1 Survival and growth of hatchery-reared Mediterranean spider crab

2 juveniles, *Maja squinado*, under different rearing conditions

3 Maria del Mar Gil^{a,b,*}, Elena Pastor^a, Juana Durán^a

4
5 ^aLIMIA, Laboratori d'Investigacions Marines i Aqüicultura (Balearic Government).

6 Eng. Gabriel Roca 69, 07157 Port d'Andratx, Balearic Islands, Spain

7 ^bIMEDEA, Institut Mediterrani d'Estudis Avançats, Ichthyology Group (CSIC-

8 UIB). Miquel Marquès 21, 07190 Esporles, Balearic Islands, Spain

9
10
11
12 *To whom correspondence should be addressed:

13 Maria del Mar Gil

14 e-mail: mmgil@dgpesca.caib.es

15 Laboratori d'Investigacions Marines i Aqüicultura, LIMIA (Balearic Government)

16 Av. Eng. Gabriel Roca 69

17 07157 Port d'Andratx, Balearic Islands, Spain

18 Phone: + 00 34 971 67 23 35

19 Fax: + 00 34 971 67 42 40

ABSTRACT

The Mediterranean spider crab, *Maja squinado* (Herbst, 1788), is of increasing interest for commercial or stock enhancement purposes, which necessitate the production of a large number of specimens in captivity. However, the growth and survival of crabs during the early juvenile phase could be affected or limited by several factors. In this study, the effects of stocking individually or in pairs, diet, shelter presence and shelter type for juveniles (from 2nd to 5th juvenile crab instar) were investigated. Survival was higher for the group with two individuals per cylinder than the group with one individual per cylinder, but the growth (carapace length and intermoult period) was not significantly different. The tested diets (fresh and dry diets) did not produce significant differences in growth, but the dry diet resulted in a higher survivorship at the end of the experiment (75%) than the fresh diet (47.5%). Finally, the presence of shelter was clearly beneficial for the groups with two individuals per cylinder, mainly in the case of natural shelter. Crabs in natural shelter had a higher survival rate (55%) and were larger, probably due to the consumption of the algae added as shelter. Thus, this study is a first approach to find the optimal rearing conditions that maximizes the production of juveniles during an especially vulnerable phase.

Keywords:

Cannibalism, diet, growth, *Maja squinado*, Mediterranean spider crab, survival, shelters

1. Introduction

The Mediterranean spider crab *Maja squinado* (Herbst, 1788) is one of the largest and most valued European crab species. This species is captured by small-scale fisheries (Torres et al., 2013) in areas such as Croatia, France or Spain (<http://www.sealifebase.org/report/FAO>), where it has a notable local gastronomic interest. In addition to being a relevant exploited resource, *M. squinado* has interesting biological characteristics that make it appropriate for aquaculture, such as a high fecundity and rapid larval development (3 larval stages in 17 days at 19 °C; Durán et al., 2012).

This species was abundant in the Spanish Mediterranean until the first half of the 20th century (Garcia, 2007). Currently, it is scarce and considered endangered (UNEP, 1996) and it has become a very rare catch in some areas (e.g., Mallorca Island, Western Mediterranean; Garcia, 2007). Environmental degradation and inappropriate fisheries management are the most common causes of the decline and even the collapse of certain fisheries (Born et al., 2004). Thus, in some areas of the Spanish Mediterranean coast, such as Balearic Islands, governmental programmes for the recovery of wild populations have been developed via the release of hatchery-reared specimens (Durán et al., 2012).

The production of a large number of specimens, for both commercial and stock enhancement purposes, requires a lot of prior knowledge. A considerable number of studies on the production, i.e., the reproduction and growth under captivity conditions, of *Maja brachydactyla* have been conducted in the last few years (e.g., Castejón et al., 2015; Domingues et al., 2012; Guerao et al., 2012; Rotllant et al., 2015; Simeó et al., 2015). However, information about *M. squinado* is scarce. Just a few studies on the

reproductive process, larval rearing and growth under intensive culture conditions have been published (Durán et al., 2012; Durán et al., 2013; Guerao et al., 2008; Guerao and Rotllant, 2010). Note that *M. brachydactyla* and *M. squinado* were considered to be the same taxon (*M. squinado*) until morphological and genetic analyses showed that the Atlantic and Mediterranean spider crabs were different species (Neumann, 1998); therefore, some of the studies presented on *M. Squinado* before this distinction was made were in fact about the Atlantic species. Furthermore, the life cycle of spider crabs is complex, and behaviour and requirements vary with age, life history and size (Alaminos and Domingues, 2008). Therefore, knowledge about the factors affecting or limiting growth and survival during the juvenile phase under captivity conditions is required.

Growth rate in crab aquaculture is affected by environmental factors, such as food availability (Alaminos and Domingues, 2008; Dubber et al., 2004), holding space (Swiney et al., 2013) and stock density (Daly et al., 2009). In addition, the major factors influencing survival seem to be cannibalism (Sainte-Marie and Lafrance, 2002; Triño et al., 1999), food availability (both quality and quantity; Penha-Lopes et al., 2006; Sheen, 2000) and overcrowding stress (Daly et al., 2009; Wilber and Payson Wilber, 1991). For example, juveniles of the snow crab *Chionoecetes opilio* investigated by Sainte-Marie and Lafrance (2002), the spider crab *Mithrax spinosissimus* by Wilber and Payson Wilber (1991) and the blue swimming crab *Portunus pelagicus* by Marshall et al. (2005), exhibit cannibalism and density limitation during early development, resulting in losses as high as 60% in experiments from 20 to 104 days. Artificial habitats and shelters significantly enhance the carrying capacity of the rearing receptacle by reducing the incidence of agonistic interactions among crabs and achieving higher productivity levels (Mann et al., 2007; Marshall et al., 2005). Other methods for reducing

cannibalism among juvenile crabs include the addition of substrate, the provision of high quality diets, the grading of size classes, and the optimization of rearing densities (Daly et al., 2009; Fernández, 1999; Marshall et al., 2005; Ut et al., 2007; Zmora et al., 2005). An alternative to avoiding cannibalistic tendencies is separating individuals into compartments or cells, which usually incurs costs due to the increased labour needed to feed and clean the holding cells and the materials and space required to build and house isolated crabs (Swiney et al., 2013). Therefore, a challenge to culturing spider crabs is providing the environment and rearing conditions that minimize mortality and maximize growth.

The objective of this study was to assess the effects of diet, individually or in pairs stocking, shelter presence, and shelter type on the growth and survival of *M. squinado* early juveniles.

2. Materials and methods

2.1. Broodstock and larval rearing

Adult ovigerous females and males of the species *M. squinado* were captured on the northwestern coast of Corsica by local fishermen using trammel nets. The crabs were then transported by vessel to LIMIA (*Laboratori d'Investigacions Marines i Aquicultura*, Balearic Government, Spain) in two 0.8 m³ tanks constantly supplied with flow-through seawater (18°C) pumped directly from the sea. At the LIMIA facilities, the wild crabs were maintained in a 5 m³ rectangular flat-bottom fibreglass tank with a controlled temperature of 18 °C and a natural photoperiod. The flow-through open system allowed a water renewal rate of 50 L min⁻¹. Four days prior to spawning

(indicated by the colour of the egg mass which change from an initial yellow-orange to a black coloration at the end of development; Durán et al., 2012), the ovigerous females were isolated in 0.2 m³ round flat-bottomed fibreglass tanks with the outflow equipped with a larval collector (500 µm mesh size). The active, newly hatched zoeae were collected and reared following the larval rearing techniques described in Durán et al. (2012). Once they reached megalopae instar, they were stocked in 1 m³ round fibreglass tanks for settlement and were reared following the mass culture protocol described by Pastor et al. (2011), until they were used in the following experiments. All laboratory experiments based on live crustaceans complied with the prevailing national laws for ethical treatment of experimental animals at that time. These experiments were carried out in strict accordance with the recommendations from the Directive 2010/63/UE, and in strict compliance with the Spanish law (RD53/2013, BOE n. 34, 8 February 2013).

2.2. Experiments design

The experiments were conducted in 10 cm diameter PVC cylinders (1.5 L, 78.54 cm²) with 1 mm mesh bottoms. The cylinders, arranged in rows of 10 cylinders (see Fig. 1), were installed in a 5 m³ fibreglass tank equipped with a tide system for the periodic renewal of the water. Tide in the tank was completed every 2 hours, and incoming water was previously UV-sterilized and filtered through 1 µm filters. The salinity was 37 g L⁻¹, and the temperature was controlled using a refrigeration system (19.01 ± 1.58 °C). The ammonium level was checked daily to ensure that it remained within a physiologically optimal range.

Prior to the start of each experiment, the recently moulted C2 instar crabs were collected from the mass culture tanks and randomly placed individually or paired in the cylinders.

During the experiments, crabs were fed daily, and cylinders were cleaned before feeding to remove waste feed and faecal matter. Additionally, all cylinders were checked daily and the exuviae of the moulted individuals and dead crabs were recorded and stored in a 10% formaldehyde solution until its analysis.

2.2.1. Experiment 1: Individually or in pairs stocking

A total of 120 *M. squinado* 25-day-old juveniles (2nd crab instar, C2) with an initial carapace length (CL) of 3.229 ± 0.038 mm were used in this experiment over a 70 day period (until the crabs reached the 5th crab instar, C5). Two stocking systems, one individual (i.e., one individual per cylinder, group G1) and the other in pairs (i.e., two individuals per cylinder, group G2), were tested (Table 1). Each group were composed by 40 cylinders; therefore 40 crabs were examined in the group G1 and 80 crabs in the group G2.

In addition to the collected data common for all groups, in the group G2 (two individuals per cylinder), cannibalism events were noted when one of the dead crabs presented clear evidence of being partially or totally consumed.

2.2.2. Experiment 2: Diet

A total of 80 *M. squinado* 25-day-old juveniles (C2) with an initial CL of 3.320 ± 0.059 mm were used in this experiment over a 70 day period (until the crabs reached the 5th crab instar, C5). The effect of diet was investigated by comparing a fresh and a dry diet; group G3 was fed pellets formulated for shrimp (Larval Plus, Catvis B.V., The

Netherlands), while group G4 was fed fresh mussel meat (Table 1). Each group was composed by 40 cylinders and crabs were stocked individually; therefore 40 crabs were examined in each group.

2.2.3. Experiment 3: Presence of shelters

A total of 160 *M. squinado* 25-day-old juveniles (C2) with an initial CL of 3.313 ± 0.035 mm were used in this experiment over a 70 day period (until the crabs reached the 5th crab instar, C5). The crabs were distributed in pairs in the cylinders (40 cylinders per group). The presence or absence of shelter was tested by providing group G5 with natural seaweed (Table 1). This group was compared with the individuals of the group G2. The seaweed (*Ulva lactuca* and *Corallina officinalis*) was replaced once a week, and the new collected seaweed was disinfected with an iodophor solution of 0.1 ppm iodine (OIE, 2016) prior to its addition to the cylinders.

2.2.4. Experiment 4: Type of shelter

This experiment, which was carried out the year after the rest of experiments, was conducted over 60 days with 25-day-old C2 instar *M. squinado* juveniles. A total of 320 individuals, with an initial CL of 3.128 ± 0.038 mm, were randomly distributed in 2 groups of 80 cylinders with a density of two individuals per cylinder.

The growth and survival associated with two different types of shelters, one natural and the other artificial, was tested in this experiment (Table 1). For that, a group (G6) were provided with an artificial substrate composed of green raffia fronds, and the other group (G7) were provided with natural seaweed (the same species used in the Experiment 3). Both types of shelter presented a similar size with a high number of hiding spaces. The aim of this experiment was to determine if the highest growth and

survival obtained in the Experiment 3 in the group with shelter presence were due to the protective effect of the shelter or the complementary feeding obtained by the ingestion of the seaweed.

2.3. Data analysis

The survival and growth were analysed for each group of all the experiments using the recorded and collected exuviae and dead crabs.

The survival rates of the different groups were analysed and compared using the survival analysis method. The survival analysis method is an analysis technique designed to investigate the factors affecting the time at which a particular event (here, the death of the crabs) occurs (Crawley, 2007). To determine the differences in the survival rates among the groups, a proportional hazards model (or Cox regression model) was applied to the data. This model describes the probability per unit time of the event occurring as a function of a basic probability (the baseline hazard) and a set of explanatory variables (here, individually or in pairs stocking, diet, presence of shelter or type of shelter). Not all the crabs died within the duration of the experiment; therefore, the partially missing data were considered right-censored observations. The *coxph* function from the *survival* library (developed by T. Therneau and T. Lumleyat; <http://cran.r-project.org/web/packages/survival/survival>) of the R package (at <http://www.r-project.org/>) was used to estimate the model parameters and likelihood ratios. The predicted survival rates at different times and treatments were estimated using the function *survfit* from the same library.

The growth was assessed by quantifying the carapace length (CL) and the duration of the intermoult period (IP). The CL was measured to the nearest 0.01 mm with an

electronic profile projector (Mitutoyo, Model PJ-A3000, Japan) as the distance between the rostral margin (excluding the rostral spines) and the posterior margin of the carapace. The IP was recorded as the number of days between two consecutive moulting events. The CL and IP were compared among the tested factors after each moult instar and at the end of the experiment (C5 instar) using one-way ANOVA or the Kruskal-Wallis test. For all ANOVA analyses, the Shapiro test for normality and the Bartlett test for homogeneity of variance were used to determine if data met the assumptions of ANOVA. When data failed these tests, the non-parametric Kruskal-Wallis test was used. These analyses were conducted in the R package.

3. Results

3.1. Effect of rearing juvenile crabs individually or in pairs

Growth (CL and IP) and survival rate of the G1 group (with one individual per cylinder) and the G2 group (with two individuals per cylinder) were compared. The growth based on the CL and IP measurements showed no obvious differences between the groups (Table 2). The C4 instar had a significantly larger CL for the group with two individuals per cylinder, but this difference was not significant at the end of the experiment (Fig. 2). In the same way, the duration of the C3-C4 IP was significantly shorter for group G2. However, in the next period (C4-C5), the group with two individuals per cylinder had higher IP values than the group with one individual per cylinder, although significant differences were not observed.

The overall observed survival at the end of the experiment was 37.5% in the group with two individuals per cylinder (G2) and 12.5% in the group with one individual per

cylinder (G1), although significant differences were not observed in the survival analysis ($P=0.126$). For that reason, the survival rate of the two individuals in each cylinder was analyzed separately, i.e., the first dead crab in each cylinder was assigned to the subgroup G2 (1st) and the second crab was assigned to the subgroup G2 (2nd), whereas if no crab died in a certain cylinder, the crabs were randomly assigned. In this second analysis, significant differences were detected (coefficient=43.34, df=2, $P<0.001$, Fig. 3). One of the crabs in the shared cylinder would have a very low survival rate, while the other crab would have a high survival rate significantly exceeding that of the group with one individual per cylinder. Furthermore, 27.7% of the crabs dead in the subgroup G2 (1st) presented clear evidences of cannibalism (i.e., they were partially or totally consumed by the other crab). Almost all of these attacks (90%) occurred the same day or the day after the moulting process.

3.2. Effect of diet type

The growth and survival of the juveniles fed with a dry pellet diet (G3) and with a fresh diet (G4) were compared. The comparison of the CL and IP measurements showed that neither tested diet was more advantageous in regard to growth (Table 3). The CL of the C3 instar was significantly higher for the group fed with a fresh diet. However, this difference disappeared in the next instar, and at the end of the experiment, the differences between groups were not significant and demonstrated similar values (Fig. 2). Similarly, the IP between the C3 and C4 instars was significantly lower for the G4 group, but at the end of the experiment, the difference was not significant.

The observed survivorship at the end of the experiment was 75% for the group of crabs fed with a dry pellet diet (G3), while the group fed with a fresh diet (G4) had a

survival of only 47.5%. Therefore, the survival rate was significantly higher for the G3 group (coefficient=4.64, df=1, $P<0.05$, Fig. 4).

3.3. Effect of the presence of shelter

The G5 group, which included two individuals per cylinder and seaweed shelter, was compared with group G2, in which the crabs were reared without shelter. The growth comparison (Table 4) showed no significant differences between the IP measurements throughout the experiment. Conversely, the CL measurements suggested that the group reared with seaweed (group G5) grew more (mean growth value of 7.493 mm) at the end of the experiment than the group without shelter (group G2, mean growth value of 6.670 mm, Fig. 2). Furthermore, the statistical comparison showed significant differences between these groups at the end of the experiment ($P=0$).

Survival was also significantly higher for group G5 than for group G2 (coefficient=7.19, df=1, $P<0.05$), showing an observed survival rate of 55% in the group with shelter and a survival rate of 37.5% in the group without shelter at the end of the experiment (Fig. 5).

3.4. Effect of type of shelter

The effect of the type of shelter, natural or artificial, was analysed in terms of growth and survival. The juvenile crabs reared with natural shelter (group G7) grew significantly more than the crabs reared with artificial shelter (group G6, Fig. 2). The CL measurements were significantly higher for the group G7 with a mean value of 7.009 mm at the end of the experiment, while the group G6 had a mean value of 6.484

mm (Table 5). Conversely, the IP measurements did not show significant differences during the experiment.

The overall observed survival at the end of the experiment was 50.0% in the group with natural shelter (G7) and 26.2% in the group with artificial shelter (G6), and a significant difference was observed with the survival analysis (coefficient=14.25, df=1, $P<0.05$; Fig. 6).

4. Discussion

The production of *M. squinado* during the first phase of the nursery cycle suffers a dramatic reduction due to the stocking density when the crabs are reared in a mass culture (Pastor et al., 2011). This reduction in production is due to lethal interactions among the population, which can be motivated by a lack of space, shelter or a proper diet. Our research focused on the effect of these factors on survivorship and growth as a first approach to find the optimal conditions for the culturing of *M. squinado* juveniles.

The quality and amount of ingested food determine the resulting growth for most crustaceans (Dubber et al., 2004), and inadequate diet has been found to decrease survival rates in several species (Dubber et al., 2004; Penha-Lopes et al., 2006). In fact, cannibalism was not detected when a proper diet was provided in a study conducted with *Maja brachydactyla* by Guerao and Rotllant (2009). In this research, the survival of *M. squinado* juvenile crabs was affected by the different diets, but growth was not affected. Both the fresh (mussel meat) and pellet (shrimp feed pellets) diets resulted in similar growth and did not produce significant differences. Conversely, other investigations have demonstrated that some commercial shrimp feed pellets are inappropriate for other crustaceans (Alaminos and Domingues, 2008; Dubber et al.,

2004; Penha-Lopes et al., 2006) because they provide a lower growth rate. However, the use of formulated pellet diets can be very beneficial because they provide all the proper proportions of required nutrients necessary for the rapid growth, high feed efficiency and health of crabs, have a high availability, and are easy to transport, store and use because they are prepared to be directly administered (Alaminos and Domingues, 2008; Penha-Lopes et al., 2006). In contrast, fresh diets can present seasonal variations in quality, storage and handling that make the fresh option unfeasible and/or uneconomical (Crear et al., 2000). Furthermore, in this study, the fresh diet produced a significantly higher mortality rate than the dry diet, which may be related to the higher disease transmission risk of the fresh diets (Cowey and Sargent, 1972) or the lack of some required nutrients in the proper proportions. However, we must bear in mind that this study only compared two types of diet. Therefore, in the future, other types of fresh and dry diets should be tested in order to find the optimal diet.

Inter-cohort cannibalism could function as a self-regulatory mechanism by a density-dependent functional response to settlement densities (Moksnes et al., 1997). Thus, one of the *M. squinado* juveniles in each shared cylinder (G2) had a high probability of dying that was much larger than that of the specimens that were alone (G1). In fact, 27.7% of the dead crabs in shared cylinders were partially or totally consumed. Cannibalistic behaviour was observed even in presence of excess food; therefore, it is possible that, similar to the pattern observed in the blue-swimmer crab, *Portunus pelagicus* studied by Marshall et al. (2005), the cannibal does not regard the victim as food for survival, but as a competitive counterpart to which it feels aggressive. Predation and cannibalism supply additional nitrogen, vitamins and other essential substances, particularly when the quality and quantity of the available food is low (Anger, 1995; Kennish, 1996). This is reflected in the higher survival rate observed in

the second juvenile crab sharing the cylinder, whose survival was significantly greater than that of the crabs reared alone.

Cannibalism was observed mainly after the moulting process, when the carapace is not completely hardened, and the crab is vulnerable to attacks by congeners. Cannibalism associated with moulting has also been observed in other crustacean species, such as, the spider crab *Maja brachydactyla* in the study carried out by Domingues and Alaminos (2008) and the red king crab *Paralithodes camtschaticus* in the study of Borisov et al. (2007). Attacking another crab poses a great risk to the cannibal; therefore, to reduce this risk, the cannibal chooses a moment when the vulnerability of the potential victim is higher, i.e., during the moulting process (Elgar and Crespi, 1992; Marshall et al., 2005).

Numerous studies have demonstrated that the presence of shelter significantly reduces cannibalism and increases the survival of juveniles (Mann et al., 2007; Moksnes et al., 1997). This occurs because shelters reduce the search and capture efficiencies of visual predators and therefore reduce encounter rates, subsequently leading to lower predation rates (Marshall et al., 2005; Moksnes et al., 1997). Consistently, in this study, a significantly higher survival rate was observed with the presence of shelter (G5), and an even better growth rate was observed. We thought that this higher growth rate was due to the consumption of the algae added as shelter. Several authors have documented the nutritional benefit of supplemented plant material in the diets of crustaceans (Arnold et al., 2006; Jones, 1995; Primavera and Gacutan, 1989). This possibility was confirmed by the Experiment 4, in which the juvenile crabs reared with the natural shelter had higher survival and growth rates than the crabs reared with the artificial shelter. Therefore, although the use of natural shelter increases handling difficulties because of the harvesting and maintenance requirements, the shelter presents clear benefits.

There were, however, two inherent limitations in the design of our experiments that restrict their direct application to large-scale aquaculture. First, the duration of the experiments was deliberately limited to the first juvenile stages because the most important population reduction has been observed during this period. Therefore, the results cannot be extrapolated to later stages. Second, the juveniles were held in small containers individually or in pairs, which allowed the growth and survival rates to be tracked for juvenile crabs. However, commercial aquaculture is likely to involve holding animals together in relatively high densities in order to maximize spatial efficiency, resulting in high cannibalism rates (Daly et al., 2009; Naranjo-Páramo et al., 2004). Nevertheless, the results presented in this study prove that, in the tested conditions, holding pairs of spider crabs in shared cells increases the overall survival rate compared to that of spider crabs in individual cells or spider crabs under mass production conditions (Pastor et al., 2011). Therefore, although more space is needed to hold crabs in pairs, the productivity is maximized during a period when they are especially vulnerable.

In conclusion, under the rearing conditions examined here, the production of early *M. squinado* juveniles is maximized when they are fed with commercial shrimp feed pellets and reared in pairs in small cylinders, with natural algae provided as shelter. However, further investigations should be carried out testing other densities, foods or shelters, in order to find the optimal rearing conditions that maximize the production. Thus, obtaining a large number of specimens, either for commercial purposes or for the recovery of the endangered populations in the wild, might be feasible in the near future.

Acknowledgements

Financial support was provided by JACUMAR, Secretaria General de Pesca Maritima, Spanish Government (“Cultivo de la centolla” and “Propuestas y mejoras para el diseño de los planes de repoblación y evaluación de los impactos de los escapes” projects), and by the Government of the Balearic Islands. We are especially grateful to the staff, scholars and trainers of LIMIA for their help and support during the development of this study.

References

- Alaminos, J., Domingues, P., 2008. Effects of different natural or prepared diets on growth and survival of juvenile spider crabs, *Maja brachydactyla* (Balss, 1922). Aquaculture International. 16, 417-425.
- Anger, K., 1995. Developmental biology of *Armases miersii* (Grapsidae), a crab breeding in supratidal rock pools. II. Food limitation in the nursery habitat and larval cannibalism. Marine Ecology Progress Series. 117, 83-89.
- Arnold, S.J., Sellars, M.J., Crocos, P.J., Coman, G.J., 2006. Intensive production of juvenile tiger shrimp *Penaeus monodon*: An evaluation of stocking density and artificial substrates. Aquaculture. 261, 890-896.
- Borisov, R.R., Epelbaum, A.B., Kryakhova, N.V., Tertitskaya, A.G., Kovatcheva, N.P., 2007. Cannibalistic behavior in red king crabs reared under artificial conditions. Russian Journal of Marine Biology. 33, 227-231.
- Born, A.F., Immink, A.J., Bartley, D.M., 2004. Marine and coastal stocking: global status and information needs. In: Bartley, D.M., Leber, K.M. (Eds.), Marine Ranching. FAO Fisheries Technical Paper, pp. 1-12.
- Castejón, D., Rotllant, G., Giménez, L., Torres, G., Guerao, G., 2015. The effects of temperature and salinity on the survival, growth and duration of the larval development of the common spider crab *Maja brachydactyla* (Balss, 1922) (Brachyura: Majidae). Journal of Shellfish Research. 34, 1073-1083.
- Cowey, C.B., Sargent, J.R., 1972. Fish nutrition. Advances in Marine Biology. 10, 383-494.
- Crawley, M.J., 2007. The R Book. John Wiley & Sons Ltd, USA.

436 Crear, B.J., Thomas, C.W., Hart, P.R., Carter, C.G., 2000. Growth of juvenile southern
 437 rock lobsters, *Jasus edwardsii*, is influenced by diet and temperature, whilst
 438 survival is influenced by diet and tank environment. *Aquaculture*. 190, 169-182.
 439 Daly, B., Swingle, J.S., Eckert, G.L., 2009. Effects of diet, stocking density, and
 440 substrate on survival and growth of hatchery-cultured red king crab
 441 (*Paralithodes camtschaticus*) juveniles in Alaska, USA. *Aquaculture*. 293, 68-
 442 73.
 443 Domingues, P., Alaminos, J., 2008. Efectos de la densidad de cultivo y de elementos de
 444 refugio en el crecimiento y supervivencia de juveniles de centollo, *Maja*
 445 *brachydactyla* (Balss, 1922). *Revista de biologia marina y oceanografia*. 43,
 446 121-127.
 447 Domingues, P., Alaminos, J., García-Garrido, S., Hachero-Cruzado, I., Rosas, C., 2012.
 448 Growth and survival of juvenile spider crabs, *Maja brachydactyla* (Balss, 1922),
 449 fed with fresh or frozen mussels. *Aquaculture Research*. 43, 167-177.
 450 Dubber, G.G., Branch, G.M., Atkinson, L.J., 2004. The effects of temperature and diet
 451 on the survival, growth and food uptake of aquarium-held postpueruli of the
 452 rock lobster *Jasus lalandii*. *Aquaculture*. 240, 249-266.
 453 Durán, J., Pastor, E., Grau, A., Valencia, J.M., 2012. First results of embryonic
 454 development, spawning and larval rearing of the Mediterranean spider crab
 455 *Maja squinado* (Herbst) under laboratory conditions, a candidate species for a
 456 restocking program. *Aquaculture Research*. 43, 1777-1786.
 457 Durán, J., Palmer, M., Pastor, E., 2013. Growing reared spider crabs (*Maja squinado*) to
 458 sexual maturity: The first empirical data and a predictive growth model.
 459 *Aquaculture*. 408–409, 78-87.

460 Elgar, M.A., Crespi, B.J., 1992. Cannibalism: Ecology and Evolution among Diverse
461 Taxa. Oxford University Press, USA.

462 Fernández, M., 1999. Cannibalism in Dungeness crab *Cancer magister*: effects of
463 predator-prey size ratio, density, and habitat type. Marine Ecology Progress
464 Series. 182, 221-230.

465 Garcia, L., 2007. Els Crancs de les Balears. Edicions Documenta Balear S.L., Spain.

466 Guerao, G., Pastor, E., Martin, J., Andrés, M., Estévez, A., Grau, A., Durán, J., Rotllant,
467 G., 2008. The larval development of *Maja squinado* and *M. brachydactyla*
468 (Decapoda, Brachyura, Majidae) described from plankton collected and
469 laboratory-reared material. Journal of Natural History. 42, 2257-2276.

470 Guerao, G., Rotllant, G., 2009. Survival and growth of post-settlement juveniles of the
471 spider crab *Maja brachydactyla* (Brachyura: Majoidea) reared under individual
472 culture system. Aquaculture. 289, 181-184.

473 Guerao, G., Rotllant, G., 2010. Development and growth of the early juveniles of the
474 spider crab *Maja squinado* (Brachyura: Majoidea) in an individual culture
475 system. Aquaculture. 307, 105-110.

476 Guerao, G., Simeó, C.G., Anger, K., Urzúa, Á., Rotllant, G., 2012. Nutritional
477 vulnerability of early zoea larvae of the crab *Maja brachydactyla* (Brachyura,
478 Majidae). Aquatic Biology. 16, 253-264.

479 Jones, C.M., 1995. Production of juvenile redclaw crayfish, *Cherax quadricarinatus*
480 (von Martens) (Decapoda, Parastacidae) II. Juvenile nutrition and habitat.
481 Aquaculture. 138, 239-245.

482 Kennish, R., 1996. Diet composition influences the fitness of the herbivorous crab
483 *Grapsus albolineatus*. Oecologia. 105, 22-29.

484 Mann, D.L., Asakawa, T., Kelly, B., Lindsay, T., Paterson, B., 2007. Stocking density
 485 and artificial habitat influence stock structure and yield from intensive nursery
 486 systems for mud crabs *Scylla serrata* (Forsskål 1775). *Aquaculture Research*.
 487 38, 1580-1587.

488 Marshall, S., Warburton, K., Paterson, B., Mann, D., 2005. Cannibalism in juvenile
 489 blue-swimmer crabs *Portunus pelagicus* (Linnaeus, 1766): effects of body size,
 490 moult stage and refuge availability. *Applied Animal Behaviour Science*. 90, 65-
 491 82.

492 Moksnes, P.O., Lipcius, R.N., Pihl, L., van Montfrans, J., 1997. Cannibal-prey
 493 dynamics in young juveniles and postlarvae of the blue crab. *Journal of*
 494 *Experimental Marine Biology and Ecology*. 215, 157-187.

495 Naranjo-Páramo, J., Hernandez-Llamas, A., Villarreal, H., 2004. Effect of stocking
 496 density on growth, survival and yield of juvenile redclaw crayfish *Cherax*
 497 *quadricarinatus* (Decapoda: Parastacidae) in gravel-lined commercial nursery
 498 ponds. *Aquaculture*. 242, 197-206.

499 Neumann, V., 1998. A review of the *Maja squinado* (Crustacea: Decapoda: Brachyura)
 500 species-complex with a key to the eastern Atlantic and Mediterranean species of
 501 the genus. *Journal of Natural History*. 32, 1667-1684.

502 OIE, 2016. *Manual of Diagnostic Tests for Aquatic Animals* (2016).

503 Pastor, E., Durán, J., Caimari, M.A., 2011. Experiences in mass production of
 504 Mediterranean spider crab *Maja squinado* juveniles for restocking studies,
 505 *Proceedings of the Aquaculture Europe Congress*. European Aquaculture
 506 Society, Greece, pp. 843–844.

507 Penha-Lopes, G., Rhyne, A.L., Lin, J., Narciso, L., 2006. Effects of temperature,
 508 stocking density and diet on the growth and survival of juvenile *Mithraculus*

509 *forceps* (A. Milne Edwards, 1875) (Decapoda: Brachyura: Majidae).

510 Aquaculture Research. 37, 398-408.

511 Primavera, J.H., Gacutan, R.Q., 1989. Preliminary results of feeding aquatic

512 macrophytes to *Penaeus monodon* juveniles. Aquaculture. 80, 189-193.

513 Rotllant, G., Simeó, C.G., Macià, G., Estévez, A., 2015. High environmental salinity

514 reduces the reproductive potential of the spider crab *Maja brachydactyla*

515 (Decapoda, Majidae). Marine Ecology. 36, 496-505.

516 Sainte-Marie, B., Lafrance, M., 2002. Growth and survival of recently settled snow crab

517 *Chionoecetes opilio* in relation to intra- and intercohort competition and

518 cannibalism: a laboratory study. Marine Ecology Progress Series. 244, 191-203.

519 Sheen, S.-S., 2000. Dietary cholesterol requirement of juvenile mud crab *Scylla serrata*.

520 Aquaculture. 189, 277-285.

521 Simeó, C.G., Andrés, M., Estévez, A., Rotllant, G., 2015. The effect of male absence on

522 the larval production of the spider crab *Maja brachydactyla* Balss, 1922.

523 Aquaculture Research. 46, 937-944.

524 Swiney, K.M., Long, W.C., Persselin, S.L., 2013. The effects of holding space on

525 juvenile red king crab, *Paralithodes camtschaticus* (Tilesius, 1815), growth and

526 survival. Aquaculture Research. 44, 1007-1016.

527 Torres, A.P., Dos Santos, A., Alemany, F., Massutí, E., 2013. Larval stages of

528 crustacean species of interest for conservation and fishing exploitation in the

529 western Mediterranean. Scientia Marina. 77, 149-160.

530 Triño, A.T., Millamena, O.M., Keenan, C., 1999. Commercial evaluation of monosex

531 pond culture of the mud crab *Scylla* species at three stocking densities in the

532 Philippines. Aquaculture. 174, 109-118.

533 UNEP, 1996. Acta final de la reunión de plenipotenciarios sobre los anexos del
 534 protocolo referente a las zonas especialmente protegidas y la biodiversidad
 535 biológica en el Mediterráneo, UNEP(OCA)/MED IG 10/4, Spain.
 536 Ut, V.N., Le Vay, L., Nghia, T.T., Hong Hanh, T.T., 2007. Development of nursery
 537 culture techniques for the mud crab *Scylla paramamosain* (Estampador).
 538 Aquaculture Research. 38, 1563-1568.
 539 Wilber, D.H., Payson Wilber, T., 1991. Environmental influences on the growth and
 540 survival of West Indian spider crabs *Mithrax spinosissimus* (Lamarck) in
 541 culture. Journal of Experimental Marine Biology and Ecology. 146, 27-38.
 542 Zmora, O., Findiesen, A., Stubblefield, J., Frenkel, V., Zohar, Y., 2005. Large-scale
 543 juvenile production of the blue crab *Callinectes sapidus*. Aquaculture. 244, 129-
 544 139.
 545
 546
 547

Tables

Table 1

Factors tested in the different experiments and rearing conditions of each group. The fresh food was fresh mussel meat, the dry diet was pellets formulated for shrimp and the mixed food was a mixture of both.

	Factor	Group	Individually or in pairs	Food	Shelter	Type of shelter
Exp. 1	DENSITY	G1	1 ind./cylinder	Fresh	No	-
		G2	2 ind./cylinder	Fresh	No	-
Exp. 2	FOOD	G3	1 ind./cylinder	Dry	No	-
		G4	1 ind./cylinder	Fresh	No	-
Exp. 3	PRESENCE/ABSENCE SHELTER	G2	2 ind./cylinder	Fresh	No	-
		G5	2 ind./cylinder	Fresh	Yes	Natural
Exp.4	TYPE OF SHELTER	G6	2 ind./cylinder	Mixed	Yes	Artificial
		G7	2 ind./cylinder	Mixed	Yes	Natural

Table 2

Growth comparison between group G1 (one individual per cylinder) and group G2 (two individuals per cylinder). The mean and SE values of carapace lengths (CL) are in mm, and those of intermoult periods (IP) are in days. Data were analysed with ANOVA if the ANOVA assumptions were met and with the Kruskal-Wallis test (K-W) if the assumptions were not met. Significant differences are denoted in bold.

Analysis	Instar	Group	Mean	SE	Statistic	F or H	P-value
CL	Initial (C2)	G1	3.195	0.053	K-W	2.657	0.103
		G2	3.249	0.052			
	C3	G1	4.228	0.070	K-W	1.628	0.202
		G2	4.329	0.064			
	C4	G1	5.350	0.090	ANOVA	6.067	0.016
		G2	5.616	0.064			

	Final (C5)	G1	6.493	0.149	K-W	1.489	0.222
		G2	6.670	0.098			
IP	C3-C4	G1	19.867	2.426	K-W	6.259	0.012
		G2	13.840	0.741			
	C4-C5	G1	19.000	2.028	ANOVA	1.109	0.302
		G2	21.450	1.268			

Table 3

Growth comparison of *M. squinado* juveniles fed a dry diet (G3) and juveniles fed a fresh diet (G4). The mean and SE values of carapace lengths (CL) are in mm, and those of intermoult periods (IP) are in days. Data were analysed with ANOVA if the ANOVA assumptions were met and with the Kruskal-Wallis test (K-W) if the assumptions were not met. Significant differences are denoted in bold.

Analysis	Instar	Group	Mean	SE	Statistic	F or H	P-value
CL	C2(Initial)	G3	3.327	0.079	ANOVA	0.025	0.874
		G4	3.308	0.092			
	C3	G3	4.365	0.108	ANOVA	4.694	0.034
		G4	4.689	0.105			
	C4	G3	5.547	0.150	ANOVA	2.407	0.126
		G4	5.827	0.107			
	C5 (Final)	G3	6.809	0.167	K-W	0.015	0.900
		G4	6.801	0.135			
IP	C3-C4	G3	17.316	0.879	ANOVA	8.21	0.007
		G4	14.227	0.654			
	C4-C5	G3	19.565	1.251	ANOVA	0.886	0.352
		G4	21.471	1.638			

Table 4

Comparison of the carapace length (CL, in mm) and intermoult period (IP, in days) measurements of *M. squinado* juveniles reared in cylinders with the presence of shelter (G5) and juveniles reared in cylinders without shelter (G2). Data were analysed with

ANOVA if the ANOVA assumptions were met and with the Kruskal-Wallis test (K-W)
if the assumptions were not met. Significant differences are denoted in bold.

Analysis	Instar	Group	Mean	SE	Statistic	F or H	P-value
CL	C2 (Initial)	G5	3.366	0.046	K-W	3.670	0.055
		G2	3.249	0.052			
	C3	G5	4.336	0.053	ANOVA	0.007	0.933
		G2	4.329	0.064			
	C4	G5	5.520	0.082	K-W	0.157	0.692
		G2	5.616	0.064			
	C5 (Final)	G5	7.493	0.175	K-W	16.389	0.000
		G2	6.670	0.098			
IP	C3-C4	G5	12.881	0.508	ANOVA	1.027	0.315
		G2	13.840	0.741			
	C4-C5	G5	19.420	0.848	ANOVA	2.224	0.140
		G2	21.450	1.268			

578

579

580 Table 5

581 Comparison of the carapace length (CL, in mm) and intermoult period (IP, in days)
582 measurements of *M. squinado* juveniles reared in cylinders with artificial shelter (G6)
583 and juveniles reared in cylinders with natural shelter (G7). Data were analysed with
584 ANOVA if the ANOVA assumptions were met and with the Kruskal-Wallis test (K-W)
585 if the assumptions were not met. Significant differences are denoted in bold.

Analysis	Instar	Group	Mean	SE	Statistic	F or H	P-value
CL	C2 (Initial)	G6	3.164	0.056	ANOVA	0.815	0.368
		G7	3.095	0.052			
	C3	G6	4.025	0.063	K-W	9.661	0.002
		G7	4.259	0.055			
	C4	G6	5.255	0.082	K-W	7.147	0.007
		G7	5.564	0.074			
	C5 (Final)	G6	6.484	0.106	K-W	14.13	0.000
		G7	7.009	0.092			
IP	C3-C4	G6	15.475	0.570	K-W	0.174	0.676
		G7	15.895	0.654			
	C4-C5	G6	17.522	0.788	ANOVA	1.284	0.259

	G7	18.759	0.687
586			
587			
588			
589			
590			

Figure captions

Fig. 1. View of the tank of the experiments with the different groups of cylinders.

Fig. 2. Boxplot of the CL measurements (in mm) at the end of the experiments for the groups defined in the Table 1. A line within the box marks the median value, the boxes span the 25 and 75% percentiles and the bars represent the minimum and maximum non-outlier values.

Fig. 3. Estimated survival rate, expressed as a decimal and obtained from the survival analysis method, for the group with one individual per cylinder (G1) and for the group with two individuals per cylinder (G2), with the survival rate of the individuals of the same cylinder analysed separately (G2 1st and G2 2nd).

Fig. 4. Estimated survival rate, expressed as a decimal and obtained from the survival analysis method, for the group of crabs fed a pellets diet formulated for shrimp (G3) and for the group fed a fresh diet composed of mussel meat (G4).

Fig. 5. Estimated survival rate, expressed as a decimal and obtained from the survival analysis method, for the group with two individuals per cylinder and presence of shelter (G5) and for the group with two individuals per cylinder without shelter (G2).

Fig. 6. Estimated survival rate, expressed as a decimal and obtained from the survival analysis method, for the group with the artificial shelter (G6) and for the group with the natural shelter (G7).

616

617

619

620

621