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# A Comparative Study of Osmoregulation in Four Fiddler Crabs (Ocypodidae: *Uca*)

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**ABSTRACT**—This study aims to give an integrative description of the correlation of physiological parameters of osmoregulation and the habitats of the four common *Uca* species in Taiwan. *Uca arcuata* inhabits areas close to fresh water in the upper beach. *Uca formosensis* is only found in the areas near the mean high water of spring tide where there is a clear dry-wet transition within a single semilunar cycle. *Uca vocans* is found in the lower intertidal zone. *Uca lactea*, the most widely distributed species, can easily be found on most muddy sand shores. The number of gills was observed and histological sectioning performed on each species. The range of salinity in which the fiddler crabs maintained their hemolymph osmolality without any significant change (i.e. osmoregulatory homeostasis) and the gill $Na^+$, $K^+$-ATPase activity were determined by transferring individuals to different salinity tanks. The results suggest that *U. formosensis* and *U. lactea* can sustain a wider range of salinity change through both modification in gill morphology and $Na^+$, $K^+$-ATPase activity. *Uca arcuata* can regulate in a hypo-osmotic condition and *U. vocans* tends to be a weak-osmoregulator.

**Key words:** euryhaline, fiddler crab, osmoregulation, gill morphology, $Na^+$, $K^+$-ATPase

## INTRODUCTION

Euryhaline fiddler crabs inhabit estuaries and intertidal regions where the salinity can fluctuate from almost fresh water to suprasaline (D'Orazio and Holliday, 1985; Rabalais and Cameron, 1985; Luquet *et al*., 1995; Zanders and Rojas, 1996). Brachyuran crabs typically have 9 pairs of gills, but the number of gills tends to decrease as the degree of terrestriality increases (Gray, 1957; McMahon and Burggren, 1988; Taylor and Taylor, 1992). In those crabs, which live in brackish water or intertidal areas, the anterior gills are usually for respiration and the posterior gills for both respiration and osmoregulation (Copeland, 1968; Luquet *et al*., 1995; Péqueux, 1995 for review; Takeda *et al*., 1996). The respiratory lamellae consist of a thin layer of epithelia without any accumulation of mitochondria. On the contrary, a lot of ionocytes with many mitochondria can be found in the epithelial layer of osmoregulatory lamellae (Greenaway, 1988; Taylor and Taylor, 1992; Luquet, 1995; Takeda *et al*., 1996).

Previous studies have reported that $Na^+$, $K^+$-ATPase located in the basal infolding system of the ionocytes will change its activity with environmental salinity, implying a role in regulation of the internal ionic composition (D'Orazio and Holliday, 1985; Holliday, 1985, 1988; Corotto and Holliday, 1996; McLaughlin *et al*., 1996). This can be correlated with the habitat of the species. When transferred to hypo- or hyper-osmotic conditions, brachyuran crabs response differently in respect of branchial $Na^+$, $K^+$-ATPase activity. An increase in $Na^+$, $K^+$-ATPase activity was observed when brachyuran crabs, including *Uca pugnax, Hemigrapsus nudus, Carcinus maenas, Uca pugilator, and Pseudosesarma moeschi,* were transferred to hypoosmotic conditions (D'Orazio and Holliday, 1985; Holliday, 1985; Péqueux, 1995; Corotto and Holliday, 1996; McLaughlin *et al*., 1996). On the other hand, when transferred to hyperosmotic conditions, *H. nudus* (Corotto and Holliday, 1996) and *C. maenas* (Péqueux, 1995) had no change in $Na^+$, $K^+$-ATPase activity, while *U. pugilator* decreased (D'Orazio and Holliday, 1985) and *P. moeschi* increased (McLaughlin *et al*., 1996). These studies, however, focused more on discussion at a physiological level rather than on the relation between physiological diversity and its ecological implications.

In this study, four of the most common *Uca* species (*U. vocans, U. arcuata, U. formosensis* and *U. lactea*) which live in mid- to supra-tidal zones in Taiwan were compared, especially in gill morphology, hemolymph osmolality and $Na^+$, $K^+$-ATPase activity.

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## MATERIAL AND METHODS

### Field Observation

Environmental parameters were recorded for the mudflat of the south Tadu estuary, Taiwan, where the four *Uca* species were collected. Four transect lines were prepared, each 50 m apart. Each transect line was 600 m long, and a sampling area ($10\times10$ m$^2$) was established every 200 m. There were four sampling areas per line. The total number of each species of crabs present during low tide, and salinity in crab burrows or on the surface, were recorded daily for 14 days. On the day after a neap tide when the tidal water first reached the burrows of *U. formosensis* in the April of 1998, salinity of water sampled from 10 burrows of each species was determined.

### Laboratory Preparation

Adult male specimens of *U. arcuata, U. formosensis, U. vocans and U. lactea* were collected from the Tadu estuary (120°30'N, 24°13'E) in mid-Taiwan. They were transported to the laboratory and three individuals per species were sacrificed immediately for gill number determination and histological sectioning of the gills. The rest were maintained in 200 L holding aquaria ($90\times45\times60$ cm$^3$, one aquarium for each species) before experiments. All animals were kept at 26–28°C under a 14 L/10 D photoperiod.

Holding aquaria had a layer of 3-cm coarse coral reef sand and contained about 80 L of 25 ppt artificial seawater (ASW) made directly by mixing local tap water and salts (Coralife scientific grade marine salt, USA). The water level was about 12–15 cm. The water was aerated and filtered continuously. Several plastic racks ($18\times12\times13$ cm$^3$) with nylon mesh were provided as a platform for the crabs to stay in air. They were allowed to enter or leave the water and acclimated at least a week (but less than one month) before the salinity transfer experiment. Salinity was monitored by refractometry (ATAGO model S/Mill-E, Japan) every other day. A stock solution of 60 ppt ASW was prepared for experimental use and subsequently diluted to 0, 5, 15, 25, 35, 45 and 60 ppt ASW. The respective osmolalities were as follows: 0, 36, 438, 736, 996, 1274 and 1679 mosmol kg$^{-1}$ $H_2O$. The 0 ppt ASW was obtained from a 2000 $\times$ dilution. This was to simulate the result of the mixing of fresh water and seawater under the natural environment.

Twenty liters of experimental saline water were introduced into 25 L white plastic tanks ($50\times20\times25$ cm$^3$) with lids. Each tank had coral reef sand on the bottom and the water was aerated and filtered continuously.

Weighed crabs were wrapped in a paper towel and kept on ice. As soon as they could no longer move, the carapace was carefully removed and the right side gills were cut off for determination of gill morphology and $Na^+$, $K^+$-ATPase activity.

### Branchial morphology

The degree of branchial development and/or reduction was compared among the four species of *Uca*, following the method of Takeda *et al*. (1996). Briefly, three of the largest individuals of each species were chosen for dissection. After the carapace was removed, the gills on the right side were removed from the articulation, appendage, or sternite, and their length, number, and arrangement were recorded. The positions and numbers of the gills with the abbreviations for them are presented in Table 1.

### Histology

To investigate the osmoregulatory function of the gills, histology of the four most prominent gills (gills 5–8) of each species was examined. The podobranchiae on the second and third appendages (gills 1 and 3) and anterior arthrobranchiae (gill 4) on the third thoracic segment were too small to examine. Excised gills were fixed in FAA fixative (formalin, acetic acid, 70% alcohol, 1:1:18) without further dissection, followed by t-butonal-alcohol series dehydration. Sections (8 μm thick) were sliced from each gill and stained with hematoxylin and eosin. Gills with a marked increase in tissue thickness were considered to be highly osmoregulatory, while those with a thin layer of epithelium underneath the cuticular layer were recorded as respiratory gills. The criteria for determination are based on the review paper by Péqueux (1995) in which a thin epithelium of 1 to 5 μm is recognized as the site of gas exchange and a thick epithelium of 10 to 20 μm as the site of ion transport. Gills with only a small increase in thickness were recorded as weakly osmoregulatory gills.

### Hemolymph osmolality (HO)

The range of salinity in which the fiddler crabs maintained their hemolymph osmolality was examined in the four species of crabs. By keeping 3-5 crabs in a mesh cage ($18\times12\times13$ cm$^3$), four to eight cages were submerged in one experimental tank. Crabs were forced to remain fully immersed in 25 ppt ASW for 72 hr and transferred to 0, 5, 15, 25, 35, 45 or 60 ppt ASW, respectively. D'Orazio and Holliday (1985) indicated that the hemolymph of *U. pugilator* would show little change if they were allowed to enter or leave the media freely. Therefore, crabs were kept submerged throughout the experiment. The HO was measured at 0, 1, 4 hr and 2, 7, 14 days after submersion. Three to five individuals were used to determine the HO at each sampling period for each species.

The hemolymph samples were taken through the arthrodial membrane at the base of the large chela with a 27-gauge needle and quickly expelled into a 0.5 ml plastic tube. Hemolymph osmolality (8 μL aliquot of hemolymph) was immediately determined by an osmometer (Wescor Model 5500 vapor pressure osmometer). Laboratory temperature was kept at 25–26°C during osmolality measurement.

### $Na^+$, $K^+$-ATPase activity

Due to mortality of individuals of some species kept at extreme salinities (0 and 60 ppt ASW) in the HO experiment, only three levels of salinity (5, 25 and 45 ppt ASW) were included in this experiment. Two sampling periods, 4 hr and 14 d, would represent respectively the time of one ebb or flow tide and of one spring tide. Similar to the preparation in the HO experiment, individuals (3 per mesh cage) were first submerged into the experimental tanks with 25 ppt ASW for 72 hr before being transferred directly to 5, 25 or 45 ppt ASW. Assays were performed and three individuals per species were collected at 0 hr, 4 hr and 14 days. The right side of the four most prominent gills (gills 5, 6, 7 and 8) was sampled for enzyme activity determination.

Each weighed gill was rinsed in ice-cold homogenizing medium (HM: 0.25 M sucrose, 6 mM EDTA). Because the gills differed in size, the amount of HM added for homogenization varied. Basically, the volume (in μL) of HM was equal to 10–20 times the mass (in mg) of the gills. The gills were homogenized by a motor-driven, glass homogenizer (Hsiangtai Model DC-2RM, Taiwan) at 600 rpm for approximately 30 sec or until lamellae were not seen. Crude homogenate was then centrifuged at 4°C for 15 min at 4000 rpm (Hettich with rotor 1412) to remove cellular debris. The supernatant was collected, diluted 10 times with HM and rehomogenized in a hand-held homogenizer. Aliquots of 66.7 μL homogenate were prepared in 1.0 ml Eppendorf vials on ice and assayed immediately.

The method to determine $Na^+$, $K^+$-ATPase enzyme specific activity (ESA) was that of Holliday (1985). One of two reaction media (200 μl each) was added to aliquots of the 66.7 μL homogenate. One reaction medium had the complete formulus of all ions (167 mM NaCl, 50 mM KCl and 33.3 mM imidazole, pH 7.2) while the other had no potassium but contained 1.67 mM ouabain (an $Na^+$, $K^+$-ATPase specific inhibitor) (217 mM NaCl, 33.3 mM imidazole and 1.67 mM ouabain (Sigma), pH 7.2). They were mixed and preincubated at 30°C for 5 min in a water bath before 66.7 μL of the starting solution (25 mM $Na_2ATP$ (Sigma) and 50 mM

$MgCl_26H_2O$ (Sigma), pH adjusted to 7.2 with crystalline imidazole-HCl) was added and mixed well. After another incubation at 30°C for exactly 15 min, the reaction was stopped by adding 1.5 ml ice-cold Bonting's reagent (560 mM $H_2SO_4$, 8.10 mM ammonium molybdate and 176 mM $FeSO_4$) and color was allowed to develop for 20 min at 20°C. Phosphate concentration was measured spectrophotometrically at 700 nm as the reduced phosphomolybdate complex.

The protein contents in the homogenates were determined spectrophotometrically at 595 nm using bovine serum albumin as a standard. Enzyme specific activity (ESA) of $Na^+$, $K^+$-ATPase was calculated as the difference between phosphate (Pi) liberated by each homogenate in the two media and expressed as mmol $PO_4^{2-}$ per mg homogenate protein per h.

### Statistics

All results were expressed as mean value ± standard error. In the hemolymph osmolality experiment, the significance of difference in transfer time in various media was determined by one-way ANOVA followed by Duncan's Multiple Range Test (SAS 6.12).

For the comparisons of $Na^+$, $K^+$-ATPase activity, because of the non-independence of the ESA of each gill within an individual and because the ESA among gills was measured at the same time, a repeated-measures ANOVA was performed (Tabachnick and Fidell, 1989) on the four most prominent gills. The grouping variable was salinity, divided into (1) control, which was crabs acclimated to 25 ppt at the beginning of the experiment, (2) 5 ppt, (3) 25 ppt and (4) 45 ppt. Three individuals of each species were collected 4 hr and 14d after transfer. SAS GLM profile analysis (SAS 6.12) was used. Additionally, pair-wise comparisons were performed between certain data subsets.

## RESULTS

### Field Observation

*Uca arcuata* was mostly found on wet muddy substrate along tidal creeks and canals. The environmental salinity of *U. arcuata* was less than 5 ppt during low tide and between 20 to 30 ppt during high tide. The salinity range for *U. formosensis* recorded in the field ranged from 0 to 38 ppt. *Uca formosensis* lives on open mudflats of supratidal regions where seawater does not reach for 3 to 5 days during neap tides. Nevertheless, water can be sampled from the bottom of their burrows and the average salinity of water collected in the burrows during neap tides was lower than 5 ppt. The species, which lives at the lowest tidal location, is *U. vocans*. Tidal water reaches here everyday and daily salinity changes during the 14 days of recording ranged from 23 to 35 ppt. The most widely distributed *U. lactea* was found in all three habitats. Since there were more *U. lactea* in the low to mid-tidal region than in the rest of the area, we recorded its habitat characteristics in this area. The salinity range of this low to mid-tidal area was between 24 to 38 ppt during the 14 days of observation.

The salinity determined from 10 burrows for each species were as follows: 11.3±2.7 ppt for *U. arcuata*, 41.9±1.1 ppt for *U. formosensis*, 39.3±0.8 ppt for *U. vocans* and 36.1± 0.8 ppt for *U. lactea*.

### Gill morphology

None of the four species had gills 2 and 9. On all four species of *Uca*, the posterior four pairs of gills (gills 5–8) were more prominent than the anterior three pairs (gills 1, 3 and 4). For all species, the anterior arthrobranchiae attached to the forth appendages (gill 6) were the longest. A species-specific variation was found in the degree of reduction in the three anterior gills (gills 1, 3 and 4) (Table 1). There were 7 complete gills in *U. vocans*. Both *U. arcuata* and *U. formosensis* had 6 complete gills. Gill 3, which was completely absent in *U. formosensis*, is reduced to a rod shape without lamellae in *U. arcuata*. The least number of gills was found in *U. lactea*, which had only 5 gills, with a reduction of gill 4 and a complete reduction of gills 3 and 9.

### Histology

Histology of the four most obvious gills (gills 5–8) of *Uca* indicated further morphological variation. In *U. formosensis*, many ionocytes, easily noticed by the tissue thickening, were observed in the lamellae of all four gills. Although a highly osmoregulatory type of gill was suggested, the degree of lamella thickening was more evident in gills 7 and 8 than in gills 5 and 6.

Highly osmoregulatory type gills were also found in gills 7 and 8 of the other three *Uca* species, except gill 7 in *U. vocans*. In *U. lactea*, respiratory and weakly osmoregulatory gills were recorded respectively for gills 5 and 6. Gill 6, but not gill 5, of *U. arcuata* was determined to be weakly osmoregulatory. Both gills 5 and 6 of *U. vocans* were classified as respiratory (Table 2).

**Table 1.** Gill morphology in the four species of *Uca*.

| Species | Thoracic segments | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | II | | III | | | IV | | V | VI | |
| | Po. | A. | Po. | A.A. | P.A. | A.A. | P.A. | Pl. | Pl. | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| *U. arcuata* | + | | − | + | + | + | + | + | | 6 (7) |
| *U. formosensis* | + | | | + | + | + | + | + | | 6 |
| *U. vocans* | + | | + | + | + | + | + | + | | 7 |
| *U. lactea* | + | | | − | + | + | + | + | | 5 (6) |

"+": gill with lamellae. "–": reduced gill without lamellae. Po.: podobranchiae, A: arthrobranchiae, A.A. : anterior arthrobranchiae, P.A.: posterior arthrobranchiae, and Pl.: pleurobranchiae. Numbers in parentheses are the number of complete and reduced branchiae.

**Table 2.** Osmoregulatory and respiratory functions in the four most apparent gills of *Uca*.

| | Gill Number | | | |
|---|---|---|---|---|
| | 5 | 6 | 7 | 8 |
| *U. arcuata* | ++ | + | ++ | ++ |
| *U. formosensis* | ++ | ++ | ++ | ++ |
| *U. vocans* | – | – | + | ++ |
| *U. lactea* | – | + | ++ | ++ |

The signs give an indication of the relative thickness of each gill from those crabs collected directly from the field without laboratory acclimation. "++": highly osmoregulatory gills, "+": weakly osmoregylatory gills, and "–": respiratory gills.

### Hemolymph osmolality

After acclimation to 25 ppt ASW for 72 hr, the four species of fiddler crabs had average hemolymph osmolality as follows: *U. arcuata* 755.8±5.5 mosmol $kg^{-1}$ $H_2O$ (N=26), *U. formosensis* 860.9±2.0 mosmol $kg^{-1}$ $H_2O$ (N=31), *U. vocans* 786.0±4.7 mosmol $kg^{-1}$ $H_2O$ (N=29) and *U. lactea* 840.1± 4.7 mosmol $kg^{-1}$ $H_2O$ (N=30). Results following transfer to different salinities are presented by species.

*U. arcuata*. Hemolymph osmolality (HO) did not change significantly within 14 days for those individuals transferred to 15 or 25 ppt ASW (Fig. 1A). Comparing the osmolality at 0 hr (crabs were collected immediately after being transferred) and 4 hr after transfer, a significant difference was found in those transferred to 0, 35, 45 and 60 ppt ASW. All individuals in 0 ppt ASW died shortly after 7-day observation. In 35 ppt ASW, HO increased significantly 4 hr after transfer, reached environmental condition at 7 d (1012.6± 2.9 mosmol $kg^{-1}$ $H_2O$) and recovered slightly thereafter. Dramatic increases in HO were recorded in those individuals transferred to 45 and 60 ppt ASW and all individuals died shortly after 2 days (Fig. 1A).

*U. formosensis*. Only individuals in 0 ppt ASW displayed a significant decrease in HO after 4 hr of transfer (Fig. 1B). HO declined to 487.2±8.9 mosmol $kg^{-1}$ $H_2O$ prior to their death on day 2. It was the seventh day before a significantly lower HO was noticed in either 5 or 15 ppt ASW (as compared to the HO at 0 hr in their respective salinity) (Fig. 1B). For those in 35 or 45 ppt ASW, maximal HO was observed on day 7 (979.8±14.6 mosmol $kg^{-1}$ $H_2O$ and 1329.6±8.4 mosmol $kg^{-1}$ $H_2O$, respectively). Although HO in

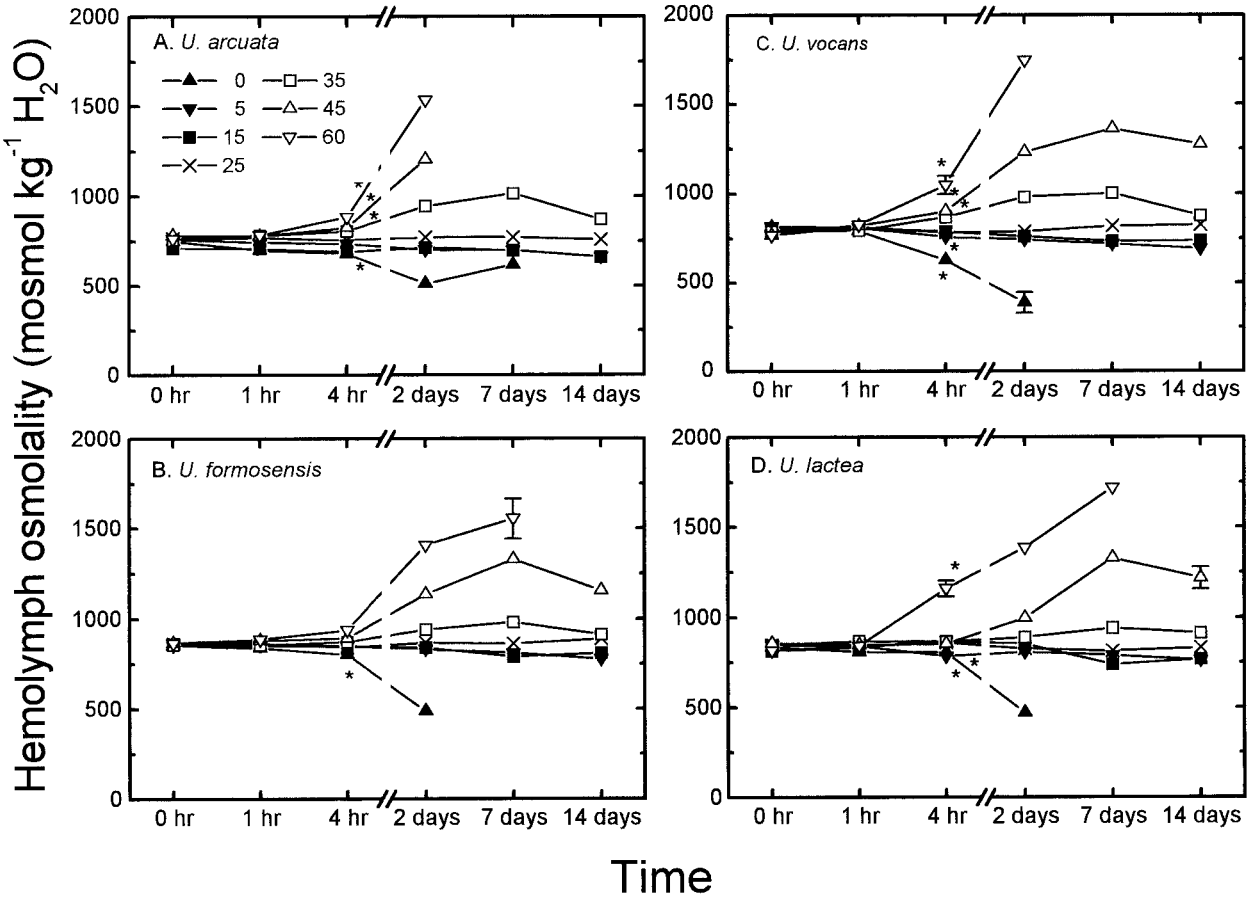


**Fig. 1.** Time course of changes in hemolymph osmolality (HO) in (A) *Uca arcuata*, (B) *U. formosensis*, (C) *U. vocans* and (D) *U. lactea* after transfer of crabs from 25 ppt to various salinities. Each symbol represents the mean value of hemolymph samples from 3 to 5 crabs. For a better recognition of the symbols, only those standard errors larger than 35 were displayed. Asterisks indicate significant differences between mean values of HO at time zero and 4 hr after transfer.

both salinities recovered significantly on day 14 (compared to day 7), they were still significantly higher than their respective controls. Individuals in 60 ppt ASW did not increase their HO until 2 days after transfer and they survived for at least 7 days.

*U. vocans*. It took only 4 hr for *U. vocans* to show a significant difference in HO after they were transferred to 0, 5, 35, 45 or 60 ppt ASW (Fig. 1C). *U. vocans* survived in 0 or 60 ppt ASW for 2 days only. There were slight variations through time in those submerged in 5, 15 and 25 ppt ASW. When kept in 35 ppt ASW, individuals of *U. vocans* had a significant rise in HO by 4 hr. Their HO was close to environmental conditions on day 2 (981.2±15.3 mosmol $kg^{-1}$ $H_2O$) and day 7 (1001.6±11.5 mosmol $kg^{-1}$ $H_2O$). By the end of the 14 days, the HO was significantly lower than that at the end of 7 days and recovered to the level recorded after 4 hr. Similarly, when *U. vocans* was maintained in 45 ppt ASW SW, a drastic increase was noticed by day 7 (1364.3±4.9 mosmol $kg^{-1}$ $H_2O$) and a slight decline thereafter (1276.6±21.3 mosmol $kg^{-1}$ $H_2O$) on day 14. *U. vocans* only survived in 60 Fppt ASW SW up to 2 days with its HO (1747.5±7.4 mosmol $kg^{-1}$ $H_2O$) almost equivalent to the

**Table 3.** Univariate repeated-measures ANOVA of the effects of salinity on the $Na^+$, $K^+$-ATPase activity in the gills of four *Uca* species.

| Source of variance | 4 hr after transfer | | | | 14 d after transfer | | | |
|---|---|---|---|---|---|---|---|---|
| | df | MS | F | P | df | MS | F | P |
| *U. arcuata* | | | | | | | | |
| Between subject effects | | | | | | | | |
| Salinity (S) | 3 | 2012.6 | 6.55 | 0.0193* | 2 | 430.4 | 3.45 | 0.1345 |
| Error | 7 | 307.3 | | | 4 | 124.6 | | |
| Within subject effects | | | | | | | | |
| Gill (G) | 3 | 400.3 | 2.30 | 0.1064 | 3 | 1080.9 | 25.70 | 0.0001* |
| S × G | 9 | 95.3 | 0.55 | 0.8230 | 6 | 64.0 | 1.52 | 0.2521 |
| Error | 21 | 173.8 | | | 12 | 42.1 | | |
| *U. formosensis* | | | | | | | | |
| Between subject effects | | | | | | | | |
| Salinity (S) | 3 | 15.3 | 7.44 | 0.0272* | 3 | 64.3 | 5.00 | 0.0306* |
| Error | 5 | 2.1 | | | 8 | 12.9 | | |
| Within subject effects | | | | | | | | |
| Gill (G) | 3 | 148.4 | 23.32 | 0.0001* | 3 | 208.3 | 35.30 | 0.0001* |
| S × G | 9 | 4.1 | 0.64 | 0.7467 | 9 | 11.3 | 1.92 | 0.0976 |
| Error | 15 | 6.4 | | | 24 | 5.9 | | |
| *U. vocans* | | | | | | | | |
| Between subject effects | | | | | | | | |
| Salinity (S) | 3 | 24.2 | 0.50 | 0.7031 | 3 | 850.0 | 11.54 | 0.0194* |
| Error | 4 | 48.6 | | | 4 | 73.7 | | |
| Within subject effects | | | | | | | | |
| Gill (G) | 3 | 311.4 | 20.08 | 0.0001* | 3 | 948.5 | 30.94 | 0.0001* |
| S × G | 9 | 10.4 | 0.67 | 0.7211 | 9 | 163.8 | 5.34 | 0.0045* |
| Error | 15 | 15.5 | | | 12 | 30.7 | | |
| *U. lactea* | | | | | | | | |
| Between subject effects | | | | | | | | |
| Salinity (S) | 3 | 154.4 | 1.05 | 0.4606 | 3 | 423.0 | 3.15 | 0.1483 |
| Error | 4 | 146.4 | | | 4 | 134.3 | | |
| Within subject effects | | | | | | | | |
| Gill (G) | 3 | 667.5 | 8.98 | 0.0021* | 3 | 570.4 | 11.01 | 0.0009* |
| S × G | 9 | 101.4 | 1.36 | 0.3016 | 9 | 85.4 | 1.65 | 0.2064 |
| Error | 12 | 74.3 | | | 12 | 51.8 | | |

"df" stands for degree of freedom, "MS" for mean square, "F" for F statistics and "P" for P value. "S×G" stands for the interactive effect of salinity and gill on the $Na^+$, $K^+$-ATPase activity.

external milieu.

*U. lactea*. Among the seven salinity levels, 25 ppt ASW was the only salinity treatment that did not cause significant change in HO within the 14-day observation. Four hours after transfer, significant changes in HO were found in organisms from 0, 5 and 60 ppt ASW (Fig. 1D). All specimens of *U. lactea* had a significantly lower HO and they died shortly after the second day in 0 ppt ASW. Although there was a significant change in HO when specimens of *U. lactea* were held in 5 ppt ASW, HO did not change further. At 35 ppt ASW, a significant rise in HO was found 2 d after transfer. In 45 ppt ASW, HO peaks in specimens of *U. lactea* on day 7 (1329.5±7.5 mosmol $kg^{-1}$ $H_2O$), thereafter declining significantly by day 14. The HO of specimens of *U. lactea* in 60 ppt ASW rose to 1157.6±43.6 mosmol $kg^{-1}$ $H_2O$ within 4 hr and was iso-osmotic to the environment by day 7 (1718.5 ±16.6 mosmol $kg^{-1}$ $H_2O$), but no specimen of *U. lactea* survived 14 days exposure to this salinity.

In summary, the salinity range for osmoregulatory homeostasis was species-specific. Upon four hours after transfer, *U. formosensis* had the widest range (5 to 60 ppt ASW), followed by *U. lactea* (15 to 45 ppt ASW) and *U. arcuata* (5 to 25 ppt ASW), and *U. vocans* had the narrowest (15 to 25 ppt ASW). Response to salinity over a longer period of time was also species-specific.

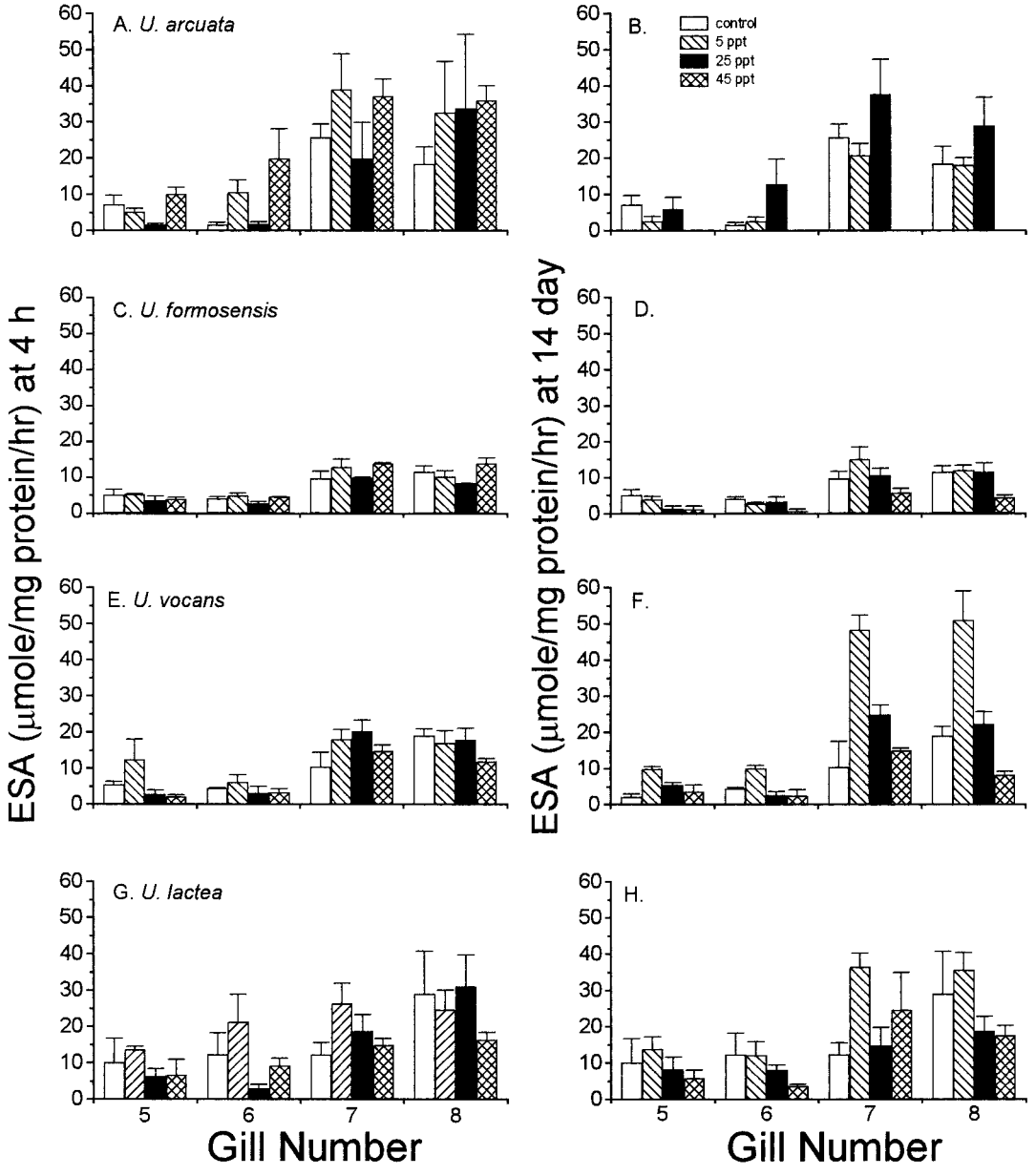


**Fig. 2.** $Na^+$, $K^+$-ATPase ESA in the gills of (A, B) *U. arcuata*, (C, D) *U. formosensis*, (E, F) *U. vocans* and (G, H) *U. lactea*. Crabs were first acclimated at 25 ppt for 72 hr before being transferred to 5, 25 and 45 ppt ASW. $Na^+$, $K^+$-ATPase ESA at 4 hr and 14 days was determined from 3 to 5 crabs. Error bars represent±1 SE.

**$Na^+$, $K^+$-ATPase activity assay (ESA)**

This experiment compared the time course of branchial $Na^+$, $K^+$-ATPase activity of the four crab species salinity transfer after salinity transfer. An increase in the $Na^+$, $K^+$-ATPase activity was taken as an indication of an active physiological regulation in response to salinity change.

Among all four species studied, the two posterior gills (gills 7 and 8) had a higher ESA than the two anterior to them (gills 5, 6). The test statistics for each species and time periods are summarized in Table 3.

*U. arcuata*. Within 4 hr after transfer to 45 ppt, salinity had a significant effect on ESA (Fig. 2A, Table 3A). But this difference disappeared by the end of the 14 d experiment (Fig. 2B, Table 3B). Instead, the difference among gills was significant. However, the standard error of each of the 4 hr treatment combinations was also high, making further comparisons insignificant. None of the individuals submerged in 45 ppt survived to the end of the 14 d experiment.

*U. formosensis*. Specimens of *U. formosensis* had the lowest ESA among the four *Uca* species (Fig. 2C, D). However, in addition to a significant difference in ESA among salinity treatments, a difference was found among gills in both sampling periods (Table 3C, D). There was no significant difference in ESA between these two sampling periods (Fig. 2C, D).

*U. vocans*. Four hours after transfer, the salinity effect on ESA was not significant, while the difference among gills was significant (Table 3E). The ESA changed significantly, especially in the two posterior gills at low salinity on day 14 (Table 3F and Fig. 2E, F).

*U. lactea*. Although the gills have significantly different ESA, salinity did not have an effect on the ESA of *U. lactea* at either 4 hr or 14 d after transfer (Table 3G, H and Fig. 2G, H).

## DISCUSSION

In this study, discussion of physiological responses to salinity fluctuation in the natural environment is possible since several species were studied at the same time and both the ecological data on salinity fluctuation and laboratory investigation on enzyme activity were included.

Gray (1957) reported that the reduction in gills was correlated with the degree of terrestrial adaptation in crabs. This correlation was observed in our study, with *U. lactea* having only 5 pairs of functional gills, *U. formosensis* and *U. arcuata* each having 6 and *U. vocans* having 7. Similar results were also reported by Takeda *et al*. (1996) in their study of 16 species of ocypodid and grapsid crabs. *Uca annulipes*, whose distribution is highest on the shore has the fewest pairs of gills (5), while *U. tetragonon* and the subspecies *U. vocans vocans*, both of which live near the low tide mark, each have 7 pairs (Takeda *et al*., 1996). However, it is inappropriate to predict the degree of terrestrial adaptation by the number of gills when the crab species vary greatly in size or are phylogenetically distant. For example, *Gecarcinus lateralis*, which lives in supratidal zone and is completely terrestrial, has 7 functional gills plus 2 vestiges (Copeland, 1968) and it would have been misleading to compare its terrestrial adaptation with those of *Uca* species. In our study, *U. lactea* is significantly smaller than the other three species and it is uncertain whether this would have any association with the extent of gill reduction.

For most terrestrial crabs, gas exchange takes place in the specialized vascular system on the inner lining of carapace, while osmoregulation is performed in thickened gill lamellae filled with ionocytes (Mantel and Farmer, 1983; Greenaway, 1988). In our study, *Uca formosensis* and *U. arcuata* had increases in thickness in the four most prominent gills. Similar results were also reported in two supratidal fiddler crabs, *U. annulipes* and *U. forcipata* (Takeda *et al*., 1996).

The four *Uca* species exhibited various degrees and ranges of osmoregulatory homeostasis when transferred to different salinities. The ability to maintain HO was well correlated with the habitat of each species described in the first paragraph in the Result. From our results for crabs submerged in various salinities for up to 14 days, *U. formosensis* is a better hyper-hypoosmoregulator than *U. lactea*, and both *U. arcuata* and *U. vocans* are hyper-osmoregulators.

For those species such as *U. longisignalis*, *U. rapax* and *U. subcylindrica* which live in a habitat similar to or more terrestrial than that of *U. formosensis*, their salinity tolerance ranges (or the salinity ranges of survival) are much larger than in other intertidal *Uca* species (Rabalais and Cameron, 1985; Zanders and Rojars, 1996). The salinity tolerance ranges of *U. longisignalis* and *U. subcylindrica* were 0.008 to 110 g $L^{-1}$ NaCl (about 0 to 110 ppt) and 2 to 90 g $L^{-1}$ NaCl (2 to 90 ppt), respectively (Rabalais and Cameron, 1985), while that of *U. rapax* was 7 to 139 g $L^{-1}$ NaCl (7 to 139 ppt)(Zanders and Rojars, 1996). Although their ranges were much larger than that of *U. formosensis*, the gradual transfer (instead of our direct transfer) to extreme salinity may explain this difference.

Several studies have indicated that enzyme activity of branchial $Na^+$, $K^+$-ATPase varies with species and their acclimation conditions (Corotto and Holliday, 1996; McLaughlin *et al*., 1996; Castilho *et al*., 2001). In brief, an increase in $Na^+$, $K^+$-ATPase ESA was found in the intertidal crustaceans exposed to diluted seawater. The increase is especially apparent in the posterior gills. Increase in $Na^+$, $K^+$-ATPase ESA at low salinity has been observed in *U. pugilator* (D'Orazio and Holliday, 1985) and *U. pugnax* (Holliday, 1985) acclimated for 21 d. *U. vocans*, which is distributed in the lower intertidal areas, had a significantly higher $Na^+$, $K^+$-ATPase ESA in the two posterior gills when they were kept at low salinity for 14 d, presumably to increase ion uptake. However, this response did not occur in the rest of the three *Uca* species in Taiwan. *Uca lactea* was collected from a similar tidal location as *U. vocans*. *Uca lactea* exhibited a similar but insignificant trend of change in $Na^+$, $K^+$-ATPase ESA when transferred to low salinity for 14 days. Neither *U.*

*arcuata* nor *U. formosensis* had a significant change in $Na^+$, $K^+$-ATPase ESA at low salinity. In addition to species-specific variation, different length of acclimation (i.e. 21 d in the studies by D'Orazio and Holliday (1985) and Holliday (1985) and 14 d in the present study) should be taken into account.

There are far fewer studies of $Na^+$, $K^+$-ATPase ESA in crabs acclimated to hyperosmotic conditions and the results are inconclusive. No significant change was observed in $Na^+$, $K^+$-ATPase ESA of *U. pugnax* at high salinity (150% or 200% SW) (about 53 or 71 ppt, respectively) (Holliday, 1985), while a significant decrease in $Na^+$, $K^+$-ATPase ESA was reported for the posterior gills of *U. pugilator* acclimated to 200% seawater (approximately 71 ppt) (D'Orazio and Holliday, 1985). In our study, the change in $Na^+$, $K^+$-ATPase ESA of both *U. formosensis* and *U. vocans* after 14 d of acclimation may not necessarily be biologically significant, due to small sample size, large standard deviation and partial mortality. In the future, it would be particularly interesting to examine the plasticity of branchial morphology and $Na^+$, $K^+$-ATPase ESA in a certain widely distributed species such as *U. lactea* from different types of habitats.

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