Lowered Salinity Tolerance in Sea Skaters *Halobates micans*, *Halobates sericeus*, and *Halobates* sp. (Heteroptera: Gerridae)

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**ABSTRACT** Adult specimens of three species of oceanic sea skater, *Halobates sericeus* Eschscholtz, *Halobates micans* Eschscholtz, and *Halobates* sp. were placed in one of four solutions of different salinity (sea water [35–36‰], sea water : fresh water = 2:1 [23–24‰], sea water : fresh water = 1:2 [11–13‰], and fresh water [0‰]) after collection from the temperate and subtropical Pacific Ocean, tropical Indian Ocean, and Tomini Gulf in Indonesia, and observed in 2-h intervals until they died. *H. micans* collected from the tropical Indian Ocean survived twice as long (50–100 h) on average as *H. sericeus* collected from the temperate and subtropical Pacific Ocean (35–45 h) under salinities of 12–36‰. Paralysis from freshwater treatment occurred within 2–9 h in all specimens of both species of *H. sericeus* from the Pacific Ocean and *H. micans* from the Indian Ocean, and all insects died within 2 h of starting the paralysis. In fresh water, oceanic sea skaters of *H*. sp. collected from the inner water of Tomini Gulf survived for ∼24 h on average, significantly longer than those collected from the open ocean. Significantly longer length of survival was shown by the three species on one-thirds, two-thirds brackish, and 100‰ sea water than on fresh water. The long length of survival shown by oceanic sea skaters even in brackish water may be an adaptation to the occasional rain fall on the sea water film.

**KEY WORDS** Oceanic sea skater, tolerance to brackish water, tolerance to fresh water
motic media, rocky tide pools, salt marshes, and desert pools because of hyper resistance to hyper salinity hemolymph (Bradley 2008).

A semiaquatic insect, *Aquarius paludum*, a freshwater and semicosmopolitan species in the Palearctic area, responds to exposure to brackish water. The limit for nymph growth was 0.9% of NaCl, and even in 0.45% salinity the insects can depress their reproductive activity (to as little as two-thirds of fecundity) in populations inhabiting freshwater habitats (Kishi et al. 2006, 2007, 2009). In the open ocean, bouts of heavy rain can lead to a rapid decrease in osmotic pressure in the sea water film. As it rains in the habitats of the sea skaters, they should be able to tolerate lower salinities. We performed a pilot experiment on the oceanic sea skater, *Halo-\text{bates}* to verify this hypothesis.

**Materials and Methods**

**Samplings.** Samples of insects were collected from the R/V HAKUOMARU, a 3991t ship owned by JAMSTEC (Japan Agency for Marine-earth Science and Technology). They were sampled in tropical Pacific Ocean (Tomini Gulf) (Fig. 1A), temperate and tropical Pacific Ocean (Routes including a straight track from Tokyo to Honolulu) (Fig. 1B) and tropical Indian Ocean (Fig. 1C).

Between 6 and 11 March 2010, samples were taken in the area of Tomini Gulf, at four locations (0°44′S, 121°00′E), (0°30′S, 121°00′E), (0°15′S, 121°30′E), and (0°15′S, 120°00′E). Insects were collected at night, under bright light with a 30-cm-diameter net fixed to a wooden stick swept through a 9-m² area of the sea along the side of the deck. (Because of positive phototaxis, large numbers of oceanic sea skaters were attracted to the boat lights.)

Samples were also collected between 3 and 7 September 2010 in temperate Pacific Ocean (along the line from 30°08′N 144°30′E to 27°02′N, 162°46′W) and between 11 and 15 November 2010 in the tropical Indian Ocean (along the line from 04°09′S, 094°26′E to 13°33′S, 070°50′E). Here, a neuston net (6 m long and 130 cm width diameter) was trailed at night under bright lights at the ship speed of 2.0 knot. Each trailing session lasted for 15 min and was repeated five or eight times in each location.
Adults of *Halobates* sp., *H. sericeus*, and *H. micans* were kept at 29 ± 2°C in white and semitranslucent aquaria (30 by 30 by 40 cm) filled with sea water, fed on adult flies of *Lucilia illustris* Meigen from 12 h to 7 d. These adults then were used in salinity tolerance experiments.

**Experiments with Salinity Tolerance.** Most specimens collected in the Tomini Gulf were *Halobates* sp. (a new species on the way of description: a key character is numerous long hairs on the whole surface of male genital organ and never in the other *Halobates* species: Andersen and Cheng 2004). The effects of water salinity on length of survival (Mean ± SD[n]) in this species were examined at low (144.72 individuals/m²), medium (192.96), and high (615.06) population density, because in several species of Gerridae (*Aquarius paludum, Gerris latiabdominis, Gerris bueinoi, Gerris pingreensis*) population density strongly affects life history traits (synchronized and rapid growth, reduced or enhanced reproduction, or dispersal characteristics) (Harada 1994, Harada et al. 1997, Harada and Spence 2000).

Most specimen collected in the central subtropical Pacific Ocean were *H. sericeus*. The effects of water salinity on length of survival (mean ± SD[n]) in this species were examined at medium (265.32 individuals/m²) and very high (928.62 individuals/m²) population density.

For the salinity tolerance experiments, adult specimens of *Halobates sericus, H. micans, and H. sp* were transferred to aquaria similar to those used for rearing and placed in the semidry laboratory of the R/V HAKUHOMARU. In each experimental series, insects were placed in one of three 1.5-liter sea water solutions (A: sea water: 36–37 ‰, B: sea water/fresh water (distilled water): 2/1 V/V: 24–25 ‰, and C: sea water/fresh water: one-half, V/V: 12–13 ‰). Control insects were placed in fresh water (0 ‰). Salinity was measured with an electrical conductivity meter. In most cases, each aquarium held eight to 17 insects. In one experiment, each aquarium held 48–51 insects. The insects were monitored for paralysis (no movement of one or more legs) and mortality (no movement even after mechanical artificial stimuli) in 2-h intervals. All salinity tolerance experiments were conducted using insects that had been starved for at least 8 h. Specimens were checked to see if they were still alive in a starved condition every 2 h continuously until all adults had died. The number of paralyzed or dead insects was measured every 2 h and these records were converted to mean time of paralysis and death.

The identification of *Halobates micans, Halobates sericus, and Halobates sp* was performed in accordance with the keys (body length, morphological...
Table 1. Effects water salinity on length of survival (mean ± SD [n]) at low (144.72 individuals/m²), medium (192.96), and high (615.06) population density in oceanic sea skaters, Halobates sp. collected from Tomini Gulf

<table>
<thead>
<tr>
<th>Density</th>
<th>SW (36–37‰)</th>
<th>2/3 SW (24–25‰)</th>
<th>1/3 SW (12–13‰)</th>
<th>Fresh water (0‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>102.0 ± 43.7 [12]</td>
<td>100.3 ± 31.9 [8]</td>
<td>25.0 ± 46.9 [9]</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>93.9 ± 44.5 [16]</td>
<td>107.1 ± 37.4 [13]</td>
<td>85.5 ± 33.2 [16]</td>
<td>24.7 ± 11.7 [17]</td>
</tr>
<tr>
<td>High</td>
<td>86.2 ± 23.6 [51]</td>
<td>92.3 ± 29.6 [52]</td>
<td>90.8 ± 33.7 [51]</td>
<td>22.1 ± 8.5 [42]</td>
</tr>
</tbody>
</table>

ANOVA on effect of salinity with covariance of density.
F-value = 102.2, df = 3, P < 0.001*
ANOVA on effect of density with covariance of salinity.
F-value = 1.5, df = 2, P = 0.213

* P < 0.01.

Table 2. Effects water salinity on length of survival (mean ± SD [n]) at medium (265.32 individuals/m²) and high (928.62) population density in oceanic sea skaters, H. micans collected from the tropical Indian Ocean.

<table>
<thead>
<tr>
<th>Density</th>
<th>SW (36–37‰)</th>
<th>2/3 SW (24–25‰)</th>
<th>1/3 SW (12–13‰)</th>
<th>Fresh water (0‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>86.2 ± 36.2 [22]</td>
<td>93.6 ± 28.1 [24]</td>
<td>97.0 ± 39.2 [20]</td>
<td>6.0 ± 0.0 [24]</td>
</tr>
<tr>
<td>High</td>
<td>86.9 ± 36.3 [231]</td>
<td>90.0 ± 36.1 [225]</td>
<td>98.3 ± 40.5 [225]</td>
<td>7.2 ± 1.8 [230]</td>
</tr>
</tbody>
</table>

ANOVA on effect of salinity with covariance of density.
F-value = 440.5, df = 3, P < 0.001*
ANOVA on effect of density with covariance of salinity.
F-value = 0.038, df = 1, P = 0.846

* P < 0.01.

characters of male genital organs, the ratio of the first segment and (the last) fourth segment of antennae, body color, and width of legs) shown in Andersen and Cheng (2004).

Statistical Analysis. Length of survival (number of hours) was analyzed among four salinity conditions with Kruskal–Wallis tests or analysis of variance (ANOVA). Mann–Whitney U-tests were used for pair-wise analysis between the population of H. sp. collected in Tomini bay and the population collected in the open Pacific Ocean. Analysis of covariance (ANCOVA) was performed for integrated analysis of the effects of salinity and population density.

Results

Behavioral paralysis which occurred especially in the mid and hind legs was induced only by freshwater in the salinity experiments. This paralysis which can be called “freshwater coma” occurred 1–30 h after transfer and all specimens died within 2 h after the start of paralysis. Osmosis of fresh water seems to occur in the hemolymph of the body of sea skaters through the femur and tarsus segments of the leg where the fresh water film touches the body. A disorder of the motor neurons in the legs may have occurred because of the rapid decrease in osmotic pressure in the hemolymph.

All species expressed high tolerance to brackish water with little or no differences among sea water, 25‰ and 12‰ brackish water (Fig. 2, Table 1 and Table 2). Population density had no effect on fresh water tolerance both in Halobates sp (Table 1) and H. micans (Table 2). Halobates sp. from Tomini Gulf (Table 1) expressed higher tolerance to fresh water than H. micans from tropical Indian Ocean and H. sericus from Pacific Ocean (Table 2 and Fig. 2). Although tolerance to fresh water differed statistically between H. micans (n = 254, 6–7 h in survival on average) from tropical Indian Ocean and H. sericus (n = 5, 2–3 h) from Pacific Ocean (Mann–Whitney U-test, z = −4.177, P < 0.001; both exhibiting “fresh water coma,” but the difference was small (4–5 h difference).

In fresh water, Halobates sp. collected from the inner water of Tomini Gulf survived for ~24 h on average (Table 1), significantly longer (Mann–Whitney U-test: z = −3.354, P < 0.001) than Halobates sp. collected from the tropical Pacific Ocean (4.0 ± 1.6, n = 4, survival hours in fresh water).

Discussion

The skaters tolerate broad range of lowered salinity in their environment. How does this correspond with information available for other insects or arthropods? 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 (as little as two-thirds of fecundity) in populations inhabiting freshwater habitats (Kishi et al. 2006, 2007, 2009).

Moreover, another fresh water Halobatinae species, Metrocoris histrio shows narrow range of higher salinity tolerance, all dead within 2 or 3 h even under 10‰ and half longevity under 5‰ (than that in fresh water) (T. S., unpublished data). This exceptional broad salinity tolerance by oceanic sea skaters could be achieved because of the both mechanisms of “osmoregulating and osmoconforming” (Bradley 1987). Namely, high salt-excretion and water absorbance mechanism (osmoregulating) and higher hemolymph osmotic pressure (10–15‰) than usual terrestrial insects (~9‰) could have developed in oceanic sea.
skaters. However, no rigorous data are available for oceanic sea skaters. Unpublished observations by Edney and Cheng (mentioned in Cheng 1989) refer to the value of 523.6 mosmol/kg (19.2‰). This value is indeed at the upper limit of the range seen in various terrestrial and freshwater insects (250–550 mosmol/kg) (Hadley 1994).

Several fresh water species of Gerridae (Aquarius paludum, Gerris latiabdominis, G. buenoi, G. pingvensis) exhibit strong effects of high population density on synchronizing and rapid growth, reduced or enhanced reproduction, and dispersal characteristics (Harada 1994, Harada et al. 1997, Harada and Spence 2000). In our experiments, population density had no effect on fresh water tolerance by oceanic sea skaters.

Oceanic sea skaters inhabiting the Tomini Gulf tolerated fresh water for significantly longer periods of time than those inhabiting the open ocean (Tables 1, 2). The higher resistance of sea skaters inhabiting Tomini Gulf may be linked to the fact that they are more often exposed to heavy rain fall especially 3 mo before end of March when the experiments were performed (Fig. 3).

H. micans were slightly more tolerant to fresh water than H. sericeus. This finding is difficult to discuss. To the best of our knowledge, there have been no studies on comparison of fresh water tolerance among various marine insect species belonging to the same genus. However, Lee and Bell (1999) showed that over the last 200 yr some marine or brackish invertebrates moved to the fresh water habitats that include 2 Cnidaria species, 1 Annelida species, 1 Mullusca species, and 4 Arthropoda (all Crustacea) species. Probably, all the species seemed to achieve adaptation to the freshwater by developing both or one of “osmoregulating and osmoconforming” functions.

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