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Effect of salinity on the physiology and bioenergetics of adult Chinese mitten crabs *Eriocheir sinensis*

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ABSTRACT

Rates of physiological processes and bioenergetics of the Chinese mitten crab *Eriocheir sinensis* were determined during a 7-day experiment on adult males (mean wet weight 147.27 ± 20.86 g) exposed to salinities of 0.5, 7 and 25. Salinity did not significantly affect food consumption rate ($p > 0.05$). The rate of faeces production rose between salinities of 0.5 and 25 ($p < 0.05$), whereas the rate of ammonia excretion decreased. At salinities of 7 and 25 crabs consumed 1.6 and 1.3 times more oxygen respectively than at a salinity of 0.5 ($p < 0.05$). *E. sinensis* was able to maintain a positive energy balance at salinities of 0.5 and 25, and the scope for growth reached 66.07 ± 45.23 and 47.79 ± 31.42 J d^{-1} g^{-1} dry wt, respectively. Although the high inter-individual variability significantly influenced the obtained results, the lower metabolic expenses and higher scope for growth indicate that freshwater environments ($S < 7$) are more "profitable" for adult of Chinese mitten crabs from a bioenergetical point of view than saline waters.

1. Introduction

The Chinese mitten crab (Crustacea: Brachyura: Varunidae) *Eriocheir sinensis*, native to the Yellow Sea Coast in China and to Korea, is listed as one of the world's worst 100 alien invasive species by the International Union for the Conservation of Nature and Natural Resources (IUCN) due to severe damage or harm to the environment and economy (Lowe et al., 2000; Veldhuizen, 2001). The invader was recorded for the first time outside its native range almost 100 years ago (Panning, 1938). It was introduced in ballast tanks into German waters, but, due to larval retention as well as the ability of juveniles and adults to complete long migrations, the crabs spread quickly across the European continent (Gilbey et al., 2008; Herborg et al., 2003; Paunovic et al., 2004). Decades later, in 1992, *E. sinensis* has been also reported along the west coast of North America, in San Francisco Bay (Cohen and Carlton, 1997; Rudnick et al., 2000). On both continents the mitten crab has successfully established populations (e.g. Klatt, 1938; Rudnick et al., 2005). However, as *E. sinensis* has a catadromous life cycle, involving several life stages, previous experimental studies have shown their different tolerances toward salinity (Anger, 1991; Cohen and Weinstein, 2001; Rudnick et al., 2005). While the younger benthic forms prefer freshwater, the older, sexually mature specimens can live in fresh and brackish water as well as in the sea (Garcia-de-Lomas et al., 2009; Panning, 1938; Veilleux and de Lafontaine, 2007). Moreover, according to Kinne (1971) mitten crabs either migrate from one habitat to the other or establish populations in all three environments simultaneously. In the brackish water of the Baltic Sea (mean salinity ca. 7), adult *E. sinensis* are found throughout the whole year, and the presence of native epibionts, e.g. the barnacle *Balanus improvisus*, may indicate their extended stay in this environment (Normant et al., 2002a; Normant et al., 2007; Ojaveer et al., 2007). In other non-native regions, i.e. in Tagus estuary (Portugal) or San Francisco Bay (North America) adult mitten crabs inhabit even more saline environments (Cabral and Costa, 1999; Rudnick et al., 2005).

Many studies show an efficient osmoregulation mechanism in *E. sinensis*, enabling it to function in a wide range of salinities (e.g. Cieluch et al., 2007; Liu et al., 2008; Olen, 1999; Pequeux et al., 1996; Rathmayer and Siebers, 2001; Silvestre et al., 2004; Whiteley et al., 2001). However, this factor also significantly affects rates of other physiological processes (i.e. oxygen uptake, food consumption and assimilation, ammonia excretion, etc.) in aquatic organisms (e.g. Guerin and Stickle, 1997; Hulathduwa et al., 2007; Kinne, 1971). Despite the fact that *E. sinensis* is a globally recognized invasive species that is continually expanding its range little is known about its physiological functioning. Therefore, our aim was to determine rates of food consumption, faeces and ammonia excretion as well as oxygen uptake at fresh ($S = 0.5$), oligohaline ($S = 7$), and polyhaline ($S = 25$) waters. As the rates of these physiological processes can be easily converted into energy equivalents, we also established an energy balance for adult *E. sinensis* and calculated energy available for individual production, i.e. growth and reproduction (Winberg, 1960). This parameter, also known as the scope for growth, is a good bioindicator of environmental stress. During exposure to less advantageous conditions, individual production

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decreases which may have profound impacts on population-level responses to newly invaded areas (e.g. Maltby et al., 1990; Normant and Lamprecht, 2006). The obtained results add to the slowly growing literature on *E. sinensis* functioning in different environments, and may also be useful for ecological modelling estimating the effect of this species on inhabited ecosystems through its contribution in energy turnover.

2. Materials and methods

2.1. Crab collection and maintenance

The experiments were conducted according to the methodology applied by Hulathduwa et al. (2007) and the scheme given by Jakubowska and Normant (2011) on eight *E. sinensis* adult males collected in the German River Havel ($T=12$ °C, $S=0.5$) during the reproductive migration. Their carapace widths ranged from 65.1 to 71.6 mm (mean 66.0 ± 3.0 mm) and the wet weights from 113.5 to 180.2 g (mean 147.3 ± 20.9 g). In the laboratory, crabs were placed individually in closed plastic boxes (22 × 32 × 16 cm) filled with aerated water ($T=15$ °C, $S=0.5$). They were fed once a week with blue mussel *Mytilus trossulus* and green algae (*Enteromorpha* sp.). Crabs were maintained in the laboratory for 2 weeks at the salinity of 0.5 ($T=15$ °C), after which they were gradually ($S=2$/day) acclimated to the higher salinities of 7 and 25 (Guerin and Stickle, 1992). Sea water solutions were prepared using commercial sea salt (Aqua Medic, Germany) and tap water. Animals were kept at the appropriate salinity (either 0.5, 7 or 25) for 1 week before experiments commenced. At this time their behavior (locomotor and feeding activity) was observed. The identity of the crabs was recorded and tracked so that paired comparisons could be made.

2.2. Oxygen consumption rate

The rate of oxygen uptake was determined in crabs starved or fed for 7 days using the method suggested by Brown and Terwilliger (1999). The crabs were placed individually in a hermetically sealed respiration chamber, completely filled with aerated water ($S=0.5$, 7 and 25; $T=15$ °C). The controls were three identical respiration chambers without crabs. Oxygen uptake was measured three times for every animal and control, after 20, 40 and 60 min of residence in the chamber using a Clark oxygen electrode OX-N connected to a PA 2000-meter (Unisense, Denmark). The rate of respiration was calculated from the difference between the initial and final oxygen concentrations in the respiration chamber, making allowance for the controls. Results were calculated according to Tian et al. (2004) and metabolic rate is given in micromoles of oxygen per day per gram wet weight of crab (μmol O_2 d^{-1} g^{-1} wet wt). The quantity of energy expended on metabolic processes (R) was then calculated using the oxycaloric equivalent 1 μmol O_2 = 0.450 J (Gnaiger, 1983).

2.3. Ammonia excretion rate

The rate of ammonia excretion was calculated in crabs starved or fed for 7 days based on Koroleff's (1976) indophenol method. Three replicate water samples were collected from each respiration chamber (both those with crabs and the controls). The results are given in micromoles of ammonium nitrogen per day per gram wet weight of crab (μmol NH_4–N d^{-1} g^{-1} wet wt). Using the equivalent 1 μmol = 0.348 J NH_4–N (Elliot and Davison, 1975), the energy excreted in the form of ammonia (U) was then calculated.

2.4. Food consumption rate

The food used during the experiments was the soft tissue of the blue mussel *M. trossulus* with an average ($n=10$) energy value of 24.4 ± 0.9 J mg^{-1} dry wt, an organic matter content of 86.6 ± 4.3% dry wt, and a water content of 84.3 ± 1.0% wet wt. The energy value was determined using a bomb microcalorimeter according to Normant et al. (2002b), whereas the organic matter content was calculated from the weight loss on ashing in a muffle furnace for 12 h at 450 °C (Gnaiger and Bitterlich, 1984).

The rate of food intake was determined from the weight loss of food given to the crabs each day for 7 days. The uneaten leftovers were successively removed from the aquaria, after which their wet and dry weights were established. The feeding rate is given in mg dry weight per day per gram wet weight of crab (mg d^{-1} g^{-1} wet wt), averaged over the 7-day period of the experiments. Then, the amount of energy acquired with the food (C) was calculated on the basis of the energy contained in the food consumed.

2.5. Faeces production rate

During the 7 days of the experiment and for 5 days after its completion (McGaw, 2006), crab faeces were collected until the alimentary canal was empty in order to calculate the rate of excretion. Faecal pellets were pipetted on to a previously ashed ($T=450$ °C, $t=12$ h) GF/C filter (Whatman 47 mm, 0.45 μm), after which the filter and excreta were dried ($T=55$ °C, $t=48$ h) and again ashed in a muffle furnace ($T=450$ °C, $t=12$ h) to determine the organic matter content (Gnaiger and Bitterlich, 1984). The rate of excretion is given in mg of dry weight per day per gram wet weight of crab (mg d^{-1} g^{-1} wet wt) averaged over 12 days.

2.6. Food absorption efficiency

Because the mass of excreta from a single crab was insufficient to determine the energy value in the bomb microcalorimeter, the food absorption efficiency (*AE*) was calculated using the formula given by Conover (1966):

$$\mathrm{AE} = (\mathrm{F} - \mathrm{E}) * \left[(1 - \mathrm{E}) * \mathrm{F}\right]^{-1} * 100 \tag{1}$$

where F is the ratio of ash-free dry weight to total dry weight in the food, and E is the ratio of ash-free dry weight to total dry weight in the faeces.

2.7. Individual production

The amount of energy assimilated from food (A) was calculated by multiplying the amount of energy consumed as food (C) by the absorption efficiency (*AE*). Then the rate of the various physiological processes, expressed in energy units joules per day per gram of wet weight of crab (J d^{-1} g^{-1} wet wt), were inserted in the transformed energy budget equation given by Winberg (1960) to calculate energy available for growth and reproduction (P).

$$P = A - U - R \tag{2}$$

2.8. Statistical analysis

The Wilcoxon matched pairs test at a confidence level of $\alpha = 95\%$ was used to test the significance of the differences between the rates of the physiological processes at the experimental salinities ($n=8$ crabs). The relationship between these processes was calculated by linear regression analysis ($y = ax + b$) with a coefficient of determination R^2 for a significance level $p<0.05$.

3. Results

The lowest rate of oxygen uptake by *E. sinensis* averaged 25.68 ± 3.06 μmol O_2 d^{-1} g^{-1} wet wt occurred at a salinity of 0.5 (Fig. 1A). Values