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First evidence of *Eriocheir sinensis* reproduction from Schleswig—Holstein, Northern Germany, western Baltic Sea

Thurid Otto[1]* and Dirk Brandis[2]

[1]*Zoological Institute, Christian-Albrechts-University Kiel Olshausenstrasse 40, 24118 Kiel, Germany*
[2]*Zoological Museum of the Christian-Albrechts-University Kiel Hegewischstrasse 3, 24105 Kiel, Germany*

E-mail: totto@zoologie.uni-kiel.de (TO), brandis@zoolmuseum.uni-kiel.de (DB)

**Corresponding author*

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Abstract

Eriocheir sinensis invaded the Baltic Sea over 80 years ago, but there is still little known about mitten crab reproduction in this region. According to present knowledge, reproduction does not occur in the central, eastern and northern parts of the Baltic Sea due to the low salinity present. Sufficient salinity levels for reproduction of the Chinese mitten crab may exist in the western part and in the Kattegat, but published evidence of reproduction is still lacking from the region. Recently however a total of 62 ovigerous females were collected in the eastern part of the Kiel Canal (connecting the North Sea with the Baltic Sea) close to the Kiel Fjord as well as in the Kiel Fjord itself (Schleswig-Holstein, German Baltic Sea coast). The eggs from some of these females were hatched in the laboratory under 'Kiel Fjord salinity conditions' and reared to third zoeal stage. Furthermore, larvae were found in plankton samples from the Kiel Fjord and juvenile crabs were present in a lake close to this watershed.

Key words: invasive alien species; catadromous life cycle; Kiel Fjord; Kiel Canal; salinity

Introduction

The native range of Chinese mitten crab *Eriocheir sinensis* (H. Milne-Edwards, 1853) (Crustacea, Decapoda, Varunidae) reaches from the eastern pacific coast of China to the Korean Peninsula. The species was likely introduced to northern Germany in the early 20th century with ballast water, the first evidence of this invasive species was a male specimen found in the River Aller during 1912 (Peters and Panning 1932). In a relatively short period of time, the Chinese mitten crab established self-sustaining populations mainly in the Elbe and Weser river systems (Boettger 1933; Marquard 1926; Peters and Panning 1932; Peters 1938). From there the species spread out across a large part of northern Europe including almost the entire coastal Baltic Sea and its adjacent rivers (Bacevičius and Gasiūnaitė 2008; Herborg et al. 2003; Normant et al. 2000; Ojaveer et al. 2007; Panning 1938; Panov et al 2003; Peters 1938), where it is now a common by-catch, albeit in much lower numbers than in adjacent rivers of countries bordering the North Sea. The Chinese mitten crab has a catadromous life cycle and presently it is generally agreed, that the German population of *Eriocheir sinensis* can only reproduce successfully in the North Sea due to the appropriate salinities - between 15 close to the river mouths and 32 offshore (Cohen and Weinstein 2001 and references therein). Anger (1991) and later also Montú (1996) reared larvae from ovigerous *Eriocheir sinensis* females dredged in the southeastern North Sea and described 1 praezoea, 5 zoea stages and 1 megalopa. Anger (1991) showed that every stage had a different tolerance in terms of temperature and salinity. From the first until the last stage, the larvae become increasingly stenohaline with an optimum in marine salinity. The survival rate of the larval stages was the highest at temperatures of ≥12°C, whereas the possible salinity range for a successful development extended at increasing temperatures. With decreasing temperature the tolerance to lower salinities also decreased and developmental time increased. In summary, the critical stages in the life cycle - embryonal and larval - require relatively high salinity levels. In contrast,

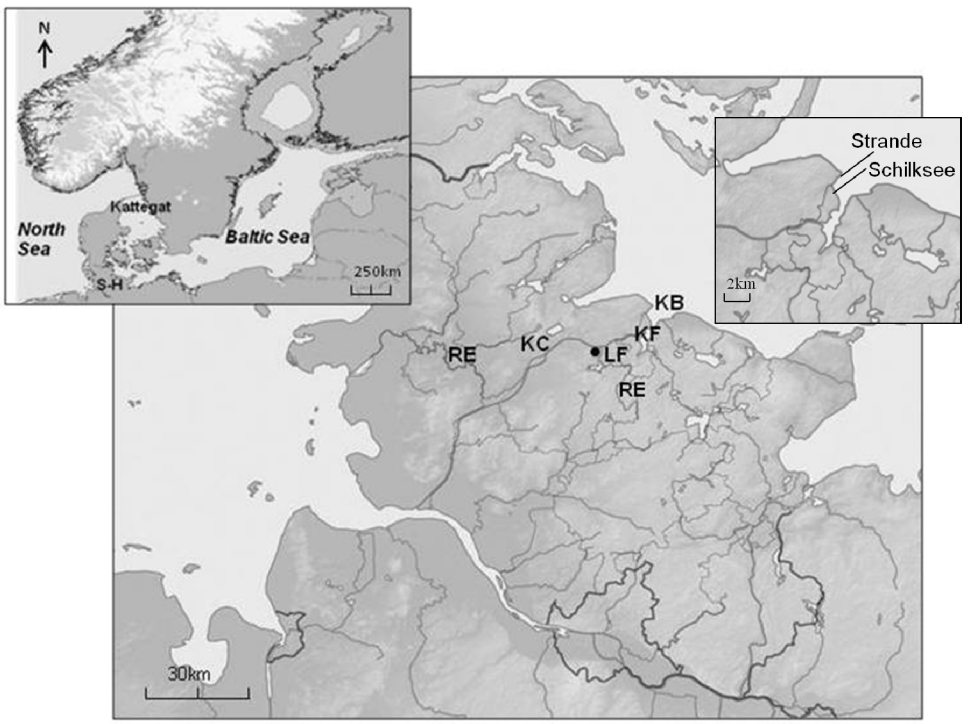

Figure 1. The present study was conducted in Schleswig-Holstein (S-H), Northern Germany, at the Baltic Sea coast (Baltic Sea map by courtesy of R.Sommer, Ecology Center Kiel). In the map of Schleswig-Holstein and in the enlargement the places of *Eriocheir sinensis*-catches are shown. KB=Kiel Bight, KC=Kiel Canal, KF=Kiel Fjord, LF=Lake Flemhude, RE= River Eider.

gonadal development, development of the megalopa, juveniles and adult growth occur in freshwater (Cohen and Weinstein 2001 and references therein). With regard to mitten crab reproduction in the Baltic Sea a number of studies have reported this as unlikely (Cohen and Weinstein 2001; Gollasch pers.comm. cited in Cohen and Weinstein 2001; Haathela 1963; Rasmussen 1987) as the salinity in this sea ranges between 1–7 in the east and north and 9 – 20 in the western part (Rönnberg and Bonsdorff 2004). In contrast, salinity in the Kattegat (Figure1) is about 12–30 at the surface and 32–34 at the bottom (Rönnberg and Bonsdorff 2004). However, reported numbers of collected male and female Chinese mitten crabs have increased from the northeastern part as well as the western part of the Baltic Sea during the last decade (e.g. Ojaveer et al. 2007 and references therein), but there have been no published data with regard to larvae and juveniles.

The purpose of this study is to present the first evidence of *Eriocheir sinensis* successfully reproducing off the German Baltic Sea coast.

Methods

The study area was the Kiel Fjord and the Kiel Bight in Schleswig-Holstein, Northern Germany (Figure 1). From April to August 2009 marine plankton sampling was vessel-based. Plankton nets of 25µm and 55µm were used for vertical net tows at different depths. The towage time was 5 minutes with a speed of 1 knot. In total, 110 samples were taken. In addition, a total of 40 plankton samples from the Institute for Marine Science, Kiel University (IFM-GEOMAR) were analyzed. These were sampled weekly (spring and summer 2009) with a 25 µm-plankton net in the Kiel Fjord and in the eastern Kiel Canal. The plankton samples were fixed with 4% seawater-formaldehyde in 200ml containers. From September to December 2007–2009 Chinese mitten crabs were collected by a fisherman as by-catch in standard fish traps in the Kiel Canal close to the Fjord. From the end of April until the end of June 2009 fish traps set in the Kiel Fjord by IFM-GEOMAR were checked 1–2 time per week for crabs. These traps were set at the

footbridge of the institute to catch fishes and crabs for the aquarium, for courses and public events. During the study period, a total of 62 ovigerous females were collected as by-catch in the Kiel Canal and 9 gravid females from the traps in the Kiel Fjord. The ovigerous female caught at the end of April 2009 has been deposited at the Zoological Museum Kiel under accession number ZMK CR2911. The obtained gravid females in May and June were kept in aquaria in the laboratory of the Zoological Museum Kiel. The aquaria were filled with seawater from the Kiel Fjord with a salinity of about 14. The water temperature was kept between 7–11°C to simulate the conditions found in the Kiel Fjord during spring and subsequently at 12–15°C as during early summer. The process of hatching was observed and the larvae were photographed. After the larvae had hatched, an initial number of 70 specimens were transferred into 100ml glass vials with wide-bored pipettes. The rearing was conducted as described in the study of Anger (1991), but using the sea water of the Kiel Fjord (see above). The larvae were fed with freshly hatched *Artemia* sp. nauplii and the water was changed every day. In addition to marine sampling, juvenile crabs were collected at a lakeside ca. 11 km from the Kiel Fjord, connected with the Kiel Canal (Lake Flemhude, Figure 1) from spring until summer 2008–2009.

Results

From the eggs of the ovigerous females collected in spring, 3 different larval stages were reared in the laboratory. On average, about 50% of larvae hatched moulted to zoea II, about one-third moulted to zoea III (Table 2). None of the zoea III survived. The eggs on females caught in early summer were mostly damaged or infected by fungi and none of the few hatched larvae survived. Seven zoea of *Eriocheir sinensis* were found in the plankton samples caught at two sample places in the Kiel Fjord where it merges into the Kiel Bight: 4 zoea were collected on 25 May 2009 at Strande and 3 zoea were found in a sample from Schilksee taken on 11 June 2009 (Figure 1, Table 1). The detailed morphological comparison with the reared larvae and the comparison with the study of Montú et al. (1996) confirmed, that the larvae caught in the Kiel Fjord were zoea II *Eriocheir sinensis* larvae. In search for juvenile crabs close to the Kiel Fjord, exuviae of juvenile crabs were found in June 2008 and May 2009 at the lakeside of Lake Flemhude (Table 1). All these findings represent the first registered records of ovigerous females and larvae in the Kiel Fjord and juveniles close to the Kiel Fjord. Together with the reared larvae in the laboratory the sampled specimens provide evidence that the full life cycle of *Eriocheir sinensis* may take place in the eastern Kiel Canal and the Kiel Fjord and therefore suggest that *Eriocheir sinensis* is successfully able to reproduce in the western Baltic Sea.

Discussion

The high numbers of ovigerous females in the eastern Kiel Canal during autumn provides evidence for a migration into the Kiel Fjord followed by mating. Furthermore, the periodical presence of ovigerous females in the Kiel Fjord during spring demonstrates active migration behaviour into the Kiel Fjord for hatching. The presence of larvae in plankton samples as well as the reared larvae in the laboratory appears to indicate successful hatching and development processes in spite of the low salinity of the Baltic Sea. Finally, the occurrence of small juvenile crabs in Lake Flemhude close to the Kiel Fjord in May and June provides further evidence to support the hypothesis for successful development of *Eriocheir sinensis* in the Kiel Fjord. Though larvae could be reared only until the third stage, all relevant findings and observations concurrently indicate that successful reproduction of the species in the western Baltic Sea is likely.

In general, salinity is the most limiting factor for a successful egg hatching and larval development of marine brachyuran crabs. According to Buhk (1938) a salinity of about 23 for hatching of *Eriocheir sinensis* larvae is required. Observations in these laboratory experiments included the hatching of eggs in Baltic Sea water with only 14–16 and furthermore a significant amount of prezoeas developing successfully to the zoea I stage. This contradicts the results of Buhk (1938) and shows that the possible salinity range for hatching appears to be larger than assumed so far. In contrast to the estuaries of the North Sea influenced by tidally fluctuating salinities, the Baltic Sea has quite constant salinity conditions. This can be an advantage even when the salinity is suboptimal. The laboratory experiments in Kiel Fjord seawater with salinity close to the lower limit (14–16) together with low temperatures (< 12°C in the fjord during spring)

Table 1: Relevant data for the specimens sampled. Sex and Museum accession number could not be given for every specimen.

Stage	Location	Coordinates	Record dates	number	sex	Museum number
Adult	Kiel Canal	54°20′36″N, 09°58′10″E	Sept.-Dec.2007-2009	62	♀	–
Adult	Kiel Inner Fjord	54°19′46″N, 10°08′58″E	April-June 2009	9	♀	ZMK CR 2911
Larvae	Kiel Outer Fjord/Strande	54°26′20″N, 10°11′51″E (starting point of towage)	May 2009	4	–	ZMK CR 4000
Larvae	Kiel Outer Fjord/Schilksee	54°25′45″N, 10°11′19″E (starting point of towage)	June 2009	3	–	ZMK CR 4001
Juvenile	Lake Flemhude	54°20′26″N, 09°57′52″E	June 2008, May 2009	2	–	ZMK CR 2912, 2913

Table 2: Relevant data for the larvae reared in the laboratory. The larvae were reared from ovigerous females caught in the Kiel Fjord in spring 2009. The eggs of the females caught in early summer were all infected by fungi.

Sampling date of ovigerous females	Initial Number of hatched larvae	Number of zoea1	Number of zoea2	Number of zoea3
29.4.2009	70	67	45	32
09.5.2009	70	64	39	24
09.5.2009	70	65	34	30
20.5.2009	70	61	33	22
22.5.2009	70	58	23	9
04.6.2009	–			
11.6.2009	–			
19.6.2009	–			

may explain the high mortality rates of larvae and the lack of success at development beyond zoea III. Cieluch et al. (2007) showed that the early larval stages of *Eriocheir sinensis* females dredged in the North Sea have a strong regulatory capacity. Hence, these stages are able to cope with the low salinities in estuaries, where the females are hatching (Cieluch et al. 2007; Rudnick et al. 2003). The subsequent larval stages, which are presumably transported to areas with higher salinities in the North Sea, keep the ability to hyper-osmoregulate, but on a lower level (Cieluch et al. 2007). Therefore they also could cope with low salinities but with a higher mortality rate. Ecological flexibility of *Eriocheir sinensis* larvae is further supported by findings of Anger (1991) and Montú (1996). Both observed that *Eriocheir sinensis* larvae exhibit a unique adaptation to produce an additional stage under unfavourable salinity conditions (zoea stage VI).

The present study suggests that the whole reproduction cycle of *Eriocheir sinensis* may take place in the western part of the Baltic Sea. However, against the background of the physiological investigations of Anger (1991) and Cieluch et al. (2007), these findings may indicate that *Eriocheir sinensis* reproduces in the western Baltic Sea, on an extreme physiological margin of the developmental range, resulting in subsequent high larval mortality.

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