

**Gonad maturity in female
Chinese mitten crab
Eriocheir sinensis from the
southern Baltic Sea –
the first description of
ovigerous females and the
embryo developmental
stage**

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Abstract

This paper describes for the first time the gonad maturity stage of *Eriocheir sinensis* females (carapace width 55.20–78.10 mm) collected in the autumns and winters of 2005–2012 in the Gulf of Gdańsk and Vistula Lagoon (southern Baltic Sea). Seventeen females had gonads in the penultimate stage, which indicates that spawning would shortly take place. Four other females had gonads in the last stage, which means they were already carrying eggs. These accounted, on average, for $17.9 \pm 2.9\%$ of female weight and were in the 3rd and 4th embryo developmental stage. The results show that the low salinity of southern Baltic Sea (≤ 7 PSU) permits mating and fertilization as well as embryo development in *E. sinensis*. It is still not clear, whether such a salinity level will enable hatching and the complete larval cycle.

1. Introduction

The Chinese mitten crab *Eriocheir sinensis* is a well-known non-native species introduced in ballast tanks to European waters almost one hundred

The complete text of the paper is available at <http://www.iopan.gda.pl/oceanologia/>

years ago (Peters & Panning 1939, Gollasch 2006). The largest self-sustained population of this species lives in the River Elbe and its tributaries (Germany). Owing to larval retention as well as the capability of juveniles and adults to migrate long distances, specimens from this population often spread into neighbouring countries (Herborg et al. 2003, Drotz et al. 2010, Czerniejewski et al. 2012). Since 1926 adult mitten crabs have been recorded in the southern Baltic Sea (Peters 1933, 1938), but in larger numbers only in recent decades (Ojaveer et al. 2007). According to Panning (1939) and Veilleux & Lafontaine (2007) sexually mature specimens can live in fresh and brackish waters as well as in the sea, but the eggs and larvae of *E. sinensis* require high salinities (ca 20 PSU) to develop successfully (Anger 1991, Montú et al. 1996). On the basis of genetic studies (Herborg et al. 2007, Ojaveer et al. 2007, Czerniejewski et al. 2012) it is assumed that this species is probably unable to reproduce in brackish Baltic waters and that the crabs living here are only an offshoot of the ‘German’ population. On the other hand, several ovigerous females, planktonic larvae and juveniles of the mitten crab were found recently in the western Baltic Sea (Kiel Fjord and neighbourhood), indicating that the completion of the whole reproduction cycle might be possible (Otto & Brandis 2011). Apart from laboratory experiments on realised fecundity (Czerniejewski & De Giosa 2013) and a brief mention about egg-carrying females (Ojaveer et al. 2007), there is no information concerning the reproduction of *E. sinensis* in the southern Baltic Sea, where the salinity is much lower than in the western Baltic.

Here, we present for the first time data on gonad maturity in *E. sinensis* females from the coastal waters of the southern Baltic Sea. Ovigerous females as well as the developmental stages of the embryos carried are described. The results provide new information on the reproductive activity of the Chinese mitten crab in the brackish waters of the Baltic Sea.

2. Material and methods

E. sinensis females were collected in the years 2005–2008 ($N = 9$) and 2012 ($N = 13$) in the Gulf of Gdańsk and Vistula Lagoon (southern Baltic Sea). The details are given in Table 1. In the laboratory carapace width, length and height were measured with slide calipers (± 0.01 mm), after which females were weighed (± 0.01 g). Then the female gonads were excised and examined under a microscope in regard to the five-scale gonad maturity stages described by Garcia-de-Lomas et al. (2010), where: G1 – no visible oocytes; G2 – oocytes visible on the surface of the gonads; G3 – oocytes forming a compact mass, but are separable from other layers of the gonad; G4 – oocytes forming a soft mass and being easily detachable from the mass; G5 – easily separable eggs, in pleopodal setae of abdomen.

Table 1. Data on the 22 females of *Eriocheir sinensis* sampled in the Gulf of Gdańsk (GG) and the Vistula Lagoon (VL) examined in study (GMS – gonad maturity stage, * – with eggs)

| Female No. | Location | Date | Carapace [mm] | | | Wet weight [g] | | GMS |
|------------|----------|-----------|---------------|--------|--------|----------------|-------|-----|
| | | | width | length | height | female | eggs | |
| 1 | GG | Dec. 2005 | 63.61 | 58.41 | 33.89 | 103.34* | 27.18 | G5 |
| 2 | GG | Dec. 2006 | 56.73 | 53.00 | 29.45 | 70.84* | 12.16 | G5 |
| 3 | GG | Dec. 2006 | 62.33 | 55.87 | 31.37 | 88.70* | 17.00 | G5 |
| 4 | GG | Nov. 2006 | 62.40 | 54.99 | 32.22 | 125.00* | 31.00 | G5 |
| 5 | GG | Jan. 2007 | 57.20 | 50.75 | 31.33 | 76.07 | – | G4 |
| 6 | VL | Oct. 2008 | 60.35 | 55.65 | 30.65 | 88.60 | – | G4 |
| 7 | VL | Oct. 2008 | 60.38 | 53.80 | 31.00 | 90.65 | – | G4 |
| 8 | VL | Oct. 2008 | 63.46 | 58.27 | 31.86 | 106.08 | – | G4 |
| 9 | VL | Oct. 2008 | 64.03 | 57.77 | 32.57 | 110.29 | – | G4 |
| 10 | VL | Nov. 2012 | 64.48 | 57.30 | 34.35 | 120.32 | – | G1 |
| 11 | VL | Nov. 2012 | 55.20 | 53.10 | 28.10 | 75.43 | – | G4 |
| 12 | VL | Nov. 2012 | 57.42 | 51.90 | 29.99 | 78.22 | – | G4 |
| 13 | VL | Nov. 2012 | 58.78 | 54.80 | 29.72 | 90.04 | – | G4 |
| 14 | VL | Nov. 2012 | 59.40 | 62.40 | 32.30 | 102.74 | – | G4 |
| 15 | VL | Nov. 2012 | 60.08 | 53.46 | 30.31 | 80.23 | – | G4 |
| 16 | VL | Nov. 2012 | 60.08 | 56.35 | 30.33 | 95.31 | – | G4 |
| 17 | VL | Nov. 2012 | 60.28 | 54.87 | 30.64 | 101.84 | – | G4 |
| 18 | VL | Nov. 2012 | 65.70 | 58.55 | 34.47 | 128.36 | – | G4 |
| 19 | VL | Nov. 2012 | 67.18 | 61.29 | 33.52 | 131.28 | – | G4 |
| 20 | VL | Nov. 2012 | 68.07 | 62.05 | 37.56 | 141.58 | – | G4 |
| 21 | VL | Nov. 2012 | 68.80 | 61.30 | 37.10 | 129.57 | – | G4 |
| 22 | VL | Nov. 2012 | 78.10 | 74.4 | 39.80 | 164.17 | – | G4 |
| Mean | | | 62.46 | 57.29 | 32.39 | 104.48 | 21.84 | |
| ±SD | | | 5.09 | 5.05 | 2.88 | 24.52 | 8.75 | |

In the case of G5 females eggs were extracted after the female had been weighed, after which the female was reweighed without eggs. The weight of eggs was determined by subtracting the mass of the female without eggs from the mass of the female with eggs on pleopods.

Eggs extracted from gravid females were examined under a microscope at x1, x2, x4 and x6.3 using reflected light. Digital photographs of eggs were taken with a DS-Fi1 5.0-megapixel digital camera (Nikon, Japan). Embryonic development was defined according to the embryo development scale for the Chinese mitten crab given by Peters (1938) and for the blue king crab *Paralithodes platypus* given by Stevens (2006).

Results were expressed as a mean with standard deviation (mean ± SD). The relationship between female carapace width and eggs wet weight was

determined by linear regression analysis ($y = ax + b$) with a coefficient of determination R^2 for a significance level $P < 0.05$.

3. Results

The carapace width of females ($N = 22$) collected in the Gulf of Gdańsk and Vistula Lagoon varied from 55.20 to 78.10 mm (mean 62.46 ± 5.09 mm). Detailed information on size, weight and gonad maturity stage of all the Chinese mitten crab females are given in Table 1. Most of the females ($N = 17$) were in the G4 gonad developmental stage; only four females had eggs on pleopods, thereby belonging to the G5 gonad developmental stage. The gonad maturity stage was not correlated with female carapace width.

The wet weight of eggs ranged from 12.16 to 31.00 g (mean 21.84 ± 8.75 g), which accounted for $17.9 \pm 2.9\%$ of the egg-carrying female weight on average. Eggs wet weight (EW) was significantly correlated ($P < 0.05$, $R^2 = 0.58$) with female carapace width (CW) according to the equation $EW = 2.16 CW - 110.78$. On the basis of photographs (Figure 1) it was found that extracted embryos were between the initial phases of the 3rd and 4th developmental stages, and characterised by a lack of visible cells and structures. The embryonic lobes would probably become visible in the following days.

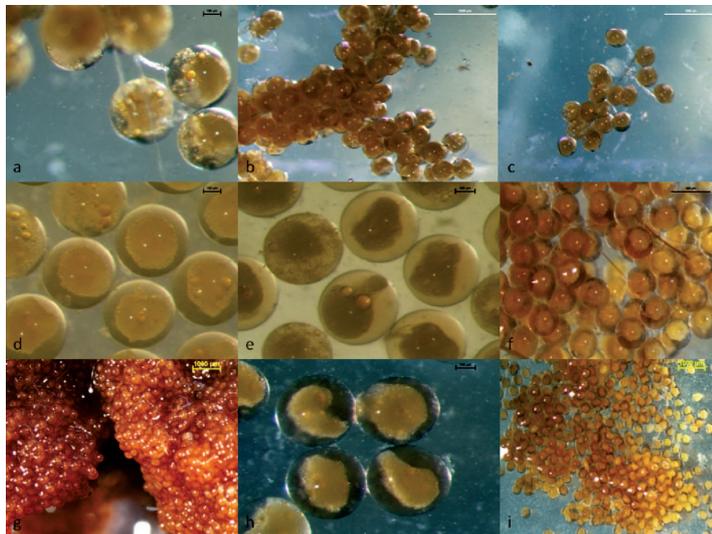


Figure 1. *Eriocheir sinensis* G5 embryo development. Eggs extracted from female: No. 1 (a, b, c), No. 2 (d, e), No. 3 (f, g) and No. 4 (h, i). Magnification: x6.3 (a, d, e, h), x4 (f), x2 (b, c), x1 (g, i)

4. Discussion

Based on the gonad maturity stage it is assumed that the females were shortly before (stage G4) or after (stage G5) copulation and the eggs were in the 3rd and 4th embryo development stage. According to Stevens (2006) the 3rd embryo stage in the blue king crab lasts about 114–156 days after copulation, whereas the 4th stage lasts about 157–170 days. Thus, based on the sampling time (November/December) as well as on the embryo development stages one can assume that the examined egg-carrying females had copulated at least 3 months previously. The eggs were tightly attached to the female pleopods and extracting them for analysis was time-consuming. This is rather surprising, because the gravid females were collected at a salinity of 7 PSU. According to Peters (1938) and Panning (1939) the ‘cement-like’ substance that attaches the eggs to the egg-carrying setae does not harden at salinities lower than 14 PSU and females lose their eggs. Although Peters (1938) conducted some successful laboratory experiments with egg-carrying females at 6.5 PSU, to date no evidence of such a situation in a natural environment has been forthcoming. Moreover, there is no information in the literature concerning egg-carrying females of *E. sinensis* found in other brackish waters. For example, in the Guadalquivir Estuary (Spain), where the salinity is 5 PSU at the time of reproductive migration, only immature females in stages G2 and G3 were caught (García-de-Lomas et al. 2010). The collection time of females in gonad maturity stages G4 and G5, i.e. in autumn and winter, is also characteristic of the reproduction cycle of *E. sinensis*. According to Peters (1938) and Anger (1991), copulation in European populations of this species takes place in autumn. Afterwards ovigerous females migrate to the sea where they bury themselves in the bottom to overwinter.

The carapace width of the females was relatively large and similar to that recorded in other waters, e.g. in the River Elbe, the Volga and the Tagus Estuary or even in the waters of North America (Cabral & Costa 1999, Herborg et al. 2003, Rudnick et al. 2003, 2005, Ruiz et al. 2006, Shakirova et al. 2007). Larger females carried a significantly greater mass of eggs on their pleopods than smaller ones. Such a relationship was reported by Czerniejewski & De Giosa (2013). According to these authors the fecundity of *E. sinensis* female ranges from 141 100 to 686 200 eggs and is much larger than for other grapsid crabs. However, other authors state that females can produce up to one million eggs (Panning 1939, Cohen & Weinstein 2001, Veilleux & Lafontaine 2007). Since the Chinese mitten crab breeds only once in its lifetime, high female fecundity is one of the keys to successful invasion.

The most significant limiting factor where egg hatching is concerned is low salinity (Panning 1939, Otto & Brandis 2011); however, as shown by Anger (1991), tolerance to this factor increases with temperature. Thus, gravid females usually wait until summer or they move to shallow waters, where temperatures become optimal for hatching, i.e. 15–25°C (Ingle 1986). On the other hand the optimum salinity for hatching and complete larval development is 20 PSU (Panning 1939, Anger 1991, Montú et al. 1996, Dittel & Epifanio 2009). This is much more than in the southern Baltic Sea, where the salinity is ca 7 PSU (Leppäranta & Myrberg 2009). Taking into account the fact that summer temperatures in the Baltic are in the 18–22°C range, it might be assumed that these conditions do not fit the requirements for the proper larval development of *E. sinensis*. It was previously speculated that the Baltic Sea is only a migration area for Chinese mitten crabs, which reproduce in the Elbe Estuary/North Sea or in the Kattegat/Skagerrak (Normant et al. 2000, Normant & Chrobak 2002, Ojaveer et al. 2007). This assumption was supported both by the lack of larvae and juveniles, as well as by genetic studies that showed a similarity between specimens from the southern Baltic Sea and from German rivers (Żmudziński 1961, Herborg et al. 2007, Czerniejewski et al. 2012). On the other hand it was recently reported by Otto & Brandis (2011) that *E. sinensis* may well reproduce in the western Baltic Sea. As already mentioned in the ‘Introduction’, these authors found both gravid females and larvae and juveniles in Kiel Fjord and in the eastern Kiel Canal, where the salinity is 12–30 PSU. It is assumed that this population may be a donor area for the crabs found in the southern and eastern Baltic Sea.

Based on these studies it might be assumed that females of *E. sinensis* follow a regular life cycle in the southern Baltic Sea, reaching sexual maturity, copulating and placing eggs on pleopods. But it is not clear whether the eggs undergo complete development and hence, whether the Chinese mitten crab is able to reproduce in the southern Baltic Sea. On the one hand there is no evidence of any larval stages, but this may be due to the lack of appropriate zooplankton studies (i.e. the use of inappropriate sampling gear at the wrong time and/or place). On the other hand, the latest studies of Otto & Brandis (2011) have shown that there is probably a chance of the larval cycle reaching completion in the Baltic Sea, because *E. sinensis* larvae can live and develop in extreme conditions as far as their physiology is concerned. Moreover, non-native species evolve quickly and are able, even in the short term, to adapt to new conditions, which may significantly differ from those in their native regions (Sax & Gaines 2003). A spectacular example is the calanoid copepod *Eurytemora affinis*. During one century the evolution of ionic regulation in this Atlantic species has

enabled it to colonise fresh waters in North America (Lee et al. 2007, Lee & Gelembiuk 2008). *E. sinensis* has inhabited the southern Baltic Sea for almost 100 years and maybe this species too, with its high phenotypic plasticity, has evolved mechanisms which in the age of global warming enable larvae to tolerate less saline waters. To confirm these assumptions more detailed studies are required: in the environment (a search for larvae) and in the laboratory (on selection response).

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