Estudi de la biologia reproductiva de la cabra de mar, *Maja brachydactyla*: aparell reproductor i qualitat de les postes en captivitat

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Apèndix

1. Llistat de publicacions inclosses a la tesi

1. **Simeó**, C.G., Kurtz, K., Chiva, M., Ribes, E., Rotllant, G. (2009) L'espermatogènesi en els crancs (Crustacea, Brachyura): un model atípic de condensació del nucli espermàtic. Treballs de la Societat Catalana de Biologia 59, 71-93.

2. **Simeó**, C.G., Ribes, E., Rotllant, G. (2009) Internal anatomy and ultrastructure of the male reproductive system of the spider crab *Maja brachydactyla* (Decapoda: Brachyura). Tissue & Cell 41, 345-361.

3. **Simeó**, C.G., Kurtz, K., Chiva, M., Ribes, E., Rotllant, G. (2010) Spermatogenesis of the spider crab *Maja brachydactyla* (Decapoda: Brachyura). Journal of Morphology 271, 394-406.

4. **Simeó**, C.G., Kurtz, K., Rotllant, G., Chiva, M., Ribes, E. (2010) Sperm ultrastructure of the spider crab *Maja brachydactyla* (Decapoda: Brachyura). Journal of Morphology 271, 407-417.

5. **Simeó**, C.G., Andree, K.B., Rotllant, G. (2011) Identification of *vasa*, a potential marker of primordial germ cells in the spider crab *Maja brachydactyla*, and its expression during early post-embryonic development. Invertebrate Reproduction & Development 55, 91-99.

6. **Simeó** C.G., Andrés, M., Estévez, A., Rotllant, G. (En revisió) The effect of male absence on the larval production of the spider crab *Maja brachydactyla*. Aquaculture Research (En revisió).

7. **Simeó** C.G., Estévez, A., Rotllant, G. (En revisió) Effect of photoperiod on larval production of the spider crab *Maja brachydactyla*. Aquaculture (En revisió).

2. Altres publicacions

1. Andrés, M., Estévez, A., **Simeó**, C.G., Rotllant, G. (2010) Annual variation in the biochemical composition of newly hatched larvae of *Maja brachydactyla* in captivity. Aquaculture 310, 99-105.

2. Guerao, G., Andree, K.B., Froglia, C., **Simeó**, C.G., Rotllant, G. (2011) Identification of European species of *Maja* (Decapoda: Brachyura: Majidae): RFLP analyses of COI mtDNA and morphological considerations. Scientia Marina 75, 129-134.

3. Guerao, G., **Simeó**, C.G., Anger, K., Urzúa, A. Rotllant, G. Nutritional vulnerability of early zoea larvae of the crab *Maja brachydactyla* (Brachyura, Majidae). Aquatic Biology (En revisió).

4. Rotllant, G., **Simeó**, C.G., Guerao, G.,Sastre, M., Cleary, D. R., Calado, R., Estévez, A. Inter-annual variability in larval production from wild ovigerous *Maja brachydactyla* (Decapoda, Majidae) reveals the need to domesticate broodstock for aquaculture. Aquaculture (En revisió).

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Annual variation in the biochemical composition of newly hatched larvae of *Maja brachydactyla* in captivity

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ABSTRACT

Quality of newly hatched larvae (NHL) of *Maja brachydactyla* in captivity has been characterized throughout the year to evaluate their availability for mass production. Spawning took place every month and NHL were collected and analyzed to estimate individual dry weight (DW) and proximate biochemical composition (protein, carbohydrate and lipids). Lipid class, fatty acid composition, amino acid profile, mineral and vitamins A, E and C contents were analyzed seasonally. NHL obtained throughout the year are a potential source for aquaculture purposes, since the increment in the relative protein and lipid (especially phospholipids and n-3 PUFA) content might compensate the decrease in DW of larvae hatched from broodstock kept during one year in captivity. However, the decrease in vitamins A and E as well as in certain essential amino acids (Lys, Val, and His) and trace elements (Cu and Fe) of NHL at the end of the year might be indicative of a nutritional deficiency in broodstock diets.

1. Introduction

Larval production is a major concern for aquaculture purposes. Not only the quantity but also the quality of the larvae obtained under captivity is of paramount importance to ensure a profitable commercial harvest of juveniles and/or adults. Larval physiological condition and performance during decapod culture has been commonly referred as larval quality, which has been studied under five general criteria: biochemical composition, morphology, behavior, production yields and survival to stress (reviewed by Racotta et al., 2003). Spider crab eggs are lecithotrophic and thus their development depends on the reserves transferred from the female. As a consequence, the initial level of these reserves in newly hatched larvae can determine their quality, and can be considered as predictive quality criteria. Recent studies in crustacean culture have been directed to improve larval quality and to establish its relationship with broodstock condition, especially in shrimps (Racotta et al., 2003). Broodstock condition or maternal effects (including nutrition, environmental conditions and reproductive exhaustion) have been proven to affect egg and larval quantity and quality in decapod crustaceans, both in the wild (Anger, 2006) and in captivity (Palacios et al., 1999; Wickins et al., 1995; Xu et al., 1994).

The spider crab *Maja brachydactyla* has a high economical value, supporting commercial captures in different countries through the NE Atlantic coasts (Spain, Portugal, France, Ireland and UK). The high fishing pressure hold up by this crab (Freire et al., 2002), together with its adequate growth and reproductive characteristics (Alaminos and Domingues, 2008; Figueiredo and Narciso, 2008; González-Gurriarán et al., 1995; Guerao and Rotllant, 2009; Iglesias et al., 2002; Palma et al., 2008; Rotllant et al., 2007; Simeó et al., 2009) have contributed to define the species as potential for aquaculture. Seminal receptacles in the female play a key role in the reproductive behavior of spider crab as a storage place of sperm from one or more copulations by several males, allowing the subsequent fertilization of consecutive oocyte batches without carrying any new copulation (reviewed by González-Gurriarán et al., 1998).

Larval culture of *M. brachydactyla* has been optimized in recent years through the study of several aspects of its rearing, including its zootechny (Andrés et al., 2007) and the study of the biochemical changes occurring during ontogeny (Andrés et al., 2008, 2010a,b). However, obtaining good quality newly hatched larvae all the year round is still of paramount importance in order to establish the basis for mass production. The aim of this work was to study the variation in the composition of *M. brachydactyla* larvae obtained along a whole year under intensive culture in order to evaluate the effects of broodstock captivity.

2. Materials and methods

2.1. Broodstock capture and maintenance

Adult *M. brachydactyla* were captured with commercial fishery boats off the coast of Galicia, northwestern Spain (November 2004), and transported to IRTA (Sant Carles de la Ràpita, Tarragona, Spain) in

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Identification of European species of *Maja* (Decapoda: Brachyura: Majidae): RFLP analyses of COI mtDNA and morphological considerations

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SUMMARY: Four species of crabs of the genus *Maja* have been described along the European coast: *M. brachydactyla*, *M. squinado*, *M. goltziana* and *M. crispata*. The commercially important species *M. brachydactyla* and *M. squinado* achieve the largest body sizes and are the most similar in morphology, and are therefore easily confused. The four species of *Maja* were identified using a novel morphometric index and a polymerase chain reaction followed by restriction fragment length polymorphism analysis (RFLP). The relationship between carapace length and the distance between the tips of antorbital spines was used to distinguish adults of *M. brachydactyla* and *M. squinado*. PCR-RFLP analysis of a partial sequence of the mitochondrial cytochrome oxidase type I (COI) revealed that the four species of the genus *Maja* can be unambiguously discriminated using the combination of restriction endonucleases enzymes *Hpy*CH4V and *Ase* I. The molecular identification may be particularly useful in larvae, juvenile and young crabs, when the morphological differences found in adults are not applicable.

Keywords: *Maja*, *M. squinado*, *M. brachydactyla*, molecular identification, morfometry, COI, RFLP.

RESUMEN: Identificación de las especies europeas del género *Maja* (Decapoda: Brachyura: Majidae): análisis de PCR-RFLP de una región del mtADN COI y consideraciones morfológicas. – Cuatro especies del género *Maja* han sido descritas en las costas europeas: *M. brachydactyla*, *M. squinado*, *M. goltziana* y *M. crispata*. Las especies *M. brachydactyla* y *M. squinado*, que tienen importancia comercial, alcanzan los tamaños más grandes y son morfológicamente muy similares, siendo muy fácil confundirlas. La identificación de las cuatro especies se ha realizado utilizando un nuevo índice morfométrico y un análisis de polimorfismos de fragmentos de restricción (RFLP). La relación entre la longitud del cefalotórax y la distancia entre los extremos distales de las espinas antorbitales se ha utilizado para la diferenciación de los adultos de *M. brachydactyla* y *M. squinado*. El análisis PCR-RFLP de una secuencia parcial de la citocromo oxidasa tipo I mitocondrial (COI) indica que las cuatro especies del género *Maja* pueden ser discriminadas usando una combinación de las endonucleasas *Hpy*CH4V y *Ase* I. La identificación molecular puede ser particularmente útil en las larvas, juveniles y cangrejos jóvenes, cuando las diferencias morfológicas encontradas en los adultos no son aplicables.

Palabras clave: *Maja*, *M. squinado*, *M. brachydactyla*, identificación molecular, morfometría, COI, RFLP.

INTRODUCTION

Four species of spider crabs of the genus *Maja* Lamarck, 1801 (Majoidea, Majidae) have been reported along the European coast: *M. brachydactyla* Balss, 1922; *M. crispata* Risso, 1827; *M. goltziana* D'Oliveira, 1888; and *M. squinado* (Herbst, 1788) (Neumann, 1998; Sotelo *et al.,* 2008, 2009). *Maja brachydactyla* and *M. squinado* are of high commercial value due to their larger size (Ševčić, 1974; Le Duff, 1990). While *M. goltziana* adults are easily identified by the presence of a strong dorso-distal

Títol: Nutritional vulnerability of early zoea larvae of the crab *Maja brachydactyla* (Brachyura, Majidae)

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Referència: acceptat a Aquatic Biology

Resum

The nutritional vulnerability of the zoea I of *Maja brachydactyla* was studied in experimental treatments with differential periods of starvation. As response variables, moulting and survival rates, the point-of-reserve-saturation (PRS), the point-of–no-return (PNR), dry mass, elemental composition (CHN), hepatopancreas lipid vacuoles, and activities of digestive enzymes were measured. Average PNR_{50} and PRS_{50} values of 2.8 and 1.9 days, respectively. In PNR treatments, complete mortality occurred only after extended initial starvation periods of ≥5 days and, in PRS treatments, the zoea I was capable to complete the moulting cycle after a short initial feeding period of only 1 day. In continuously starved control larvae, the moulting cycle was arrested at the onset of apolysis. Concomitantly, considerable amounts of biomass were lost and the C:N ratio decreased, indicating lipid degradation during starvation. This effect was also microscopically visible as a decline of lipid vacuoles in the hepatopancreas. Feeding after previous starvation periods of <7 days resulted in a reestablishment of the lipid vacuoles, indicating successful capture and ingestion of food, but this did not necessarily allow for completing the moulting cycle. In fed zoea-I larvae, digestive enzyme activities increased during the moulting cycle, while a significant decrease occurred under starvation conditions. In newly moulted zoea II, biomass values as well as enzymes activities increased with the duration of initial feeding periods in the zoea I. This study shows that biomass, elemental composition, the occurrence of lipid vacuoles in the hepatopancreas, and activities of digestive enzymes are suitable indicators of the nutritional condition of early zoeal stages of *M. brachydactyla*.

Títol: Inter-annual variability in larval production from wild ovigerous *Maja brachydactyla* (Decapoda, Majidae) reveals the need to domesticate broodstock for aquaculture

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- Daniel R. Cleary i Ricardo Calado; Departamento de Biologia & CESAM, Universidade de Aveiro

Referència: enviat a Aquaculture

Resum

The spider crab *Maja brachydactyla* is considered one of the most promising species of crustaceans for aquaculture in Europe. As with most cultured species, the availability of high quality larvae is of paramount importance for its successful production. However, the culture of *M. brachydactyla* shares a bottleneck that affects the culture of other crab species across the globe: larval supply relies almost exclusively on the collection of ovigerous wild broodstock. Because interannual shifts in larval quality are known to affect the survival and growth performance of cultured specimens, a field survey was performed to assess interannual (2005-2010) variation in the quantity and biochemical composition of newly hatched larvae (NHL) of *M. brachydactyla*. With the exception of NHL sampled in 2005, larval biochemical profiles differed significantly among years and there were pronounced differences in the intra-annual variation with the year 2010 exhibiting particularly variations compared to other years. Differences recorded among batches of NHL were mainly explained by the contribution of triacylglycerols (TAG) and, to a lesser degree, by larval protein and lipid content. While intraspecific and interannual variability in brachyuran crab larval production has already been reported, the present work is the longest consecutive interannual survey performed on the biochemical composition of NHL. Larval production in *M. brachydactyla* was quantitatively and qualitatively (evaluated through elemental and biochemical composition) variable and rather unpredictable, even though surveyed broodstock was obtained from the same local population. Available data on the biochemical composition of gonads, embryos and

NHL of *M. brachydactyla* can now provide researchers with part of the basic knowledge needed to develop adequate broodstock diets and thus contribute towards an aquaculture that does not depend on wild broodstock to provide high quality NHL.

3. Comunicacions a congressos

3. 1 Llistat de les comunicacions a congressos

1. **Simeó**, C.G., Andrés, M., Estévez, A., Rotllant, G. Effect of photoperiod and sex-ratio on larval hatching of spider crab *Maja brachydactyla*. Pòster. Australasian Aquaculture. Adelaida (Australia), 27-30 d'agost, 2006.

2. **Simeó**, C.G., Rotllant, G., Ribes, E. Morfología del aparato reproductor masculino de la centolla, *Maja brachydactyla* (Crustacea: Decapoda). Comunicació oral. XIV Simposio Ibérico de Estudios de Biología Marina. Barcelona (España), 12-15 de setembre, 2006.

3. **Simeó**, C.G., Rotllant, G., Ribes, E. Formació de l'espermatòfor a la cabra de mar *Maja brachydactyla* (Decapoda, Brachyura). Comunicació oral. X Jornades de la Biologia de la Reproducció. Barcelona (España), 25 d'abril, 2007.

4. **Simeó**, C.G., Andree, K., Rotllant, G**.** Identification and investigation of a *vasa*-like gene expression in the spider crab, *Maja brachydactyla*. Comunicació oral. International Conference of Invertebrate Reproduction and Development. Panama City (Panamá), 6-9 d'agost, 2007.

5. **Simeó**, C.G., Rotllant, G., Ribes, E. The vas deferens of the spider crab *Maja brachydactyla* (Crustacea: Decapoda): morphology and function. Comunicació oral. International Conference of Invertebrate Reproduction and Development. Panama City (Panamá), 6-9 d'agost, 2007.

6. **Simeó**, C.G., Andrés, M., Estévez, A., Rotllant, G. Efecto de la proporción de sexos, el fotoperíodo y la salinidad en la calidad de la puesta de la centolla, *Maja brachydactyla*. Pòster. XI Congreso Nacional de Acuicultura. Vigo (España), 24- 28 de setembre, 2007.

7. Andrés, M., Estévez, A., **Simeó**, C.G., Rotllant, G. Annual variations in quantity and quality of newly hatched larvae of *Maja brachydactyla* in captivity. Comunicació oral. IX Colloquium Crustacea Decapoda Mediterranea. Torino (Italia), 2- 6 de setembre, 2008.

8. **Simeó**, C. G., Kurtz, K., Chiva, M., Ribes, E., Rotllant, G. Spermatogenesis of the spider crab,*Maja brachydactyla*. Comunicació oral. IX Colloquium Crustacea Decapoda Mediterranea. Torino (Italia), 2- 6 de setembre, 2008.

9. **Simeó**, C. G., Andree, K.B., Rotllant, G. Aïllament, seqüenciació i expressió del gen homòleg al vasa a la cabra de mar, *Maja brachydactyla*. Pòster. II Simposi d'Aqüicultura de Catalunya. Sant Carles de la Ràpita (España), 15- 16 d'octubre, 2009

10. **Simeó,** C.G., Estévez, A., Rotllant, G. Efecto del desplazamiento de fotoperiodo en la inducción y calidad de la puesta de la centolla, *Maja brachydactyla*. Pòster. XII Congreso Nacional de Acuicultura. Madrid (España), 24- 26 de novembre, 2009.

11. Guerao, G., Andree, K.B., Froglia, C., **Simeó**, C.G., Rotllant, G. Identificación morfológica y molecular del Centollo Mediterráneo *Maja squinado* (Decapoda, Brachyura, Majidae). Pòster. XII Congreso Nacional de Acuicultura. Madrid (España), 24- 26 de novembre, 2009.

12. Rotllant, G., **Simeó**, C.G., Guerao, G., Sastre, M., Estévez, A. Inter-annual variations in quantity and quality of newly hatched larvae of *Maja brachydactyla*. Pòster. Asian Pacific Aquaculture Conference. Kochi (India), 17- 20 de gener, 2011.

13. Guerao, G., **Simeó**, C.G., Urzúa, A., Anger, K., Sastre, M., Rotllant, G. Estudio sobre la plasticidad nutricional en el primer estadio larvario de *Maja brachydactyla* (Brachyura, Majidae). Pòster. XIII Congreso Nacional de Acuicultura. Castelldefels (España), 21-24 de novembre, 2011.

14. Guerao, G., **Simeó**, C.G., Anger, K., Macià, G., Rotllant, G. Efecto del ayuno en el estado nutricional de las megalopas de *Maja brachydactyla* (Brachyura, Majidae). Pòster. XIII Congreso Nacional de Acuicultura. Castelldefels (España), 21-24 de novembre, 2011.

3. 2 Resums de les comunicacions a congressos

1. Effect of photoperiod and sex-ratio on larval hatching of spider crab *Maja brachydactyla*.

The spider crab *Maja brachydactyla* (Decapoda, Majidae) is the target of an intensive fishery in several areas of the NE Atlantic and some stocks show signs of overfishing. Hence, production of the spider crab in captivity might be a solution for the sector. In the wild females spawn 2 or 3 times along their annual reproductive and up to 6 times in captivity. The fisheries of a similar species of the same family, the snow crab *Chionocetes opilio* is mainly targeted on large males producing high quality sperm. Recent studies have shown that the reduction in the sperm quality of this species is the main cause of population decline. Two experiments were carried out to evaluate the quality of newly hatched larvae of *M. brachydactyla* when broodstock was exposed to different conditions throughout the year.

Six broodstock groups were kept at 18±1ºC and 35±1‰ salinity. In experiment 1, natural photoperiod was used and 2 replicated sex-ratios were tested: 3 female: 1 male and 3 female: 0 male. In experiment 2, sex-ratio was 3 female: 1 male, and 3 replicated fixed photoperiods were tested: long (18hL/6hD), medium (12hL/12hD) and short (6hL/18hD). Larval samples from every spawning of the broodstock groups were collected throughout the year. Fresh and dry weight, ash and organic matter content (protein, carbohydrates and lipids) were measured for each sample.

In the wild, spawning season of the spider crab is restricted to March-September. In our conditions spawning occurred every month and the sex-ratio did not have any influence in the larval production (Fig.1).

Significant differences in protein content and dry weight (N=117, P=<0.001) were observed between the newly hatched larvae collected in autumn and those collected in other months. On the other hand the use of fixed photoperiod seems to have a negative effect on spawning. Although eggs in different stages of fertilization and embryonic development were released no newly hatched larvae was obtained.

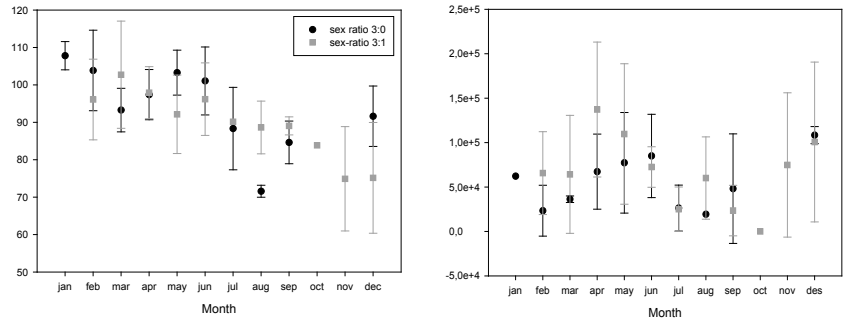

Fig 1. Variation in individual dry weight and number of newly hatched larvae along the year.

2. Morfología del aparato reproductor masculino de la centolla, *Maja brachydactyla* (Crustacea: Decapoda).

El aparato reproductor masculino de la centolla, *Maja brachydactyla*, se aloja en el cefalotórax y está formado por un par de testículos y conductos deferentes. Los testículos son un par de largos túbulos, situados en la mitad anterior del cuerpo. El extremo se encuentra en la inserción del primer pereiópodo y avanza anteriormente hasta la base de los pedúnculos oculares. Luego, ambos túbulos corren paralelos y se colocan entre los tendones mandibulares y de ahí se separan alrededor del estómago finalizando cerca del corazón, dando comienzo los conductos deferentes. Éstos se localizan en la mitad posterior del cuerpo y se dividen, atendiendo a criterios morfológicos y funcionales, en tres partes: anterior (CDA), medio (CDM) y posterior (CDP). El CDA es un corto conducto que se dispone por debajo del corazón. El CDM se caracteriza por una apariencia rugosa, y los conductos se distancian lateralmente. El CDP se encuentra en el extremo caudal del cefalotórax, presenta un aspecto de masa glandular, y se une a un conducto eyaculatorio, por el que se vierte el contenido seminal al gonoporo, situado en el quinto segmento torácico. El gonoporo conecta con la base de los gonopodios, los dos apéndices del primer segmento del abdomen modificados para la función copulatoria.

Los testículos están constituidos por un único túbulo seminífero, enrollado sobre sí mismo y envuelto por una fina capa de tejido conjuntivo. Un septo divide el interior del túbulo seminífero en dos zonas: en la de mayor tamaño se encuentran células gaméticas en diferentes fases de maduración y la de menor tamaño contiene espermatozoides maduros. Los conductos deferentes

están formados por un túbulo de mayor diámetro que el túbulo seminífero cuya pared está formada por un epitelio secretor, una lámina basal y una capa externa de tejido conjuntivo. El CDA tiene un aspecto de túbulo enrollado sobre sí mismo, con la superficie lisa en la zona próxima al testículo. En la parte posterior del CDA la pared tiene una serie de cortos conductos ciegos que surgen del conducto principal y cuyo número se incrementa en el CDM y CDP. El CDM deja de estar enrollado sobre sí mismo y presenta gran cantidad de tubúlos ciegos en su superficie. El CDP presenta una morfología tubular compleja propia de una glándula, se sitúa entre el CDM y el conducto eyaculatorio. El epitelio del CDA se encarga de la secreción de unas sustancias que provocan la aglutinación de los espermatozoides libres y la formación de la pared de los espermatóforos. En el CDM se finaliza la formación de la pared de los espermatóforos. El CDP segrega una sustancia que engloba a varios espermatóforos con la finalidad de ser transportados a la espermateca de la hembra. Por último, el conducto eyaculatorio es un túbulo liso, cuya pared está formada por un epitelio, una capa media de musculatura estriada y una capa externa de tejido conjuntivo. La luz del conducto presenta un contenido espermático que varia en función de la última cópula.

3. Formació de l'espermatòfor a la cabra de mar *Maja brachydactyla* (Decapoda, Brachyura).

La formació de l'espermatòfor de *Maja brachydactyla* i el paper del conducte deferent s'ha descrit mitjançant tècniques de microscòpia òptica i electrònica. L'epiteli del conducte deferent anterior produeix les secrecions que formen la paret de l'espermatòfor. El conducte deferent mitjà conté els espermatòfors envoltats de fluids seminals. El conducte deferent posterior continua la producció i magatzem de secrecions seminals. L'espermatòfor *M. brachydactyla* és el·lipsoïdal i està constituït per una fina paret acel·lular que envolta els espermatozous que es troben embeguts en una matriu. El procés de formació, estructura i funció de l'espermatòfor és equivalent al d'altres espècies de Braquiura prèviament descrites.

4. Identification and investigation of a *vasa*- like gene expression in the spider crab, *Maja brachydactyla*.

In sexually reproducing organisms germline cells are developed from a single population of undifferentiated cells which become specified as primordial germ cells (PGCs) during embryonic development due to the activities of vasa gene product homologs and other cellular determinants. The *vasa* gene en-

codes a RNA helicase which has been demonstrated to be involved in the PGCs development in early embryogenesis of many metazoans, both vertebrate and invertebrate, and are equally expressed in the adult gonads during gametogenesis. Therefore, the conserved nature of these *vasa*-like genes has been suggested as a molecular marker of germline cell development. Of the few histological and morphological studies on gonad ontogeny in decapod crustaceans that have been performed to date, and fewer still have investigated the role of *vasa* in gonad development. In this study we are examining the changes in expression of *vasa* during early development of *M. brachydactyla*. Total RNA was isolated from testis of adult *M. brachydactyla*, and reverse transcription was carried out to synthesize cDNA. Single fragments of approximately 900 bp and 500 bp were amplified by PCR using degenerate primers for *vasa* and *beta-actin*, respectively. A set of specific primers were designed based on the sequences obtained for each gene to analyze the expression of the *vasa*-like gene by RT-PCR, using *beta-actin* as a reference, in different adult tissues (hepatopancreas, heart, gill, ovary and testis). Specific expression of the *vasa*-like gene was positive in the adult ovary and testis. Preliminary results of *vasa* expression in larval development will be presented.

5. The vas deferens of the spider crab *Maja brachydactyla* (Crustacea: Decapoda): morphology and function.

The male reproductive system of *Maja brachydactyla* is composed of paired testes, vasa deferentia and ejaculatory ducts. In this study, the vas deferens has been described by light and electron microscopy, in order to understand its reproductive biology. Vas deferens of *M. brachydactyla* has been divided in three regions. The anterior (AVD) and median vas deferens (MVD) present a single layer of prismatic or cuboidal epithelial cells showing secretory activity. Cytoplasm is filled by rough endoplasmic reticulum and Golgi complexes produce vesicles of high electron dense material, which are secreted in the apical region of the plasma membrane, brushed with microvilli. AVD shows two secretions of different nature involved in the spermatophore formation; meanwhile MVD secretes substances in which spermatophores are stored. The posterior vas deferens (PVD) is lined by a pseudo-stratified prismatic epithelium surrounded by a thick muscular layer. Epithelial cells cytoplasm present few Golgi complexes and ramified microvilli are seen in the apical region of the plasma membrane, and no secretory activity has been reported. Large ramified diverticula are associated to the PVD and are lined by a secretory cuboidal epithelium. Few Golgi complexes and a quite developed endoplasmic reticulum are present in the cytoplasm. Lumen is filled by heterogeneous substance, which

will be poured out to the PVD at copulation, surrounding spermatophores. The ejaculatory duct has not been included as part of the vas deferens, although its histological similarity with PVD, due to the lack of secretory activity and its extrusion role of seminal fluids and spermatophores.

6. Efecto de la proporción de sexos, el fotoperíodo y la salinidad en la calidad de la puesta de la centolla, *Maja brachydactyla*.

El efecto de la proporción de sexos, fotoperíodo y salinidad sobre el número y la calidad de las puestas en cautividad de la centolla *Maja brachydactyla* se ha evaluado en tres experimentos de dos años de duración. El experimento I evaluó el efecto de la ausencia (3:0) o presencia (3:1) de machos. En el experimento II, los reproductores se sometieron a 4 fotoperiodos (16hL/8hO, 12hL/12hO, 8hL/16hO y natural). El experimento III valora el efecto de la salinidad (34 y 38‰). El efecto de estos factores sobre la calidad de la puesta se cuantificó mediante el recuento del número de puestas por hembra, número de larvas por puesta y el peso seco de las larvas. Los resultados muestran un efecto negativo de la ausencia de machos en el número de larvas por puesta y hembra en el segundo año. El número de puestas por hembra, así como el peso de las larvas disminuyó en los fotoperiodos fijos. La salinidad afectó al número de puestas por hembra, número de larvas por puesta y hembra y peso seco de las larvas.

7. Annual variations in quantity and quality of newly hatched larvae of *Maja brachydactyla* in captivity.

The spider crab, *Maja brachydactyla*, has been considered a potential species for aquaculture. Obtaining larvae all the year round is of paramount importance in terms of mass production. Thus, the quality of newly hatched *M. brachydactyla* larvae was studied for a year in order to evaluate the effects of broodstock captivity. Adults of *M. brachydactyla* were captured from the Atlantic NW coast of Spain and transported to IRTA where they were kept in 2000 L tanks at 36‰ salinity and a constant temperature of 18°C. The brooding females were fed on fresh mussels and frozen crab. Spawning took place every month and newly hatched larvae were collected, counted and analysed throughout the year. Individual dry weight (DW), ash (A) content and proximate biochemical composition (protein, PR; carbohydrates, CH; lipids, LP) were measured for each sample whereas lipid class composition, amino acid profile, and the content in vitamins A, E and C were analysed from

pooled samples of the larvae obtained during spring (SP), summer (SU) and autumn + early winter (AU+WI).

DW (μg ind^{-1}) and organic matter content (OM=ash free DW) of newly hatched larvae decreased significantly during the year (from 98.5±10.9 in February to 74.9±13.9 in November; ANOVA $p<0.001$). Relative content of PR and LP (%DW) increased significantly in the larvae at the end of the year (PR from 30.0±3.4 in February to 49.9±13.3 in November; ANOVA $p<0.001$), resulting in the same absolute (μg ind^{-1}) PR and LP content than the larvae hatched at the beginning. Carbohydrate content did not vary throughout the year, nor did the relative fractions LP/PR (0.2) and A/OM (0.3). No seasonal differences were found in the major lipid classes. Amino acid content (%DW) decreased significantly in AU+WI, especially in terms of the essential (Lys_{SP}=4.67±0.12, Lys_{AU+WI}=2.93±0.06; Val_{SP}=1.96±0.01, Val_{AU+WI}=1.52±0.06; Ile_{SP}=1.27±0.06, Ile_{AU+WI}=1.02±0.13; Hys_{SP}=1.05±0.07, Hys_{AU+WI}=0.73±0.03; $p<0.05$). A decrease in vitamins A and E was observed at AU+WI larvae whereas a significant increase in vitamin C was detected throughout the year (SP: 3.09±0.01 mg kg^{-1}, AU+WI: 4.38±0.06 mg kg^{-1}; ANOVA $p<0.001$).

A negative effect of captivity in the quality of the larvae, especially in terms of essential amino acids and vitamins was observed. Better feeding conditions and food quality should be considered in order to maintain good larval quality year round.

8. Spermatogenesis of the spider crab, *Maja brachydactyla*.

Crustacean spermatogenesis is very interesting since chromatin in spermatozoa is not condensed. Furthermore, brachyuran spermatozoa have been studied for systematical and phylogenetic purposes. However, few studies have dealt with differentiation of sperm cells. In this study, spermatogenesis, with special reference to spermiogenesis, of the spider crab *Maja brachydactyla* is described using electron microscopy. Spermatogenesis occurs in the transformation zone, which is the central region of the seminiferous tubule. Spermatogonia are located in the germinal zone, at one pole of the seminiferous tubule, whereas mature spermatozoa are founded in the evacuation zone, opposite the germinal zone. Earliest spermatocytes are spherical cells which have a central voluminous nucleus. The cytoplasm contains few mitochondria and irregular vesicles of light electron dense material. Later, nuclei present structures of different stages of the first meiotic division, such as synaptonemal complexes in pachytene phase. The cytoplasm contains scarce

small mitochondria and a prominent nucleolus-like body or *nuage*. An endomembrane system progressively develops, showing concentric flattened cisterns with lateral dilations in zygotene and flattened cisterns extending longitudinally in pachytene stages. Early spermatids have a central nucleus and loosely condensed chromatin. The cytoplasm contains few mitochondria and a developing endomembrane system composed of longitudinal flattened cisterns. Later, chromatin decondenses and the nucleus takes on a homogeneous appearance with a few clumps of lightly condensed chromatin. In the cytoplasm, the endomembrane complex increases giving an enlarged endoplasmic reticulum (ER) and Golgi complex (GC). Vesicles of the GC merge at one pole of the spermatid, developing the proacrosomal vesicle (PV). Middle spermatids contain both a semispherical nucleus and PV, the latter which includes an electron dense granule in the apical region. The ER and GC are smaller, filling up the cytoplasm located peripherally between the nucleus and the PV. In late spermatids, the nucleus surrounds the PV and the chromatin becomes more electron dense. The ER and GC are found at both sides of the nucleus as a membranous system surrounding degenerated mitochondria. In the PV, the acrosomal cap (or *operculum*) appears over the electron dense granule. The acrosomal tubule (or *perforatorium*) is developed basally from a projection of cytoplasm. Finally, the material of the PV is condensed. Mature spermatozoa possess a globular, complex acrosome and cup-shaped nucleus with 4-5 radial extensions containing slightly condensed chromatin. The membranous system is located at the base of radial extensions and contains degenerated mitochondria and microtubules.

9. Aïllament, seqüenciació i expressió del gen homòleg al vasa a la cabra de mar, *Maja brachydactyla*.

El gen *vasa* és un marcador de les cèl·lules de la línia germinal, donat que s'expressa específicament en aquestes cèl·lules en embrions, larves i adults dels metazous. La seva caracterització a la cabra de mar, *Maja brachydactyla*, facilitaria el control de la reproducció en cultius en captivitat. Els objectius d'aquest estudi foren l'aïllament i seqüenciació del gen *vasa* a *M. brachydactyla* i la caracterització de la seva expressió durant el desenvolupament larvari i primer juvenil mitjançant PCR quantitativa. Un fragment (*Mb vasa, Maja brachydactyla vasa*) de 865 parells de bases fou aïllat del testicle, clonat i seqüenciat. La presència a la seqüència proteica deduïda de set dominis conservats a la família de proteïnes DEAD-box, un grup de RNA helicases entre les quals es troba vasa; l'anàlisi filogenètic de la seqüència proteica i l'especificitat de l'expressió de *Mb vasa* al testicle i ovari confirmen que el

fragment aïllat és homòleg de *vasa* a la cabra de mar. *Mb vasa* s'expressa durant tot el desenvolupament larvari i primer estadi juvenil a nivells baixos, però detectables mitjançant PCR quantitativa. Al llarg del desenvolupament larvari, l'expressió de *Mb vasa* augmenta lleugerament, mentre que l'expressió després de la metamorfosi al primer juvenil és significativament major respecte tots els estadis larvaris. En conjunt, l'expressió de *Mb vasa* durant les primeres fases del desenvolupament postembrionari s'ajusta a una corba de creixement exponencial (y=4,458E-07$e^{0.992x}$, R^2=0,997). Aquest increment podria ser degut al desenvolupament de les gònades, especialment després de la metamorfosi al primer juvenil.

10. Efecto del desplazamiento de fotoperiodo en la inducción y calidad de la puesta de la centolla, *Maja brachydactyla*.

Induction of the off-season spawning and larval hatching under delayed photoperiod regimes has been studied in the spider crab, *Maja brachydactyla*. Broodstock has been subjected to two experimental treatments, in which natural photoperiod was delayed 3 months (group 3-) and 6 months (group 6-). Natural photoperiod was used as control. Number of batches of newly hatched larvae per female, number of larvae per hatch and female, larval individual dry weight, and proximate biochemical content (protein, carbohydrate and lipid) of newly hatched larvae were determined monthly for each treatment. No significant differences between the experimental treatments and the control group were found in any of the parameters measured. These results suggest that the use of photoperiod is not enough to induce off-season spawning and larval hatching in the spider crab.

11. Identificación morfológica y molecular del Centollo Mediterráneo *Maja squinado* (Decapoda, Brachyura, Majidae).

The present work provides morphological and molecular information allowing easy recognition of the European species of the genus *Maja* (except *M. goltziana*). *M. brachydactyla* and *M. squinado* are commercial species that can be easily confused. The relationship between LC and LP is a useful morphometric index to identify *M. brachydactyla* and *M. squinado* species. At the molecular level, using the restriction enzymes Spe I and Ase I with the mitochondrial gene COI, allows rapid identification of species (including *M. crispata*). The molecular identification may be particularly useful in juvenile and young crabs, when the morphological differences found in adults are not applicable.

12. Inter-annual variations in quantity and quality of newly hatched larvae of *Maja brachydactyla*.

The spider crab *Maja brachydactyla* (Decapoda, Majidae) is the target of an intensive fishery in several areas of the NE Atlantic and some stocks show signs of overfishing. Hence, production of the spider crab in captivity might be a solution for the sector and research in this topic has been carried out in Spain. Quality of newly hatched larvae was evaluated throughout a period of six years (2005-2010).

Every year from 2005 to 2010, broodstock from Ría de A Coruña (NE Atlantic, Galicia, Spain) was transported to IRTA (Tarragona, W Mediterranean Sea, Spain) and kept in 2000 L tanks connected to a recirculation unit providing constant conditions of salinity (35±1‰) and temperature (18±1ºC) with a sex-ratio of 3 female – 1 male under natural photoperiod (12hL/12hD). Newly hatched larvae (NHL) of each year 10 first batches were collected and fresh and dry weight (DW), ash content, proximal composition (protein, carbohydrates and lipids), lipid classes and fatty acids were measured.

Total number of NHL presented high variations among batches even during the same year. Nevertheless, the production of NHL was the lowest in 2007 and the highest in 2008 (25920 *vs* 132340 NHL). In addition, NHL of 2007 were the smallest, reaching only 70 µg DW whereas those obtained in 2005 were the heaviest (99 µg DW) and presented the highest levels of proteins (303 µg mg tissue^{-1}), carbohydrates (17 µg mg tissue^{-1}) and lipids (76 µg mg tissue^{-1}). However, in the following years no clear relationship between DW and proximal composition could be found (Table 1).

Preliminary data on lipid class and fatty acid composition was obtained for 2005 NHL. Neutral lipids were the main components in all the samples analyzed, accounting for 59.95% of total lipids. Cholesterol represented an important fraction (19.15%) confirming its key role in growth, metabolic maintenance, and the regulation of moulting. The major phospholipids were phosphatidylcholine (17.70%) and phosphatidylethanolamine (13.81%), whereas in the case of fatty acids, a high polyunsaturated content (especially in EPA and DHA) are an indication ofhigh dietary PUFA requirement of NHL. The samples of 2006-2010 period are being processed to be presented during the congress.

TABLE 1. Dry weight (µg larvae^{-1}), ash content and proximal composition (µg mg tissue^{-1}) of newly hatched larvae of *Maja brachydactyla* in early season during six consecutive years. dbp= data being processed.

	No. larvae batch^{-1}	Dry weight	Ash	Proteins	Carbohydrates	Lipids
2005	66390±43590	98,68±11,33	26,65±2,58	303,35±37,13	17,15±4,58	75,90±15,33
2006	44740±38805	80,70±10,66	29,94±4,20	220,52±87,50	16,03±8,85	63,94±20,28
2007	25920±32443	69,97±16,81	29,88±3,10	248,91±104,89	17,49±6,03	47,75±14,76
2008	132340±50583	87,79±6,82	30,10±2,34	196,81±40,71	10,85±4,45	49,61±10,66
2009	81300±29417	84,66±9,28	dbp	dbp	dbp	dbp
2010	62908±21138	90,61±19,86	29,42±5,22	dbp	dbp	dbp

13. Estudio sobre la plasticidad nutricional en el primer estadio larvario de *Maja brachydactyla* (Brachyura, Majidae).

The nutritional vulnerability of the first zoeae of *Maja brachydactyla* was evaluated in terms of the point-of-reserve-saturation (PRS), the point-of–no-return (PNR), moulting capacity, dry mass, carbon (C), hydrogen (H), nitrogen (N), digestive gland morphology and digestive enzymatic activities. Continuously starved larvae did not moult to the zoea II. The PSR_{50} and PNR_{50} were 1.8 and 2.8 days, respectively. Dry mass and CHN composition are the most informative nutritional indicators.

14. Efecto del ayuno en el estado nutricional de las megalopas de *Maja brachydactyla* (Brachyura, Majidae).

The nutritional flexibility of the megalopae of *Maja brachydactyla* was evaluated in terms of dry mass (PS), carbon (C), hydrogen (H), nitrogen (N), moulting cycle, and digestive gland morphology. The study of the moult cycle shows that development of continuously starved megalopae is arrested in the premoult stage. The number and size of the vacuoles in the digestive gland might be considered as a good indicator of ingestion but not of the nutritional status. PS and CHN composition appeared to be good indicators of the nutritional condition of megalopae.