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Original Communication

Do sea-skaters of *Halobates* inhabiting a high precipitation area around the equator have higher resistance to lower temperatures?

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ABSTRACT

This study aims to test a hypothesis that the hardiness to lower temperatures of oceanic sea-skaters (*Halobates germanus* and *H. micans*) inhabiting the highest annual precipitation areas around the equator in the Pacific Ocean is greater than that of sea-skaters inhabiting nearby areas where there is less precipitation. This hypothesis is related to the sudden decrease in air temperature by about 5 ºC near the sea surface when it is rainy. Samples were taken from two sampling areas in the tropical Pacific Ocean (high annual precipitation area: 8ºN-5ºS, 136ºE-156ºE; low annual precipitation area: 12ºN, 135ºE). Semi-cool-coma temperature (SCCT) and cool-coma temperature (CCT) were recorded. The gap temperature for cool-coma (GTCC) was calculated as the difference between the surface sea water temperature at sampling sites and the CCT. The CCT (mean ± SD [n] = 12.8 ± 3.3 ºC [179]) and SCCT (17.1 ± 1.8 ºC [179]) of the specimens collected from the high precipitation area around the equator were significantly lower (CCT: $p < 0.001$, SCCT: $p = 0.001$) than the CCT (mean ± SD [n] = 17.9 ± 1.8 ºC [35]) and SCCT (18.0 ± 1.8 ºC [35]) found in specimens collected outside the high precipitation area. The GTCC (16.8 ± 3.4 ºC [179]) shown by the specimens collected from the high precipitation area was significantly higher ($p < 0.001$) than that of those collected from an area outside the high precipitation area (10.9 ± 1.9 ºC [35]). These results support our hypothesis.

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KEYWORDS: cool-coma, oceanic sea-skaters, tropical Pacific Ocean, high precipitation area

INTRODUCTION

Chill-coma study on *Drosophila*

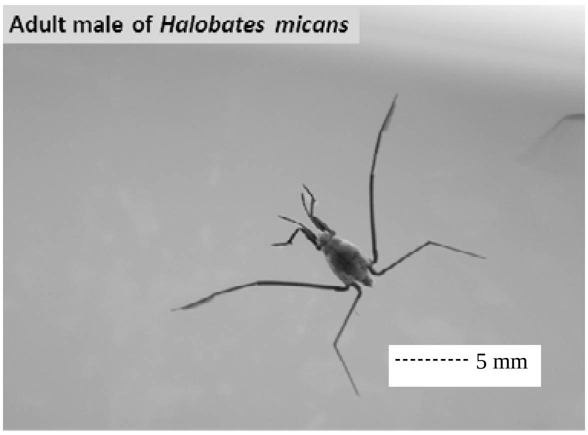
The phenomenon of ‘chill-coma’ in insects has been studied mainly using *Drosophila* [1]. The beginning of chill-coma is indicated by the loss of normal behavioral responses to external stimuli and the loss of strength or coordination required to stand [1]. The relationship between exposure time to CTmin (critical thermal minimum: the highest temperature to trigger the chill-coma) and the time taken for recovery from the coma has also been studied mainly in *Drosophila*; long exposure times being associated with longer recovery times [2, 3, 4].

Chill-coma studies on other taxa and temperature acclimation effects

The resistance to lower temperature has also been studied in other insects. For example, populations of tse-tse flies, *Glossina pallidipes*, collected from the field (located at 1ºN to 13ºS, with annual mean temperatures of 16 º - 25 ºC) showed 50% probabilities of survival when exposed to low temperatures of 3.7 ºC, 8.9 ºC and 9.6 ºC for 1-h, 2-h and 3-h, respectively [5]. The parasitoid wasp *Venturia canescens* showed the effect of temperature acclimation on recovery time from chill-coma [6]. The arrhenotokous female wasps which were grown at 17 ºC, 25 ºC and 30 ºC showed recovery times of about 5 min, 17 min and 50 min, respectively, whereas thelytokous females grown at 17 ºC, 25 ºC and 30 ºC recovered from the chill-coma after average times of 5 min, 90 min and 120 min, respectively [6]. The results indicate that lower temperatures during growth make recovery times shorter. The cricket *Acheta domesticus* also showed the effect of temperature acclimation on recovery time from chill-coma induced by exposure to -5 ºC for 1 hour [7]. When specimens of cricket were kept at 33 ºC, 29 ºC and 25 ºC for 7-9 days just after adult emergence, the time for recovery from chill-coma was around 2200 sec, 1600 sec and 800 sec, respectively [7]. Also for the temperate ant *Temnothorax nylanderi* cold acclimation shortened the recovery time from 8-9 min to 3-4 min on average [8].

Temperature dynamics in the tropical ocean and heat resistance of oceanic sea-skaters

In the case of oceanic sea-skaters inhabiting the tropical Pacific and Indian Oceans, the surface sea water temperature is very stable, around 30 ºC throughout the year. Therefore, the resistance to temperature change can be speculated to be very low. For instance, the heat coma temperatures (HCT) of three species of oceanic sea-skaters, *Halobates micans* (Photograph 1), *H. germanus* and *H. sericeus* in the normal sampling area at the time of collection (August) in the tropical Pacific Ocean (around 12ºN, 135ºE) are about 32 ºC, 33 ºC and 35 ºC, respectively [9], values which are near to the sea water temperature of 28 ºC - 30 ºC. However, exceptionally high heat tolerance was shown by sea-skaters collected from the area 08º00'S, 80º30'E (fixed observing locality during the cruise, MR11-07) in the tropical Indian Ocean. The average HCT was very high - about 40 ºC and 39 ºC for *H. germanus* and *H. micans*, respectively [10]. This high HCT, around 40 ºC, is similar to the value for *Metrocoris histrio*, a fresh water Halobatinae species (Harada *et al*., unpublished), which inhabits fresh water springs and is exposed to seasonal fluctuation of air temperature (-3 ºC to 35 ºC in Kochi, Japan: 33ºN, 133ºE).



Photograph 1. Male adult of an oceanic sea skater *Halobates micans.*

This value of HCT shown by *H. germanus* and *H. micans* was higher by 3-5 ºC than that shown by specimens inhabiting other subtropical and tropical open ocean areas in the Indian and Pacific Oceans [9]. This high heat-resistance coincided with high heat coma temperatures (averaging 39 ºC) shown by specimens collected at 06º25'S, 89º00'E in the tropical Indian Ocean [11]. The chief scientist of the cruise MR11-07, who is also a meteorologist, Prof. Kunio Yoneyama (personal communication) was kind enough to give us the very important information that this fixed position (08º00'S, 080º00'E) is inside a special area (5º-10ºS, 75º-95ºE) where intra-seasonal temperature variations are the highest found in the world's open oceans.

This special area is a site where a large low-pressure weather system develops due to the Madden-Julian Oscillation (MJO) (Yoneyama, personal communication). The extremely high resistance to high temperature around 40 ºC shown by *H. micans* and *H. germanus* might have developed in this area over generations. On the other hand, the exposure to wide temperature fluctuations during the larval and adult stages might have led to the effects of temperature acclimation on the resistance to increased temperature. However, no studies have been done on the relationship between temperature dynamics in the tropical ocean and the resistance to the lower temperatures.

The areas around the equator in the central tropical Pacific Ocean is the place where the annual precipitation is the highest in the world (Fig. 1). The hypothesis to be tested is that the resistance to lower temperatures of oceanic sea-skaters *Halobates germanus* and *H. micans*, inhabiting the areas of highest annual precipitation around the equator is greater than that shown by sea-skaters inhabiting regions outside this high precipitation. The theoretical

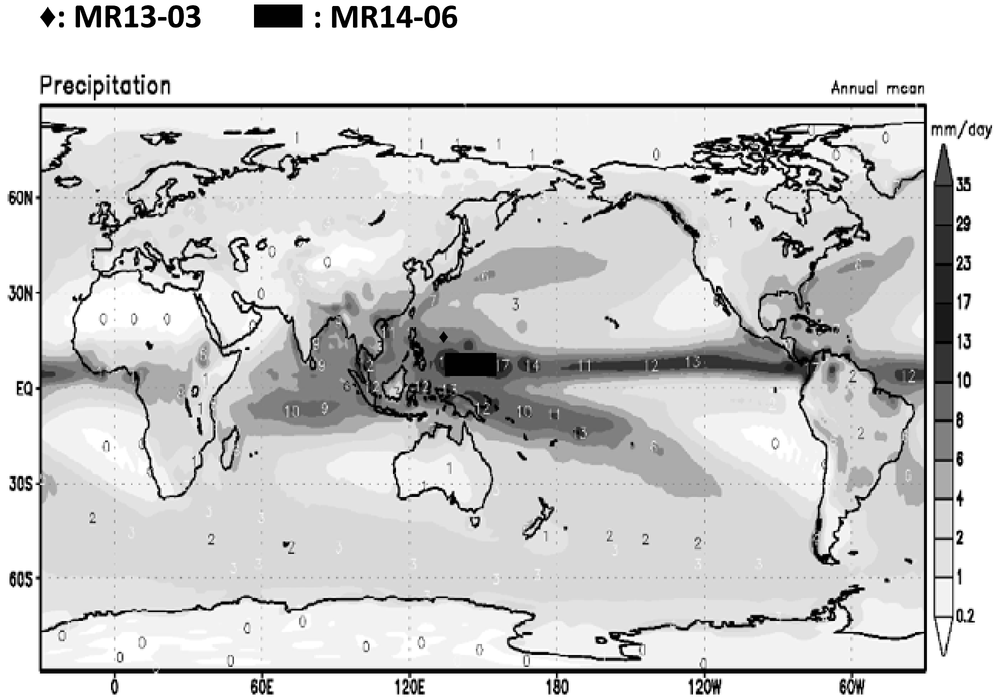


Fig. 1. Mean annual precipitation map, with the sampling areas superimposed (Modified adaptation from http://research.jisao.washington.edu/legates_msu/).

basis of this hypothesis is that the sudden decrease in air temperature, by about 5 ºC near the sea surface [11], could lead to selection of animals with higher genetic resistance to decreased temperature; alternatively, it might be that the lower temperatures which accompany the rainfall lead to the development of a more marked resistance to lower temperatures - a ‘cool acclimation effect’. The purpose of this study is to test this hypothesis.

MATERIALS AND METHODS

Samplings

During the two cruises (cruise no.: MR13-03 and cruise no.: MR14-06 by R/V Mirai [8687t] owned by JAMSTEC [Japan agency of marine-earth science and technology]), samplings were performed mostly at night with a Neuston net (an open box-typed frame and inner net with a 1.3 m wide opening and 6 m in length), between 18:24 and 22:15 h. The net was towed to the starboard side for 15 min during each sampling period. Towing was repeated twice with the ship cruising at a speed of 2 knots. The Neuston net was trailed on the sea surface for the 3 sampling periods (each 15 min x 3 times), to enable the collection of living sea-skaters from the bottom of the net. These skaters were used in the cool-coma experiments (see below). The surface area swept by the nets was expressed as the value of the flow-meter × the front width of the nets.

Treatments of specimens after collection and before the cool-coma experiments

Sea-skaters trapped in the plastic bottle fixed at the end of the Neuston net were paralyzed by the physical shock of trailing the net. Such paralyzed sea-skaters were transferred to a piece of paper towel to enable them to respire, some of which recovered from paralysis after 20 min. When sea-skaters were trapped in the jelly of jelly fish, the jelly was removed from their body by hand, very carefully and quickly, to enable them to recover from paralysis.

Adults that recovered from paralysis were placed in sea water in the laboratory ‘holding’ aquaria in preparation for cool-coma experiments. Those that did not recover were discarded. The aquaria were cube shaped (30 cm × 30 cm × 40 cm) and were used for rearing the adults. Each aquarium contained ten to thirty adult *Halobates*. Both the room temperature and the sea water temperature in the aquaria were kept at 28 ± 2 ºC, and the sea-skaters were kept in these conditions for at least 11-12 hours after collection. When the sea-skaters were used for the experiments during the daytime of two consecutive days, they were fed with adult flies of *Lucillia illustris* continuously prior to 11-12 hours before the experiments. However, no food was given for 11-12 hours prior to the experiments. Food was given only between 10:00-18:00 h and the sea water in the aquaria was replenished at 08:00 and 18:00 hours to avoid pollution from the food.

Cool-coma experiment

A transparent, circular aquarium was used for the experiments, and the sea water in it was kept at the same temperature (28 ± 2 ºC) as that in the holding aquaria. Ten or eleven adult specimens were moved to low temperature thermostatic water bath (Thomas: T22LA) measuring 55 cm × 40 cm × 35 cm. The temperature was very precisely controlled by the automatic thermostat of the water bath. Sea water temperature was decreased stepwise by 1 ºC every 15 min until cool-coma occurred in all the specimens. Temperatures at which semi-cool-coma (SCC) and cool-coma (CC) occurred were recorded. The SCC temperature and CC temperature are defined as follows. SCCT: The temperature at which skating behavior stopped completely for more than 5 seconds; CCT: The temperature when ventral surface of the body was caught in the sea water film and the ability to skate was lost, or when abnormal postures on the sea water were observed, for example, one leg sunk in the water, the body was upside down, or the mid-leg moved backwards and attached to hind leg. The gap temperature for cool-coma (GTCC) was calculated as the difference between the baseline temperature (to which the specimens had been adapted in the holding aquaria for at least 11 hours) and the CCT.

The results of SCCT, CCT and GTCC were expressed as mean ± standard deviation (SD) and Mann-Whitney U-test for non-parametric analysis was performed because the value of SCCT, CCT and GTCC did not always show the normal distribution. For the statistical analyses in this study, a software (SPSS 12.0 J for Windows, SPSS Inc., Chicago, IL, USA) was used.

RESULTS

Samplings

At the fixed station of 12ºN, 135ºE, both *H. micans* and *H. germanus* were co-dominant species showing population densities around 10,000/km^2 as estimated (Table 1). In the high precipitation sampling area (8ºN-5ºS, 136º-156ºE), *H. germanus* was the dominant species with a population density of 17,000/km^3, whereas the density of *H. micans* was around 4,000/km^3 (Table 1).

Cool-coma experiment

The CCT (mean ± SD [n] = 12.8 ± 3.3 ºC [179]) and SCCT (17.1 ± 1.8 ºC [179]) for specimens of *H. germanus* collected from the high precipitation area around the equator were significantly lower (CCT: $p < 0.001$, SCCT: $p = 0.001$) than the CCT (17.9 ± 1.8 ºC [35]) and SCCT (18.0 ± 1.8 ºC [35]) shown by the specimens collected from a region away from the high precipitation area (Figs. 2 and 3). The GTCC (16.8 ± 3.4 ºC [179]) shown by the specimens collected from the high precipitation area was significantly higher ($p < 0.001$) than that of those collected from outside this area (10.9 ± 1.9 ºC [35]) (Fig. 4). All the results for *H. micans* were similar to those for *H. germanus* (Figs. 2, 3, 4).

DISCUSSION

Cool-coma temperature higher than chill-coma in terrestrial species

A remarkable observation of this study is that the ‘cool-coma’ occurred around 13 º - 20 ºC and the coma appeared at higher temperatures than the ‘chill-coma’ phenomenon found in terrestrial insects, which occurs at around 0 º - 10 ºC. For example, even in tropical tse-tse flies, a low-temperature coma occurs at 3.7 º - 9.6 ºC (Terblanche *et al*., 2008) [5]. The observation that the cool-coma occurs in tropical oceanic sea-skaters at such higher temperature seems to be related to the relatively stable sea water temperatures (around 29 ºC) that exist in this environment.

Chill-coma temperature in oceanic sea-skaters *Halobates germanus and H. micans* inhabiting areas with highest annual precipitation

A relatively higher population density of *H. germanus* inhabiting the high precipitation area around equator was found and is compared with the population at

Table 1. The oceanic sea-skaters *Halobates* collected from two areas, 12ºN 135ºE (Cruise: MR13-03) and 8ºN-5ºS 136º-156ºE (Cruise: MR14-06) in June 2013, December 2014 and January 2015. (T: Total number of individuals collected; H.m.: *Halobates micans*; H.g.: *Halobates germanus*; H.s.: *Halobates sericeus*; H. spp.: *Halobates* spp.; WT: Water temperature (ºC); AT: Air temp (ºC); L: Larvae; A: Adults; E: No. of exuviae; Date: Sampling date; three sampling units were done per night both for MR13-03 and MR14-06. A: Number of individuals collected; B: Density of individuals (number per 1 km²) EG: number of eggs collected. *Samplings were performed in the daytime (13:09-15:32) only on 25th Dec 2014 and 2nd Jan 2015.

	Cruise No.	Sampling sites	T	L	A	H.m.	H.g.	H.s.	H.spp.	EG	E	WT	AT	Time	Date
A	MR13-03	12ºN, 135ºE	603	484	119	276	327	0	0	0	1	28.4-29.7	29.3-29.6	20:32-22:12	13-27 Jun 2013
	MR14-06	8ºN-5ºS, 136º-156ºE	633	266	367	112	521	0	0	0	9	26.0-26.8	24.1-26.1	18:24-19:51*	25 Dec 2014-16 Jan 2015
B	Cruise No	Sampling sites	T	L	A	H.m.	H.g.	H.s.	H.spp.	EG	E	WT	AT	Time	Date
	MR13-03	12ºN, 135ºE	21516.4	17270.2	4246.2	9848.3	11688.1	0	0	0	35.7	28.4-29.7	29.3-29.6	20:32-22:12	13-27 Jun 2013
	MR14-06	8ºN-5ºS, 136º-156ºE	22586.9	8761.5	12088.2	3689.1	17160.6	0	0	0	296.7	26.0-26.8	24.1-26.1	18:24-19:51*	25 Dec 2014-16 Jan 2015

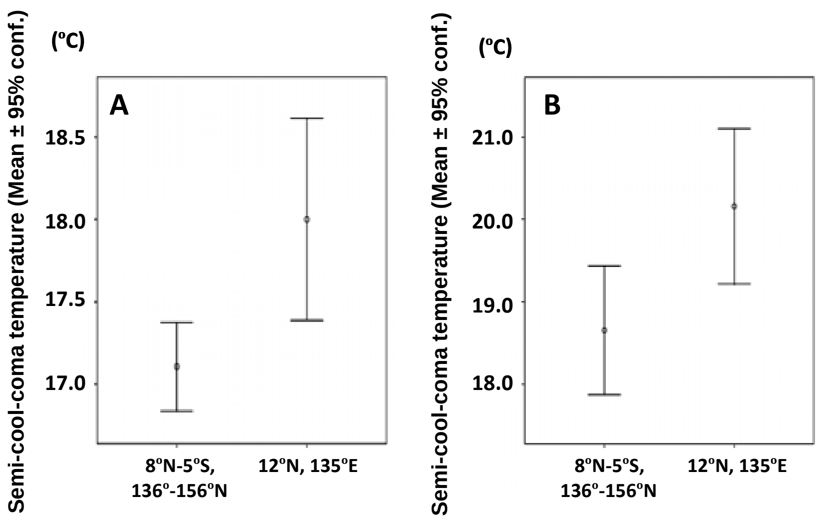


Fig. 2. Comparison of semi-cool-coma temperatures (SCCT) shown by specimens collected from 12ºN, 135ºE and those collected from 8º-5ºS, 136º-156ºE around the equator. Results from two species of oceanic sea-skaters are shown: A. *Halobates germanus* (Mann-Whitney U-test: z = -3.185, $p < 0.001$) and B. *H. micans* (Mann-Whitney U-test: z = -2.317, p = 0.020).

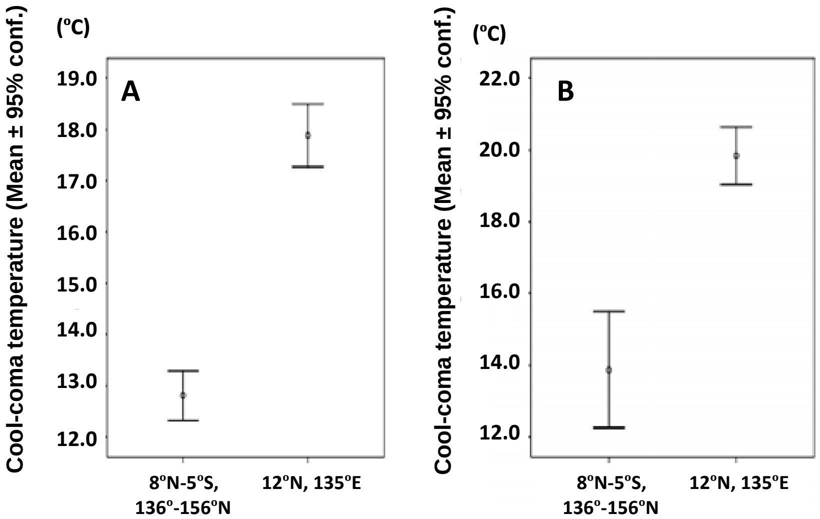


Fig. 3. Comparison of cool-coma temperatures (CCT) shown by the specimens collected from 12ºN, 135ºE and those collected from 8º-5ºS, 136º-156ºE, around the equator. Results from two species of oceanic sea-skaters are shown: A. *Halobates germanus* (Mann-Whitney U-test: z = -7.890, $p < 0.001$) and B. *H. micans* (Mann-Whitney U-test: z = -4.962, $p < 0.001$).

the station (12ºN, 135ºE) which is located outside this high precipitation area. This high population density at the high precipitation area might be related to the higher resistance to lower salinity that is found in *H. germanus* (Sekimoto *et al*., unpublished.).

Our hypothesis that 'the resistance to lower temperatures of oceanic sea-skaters, *Halobates germanus* and *H. micans*, inhabiting the areas of highest annual precipitation around the equator is greater than that shown by sea-skaters inhabiting

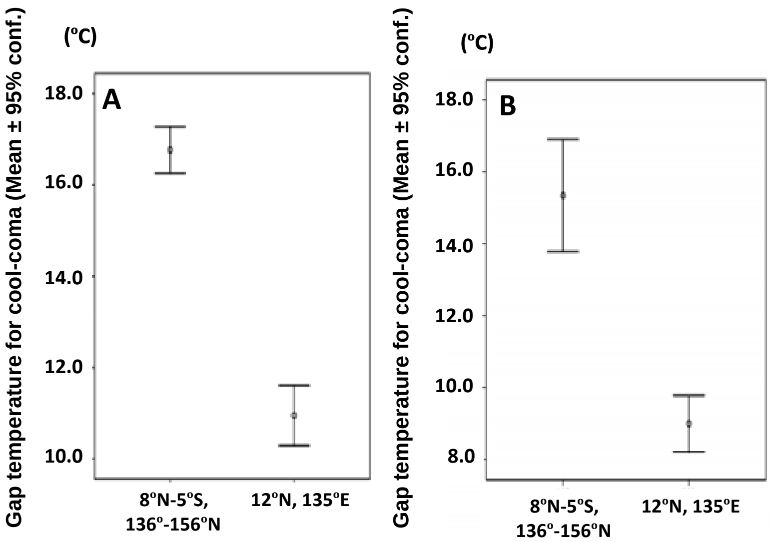


Fig. 4. Comparison of gap temperature for cool-coma (GTCC) shown by the specimens collected from 12ºN, 135ºE and those collected from 8º-5ºS, 136º-156ºE, around the equator. Results from two species of oceanic sea-skaters are shown: A. *Halobates germanus* (Mann-Whitney U-test: $z = -8.331$, $p < 0.001$) and B. *H. micans* (Mann-Whitney U-test: $z = -5.256$, $p < 0.001$).

regions outside this area of high precipitation', is supported by the results of the present study. However, it remains unknown, and requires a future study, if such high resistance to lower temperatures is genetic and is due to natural selection and/or if it is an acclimation effect due to regular exposure to the decrease in temperature that accompanies the frequent rainfalls in the area around the equator.

CONCLUSION

The hardiness to lower temperatures of oceanic sea-skaters (*Halobates germanus* and *H. micans*) inhabiting the highest annual precipitation areas around the equator in the Pacific Ocean is greater than that of sea-skaters inhabiting nearby areas where there is less precipitation.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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