

Correlation analysis of heat hardiness and super-cooling point in the oceanic sea skaters, *Halobates*

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ABSTRACT

The only insects known to live in the open ocean are oceanic sea skaters of the genus *Halobates* (Heteroptera: Gerridae). We have collected three species in the temperate and tropical western Pacific Ocean, and also tropical Indian Ocean and measured heat coma temperature (HCT) and super cooling point (SCP) in 503 specimens. The SCPs and HCT were widely ranged from -4°C to -21°C and 26°C to 41°C, respectively. Significant negative correlation between HCT and SCP was shown by *Halobates sericeus* Eschscholtz 1883 inhabiting temperate (30°-35°N) and subtropical (20°-30°N) Pacific Ocean, whereas no significant correlation was shown by *Halobates germanus* White, 1883 in and along Kuroshio current in the temperate Pacific Ocean. In the fixed point of 5°N, 140°E in the tropical Pacific Ocean, *Halobates micans* Eschscholtz, 1822 is the dominant species and only females showed significant negative correlation between HCT and SCP. At the fixed point at 8°S, 80°E in the tropical Indian Ocean, *H. micans* is again the dominant species and showed no correlation between HCT and SCP and extremely high HCT with the average of 39°C. SCP can be an index of cold hardiness.

Halobates sericeus distributes in wide latitude range from 0°N to 40°N in the Pacific Ocean probably using several currents and might have developed the cross tolerance of higher and lower temperature resistance with a common mechanism including for example “heat shock proteins” as a speculation.

KEYWORDS: temperature dynamics, distribution, heat tolerance, cold hardiness, cross tolerance, super cooling point

INTRODUCTION

The cross tolerance has been shown mainly between cold hardiness and drought hardiness in the crass, insects [1]. However, there has been a few reports on the cross tolerance between low and high temperature hardiness and one of these reports was on a fruit fly, *Drosophila melanogaster* [2]. Harada *et al.* [3] reported that a fresh water species of water strider, *Aquarius paludum paludum* developed the cross tolerance of lower and higher lethal temperatures via the growth under relatively low ambient temperature of 20°C than a higher temperature of 25°C. However, the cross tolerance of hardiness to low and high temperatures in the oceanic sea skaters has been recently shown only by a short report on an experiment during one science cruise in the tropical Pacific Ocean [4].

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Just a few thousand species are marine [5, 6, 7, 8], despite the enormous biodiversity of insects in terrestrial and freshwater areas. The only insects known to live in the open ocean are sea skaters of the genus *Halobates* (Heteroptera: Gerridae). The 47 species of *Halobates* have been described so far and most of them occur in near-shore areas of the tropical seas. They are often incorporated in mangrove ecosystem and many of them are endemic to specific islands or island groups [7]. Five species of sea skaters: *Halobates micans* Eschscholtz, 1822, *Halobates sericeus* Eschscholtz, 1822, *Halobates germanus* White, 1883, *Halobates splendens* Witlaczil, 1886, *Halobates sobrinus* White, 1883 are considered as true open ocean inhabitants [6]. One additional species is now under the process of description [9]. Fruitful information has been accumulated and now available on distribution, phylogeny, life history and ecology of oceanic sea skaters [6, 7, 10, 11, 12, 13, 14]. Much less has been reported, however, on specific behavioral and physiological adaptations that allowed their unique (among insects) evolutionary transition to oceanic surface as habitat. Cheng [7] raised, in her excellent review paper, numerous questions which could be condensed into a single question: "How does *Halobates* succeed in the open ocean where others have failed?"

In this paper, physiological data are presented concerning three different sea skaters from temperate, subtropical and tropical western Pacific Ocean and also from tropical Indian Ocean. The heat coma temperature (HCT) and ice crystallization temperature (super cooling point, SCP) which can be a possible index for cold hardiness [15] were measured in the specimens that were collected during 4 science cruises which were administered in 2009 to 2011. The three species of *Halobates* as oceanic sea skaters show distinct geographical distributions [6, 7]. *Halobates micans* (biggest species among three) is the only species found in three oceans (Pacific, Indian and Atlantic) as a "cosmopolitan" species. In the Pacific Ocean, this species is mainly associated with the equatorial zone between 20°N and 20°S. *Halobates sericeus* (smallest species) has been reported to be absent in the equatorial zone of Pacific but it extends much further north (up to 45°N) and south (up to 30°S) than *H. micans* [6, 7]. However, our recent

data show that *H. sericeus* may occur as far south as 5°N, at least occasionally [16]. *Halobates germanus* (medium size among the three) is missing in the eastern Pacific and, it seems to be more closely associated with coasts of islands than other two species in the western Pacific Ocean (Harada *et al.*, submitted).

The main objective of our study was to test the two hypotheses: (1) that the levels of HCT correlate with the levels of SCP reflecting some physiological linkage between the two temperature thresholds; and (2) whether this correlation pattern differs among species and also habitat latitudes.

MATERIALS AND METHODS

This study represents part of research program conducted during the cruise no. KT-09-20 (Fig. 1) of the R/V Tanseimaru ship (606t, owned by JAMSTEC: Japan Agency for Marine-Earth Science and Technology) in and around the Kuroshio current in temperate Pacific Ocean in Sep 2009, during the cruise no. KH-10-04-Leg1 (Fig. 2) of the R/V Hakuohomaru ship (3991t, owned by JAMSTEC) from Tokyo to Honolulu in temperate and subtropical Pacific Ocean in Sep 2010, and during the cruises no MR-10-03 (Fig. 3) and MR-11-07 (Fig. 4) of the R/V MIRAI ship (8687t, owned by JAMSTEC) in western tropical Pacific Ocean in May-Jun 2010 and in eastern tropical Indian Ocean in Sep-Oct 2011, respectively. In total, 487 living sea skaters were collected from the surface of oceans by three or six replicates of 15 min-trailing the Neuston Net (diameter 1.3 m, length 6.0 m) in each sampling location (ship speed of 2.0 knot to the sea water). The temperature of ocean surface was between 27-29°C. Three well established species were distinguished: *Halobates micans*, *H. sericeus* and *H. germanus* [9]. All specimens were immediately moved to aquaria with sea water, where temperature was maintained at $28 \pm 1^\circ\text{C}$. The sea skaters were supplied with adult flies, *Lucillia illustris* as food and were acclimated in the aquaria for 12-180 h prior to the experiments.

The *heat coma temperature* (HCT) was measured inside the transparent aquarium where temperature was increased stepwise from initial 27-29°C by 1°C every 1 h (fluctuations were within the limits of $\pm 0.3^\circ\text{C}$). HCT was defined as

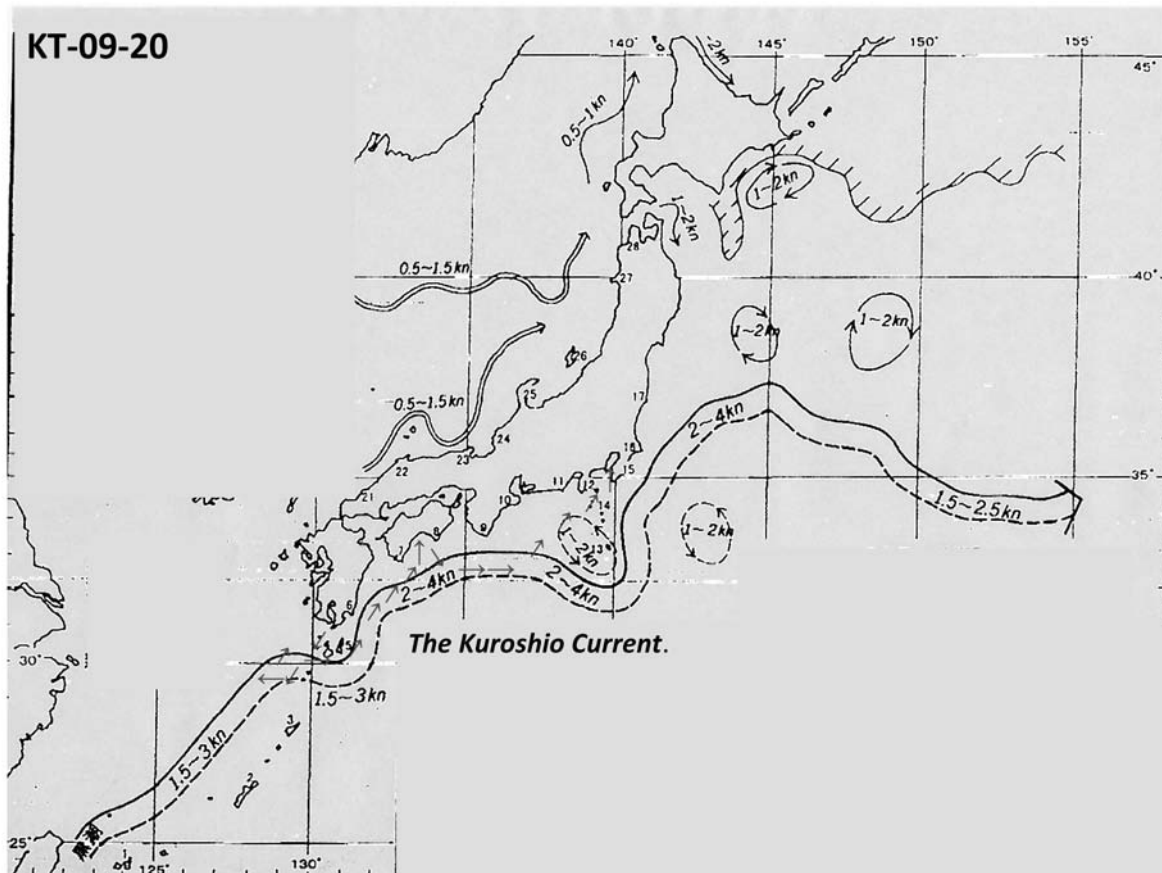


Fig. 1. Track of the cruise, KT-09-20 by the Research Vessel, Tanseimaru.

the temperature at which ventral surface of sea skater's body was caught by water film. The same specimens that were previously used for HCT measurement were further used for determination of their *super cooling point* (SCP). Surface of each insect was dried with filter paper, and nickel/bronze thermocouple was attached to the ventral surface of abdomen. The insect, inside double-wall styrofoam insulating box, was cooled at a rate of approximately 1°C/min in the freezer where temperature was set to -35°C. The lowest temperature which was reached prior to occurrence of freeze exotherm (release of latent heat of ice crystallization) was recorded as the SCP.

All statistical analyses of data were performed using SPSS Version 12.0 statistical software.

RESULTS

The sea skaters regularly ceased their movements prior to reaching the HCT. Typically, the movements

became very slow or ceased completely at temperatures that were 2-3°C below the HCT. However, the assessment of movements is ambiguous. The periods of inactivity/rest could be confused with paralysis by heat. Therefore, only the HCT data were decided to be analyzed in this study.

Table 1 summarizes data on HCT and SCP for all 487 measured adult specimens belonging to three species. Both datasets (HCT and SCP) are presented as means \pm SD because they passed normality tests in all species (at $\alpha = 0.05$). Data sets were analyzed for between-species differences by one-way ANOVA. No differences in the HCT and SCP values were found between sexes (Table 1). The means of HCT and SCP ranged between 32.1°C and 39.9°C and between -11.3°C and -16.8°C, respectively, in individual species. SCP values (-11.3 ~ -14.1°C on average) shown by individuals in and around the Kuroshio current which were

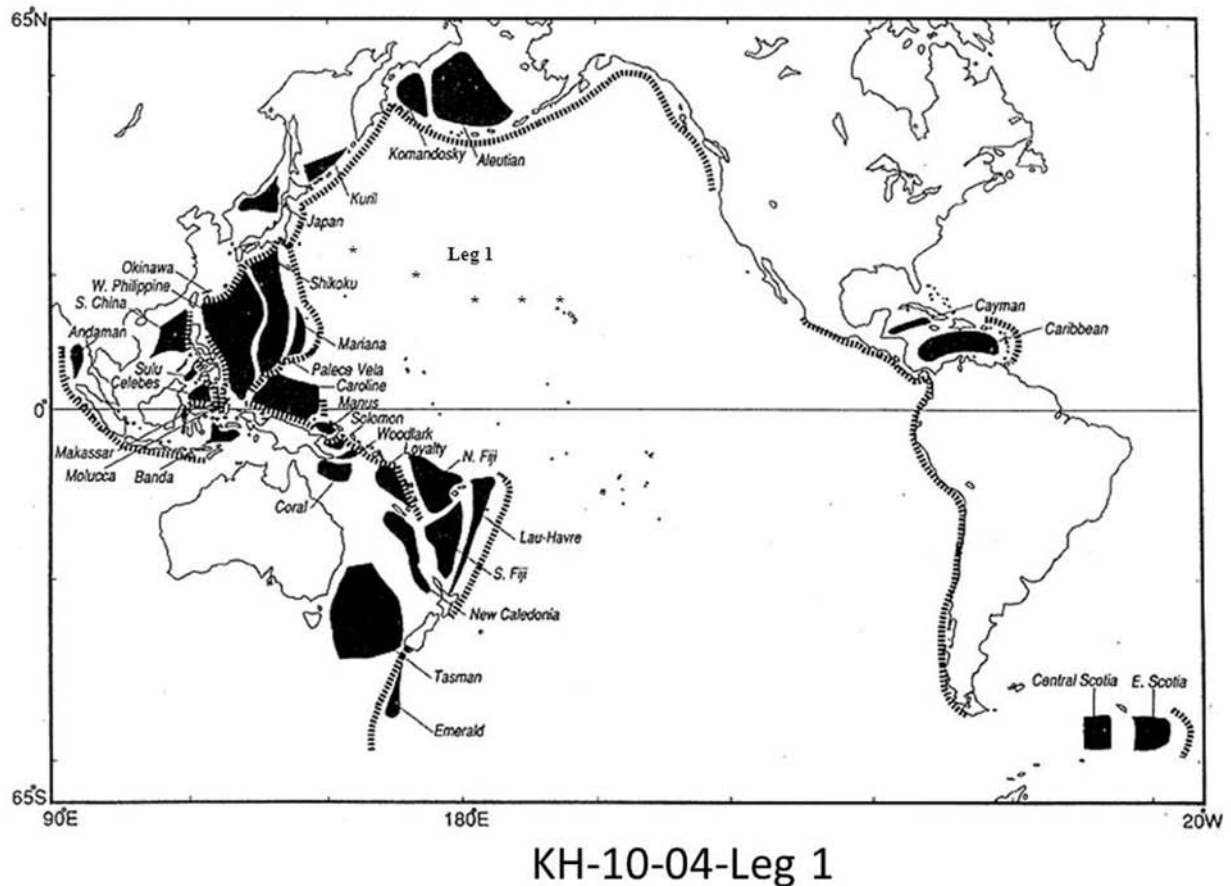


Fig. 2. Track of the cruise, KH-10-04-Leg 1, by the Research Vessel, Hakuohomaru.

flowing near to the Japan Islands were significantly higher than those ($-14.9^{\circ}\text{C} \sim -16.8^{\circ}\text{C}$) by individuals collected in the mid Ocean area far from the islands (Table 1) (Mann-Whitney U-test: $z = -6.526$, $p < 0.001$). Individuals collected in and around the Kuroshio current showed lower HCT ($32.1^{\circ}\text{C} \sim 35.8^{\circ}\text{C}$) than those in mid-Oceans ($36.4^{\circ}\text{C} \sim 39.9^{\circ}\text{C}$) (Mann-Whitney U-test: $z = -7.580$, $p < 0.001$). At the fixed point of 8°S 80°E in the tropical Indian Ocean, both of *Halobates germanus* and *H. micans* showed extremely high HCT of 39°C - 40°C on average (Table 1).

Correlation analysis was performed between HCT and SCP in each of 4 habitats and also each of 3 species, individually. Significant negative correlations were detected for adult specimens collected on or around the Kuroshio current (Fig. 5) and only male specimens (Fig. 6) collected during the cruise from Tokyo to Honolulu in *Halobates sericeus*. Only female specimens of

H. micans showed significant negative correlation between HCT and SCP values in the tropical Pacific Ocean (Fig. 7). Correlative analysis on all specimens of *Halobates micans* collected at the fixed point of 8°S , 80°E in the tropical Indian Ocean during the cruise, MR-11-07 showed no correlation (Fig. 8).

DISCUSSION

The surface temperature of tropical western Pacific Ocean is maintained relatively constant between 27 - 29°C throughout the year [17, 18]. In this study, significant differences in HCT were shown among the habitats. Significant lower HCT by *Halobates sericeus* was shown by the specimens living on or around the Kuroshio current flowing near to the Japan Islands. Such specimens of *H. sericeus* were speculated to be grown in or around the current in July or August where the water temperature in the Kuroshio

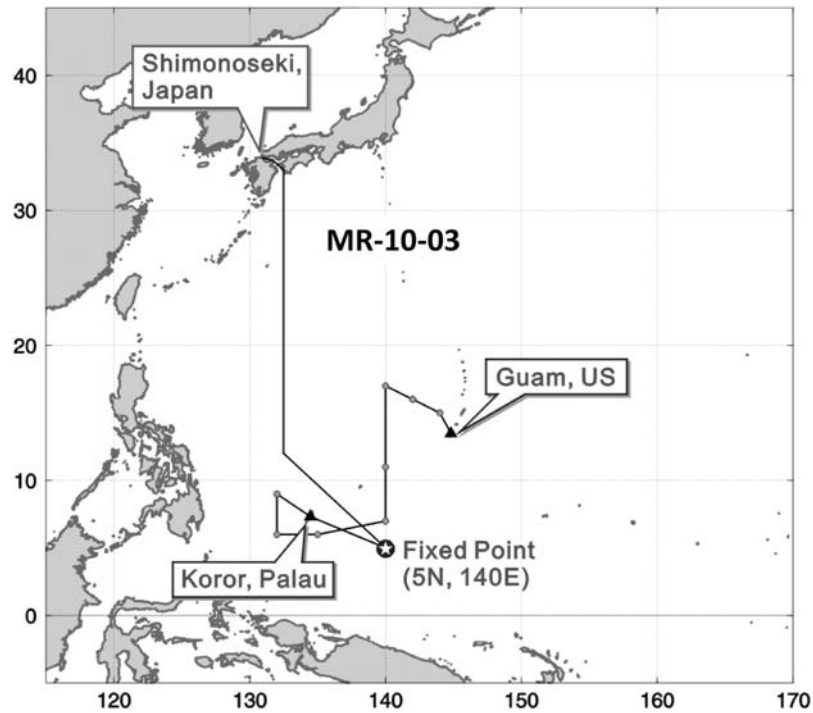


Fig. 3. Track of the cruise, MR-10-03, by the Research Vessel, Mirai. All samplings of oceanic sea skaters were performed at the fixed point of 5°N, 140°E in the tropical Pacific Ocean.

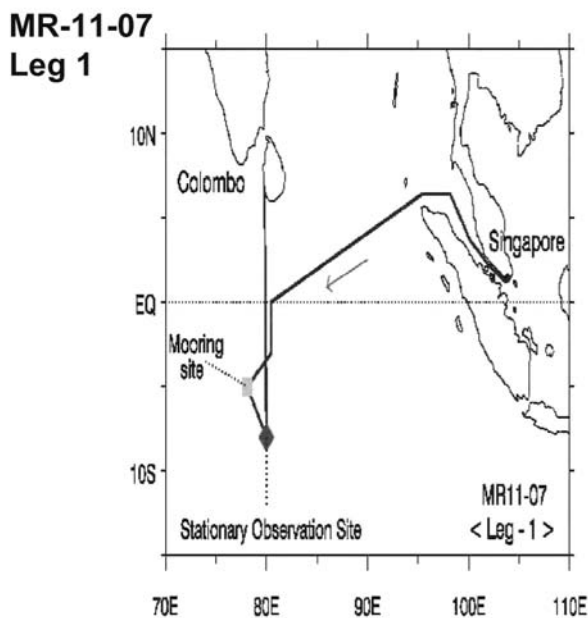


Fig. 4. Track of the cruise, MR-11-07, by the Research Vessel, Mirai. All samplings of oceanic sea skaters were performed at the fixed point of 8°S, 80°E in the tropical Indian Ocean.

might be stable around 30°C. Such stable summer water temperature in the Kuroshio might be related to the lower resistance to temperature change by *H. sericeus* which prefers to ride on the Kuroshio current (Harada *et al.*, submitted).

Exceptionally high heat tolerance was shown in the area of 08°00'S, 80°30'E in the tropical Indian Ocean. Average of HCT was about 40°C and 39°C for *H. germanus* and *H. micans*, respectively. This high value by both species is much higher than that in any other subtropical and tropical open ocean area in Indian and Pacific Oceans [19, 20] by 3-5°C. Such high heat resistance coincides with high heat coma temperature of 39°C as average value at 06°25'S, 089°00'E in the tropical Indian Ocean [20]. Harada *et al.* [20] discussed on such extreme high heat resistance that currents get together from North and South in this area and some special temperature dynamics appearing here can be related to the extreme high temperature hardiness. The chief scientist of the cruise, MR-11-07 and a Meteorologist, Dr. Kunio Yoneyama kindly gave us a very important

Table 1. Average values of heat coma temperature (HCT) and super cooling point (SCP) shown by oceanic sea skaters as three species which inhabit temperate, subtropical and tropical Pacific Ocean and pacific Indian Ocean. (mean \pm SD, n) “The Kuroshio”: Temperate Pacific Ocean, Sep 2009, KT-09-20; “Temp. to subtropical Pacific”: Sep 2010, KH-10-04-Leg 1; “Tropical Pacific”: May-Jun 2010, MR-10-03; “Tropical Indian”: Sep-Oct 2011, MR-11-05-Leg 1.

	HCT		SCP	
	Females	Males	Females	Males
<i>Halobates sericeus</i>				
The Kuroshio	33.6 \pm 3.3(28)	32.1 \pm 2.8(14)	-13.6 \pm 4.8(28)	-14.1 \pm 5.1(14)
Temp. to sub-Tropical Pacific	36.8 \pm 1.2(62)	36.4 \pm 1.4(66)	-16.3 \pm 2.1(61)	-16.3 \pm 1.8(66)
<i>Halobates germanus</i>				
The Kuroshio	34.8 \pm 3.8(31)	35.8 \pm 3.8(17)	-12.6 \pm 3.7(31)	-11.3 \pm 4.3(17)
Tropical Indian	39.9 \pm 1.1(9)	39.3 \pm 1.0(6)	-14.9 \pm 3.7(9)	-16.8 \pm 2.5(6)
<i>Halobates micans</i>				
Tropical Pacific	37.0 \pm 2.9(86)	37.1 \pm 2.9(74)	-16.5 \pm 2.4(86)	-16.8 \pm 2.2(74)
Tropical Indian	39.0 \pm 1.9(46)	38.5 \pm 1.4(48)	-16.0 \pm 2.9(46)	-16.3 \pm 2.3(47)
<i>One-Way ANOVA (F, p, df)</i>				
Sex	0.03, 0.862, 1		2.03, 0.154, 1	
Species	22.4, <0.001, 2		34.5, <0.001, 2	
Habitats	58.5, <0.001, 3		37.8, <0.001, 3	

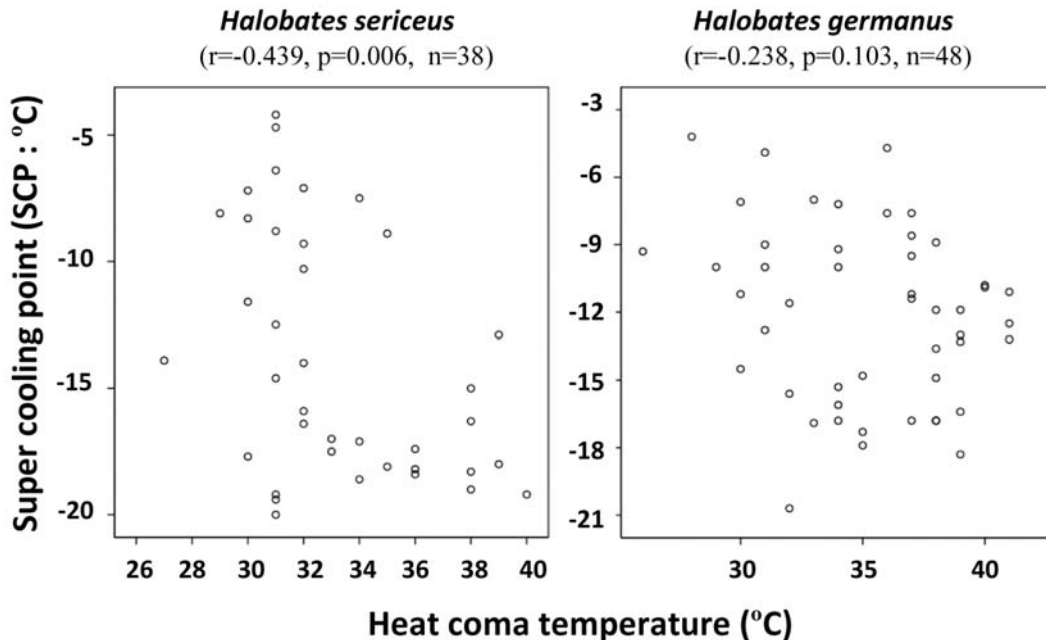


Fig. 5. Significant exhibition of “cross tolerance” between super cooling points (SCP:°C) and heat coma temperature (HCT: °C) shown not by adult *Halobates germanus* (Pearson’s correlation analysis: $r = -0.238$, $p = 0.103$, $n = 48$) but by adults of *Halobates sericeus* ($r = -0.439$, $p = 0.006$, $n = 38$) inhabiting Kuroshio and area around that in the temperate Pacific Ocean, during the cruise, KT-09-20 (30°N-34°N, 129°E-140°E).

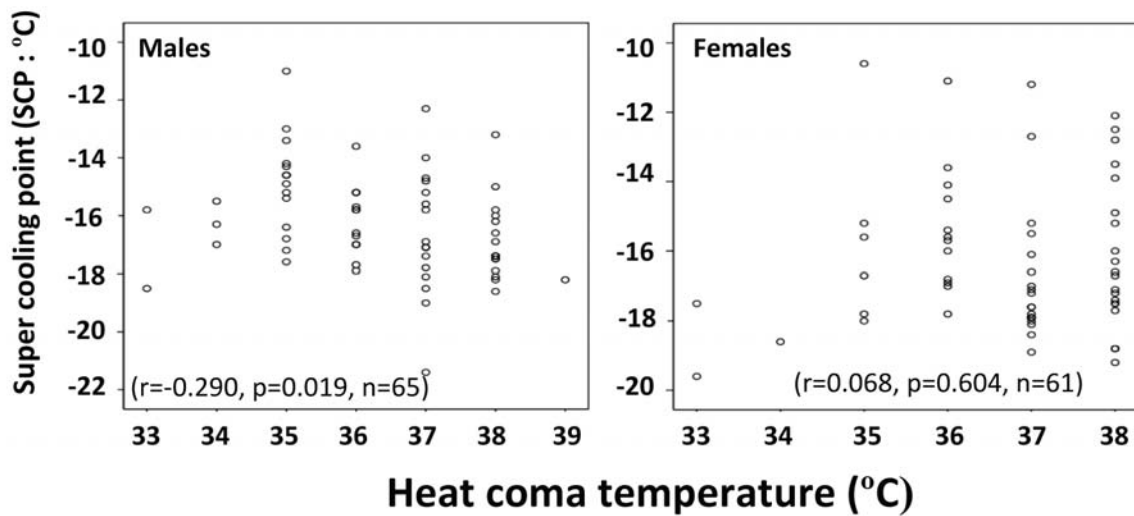


Fig. 6. Significant exhibition of “cross tolerance” between super cooling points (SCP:°C) and heat coma temperature (HCT:°C) shown not by female-adults (Pearson’s correlation analysis: $r = 0.068$, $p = 0.604$, $n = 61$), but by male-adults ($r = -0.290$, $p = 0.019$, $n = 65$) of *Halobates sericeus* inhabiting in the temperate and subtropical Pacific Ocean along the cruise track from Tokyo to Honolulu. The cruise number: KH-10-04 leg1 (140°E~163°W, 34°N~19°N).

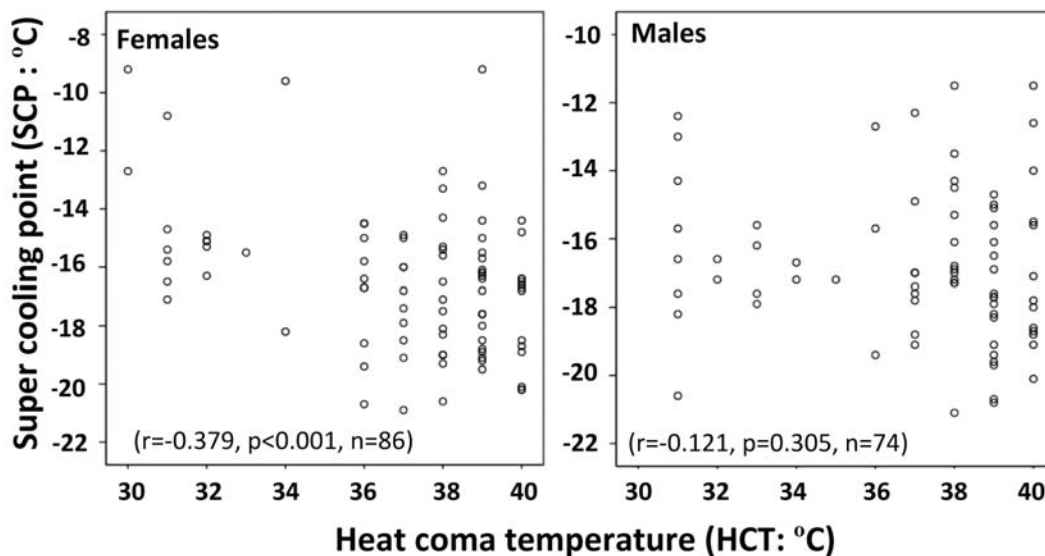


Fig. 7. Exhibition of “cross tolerance” between super cooling points (SCP:°C) and heat coma temperature (HCT:°C), shown not by adult males but by adult females of *Halobates micans* inhabiting in the tropical Pacific Ocean at the fixed point of 5°N, 140°E (The cruise number: MR-10-03). (Pearson’s correlation analysis: females, $r = -0.379$, $p < 0.001$, $n = 86$; males, $r = -0.121$, $p = 0.305$, $n = 74$).

comment that this fixed position of 08°00’S, 080°00’E is included in the special area of 5-10°S, 75°-95°E where intra-seasonal temperature variation is highest in the world open oceans. This special area is predicted to be a site for developing

a large low pressure mass due to the Madden-Julian Oscillation (MJO) (Yoneyama, personal communication). This high resistance to high temperature shown by *H. micans* might be naturally selected within one generation which is

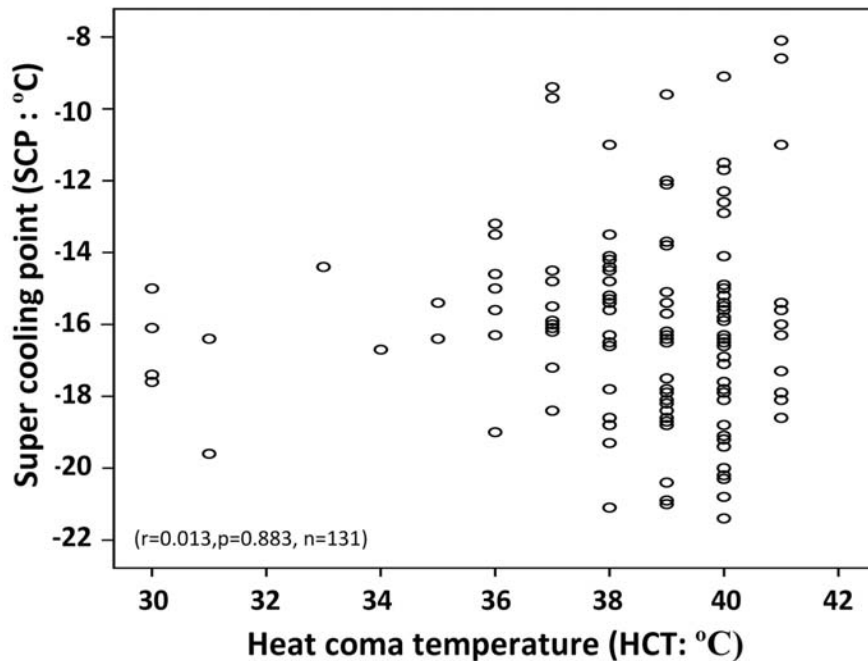


Fig. 8. Correlative analysis of Super-cooling point (SCP) and Heat Coma Temperature shown by all specimens of *Halobates micans* collected at the fixed point of 8°S 80°E in the tropical Indian Ocean (the cruise number: MR-11-07). No correlation was shown (Pearson's correlation test: $r = 0.013$, $p = 0.883$, $n = 131$).

proposed within 2-3 months or also over generations by such dynamic air and water temperatures around sea surface. However, the relationship between heat tolerance of sea skaters and meteorological data are currently remaining to be analyzed in the near future.

The ice crystallization does not necessarily occur accompanying with the lower lethal temperature. Most insects are dead because of chill-injuries at temperatures far above the SCP [21, 22, 23, 24]. On the other hand, the meta-analysis of data collected for over 350 terrestrial insect species revealed that some correlation between the SCP and cold tolerance exists. The more severe (colder) was the habitat (scored on an 11-point scale from tropics to poles), the lower was the SCP of insects living there [25]. All three sea skater species inhabiting open oceanic seas showed very similar SCP levels ranging between -15°C and -18°C on average. This value is unusually low for active insects and resembles rather the values characteristic for winter-acclimated terrestrial insects in temperate regions [26]. The average value of SCP ranges between -4°C to -8°C in the adults of closely related temperate freshwater halobatine species,

Metrocoris histrio, irrespective of season (summer, fall, winter), for comparison (Harada unpublished observations). Another freshwater strider, *Aquarius paludum* (Heteroptera, Gerridae) also shows relatively high SCP, ranging between the average value of -8°C in summer and -16°C in winter depending on season [27, 28].

Relatively higher SCP (-11.3°C ~ -14.1°C on average) was shown by the oceanic sea skaters collected from the Kuroshio area near to Japan Islands. This higher SCP might be related to the lower salinity in the surface sea water which can be estimated because of the heavy rains by typhoon (One typhoon attacked the cruise track during the cruise period of KT-09-20). Moreover, the influence of the flowing of fresh waters from the river mouths along the shores of Japan Islands. Conversely, the lower SCP shown by the specimens collected in the open oceans could be related to the long distance of sampling area from the lands and little influence from the freshwater flowing from rivers.

Some linkage might exist between SCP and exceptional feature possibly as high osmotic pressure of haemolymph due to surrounding sea

water salinity for oceanic sea skaters, though there have been no evidence. There is statistically significant correlation between SCP and HCT, even in such extremely low and limited SCPs range. At a level of individual insects, the higher was the level of HCT, the lower was the level of SCP. Similar relationship was observed also at a species level: while *H. sericeus* had the highest HCT and the lowest SCP, *H. micans* had the lowest HCT and the highest SCP among all four species (Harada *et al.*, submitted) during another cruise (MR-09-04) in tropical Pacific Ocean. In this study, the influence of ocean dynamics (typhoons and the Madden-Julian Oscillation:MJO) could strongly modify the basis of values of HCT and SCP depending on “species”.

Because the temperature of SCP correlates with the temperature of melting (m.p.), which is, in a colligative manner, influenced by osmolality of body fluids [29], the osmolality might be a good candidate for a linking physiological trait which is responsible for relatively low SCP levels. High body fluid osmolality might be expected in insects inhabiting marine waters with the mean salinity of 3.5% (approximately 1000 mosmol/kg). No rigorous data are available for oceanic sea skaters, unfortunately. Unpublished observations by Edney and Cheng (mentioned in Cheng [7]) refer to the value of 523.6 mosmol/kg. This value is at the upper limit of a range shown by various terrestrial and freshwater insects (250-550 mosmol/kg) [30], which may explain why the SCP values of oceanic sea skaters were so low.

What is the physiological mechanism of the correlation between SCP and HCT? Heat shock protein (HSP) was well known to be in transcript and translated several times higher in response to the exposure to heat in insects, especially in *Drosophila* [31, 32]. Heat shock protein protects from the damage of functional or constructive protein due to heat, for example [33]: the *HSP70* protein may ‘chaperone’ the protein against the lysosome for degradation [34] or help the protein-re-fold after return to favorable temperatures [35]. The over expression of HSP might be speculated to promote the expression of other substances to increase the osmotic pressures in the case of oceanic seaskaters, because heat shock proteins accelerate the synthesis of sorbitol in white flies,

polyol in whiteflies and aphids and also promote the trehalose synthesis in yeast [36, 37].

It was suggested that the data in this study can be further extended and bring interesting knowledge in future on a relationship between the thermal characteristics as ocean dynamics (typhoon, currents and MJO for example) of sea skater habitat on one side and linked physiological parameters which are complex on the other side. This study may serve to better future understanding the distribution and biology of these unique insects.

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