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Realized Fecundity in the First Brood and size of Eggs of Chinese Mitten crab (*Eriocheir sinensis*)- Laboratory Studies

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Abstract

The Chinese mitten crab was collected from north part of Szczecin Lagoon (The Odra river estuary) within 12 October–6 December 2008 and 5 October–3 December 2010, to investigate some reproduction aspects in laboratory. This study was carried out to estimate the fecundity during oviposition period in first brood of Chinese mitten crab, eggs size and describe relation to various morphometric characters such as carapace width and height and body weight. The results showed that the fecundity of Eriocheir sinensis range from 141100 to 686200 eggs (mean 461100 eggs per female) with first brood, and mean eggs size were 371,4 µm (range 361 - 375µm). All the morphometric characteristics studied showed positive correlation with fecundity and the degree of relationship varied considerably. Among these, the carapace width was found to be the best morphometric character for predicting the fecundity of Chinese mitten crab.

Keywords: Chinese mitten crab, realized fecundity, morphometric characters, size of eggs.

Introduction

The study of fecundity of any species is important to have a full understanding of its biology and population dynamics. Fecundity is in general used to describe the number of eggs produced by an individual female[1]. However, a more complete definition has been given by Corey[2] and Stechey and Somers[3] for crayfish, who defined fecundity as: i. potential fecundity, that is the number of oocytes counted in the ovaries; ii. realized fecundity, that is the number of eggs actually extruded and attached to pleopod; and iii. actual fecundity, which refers to the number of juveniles hatched.

Fecundity of crabs varies from species to species and also varies within the same species due to different factors such as age, size, nourishment, ecological conditions of the water body[4,5,6].

In crabs, studies on the relationship between size of some morphological features and fecundity are scanty. Positive correlation between carapace width and fecundity was reported for crabs such as *Paralithodes camtschatica*[7], *Clibanarius antillensis*, *C. sclopetarius* and *C. vittatus*[5] and *Carcinoplax vestita*[8]. Similar relationship has been observed between carapace length and fecundity in *Platyxanthus patagonicus*[9].

According to the Global Invasive Species Database, the Chinese mitten crab is one of the 100 "World's Worst" invaders. Because of the profound economic and ecological effects of the invasive populations in Europe and in the San Francisco Bay area, there is much concern about the potential impacts of this species in Europe and along the east coast of the USA[10,11]. Despite its potential commercial importance as a billion dollar aquaculture species in China[12], and a global history of aquatic invasion[13], the scientific literature on the fecundity, reproduction and relationship between the morphological features and fecundity is still limited. Only Kobayashi and Matsura[14], and Kobayashi[15] gave some interesting data about reproduction in *E. japonicus* (genus *Eriocheir*).

The present study was carried out to investigate realized fecundity in the first brood of Chinese mitten crab and its relationship to various morphometric characters such as carapace width and height and body weight.

Material and Methods

A total of 259 Chinese mitten crabs were caught as a by- catch in fyke nets, within 12 October – 6 December 2008 and 5 October – 3 December 2010 in the north of Szczecin Lagoon (figure 1). Crabs were sexed, resulting in 115 males and 144 females. Live crabs were delivered to the laboratory and weight (W) was measured to 0.1 g on an Axis B2000B (Axis Co., Poland) electronic scales. Carapace width (CW) and height (CH) were measured to 0.01 mm, with computer–interfaced electronic callipers. The collected animals were divided into four separate holding (1000l) tanks for both sexes at salinity 15 ppm, and temperature 8-12^{0}C. After 14-21 days, a pair of crabs (female and male) were transferred from tanks to smaller aquaria (40 x 40 x45 cm) containing seawater at salinity 25 ppm. The animals were maintained at a 12 h:12 h light-dark cycle[16]. The water quality in the tanks and aquaria was maintained using a flow-through system (2L/min) and gentle aeration. Observations were made over a 60 min. period, and types of mating interaction were noted[16]. When crabs had no

physical contact, or had contact without mating after 5 days, female and male were changed. After mating males were replaced from aquaria, and females were monitored for spawning six times a day. Eggs were removed from the pleopods of ovigerous female using a soft-hair brush, and the collected egg mass was preserved. Fecundity (FEC) was estimated by the gravimetric method[17], using a subsample of 5% by weight of the egg mass. A sample of 5 eggs was taken from different regions of pleopods, and the egg diameters (ES) were measured using an ocular micrometer scale under a microscope stereoscope.

An explorative data analysis process was applied to data to investigate the presence of outliers and the type of relationships between variables.

The relationship between FEC and each in turn of the explanatory variables CW, CH and W was modeled by fitting normal linear regression to data. For each model, significance of the regression line slope was verified using the t-test procedures[18]. Models with different explanatory variable were compared using the determination coefficient r-squared: bigger the r-squared, better the model.

A graphical validation process[19] was applied to the choosen optimal model to assess normal linear regression assumptions: homogeneity, normality, independence. Influence of observations was evaluated on the basis of Cook's distance. The relationship between ES and explanatory variables was also investigated following the same procedures.

Results and Discussion

Of the 259 crabs collected from the Szczecin Lagoon, 144 were females and 115 were males, giving a sex ratio of 1:0.80. The monthly variation in sex ratio is shown in table 1. Females were more abundant than males in the autumn months of October and November and males were dominant in December. However a Chisquare test (Chi-Sq = 1.604; d.f. = 2; p-value = 0.448) showed that sex ratio may be considered independent of the catching month.

Out of a total of 144 females catching in 2009-2010, mating occurred only for 42 individuals (29,8%). Individual fecundity of *Eriocheir sinensis* on the first brood ranged from 141 100 to 686 200 eggs per female. Mean fecundity was 461 100 ± st.err. 16871.65 eggs. Egg size ranged from 361 to 378 µm. Mean egg size was 371.40 ± st. err. 0.64 µm. The carapace width of the crab specimens used for fecundity prediction ranged from 46.68 to 80.44 mm (mean 68.17 mm ± st. err. 1.04), the carapace height ranged from 22.11 to 43.31 mm (mean 35.71 mm ± st. err. 0.61), and weight ranged from 47.10 to 219.30 g (mean 142.40 g ± st. err. 6.06). A summary of the data for each variable is shown in table 2.

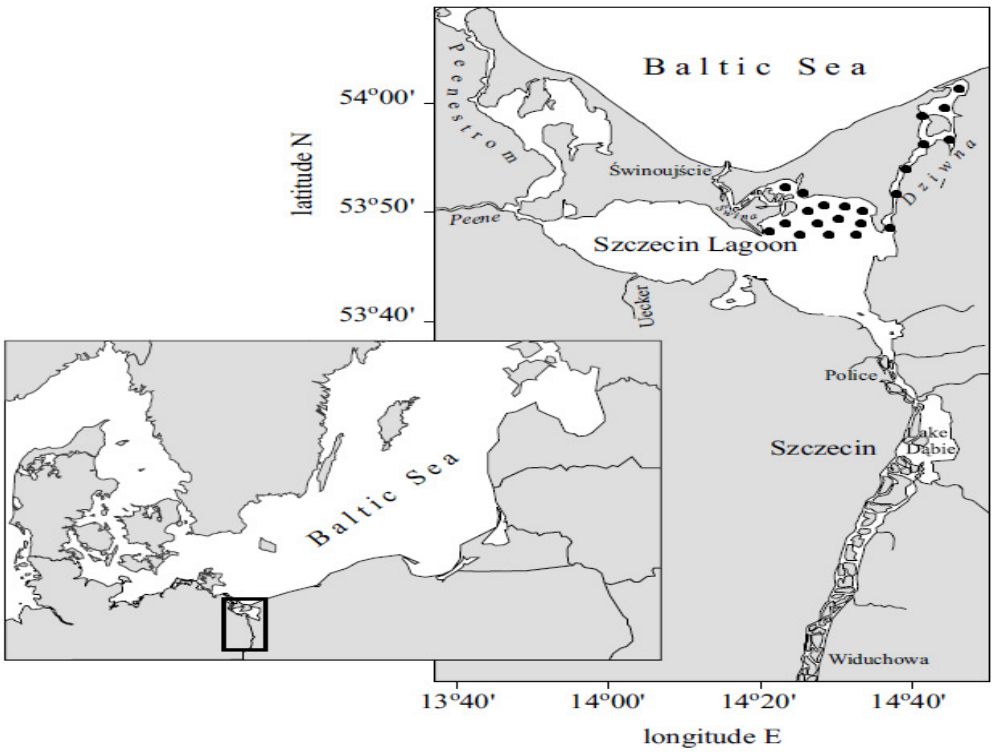

Figure-1
Location of Chinese mitten crab collection sites in the Szczecin Lagoon

Table-1
Sex ratio of the Chinese mitten crab

Month	Females	Males
October	42	36
November	86	61
December	16	18
Together	144	115

Table-2
Summary statistics for FEC, ES, CW, CH and W

	Min.	1st quart.	Median	mean±st.err.	3rd quart.	max.
FEC	141100	397800	479500	461100±16871.65	540900	686200
ES	361	369	372	371.40± 0.64	375	378
CW	46.68	64.78	69.47	68.17±1.04	73.85	80.44
CH	22.11	33.18	36.48	35.71±0.61	38.42	43.31
W	47.10	116.90	148.80	142.40±6.06	172.50	219.30

Boxplots and Cleveland dotplots (not shown here) didn't show presence of outliers in any of the considered variables. Explorative plots of fecundity (FEC) against each of the considered explanatory variables showed a strong linear relationship. Three linear regression models were fitted to data. FEC was assumed as the dependent variable and each time one of carapace width (CW), carapace height (CH) and crab weight (W) was assumed as the explanatory variable in turn.

In figure 2, plots of data with estimated regression lines are represented. Estimated slope, standard error, t statistic, p-value and r-squared are reported in table 3 for each considered model. All linear relationships were positive and significant ($p<0.0001$). Based on the r-squared values, we concluded that the optimal fitted model was that assuming CW as the explanatory variable:

$$FEC = -572757.4+15165.8 * CW+N(0,\sigma^2)$$

with estimated standard error σ = 38230 on 40 degrees of freedom and r-squared = 0.8807.

Model validation was based on the inspection of four plots: residuals against fitted values plot, normal Q-Q plot (standardized residuals versus theoretical quantiles), square root of the absolute value of standardized residuals against fitted values and standardized residuals against leverages. All investigated plots (not shown here) did not indicate any problem with model assumptions (homogeneity, independence, normality) and influential observations.

Explorative plots of ES data against each of the considered explanatory variable data didn't suggest any particular relationship (figure 3). Three linear regression models were fitted to data. ES was assumed as the dependent variable and each time one of CW, CH and W was used as the explanatory variable in turn. For all models, the slopes were found to be not significantly different from 0 (at 5%).

A large part of the benthic fauna associated with estuarine and marine waters is formed by brachyuran crustaceans. Some authors suggested that it is caused by high fecundity of that species [20]. Kwei[21] reported 1.9-2.8 million eggs in *Callinectes latimanus*, while Guillory et. al.[22] documented mean fecundity of 3.2 million eggs for *Callinectes sapidus*. However, some of the brachyuran crabs are characterized by lower fecundity[4,23]. Some species of the *Majidae* had significantly lower numbers of eggs per brood than others *Brachyura* crabs but the majid's lower number of eggs per brood was compensated by a greater than average number of broods per year, resulting in approximately the same average fecundity per year[4]. Multiparous brachyurans are known to release up to 10 broods annually[4,24,25], and one brood per instar per season appears to be the rule in most species of Cancer[26]. In genus *Eriocheir*, female produce multiple broods during the reproductive season[27]. Kobayashi[15] found that *Eriocheir japonicus* had up to 3 broods in a single reproductive season, but fecundity in the first brood is more than in the later oviposition. In *Eriocheir japonicus*, total fecundity ranged from 230 000 to 980 000 eggs/female, but in first brood from 120 000 (40mm in CW) to 600 000 (70mm)[15]. Female mitten crabs are highly fecund too, producing between 100 000 and 1 milion eggs[13]. In our experiment, fecundity in first brood ranged from 141100 to 686200 eggs per female (mean 461100 eggs per female). When compared to other grapsid crabs, size and fecundity of the first oviposition of *E. sinensis* are larger[4,24,28]. Probably, large fecundity per brood with large maturity size compensates for the decrease in their brooding time and lifetime fecundity[15]. The reproductive characteristics of a species are a result of the interaction between various endogenous and exogenous factors[26], such as temperature, salinity, food availability, photoperiod and lunar cycles[29,30].

Strong size fecundity relationships are found in brachyuran families[4]. Fecundity of Chinese mitten crab is significantly linearly correlated with carapace width and height and weight. The fecundity in first brood of *Eriocheir sinensis* increased with increase in carapace width (CW) and height (CH) and weight of

crabs (W). Similar relationship was observed between CW and fecundity in *Geryon fenneri*[31], in *Scylla olivacea*[32] and in *Cancer paguru*[6]. In a study on the reproductive cycle of *P. pelagicus* off southern Australia, Kumar et al.[33] reported a rise in fecundity by 83.9% with an increase in carapace width from 105 mm to 125 mm. Positive correlation between weight and fecundity with high r-squared value (0.908) was reported by Haddon[34] in *Ovalipes catharus*. Villegaset al.[35] analyzed that relationship on the shrimp *Penaeus monodon* and Sharma and Subba[36] on the freshwater prawn *Macrobrachium lamareii* also showed positive relationship between body weight and fecundity, but with low r-squared. However, in Chinese mitten crab, based on the r-squared values, the carapace width was found to be the most suitable characteristic for predicting fecundity. The strong positive correlation between fecundity and carapace width of Chinese mitten crab leads us to the contention that the animal size could be a potential allometric constraint on the reproductive output, as postulated in other brachyurans[4].

Fecundity is determinate not only by female body size, but also by average egg size[4]. Egg size in brachyurans is not generally correlated with adult size[37]. In most of species of crabs egg size ranged between 250µm and 450µm, but in *S. curacaoense* and *A. miersii* have much larger eggs (600 -700 µm), which explain their low fecundity[38]. The measurement of the egg diameter of Chinese mitten crab showed variations in egg sizes. The diameter ranged from 361 to 375 µm with a mean of 371.4 µm (±0,64). According to Dittel and Epifanio[13] eggs of Chinese mitten crab are about 350-380 µm in diameter. There was not significant correlation between egg size and carapace width (CW) and height (CH), and individual weight in this species.

Sex ratio in a majority of species is close to unity, despite some variations between populations of a species, and from year to year in the same population[39]. The overall sex ratio in *Eriocheir sinensis* differed from the expected 1:1 ratio, but not significantly. In Odra estuary in autumn months were higher occurrences of male than female[40].

Table-3
Explanatory variable, estimated slope, standard error, t-statistic, p-value and r-squared for each considered regression model

Explanatory Variable	Estimated Slopes	Standard Error	t-statistic	p-value	r-squared
CW	15165.8	882.5	17.185	<0.0001	0.8807
CH	24907	1961	12.704	<0.0001	0.8014
W	2491.6	196.7	12.665	<0.0001	0.8004

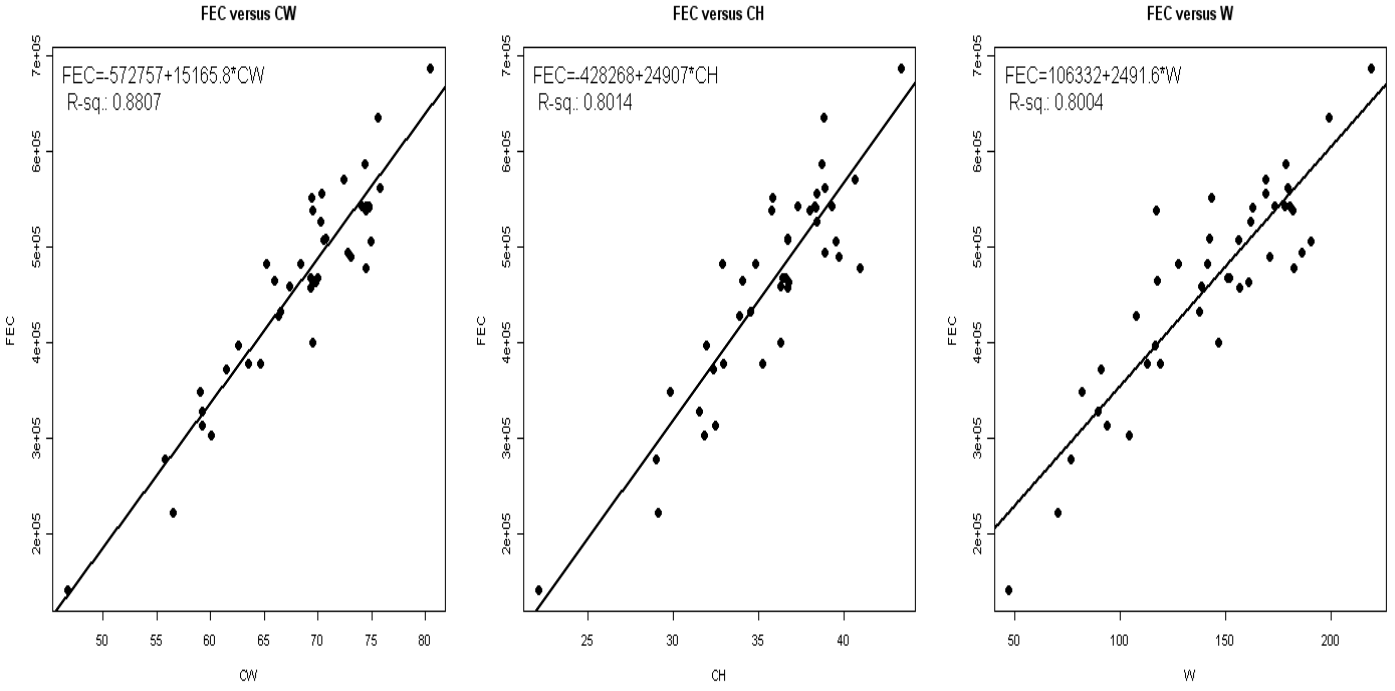

Figure-2
Plots of FEC against each explanatory variable (CW, CH and W) with estimated regression lines

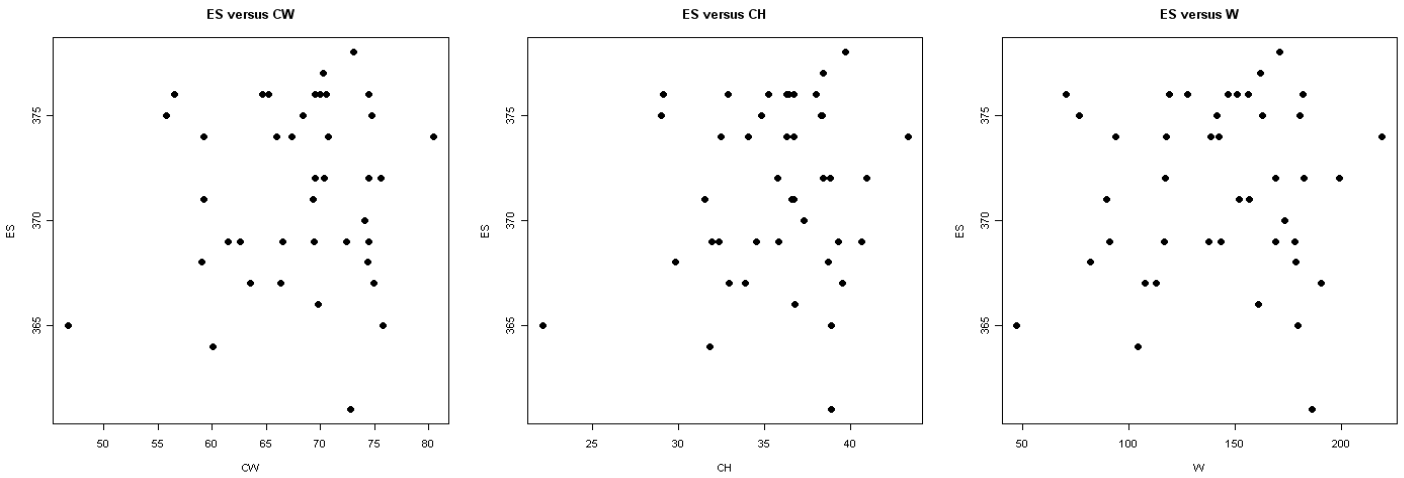

Figure-3
Plots of ES against each explanatory variable (CW, CH and W)

Conclusion

Female mitten crabs are highly fecund, producing between 141100 and 686200 eggs (mean 461100 eggs per female) in first brood. Fecundity in *E. sinensis* increased with female size. The optimal fitted model was that assuming CW as the explanatory variable: FEC = -572757.4+15165.8 * CW+N(0,σ^2) with estimated standard error σ = 38230 on 40 degrees of freedom and r-squared = 0.8807. The measurement of the egg diameter showed variations in egg sizes. The diameter ranged from 361 - 375µm (mean eggs size were 371,4 µm). All the morphometric characteristics studied showed positive correlation with fecundity and the degree of relationship varied considerably. Among these, the carapace width was found to be the best morphometric character for predicting the fecundity of Chinese mitten crab.

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