Characterization of larval moulting cycles in *Maja brachydactyla* (Brachyura, Majidae) reared in the laboratory

Guillermo Guerao^{a*}, Guiomar Rotllant^a, Klaus Anger^b

^aIRTA, Unitat de Cultius Experimentals. Ctra. Poble Nou, Km 5.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain.

^bAlfred-Wegener-Institut für Polar- und Meeresforschung; Biologische Anstalt Helgoland, Meeresstation, 27498 Helgoland, Germany.

* **Corresponding author:** E-mail address: guillermo.guerao@irta.cat (G. Guerao)

Fax: +34-977 74 41 38

Abstract

The moulting cycles of all larval instars (zoea I, zoea II, megalopa) of the spider crab *Maja brachydactyla* Balss 1922 were studied in laboratory rearing experiments. Morphological changes in the epidermis and cuticle were photographically documented in daily intervals and assigned to successive stages of the moulting cycle (based on Drach's classification system). Our moult-stage characterizations are based on microscopical examination of integumental modifications mainly in the telson, using epidermal condensation, the degree of epidermal retraction (apolysis), and morphogenesis (mainly setagenesis) as criteria. In the zoea II and megalopa, the formation of new setae was also observed in larval appendages including the antenna, maxillule, maxilla, second maxilliped, pleopods, and uropods. As principal stages within the zoea I moulting cycle, we describe postmoult (Drach's stages A-B combined), intermoult (C), and premoult (D), the latter with three substages (D₀, D₁, D₂). In the zoea II and megalopa, D₀ and D₁ had to be combined, because morphogenesis (the main characteristic of D₁) was unclear in the telson and did not occur synchronically in different appendices. The knowledge of the course and time scale of successive

moult-cycle events can be used as a tool for the evaluation of the developmental state within individual larval instars, providing a morphological reference system for physiological and biochemical studies related to crab aquaculture.

Key words: *Maja brachydactyla*, zoea, megalopa, moult cycle, apolysis, setagenesis.

1. Introduction

Moult staging has important practical applications as a routine procedure in decapod crustacean aquaculture (Robertson et al., 2007), because numerous physiological, biochemical, behavioural, and other traits and responses change markedly during the course of each moulting cycle (Chang, 1995). In order to take such intrinsic variations into account, it is first necessary to characterize the morphologically recognizable stages of the moulting cycle, which may then be used as a reference system for other studies, e.g. of larval physiology.

Drach (1939) studied the moult cycle in adult brachyuran crabs, *Cancer pagurus* Linnaeus, 1758 and *Maja brachydactyla* (as *Maia squinado*), providing the first classification system, which comprised five principal stages (A-E) and various subdivisions. Following this pioneering study, the moulting cycle is now generally divided into the following principal stages: (1) metecdysis or postmoult (Drach's moult-stages A and B), the period immediately following ecdysis; (2) anecdysis or intermoult (stage C), the main period of tissue growth; (3) proecdysis or premoult (stage D), a period of conspicuous morphological and physiological changes in preparation for the next moult; (4) ecdysis (stage E), the short event of shedding the old cuticle (Skinner, 1962; Drach and Tchernigovtzeff, 1967; Stevenson, 1985; Chan et al., 1988).

The spider crab *Maja brachydactyla* Balss, 1922 (Brachyura, Majidae) has recently been proposed as a potential candidate for aquaculture, due to its high fecundity and a relatively rapid larval

development (Iglesias et al., 2002; Guerao and Rotllant, 2008). These studies have shown that its larvae can successfully be reared under intensive and semi-extensive cultivation conditions, allowing for satisfactory growth and survival (Andrés et al., 2007). The larval development of this species comprises two zoeal stages and a megalopa, which were morphologically studied by Lebour (1927, 1928), Paula (1985), Clark (1986), Ingle (1992) and Guerao et al. (2008). Under constant experimental conditions of salinity (36 ‰) and temperature (18°C), metamorphosis to the first juvenile crab instar is reached about 18 days after hatching (Andrés et al., 2007, 2008; Guerao et al., 2008; Palma et al., 2008).

Moult-stages of brachyuran crab larvae have morphologically been described in only a few previous investigations (McConaughy, 1982; Anger, 1983; Brumbaugh and McConaughy, 1995; González-Gordillo et al., 2004). The principal aim of the present study is to identify in the spider crab *Maja brachydactyla* a rapidly recognizable chronological series of integumentary changes occurring during the developmental period from hatching of the zoea I to metamorphosis of the megalopa to the first juvenile crab instar. The knowledge of this sequence of moult-cycle stages, and of their time scale, provides a useful tool for an evaluation of the developmental state within successive larval instars, enhancing the temporal resolution of larval studies and serving as a reference system for future physiological and biochemical studies of larval growth and nutrition.

2. Materials and methods

2.1. Larval culture

Adult *M. brachydactyla* (3 females and 2 males of 150 to 170 mm of carapace length) were captured from the NE Atlantic (Galicia, Spain) and transported to the IRTA (Sant Carles de la Ràpita). The broodstock was kept in 2000 L tanks connected to a recirculation unit (for more details, see Andrés et al., 2007). Actively swimming newly hatched larvae were collected from broodstock tanks and

transferred to 500 mL beakers (n=30). The subsequent rearing experiments were conducted at a constant salinity of 36 ‰ and two constant temperatures (18 ± 1 and 15 ± 1 °C), a natural photoperiod of ca. 12 h light per day (early spring condition), and freshly hatched *Artemia* nauplii provided daily as food (after each water change). Larvae that moulting the same day to the same instar (zoea II, megalopa) were grouped into the same beaker. Five larvae were sampled at 24 h intervals and immediately mounted for microscopical observation. The experiment finished when most megalopae (>90 %) had moulted to juveniles, so that the final morphological changes immediately before the metamorphosis could be recorded.

2.2. Morphological analysis

Morphological details were studied under 40X and 63X magnification using a Leica DM LB microscope equipped with an Olympus DP 70 digital photographic camera. The analysis concentrated mainly on the telson, because this part of the larval body is easy to examine without requiring dissection. However, in order to study also the degree of synchronization of the moulting cycle in different structures, we also considered changes in the antenna (zoea II), the endites of the maxillule, the scaphognathite of the maxilla, the endopod dactyl of the second maxilliped (megalopa), pleopods (zoea II, megalopa), uropods (megalopa), and rostral and dorsal spines of the carapace (zoea II).

The term “stage” is in the literature commonly used to denote a larval or juvenile stage (also termed “instar”) such as zoea I, megalopa, first crab stage, but also for a stage of the moulting cycle within a given instar. In order to avoid ambiguity and confusion, we use here the terminology proposed by Anger (2001): Whenever “stage” is used, it should be accompanied by a determiner, e.g. “larval stage”, “zoeal stage”, “moult-stage” or “stage of the moulting cycle”. To enhance clarity, “larval stage” will be avoided in this paper and replaced with the synonym “instar”, which always means a

complete moulting cycle. Furthermore, Drach's classification system, which is largely adopted in this paper, comprises both the principal phases (stages A-E) and subdivisions thereof (e.g. "stages" D₀, D₁ etc.); for sake of clarity, the latter will here be referred to as "substages".

3. Results

At the two experimental temperatures (15° and 18°C), the successive larval moulting cycles of *M. brachydactyla* lasted on average 5-6 days in the zoea I, 4-5 days in the zoea II, and 7-10 days in the megalopa, i.e. complete larval development from hatching to metamorphosis took 16-21 days (Table 1). Drach's metecdysis stages A and B were generally short, and their transition was very gradual, so that they had to be combined here, considered together as "premoult" (A-B). Since also the transition between stages B and C was not characterized by marked morphological changes, the postmoult and intermoult stages A-C are in this study considered as one combined period. Similarly, the substaging of the intermoult and premoult periods (stages C, D) could not follow the detailed system proposed by Drach (1939) and Drach and Tchernigovtzeff (1967), which can only be used in studies of the much thicker and structurally more differentiated epidermal and cutical layers of juvenile and adult crabs, requiring also histological techniques. Nevertheless, the premoult phase of first-instar larval spider crabs could be divided into three substages, D₀ (early premoult), D₁ (intermediate premoult), and D₂ (late premoult, including also Drach's substages D₃ and D₄). In the zoea II and megalopa, however, substages D₀ and D₁ also had to be combined, because setagenesis and other morphogenetic events, which are the main criteria for their distinction, could not be observed in the telson; moreover, morphogenesis did not occur synchronically in the various other appendages and spines examined. The characteristic events of the moult-stages and substages within the successive larval instars may be described as follows (for durations, see Table 1).

3.1. Description of moult_stages

130 3.1.1. Zoea I

131 **A-C.** Immediately after hatching (stage A), the exoskeleton is very thin and the larval body is soft
132 (which can be ascertained with forceps). The epidermal tissues have a spongy structure with
133 numerous interconnected lacunal spaces, where hemolymph circulates, transporting microscopically
134 visible hemocytes. Subsequently (stage B), the tissues begin to concentrate along the inner cuticle
135 surface, becoming gradually denser in appearance (Fig. 1a). A-B is a brief period lasting ca. 1 day.

136

137 When the reduction of the epidermal lacunae has ceased and the cuticle has become conspicuously
138 thicker, more rigid, and elastic, we considered these changes as an indication for the transition to
139 stage C. Also, the thickness of the cuticle reaches its maximum at the beginning of the intermoult
140 period. Throughout this stage, the epidermal tissues continue to grow, showing a gradually
141 increasing density and extension, while no conspicuous morphological changes occur (Fig. 1b). Like
142 postmoult (A-B), stage C is a brief period (taking ca. 1 day at both temperatures). Since also the
143 transition between postmoult and intermoult is gradual rather than distinct, we combine here stages
144 A-C (in total 2 days at 15-18°C).

145

146 **D₀.** The beginning of early premoult is clearly visible, indicated by an incipient separation of the
147 epidermis from the cuticle (apolysis). This process can first be observed near the base (articulation)
148 of the serrulate setae of the telson (Fig. 1c), before it proceeds along the whole epidermis-cuticle
149 interface. The separation of the epidermis from the old cuticle is a crucial precondition for all
150 subsequent processes of morphogenesis (see below). Substage D₀ lasted at both experimental
151 temperatures about 2 days.

152

153 **D₁.** Morphogenesis, which is the characteristic event of the intermediate premoult substage, begins at
154 the base of the retracted setae, visible as a circular epidermal invagination (Fig. 1d). Subsequently,

155 apolysis occurs in all setae, spines, and the outer rami of the bifurcated telson, advancing gradually
 156 in a proximal direction, while new setules and secondary spinules appear on the increasingly
 157 retracted epidermal surfaces. Deep folds of epidermal invagination (comparable to a glove turned to
 158 the inside) are a prerequisite for an enlargement of setal size as well as a formation of new setae
 159 required for the following larval instar (here: the zoea II). Altogether, the intermediate premoult
 160 lasted in the zoea I instar for about 3 days.

161

162 **D₂-E.** The beginning of late premoult is characterized by the appearance of a thin, yellowish new
 163 cuticle on the epidermal surface of the setae and spines, which reach their gratest retraction from the
 164 old cuticle sheath. The new (plumodenticulate) setae of the telson are completely formed, and their
 165 setules and secondary spinules are clearly visible, due to the distinct lining with a thin but
 166 microscopically conspicuous new cuticle on their surface (Figs. 1e,f). Hence, the main characteristics
 167 of substage D₂ is the termination of morphogenesis and the protection of the newly formed structures
 168 with a new exoskeleton. The thickness and visibility of the new cuticle increase considerably
 169 throughout this substage, which took 4-5 days (Table 1).

170

171 Ecdysis (Drach's moult-stage E) is preceded by maximal epidermal retraction from the old cuticle
 172 sheaths and an apparent uptake of water, which inflates the spaces between the old and new cuticle.
 173 The old cuticle eventually ruptures between the cephalothorax and the first pleomere (observed
 174 under a stereo microscope), and this is followed by further water uptake (swelling) and rapid
 175 shedding of the old cuticle (within a few minutes).

176

177 3.1.2. Zoea II

178 **A-C.** The postmoult and intermoult stages are morphologically very similar to those described for
 179 the zoea I instar (Figs. 2a,b).

180

181 **D₀-D₁.** As in the zoea I, apolysis was first observed at the base of the terminal setae of the telson
 182 (Fig. 2c), clearly indicating the beginning of substage D₀. Substage D₁, however, could not safely be
 183 differentiated in the second zoeal instar, because its morphogenesis differs greatly from that observed
 184 near the end of the zoea I instar. Since the telson of the subsequent instar, the megalopa, requires a
 185 reduction rather than a formation of new or larger setae, the intermediate premoult of the zoea II is
 186 characterized by setal degeneration rather than basal invaginations. Instead of enlarging its surface
 187 through invagination processes, the epidermal matrix degenerates, beginning from the distal tips of
 188 the rami and the large terminal setae (no longer needed in the following instar; Fig. 2d), so that
 189 gradually the typical round or slightly bilobed megalopal telson is shaped, which completely lacks a
 190 furca. Similarly, the epidermis begins to retract and degenerate also in the rostral and dorsal carapace
 191 spines, as well as in the protopod process and the exopod of the antenna. Morphogenetic processes
 192 observed in other body parts including the maxillules, pleopods and uropods, showed poor
 193 synchrony and could therefore not be used as criteria for substaging of the premoult stage.

194

195 **D₂.** The epidermal matrix has completely retracted from the zoeal furca, and the posterior margin of
 196 the megalopal telson becomes clearly visible, now covered by a new cuticle (Fig. 2e). Similarly, the
 197 epidermis has completely retracted from the protopodal process and the exopod of the antenna (Fig.
 198 2f), and the new setae of the maxillule appear (Fig. 3a). During this phase, also the characteristic
 199 coupling hooks in the endopods of pleopods 1-4 become visible.

200

201 3.1.3. Megalopa

202 **A-C.** Similar to the situation in the previous stages (Figs. 3b,c).

203

204 **D₀- D₁.** Apolysis begins along the terminal margin of the telson (Fig. 3d), but can be observed also in
 205 a maxillule, the endopod of the second maxilliped, and the pleopods. As in the zoea II, moult stage
 206 D₁ is not clearly separable. Examination of the endopod of the second and third maxillipeds showed
 207 that setagenesis begins in these appendages near the middle of the early to intermediate premoult
 208 phase, while apolysis occurs in the distal parts of the dactyls of pereopods 2-5, and the epidermis of
 209 their proximal portion begins to form invaginations.

210

211 **D₂.** As in the previous larval instars, this substage is characterized by the appearance of a new
 212 cuticle. The epidermis in the posterior parts of the megalopal telson appears strongly retracted, so
 213 that the characteristic margin of the juvenile telson is formed (Fig. 3e). The new setae of the maxilla
 214 and second maxilliped are formed, and the setules and the proximal end become clearly visible (Fig.
 215 3f). The epidermal matrix retracts from the degenerating uropods and, shortly before moulting to the
 216 first juvenile crab instar, the exopods become transparent and eventually void of epidermal tissues.
 217 The epidermis of the pereopods, by contrast, appears strongly folded and invaginated (allowing for
 218 an enlargement of the walking legs and chelae), while the distal parts of the dactyls of pereopods 2-
 219 5, with completely formed new juvenile spines and setae, are retracted from the megalopal cuticle,
 220 facilitating the imminent terminal larval moult.

221

222 4. Discussion

223 In the literature, the number of moult-cycle stages distinguished by different authors varies greatly
 224 due to differences in the examined appendages or the time scale of observations (e.g. Drach and
 225 Tchernigovtzeff, 1967; Davis et al., 1973; Hatfield, 1983; Lipcius et al., 1990; Brumbaugh and
 226 McConaughy, 1995). The course of the moulting cycle of larval spider crab, *Maja brachydactyla*, is
 227 similar to that previously described by Anger (1983) for another majid, *Hyas araneus*, and most of
 228 the few other species of decapod crustaceans, for which comparable data have become available

(Anger, 2001). In juvenile and adult crabs, long intermoult periods, as well as thick, calcified, and highly complex integumental structures allow for a high temporal resolution of observations and an identification of numerous morphologically defined stages and substages of the moulting cycle (Drach, 1939; Drach and Tchernigovtzeff, 1967; Stevenson, 1985; Chan et al., 1988). In planktonic crustacean larvae, by contrast, the moulting cycles are generally short, and the epidermal and cuticular structures are thin and little structured. As a consequence, the temporal resolution of experimental observations is low, it is often difficult to detect morphological changes, and sometimes it would be arbitrary to define the transitions between putative stages or substages within a larval instar. This requires a strong simplification of Drach's classification system. As in previous studies with larval decapods (Anger, 2001; Hayd et al., 2008), we therefore had to combine all postmoult and intermoult stages (A-C) as well as Drach's substages D₃-D₄ within the premoult period, stage D); also substages D₀-D₁ in the zoea II and the megalopa could not be separated due to lacking setagenesis in the larval telson. Other appendages and carapace spines could not be used as suitable alternative structures, due to poor synchronization of moult-cycle events occurring in the various parts of the larval body. Apolysis and setagenesis, for instance, could first be observed in the pleopods and pereopods, and only later in other appendages and in the telson of larval *M. brachydactyla*. In the megalopa, it also remains difficult to assign the moult-stages observed in our study to those proposed by González-Gordillo et al. (2004) for *Carcinus maenas* (L., 1758). The sequence of morphological changes may differ between these two crab species, but we also suggest that a distinction of too many stages may not be practicable, at least for aquaculture purposes, which are a main focus in our research. Future comparative studies on the moulting cycle in larval decapods may show the degree of interspecific and instar-specific variation and identify further, possibly more reliable morphological criteria.

In spite of all technical limitations for moult-staging in crustacean larvae, even a simplified system with only 3-4 clearly separated and well defined developmental periods (A-C, D₀, D₁, and D₂) may already be a valuable reference system for physiological, biochemical, ecological, and behavioural studies of larval biology, and it may be a tool for evaluating and predicting larval growth, nutrition, and moulting in the field and in aquaculture (see e.g. van Herp and Bellon-Humbert, 1978; Sasaki et al., 1986; Lipcius et al., 1990; Wolcott and de Vries, 1994; Gebauer et al., 2004; Schmidt et al., 2004). Various environmental conditions such as temperature (present study), light (Bermudes and Ritar, 2008; Bermudes et al., 2008), salinity (Romano and Zeng, 2006), or feeding treatments (Minawaga and Murano, 1994) can modify the moulting cycle, which implies changes in numerous physiological, biochemical, and behavioural parameters (see Chang, 1995; Anger, 2001). Hence, moult-staging is, in addition to information on larval age, instar, size, and biomass, another independent tool for the evaluation of larval condition, aiding to an optimization of rearing conditions in aquaculture and basic research cultivation of decapod crustaceans.

5. Acknowledgements

Financial support was provided by the Ministry of Science and Research to GG (post-doctoral fellowship; INIA) and GR (Ramón y Cajal contract). Bench fees were funded by INIA (RTA2008-00003-00-00 project) to GR. The authors would like to thank Glòria Macià and Sandra Molas for their support as hatchery and laboratory technicians at IRTA, Sant Carles de la Ràpita.

6. References

Aiken, D.E., 1973. Proecdysis, setal development, and molt prediction in the American lobster (*Homarus americanus*). J. Fish. Res. Bd Can. 30, 1337-1344.

- 278 Andrés, M., Estévez, A., Rotllant, G., 2007. Growth, survival and biochemical composition of spider
279 crab *Maja brachydactyla* (Balss, 1922) (Decapoda: Majidae) larvae reared under different
280 stocking densities, prey: larva ratios and diets. *Aquaculture* 273, 494–502.
- 281 Andrés, M., Estévez, A., Anger, K., Rotllant, G., 2008. Growth and biochemical composition during
282 larvar and early juvenile development of the spider crab, *Maja brachydactyla* (Decapoda:
283 Majidae). *J. Exp. Mar. Biol. Ecol.* 357 (1), 35-40.
- 284 Anger, K., 1983. Moults cycle and morphogenesis in *Hyas araneus* larvae (Decapoda, Majidae),
285 reared in the laboratory. *Helgoländer Meeresuntersuchungen* 36, 285-302.
- 286 Anger, K., 2001. The biology of decapod crustacean larvae. *Crustacean Issues* Vol. 14. A.A.
287 Balkema, Lisse, The Netherlands, 420 pp.
- 288 Bermudes, M., Ritar, A.J., 2008. Response of early stage spiny lobster *Jasus edwardsii* phyllosoma
289 larvae to changes in temperature and photoperiod. *Aquaculture* 281, 63-69.
- 290 Bermudes, M., Ritar, A.J., Carter, C.G., 2008. The ontogeny of physiological response to light
291 intensity in early stage spiny lobster (*Jasus edwardsii*) larvae. *Comp. Biochem. Physiol. A*
292 *Comp. Physiol.* 150, 40-45.
- 293 Brumbaugh, R.D., McConaughy, J.R., 1995. Time to metamorphosis of blue crab *Callinectes*
294 *sapidus* megalopae: effects of benthic macroalgae. *Mar. Ecol. Prog. Ser.* 129, 113-118.
- 295 Chang, E.S., 1995. Physiological and biochemical changes during the molt cycle in decapod
296 crustaceans: an overview. *J. Exp. Mar. Biol. Ecol.* 193, 1-14.
- 297 Chan, S.-M., Rankin, S.M., Keeley, L.L., 1988. Characterization of the molt stages in *Penaeus*
298 *vannamei*: setogenesis and hemolymph levels of total protein, ecdysteroids, and Glucose.
299 *Biol. Bull.* 175, 185-192.
- 300 Clark, P.F., 1986. The larval stages of *Maja squinado* (Herbst, 1788) (Crustacea: Brachyura:
301 Majidae) reared in the laboratory. *J. Nat. Hist.* 20, 825-836.
- 302 Davis, C.W., Fyhn, U.E.H., Fyhn, H.J., 1973. The intermolt cycle of cirripeds: criteria for its stages
303 and its duration in *Balanus amphitrite*. *Biol. Bull.* 145, 310-322.

- 304 Drach, P., 1939. Mue et cycle d'intermue chez les crustacés décapodes. Annales de l'Institut
305 Oceanographique 14, 103–391.
- 306 Drach, P., 1944. Etude préliminaire sur le cycle d'intermue et son conditionnement hormonal chez
307 *Leander serratus* (Pennant). Bulletin Biologique de la France et de la Belgique 78, 40–62.
- 308 Drach, P., Tchernigovtzeff, C., 1967. Sur la méthode de détermination des estades d'intermue et son
309 applications general aux Crustacés. Vie et Milieu 18, 595–607.
- 310 Gebauer, P., Paschke, K., Anger, K., 2004. Stimulation of metamorphosis in an estuarine crab,
311 *Chasmagnathus granulata* (Dana, 1851): temporal window of cue receptivity. J. Exp. Mar.
312 Biol. Ecol. 311, 25–36.
- 313 González-Gordillo, J.I., Rodríguez, A., Queiroga, H., 2004. Characterization of the megalopal
314 premoult stages of the green crab, *Carcinus maenas* (Decapoda, Portunidae), from laboratory
315 culture. J. Crust. Biol. 24(3), 502–510.
- 316 Guerao, G., Pastor, E., Martin, J., Andrés, M., Estévez, A., Grau, A., Duran, J., Rotllant, G., 2008.
317 The larval development of *Maja squinado* and *M. brachydactyla* (Decapoda, Brachyura,
318 Majidae) described from plankton collected and laboratory-reared material. J. Nat. Hist. 42
319 (33–34), 2257–2276.
- 320 Guerao, G., Rotllant, G., 2009. Survival and growth of post-settlement juveniles of the spider crab
321 *Maja brachydactyla* (Brachyura: Majoidea) reared under individual culture system.
322 Aquaculture 289, 181–184.
- 323 Hatfield, S.E., 1983. Intermolt staging and distribution of dungeness crab, *Cancer magister*,
324 megalopae. Fisheries Bulletin (U.S.) 172, 85–96.
- 325 Hayd, L.A., Anger, K., Valenti, W.C., 2008. The moulting cycle of larval Amazon River prawn
326 *Macrobrachium amazonicum* reared in the laboratory. Nauplius 16, 55–63.
- 327 Iglesias, J., Sánchez, F.J., Moxica, C., Fuentes, L., Otero, J.J., Pérez, J.L., 2002. Datos preliminares
328 sobre el cultivo de larvas y juveniles de centolla *Maja squinado* Herbst, 1788 en el Centro

- 329 Oceanográfico de Vigo del Instituto Español de Oceanografía. Boletín del Instituto Español
330 de Oceanografía 18, 25–30.
- 331 Ingle, R.W., 1992. Larval stages of Northeastern Atlantic Crabs. An Illustrated Key. Chapman and
332 Hall, London, 1-363.
- 333 Kurup, N.G., 1964. The intermolt cycle of an anomuran, *Petrolisthes cinctipes* Randall (Decapoda).
334 Biol. Bull. 127, 97-107.
- 335 Lebour, M.V., 1927. Studies of the Plymouth Brachyura. I. The rearing of crabs in captivity, with a
336 description of the larval stages of *Inachus dorsettensis*, *Macropodia longirostris* and *Maja*
337 *squinado*. J. Mar. Biol. Ass. UK., 14, 795-820.
- 338 Lebour, M.V., 1928. The larval stages of the Plymouth Brachyura. Proc. Zool. Soc. London, 2, 473-
339 560.
- 340 Lipcius, R.N., Olmi III, E.J., van Montfrans, J., 1990. Planktonic availability, molt stage and
341 settlement of blue crab postlarvae. Mar. Ecol. Prog. Ser. 58, 235-242.
- 342 Longmuir, E., 1983. Setal development, moult-staging and ecdysis in the banana prawn *Penaeus*
343 *merguiensis*. Mar. Biol. 77, 183-190.
- 344 McConaughy, J.R., 1982. Regulation of crustacean morphogenesis in larvae of the mud crab,
345 *Rhithropanopeus harrisi*. J. exp. Zool. 223, 155-163.
- 346 Minagawa, M., Murano, M., 1993. Larval feeding rhythms and food consumption by the red frog
347 crab *Ranina ranina* (Decapoda, Raninidae) under laboratory conditions. Aquaculture 113,
348 251-260.
- 349 Palma, J., Correia, M., Andrade, J.P., 2008. Usefulness of flat bottom tanks on the settlement of
350 spider crab (*Maja squinado*, Herbst) larvae. Aquaculture Res. 39, 1005-1008.
- 351 Paula, J., 1985. The first zoeal stages of *Polybius henslowi* Leach, *Maja squinado* (Herbst),
352 *Pachygrapsus marmoratus* (Fabricius) and *Uca tangeri* (Eydoux) (Crustacea, Decapoda,
353 Brachyura). Arq. Mus. Boc. (Série B) 2, 137-147.

- 354 Robertson, L., Bray, W., Leung-trujillo, J., Lawrence, A., 2007. Practical molt staging of *Penaeus*
355 *setiferus* and *Penaeus stylirostris*. Journal of the World Aquaculture Society 18(3), 180-185.
- 356 Romano, N., Zeng, C., 2006. The effects of salinity on the survival, growth and haemolymph
357 osmolality of early juvenile blue swimmer crabs, *Portunus pelagicus*. Aquaculture 260, 151-
358 162.
- 359 Sasaki, G.C., Capuzzo, J.M., Biesiot, P., 1986. Nutritional and bioenergetic considerations in the
360 development of the American lobster *Homarus americanus*. Can. J. Fish. Aquat. Sci. 43,
361 2311-2319.
- 362 Schmidt, K., Tarling, G.A., Plathner, N., Atkinson, A., 2004. Molt cycle-related changes in feeding
363 rates of larval krill *Meganyctiphanes norvegica* and *Thysanoessa* spp. Marine Ecology-
364 Progress Series 281, 131-143
- 365 Skinner, D.M., 1962. The structure and metabolism of a crustacean integumentary tissue during a
366 molt cycle. Biol. Bull. Mar. Biol. Lab. Woods Hole 123, 635-647.
- 367 Stevenson, J. R., 1985. Dynamics of the integument. Pp. 1-42 in D. E. Bliss and L. H. Mantel, eds.
368 The Biology of Crustacea. Vol. 9. Integument, Pigments, and Hormonal Processes. Academic
369 Press, New York.
- 370 van Herp, F., Bellon-Humbert, C., 1978. Setal development and molt prediction in the larvae and
371 adults of the crayfish, *Astacus leptodactylus* (Nordmann, 1842). Aquaculture 14, 289-301
- 372 Vranckx, R., M. Durliat, 1978. Comparison of the gradient of setal development of uropods and of
373 scaphognathites in *Astacus leptodactylus*. Biol. Bull. 155, 627-639.
- 374

375 **Table 1**

376 *Maja brachydactyla*. Characterization of moult-stages and their duration at two different

377 temperatures.

Larval instar	Moult stage	Morphological observations on telson	Age (days) 15 °C	Age (days) 18 °C
Zoea I	A-B	Spongy epidermis	0	0
	C	Condensation of the epidermal tissues	1	1
	D ₀	Apolysis	2	2
	D ₁	Setogenesis	3	3
	D ₂	Appearance of new cuticle	5	4
	E	Ecdysis	6	5
Zoea II	A-B	Spongy epidermis	0	0
	C	Condensation of the epidermal tissues	1	1
	D ₀	Apolysis	3	2
		Advanced apolysis	4	3
		Branches with all spines and setae degenerate	5	4
	D ₂	Appearance of new cuticle	6	5
Megalopa	E	Ecdysis	7	6
	A-B	Spongy epidermis	0	0
	C	Condensation of the epidermal tissues	1	1
	D ₀	Apolysis	3	2
		Advanced apolysis	6	4
		Maximum retraction of the epidermis	7	5
	D ₂	Appearance of new cuticle	9	6
	E	Ecdysis	10	7

378

379

Figure captions

Figure 1. *Maja brachydactyla*, zoea I, telson. (a), moult-stage B, postmoult; (b), moult stage C, intermoult; (c), apolysis, moult-stage D₀, early premoult; (d), setagenesis, moult-stage D₁, intermediate premoult; (e), moult-stage D₂, late premoult; (f), advanced stage D₂, detail of the articulation of plumodenticulate setae. ap, apolysis; co, epidermal condensation; in, epidermal invagination. Scale bars 100 µm.

Figure 2. *Maja brachydactyla*, zoea II. (a), telson, moult-stage B, postmoult; (b), telson, moult-stage C, intermoult; (c), telson, apolysis, moult-stage D₀, early premoult; (d), telson, retraction of furcal tissues, moult-stages D₀-D₁, early to intermediate premoult; (e), telson, moult-stage D₂, late premoult; (f), antenna, advanced moult-stage D₂. en, endopod; ex, exopod; pr, spinous process of the protopod; re, retraction of tissues. Scale bars 100 µm.

Figure 3. *Maja brachydactyla*. Zoea II. (a), maxillule, moult-substage D₂. Megalopa. (b), telson, moult-stage B; (c), telson, moult-stage C; (d), telson, moult-substage D₀, early apolysis; (e), telson moult-substage D₂; (f), dactyl of the endopod of the second maxilliped, moult-sudstage D₂. Scale bars 100 µm.

400

401

402 Figure 1

403

Figure 2

408

409

410 Figure 3