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

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**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 (McConaugha, 1982; Anger, 1983; Brumbaugh and McConaugha, 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 Rápita). 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
3.1.1. Zoea I

A-C. Immediately after hatching (stage A), the exoskeleton is very thin and the larval body is soft (which can be ascertained with forceps). The epidermal tissues have a spongy structure with numerous interconnected lacunal spaces, where hemolymph circulates, transporting microscopically visible hemocytes. Subsequently (stage B), the tissues begin to concentrate along the inner cuticle surface, becoming gradually denser in appearance (Fig. 1a). A-B is a brief period lasting ca. 1 day. When the reduction of the epidermal lacunae has ceased and the cuticle has become conspicuously thicker, more rigid, and elastic, we considered these changes as an indication for the transition to stage C. Also, the thickness of the cuticle reaches its maximum at the beginning of the intermoult period. Throughout this stage, the epidermal tissues continue to grow, showing a gradually increasing density and extension, while no conspicuous morphological changes occur (Fig. 1b). Like postmoult (A-B), stage C is a brief period (taking ca. 1 day at both temperatures). Since also the transition between postmoult and intermoult is gradual rather than distinct, we combine here stages A-C (in total 2 days at 15-18°C).

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

D₁. Morphogenesis, which is the characteristic event of the intermediate premoult substage, begins at the base of the retracted setae, visible as a circular epidermal invagination (Fig. 1d). Subsequently,
apolysis occurs in all setae, spines, and the outer rami of the bifurcated telson, advancing gradually in a proximal direction, while new setules and secondary spinules appear on the increasingly retracted epidermal surfaces. Deep folds of epidermal invagination (comparable to a glove turned to the inside) are a prerequisite for an enlargement of setal size as well as a formation of new setae required for the following larval instar (here: the zoea II). Altogether, the intermediate premoult lasted in the zoea I instar for about 3 days.

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

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

3.1.2. Zoea II

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

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

3.1.3. Megalopa

A-C. Similar to the situation in the previous stages (Figs. 3b,c).
Apolysis begins along the terminal margin of the telson (Fig. 3d), but can be observed also in a maxillule, the endopod of the second maxilliped, and the pleopods. As in the zoea II, moult stage D1 is not clearly separable. Examination of the endopod of the second and third maxillipeds showed that setagenesis begins in these appendages near the middle of the early to intermediate premoult phase, while apolysis occurs in the distal parts of the dactyls of pereiopods 2-5, and the epidermis of their proximal portion begins to form invaginations.

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

4. Discussion

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

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**Table 1**

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

| Larval instar | Moul 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<sub>0</sub> | Apolysis                            | 2               | 2               |
|               | D<sub>1</sub> | Setogenesis                         | 3               | 3               |
|               | D<sub>2</sub> | 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<sub>0</sub> | Apolysis                            | 3               | 2               |
|               |            | Advanced apolysis                   | 4               | 3               |
|               | D<sub>2</sub> | Appearance of new cuticle           | 5               | 4               |
|               | E          | Ecdysis                             | 6               | 5               |
| **Megalopa**  | A-B        | Spongy epidermis                    | 0               | 0               |
|               | C          | Condensation of the epidermal tissues | 1               | 1               |
|               | D<sub>0</sub> | Apolysis                            | 3               | 2               |
|               |            | Advanced apolysis                   | 6               | 4               |
|               | D<sub>2</sub> | Maximum retraction of the epidermis | 7               | 5               |
|               | E          | Ecdysis                             | 9               | 6               |
|               |            | Appearance of new cuticle           | 10              | 7               |
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*. Zoa 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.
Figure 1
Figure 2
