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Research Article

Effects of Different Salinities on Juvenile Growth of *Gammarus aequicauda* (Malacostraca: Amphipoda)

Lidia Delgado,¹ Guillermo Guerao,² and Carles Ribera¹

¹ Institut de Recerca de la Biodiversitat (IRBio) y Departament de Biologia Animal, Universitat de Barcelona, Avinguda Diagonal 645, 08028 Barcelona, Spain

² IRTA, Unitat de Cultius Aquàtics (UCA), 43540 Sant Carles de la Ràpita, Tarragona, Spain

Correspondence should be addressed to Guillermo Guerao, guillermo.guerao@irta.cat

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Gammarus aequicauda is a euryhaline amphipod that is a common inhabitant of brackish environments of the Mediterranean Sea. In the Ebro delta, the population density of *G. aequicauda* is highly variable throughout the year. The main objective of this study is to investigate the effect of salinity on the growth of *G. aequicauda* juveniles. *G. aequicauda* embryos and juveniles can survive and grow in the laboratory between 2 psu and 40 psu salinity, depending on the previous acclimation period for the reproductive individuals. Adults acclimated at 34 psu produced embryos and juveniles that survived and developed at salinities between 9 psu and 40 psu; adults acclimated at 9 psu produced embryos and juveniles that could develop in oligohaline conditions. The lower growth rate values were $10.9 \mu\text{m d}^{-1}$ and $13.5 \mu\text{m d}^{-1}$ at 40 psu and 2 psu, respectively, with the higher values of $18.0 \mu\text{m d}^{-1}$ and $18.5 \mu\text{m d}^{-1}$ at 19 and 34 psu, respectively.

1. Introduction

Salinity is one of the main environmental factors that exerts an intense pressure on crustaceans by controlling their distribution. Gammaridean amphipods living in coastal, estuarine, and adjacent freshwater environments tolerate highly variable salinities, including hypo-osmotic conditions [1–7]. Understanding the tolerance limits in the different life cycle phases of amphipods will be helpful in further studies on their life history and population distribution.

G. aequicauda (Martynov, 1931) is one of the most common and abundant amphipods from lagoons and brackish environments of the Mediterranean and the Black Sea [8–17]. *G. aequicauda* is a euryhaline species, being very resistant in isolate habitats from the sea with extensive ranges of salinity. This species has an important trophic role in the transport of energy to a higher consumer level, and its feeding activities contribute greatly to macrophyte fragmentation, thus enhancing microbial colonization and macrophyte decomposition [9–12, 18–22]. Although the distribution, life cycle, reproductive biology, and population dynamics of *G. aequicauda* have been studied in several

coastal environments [8, 13, 14, 18, 22, 23], laboratory studies on the effect of salinity on survival and growth are scarce [24].

The Ebro delta is an estuarine environment that is influenced by rice crops. Agricultural practices regulate the hydrological cycles of the system, inducing periods of desalination and salination that are inverted in comparison with natural estuaries [25–27]. The Encanyissada lagoon is a shallow eutrophic coastal lagoon in the Natural Park on the right-hand semidelta of the Ebro delta [28]. The lagoon comprises a fluctuating ecosystem that receives fresh water drainage from irrigated lowland rice fields from April to October [25].

G. aequicauda is a characteristic and abundant macrofaunal species in the Ebro delta [9, 13]. *G. aequicauda* individuals are subject to large variations in salinity. For example, the salinity in Encanyissada lagoon fluctuates between 4 and 37 psu, with lower concentrations near the shore, where sudden drops of salinity down to 2–3 psu are observed during fresh water “discharge”. In this habitat, population densities of *G. aequicauda* vary greatly throughout the year [13], and the most important factors that regulate population density

of this amphipod are unknown Prato et al. [24] showed that the survival of *G. aequicauda* was affected by salinity with the optimal range of 15 psu to 36 psu. Kevrekidis et al. [14] concluded that life history, growth, and reproduction are not markedly affected by low salinity (0.3–5.7 psu) although low salinity does affect embryo viability. Previous studies on the biology and population dynamics in the Ebro delta showed that abundance is not correlated with changes in salinity [13]. Researchers argue about the ontogenetic variations in the osmoregulatory ability in some species of amphipods [6, 29–32], whereas in *G. aequicauda*, the salinity conditions in which embryos and juveniles can survive and develop are currently unknown.

The present work was aimed at studying the effects of salinity conditions on the survival and growth of juvenile *G. aequicauda* to provide information on their distribution range, growth, and ecology in the Ebro delta.

2. Materials and Methods

2.1. Collection and Acclimation of Amphipods. Amphipods were collected in February 2008 from Encanyissada lagoon at the communication channel of the lagoon with Alfacs Bay (Ebro Delta; 40°37' N 0°36' E) on the NW Mediterranean coast [13]. Collection was done with a hand-held net with a mesh size of 500 μm and a mouth aperture 35 cm in diameter. Water temperature at the collection site was 17°C, and salinity was 34 psu. Before experiments, animals were held in the laboratory for two days at this temperature and salinity.

After transferring the animals to the laboratory, the individuals were divided into two groups. Group 1: individuals were maintained in a 100 L aquarium provided with aeration and with natural sea water at the same conditions of the collection site (17°C and 34 psu salinity) and under an artificial 12:12 h light:dark cycle. Group 2: individuals were acclimated in a 100 L aquarium with a salinity of 9 psu stepwise to increasingly dilute media (decrements of ≤ 3 psu, at intervals of 1–2 days) about 2–3 weeks before any experiment was undertaken. The temperature and photoperiod were identical to that in group 1. The different experimental salinities were obtained by diluting filtered seawater from Alfacs Bay (34 psu) with appropriate quantities of freshwater (conductivity: 300 $\mu\text{S}/\text{cm}$). Hyperhaline conditions (40 to 50 psu) were obtained by adding artificial seawater at a salinity of 70 psu. Salinity was checked by a WTW InoLab Level 3 refractometer. Both groups were fed the macroalgae (Chlorophyta) *Ulva* sp. (in excess) obtained in the collection sites. Twenty percent of the water from the aquariums was changed every 48 hours.

2.2. Experiments. To determine the effect of salinity on juvenile growth, brooding females from group 1 were directly transferred from water with the acclimation salinity (34 psu) to water with constant salinities of 0 (300 $\mu\text{S}/\text{cm}$), 2, 4, 9, 19, 34, 40, and 50 psu. The mean brood size of *G. aequicauda* from the Ebro delta populations was 23.6 [13]. Females with a brood size <15 were discarded. Three brooding females were placed individually in 10 L aquariums for each

treatment (three replicate). After hatching, females were removed, and 15 recently hatched juveniles were maintained in each aquarium and were reared until the end of juvenile development. The experiments were conducted under a 12:12 h light:dark regime. Temperature was maintained at $17 \pm 1^\circ\text{C}$ ($\pm\text{SE}$), *Ulva* sp. were provided as food, and 50% of the water was exchanged every 48 hours. Five live juveniles from each treatment group and a replicate were measured cephalon length every 7 days for a total of 42 days.

To compare the effect of acclimation on juvenile growth at oligohaline conditions (<5 psu), brooding females of group 2 were directly transferred from the acclimation salinity (9 psu) to water with the following constant salinities: 0 (300 $\mu\text{S}/\text{cm}$), 2, 4, and 9 psu. The procedure was identical to the first experiment.

2.3. Measurements. Cephalon length (CL) was measured from the anterior margin (front) to the posterior dorsal margin of the cephalon. Body length (BL) was measured from the front to the base of the telson. CL was used as an individual size reference, because BL is difficult to measure, especially in live individuals; however, CL is an appropriate measure to estimate the size of the amphipods. To determine the relationship between BL and CL, 96 individuals were measured. The relationship between CL and BL was studied by a regression analysis. To measure CL during the experiments, each amphipod was placed on a glass slide and was examined for <1 min to minimize the effects of hypoxia and handling stress. Measurements were taken with an image analyzing system (AnalySIS, Münster, Germany) connected to a stereomicroscope (Nikon SMZ800).

2.4. Data Analysis. The relative growth of body parts was determined using the allometric equation $\text{BL} = a\text{CL}^b$. Using the transformed variables $\log_{10} \text{BL}$ and $\log_{10} \text{CL}$ (logarithmic equation): $\log_{10} \text{BL} = \log_{10} a + b \log_{10} \text{CL}$, tests for departures from isometry ($H_0: b = 1$) were performed on the slope values obtained by the Student's *t*-test ($P < .001$). The statistical analysis of the growth data in the first and second experiments was performed by one-way ANOVA using the SigmaStat 3 (Systat Software Inc., USA) software package.

3. Results

3.1. Measurements. There exists a positive correlation between BL and CL ($r^2 = 0.9795$ and $n = 96$). The regression equation was:

$$\log_{10} \text{BL} = 1.211 \log_{10} \text{CL} + 0.2197. \quad (1)$$

(See Figure 1).

The relationship between BL and CL shows positive allometric growth ($b = 1.211$; $H_0: b = 1$; $t = -16.23$; $P < .001$). Therefore, body length (BL) was between 5 and 7 times greater than cephalon length (CL), depending on the size of the amphipod.

3.2. Experiment 1. *G. aequicauda* embryos and juveniles from brooding females acclimated at 34 psu salinity can survive and grow at salinities between 9 psu and 40 psu

TABLE 1: The survival, mean size (CL), mean CL growth rate (GR), and estimated BL growth rate (GR_e) (estimated from mean GR) at 42 d in *Gammarus aequicauda* at different salinity conditions. Abbreviations: AC, acclimation conditions.

AC (psu)	Treatment (psu)	CL \pm SD (μm)	GR \pm SD (CL, $\mu\text{m d}^{-1}$)	GR_e (BL, $\mu\text{m d}^{-1}$)	Survival (%)	Precopula pairs
34	0	—	—	—	0	—
34	2	—	—	—	0	—
34	4	—	—	—	0	—
34	9	974.6 \pm 51	14.9 \pm 1.2	120	93.3	+
34	19	1015.2 \pm 110	18 \pm 2.6	140	88.0	+
34	34	1040.1 \pm 36	18.5 \pm 1.0	143	90.0	+
34	40	713.7 \pm 24	10.9 \pm 0.6	80	82.0	—
9	0	—	—	—	0	—
9	2	827.5 \pm 50	13.5 \pm 1.2	102	78.3	—
9	4	976.9 \pm 37	17.3 \pm 1.0	132	88.0	+
9	9	924.4 \pm 49	16.7 \pm 1.2	125	81.6	+

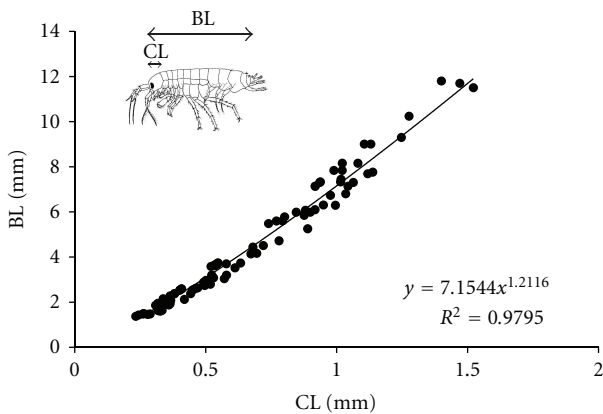


FIGURE 1: Dispersion diagram of BL (body length in mm) in relation to CL (cephalic length in mm) in 96 individuals of *Gammarus aequicauda*.

(Table 1; Figure 2(a)). At 4 psu, juveniles only survived 16 d. All ovigerous females died after 12–24 h at 0 psu and 2 psu. At 50 psu, brooding females survived, but no juveniles were observed. The size of individuals reared at 40 psu salinity was significantly lower after 42 d than those that received the other treatments ($P < .004$, ANOVA). At salinities between 9 and 34 psu, mature individuals (precopula pairs) were observed at the end of experiment after 42 d of culture. The lower growth rate was $10.9 \mu\text{m d}^{-1}$ (CL) at 40 psu, and the higher growth rate was $18.5 \mu\text{m d}^{-1}$ at 34 psu (Figure 3).

3.3. *Experiment 2.* *G. aequicauda* embryos and juveniles derived from females acclimated at 9 psu salinity can survive and grow in oligohaline conditions (2 psu and 4 psu) but not in freshwater (Table 1; Figure 2(b)). At 0 psu salinity, the ovigerous female died at 96–108 h, and no juveniles were observed. After 42 d, individuals reared at 2 psu salinity were smaller than those reared at 4 psu and 9 psu, but the differences were significant only for the 4 psu treatment group ($P < .018$, ANOVA). At 4 and 9 psu salinity conditions, precopula pairs were observed at the end of experiment

(42 d). The growth rate values (CL) obtained were 13.5–17.3 $\mu\text{m d}^{-1}$ (Figure 3).

4. Discussion

The relative growth of *Gammarus aequicauda* was previously studied by Kevrekidis et al. [33]. These authors showed that there is a positive correlation between cephalic length and body length. However, Kevrekidis and Lazaridou-Dimitriadou's equation differs from the equation found in the present study in terms of the slope ($b = 1.237$ versus $b = 1.211$, resp.). Both equations give similar results only for small sizes. The differences may be due to the geographically variability in the allometric growth.

Salinity as an environmental factor has been considered mostly in terms of its effects on survival, distribution, and reproductive strategies in marine and brackish-water amphipods [1, 3, 34–36]. The determination of the potential capacities of a population in relation to salinity conditions is an important prerequisite for assessing more complicated ecological situations. As expected, *Gammarus aequicauda* shows a high resistance to abrupt changes in salinity. The present study shows that *G. aequicauda* can survive and grow in a wide range of salinities between 2 psu and 40 psu. These values are similar to those reported in other euryhaline peracarida species, such as the isopod *Sphaeroma serratum* Fabricius [37], the tanaidacea *Tanais cavolinii* Milne-Edwards [38] and the amphipods *Hyale crassicornis* Haswell [39], *Traskorchestia traskiana* Stimpson [40], *Orchesia gammarellus* Pallas [41], *Orchesia chiliensis* Milne-Edwards [42], *Cyathura polita* Stimpson [6, 43] and *G. duebeni* Lillgeborg [44].

The limits of tolerance depend upon the conditions of acclimation. When *Gammarus aequicauda* has been acclimated at low salinity (9 psu), survival at oligohaline conditions is greatly increased. Gradual acclimation over long intervals of time resulted in better survival in amphipods [29]. The present results contrast with those obtained by Prato et al. [24]. According to these authors, a high percentage of *G. aequicauda* acclimated to 36 psu can survive

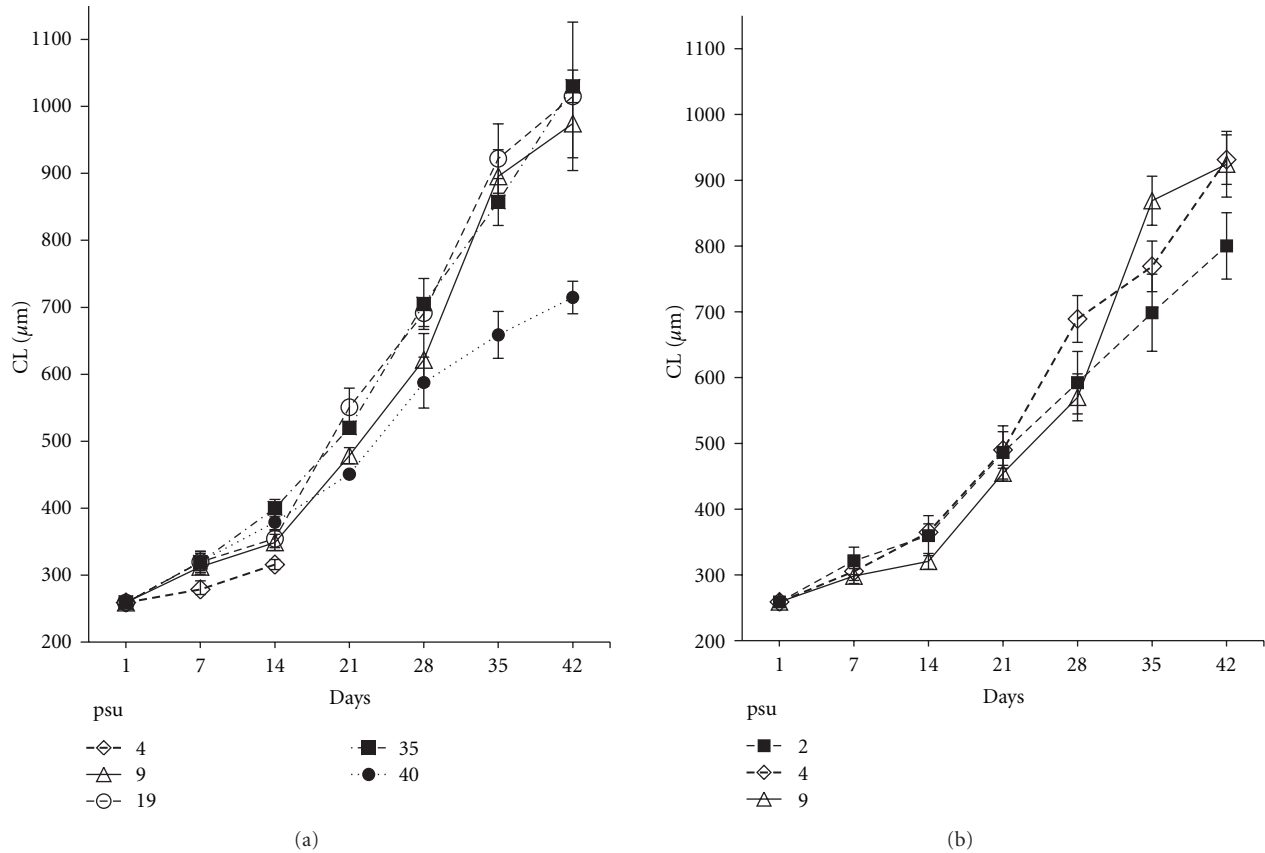


FIGURE 2: Growth of juveniles of *Gammarus aequicauda* (CL, μm). (a) First experiment: juveniles from ovigerous reproductive adults acclimated at 34 psu. (b) Second experiment: juveniles from reproductive adults acclimated at 9 psu.

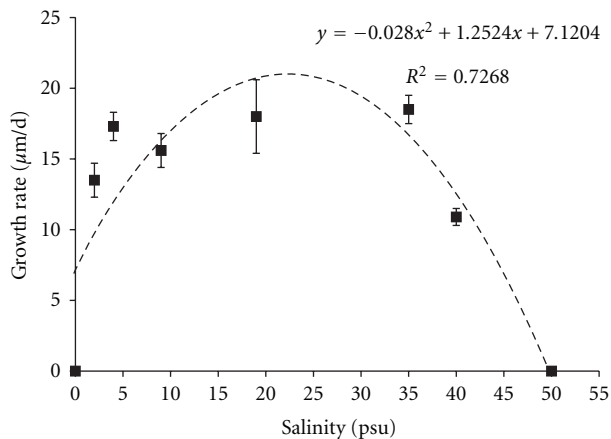


FIGURE 3: Growth rate of juveniles (CL, $\mu\text{m d}^{-1}$) of *Gammarus aequicauda* at different tested salinities (0, 2, 4, 9, 19, 34, 40, and 50 psu).

at low salinities (0 psu and 3 psu) for 10 days without any prior gradual acclimation to lower salinities. We have not been able to repeat Prato et al. results with individuals (adults or juveniles) of the Ebro delta population, perhaps due to possible intraspecific differences. Intraspecific geographical variations have been observed in other gammarid species,

such as physiological and geographical differences between Ireland and Britain populations of *G. duebeni* [44]. It is possible that populations of *G. aequicauda* from Mar Piccolo (Italy) have a higher capability for compensatory adjustments to rapid salinity changes than *G. aequicauda* from Ebro delta.

Kevrekidis et al. [14] suggested that the growth and reproductive biology of *G. aequicauda* are not markedly affected by low salinities. According to the present results, *G. aequicauda* could well tolerate the salinities that were reported in the study area (4–37 psu) [13]. Within this range of salinities, this species can survive, reproduce, and grow in the laboratory. These results are consistent with those of Delgado et al. [13] who did not find a correlation between monthly *G. aequicauda* abundance and salinity values. It is known that parameters such as temperature and infection with parasites can change the range of salinity tolerance in amphipods [29, 39, 45]. Therefore, other factors such as temperature, oxygen concentration, predation, and pollution should also be considered in future studies.

The daily increases in BL obtained in this study ($80\text{--}143 \mu\text{m d}^{-1}$; estimated from $\log\text{BL} = 1.211\log\text{CL} + 0.2197$) are high compared with the values previously recorded by Delgado et al. [13] from their polymodal frequency distribution analyses ($21\text{--}99 \mu\text{m BLd}^{-1}$). This discrepancy is likely due to sampling biases that interfere with the

frequency distribution analyses. Although the growth rate values obtained in this study agree with those recorded by Kevrekidis et al. [14] ($50\text{--}150\ \mu\text{m d}^{-1}$) and Greze [18] ($80\text{--}150\ \mu\text{m d}^{-1}$), these values are comparable to those reported in other amphipods. For instance, the growth rates of *Hyale crassicornis* were between $44\ \mu\text{m d}^{-1}$ and $114\ \mu\text{m d}^{-1}$ [39]. The lower growth rate values for *G. aequicauda* were obtained at extreme salinities (2 psu and 40 psu). It is likely that there is a higher energy requirement for osmoregulation under osmotic stress, which reduces the energy available for growth. D. H. Steele and V. J. Steele [3] observed a reduced growth rate to maturity in *Gammarus lawrencianus* with decreasing salinity (from 15–20 psu to 2.5 psu). Normant et al. [46] reported similar values for *G. oceanicus*.

Under laboratory conditions, *G. aequicauda* reaches sexual maturity at 42 d when females reach 6–7 mm in BL. Delgado et al. [13] reported that the minimum size of an ovigerous female was 5 mm. These results are similar to those obtained for other *Gammarus* species. For example, *G. locusta* reared in the laboratory at 20°C and 33 psu salinity becomes sexually mature at 35 d, whereas at 15°C and 20–33 psu salinities, age at maturity was estimated to be 49 d [47].

In conclusion, *Gammarus aequicauda* can adapt to a wide range of salinity conditions, allowing juveniles to grow in many habitats under natural conditions. Growth of *G. aequicauda* juveniles is optimal at 4–34 psu salinities and 17°C–18°C. Thus, the *G. aequicauda* life history was not markedly affected by salinity changes if changes are not extremely sharp.

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