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EVIDENCE THAT SALT WATER MAY NOT BE A BARRIER TO THE DISPERSAL OF ASIAN FRESHWATER CRABS (DECAPODA: BRACHYURA: GECARCINUCIDAE AND POTAMIDAE)

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ABSTRACT. **–** The true freshwater crabs are a strictly freshwater circumtropical group that is found throughout the inland waters of the continents as well as on numerous islands. We tested competing biogeographic hypotheses for how this distribution pattern arose. Vicariant explanations, based on the belief that true freshwater crabs are unable to tolerate exposure to high salinities, assume that stretches of seawater are significant barriers to dispersal. Dispersalist explanations, based on phylogenetic and fossil evidence, assume that freshwater crabs are capable of at least limited trans-oceanic dispersal. We evaluated the physiological tolerance to sea water of two Thai freshwater crab species held for two weeks in either shallow or deep sea water. Both species survived in full-strength sea water for at least 14 days. Survival rates in high salinities were not affected by body size, gender, or whether crabs were fully or partially submerged. Although freshwater crabs are never found naturally in any aquatic saltwater ecosystem, physiologically they are euryhaline osmoregulators that do not die immediately in full-strength sea water. These strictly freshwater decapods can survive in sea water for long enough to disperse across at least short distances between continents and islands. Our findings support dispersalist rather than vicariant explanations for present-day freshwater crab distribution patterns.

KEY WORDS. – osmoregulation, salinity tolerance, freshwater crabs, Thailand, marine dispersal, biogeography, Gecarcinucidae, Potamidae.

INTRODUCTION

The freshwater crabs are a highly diverse and strictly freshwater group of tropical heterotreme brachyurans that lack free-living larval stages and reproduce by direct development (Cumberlidge, 1999; Yeo et al., 2008). These crabs have a global distribution in the warm inland waters of continents and tropical islands, and the fact that they are never found naturally in any aquatic ecosystem where the water has even low levels of salt raises interesting questions about how they came to be present on hundreds of islands around the world (Rodriguez & Lopez, 2003; Cook et al., 2008; Cumberlidge, 2008; Cumberlidge & Ng, 2009; Yeo et al., 2008; Cumberlidge et al., 2009; Klaus et al., 2009; Klaus et al., 2010; Jesse et al., 2011).

The central biogeographic question posed in the present work on osmoregulatory physiology centers on the ability of freshwater crabs to tolerate exposure to salt water long enough to cross short stretches of ocean to reach offshore islands. It has long been hypothesized circumstantially (without physiological evidence) that Asian potamiscine freshwater crabs (Dai, 1999; Shih et al., 2004; Shih et al., 2007) and Asian gecarcinucine freshwater crabs (Klaus et al., 2009; Klaus et al., 2010) found on islands must have crossed salt water. Similarly, other workers have suggested that species of European freshwater crabs (Potaminae) may have crossed stretches of salt water in the Mediterranean and Aegean Seas (Jesse et al., 2009; Jesse et al., 2011). In addition, transoceanic rafting has been postulated to explain the presence of African potamonautid freshwater crabs on the volcanic islands of Príncipe and São Tomé (Cumberlidge, 2008) and on the gondwanan islands of Madagascar and the Seychelles (Haig, 1984; Daniels et al., 2006; Cumberlidge & Ng, 2009; Cumberlidge et al., 2008).

While there have been only a few studies on the osmoregulatory abilities of freshwater crabs in salt water (Shaw, 1958a, b; Morris & van Aardt, 1998), or on air breathing and resistance to dehydration (Lutz, 1969; Cumberlidge, 1986; Greenaway, 1986; Daniels et al., 2002), none of these works have focused on Asian species until recently (Esser, 2007). These physiological considerations are important because freshwater crabs clinging to drifting vegetation would either be immersed in sea water underneath the floating mat, or out of the water on top of the vegetation. Rafting freshwater crabs would therefore need to be able to survive either extended immersion in deep sea water or partial immersion in shallow sea water, as well as exposure to air and extensive dehydration.

Here we compare osmoregulation and survival times in salt water of two species of Asian freshwater crabs, *Esanthelphusa dugasti* Rathbun, 1902 (Gecarcinucidae) and *Eosamon smithianum* Kemp, 1923 (Potamidae: Potamiscinae) (Ng et al., 2008). *Esanthelphusa dugasti* occurs in Thailand (Chonburi and Nakhon Phanom Provinces) and in the Lao People's Democratic Republic (Yeo, 2004), while *E. smithianum* is found only in Chon Buri, Chachoengsao, and Chantaburi Provinces in Thailand (Yeo & Ng, 2007). *Esanthelphusa dugasti* is semi-terrestrial and frequently found in rice fields and associated habitats, dividing its time between water, land, and humid burrows, while *E. smithianum* prefers well-oxygenated rivers and waterfalls (Ng, 1988; Yeo et al., 2008). The contrasting habitat preferences of these two species may have some bearing on their abilities to osmoregulate, breathe air, resist dehydration, and survive in harsh conditions (Yeo et al., 2008).

Like all freshwater crabs, these species are strictly freshwater animals and it has long been assumed that they cannot survive for long in sea water (Cumberlidge, 2008; Yeo et al., 2008). This assumption has led to vicariant biogeographical hypotheses to explain the origins of some island species (Bott, 1970; Banarescu, 1990; Apel & Brandis, 2000; Brandis, 2002). However, it is still not known whether or not these animals can withstand prolonged exposure to salt water for any length of time because the salinity tolerance of Asian freshwater crabs has only begun to be evaluated experimentally (Esser, 2007). Further, it is not clear whether the osmoregulatory abilities of adult and sub-adult freshwater crabs are better developed that those of juveniles, and whether these abilities are reflected in longer survival times in high salinity. Similarly, it is not clear whether male freshwater crabs survive longer than females in high salinity environments given that adult females must devote more energy to egg development than do males.

The few studies available have shown that the osmolality of the hemolymph and interstitial fluids of potamonautid and potamid freshwater crabs in fresh water is maintained at a level (350–500 mmol kg^{-1}) that is always hyperosmotic to the environmental fresh water (<50 mmol kg^{-1}) (Shaw, 1958a, b; Morris & van Aardt, 1998). This means that freshwater crabs in fresh water face two osmoregulatory problems that threaten to dilute their body fluids: a constant osmotic influx of water and a constant diffusive loss of salts to the surrounding water (Randall et al., 2001). Freshwater crabs in brackish water and sea water are similarly challenged by conditions that radically alter their ionic and osmotic relationships with their external media, which may be expected to reduce their survival time in high salinity water. The present study quantifies experimentally the osmoregulatory abilities and survival times of two Asian freshwater crab species of different ages and both genders subjected to a range of salinities in both deep and shallow sea water. In addition, osmoregulation in freshwater crabs is compared to that of other decapods found in freshwater habitats, and the biogeographic implications of these results are discussed.

MATERIAL AND METHODS

Specimens of *Esanthelphusa dugasti* (n = 570) were collected from a rice field in Chonburi Province, Thailand in Jul.2006, sorted by gender and age (adults, sub-adults, juveniles) (Table 1) and maintained in aerated freshwater tanks (1.2 or 1.5 L) at 30 ± 2°C. Sub-adult crabs ranged from carapace width (cw) 25 to 40 mm, and juveniles from cw 18 to 28 mm; there were no adults (cw > 40 mm) in the samples available. Specimens of *Eosamon smithianum* (n = 120) were collected by hand in Jul.2006 from two waterfall streams in the Klung District of Chantaburi Province, Thailand. To reduce possible injuries from fighting (Ng, 1988) adult specimens of *E. smithianum* were held in individually aerated aquaria: adults (≥ cw 57 mm) in 1.2 or 1.5 L tanks, smaller adults (≤ cw 34 mm) in 0.4 L tanks, juveniles (cw 18 to 28 mm) in 0.125 L tanks, and hatchlings (cw 4 to 16 mm) in 0.25 L glass jars.

Seawater concentrations were determined using a salinometer and recorded in parts per thousand (ppt). The water in the experimental tanks in the laboratory was maintained at 30° ± 2°C with salt concentrations ranging from 7 ppt (brackish water) to 33 ppt (full strength sea water in the Gulf of Thailand). Hemolymph osmolality (mmol kg^{-1}) was determined by analyzing 10–50 µl of hemolymph drawn from each animal using a latex free syringe (BD with Precision Glide Needle 0.5 × 16 mm) inserted into the arthrodial membrane at the base of a posterior pereiopod. The osmolality (mmol kg^{-1}) of 10 µl samples of hemolymph and of aquarium water was analysed with an automatic micro-osmometer (Wescor Vapro 5520).

Osmoregulatory abilities (as reflected by changes in hemolymph osmolality) and survival times were determined for groups of intermolt crabs belonging to both species subjected to a range of salinities in two experimental series, one using deep water aquaria, the other using shallow water aquaria (Table 1). These experimental protocols were designed to replicate the conditions faced by crabs that are either fully immersed in sea water when clinging underneath drifting vegetation ('deep water'), or that are on top of the vegetation and only partly immersed and partly out of the water ('shallow water'). Control groups were maintained in aerated freshwater tanks throughout the experimental period (up to 14 days) and their hemolymph osmolality determined. Animals in shallow water aquaria were only partially immersed and could breathe air as well as water, while animals immersed in deep aerated water were prevented from breathing air by filling their aquaria to the top. Groups of adult, subadult, and juvenile *E. dugasti* and *E. smithianum*

Table 1. Summary of all experimental groups and their numbers sampled (with genders pooled). FW = freshwater; ppt = parts per thousand. Only sub-adults and juveniles of *E. dugasti* were available. Data for males and females pooled.

Family, species and age group	**Water depth**												
	Shallow water (2 cm) (salinity, ppt)									**Deep water (30 cm) (salinity, ppt)**			
	FW	7	13	15	22	25	28	30	33	FW	22	25	30
GECARCINUCIDAE	**Number of specimens sampled**												
E. dugasti sub-adult CW 25–40 mm	27	30	23	29	27	14	12	27	14	28			26
E. dugasti juvenile CW 18–28 mm	26		10		26			28	5		34		17
POTAMIDAE	**Number of specimens sampled**												
E. smithianum adult CW 34–57 mm	20				17		22			15	7	13	11
E .smithianum juvenile CW 18–28 mm	10				15								
E. smithianum hatchling CW 4–16 mm					20								

were placed directly in aquaria containing deep or shallow water of a particular salinity (e.g., 7, 13, 15, 22, 30 and 33 ppt) for up to 14 days (or as long as they survived) and their hemolymph osmolality was sampled periodically (e.g., after 1, 3, 6, 9, 12 and 14 days) (Table 2). The osmolality of the water in each of these salinity regimes was also determined. The details of the different protocols for each salinity plus exposure time and water depth for each species are given in Tables 1 & 2. Depending on protocol, individual crabs either remained in a particular salinity for the entire duration of the experiment, or were subsequently transferred after a prescribed number of days to a different salinity. The survival times of individual crabs in different salinity regimes were recorded after transferring freshwater-acclimated crabs to their experimental tanks and percent survival rates were calculated for groups according to experimental protocols (Table 3). In those cases where individual crabs survived experimental exposure to high salinities for more than 14 days, they were returned stepwise to freshwater via a series of aquaria containing intermediate salinities.

Repeated measures ANOVAs were performed using SPSS Version 13.00 for Windows. Hemolymph osmolality after 5, 9, and 13 days was the within-subjects factor for the crabs, while gender, age, water salinity, and water depth were the among-crab factors. The significance level for all tests was $p \leq 0.05$; groups with less than three osmolality readings were excluded due to limitations of the statistical test. Mean hemolymph osmolalities between the two species were compared with independent t-tests using pooled data from groups of crabs of different genders and ages for each salinity level and each day.

RESULTS

The mean hemolymph osmolalities of groups of *E. dugasti* and *E. smithianum* did not vary significantly with gender, and no differences were found in independent t-tests between males and females subjected to the same salinity and water depth for the same length of time. The mean normal hemolymph osmolalities in control groups of *E. dugasti* (358.61–394.14 mmol kg^{-1}) and *E. smithianum* (440.95–459.75 mmol kg^{-1}) held in deep fresh water for two weeks although different for each species, remained hyperosmotic to fresh water throughout (Figs. 1, 2, Table 3). In general, increases in external salinity resulted in proportional increases in hemolymph osmolality whether in deep or shallow sea water, with a significant and immediate increase when crabs were first subjected to the salinity change (Figs. 1, 2). Both species were found to be active osmoregulators (rather than osmoconformers) and continued to regulate their internal

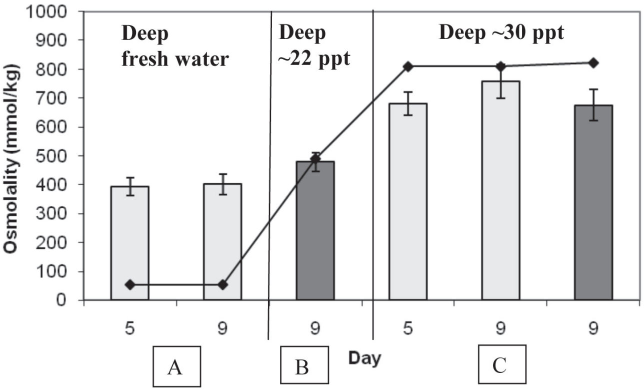

Fig. 1. Hemolymph osmolality of three experimental groups (A–C) of *Esanthelphusa dugasti* (Gecarcinucidae) held for up to 9 days in deep 30 cm fresh water and in 22 and 30 ppt salt water. Line with diamonds = water osmolality. Columns = hemolymph osmolality, light = sub-adults; dark = juveniles; error bars = standard deviation. The x-axis shows the number of days that crabs were subjected to a particular salinity. A. Sub-adults in fresh water (n = 27 and 24, respectively). B. Juveniles in 22 ppt salt water (n = 25). C. Sub-adults (n = 22 and 8, respectively) and juveniles (n = 12) in 30 ppt water. Dark horizontal bar = salinity at which hemolymph is hyperosmotic to the external water.

Table 2. Significant differences in hemolymph osmolality (mmol kg^{-1}) between *E. dugasti* and *E. smithianum* held immersed for up to 13 days in 30 cm of fresh water, brackish water (22 ppt), and full strength sea water (30 ppt) based on independent t-test results. FW = fresh water; ppt = parts per thousand.

Salinity	**Sample period (No. of days immersed)**	**Hemolymph osmolality mmol kg^{-1}**		**t-test**	**d.f.**	**p-value**
		Gecarcinucidae *E. dugasti*	**Potamidae *E. smithianum***			
FW	5	385.27	440.95	–5.4	69	0.000
FW	9	394.14	448.52	–4.679	59	0.000
FW	13	358.61	459.75	–6.5	44	0.000
22 ppt	9	506.68	602.33	–4.89	50	0.000
30 ppt	13	783.88	721.88	3.592	42	0.001

Table 3. Percent survival of experimental groups of *Esanthelphusa dugasti* (Gecarcinucidae) of different ages subjected to different salinities and water depths, ppt = parts per thousand, d = day.

JUVENILES

Salinity (ppt)	**Water depth (cm)**	**No. of animals at start**	**Day when ≥ 50% or more dead**	**% survival (after 9, 11, or 14 d)**
22	2.5	26		76.9% – 14 d
30	2.5	28	14 d	50% – 14 d
33	2.5	5	8 d	40% – 9 d
22	30	34	11 d	38.2% – 11 d
30	30	17	10 d	5.9% – 11 d

SUB-ADULTS

Salinity (ppt)	**Water depth (cm)**	**No. of animals at start**	**Day when ≥ 50% or more dead**	**% survival (after 9, 11, or 14 d)**
7	2.5	29	7 d	13.8% – 14 d
15	2.5	29	10 d	20.7% – 14 d
22	2.5	32	6 d	15.6% – 14 d
25	2.5	10	9 d	10%% – 14 d
28	2.5	12	13 d	41.7% – 14 d
33	2.5	14	4 d	7.1% – 11 d
30	30	26	9 d	19.2% – 14 d

osmolality after several days in a new salinity regime crabs, albeit at higher levels than when they were in more dilute media.

Deep water, different salinities* (Table 2, 3, Figs. 1, 2*). – The mean hemolymph osmolality of sub-adult *E. dugasti* in deep 22 ppt sea water after 9 days was maintained at around 506.68 mmol kg^{-1}, and was higher after 13 days in deep 30 ppt sea water (783.88 mmol kg^{-1}) (Fig. 1, Table 2). Similarly, the mean hemolymph osmolality of adult *E. smithianum* after 9 days in deep 22 ppt sea water was 602.33 mmol kg^{-1} (higher than in *E. dugasti*), and it was even higher (721.88 mmol kg^{-1}) after 13 days in deep 30 ppt sea water (Fig. 2, Table 2). The two species were found to have different survival rates in water of different depths, with *E. smithianum* surviving better than *E. dugasti* in both deep and shallow sea water. Age also played a part in survival times in sea water (Figs. 1–4). Sub-adult *E. dugasti* survived better than juvenile *E. dugasti* in deep water, but the reverse was the case in shallow water, whereas juvenile and hatchling *E. smithianum* survived longer than adults in both deep and shallow water.

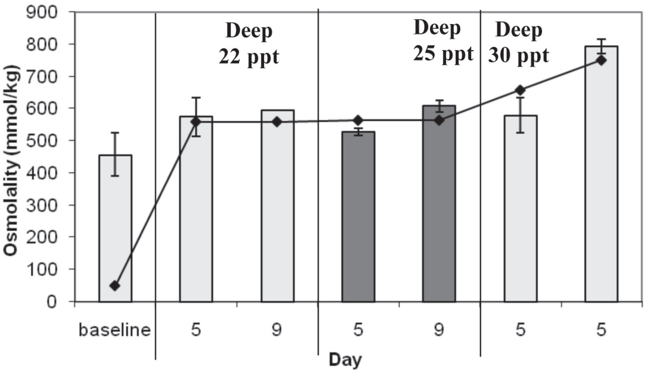

Fig. 2. Hemolymph osmolality of adult *Eosamon smithianum* (Potamidae) held for up to 9 days in deep 30 cm fresh water and in 22, 25, and 30 ppt salt water. Line with diamonds = water osmolality. Columns = hemolymph osmolality, light = sub-adults; dark = juveniles; error bars = standard deviation. The x-axis shows the number of days that crabs were subjected to a particular salinity. Adults (n = 5) and juveniles (n = 17, 5) in fresh water ('baseline'), and in deep sea water at 22 ppt (n = 5, 1, respectively), 25 ppt (n = 5), and 30 ppt (n = 3). Dark horizontal bar = salinity at which the hemolymph is hyperosmotic to the external water.

Table 4. Percent survival of experimental groups of *Eosamon smithianum* (Potamidae) of different ages subjected to different salinities and water depths; ppt = parts per thousand, d = day.

Salinity (ppt)	Water depth (cm)	No. of animals at start	Day when ≥ 50% or more dead	% survival (after 9, 11, or 14 d)
JUVENILE				
22	2.5	20 (hatchlings)	–	65% – 14 d
22	2.5	15	7 d	33.3% – 14 d
22	30	7	10 d	14.3% – 11 d
ADULTS				
Salinity (ppt)	**Water depth (cm)**	**No. of animals at start**	**Day when ≥ 50% or more dead**	**% survival (after 9, 11, or 14 d)**
2	2.5	39'	–	71.8% – 14 d
22	30	5	9 d	40% – 11 d
25	30	13	–	92.3% – 9 d
30	30	11	–	54.5% – 9 d

Shallow water, different salinities **(Table 3, Figs. 3, 4). –** In shallow brackish water (13 ppt or 15 ppt), the mean hemolymph osmolality (476.44 mmol kg^{-1}) of groups of sub-adult and juvenile *E. dugasti* after 9 days increased to levels well above the range seen in fresh water, and was close to the osmolality of the environmental water. In shallow 22 ppt sea water, the mean hemolymph osmolality of groups of sub-adult and juvenile *E. dugasti* after 13 days increased to between 543.95 and 637.33 mmol kg^{-1}, which was either just below, or approximately equal to, that of the holding water (623 to 680 mmol kg^{-1}). In shallow sea water of even higher salinities, the mean hemolymph osmolality of groups sub-adult and juvenile *E. dugasti* (770.42 mmol kg^{-1} in 30 ppt) was high but was still hyposmotic (less than that of the external medium) (post-hoc Tukey tests, harmonic mean sample size = 5.697). The mean hemolymph osmolality of groups of *E. dugasti* held for 5 days in shallow 30 ppt sea water (Fig. 3) was high, significantly higher than that for groups of crabs kept for the same time in deep sea water at 30 ppt (t = 4.212 df = 45, p = 0.00).

In shallow sea water at 22 ppt, the mean hemolymph osmolality of groups of adults, juveniles, and hatchlings of *E. smithianum* was maintained at hypo-isosmotic levels after 9 days (Fig. 4) while the hemolymph osmolality of hatchlings was high but varied a great deal (606 ± 120 mmol kg^{-1}, Fig. 4) and they had the longest survival time (27 days). In shallow sea water at 28 ppt, the hemolymph osmolality of adult *E. smithianum* was hyposmotic after 9 days but gradually increased so that it was similar to the external medium after 13 days (Fig. 4).

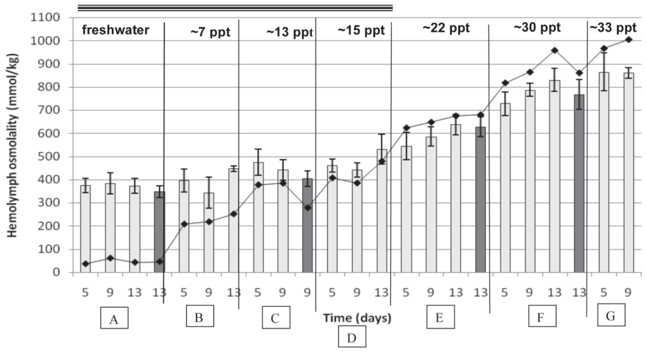

Fig. 3. Hemolymph osmolality of *Esanthelphusa dugasti* (Gecarcinucidae) in shallow 2.5 cm water at salinities of 0, 7, 13, 15, 22, 30 and 33 ppt. The x-axis shows the number of days (5, 9, or 13) that 7 groups of crabs (A–G) were subjected to a particular salinity. Line with diamonds = water osmolality. Columns = hemolymph osmolality, light = sub-adults; dark = juveniles; error bars = standard deviation. Hemolymph osmolality of: A, sub-adults (n = 24, 17, and 14, respectively), and juveniles (n = 20) in fresh water; B, sub-adults (n = 26, 10, 4, respectively) in 7 ppt salt water. C, sub-adults (n = 19 and 14) and juveniles (n = 10) in 13 ppt salt water. D, sub-adults in 15 ppt salt water (n = 25, 17 and 6, respectively). E, sub-adults (n = 20, 9, 6, respectively) and juveniles (n = 22) in 22 ppt salt water. F, sub-adults (n = 26, 7, 7, respectively) and juveniles (n = 19) in 30 ppt salt water. G, juveniles in 33 ppt salt water (n = 4, 2, respectively). Dark horizontal bar = salinities at which the hemolymph is hyperosmotic to the external water.

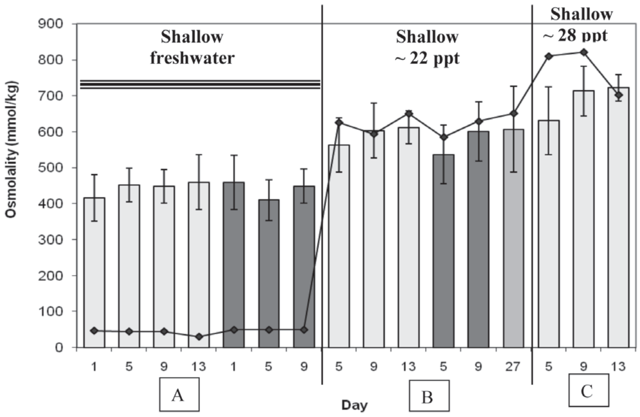

Fig. 4. Hemolymph osmolality of three experimental groups (A–C) of *Eosamon smithianum* (Potamidae) held in shallow 2.5 cm water of different salinities for up to 13 days. Line with diamonds = water osmolality. Columns = hemolymph osmolality, light = sub-adults; dark = juveniles; error bars = standard deviation. The x-axis shows the number of days that crabs were subjected to a particular salinity. A. Adults (n = 20, 15, 15, 12, respectively) and juveniles (n = 10, 5, 3, respectively) in fresh water. B. Adults (n = 16, 12, 10, respectively), juveniles (n= 12, 6, respectively) and hatchlings (n=12) in 22 ppt salt water. C. Adults in 28 ppt. salt water (n = 22, 20, 18, respectively). Dark horizontal bar = salinity at which the hemolymph is hyperosmotic to the external water.

Survival times in salt water. **–** Survival rates were found to be significantly different between the two species: groups of *E. smithianum* survived longer than groups of *E. dugasti* in both deep and shallow sea water. Age also made a difference to survival times in sea water. For example, adult and juvenile *E. smithianum* survived longer in deep full strength sea water after 9 days (55% and 14%, respectively) than did sub-adult and juvenile *E. dugasti* (19% and 6%, respectively). Similarly, adult and juvenile *E. smithianum* survived longer in shallow full strength sea water after 9 days (72% and 33%, respectively) than did sub-adult and juvenile *E. dugasti* (7% and 40%, respectively) and juvenile *E. dugasti* survived longer than sub-adults (Table 3). Interestingly, juveniles of *E. smithianum* did not survive as well as adults in either deep sea water (22 ppt) where only 14% survived after 11 days, or in shallow sea water (22 ppt) where 33% survived after 14 days. But 65% of *E. smithianum* hatchlings in shallow sea water at 22 ppt were still alive after 14 days, and 60% were still alive after 20 days.

DISCUSSION

The expected differences in the osmoregulatory abilities and survival times of male and female freshwater crabs were not found here, and hemolymph osmolality levels (that reflect osmoregulatory energy expenditure) in either *Esanthelphusa dugasti* or *Eosamon smithianum* were not significantly different. This may be due to the lack of ovigerous females in the present study, which would be expected to have higher energy demands when producing and caring for eggs. The lack of a gender difference in the osmoregulatory abilities of these Asian freshwater crabs agrees with studies on the potamonautid *Sudanonautes aubryi* (misidentified as *S. africanus*) (Lutz, 1969) and the sesarmid *Armases roberti* where plasma ion levels in challenging salinities also did not correlate with gender (Schubart & Diesel, 1998).

After 24 hours exposure to a range of deep water salinity regimes both *E. dugasti* and *E. smithianum* responded with a proportional increase in hemolymph osmolality. In low and medium salinity deep brackish water (8 to 20 ppt), crabs are hyperosmotic and maintain their internal osmotic concentration above that of the external water. In medium salinity deep brackish water of between 22–24 ppt, crabs are isosmotic, and maintain their hemolymph osmolality close to that of the external medium. This is the point at which *E. dugasti* and *E. smithianum* switch from hyperosmoregulation (their normal resting state) to iso-hyposmoregulation (a physiological response to high salinity). In full-strength sea water (30–33 ppt), crabs show further increases in their internal osmolality, which approaches (but does not exceed) that of the external medium. The internal adjustment to high salinity involves a change in the direction of movement of ions by the pumps on the gill membranes from hyperosmoregulation (influx, moving ions back into the hemolymph) in medium salinities to hyposmoregulation (efflux, expelling ions from the hemolymph) in full strength sea water (Pequeux, 1995). Tolerance by freshwater crabs of a high internal hemolymph osmolality in deep water of high external salinities for several days is an osmoregulatory strategy that is unexpected in these specialist freshwater species, and one that appears to be more typical of sesarmid and varunid euryhaline species (Schubart & Diesel, 1998).

The gradual increase in their hemolymph osmolality over time shown by crabs in deep water aquaria with challenging external media (i.e., salinities over 22 ppt) was often correlated with a decrease in survival that may be associated with a gradual weakening of their energy-consuming gill ion pumps. Similarly, a failure of ion regulation has been demonstrated in the potamonautid *Potamonautes warreni* after one week in high salinity (80% sea water, ~ 22.8 ppt) with Cl^- ion regulation failing first, followed by the failure of Na^+ and Mg^{2+} ion regulation after two weeks (Morris & van Aardt, 1998).

The expectation that the semi-terrestrial *E. dugasti* would survive longer in shallow sea water than the more aquatic *E. smithianum* was not found to be the case here, where both adults and juveniles of *E. smithianum* survived much better than those of *E. dugasti*. Although both *E. dugasti* and *E. smithianum* have been shown here to be capable osmoregulators that can survive for more than a week in either deep or shallow brackish water, we have also demonstrated that the accompanying increase in hemolymph osmolality places osmoregulatory energy demands that ultimately limit how long they can survive in full-strength sea water. In general, survival times of *E. dugasti* and *E. smithianum* in sea water decreased as salinity levels increased, presumably because the steep osmotic gradients between internal and external environments require active osmoregulation to maintain higher than normal internal osmolalities at lower than external values (Table 2). In many cases, crabs could only maintain this level of osmoregulatory energy expenditure for a short time before eventually succumbing to the salinity stress.

Freshwater crabs in shallow sea water face increased physiological challenges not least because important osmoregulatory processes are compromised when the ion pumps on the dorsal gill surfaces are exposed to air rather than immersed in water. In addition, reduced survival is seen in crabs in shallow water that are in danger of evaporative loss of water from the hemolymph and the accompanying concentration increases of most internal solutes. For example, in *S. aubryi,* the concentrating effect of increased evaporation on the hemolymph resulted in an increase in the percentage of plasma ions after 24 hours out of water (Lutz, 1969). This is not always the case because the hemolymph osmolality of *P. warreni* out of water did not increase significantly (Morris & van Aardt, 1998).

Not all species or families of freshwater crabs are similarly tolerant of sea water. For example, the African potamonautid *Potamonautes niloticus* survived only four days in 100% sea water (Shaw, 1958b), whereas the Asian potamid *E. smithianum* had strong survival rates after at least 9 days (and often longer) in either deep or shallow full-strength sea water. The longest documented survival of a freshwater crab immersed in full-strength sea water is for the European potamid *Potamon fluviatile* which lived for at least a month, although the percentage of animals that survived was not reported (Shaw, 1958b).

When experimental crabs were returned to freshwater in the present study it took three days for their hemolymph osmolality to revert back to original freshwater levels, indicating that adaptation to a change in external salinity is not instantaneous, and that exposure to saltwater for two weeks is not necessarily lethal in freshwater crabs. The response time to changes of salinity found here is slower than that reported for other osmoregulating brachyuran crabs, which typically take up to 48 hours to return to normal after exposure to salinity changes (Charmantier et al., 1998).

Age was found to be an important factor affecting survival time in salt water depending on species (adults of *E. smithianum* survived longer than juveniles, but juveniles of *E. dugasti* survived longer than sub-adults). Hatchlings of *E. smithianum* were shown here to have survival abilities in sea water equal to, or even superior to, those of adult crabs. It is perhaps surprising that hatchlings (cw 10–15 mm) of *E. smithianum* survived as well as they did, and it is interesting that juveniles of the Thai potamid *Larnaudia chaiyaphumi* held in sea water at 22 ppt and 30 ppt also showed impressive survival rates after 13 days: 90% (fresh water), 100% (22 ppt) and 70% (30 ppt) (unpublished data). This ability of juvenile freshwater crabs to osmoregulate at least as well as adults is unexpected given the high surface-to-volume ratio of small specimens whose gills have a relatively large surface area, faster weight-specific diffusion rates, and high desiccation rates. It is therefore possible that the osmoregulatory abilities of freshwater crabs that allow them to survive in dilute media are fully developed when they hatch (although this has yet to be confirmed), given that during direct development the developing embryo passes through all normal brachyuran larval stages inside the egg case (Wu et al., 2009; Xue et al., 2010). Preliminary investigations on the crayfish *Orconectes rusticus* indicate that newly hatched juveniles maintained in aquaria containing 50% seawater can survive for at least four weeks during which time they moulted several times (Esser & Cumberlidge, unpublished).

In contrast, the osmoregulatory abilities of adult euryhaline brachyurans in dilute media appears to be better developed than that of their larval stages and juveniles. For example, the euryhaline abilities in the larvae and juveniles of the marine-breeding catadromous varunid *Eriocheir sinensis* develop over a period of two moults from a moderately hyper-isosmoregulating zoeal phase to a moderately hyper-hyposmoregulating first juvenile crab stage (Cieluch et al., 2007). Adult *E. sinensis* can cope equally well in fresh water and sea water. Similar ontogenetic development of osmoregulatory ability has been reported for other euryhaline species belonging to four different marine brachyuran families (Varunidae, Sesarmidae, Portunidae, and Ocypodidae) (Rabalais & Cameron, 1985; Charmantier et al., 1998, 2002; Anger & Charmantier, 2000; Cieluch et al., 2004, 2007).

This study provides the first hemolymph osmolality values for any species of Asian freshwater crab when in fresh water and allows comparisons with published values for freshwater crab families from different continents, and other freshwater decapods in general (Fig. 5). One unexpected finding is that the semi-terrestrial *E. dugasti* has a mean hemolymph osmolality (374 ± 32 mmol kg^{-1}) in fresh water that is significantly lower than that of the more aquatic *E. smithianum* (415 ± 64 mmol kg^{-1}) (Table 2). Despite these differences, both of these values are in a similar range to that of South American trichodactylid freshwater crabs (386mmol kg^{-1}, Onken & McNamara, 2002), and freshwater crayfish (Astacidae and Cambaridae) in fresh water (Holdich et al., 1997; Susanto & Charmantier, 2000). All of these decapods in fresh water have a hemolymph osmolality of about one third that of full-strength sea water (950–1100 mmol kg^{-1}) (Fig. 5).

Interestingly, higher hemolymph osmolalities in fresh water have been reported for European potamids (540 mmol kg^{-1}) (Harris & Micallef, 1971), African potamonautids (553 mmol kg^{-1}) (Shaw, 1958a, b), and the Australian gecarcinucid *Austrothelphusa transversa* (620 mmol kg^{-1}) (Greenaway, 1986). The higher hemolymph osmolality values for this latter species may be an adaptation to its seasonally arid habitat where dehydration would tend to concentrate body

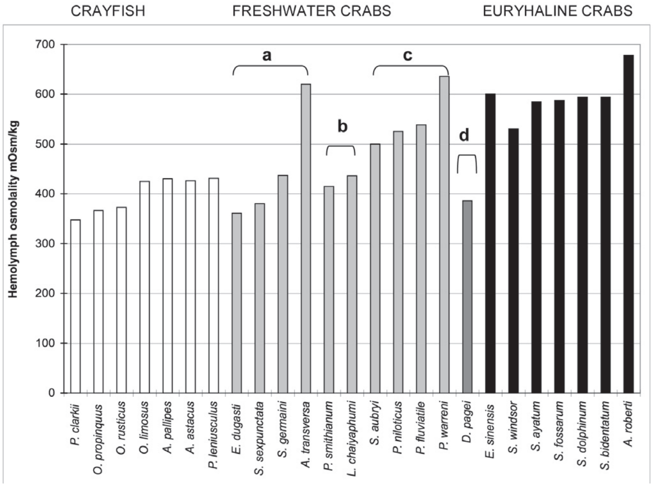

Fig. 5. Comparison of hemolymph osmolalities of eight species of freshwater crayfish (white columns), 11 species of true freshwater crabs (grey columns), and eight species of freshwater grapsoid crabs (black columns) (Harris & Micallef, 1971; Mantel & Farmer, 1983; Holdich et al., 1997; Morris & Van Aardt, 1998; Schubart & Diesel, 1999; Onken & McNamara, 2002). Freshwater crabs: a. Gecarcinucidae (Oriental). b. Potamidae (Oriental and Palaearctic). c. Potamonautidae (Afrotropical). d. Trichodactylidae (Neotropics). *P. clarkii* = *Procambarus* (Cambaridae), *O. rusticus, O. limosus* = *Orconectes* (Cambaridae), *A. pallipes, A. astacus, A. leptodactylus* = *Astacus* (Astacidae), *P. leniusculus* = *Pacifastacus* (Astacidae), *E. dugasti* = *Esanthelphusa* (Gecarcinucidae), *S. sexpunctata, S. germaini* = *Sayamia* (Gecarcinucidae); *A. transversa* = *Austrothelphusa* (Gecarcinucidae), *E. smithianum* = *Eosamon* (Potamidae), *L. chaiyaphumi* = *Larnaudia* (Potamidae), *S. aubryi* = *Sudanonautes* (Potamonautidae), *P. niloticus, P. warreni* = *Potamonautes* (Potamonautidae), *P. fluviatile* = *Potamon* (Potamidae), *D. pagei* = *Dilocarcinus* (Trichodactylidae), *S. windsor, S. ayatum, S. fossarum, S. dolphinum, S. bidentatum* = *Sesarma* (Sesarmidae), *A. roberti* = *Armases* (Sesarmidae).

fluids (Mantel & Farmer, 1983). Hemolymph osmolalities that are close to sea water have been reported in the New World euryhaline sesarmids *Armases* and *Sesarma*, and the Old World varunid *E. sinensis* when in fresh water (Schubart & Diesel, 1998, 1999). The high hemolymph osmolalities of sesarmids and varunids in fresh water imply a more recent invasion of fresh water from sea water than for true freshwater crabs and crayfish.

Biogeographical implications. **–** We have shown here that adults and juveniles of two species of Asian freshwater crabs (especially the potamid *E. smithianum*) can survive in sea water for at least one week (and much longer in some cases). It is significant that both species survived for more than a week in both deep and shallow warm full-strength water regimes because these mimic the harsh conditions that may be experienced by a freshwater crab clinging to floating vegetation and driftwood in the surface waters of the ocean. Despite reduced survival rates of freshwater crabs in high salinities, survival times of one to two weeks would be long enough for crabs to be carried short distances in sea water (of 100 km or more), for example, from the mainland to nearby islands. Direct development in freshwater crabs means that only a few individuals (including ovigerous females) would be needed to successfully establish a viable breeding population in a new environment (Cumberlidge & Ng, 2009). Freshwater crabs are not the only groups of island animals with an aversion to saltwater whose origins have been explained by overseas dispersal. For example, a combination of floating rafts, favorable surface currents, and a possible reduction of surface water salinity has been proposed to explain how Indian rice frogs (*Rana limnocharis*) reached Lanyu and Lyudao Islands from Taiwan (Toda et al., 1998), how green iguanas reached the Caribbean island of Anguilla from Guadeloupe (Censky et al., 1998), and how amphibians reached the Gulf of Guinea islands of São Tomé and Príncipe from mainland Africa (Measey et al*.,* 2007).

The findings presented here on osmoregulatory abilities and survival in sea water of two species of Asian freshwater crabs do not in themselves discount vicariant biogeographic hypotheses for the origins of island species (Bott, 1970; Banarescu, 1990; Apel & Brandis, 2000; Brandis, 2002). However, because vicariant hypotheses explain the presence of freshwater crabs on islands almost entirely by overland migration during past episodes of lowered sea levels, they are based on the assumption that freshwater crabs have a poor tolerance to saltwater exposure that makes sea water a barrier to their dispersal (Bott, 1970; Banarescu, 1990; Apel & Brandis, 2000; Brandis, 2002). The evidence presented here favours dispersalist hypotheses that postulate trans-oceanic rafting as explanations of the presence of freshwater crabs on islands, at least those that are close to either the continental mainland or other islands (Shih et al., 2004; Klaus et al., 2006; Cumberlidge, 2008; Daniels et al., 2006; Cumberlidge & Ng, 2009; Cumberlidge et al., 2008).

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