JOURNAL OF EXPERIMENTAL ZOOLOGY 303A:393–405 (2005)

Ontogeny of the Marmorkrebs (Marbled Crayfish): a Parthenogenetic Crayfish With Unknown Origin and Phylogenetic Position

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ABSTRACT Development, growth, and egg production of the Marmorkrebs (marbled crayfish), a crayfish with parthenogenetic reproduction, uncertain geographic origin, and taxonomic position, was studied under laboratory conditions. Length and weight increments strongly depended on temperature being highest at 30°C, and lowest at 15°C. At 25°C, cephalothorax length and weight increased by 17.5 mm and 1700 mg, respectively, in the course of 150 d, whereas at 15°C these parameters increased by only 7 mm and 100 mg during the same period of time. Photoperiod slightly affected growth at 25°C. During growth experiments, mortality was lower at 20°C compared to higher (25°, 30°C) or lower temperatures (15°C), and lower under short-day than under long-day conditions. Females matured early (at an age of 141–255 d, a cephalothorax length of 14–21.5 mm, and a weight of 0.63–2 g) compared to other crayfish species. Reproductive females with a cephalothorax length of between 25–35 mm produced large clutches (up to 416 eggs) and brooding periods varied between 22 and 42 d. In order to establish a staging scheme for Marmorkrebs embryos, embryos were photographed, externally visible ontogenetic events charted, and dissected embryos stained with a nuclear dye. These experiments indicate that their development is virtually identical to that of other crayfish. In conclusion, these results and others show that the Marmorkrebs may be taken as a representative valid model organism for future developmental studies on Crustacea. *J. Exp. Zool. 303A: 393–405, 2005.*

INTRODUCTION

The Marmorkrebs (marbled crayfish; Decapoda, Astacida, Cambaridae; Fig. 1) is an unidentified crayfish of uncertain geographical origin that was introduced into the German aquarium trade in the mid–1990s (Scholtz et al., 2003). Based on the comparison of partial sequences of two mitochondrial genes, Scholtz et al. suggested a close relationship of the Marmorkrebs to species of the crayfish genus *Procambarus*. The Marmorkrebs is unusual in that sexing laboratory populations (more than 140 specimens) revealed only animals with female morphology and that under laboratory conditions these females repeatedly laid eggs in the absence of any spermotophores in the spermatheca (Scholtz et al., 2003). These results, along with additional data on their reproductive system (Vogt et al., 2004), suggest that the Marmorkrebs provides the first example for parthenogenesis within the decapod crustaceans. Generally, freshwater decapods are bisexual (gonochor); hermaphrodism has been described for members of the family Parastacidae (von Martens, 1869; Faxon, 1898; Lönnberg, 1898). Parthenogenetic reproduction is common in several crustacean groups such as Cladocera, Ostracoda, and others (e.g., Gruner, '93; Flößner, 2000;

Grant support: Deutsche Forschungsgemeinschaft; Grant number: Ha 2540/4.
S.H. is a Heisenberg Fellow of the Deutsche Forschungsgemeinschaft.
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Received 24 October 2004; Accepted 13 November 2004
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.a.143.

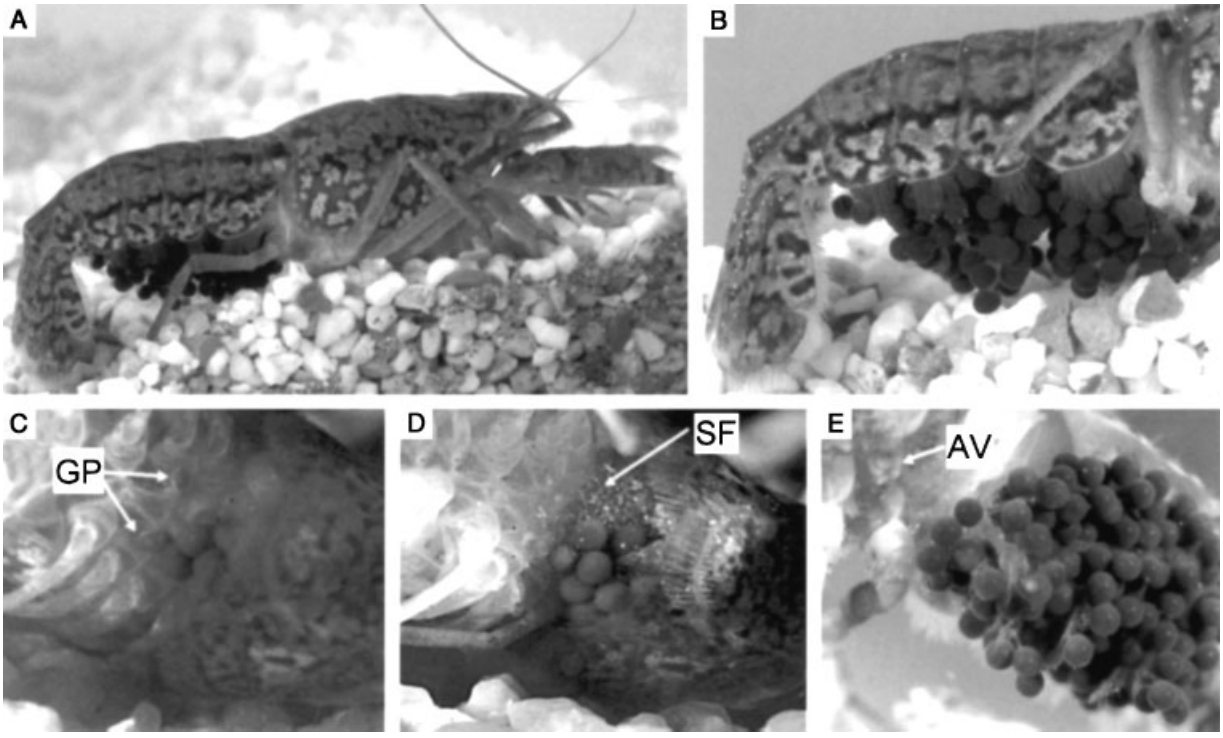

Fig. 1A, B. Ovigerous female of the Marmorkrebs showing eggs attached to the pleopods. **C.** Egg deposition: deformed eggs emerge from the gonopores (GP). **D.** Eggs become ensheathed in a cyst of secretory fluid (SF). E. A batch of freshly deposited eggs (AV: annulus ventralis).

Meisch, 2000) but not in Decapoda. Although the principal reproductive mode of the Marmorkrebs is elucidated now, growth and reproductive parameters are insufficiently understood. Therefore, growth and clutch production patterns were further explored under artificial conditions in laboratory experiments. Since parthenogenesis points to r-selected traits, rapid growth and clutch production patterns, which are typical for r-selected species, were expected.

Due to its unusual reproductive mode, it was suggested that the Marmorkrebs may be an interesting organism for physiological, evolutionary, and developmental studies (Scholtz et al., 2003). However, a prerequisite for ontogenetic studies is the presence of convenient methods to stage the embryonic development, if possible, by using criteria that are readily observable in living embryos. Within the Decapoda, versatile embryonic staging systems based on the percentage of total time from the deposition of eggs to hatching exist, for example, for the American lobster *Homarus americanus* (Reptantia, Homarida; Helluy and Beltz, '91), the Australian crayfish *Cherax destructor* (Reptantia, Astacida; Sandeman and Sandeman, '91), the prawn *Macrobrachium olfersi* (Palaemonidae; Müller et al., 2003), and the shrimp *Palaemonetes argentinus* (Caridea; Harzsch et al., '97). Therefore, the second goal of the present study was to establish such a staging scheme for the Marmorkrebs, that may serve as a basis for future developmental studies aimed at, for instance, examining cell genealogy (Scholtz, '92; '93) or exploring mechanisms of neurogenesis in decapod crustaceans (reviews e.g., Harzsch, 2002, 2003; Scholtz and Gerberding, 2002; Whitington, 2004). To this end, embryos were sampled and photographed at two-day intervals, and several externally visible ontogenetic events such as gastrulation, germ band formation, onset of heartbeat, appearance of ectodermal pigments, and eye pigmentation were charted. Furthermore, dissected embryos were stained with a nuclear dye to examine their morphology and to explore if certain aspects of embryonic development in this parthenogenetic species correspond to those examined in other decapod crustaceans.

METHODS

Mothers of all experimental animals were purchased at the Kölle-Zoo (aquarium trade near

Stuttgart, Southern Germany). They were kept in aquaria filled with aged tap water (total hardness 6–7 mval l^{-1}) at the University of Ulm.

Parthenogenesis or hermaphroditism? Reproduction of females after isolated rearing and gonad morphology

In order to compile additional evidence supporting the claim of parthenogenesis (Scholtz et al., 2003), four individuals were separated from their mothers immediately after the molt from the second postembryonic stage to the juvenile and reared in isolation at 20°C (all other rearing parameters as described in the next section). This experiment was carried out to explore if, in the absence of any contact with conspecifics, isolated individuals produced normally developing embryos. Furthermore, to determine if the females possess male reproductive organs in addition to ovaries (as evidence for hermaphroditism), the reproductive systems of three females (between 19 and 54 mm total length) were dissected.

Growth experiments

For the growth experiments, juveniles were isolated immediately after they had left the mother and kept individually in temperature chambers (Heraeus BK 5060 EL and Danfoss SC 18) at four different temperatures (15°, 20°, 25°, and 30°C) and two different photoperiods (18:6 and 6:18 light : dark). Individuals with a cephalothorax length of up to 8 mm were kept in $100 \times 75 \times 25$ cm, those with 8–10 mm in $145 \times 70 \times 50$ cm and those >10 mm in $180 \times 120 \times 120$ mm vessels. The bottom of the culture vessels was stocked with gravel (grain size 2–5 mm) and larger stones (2–5 cm diameter); broken pieces of pottery were introduced to provide shelter. The tanks were covered with sheets of plastic to prevent the animals from escaping. All vessels were aerated by WISA aquarium pumps. Culture water was aged tap water. The water in the culture vessels was replaced every second day. All individuals were fed with food pellets from the aquarium trade and chironomid larvae. The composition of the pellets was 47.5% raw protein, 6.5% raw fat, 2% raw fibre, 10.5% raw ash, and 6% water, added vitamins kg^{-1} pellets were A, 29 770 IE, D_3, 1 860 IE, E, 200 mg and L-Ascorbyl–2–polyphosphate, 137 mg (Tetra company). The weight of one pellet was 3.25 mg, the dry weight of one chironomid larva was approximately 0.95 mg. Preliminary experiments showed that both pellets and chironomid larvae were consumed eagerly. Food was added in excess. Every other day, the food was renewed and remaining food fragments were removed. Cephalothorax length, total length, and weight were measured during each intermoult period. Lengths were measured with a binocular microscope at 4.8–16 × magnification and weight was determined with a Sartorius 2004 MP balance.

Reproductive parameters

To determine reproductive parameters, adult females were isolated and kept individually in $180 \times 120 \times 120$ cm aquaria at 20–25°C and a photoperiod of between 18:6 and 6:18 light : dark. Other rearing conditions were as described for the growth experiments. Clutch size, interclutch periods, egg diameter and viability, brooding period (from the appearance of eggs to when juveniles left the mother), age, length, and weight at (first) reproduction, as well as lengths and weights of juveniles when they left the mother, were monitored during an experimental period of 200 d. To determine clutch size, eggs were removed from the mother and counted. In cases where eggs were needed for further experiments (e.g., growth experiments) clutch size was estimated (to approximate the nearest 20%), since preparation of eggs resulted in their death. The above described tools (microscope, balance) were used to measure lengths and weights.

Cold adaptation

To test whether the Marmorkrebs can survive at low temperatures, eight individuals with a cephalothorax length between 5 and 19.5 mm were successively acclimated to temperatures between 10° and 8°C. At first, they were kept for 22 d at 15°C and a photoperiod of 14:10 light : dark. Then they were exposed to a temperature of 10° to 8°C at a photoperiod of 6:18 light : dark for 62 and 46 d, respectively. Low temperature and short-day conditions were intended to mimic winter conditions. The entire experiment lasted 130 d. The temperature of 8°C at the end of the experiment was selected, since it corresponds to groundwater temperature in central Europe. Culture conditions and food were as in the growth experiments.

Development of the embryos

To examine the embryonic development, females were reared separately in 27 or 40 cm long, 19 or 25 cm broad, and 14.5 cm high plastic tanks that were stocked with gravel, aerated, and

otherwise equipped as described above. The water temperature was around 20°C. Light : dark cycle was 10:14 hours (light source : Solar, Natur 9000K LT 15 W). The water was changed twice weekly. Animals were fed three times a week with chironomid larvae, fresh carrots, lettuce, and Tetra Wafer Mixfood for ground-living fish and crabs. Eggs were removed either daily or every third day from egg-bearing females and the embryos were examined with a Leica MS 5–Bino stereomicroscope and photographed with a polaroid DC10 digital camera.

To reveal the morphology of the early embryos, specimens were dissected out of the chorion and the yolk was removed. Specimens were then fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (1 h, room temperature) and stained with the nuclear dye bisbenzimide (0.1%, 15 min. at room temperature; Hoechst H 33258), washed in buffer over night, and mounted in Fluoromount (Sigma). Specimens were viewed with a fluorescent microscope (Axioskop). Nomenclature of the morphology of the embryos is according to Sandeman and Sandeman ('91).

RESULTS

Parthenogenesis?

All four individuals that were reared in isolation immediately after they had been separated from their mothers produced eggs (compare Fig. 1) within 245–255 days. The total length of these four females at the first clutch ranged from 40 to 45 mm, their wet weight from 1440 to 2000 mg (see below for more details). Between 20% and 70% of the embryos died, the others developed normally. All animals of this population (about 30 screened) showed female genitalia with gonopores (Fig. 2A) located on the coxae of the third walking limb (pereiopod). All three dissected females showed typical ovaries (Fig. 2B1, B2) but not any characters of a male reproductive system, such as a vas deferens or testes.

Growth experiments

Length and weight increments, i.e. growth of the juvenile animals, depended on the range of temperature, from highest to lowest (Fig. 3). For example, at 25°C, cephalothorax length and total weight increased by 17.5 mm and 1700 mg, respectively, in the course of 150 d, whereas at 15°C these parameters increased by 7 mm and 100 mg, respectively, during the same period of time.

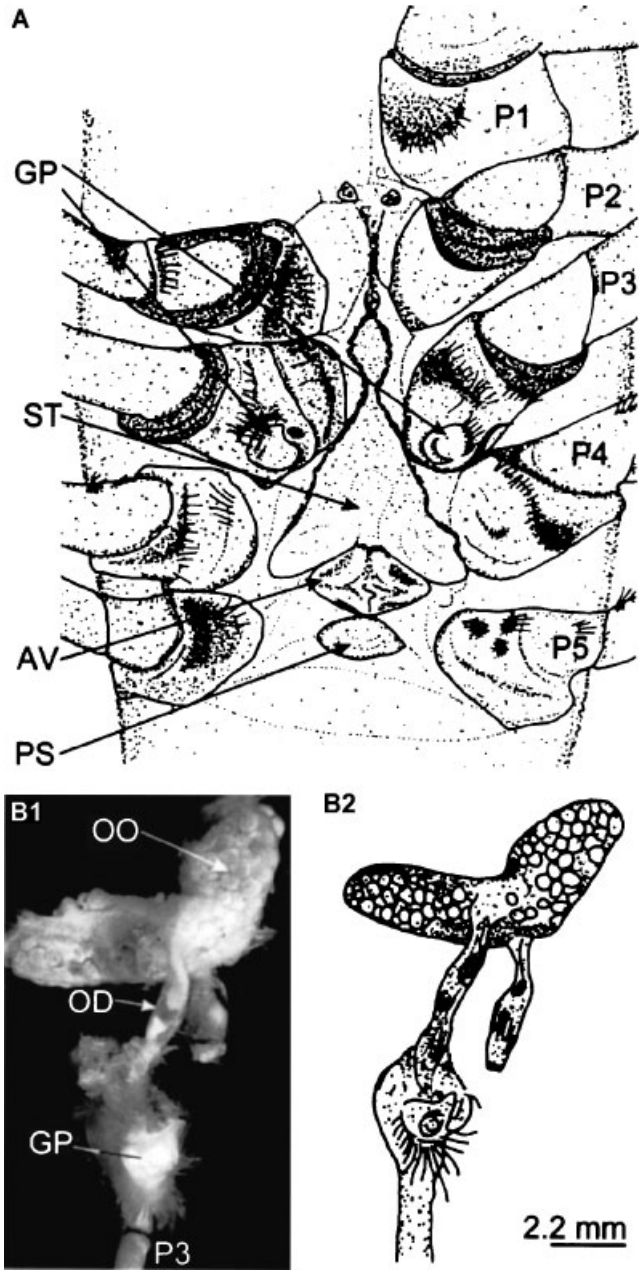

Fig. 2A. Schematic drawing of the ventral aspect of a female showing the genitalia. AV, annulus ventralis, GP: gonopores, P1–5: pereiopods one to five, PS: postannular sclerite, ST: sternum. **B1, B2.** ovary of a female at 56 mm total length. OO: oocytes, OD: oviduct, GP: Gonopore, P3: pereiopod 3.

Weight increased exponentially with length. Photoperiod slightly affected growth at 25°C (Fig. 4); animals grew slightly faster under long-day than under short-day conditions. Mortality was lowest at 20°C and lower under short-day rather than under long-day conditions (Fig. 5). At 20°C under long-day conditions, 80% of the test

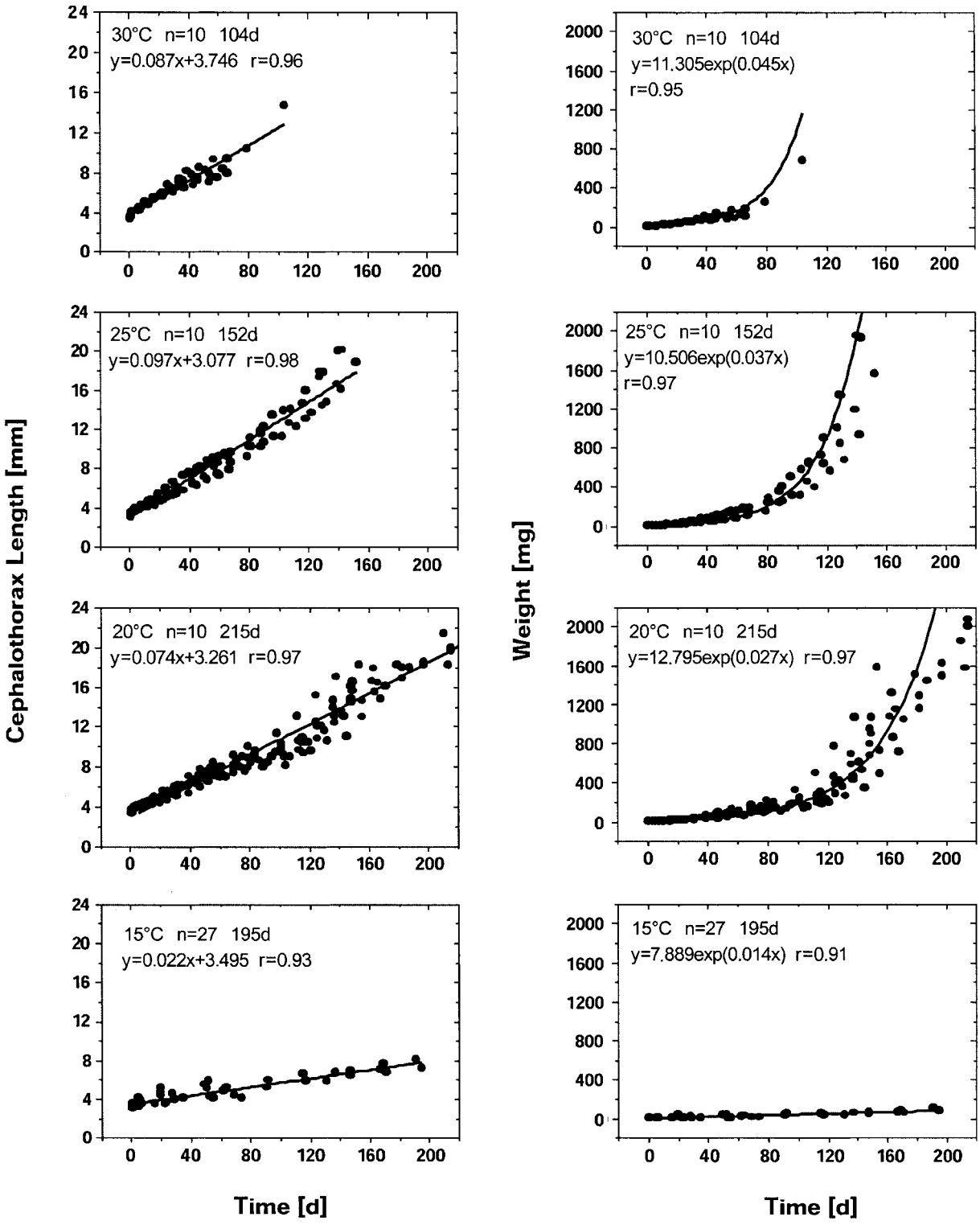

Fig. 3. Growth (increase of cephalothorax length and weight vs time) of the Marmorkrebs at 4 different temperatures.

individuals survived to the end of the experimental period of 200 d, while at lower and higher temperatures 50% had died after 50–80 d. Under short-day conditions at 25°C, 40% survived for 200 d, whereas all individuals had died after 160 d under long-day conditions.

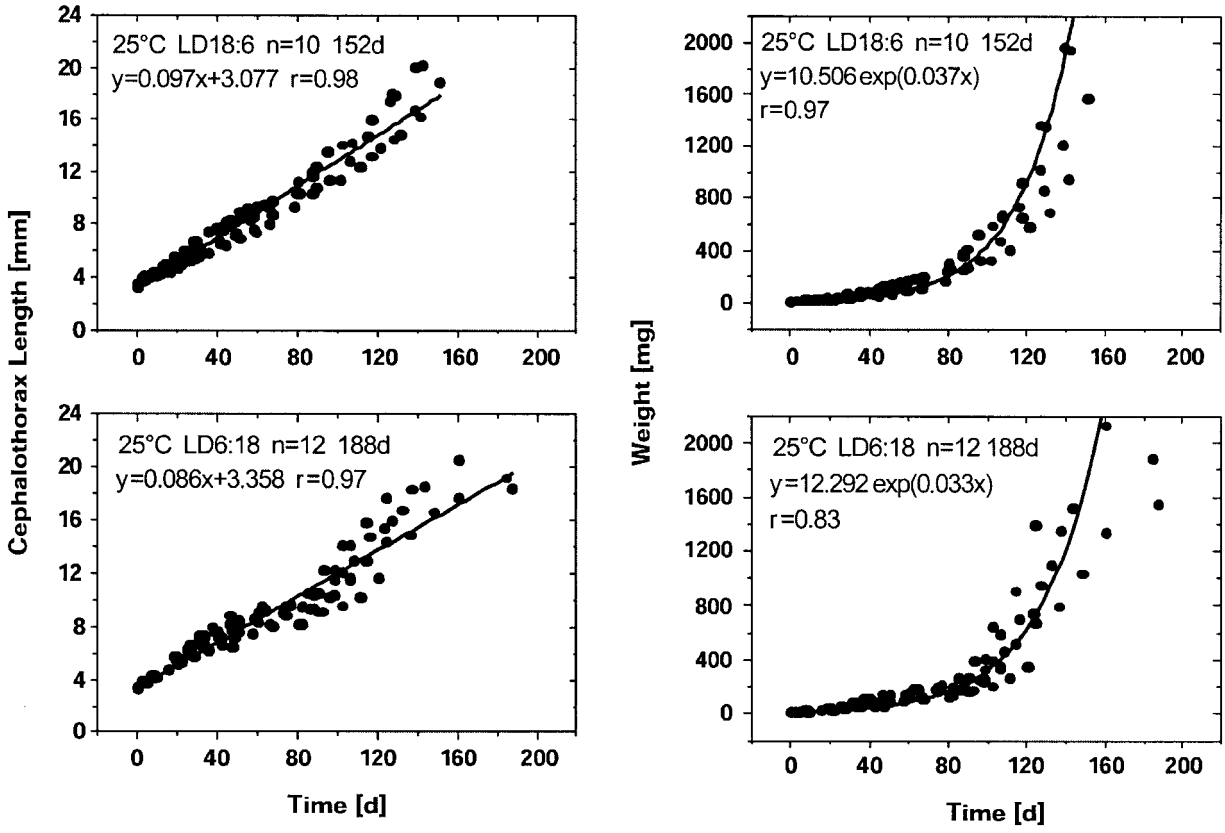

Fig. 4. Growth of the Marmorkrebs at two different photoperiods.

Reproductive parameters

Marmorkrebs females kept at 20–25°C started reproducing at an age of 141–255 d (cephalothorax length of 14 to 21.5 mm, weight of 0.63–2 g; Table 1). Ovigerous females took up only small amounts of food, or in some cases completely ceased feeding. Clutch size of females with a cephalothorax length between 1.4 and 3.5 cm varied between 10 and 416 eggs. However, the specimen with only 10 eggs seems to be an exception; in most cases individuals started reproduction with a minimal number of about 45 eggs. Clutch size increased with the size/age of the mother (Fig. 6). Interclutch periods varied between 50 and 85 d and brooding periods between 22 and 42 d. Animals molted in the interclutch period. Mean intermolt periods were 53 d (SD$\pm$ 38.5; n=49). Mean intermolt periods of reproductive females were significantly longer (t-test: $t=2.8_{(47)}$; $P<0.008$) than intermolt periods of juveniles (62 vs. 30 d). Mean egg diameter was 1.6 mm and mean egg viability 32%. When they left the mother, mean cephalothorax length of juveniles was 3.1 mm, mean weight 5.3 mg (Table 1).

Cold adaptation

The majority of animals exposed to low temperature (8°, 10°C) survived these conditions. Only two of eight individuals died at 8°C; six individuals survived longer than 40 d (i.e. the experimental period) when they where exposed to 8°C and longer than 100 d when they experienced temperatures of 10° to 8°C, respectively. Three specimens moulted at 10°C but none of the females reproduced. The females ceased to reproduce at temperatures of 15°C or below (data not shown).

Embryonic development

In this set of experiments, the brooding period on average was 27 days at 18–20°C. The developmental time span varied considerably (up to 40%) among the experimental animals depending on their size, as well as the number of eggs within a clutch and the number of clutches a particular female had already produced. Furthermore, the embryos from one female did not seem to develop in perfect synchrony. Therefore, duration of embryonic development within the clutch of an

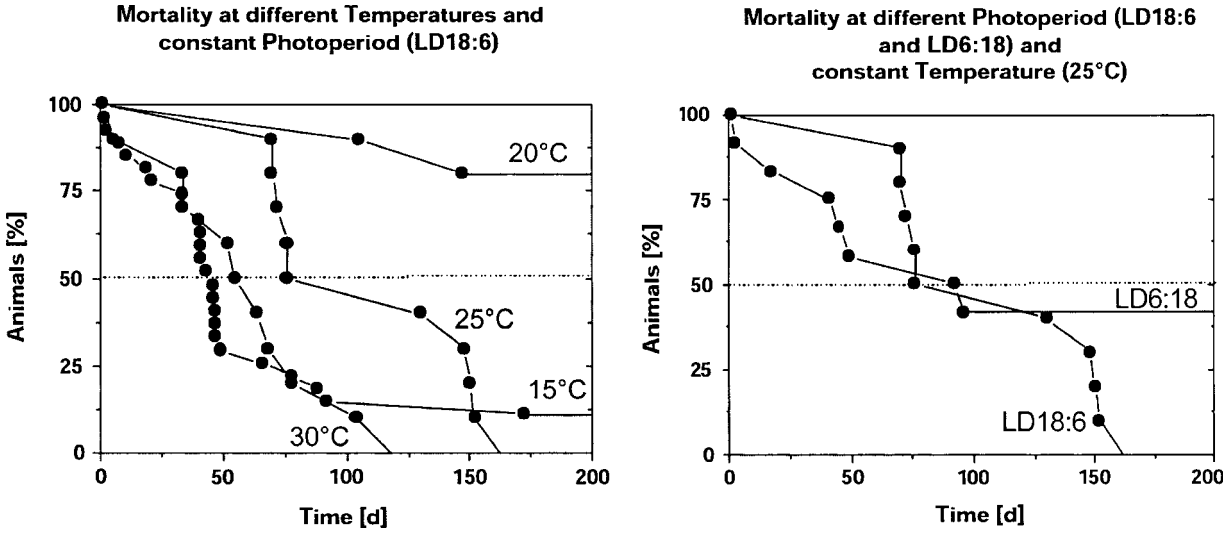

Fig. 5. Mortality of the Marmorkrebs during growth experiments at different temperatures and photoperiods.

TABLE 1. Summary of reproductive characteristics of the "Marmorkrebs" (measured at a temperature of 20–25°C and a photoperiod between 8:16 and 16:8 light:dark), with age, length and weight at first reproduction, and juvenile length/weight after separation from the mother

Parameter	Mean ± SD (n)	Range
Age at first reproduction (d)	210 ± 41.7 (9)	141–255
Cephalothorax length at first reproduction (mm)	18.4 ± 1.87 (9)	14–21.5
Weight at first reproduction (mg)	1537 ± 388.5 (9)	633–2003
Clutch size (no. ov. female^{-1})	–	(10)–416
Interclutch period (d)	67.2 ± 10.6 (9)	50–85
Brooding period (d)	30.7 ± 5.95 (10)	22–42
Egg diameter (mm)	1.62 ± 0.107 (66)	1.5–1.9
Egg viability (%)	32 ± 36.7 (15)	0–88
Cephalathorax length of juveniles (mm)	3.1 ± 0.12 (53)	2.9–3.3
Weight of juveniles (mg)	5.3 ± 0.46 (53)	4.7–5.9

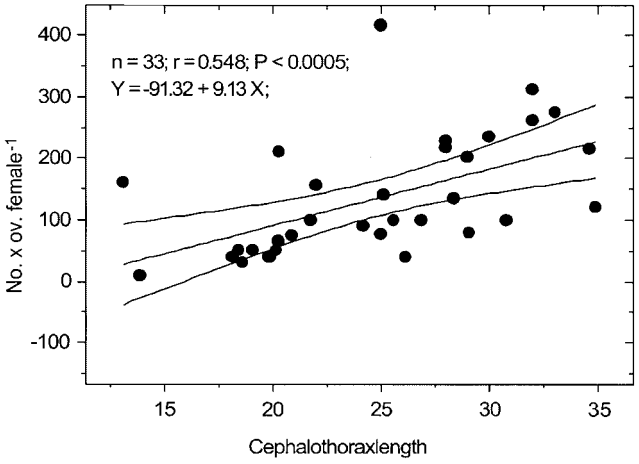

Fig. 6. Relation between clutch size and cephalothorax length (in mm) of the Marmorkrebs.

individual female was averaged and normalized to a percentage scale. For example E32% (32% of embryonic development) denotes an embryo at day 9 after egg deposition from a clutch that on average hatched after 28 days (compare Sandeman and Sandeman, '91).

The newly deposited eggs were uniformly coloured. The yolk was visible through the transparent chorion (Figs. 1, 7). Cleavage in the large yolky eggs was intralecithal. By E7% incipient blastoderm cells containing yolk have appeared at the surface of the eggs and the specimen in Fig. 7 (E7%) represents the 256 cell stage. The number of generated blastomeres increased and the cleavage furrows deepened with repeated cell divisions (Fig. 7, E11%). By E29% gastrulation had begun and the blastoporus was visible at the egg surface as a dark ovoid structure around 440 μm in length. At 32% the blastoporus had enlarged and attained the shape of a water drop. By E36% the embryo was visible as a sheet of milky to transparent tissue (asterisk in Fig. 7, E36%). The yolk of the egg was dark and opaque (Fig. 7, E36%, E39%). Staining an E38% embryo with the nuclear dye bisbenzimide revealed the typical stage of the egg nauplius (Fig. 8A), which

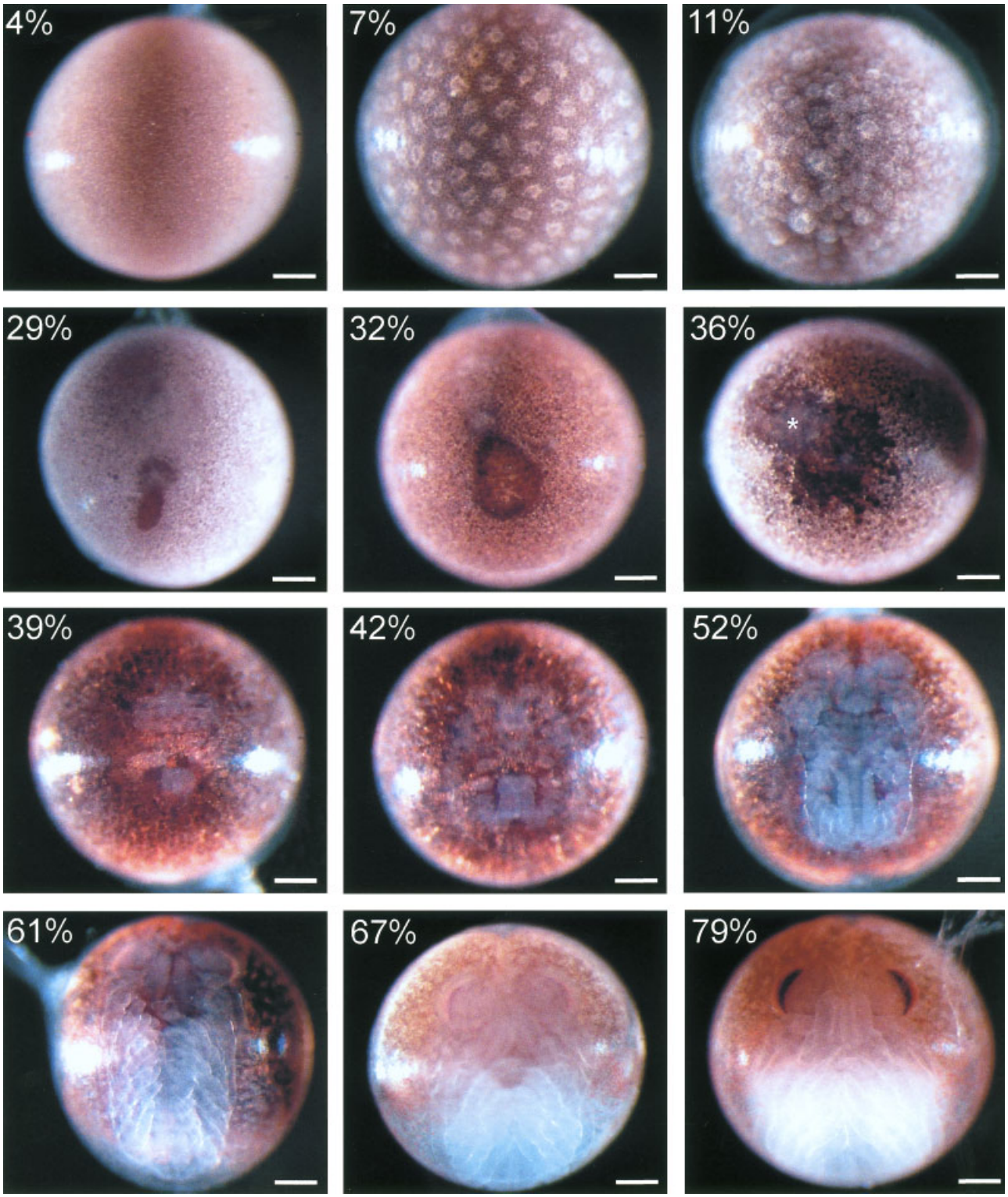

Fig. 7. Embryos of the Marmorkrebs. Percentage values in the upper left corner denote % of embryonic development on a normalised scale. See text for further details. Asterisk in E36% labels the embryonic tissue. Scale bars: 250 μm.

measured around 663 μm in length. At this stage, the optic anlagen, the anlagen of antennae one and two and of the mandible, as well as the caudal papilla were developed as well defined clusters of cells. The anlagen of the second antenna flanked a small invagination, the developing stomodeum.

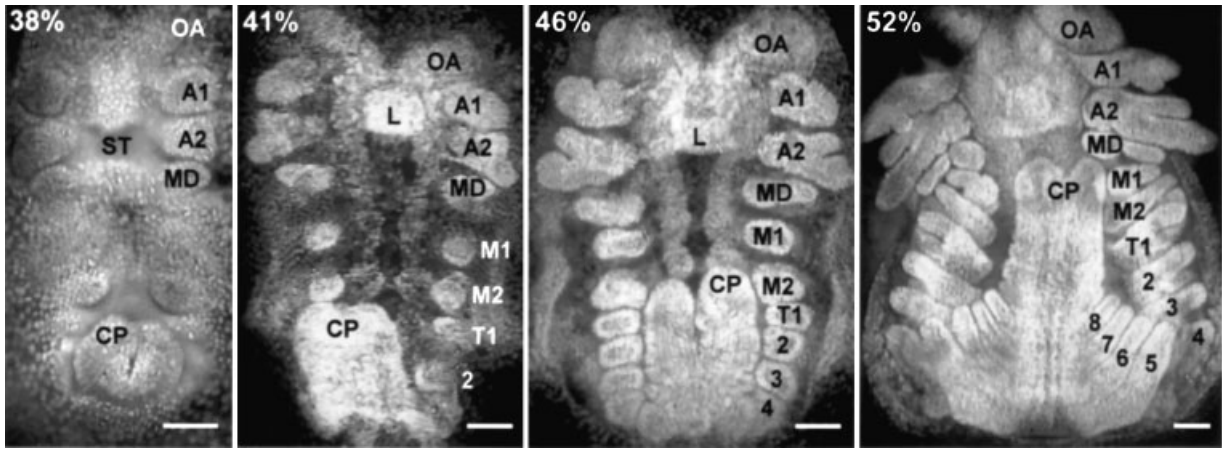

Fig. 8. Dissected embryos of the Marmorkrebs stained with a fluoresent nuclear dye. Percentage values in the upper left corner denote % of embryonic development. Anterior is to the top. Abbreviations: A1, A2: antenna one and two; CP: caudal papilla; L: labrum; M1, M2: maxilla one and two; MD: mandible; OA: optic anlagen; ST: stomodeum; T1–8: anlagen of thoracic limbs one to eight. Scale bars: 100 μm.

The caudal papilla (Fig. 7, E39%) was located close to the closing blastoporus. The mandibles were still slightly shorter and less well-developed than the other two pairs of appendages. By E41% (Fig. 8B) the optic anlagen and the labrum in front of the stomodeum were differentiated more clearly. Limb buds of the first and second maxillae and of the first two maxillipeds were present. The heart started beating at this stage. New segments were generated by a growth zone, which was composed of about 40 large stem cells, the ectoteloblasts (data not shown) located anterior to the caudal papilla. Concurrent with the addition of new segments, the caudal papilla and the growth zone bent anteriorly so that the germ band now elongated in the anterior direction (Fig. 8, B–D). The extraembryonic ectoderm started to develop and grew out to surround the entire yolk. By E46% limb buds were present on thoracomeres one to four and several more anlagen of thoracic segments had been laid down (Fig. 8C). The cells of the newly generated segments were arranged geometrically in transverse rows. The second antenna started to differentiate into two separate branches. By E52% the heartbeat was strong and regular (Fig. 7 E52%, Fig. 8D) and the embryos had grown significantly and achieved a length of around 1200 μm. At the dorsal side of the eggs, the yolk, which was completely enclosed by the extraembryonic ectoderm, was restricted into a left and a right portion, separated by a longitudinal groove. The tissue of the embryos was now opaque. The tissue of the optic anlagen was well differentiated. The first and second antennae pointed caudally (Fig. 8D, E52%). The mandibles, the first and second maxillae, three maxillipeds, and the chelipeds elongated more and more. By E52% (Fig. 8D) all eight thoracomeres were equipped with limb buds and several anlagen of pleomeres were present. The caudal papilla extended forward as far as the first maxillae. By E61% (Fig. 7) the embryos had reached around 1406 μm in length. All segments were present and the growth of appendages had proceeded further. Conspicuous red pigment spots were visible in the developing carapace. Furthermore, the first orange pigment had appeared in the retina of the developing eyes. During subsequent development, the optic anlagen extended successively into a lateral direction (Fig. 8, E67%, E79%) and the retinal pigments became dark brown. The appendages grew considerably while the yolk supply was gradually reduced. Embryos hatched with well-developed eyes, with a full set of segments, and of appendage anlagen (Fig. 9A). This stage is termed the postembryonic stage I (POI; compare with Sandeman and Sandeman, '91) and was still attached to its mother by a ligament extending from its telson. This stage had a paired dorsal ''hunchback'' containing yolk to supply nourishment (Fig. 9B). The postembryonic stage I molted to the postembryonic stage II (POII; Fig. 9D) after one to three days. The postembryonic stage II now was free but still held on to the mother's pleopods (Fig. 9C). Its eyes were stalked. Like the POI, the POII was exclusively nourished by its yolk supply. The POII went through the second postembryonic molt to become a miniature adult crayfish, called

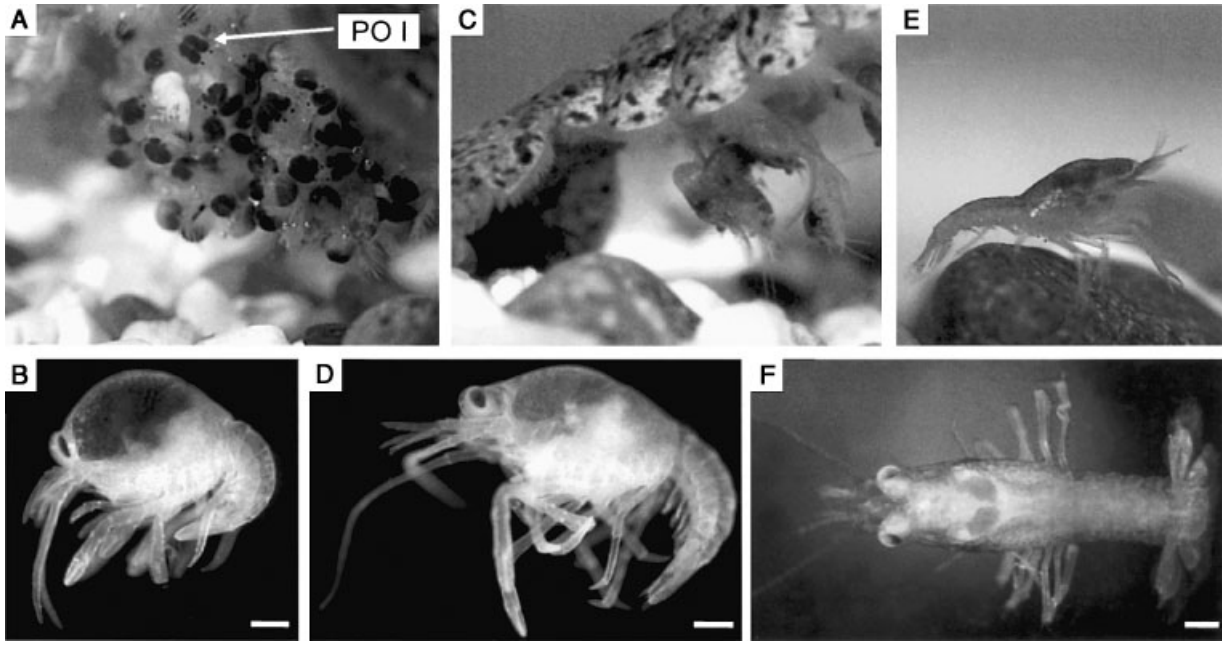

Fig. 9A. Embryos shortly before hatching and newly hatched first postembryonic stages (POI). **B.** Newly hatched first postembryonic stage. **C.** Postembryonic stages II clinging to the mother's pleopods. **D.** Postembryonic stage II. **E, F.** First juvenile stage. Scale bars: 500 μm.

1st immature adult stage (Sandeman and Sandeman, '91) or juvenile (Fig. 9E, F). Individuals left their mothers to obtain food but still sought shelter beneath the mother's pleon.

DISCUSSION

Parthenogenesis

Scholtz et al. (2003) reported that females of the Marmorkrebs laid eggs after having molted, a process that clears out any remnants of sperm from spermathecae. Our observations provide evidence that animals reared in isolation, immediately after their independence from the mother, develop into fertile females and produce viable embryos. Results also confirm that the animals have only female reproductive organs, which most likely excludes hermaphroditism (compare Vogt et al., 2004). Therefore, our results lend additional weight to Scholtz et al.'s (2003) suggestion that the Marmorkrebs has, in fact, a parthenogenetic mode of reproduction and provides the first example for parthenogenesis within the decapod crustaceans. Vogt et al. (2004) suggested that parthenogenesis in this species may have arisen spontaneously or by interspecific hybridisation, and they have investigated the architecture of the ovary to gain more insight into the reproductive strategy of the Marmorkrebs.

Growth

Measurements of the length-weight relationship show that growth in the Marmorkrebs is not isometric. This coincides with the results of Abrahamsson ('71) in *Astacus astacus* and *Pacifastacus leniusculus,* but disagrees with results of, for example, Rodriguez-Serna et al. (2000), who observed isometric growth in *Procambarus llamasi.* Temperature and photoperiod are important determinants for growth and survival of freshwater crayfish (e.g., Mason, '78; Castanon-Cervantes et al., '95). The results of the present study, demonstrating optimal growth conditions between 20–25°C and lowest mortality at 20°C, suggest a preference of the Marmorkrebs for higher temperatures, compared to European species (cf. Bohl, '89; Hager, '96).

Reproduction

Clutch size and egg size of freshwater crayfish vary between and within species (e.g., Pöckl, '98 and other works cited there). European species such as *Astacus astacus*, *Austropotamobius pallipes,* and *Austropotamobius torrentium* produce clutches with 70–200, 50–120, and 50–100 eggs,

respectively, with an egg diameter of 2.2–3 mm (e.g., Pöckl, '98; Hager, '96). The North American crayfish *Orconectes limosus* and *Procambarus clarkii*, which have been introduced into European waters, produce clutches of up to 400–600 eggs (Penn, '50; Müller, '73; Stypinskaya, '78) whereas the Australian species *Cherax quadricarinatus* can produce clutches of up to 1000 eggs (Jones, '95b). The within-species variation of clutch size can be attributed to environmental factors such as food and temperature (Lathi and Lindqvist, '83; Jones, '95; Yeh and Rouse, '95), and to the evidence that clutch size increases with increasing size/age of the mother (Abrahamson, '71; Brewis and Bowler, '85; Skurdal and Qvenild, '86; Rodriguez-Serna, 2000). However, egg size does not increase with the size of the mother (Skurdal and Qvenild, '86), but can depend on clutch size and/or the geographic origin. Lathi and Lindqvist ('83) reported that *Astacus astacus* from Finland carried more but smaller eggs than *A. astacus* from Sweden. There is also great variation between and within species regarding the size and age at maturation and duration of brooding period, which can be attributed to the different geographical ranges and differences in environmental conditions (e.g., Suko, '58; Aiken, '69; Money, '88; Daniels et al., '94; Yeh and Rouse, '95; Gutierrez-Yurrita, '97). For example, free-living *A. astacus* matures at an age of 2–3 years and a size of 62–95 mm (Abrahamsson, '71; Skurdal and Qvenild, '86). By contrast, *Orconectes limosus* matures at an age of approximately one year at a size of 60 mm (Müller, '73). *Procambarus lamasi* and *Procambarus clarki* however, start reproduction at a size of 40–60 mm and an age of approximately 100–120 d when kept at a temperature of 22–26°C (Castanon-Cervantes et al., '95; Rodriguez-Serna et al., 2000). Brooding period is 200–270 d in *A. astacus* and *Austropotamobius pallipes* (Müller, '73; Holdich and Lowery, '88), 150–240 d in *O. limosus* (Müller, '73), 35–40 d in *Cherax quadricarinatus* (Jones, '95a), 27–36 d in *Orconectes nais* and *Procambarus lamasi* (Money, '88; Rodriguez-Serna et al., 2000), 10–30 d in *P. clarki* (Castanon-Cervantes et al., '95; Gutierrez-Yurrita, '97). Juveniles leaving their mothers are between 8–10 mm in *A. astacus* (Pöckl, '98; Pekny and Pöckl, 2000) and 4.5 mm in *O. limosus* (Müller, '73). The longer intermolt periods of adult females compared to those of juveniles may reflect the high energy costs allocated to reproduction.

Results show that reproductive characteristics of the Marmorkrebs resemble those of *Procambarus* species. The Marmorkrebs matures early at small body size; egg diameter is small, brooding period short, and clutch size larger when compared with most European species. These results, together with its parthenogenetic reproduction, point to r-selected traits, which are typical of species living in extreme habitats. Egg viability was low, but within the range of that observed in other crayfish (Rodriguez-Serna, 2000). The low egg viability may be attributed to the fact that many crayfish loose eggs between the time of spawning and hatching of juveniles.

Cold adaptation

The fact that individuals of the Marmorkrebs could withstand low temperatures of 8–10°C for several months suggests that they can live in some European waters and might compete with European species. In fact, there are two recent reports about new field records of the Marmorkrebs in Germany, indicating that this animal can successfully survive and perhaps even reproduce in Europe (Blanke and Schultz, 2003, Marten et al., 2004).

Embryonic development

Due to its high fertility and its rapid embryonic development the Marmorkrebs is an interesting organism for developmental studies, such as examining cell genealogy (Scholtz, '92, '93) or exploring mechanisms of neurogenesis (reviews e.g., Harzsch, 2002; Scholtz and Gerberding, 2002; Harzsch, 2003; Whitington, 2004). The present account establishes a simple staging scheme for the Marmorkrebs by using criteria that are readily observable in living embryos. Therefore, this scheme may serve as a convenient basis for future developmental studies. Furthermore, these results indicate that the sequence of developmental events is very similar in Marmorkrebs embryos and embryos of, for example, the crayfish *Cherax destructor* (Sandeman and Sandeman, '90) or *Astacus fluviatilis* and *Astacus torrentium* (Zehnder, '34A, B), in which ontogeny has been examined in much more detail. For example, preliminary data indicate that germ band elongation in Marmorkrebs embryos is achieved by a number of ectoteloblasts similar to that in other crayfish (Scholtz, '92; '93; Scholtz and Kawai, 2002). Although this aspect warrants a more detailed analysis, results show that cellular developmental processes are similar in Marmorkrebs embryos and postembryonic stages, and those of other Decapoda, so that this animal may be taken

as a valid representative model organism for future developmental studies on crayfish.

ACKNOWLEDGMENTS

The authors gratefully acknowledge H. Wolf and U. Rose for their thoughtful comments on the manuscript.

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