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Blood Osmoregulatory Type and Gill Ultrastructure of an Estuarine Crab *Hemigrapsus penicillatus* (de Haan) (Crustacea; Brachyura)

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Abstract

The type of blood osmolarity control and the gill ultrastructure of an estuarine grapsid crab *Hemigrapsus penicillatus* were examined in order to clarify its physiological and morphological characteristics as bases for the life in estuarine environments. Those of a marine stenohaline species *Pugettia quadridens* were also investigated for the sake of contrast. The responses of the blood osmolarities to various external media of osmolarity ranging from 400 to 1600 mOsm/kg showed that *Hemigrapsus* was a hyper-osmoregulator, and *Pugettia* an osmoconformer. The survival rate was higher in *Hemigrapsus* than in *Pugettia*, not only in the most diluted external medium but also in the most concentrated one. The electron microscopic observation revealed that the three most posterior gill pairs of *Hemigrapsus* exhibited a thick ion-transporting epithelium in which well-developed apical infoldings and basolateral infoldings associated with many large mitochondria were observed. In contrast, *Pugettia* did not have differentiated gills, and all gill pairs exhibited respiratory epithelia which were poor in organelles. These results indicate that the life of *Hemigrapsus* within estuaries is supported by its hyper-osmoregulatory capability in external dilute media, and the ion-transporting epithelium of the gills plays an important role in the osmoregulation. It is considered that the osmoregulatory capability of *Hemigrapsus* limits its penetration into fresh water; however, other factors determine the seaward limit of the crab distribution.

Introduction

An estuary is a place where fresh water and sea water meet, and many biogeochemical processes occur at this freshwater-seawater interface. The estuaries are generally rich in nutrients due to these processes, but the environments fluctuate greatly with tide and river currents (see Kurihara, 1988; McLusky, 1989). These severe environments prevent most organisms from entering estuaries from the adjacent sea and rivers. Thus, ecologically, the estuarine habitat is characterized by its relatively low biodiversity, although the densities of these species are usually very high. The organisms living in an estuary, especially the so-called 'true

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estuarine organisms' which typically appear in estuaries, have been interesting subjects of physiological as well as ecological studies (McLusky & Elliott, 1981; Lockwood & Bolt, 1989; Kikuchi, 1992).

A grapsid crab *Hemigrapsus penicillatus* (de Haan) is a true estuarine organism which is widely distributed in estuaries in Japan (Ono, 1959; Goshima *et al.*, 1978; Ogura & Kishi, 1985; Fukui & Wada, 1986; Okamoto & Kurihara, 1987). Such ecological characteristics as the distribution, reproduction, and population structure have been investigated, but there is little information concerning its physiological traits. In order to clarify the physiological characteristics of this crab for living in estuarine environments, this study examined the type of blood osmolarity control, which is essential for the adaptation to change in the salt concentrations of external media (Gilles & Pequeux, 1983). We contrasted this blood osmolarity with that of a stenohaline marine crab *Pugettia quadridens* (de Haan). In addition, the ultrastructures of gill epithelia and the changes in response to external salinities were investigated, since it is well known that crustacean gills play a very important role in blood osmolarity control (Gilles & Pequeux, 1985; Pequeux and Gilles, 1988).

Materials and Methods

Hemigrapsus penicillatus and *Pugettia quadridens* were collected from the estuary of the Nanakita River, Sendai, Miyagi Prefecture and Otsuchi Bay, Otsuchi, Iwate Prefecture, respectively. Only adult (>15mm, carapace width) males and non-ovigerous females were used. Crabs were maintained in large plastic containers with an artificial SW (sea water) (Yashima Pure Chemicals Co., Ltd; salinity, ca., 35‰) for *Pugettia* and with 50% SW for *Hemigrapsus* at 20± 1℃ under a 14:10 hr light: dark cycle for 3 days prior to the experiments. Then, 30 individuals of each crab species were transferred to containers with 40, 70, 100, 130, and 160% SW. After 3 days, blood samples were removed with a syringe from the arthrodial membrane at the base of the fifth pereiopod, and allowed to clot. Osmolarity of serum and external water samples was determined using a freezing point micro-osmometer (Hermann Roebling, Type 13DR).

For investigating the ultrastructure of gill epithelia, pieces of gills were fixed with cold 3% glutaraldehyde buffered at pH 7.4 with a 0.1 M sodium mono-and diphosphate mixture. After 30 sec. of microwave irradiation in an ice-cold water bath in a microwave oven, the gills were replaced in the ice-cold new fixative for 2 hr at 4℃ with moderate shaking. Subsequetly, they were briefly washed in the same buffer solution, post-fixed with ice-cold 1% osmium tetroxide in the buffer solution for 1 hr. After a brief rinse in the buffer solution, they were dehydrated in a series of graded ethanol, infiltrated with n-butyl glycidyl ether (QY-2), and embedded in a low viscosily resin (SPURR). Thin sections were cut with a diamond knife on a Reichert-Jung OmU-4 ultramicrotome, mounted on copper grids, double stained with 1% aqueous uranyl acetate for 1 hr at 50℃ and lead citrate for 5 min. at room temperature, and examined with a transmission electron microscope (JEM 100B) operated at 80 kV.

Results

The survival of *Hemigrapsus* was higher than of *Pugettia* both in the low and high salinity of external media (**Fig. 1**). The blood of *Pugettia* remained isosmotic to the external medium in all salinities down/up to the lethal limits (i.e., osmoconformer) (**Fig. 2**). On the other hand, *Hemigrapsus* maintained a hyperosmotic state in the dilute media, but in more concentrated media, the blood osmolarity strictly followed the osmolarity of the external environment (i.e., hyper-osmoregulator). The blood was maintained at 750 mOsm/kg even in 400 mOsm/kg external media, and became isosmotic to the external medium above 800-900 mOsm/kg.

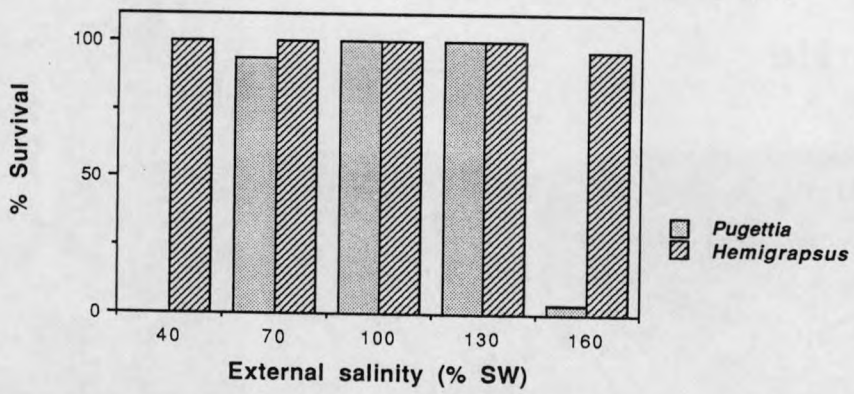

Fig. 1 The % survival of an estuarine crab *Hemigrapsus* and a marine crab *Pugettia* exposed to various external salinities (% SW = % sea water) for 3 days.

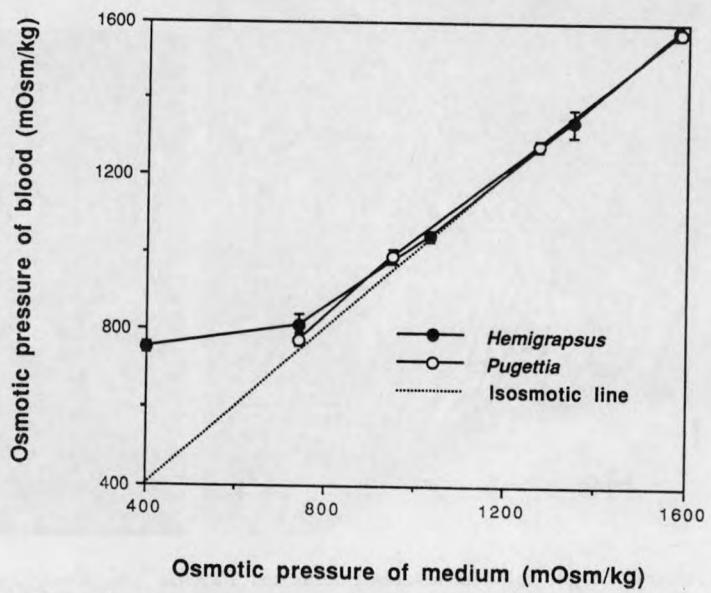

Fig. 2 The blood osmolarity controls of *Hemigrapsus* and *Pugettia*. Vertical bars show ± 2SE (n=5). The value for *Pugettia* is missing at 40% SW (400 mOsm/kg) and is based on one sample at 160% SW (1580 mOsm/kg), since none survived at the lowest salinity and only one individual at the highest.

Hemigrapsus possesses eight pairs of gills. Of these gill pairs, the three most posterior pairs of gills were characterized by thicker epithelia (ca., 6-10 μm) than the other anterior gills (thickness of epithelium, 0.6-0.8 μm) (**Fig. 3**). Under the thin cuticle (0.3-0.4 μm), the apical cell

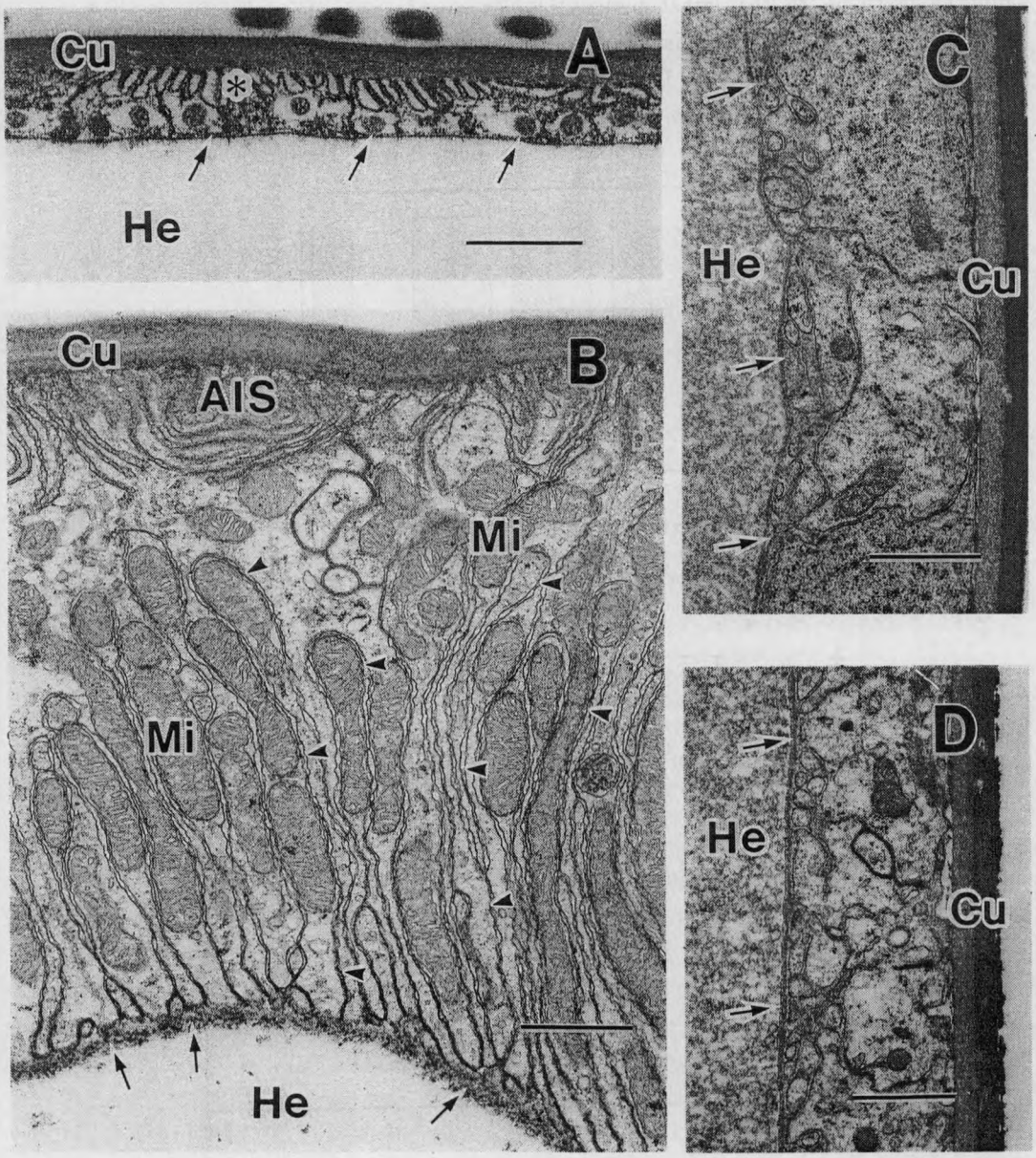

Fig. 3 Ultrastructures of gill epithelia of *Hemigrapsus* and *Pugettia*. (A) *Hemigrapsus*, the anterior arthrobranch of Maxilliped III (anterior gill); (B) *Hemigrapsus*, the posterior arthrobranch of Cheliped (posterior gill); (C) *Pugettia*, the anterior arthrobranch of Maxilliped III; (D) *Pugettia*, the posterior arthrobranch of Cheliped. AIS, apical infolding system; Cu, cuticle; He,hemocoel; Mi, mitochondria. Arrows indicate thin basal lamina and arrowheads infolding basal and lateral cell membranes. Notice that the AIS regresses in the anterior gill (asterisk). Scale bars indicate 1.0 μm.

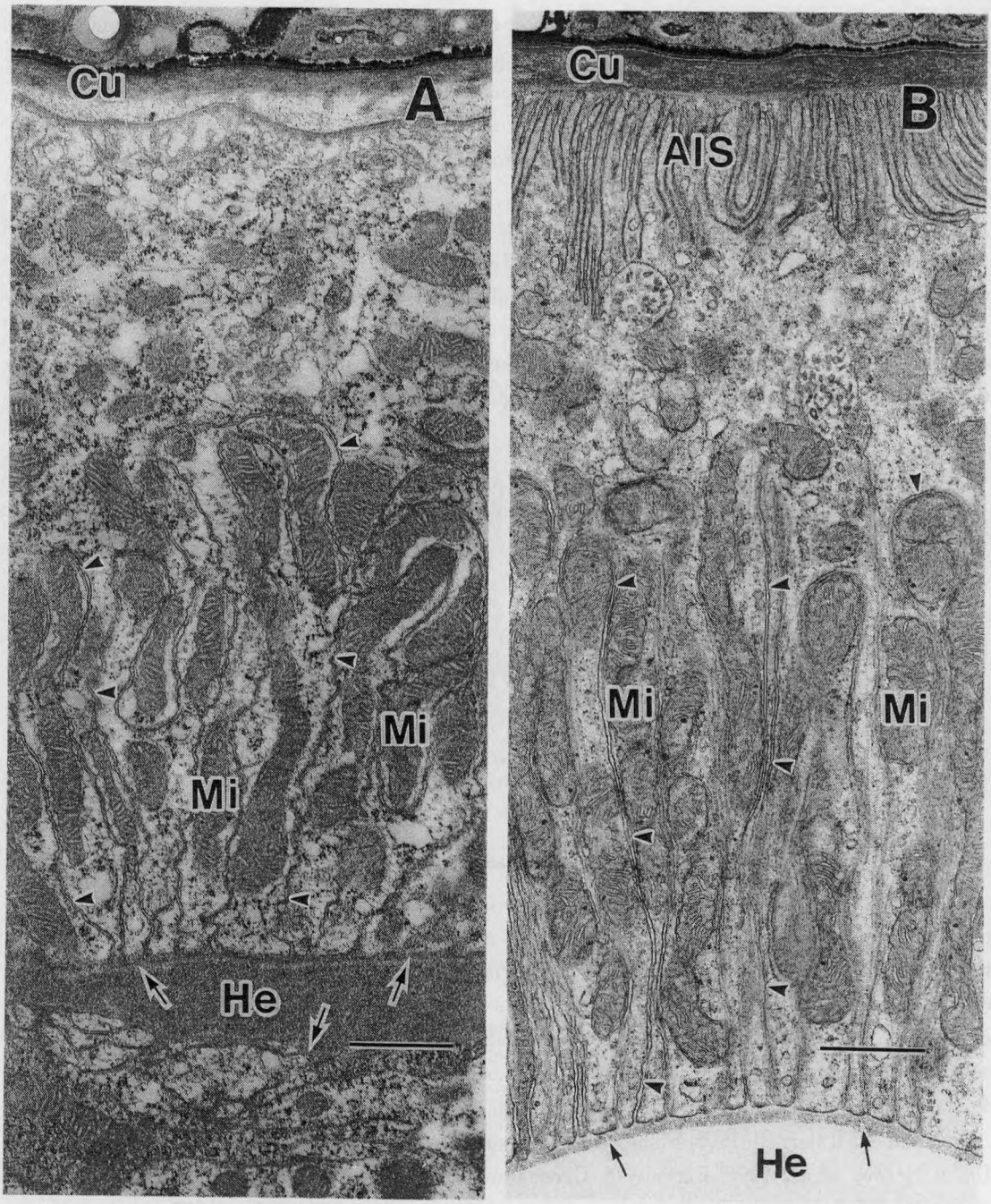

Fig.4 Changes in the ultrastructure of the posterior gill epithelium of *Hemigrapsus* acclimated from 50% SW to 160% SW (A) and to 40% SW (B) for 3 days. The lagends are same as those in Fig.3. Scale bars indicate 1.0 μm.

membrane of the posterior gill epithelia exhibited the so-called apical infolding system (AIS), that is, frequent and deep (ca., 0.7-1.2 μm) infoldings of the apical cell membrane (**Fig. 3B**). Basal and

lateral cell membranes also infolded deeply (ca., 3.5-7 μm) and contained large mitochondria between them. In the thin epithelium of anterior gills, the AIS was shallow (0.2-0.3 μm), and the infoldings of basolateral cell membranes and mitochondria were few (**Fig. 3A**). On the other hand, characteristics of gill epithelium did not differ among gill pairs of *Pugettia* (**Fig. 3C, D**). The epithelial cells neither exhibited AIS nor the infoldings of basolateral cell membranes containing mitochondria between them.

When *Hemigrapsus* was acclimated to the high environmental salinity (160% SW), a considerable regression of the well-developed AIS occurred in the posterior gills (**Fig. 4**). There were little changes in structural organization of the basolateral infoldings and the associated mitochondria, although these characteristics were somewhat inconspicuous in 160% SW acclimation. The regression of AIS was also observed in the anterior gill epithelium. No change in the ultrastructure of *Pugettia* gill epithelium responding to external salinity was recognized (**Fig. 5**).

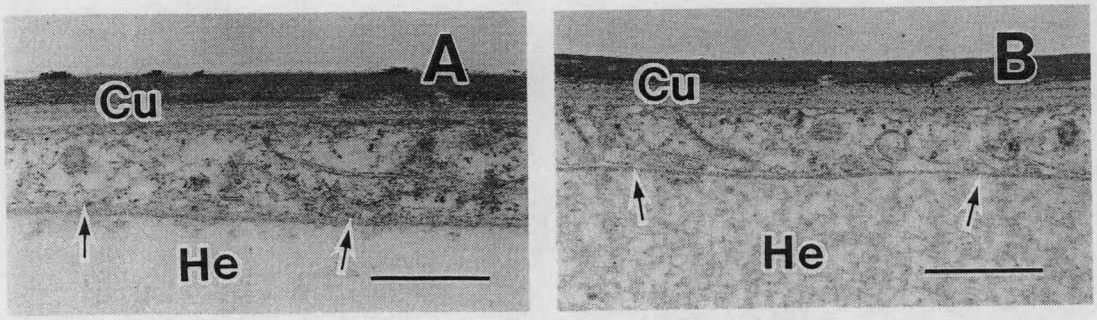

Fig. 5 The gill epithelium of *Pugettia* acclimated from SW to 130% SW (A) and to 70% SW (B) for 3 days. The legends are same as those in Fig.3. Scale bars indicate 1.0 μm.

Dscussion

The estuarine crab *Hemigrapsus penicillatus* was a hyper-osmoregulator (**Fig. 2**). The posterior gill epithelia of *Hemigrapsus* exhibited a well-developed AIS, and the deep basolateral infoldings were associated with large mitochondria (**Fig. 3**). In addition, the AIS was deeper and more conspicuous in the lower external salinities than in the higher ones (**Fig. 4**). A number of extremely well-developed examples of the AIS are known in the gill epithelia of many osmoregulating crustaceans such as amphipods (Lockwood *et al.*, 1973; Milne & Ellis, 1973; Kikuchi & Matsumasa, 1993a; Kikuchi *et al.*, 1993), a tanaid (Kikuchi & Matsumasa, 1993b), decapods (Copeland & Fitzjarrell, 1968; Fisher, 1972; Nakao, 1974; Finol and Croghan, 1983; Compere *et al.*, 1989; Dickson *et al.*, 1991; Farrelly & Greenaway, 1992), isopods (Bubel & Jones, 1974), and syncarids (McConnell, 1987). Furthemore, the significant enlargements of the AIS due to reduced salinities were reported in some euryhaline amphipods (Lockwood *et al.*, 1973; Milne & Ellis, 1973) and decapods (Foster & Howse, 1978; Pequeux & Gilles, 1988; Compere *et al.*, 1989). Based on physiological and biochemical evidence, it has been considered that this type of gill epithelium with AIS is involved in the salt absorption in hypo-osmotic environments (Mantel & Farmer, 1983; Gilles & Pequeux, 1985; Pequeux & Gilles, 1988). Hence, the epithelium of the posterior

gill of *Hemigrapsus*, a hyper-osmoregulator, may be an important site of salt absorption in dilute media in a similar manner to those of the other osmoregulating crustaceans. The epithelium of the anterior gill also possessed a shallow AIS; however, there were few mitochondria between the basolateral infoldings of the cell membrane. Thus, the contribution of this epithelium to active ion-transporting seems to be marginal.

The % survival of the estuarine *Hemigrapsus* was higher than the *Pugettia* even in the highest external salinity (160% SW) as well as in the lowest external salinity (40% SW) (**Fig. 1**). In addition, Okamoto & Kurihara (1987) reported that the survival rate of this crab exposed to SW (salinity, 32‰) was very high (above 90%) for 40 days, and did not differ from crabs exposed to dilute SW with salinities between 13 and 25‰. Therefore, the seaward distribution of this estuarine crab probably was not limited by the osmolarity of the external medium. Similar circumstances have been recognized for the other hyperregulators and hyper-hyporegulators (see Gilles & Pequeux, 1983), and that both abiotic (e. g., physical environments such as wave action and substrates for living sites) and biotic (e. g., predation and competition) factors are probable causes for limiting the seaward distribution of the true estuarine species including *Hemigrapsus penicillatus*.

The salinity tolerance experiment conducted by Okamoto & Kurihara (1987) has also shown that the survival rate of *Hemigrapsus* is much lower in the dilute SW (salinity, 0.5‰) than in the other more concentrated media (salinity, 3-32 ‰). Their results account for the fact that the crab does not penetrate true fresh water, that is, the distribution is limited within the estuary. On the other hand, there are some hyper-osmoregulating crabs penetrating fresh water habitats. For instance, an euryhaline crab *Eriocheir japonica* (de Haan) migrates between marine and fresh water habitats, and a limnetic crab *Geothelphusa dehaani* (White) is a 'true fresh-water crab.' Differences in blood osmoregulatory capability among these hyperregulating species seem to be a determinant of the extent of penetration into the fresh water. It was observed that the anterior gills of *Geothelphusa* exhibit a well-developed ion-transporting epithelium in addition to the posterior gills (Kikuchi, 1992). The lower tolerance to fresh water of *Hemigrapsus* than *Eriocheir* and *Geothelphusa* may be also related to the fact that it maintains a relatively high blood osmolarity in the dilute SW (**Fig. 2**). *Hemigrapsus* maintained a blood osmolarity of 750 mOsm/kg even when exposed to an external medium of 400 mOsm/kg. In contrast, another euryhaline crab *Eriocheir* and a fresh-water crab *Geothelphusa* maintained their blood osmolarity at 550-650 mOsm/kg, which is somewhat lower than that of *Hemigrapsus*, in the external medium of the same osmolarity (unpublished data). In addition to the blood osmolarity control, some other physiological mechanisms (e.g., intracellular fluid osmoregulation) must be taken into consideration to account for the tolerance to reduced salinity.

In conclusion, the life of *Hemigrapsus penicillatus* within estuaries is supported by its hyper-osmoregulatory capability in dilute media. The ion-transporting epithelium of the gills plays an important role in the osmoregulation. However, the crab's penetration of fresh water habitats seems to to be limited due to the blood osmoregulatory capability and/or other physiolgical mechanisms in osmoregulation. On the other hand, the seaward limit of the *Hemigrapsus* distribution may be determined by other abiotic factors (e.g., wave action) or biotic ones such as

competition and predation.

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