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Ionic balance in the freshwater-adapted Chinese crab, *Eriocheir sinensis*

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**Abstract** Ionic regulation by the gills of the freshwater-adapted Chinese crab, *Eriocheir sinensis*, was examined. The balance of uptake and loss of NaCl in crabs living in freshwater was established. Urine production was measured directly by cannulating the nephropores. Daily urinary loss of Na⁺ is equivalent to 16% of the haemolymph Na⁺ content and is substantially higher than that based on data from indirect measurements reported in the literature. Weight and area of anterior and posterior gills are proportional to body weight. The role of the gills in compensating urinary loss by uptake was determined by analysing changes in Na⁺ and Cl⁻ concentrations in the external medium in which isolated perfused gills were suspended. In posterior gills, salt loss is quantitatively balanced by NaCl net uptake from an external concentration of 1.3 mmol l⁻¹ NaCl upwards. The transport constant (Kₛ) for half maximum saturation of net uptake and saturation of NaCl uptake are 1.5 mmol l⁻¹ and 4 mmol l⁻¹, respectively. In contrast to previous studies in which tracer fluxes or transepithelial short-circuit currents were determined, our method of direct ion determination shows that no net uptake of Na⁺ or Cl⁻ occurs in posterior gills in the absence of the respective counter ion, or when uptake of one ion is blocked by a specific inhibitor. Net uptake of Na⁺ and Cl⁻ was about equal. We conclude that the uptake of the two ions is coupled. The properties of the branchial ion uptake of *E. sinensis* correlates with the distribution of this crab in river systems.

**Keywords** Crustacea · Urine production · Ion regulation · Gills · Ecophysiology

**Abbreviations** ALW artificial lake water · bw body weight · gw gill weight · Kₛ transport constant · LW lake water · TEC transepithelial current · TEP transepithelial potential

**Introduction**

The Chinese mitten crab, *Eriocheir sinensis*, is well known for its extraordinary capacity of osmoregulation, which permits its survival in environments ranging from seawater to freshwater. In crabs living in freshwater, the loss of NaCl through the body surface and the urine is thought to be compensated entirely by uptake through the gills (Krogh 1938, 1939; Potts 1954; Shaw 1961; Lockwood 1964, 1977; Potts and Parry 1964; for review see Péqueux 1995). To understand the underlying physiological adaptations, uptake and loss of ions have been investigated. Uptake was determined in whole animals by direct measurement of changes in ionic concentrations of external media (Krogh 1938; Koch et al. 1954; Koch and Evans 1956; Shaw 1959, 1960), or by flux measurement using radioactive tracers (Shaw 1961). For determining total salt loss, crabs were placed in distilled water and urinary loss was measured indirectly using two methods (Scholles 1933; Krogh 1938) which, however, yielded different results.

A major advance in the study of crustacean gill function was the technique of perfusing isolated gills to determine transepithelial potentials (TEP) and to measure ion uptake using radioactive tracers (Habas and Prosser 1963; Mantel 1967; King and Schoffeniels 1969; Schoffeniels and Gilles 1970; Gilles and Péqueux 1978; Péqueux and Gilles 1981; Siebers et al. 1985). More recently, a “split-gill lamella” preparation, placed in a modified Ussing chamber, was developed to determine the transepithelial current, TEC (Schwarz and Graszyński 1989; Schwarz 1991). This technique allows
the determination of area-specific ion fluxes across the branchial epithelium (Risteenpatt et al. 1994, 1996; Onken 1996).

The reported net uptake of Na\(^+\) and Cl\(^-\) from freshwater across the gills of *Eriocheir*, deduced from isotope studies (Péqueux and Gilles 1981; Gocha et al. 1987; Risteenpatt et al. 1996) and from measurements of transepithelial current, TEC (Schwarz 1991; Risteenpatt et al. 1994) does not indicate that the gills are effective enough to balance the urinary loss by uptake from freshwater. In fact, the gills investigated (antero- and posterior) showed net loss rather than net uptake of Na\(^+\) and Cl\(^-\) in freshwater with a NaCl concentration of 1–2 mmol l\(^{-1}\) (Péqueux and Gilles 1981; Gocha et al. 1987) and even of 10 mmol l\(^{-1}\) (Risteenpatt et al. 1996).

The kinetic data on Na\(^+\) influx in isolated posterior gills of *Eriocheir* obtained from tracer experiments (Péqueux and Gilles 1981), and from TEC measurements (Risteenpatt et al. 1994) differ substantially from data published by Shaw (1961) for whole crabs and would not permit the crabs to live in freshwater. In addition, calculation of an ion balance for the living animal from the uptake rates of isolated gills is not possible because the flux rates measured were related to gill weight or gill area without correlation to body weight (Péqueux and Gilles 1981; Gocha et al. 1987; Risteenpatt et al. 1994).

The aim of the present study is to investigate the role of the gills in ionic regulation by freshwater-adapted *Eriocheir* and to establish quantitatively the balance of branchial ion uptake from freshwater and urinary loss. The relation between gill and body weight was used to convert the uptake across the isolated gills to that of intact crabs. In contrast to the indirect methods used in previous studies, the urine production was measured directly by cannulation of the nephropores. We show that in freshwater with 1.3 mmol l\(^{-1}\) NaCl upwards, the gills are capable of balancing urinary loss, and that the net uptake of Na\(^+\) and Cl\(^-\) is coupled. This is in contrast to the findings of other authors (Risteenpatt et al. 1994, 1996; Onken and Risteenpatt 1998). The results cast doubt on the suitability of using flux measurements by means of tracers or measurement of TEC to determine branchial net uptake of NaCl under highly asymmetric conditions.

**Materials and methods**

**Animals**

Chinese crabs, *E. sinensis*, were collected in autumn in the river Elbe near Hamburg, Germany. The Na\(^+\) concentration of the Elbe in this area, measured in our laboratory, was 7 mmol l\(^{-1}\). The crabs were kept at the animal facilities of the University of Konstanz in 700-l tanks filled with running, aerated freshwater pumped in from Lake Konstanz. The temperature was around 10\(^\circ\)C during the winter months, and about 15\(^\circ\)C in summer. The animals were fed regularly with fish. The lake water had the following composition (mmol l\(^{-1}\)): Na\(^+\) 0.18, K\(^+\) 0.04, Ca\(^{2+}\) 1.25, Mg\(^{2+}\) 0.31, SO\(_4\)\(^{2-}\) 0.36, Cl\(^-\) 0.16, HCO\(_3\)\(^-\) 2.44. Crabs kept in this water survived for up to 8 months.

**Ion concentration and osmolality**

Haemolymph was obtained by puncturing the arthrobranch membrane at the base of a walking leg with a finely drawn Pasteur pipette. Urine was collected by suction after inserting the tip of a glass tube through one of the two nephridial openings of a crab (see Schölles 1933). Na\(^+\) concentrations in haemolymph and urine were determined by flame spectrophotometry (PMQ-2 Zeiss, Stuttgart, Germany) and, as a routine procedure, by measurement of osmolality with a semi-micro-osmometer (Knauer, Berlin, Germany). Figure 2 shows that the values obtained by the two methods do not differ.

For flux measurements, ions were determined by sampling the bathing medium of the gills and analysing the samples for Na\(^+\) by flame spectrophotometry, using a propane burner to yield highest sensitivity. Cl\(^-\) was determined by titration. Sample size was 100 µl for Na\(^+\), and 2 ml for Cl\(^-\) determinations. Chloride was titrated at 15\(^\circ\)C according to Mohr (1995), using 0.0025 mmol l\(^{-1}\) instead of 0.1 mmol l\(^{-1}\) AgNO\(_3\); 50 µl of 5% K\(_2\)CrO\(_4\) was added to each sample. All determinations were done in duplicate. To minimize contamination of the external bathing medium with ions leaking from the agar bridge which connected the electrode used for TEG measurements with the bath, the bridge was removed from the external bath except for the brief periods of measurement before and after the sampling period.

**Urine production**

The legs of the crab were bound with elastic bands, and polyethylene tubes of 1 mm outer diameter were secured with dental wax over the two nephridial pores, permitting the opercula to open freely. The free ends of the tubes were inserted into a 5-ml volumetric flask, taking care that the tubing did not touch its walls. The flask was set on a balance connected to a chart recorder. The animal was held with a clamp and submerged in a 6-l glass aquarium filled with lake water. Evans blue was added to the water to mark the legs in the sealing. To avoid a possible siphoning effect, the ends of the tubes in the collecting flask were set at the same level as the water surface of the aquarium. The setup was kept in a constant-temperature room (15\(^\circ\)C) with high humidity. Recording began when the urine started to flow into the flask. If the fluid showed even traces of blue colour, the experiment was terminated.

**Gill weights and surface areas**

Before determining the gill weights, the crab’s haemolymph was replaced by haemolymph-like saline (see below). The gills were cut at their base, blotted on filter paper and weighed. The gills used in ion uptake experiments were also weighed and included in the statistics. To determine gill surface areas, the gills were cut at their base and fixed in 2% glutaraldehyde in 0.1 mol l\(^{-1}\) phosphate buffer overnight in the cold. The gill was subdivided into portions of ten leaflets. The middle pair of leaflets of each segment was removed and placed on a microscope slide and drawn (camera lucida) on cardboard. The area (minus that of the gill stem) was then cut out and weighed. The weight was compared with that of a 1-cm\(^2\) square of the same cardboard. Knowing the magnification, the area of the leaflets was calculated. Since every tenth leaflet was measured, and each leaflet has two surfaces, the area thus obtained was multiplied by 20. Table 1 shows the surface areas of anterior (3–5) and posterior (6–8) gills in two crabs of different weight.

**Gill perfusion and measurements of flow resistances and transepithelial potential differences**

We use the nomenclature of Péqueux and Gilles (1981), who refer to pairs 3–5 as anterior, and to pairs 6–8 as posterior gills. To prevent blood clotting, the gills were taken from crabs in which the haemolymph had been replaced by saline. This was accomplished.