

Effects of prey: larva ratio on larval development and survival rate of *Maja crispata* (Risso 1827) (Crustacea: Majidae)

Maja crispata (Risso 1827) (Crustacea: Majidae)'nın larval gelişimi ve yaşama oranı üzerine yem: larva oranının etkileri

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Özet: *Maja crispata* (Risso 1827)'nin larval gelişimi ve yaşama oranı üzerine yem:larva oranının etkileri incelenmiştir. Denemeler 21,6 ± 0,5°C su sıcaklığında yapılmıştır ve larvalar beş yem:larva oranı [5 Artemia/larva (A1), 10 Artemia/larva (A2), 15 Artemia/larva (A3), 20 Artemia/larva (A4) ve 25 Artemia/larva (A5)] ile beslenmişlerdir. Tüm gruplarda, zoe I safhası 3 gün, zoe II safhası 5 gün sürmüştür. Megalop evresi tüm gruplarda 6 günde tamamlanmıştır ve larvaların juvenil evresine geçişleri A1 ve A2 gruplarında 13-14 günde; A3, A4 ve A5 gruplarında ise 12-14 günde tamamlanmıştır. Bu çalışma yem:larva oranının *M. crispata* larvalarının yaşama oranlarını olumsuz etkilediğini ($P<0.05$), ancak gelişim sürecini etkilemediğini göstermiştir.

Anahtar kelimeler: *Maja crispata*, örümcek yengeci, besleme, gelişim, yaşama oranı, larval yetiştiricilik

Abstract: Effects of prey:larva ratio on larval development and survival rate of *Maja crispata* (Risso 1827) were investigated. The experiments were performed at 21.6±0.5°C, and the larvae were fed with five prey:larva ratios [5 Artemia/larva (A1), 10 Artemia/larva (A2), 15 Artemia/larva (A3), 20 Artemia/larva (A4) and 25 Artemia/larva (A5)]. The stage zoea I lasted within 3 days and zoea II stage occurred in 5 days in the all experimental groups. Furthermore, the megalopa stage took place at the same duration (6 days) for all groups. Although, the larvae reached to the juvenile stage in 13-14 days at A1 and A2 groups, within 12-14 days at A3, A4 and A5 groups. This experiment presented that prey:larva ratio affected to the larval survival rates in *M. crispata* ($P<0.05$), but did not affect the developmental duration.

Keywords: *Maja crispata*, spider crab, feeding, development, survival rate, larval rearing

INTRODUCTION

The lesser spider crab, *Maja crispata* (Risso 1827) is the smallest species belongs to family of Majidae and lives in rocky and sandy intertidal and shallow waters with rich in algae to a depth of 20 m. The spider crabs distribute from the eastern Atlantic (the coast of Portugal to Cabo Blanco) to the Cape Verde Islands in Africa, and along the Mediterranean (Monod, 1956; Zariquiey-Alvarez, 1968 (as *Maja verrucosa*); Manning and Holthuis, 1981; Števcic and Galil, 1993; Udekem D'acoz, 1999; Türkay, 2001). This species reaches a maximum size much smaller than *Maja squinado* and *Maja brachydactyla* (Sampedro et al., 2003). Although, *M. crispata* are captured by trammel nets and are consumed freshly in some European countries (Fisher et al., 1987), this species are discarded by set netters along the shallow waters in Turkey.

Artemia sp. have been identified as the most suitable prey for brachyuran crab larviculture (Bigford, 1978) due to their wide acceptance by the larvae (Anger, 2001), and their easy handling as commercially available inert cysts. The live prey

concentration used during larval rearing is of primary significance for larval survival due to most brachyuran crab larvae are not selective feeders, and their feeding efficiency depends on zooplankton encounter rates. As yet, using *Artemia* density for crab larval rearing varies from 0.5 to 30 prey.ml⁻¹ (Bigford, 1978; Heasman and Fielder, 1983; Iglesias et al., 2002; Rhyne et al., 2005). However, there is no longer available data related to feeding with *Artemia* and minimum feeding ratio on larval development and survival rate of *M. crispata* in terms of rearing or culture conditions. The aim of the present study was to determine the effects of prey density on larval development and survival rate of *M. crispata* in captive conditions.

MATERIALS AND METHOD

Maintenance of broodstock

A total of 67 *M. crispata* adults (48 males and 19 females) were captured by trammel net at depth between 2 and 8 m from the Iskele harbour at Urla, Izmir on May 24, 2012. Seven

ovigerous females (the mean carapace width was 55.1 ± 1.89 mm and the mean body weight was 71.7 ± 6.9 g) were selected and placed individually in the baskets (10 l of volume and 150μ of mesh size) in an open flow-through filtered sea water system two cylindrical polyester tanks (450 l volume) in indoor facilities of the Fisheries Faculty of Ege University (Urla, Izmir). Natural photoperiodicity was adjusted. The average temperature, pH, salinity and dissolved oxygen in the tanks was measured as $20.1 \pm 2^\circ\text{C}$, 8.0 ± 0.2 , $37 \pm 0.1\text{‰}$ and $6 \pm 0.2 \text{ mg.l}^{-1}$, respectively. The specimens were fed ad libitum with piece's fish species (i.e. *Sardina pilchardus*, *Engraulis encrasicolus*) and cuttlefish (*Sepia officinalis*) by hand. The following day, uneaten part or remains were removed by siphoning.

Larval rearing

For the experiments, totally 450 newly hatched zoeae were selected from 2500 sprightly individuals spawned by the one adult female crab in the laboratory on June 26th. Except the experimental larvae, the remainders were reared to metric measurements depend on the developmental stages in a plastic basket of 10 l well-supplied filtered sea water and aeration, which was placed in a 450 l of polyester tank. By this way, 10 larvae for each developmental stage were measured as the nearest 0.1 mm by millimetric ocular (Figure 1). The larvae (30 larva.l⁻¹) were put into 1300 ml transparency plastic beakers containing about 1000 ml filtered sea water. For maintenance of the larvae, seawater was changed on a daily basis by replacing 100% of the beaker volume with fresh sea water that was regulated to experimental conditions and supplied with continuous aeration. The experiments were established in triplicate and began at the same water temperature (20.1°C), and were maintained at ambient temperatures at the end of the experiment. The all larvae were reared from zoea I to first juvenile stage. Daily controls were carried out for calculating survival rates [(initial individual number - dead individual number / initial individual number) \times 100] of the larvae based on developmental stages. By the way, all developmental stages of the *M. crispata* larvae were identified according to the Rodríguez (2002).

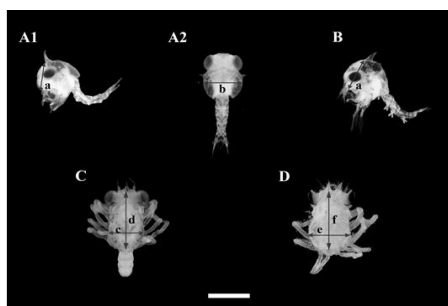


Figure 1. Metric measurements of *M. crispata* larvae based on developmental stages (scale bar: 1 mm): A1; lateral view of ZI, A2; dorsal view of ZI, B; ZII, C; megalopa, D; juvenile, a; rostradorsal length (RDL) at ZI and ZII stages, b; carapace width (CW) at ZI stage, c; carapace width of megalopa stage, d; carapace length (CL) at megalopa stage, e; carapace width (CW) at the juvenile stage, f; carapace length (CL) at the juvenile stage.

Feeding protocol

The crab larvae were fed once in a day with five prey: larva ratios [5 *Artemia.larva*⁻¹ (A1), 10 *Artemia.larva*⁻¹ (A2), 15 *Artemia.larva*⁻¹ (A3), 20 *Artemia.larva*⁻¹ (A4) and 25 *Artemia.larva*⁻¹ (A5)]. Furthermore, *Artemia* nauplii were used for zoea I (ZI) and zoea II (ZII) stages, at following stage megalopa (M) were fed with *Artemia* metanauplii enriched with mix algae powder of the Algamac®, for 24 hours. *Artemia* nauplii were counted using volumetric procedures. Illumination was adjusted 24 hours by using 40W fluorescent lamp during the incubation and enrichment period.

Furthermore, throughout the experiment, daily water parameters such as temperature, pH, oxygen and salinity were monitored using Extech® DO700 Multiparameter.

Statistical analysis

The data were given as mean \pm S values in the text. The survival rates were compared using one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test to determine significant differences among means ($P < 0.05$). Normal percentage transformation to arcsine was carried out prior to analysis of variance. All statistical analyses were carried out using the SPSS15.0 package program.

RESULTS

During the experiment, the mean water parameters were measured as $21.6 \pm 0.5^\circ\text{C}$, pH 8.1 ± 0.1 , $64.7 \pm 0.4\%$ of dissolved oxygen and $37 \pm 0.2\text{‰}$ of salinity (Fig 2). Fully larval development of *M. crispata* lasted 14 days (Table 1). Considering the experiments carried out as a whole, mean survival rates were 100% for ZI at between 0 and 3 days, $95.2 \pm 4\%$ for ZII at between 5 and 9 days, $40.7 \pm 11.8\%$ for M at between 8-13 days, and $7.76 \pm 5.4\%$ for first juvenile stage at 14 days of the experiment. Significant differences were found in the survival rates among the trials ($P < 0.05$) (Table 2). Additionally, cannibalism were determined at the M stage; the megalopa larvae eaten the live ZII stage larvae. Morphometric measurements of the *M. crispata* larvae were presented in Table 3. Furthermore, the differences, if existed, between the measurements of larvae were neglected in this study.

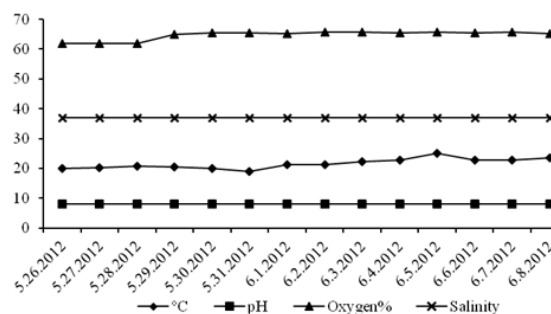


Figure 2. The water quality parameters during the experiment.

Table 1. Larval developmental duration of *M. crispata*.

Date	A1	A2	A3	A4	A5
26.05.2012	ZI	ZI	ZI	ZI	ZI
27.05.2012	ZI	ZI	ZI	ZI	ZI
28.05.2012	ZI	ZI	ZI	ZI	ZI
29.05.2012	ZI + ZII	ZI + ZII	ZI + ZII	ZI + ZII	ZI + ZII
30.05.2012	ZII	ZII	ZII	ZII	ZII
31.05.2012	ZII	ZII	ZII	ZII	ZII
01.06.2012	ZII	ZII	ZII	ZII	ZII
02.06.2012	ZII + M	ZII + M	ZII + M	ZII + M	ZII + M
03.06.2012	M	M	M	M	M
04.06.2012	M	M	M	M	M
05.06.2012	M	M	M	M	M
06.06.2012	M	M	M + J	M + J	M + J
07.06.2012	M + J	M + J	M + J	M + J	M + J
08.06.2012	J	J	J	J	J

ZI: zoe-I; ZII: zoe-II; M: megalopa; J: juvenile

Table 2. The mean survival rates of the *M. crispata* larvae

Stage	A1 (%)	A2 (%)	A3 (%)	A4 (%)	A5 (%)
Zoea I	100±0.0 ^{ns}	100±0.0 ^{ns}	100±0.0 ^{ns}	100±0.0 ^{ns}	100±0.0 ^{ns}
Zoea II	90.0±0.0 ^{ns}	93.3±1.0 ^{ns}	96.7±1.0 ^{ns}	97.8±10.7 ^{ns}	86.7±1.0 ^{ns}
Megalopa	25.3±3.8 ^{ns}	25.7±4.0 ^{ns}	28.8±4.7 ^{ns}	35.5±5.7 ^{ns}	40.0±5.7 ^{ns}
Juvenile	2.2±0.7 ^a	2.2±0.7 ^a	8.9±1.2 ^{a,b}	11.1±1.7 ^b	14.4±1.7 ^b

Values in the same row that do not share a common superscript are significantly different; ns: no significance, (P<0.05).

DISCUSSION

This type of abbreviated development is common to all Majoidea species that have been studied larval development (Guerao et al., 2008). In any case, larval development of *M. crispata* has been studied by Rodriguez (2002). Andrés et al. (2007) showed that in *M. brachydactyla* under constant conditions of salinity (36‰) and temperature (18°C), larval developmental stages take place 18 days; ZI at 3±1 days, ZII at 8±1 days, M at 12±2 days, and first juvenile at 18 days. Also, Palma et al. (2008) presented that in *M. brachydactyla*

(as *M. squinado*) at 19.9±0.1°C and 37.6±0.2‰, this period lasted 22 days within the larval stage each molting phase (ZI, ZII and M) lasted 7-8 days. However, in this study, the larval period completed in shorter times (14 days) even the groups of feed with low prey density (5 prey.larva⁻¹) than that of the researchers' findings for *M. brachydactyla* and *M. squinado*. It might be stemming from water temperature (21.6±0.5°C in the present study) and species specific characteristics.

The notable differences between the sizes of the *M. crispata* larvae in the present study and in that by Rodríguez (2002) may be attributable to laboratory rearing conditions. The larvae of *M. crispata* at ZI and ZII stages can be easily separated from the larvae of *M. squinado*, *M. brachydactyla*, and *M. goltziana* by measurements (Table 3).

According to the present results, the entire larvae even the group feeding with 5 Artemia.larva⁻¹ completed to ZII stage with over 90% of survival rate. From this point, differences between the survival rates of the larvae at first juvenile stages among the trials significantly changed (P<0.05). It is thought to be that this was the prey density effect on survival rates of *M. crispata* larvae. Anyhow, the larval survival rates were found relatively higher than that of Andrés et al. (2007) for *M. brachydactyla* (87.1% for ZI, 64.7±4.8% for ZII, 24.8±1.5% for M, and 11±0.8% for first juvenile) and of Palma et al. (2008) for ZII and M stage of *M. squinado* (100% for ZI, 55.2±10.1% for ZII, 32.4±6.4% for M, and 13.8±3.3% for first juvenile). The settlement and molt to first juvenile period was found to be critical in *M. brachydactyla* (Iglesias et al., 2002; Palma et al., 2008) and *M. squinado* (Guerao and Rotllant, 2010). However, this study showed that not only settlement and molt to first juvenile period, but also zoea II to megalopa stage is critical at least for *M. crispata*.

Sampedro and Gonzalez-Gurriarán (2004) and Palma et al. (2008) pointed out that spider crab is a non-cannibalistic species. However, cannibalism was observed at the beginning of M stage in the present study. It's thought to be that this phenomenon might be stemming from low prey density, and also species specific features.

Generally, the use of high densities of live prey in the rearing water enhanced both survival and growth of the larvae (Andrés et al., 2007). Brick (1974) showed that survival of *Scylla serrata* (Decapoda: Portunidae) up to megalopa stage increased in parallel to Artemia concentration.

Table 3. Comparing of morphometric characteristics in the larvae of *M. crispata*, *M. squinado*, *M. brachydactyla*, and *M. goltziana* from present and previous studies

Stage	Feature (mm)	<i>M. crispata</i> ¹	<i>M. crispata</i> ²	<i>M. squinado</i> ³	<i>M. squinado</i> ⁴	<i>M. brachydactyla</i> ⁵	<i>M. goltziana</i> ⁶
Zoea I	RDL	1.29±0.05	1.4-1.5	1.95	1.93±0.03	1.87±0.05	2.03-2.15
	CW	1.20±0.02	-	-	1.18±0.02	1.12±0.04	1.00-1.08
Zoea II	RDL	1.67±0.04	1.61-1.72	2.1	2.19±0.04	2.02±0.05	2.03-2.27
	CW	1.13±0.02	-	-	-	1.30±0.05	1.19-1.27
Megalopa	CL	1.80±0.02	1.72-1.90	1.4-1.5	1.88±0.03	1.67±0.07	1.79-1.91
	CW	0.92±0.02	-	-	-	-	-
Juvenile	CL	1.84±0.01	-	-	-	-	-
	CW	1.20±0.02	-	-	-	-	-

Also, Bigford (1978) reported that the survival rate of the *Libinia emarginata* zoea I was high when cultured using 6 prey.ml⁻¹ than with only 3 prey.ml⁻¹. Andrés et al. (2007) found that the same results for *M. brachydactyla* at ZI and ZII stages. Also, the current results agreed with the previous findings.

In conclusion, *M. crispata* larvae can be easily reared under controlled conditions, and keep going of ZI and ZII stages of the larvae with a minimum adequate *Artemia* ratio as 5 *Artemia.larva*⁻¹ (0.15 prey.ml⁻¹). On the other hand, the cannibalism existence in *M. crispata* larvae was shown firstly. However, the present study suggested that for obtaining of high larval survival rate, the prey density should be increased

since starting of M stage, especially. Furthermore, this experiment presented that prey density did not affect the developmental duration of *M. crispata* larvae, but survival rates. However, detailed studies should be carried out for clear understanding of its bio-ecological characteristics. of *M. crispata* larvae on optimum prey density, prey size, foraging behaviour, feeding physiology, etc.

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