

Comparative Study of Salinity Tolerance an Oceanic Sea Skater, *Halobates micans* and Its Closely Related Fresh Water Species, *Metrocoris histrio*

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Abstract

This study aims, first, to examine the limit for tolerance to lower salinity by an oceanic sea skater, *Halobates micans*, and, second, to make it clear whether exclusively fresh water Halobatinae species, *Metrocoris histrio* has salinity tolerance. Adults of *H. micans* were collected using Neuston Net from the starboard side of R/V MIRAI on a fixed station at 8°S, 80°E, whereas those of *M. histrio* were collected from a small pond filled with a spring fresh water in Kochi (33°N, 133°E), Japan. Time in survival was measured in starved condition under several salinity conditions: 0‰, 2‰, 4‰, 6‰, 8‰, 9‰ and 10‰ for *H. micans*; 0‰, 5‰, 10‰, 12.5‰, 15‰ for *M. histrio*. Half of adults were in coma due to lower salinity under 10‰ and time in survival was less than 10 hours under less than 4‰ for *H. micans*. Time in survival was half at 5‰ of 80 hours on average at 0‰ as a control and less than 10 hours at 10‰ or higher salinity for *M. histrio*. Relatively flexible osmo-regulation ability by *H. micans* would be related to wide variety of salinity condition of surface oceanic water, whereas very limited tolerance even to lower salinity of 5‰ may be permitted by the no chances to be exposed to brackish water in natural habitats of *M. histrio*. This study showed that salinity tolerance of Halobatinae species would reflect, directly, the salinity condition of their habitats.

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Keywords

Oceanic Sea Skaters, Fresh Water Halobatinae Species, Salinity Tolerance, Indian Ocean, Spring Water Pond

1. Introduction

In the last decade, salinity tolerance has been studied on a wide variety of organisms living in the coast: plant [1], mollusks [2], macro-invertebrates [3], fishes [3]-[7], crabs [8]-[11] and shrimps [12]-[17], spiders [18] and even in insects [19] [20].

1.1. Hyper-Osmoregulator? Hypo-Osmoregulator? or Both?

How great is the variation of the salinity tolerance for the organisms? In a specialist salt marsh species of a wolf spider, *Arctosa fulvolineata*, an increase in the hyper salinity (70‰) exposure for 12 days increased the salinity tolerance showing “hypo-osmoregulator” [18]. As another hypo-osmoregulator, a population of the ground beetle, *Merizodus soledadinus*, which has been introduced at the Kerguelen Islands a century ago, survived for 15.9 or 19.5 days on average even under 70‰ in comparison with 21.4 or 27.0 days under 35‰ [20]. Dhaneesh *et al.* [5] showed that larvae of the tropical reef dwelling skunk clownfish, *Amphiprion akallopisos* survived and showed no signs of stress both in high up to 53‰ and low saline up to 6‰, at least as “hypo” regulator. Juvenile abalones, *Haliotis diversicolor* showed the maximum range of 11.2‰ - 41.8‰ of salinity tolerance as critical salinity minimum and maximum [2]. The euryhaline white shrimp, *Litopenaeus vannamei*, inhabits both coastal and oceanic areas through ontogeny. Both larvae and adults of this species have high function of hyper- and hypo osmo-regulation. For example direct exposure from sea water to several salinities of 5‰, 10‰, 20‰, 32‰, 45‰ and 60‰ permitted all 17 stages larvae survive even 5 hours after the exposure to 20‰, 32‰ and 45‰ [17].

As an example of “hyper-hypo-osmoregulator”, adults of the graspid crab, *Sesarma curacaoense* is mangrove species and survived in a saline range from at least 1‰ to 44‰ meaning “hyper-hypo-osmoregulators” based on laboratory experiments [8]. When a mysids shrimp, *Mesopodopsis africana* held at a salinity of 14 (salinity at the Mouth) was either directly transferred to 0‰, 2.5‰, 5‰, 25‰, 40‰, 50‰ and 60‰, 80% to 95% of specimen under wide range of salinity of 0‰ - 40‰ survived more than 70 hours if the temperature was 20°C, whereas the survival was extremely low under every salinity in the case of 10°C, 30°C and 40°C [13].

Bodinier *et al.* [7] showed that even larval fish of euryhaline sea bream, *Sparus aurata* could be survival under long salinity range of 5.1‰ to 39.1‰ at day 1 after hatching and the range of 1.0‰ to 45.1‰ at 18°C. This high tolerance to salinity change would be related to a dynamic life history with hatching in the open sea followed by the larvae drifting to the coast and juveniles migrating to estuaries and lagoons where the salinity in the open sea. An estuarine grass shrimp, *Palaemonetes pugio*, has a symbiont as a apistome ciliate, *Hyalophasa chattoni*. This symbiont showed the growth and reproduction under the wide salinity range of 0.1‰ to 55‰ [12].

Adult intertidal crab, *Hemigrapsus edwardisii* and *H. crenulatus*, is euryhaline animal and strong hyper-osmoregulator [9]. Embryos are also highly tolerance to wide range of salinity: Even after 96 hours under the fresh water conditions, more than 80% of embryos at 2nd to 5th (last) stages were in survival, whereas only 15 or 20% of embryos at the first stage were survived. This hyper osmoregulation ability by the embryos might be related to the transference by the mother crabs on whose abdominal pleopods eggs are attached.

There is a mangrove crab which has not developed at least the hyper-osmoregulation. This crab is the Brazilian mangrove crab larvae, *Ucides cordatus* (Ocypodidae). They showed relatively lower tolerance to salinity less than 15‰ with 70% survival at 30‰, 16% at 15‰, lethal in many occasions under less than 10‰. Diele and Smith [10] proposed that this lower tolerance to lower salinity might need larval exports to offshore by currents with more salinity to keep populations of the commercially important species.

1.2. Correlation of Salinity Tolerance and Habitat Salinity

Kefford *et al.* [3] measured the acute salinity tolerance (LC50) of fresh water animals as macro-invertebrates and fishes collected from habitats in which salinity of water were different. The LC50 was positively correlated

with the maximum salinity value at which a species had been collected. They concluded that laboratory measures of acute salinity tolerance could reflect the maximum salinity of these fresh water species in the field.

Pham *et al.* [16] reported that the Caledonian blue shrimp, *Litopenaeus stylirostris* had optimum condition under 35‰, while the optimum shifted to lower salinity of 27‰ in post larval stage. This result would be correspondent with the shift of ambient salinity with larvae released to the Ocean and with post larval juveniles being in the coastal area. The salinity tolerance could be related to the habitat salinity characteristics. A water beetle, *Nebrioporus ceresyi* which inhabits standing (lentic) waters such as salt pans located areas near the coast showed the critical survival value (the half lethal time) at more than 80EC, whereas another relative species, *N. baeticus* which inhabits lotic streams situated far from the coast showed the value at less than 80EC [19].

1.3. Salinity Tolerance by Oceanic Sea Skaters in Halobates and Fresh Water Species in Gerridae

Kishi *et al.* [22]-[24] demonstrated that the limit for nymph growth of a fresh water species of *Aquarius paludum* was 9‰ of NaCl, and even in 4.5‰ salinity the insects can depress their reproductive activity (to as little as 2/3 of fecundity) in populations inhabiting freshwater habitats.

Sekimoto *et al.* [25] reported that oceanic sea skaters of *Halobates micans*, *H. sericeus* and *H. sp* had high hyper osmoregulation ability, because they show no stress at least between 12‰ to 36‰ of salinity. However, the lower limit of hardiness to lower salinity of habitat waters is unknown. Moreover, another fresh water Halobatinae species, *Metrocoris histrio* can be proposed to show narrow range of higher salinity tolerance because of no chances to inhabit brackish waters in their ecological scene: they exclusively inhabit ponds which are filled with underground waters near mountain area or “semi-lentic” area in lotic small mountain streams which are with underground waters.

This study aims, first, to examine whether salinity tolerance of Halobatinae species would reflect, directly, the salinity condition of their habitats, using an oceanic sea skater, *Halobates micans*, and a fresh water Halobatinae species, *Metrocoris histrio*.

2. Materials and Methods

2.1. Sampling

2.1.1. *Halobates micans*

All samples were collected using a Neuston Net during a cruise (MR-11-07 cruise) by the R/V MIRAI owned by JAMSTEC (Japan Agency for Marine-earth Science and Technology) on a fixed station at 8°S, 80°E in the Indian Ocean on the 6th, 12th, 21st November, 2011. The Neuston Net (6 m length and 130 cm width) was trailed at night under bright lights at the ship speed of 2.0 knot from starboard side of the ship. Each trailing session lasted for 15 min and was repeated 2 times in each day. Adults of *H. micans* were kept at 29°C ± 2°C as both air and water temperatures in white and semi-translucent aquaria (30 cm × 30 cm × 40 cm) filled with sea water. They were fed on adult flies of *Lucilia illustris* for 12 hours to 8 days after the collection. Then, they were starved for 8 hours just before the experiment.

2.1.2. *Metrocoris histrio*

All samples were collected from spring water in AMENOMIKUMARI shrine (33°53'N 133°53'E) nearby Kochi University on 11th June 2011. A round shaped net with 30 cm diameter attached to the 1m stick was used for the sampling. Samples were moved to an incubator in the laboratory of Kochi University. They had been kept there at 20°C ± 2°C and fed on adult flies of *Lucilia illustris* for 8 - 12 hours. Then they were starved for 8 hours and used for the tolerance experiment.

2.2. Salinity Tolerance Experiment

2.2.1. *Halobates micans*

Seven salinity conditions (0‰, 2‰, 4‰, 6‰, 8‰, 9‰ and 10‰) were prepared for the experiment. Salinity was measured with an electrical conductivity meter. Each of the semi-translucent aquaria included 5 - 8 adults. The specimens were monitored for coma (no movement of one or more legs) and mortality (no movement even after mechanical artificial stimuli) in an hour intervals. The specimens were checked to see if they were still

alive in a starved condition every hour continuously until all adults had died. The number of specimens with coma or death was counted every hour. The experiment was performed under $29^{\circ}\text{C} \pm 2^{\circ}\text{C}$ which is similar to the water and air temperatures in the station where the samples were collected.

2.2.2. *Metrocoris histrio*

Experimental methodology mostly follows that for *H. micans*. However, number of salinity conditions was only four (5‰, 10‰, 12.5‰, 15‰). The solutions with the 4 concentrations were made by the combination of distilled waters and 100% salt (NaCl powder). Air and water temperatures were kept at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ which is similar to the water and air temperatures in the spring waters which are with relatively stabilized temperature ($17^{\circ}\text{C} - 23^{\circ}\text{C}$) through the year.

3. Results

3.1. *Halobates micans*

All the specimens in the salinity conditions of 0‰ to 9‰ showed a behavioral paralysis which can be called fresh water coma (Sekimoto *et al.*, 2013) that eventually results in death. The heavier the extent of coma, the shorter the time required for triggering coma, and the shorter the time in survival (Figure 1) (Table 1), the lower the salinity. In the salinity of 10‰, half of the specimens showed a slight sign of low salinity water coma but it did not seem to be lethal but was recovered to normal behavior. The average time in survival under the salinity of 0‰, 2‰, 4‰, 6‰, 8‰, 9‰ and 10‰ was 4.16, 5.42, 7.39, 18.18, 31.00, 38.53 and 46.33 hours, respectively.

Table 1. Statistical analysis on the effects of salinity of habitat water on hours in survival of oceanic sea skater, *Halobates micans* and a fresh water species in Halobatinae, *Metrocoris histrio*. DMV: difference in mean value.

1. <i>Halobates micans</i>												
1) Kruskal-Wallis test: χ^2 -value > 100, df = 6, p < 0.001												
2) Bonferroni i-test												
	2‰		4‰		6‰		8‰		9‰		10‰	
	DMP	p	DMP	p	DMP	p	DMP	p	DMP	p	DMP	p
0‰	-1.26	1	-3.23	1	-14.02	0.034*	-26.84	<0.001*	-34.27	<0.001*	-42.17	<0.001*
2‰	-	-	-1.97	1	-12.76	0.078	-25.58	<0.001*	-33.1	<0.001*	-40.91	<0.001*
4‰	-	-	-	-	-10.79	0.35	-23.61	<0.001*	-31.14	<0.001*	-38.94	<0.001*
6‰	-	-	-	-	-	-	-12.81	0.163	-20.35	0.002*	-28.15	<0.001*
8‰	-	-	-	-	-	-	-	-	-7.53	1	-15.33	0.026*
9‰	-	-	-	-	-	-	-	-	-	-	-7.8	1
2. <i>Metrocoris histrio</i>												
1) Kruskal-Wallis test: χ^2 -value > 100, df = 6, p < 0.001												
2) Bonferroni i-test												
	0‰		5‰		10‰		12.5‰		15‰			
	DMP	p	DMP	p	DMP	p	DMP	p	DMP	p		
0‰	-	-	34.55	0.396	67.94	0.001*	71.97	<0.001*	79.966	<0.001*		
5‰	-	-	-	-	33.39	0.44	37.42	0.281	40.416	0.179		
10‰	-	-	-	-	-	-	4.03	1	7.03	1		
12.5‰	-	-	-	-	-	-	-	-	3	1		

*: p < 0.05.

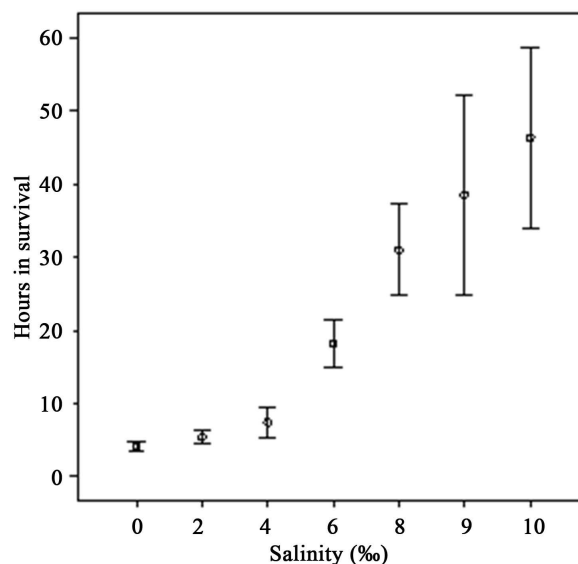


Figure 1. Hours in survival under several salinity conditions in a oceanic sea skater, *Halobates micans*.

3.2. *Metrocoris histrio*

Specimens under all four conditions did not show visible behavioral abnormality like a freshwater coma.

Averaged time in survival under 0‰ salinity as a control was 76.9 hours, whereas it was only 44.1 hours under 5‰ (Figure 2). Under more than 10‰, it was only less than 11 hours (10‰: 10.71 hours on average; 12.5‰: 6.68 hours; 15‰: 3.68 hours). Time in survival was significantly lowered in accordance with the increase in salinity.

4. Discussion

The critical value for the hardiness to lower salinity of habitat waters might be around 10‰ for an oceanic sea skater, *H. micans*, because half of specimens at 10‰ showed a coma due to lower salinity. This value might be related to the salinity level of haemolymph of this species, although we have no data on the haemolymph salinity. So far, no rigorous salinity data have been available for haemolymph of oceanic sea skaters. Unpublished observations by Edney and Cheng (mentioned in Cheng, [21]) refer to the value of 523.6 mosmol/kg. However, this value seems to be too high and indeed at the upper limit of the range seen in various terrestrial and freshwater insects (250 - 550 mosmol/kg) [26]. The salinity value for *H. micans* would be speculated as 11‰ or 12‰ which is a little bit higher than 9‰ as the averaged value by terrestrial insects, because they inhabit sea water which has a salinity of 36‰. If it would be true, the oceanic sea skaters would be possible to be not good at “hyper-osmo-regulation”, but good at “hypo-osmo-regulation”.

In a fresh water Halobatinae species, *Metrocoris histrio*, they might be exclusively hyper-osmo-regulative species and the osmotic regulation has little flexibility but “fixed”, because hours in survival are only half even under relatively low salinity of 5‰. On the other hand, oceanic sea skaters, *H. micans* have a flexible regulating ability into wide environmental salinity from 11‰ to 36‰.

From ecological points of view, natural pressure from the exposure to wide variety of salinity conditions in surface waters might select such flexible osmo-regulating ability for the oceanic sea skaters. No chances of the exposure to the brackish waters might be related to such fixed regulation ability of hyper-osmo-regulation by *M. histrio*. Another fresh water species included in Gerridae, *Aquarius paludum*, the higher limit for larvae growth is 9‰ and harder to salinity than *M. histrio* [22]-[24]. This hardiness by *A. paludum* might be related to that they can inhabit a wide variety of habitats from lentic to lotic including the estuary part.

As a limitation of this study, salinity accumulation experiments are needed to understand the detailed osmotic regulating function both for oceanic and fresh water species. Acclimation to 2‰ to 5‰ for several hours are hypothesized to make *M. histrio* harder to brackish waters, whereas another acclimation to 11‰ - 12‰ for sev-

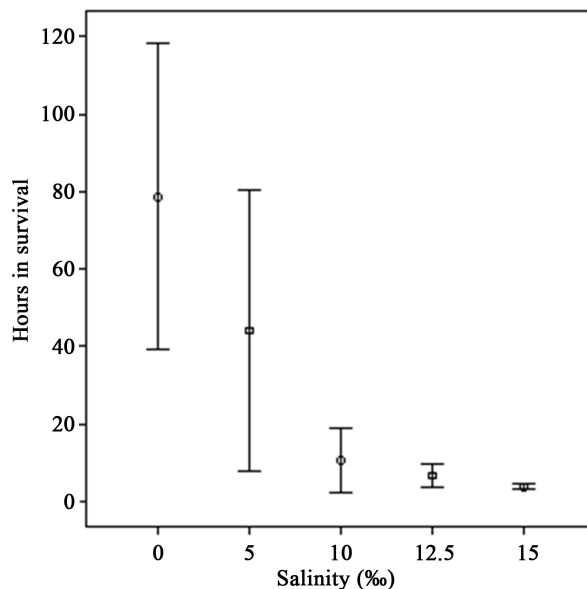


Figure 2. Hours in survival under several salinity conditions in a fresh water species, *Metrocoris histrio*.

eral hours hypothesized to make *H. micans* show no coma to lower salinity. However, such acclimation studies are remained to the future.

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