between the number of newborns and the length of the female.

RESULTS

The average time spent by a *G. aequicauda* male and female in precopula, when cultured at a temperature of 18°C and a salinity of 36‰, was 1.88 ± 0.87 (range, 1-3) d. The duration of embryonic development was 6.25 ± 0.97 (range, 5-8) d. The relationship between the number of juveniles (NJ) per brood and female head length (HL) was statistically significant (Fig. 1; $NJ = -17.2 + 36.18HL$; $R^2 = 0.48$, $n = 33$, $p < 0.01$). One female with a head length of 1.25 mm (corresponding to 10.9 mm in total length) reared under the present experimental conditions released 53 juveniles, the largest number observed. Females produced at least 3 consecutive broods, with a mean number of 19.3 ± 13.3 offspring.

Head length (mm) and dry weight (mg) increased with age, and differed with life stage ($n = 141$; $r = 0.96$, $p < 0.01$; and $r = 0.97$, $p < 0.01$, respectively) (Fig. 2). Furthermore, there was a strong correlation between mean head length (mm) and the mean number of antennular flagellum segments ($n = 141$; $r = 0.98$, $p < 0.01$).

Newborns at hatching measured about 0.24 ± 0.1 mm in head length (corresponding to 2.17 mm in total length) with 4 ± 0.3 antennular segments. Individuals reached a maximum length of 1.8 ± 0.1 mm for males (corresponding to 15.9 mm in total length) with 35 ± 0.2 antennular segments and a maximum length of 1.6 ± 0.5 mm for females (corresponding to 14.1 mm in total length) with 26 ± 0.1 antennular segments at an age of approximately 140 d.

The growth rate was low during the 1st few weeks of culture, became moderately high by the 56th d, roughly increased to the 98th d (0.21 mg/d), had dropped to 0.08 mg/d by the 126th d, and subsequently continued to decrease (Fig. 3). There was relatively high mortality during first 6 wk of life, and a lower mortality during their remaining

life span. In addition to the occurrence of survival oscillations during their life span, there was a linear tendency of survival over time for *G. aequicauda* ($p < 0.01$). The total lifespan of the animals was estimated to be 145 ± 2 d (Fig. 4).

The sex of individuals was determined at 30-37 d, and precopulatory pairing behavior began after 44 d. Female pairing success was dependent on its size, with pairing success increasing with size up to some point, whereas larger females suffered decreasing pairing success. The number of precopula pairs reached a maximum at 84-88 d, corresponding to 46% of the existing population (except for newborns) (Table 2). Correlation analysis showed that males tended to hold females that were smaller than themselves in precopula ($y = 0.3131x + 0.335$; $r = 0.47$, $n = 53$, $p < 0.01$) (Fig. 5).

The increase in volume of eggs during development was not significant ($p > 0.05$): the average volume of early eggs (stage A) was 0.23 mm^3 , while the average volume of later-stage eggs (stage D) was 0.30 mm^3 (Fig. 6).

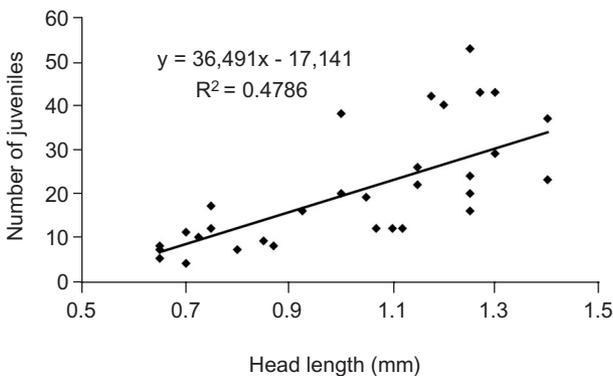


Fig. 1. Relationship between the number of juveniles released and head length (mm) of females.

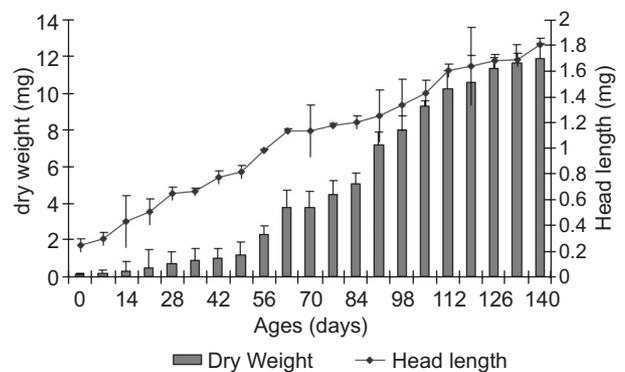


Fig. 2. Mean length (mm \pm SD) and dry weight (mg) for *Gammarus aequicauda* of different ages.

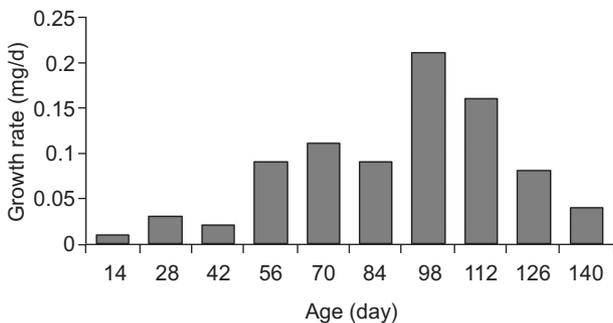


Fig. 3. Growth rate (mg/d) of *Gammarus aequicauda* of different ages.

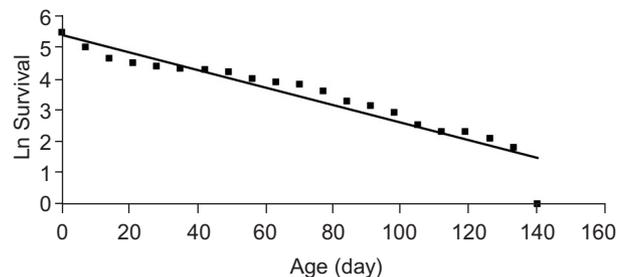


Fig. 4. Survival of *Gammarus aequicauda* cultured in the laboratory.

DISCUSSION

The duration of precopula was 1-3 d, shorter than that observed by Shuhaimi and Pascoe (2001) reporting on *Hyalella azteca*, cultured in the laboratory, with a mean time of 4 (range, 1-7) d. Males (small and large) usually preferred to enter into precopula with females smaller than themselves. In the field, the duration of precopula is also influenced by predation, so that in areas with a heavy predation, females begin precopula after about 90% of the way through the molting cycle, while in the absence of predators, precopula begins about halfway through the molting cycle (Shuhaimi and Pascoe 2001). From studies with *G. pulex* and *G. duebenii*, Adams et al. (1989) suggested that mating success was influenced by both sexual selection and loading constraints.

Brood size increases with body size among female amphipods (Steele and Steele 1991). A linear correlation between mean brood size and female size is a common feature in amphipods, e.g., *Pontocrates arenarius*, *P. altamarinus* (Beare and Moore 1996), *G. locusta* (Costa and Costa 1999), *Orchestia gammarellus* (Persson 1999), *Corophium multisetosum* (Cuhna et al. 2000), and *Echinogammarus marinus* (Maranhão and Marques 2003). Larger females generally carry more eggs than smaller ones because of the greater body length, as the marsupium capacity is proportional to body size (Sheader 1977).

In *G. aequicauda*, as in other amphipods, development is direct, and newly hatched juveniles possess all the structures typical of the adult. In

this study, laboratory-reared females of *G. aequicauda* produced broods similar to those produced by females collected in the field (Prato and Biandolino 2003). In the laboratory, it was observed that females could produce at least 3 consecutive broods. These results are similar to those obtained by Skadsheim (1982) for *Chaetogammarus marinus* and *C. stoerensis* and by Maranhão and Marques (2003) for *Echinogammarus marinus*.

In general, eggs are not produced in the absence of a male. This is true for the majority of amphipods; in our case it was observed that females of *G. aequicauda* sometimes produced unfertilized eggs. These observations are similar to those by Sexton (1924), who found unfertilized eggs in *G. chevreuxi*.

The growth of *G. aequicauda* individuals was found to be continuous throughout life under our laboratory conditions; nevertheless, growth rates were lower in the early weeks. The maximum body size attained by individuals was higher than the body size attained by organisms in the field (Prato and Biandolino 2003). In the Baltic Sea, Kolding and Fenchel (1979), studying life cycle of

Table 2. Age (d) and size (mm) at which *Gammarus aequicauda* was first observed in precopulatory pairs

| Age (days) | Precopula pairs (%) | HL ♂ (mm) | HL ♀ (mm) |
|------------|---------------------|-----------|-----------|
| 49-53 | 1 | 0.84 | 0.76 |
| 54-58 | 1 | 1.07 | 0.85 |
| 59-63 | 2 | 1.21 | 0.87 |
| 64-68 | 3 | 1.24 | 0.92 |
| 69-73 | 3 | 1.26 | 0.94 |
| 74-78 | 4 | 1.27 | 0.95 |
| 79-83 | 10 | 1.3 | 0.98 |
| 84-88 | 46 | 1.35 | 1.11 |
| 89-93 | 36.4 | 1.42 | 1.18 |
| 94-98 | 33.3 | 1.51 | 1.25 |
| 99-103 | 26.6 | 1.66 | 1.32 |
| 104-108 | 20 | 1.77 | 1.5 |
| 109-113 | 10 | 1.8 | 1.57 |

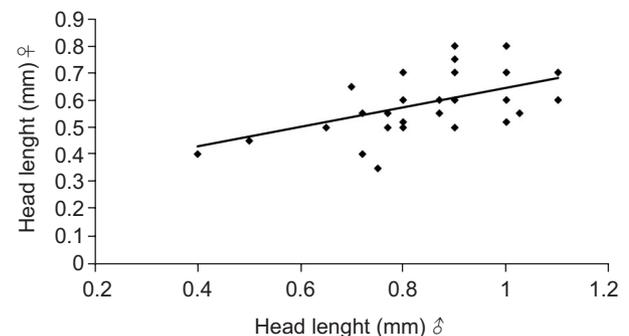


Fig. 5. Relationship between head length of males and head length of females engaged in precopula.

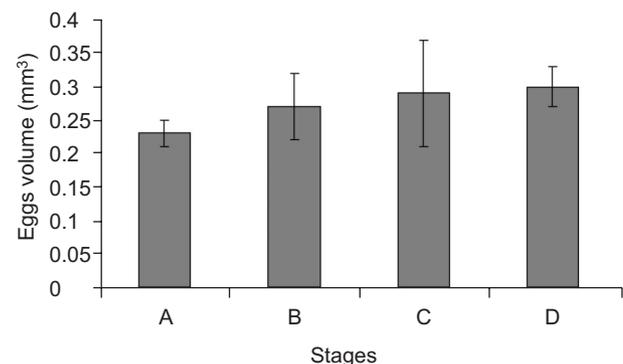


Fig. 6. Eggs volume (mean \pm SD) during various development stages.

gammarid amphipods, observed that the growth rate decreases when they mature and begin to reproduce. It is possible that favorable conditions (an ad libitum food supply, stable rearing conditions, and the absence of predators) might well benefit individual growth rates.

Several authors have reported that density, temperature, salinity, food, substrate, and oxygen concentration may contribute to differences in growth and sexual reproduction (Moore and Farrar 1996, Sheader 1996, Maranhão et al. 2003). For example, animals reared at lower temperatures may take longer to mature and are larger than animals reared at higher temperatures. Neuparth et al. (2002) gave values of maturity for *G. locusta* reared in the laboratory at 20°C and 33‰ salinity. These individuals became sexually mature at 35 d, while at 15°C and 20‰-33‰ salinities, age at maturity was estimated as 49 d. These results can be compared to the ones we obtained in our study.

An increase in egg volume during development is a common feature of many crustaceans (Davis 1981), although in our study, the increase in the volume of eggs during development was not significant. It seems that the change in volume is the result of water uptake (Sheader and Chia 1970) together with the conversion of yolk reserves into the formation of the major body regions and the development of organs and appendages (Sheader 1983 1996). On the basis of field data alone, it is difficult to come to any conclusion about the processes governing the life history traits. A combination of field and laboratory experiments may provide adequate estimates and would add pieces of our understanding of population dynamics and biological traits that can be difficult to follow in the field.

Findings from this study have implications for future developments in ecotoxicological testing with *G. aequicauda*. The advantages of laboratory-cultured populations are that individuals are close to a normal physiological state and are capable of growing and reproducing in captivity; the individuals are of known ages; a biochemical comparison is possible between laboratory populations and those suspected of existing in a polluted environment; and animals are available throughout the year. A laboratory toxicity test requires a large number of organisms, and field populations often exhibit significant variations in abundance, thus experiments may thus be interrupted because test organisms from the field are unavailable.

Test organisms used in chronic studies should grow and reproduce freely under laboratory condi-

tions and have a high reproductive potential and a short life cycle. In this study, *G. aequicauda* met these requirements. The preliminary approach used in this study thus represents a first step in evaluating the effects of contaminants upon the growth and reproduction of this species.

REFERENCES

- Adams J, PJ Watt, CJ Naylor, PJ Greenwood. 1989. Loading constraints, body size and mating preferences in *Gammarus* species. *Hydrobiologia* **183**: 157-164.
- Beare DJ, PG Moore. 1996. The distribution and reproduction of *Pontocrates arenarius* and *P. altamarinus* (Crustacea: Amphipoda) at Millport, Scotland. *J. Mar. Biol. Assoc. UK* **76**: 931-950.
- Brun B. 1975. Quelques aspects des cycles biologiques de deux Gammarens (Crustacés, Amphipodes) des eaux saumâtres du littoral Méditerranéen français. *Arch. Zool. Exp. Gén.* **116**: 343-358.
- Cesar A, L Marin-Guirao, R Vita, A Marin. 2002. Sensitivity of Mediterranean amphipods and sea urchins to reference toxicants. *Cienc. Mar.* **28**: 407-417.
- Chassany de Casabianca ML. 1979. Influence de la mise en valeur agricole sur l'évolution biocénétique des bords d'étangs corses. *Rev. Biol. Ecol. Méditerr.* **5**: 211-226.
- Costa FO, MH Costa. 1999. Life history of the amphipod *Gammarus locusta* in the Sado estuary. *Acta Oecol.* **24**: 305-314.
- Cunha MR, JC Sorbe, MH Moreira. 2000. The amphipod *Corophium multisetosum* (Corophiidae) in Ria de Aveiro (NW Portugal). I. Life history and aspects of reproductive biology. *Mar. Biol.* **132**: 637-650.
- Davis CC. 1981. Mechanisms of hatching in aquatic invertebrate eggs. II. *Oceanogr. Mar. Biol. Annu. Rev.* **19**: 95-123.
- Diviacco G. 1983. Distribution of the crustacean amphipods in the east Tyrrhenian lagoons. *Rapp. Procès Verbaux Reunions Comm. Int. Explor. Sci. Mer Méditerr.* **28**: 315-318.
- Janssen H, M Scheepmaker, M Van Couwelaar, S Pinkster. 1979. Biology and distribution of *Gammarus aequicauda* and *G. insensibilis* (Crustacea: Amphipoda) in the lagoon system of Bages-Sigean (France). *Bijdragen Dierkunde* **49**: 42-70.
- Kevrekidis T, A Koukouras. 1989. Seasonal variation of abundance of *Gammarus aequicauda* (Crustacea: Amphipoda) in the Evros Delta (NE Greece). *Israel J. Zool.* **36**: 113-123.
- Kolding S, TM Fenchel. 1979. Coexistence and life cycle characteristics of five species of the amphipod genus *Gammarus* in the Baltic. *Oikos* **33**: 323-327.
- Maranhão P, JC Marques. 2003. The influence of temperature and salinity on the duration of embryonic development, fecundity and growth of the amphipod *Echinogammarus marinus* Leach (Gammaridae). *Acta Oecol.* **24**: 5-3.
- Moore DW, JD Farrar. 1996. Effect of growth on reproduction in the freshwater amphipod, *Hyalella azteca* (Saussure). *Hydrobiologia* **328**: 127-134.
- Neuparth T, FO Costa, MH Costa. 2002. Effects of temperature and salinity on life history of the marine Amphipod *Gammarus locusta*. Implications for ecotoxicological test-

- ing. *Ecotoxicology* **11**: 61-73.
- Persson LE. 1999. Growth and reproduction in two brackish water population of *Orchestia gammarellus* (Amphipoda: Talitridae) in the Baltic Sea. *J. Crustacean Biol.* **19**: 53-59.
- Porcu M, ML Tagliasacchi Masala. 1983. Ecologie trophique des Crustacés et pollution par le mercure dans un étang saumâtre méditerranéen (Santa Gilla, Sardaigne). *Cah. Biol. Mar.* **24**: 159-175.
- Prato E, F Biandolino. 2003. Seasonal changes in population of the Amphipod *Gammarus aequicauda* (Martynov, 1931). *Mediterr. Mar. Sci.* **4**: 49-56.
- Prato E, F Biandolino. 2005. *Gammarus aequicauda* (Crustacea: Amphipoda): a potential species test in marine sediment toxicity assessment. *Aquat. Ecosyst. Health Manage.* **8**: 475- 482.
- Sexton EW. 1924. The moulting and growth stages of *Gammarus* with descriptions of normals and intersexes of *Gammarus chevreuxi*. *J. Mar. Biol. Assoc. UK* **13**: 340-401.
- Sheader M. 1977. Production and population dynamics of *Ampelisca tenuicornis* (Amphipoda) with notes on the biology of its parasite *Sphaeronella longipes* (Copepoda). *J. Mar. Biol. Assoc. UK* **57**: 955-968.
- Sheader M. 1983. The reproductive biology and ecology of *Gammarus duebeni* (Crustacea, Amphipoda) in southern England. *J. Mar. Biol. Assoc. UK* **63**: 517-540.
- Sheader M. 1996. Factors influencing egg size in the gammarid amphipod *Gammarus insensibilis*. *Mar. Biol.* **124**: 519-526.
- Sheader M, FS Chia. 1970. Development, fecundity and brooding behaviour of the amphipod *Marinogammarus obtusatus*. *J. Mar. Biol. Assoc. UK.* **50**: 1079-1099.
- Shuhaimi MO, D Pascoe. 2001. Growth, development and reproduction of *Hyalella azteca* (Saussure, 1858) in laboratory culture. *Crustaceana* **74**: 171-181.
- Skadsheim A. 1982. The ecology of intertidal amphipods in the Oslofjord. The life cycle of *Chetogammarus marinus* and *C. stoerensis*. *Mar. Ecol.* **3**: 213-224.