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Comparative study of cool coma temperature between two populations of oceanic sea skaters, *Halobates sericeus* (Heteroptera: Gerridae), located at 24-25ºN and 138ºE or 160ºE in the Pacific Ocean

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ABSTRACT

Terrestrial insects exhibit a state called 'chill coma' (CC) at around 0 ºC. In this study, we tested the hypotheses that 'cool coma' (COC) occurs at a far higher temperature than chill coma, and that sea skaters collected near the *Kuroshio* current show a higher resistance to lower temperature than those collected far from the current. The temperature at which COC occurs (cool coma temperature: COCT) was recorded on a low-temperature thermostatic water bath in which the temperature was decreased by 1 ºC every 15 min until COC occurred. After measurement of COCT, the super-cooling point (SCP) was measured. COC occurred at 19.01 ºC for adults collected at 25ºN, 160ºE and at 17.5 ºC for those collected at 24ºN, 138ºE, both of which are far higher than the CCT. The COCT and SCP (-14.8 ºC) values for those collected at 25ºN, 138ºE near the *Kuroshio* current were lower than the values for animals collected at 24ºN, 160ºE (COCT: $p = 0.005$; SCP: -14.0 ºC, $p = 0.115$). These results support both hypotheses.

KEYWORDS: chill coma temperature, super cooling point, oceanic sea skaters, the *Kuroshio* current

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INTRODUCTION

For terrestrial insects, a coma induced by lower temperatures occurs at around 0 ºC and is called a 'chill coma'. The chill coma was reviewed recently by Macmillan *et al*. [1]. As the temperature decreases, the locomotion of the insect slows and eventually stops, but recovers after the temperature increases again. This cessation of movement is described as chill coma (CC) [2]. The chill coma temperature (CCT) is defined as the temperature at which CC is triggered [3]. CC is indicated by the loss of normal behavioral responses to stimuli and by the loss of strength and coordination, resulting in the inability to stand. The highest temperature that triggers CC is called the critical thermal minimum (CTmin). This temperature is also called the knockdown or cold stupor temperature in insects [4, 5]. If the CC does not reach the 'chill injury' stage, the physiological changes can be reversed by re-warming [6]. The relationship between exposure time to the CTmin and the time required for recovering from CC has been studied mainly in Drosophila [7, 8, 9].

Water striders which inhabit fresh water are included in the Gerridae, and they appear to be able to survive between temperatures of -3 ºC (the lower limit) and 42 ºC (the higher limit) [10].

This large range may be the result of large daily and seasonal variations in water temperature in freshwater ponds and rivers. In contrast to terrestrial freshwater bodies, the water temperature in tropical ocean areas is much more stable at around 30 ºC. This could be the reason why the tropical oceanic sea skaters, *Halobates micans*, *H. germanus* and *H. sericeus*, show lower tolerances to temperature increases than does a Halobatinae species, *Metrocoris histrio*, that inhabits terrestrial freshwater bodies [11, 12], (Harada *et al.*, unpublished). However, it is pertinent to examine what is the lower temperature limit for these creatures that colonize temperate and subtropical ocean areas. During a cruise by the R/V Hakuhomaru in May 2003, the oceanic sea skater species, *H. sericeus*, was sampled at 18 locations in the East China Sea (27°10'N-33°24'N, 124º57'E-129º30'E) [13]. One or both of the other two species, *H. micans* and *H. germanus*, were collected at only 8 locations in the area south of 29°47'N, where the water temperatures were higher than 25 ºC. At three locations where the water temperature was less than 23 ºC, the samples did not contain either *H. micans* or *H. germanus*, suggesting that these two species cannot inhabit areas where the water temperature is lower than this value. Adult *H. sericeus* specimens were collected at 26º55'S, 165º34'E in the southern Pacific Ocean, with a relatively high density of 40,000 individuals per km^2 where the temperature was under 23.2 ºC [14]. It is possible that only *H. sericeus* is resistant to these lower temperatures but, so far, their hardiness to cold temperatures has not been examined.

Another possible explanation of these findings is that the *Kuroshio* current can move the oceanic sea skaters, especially *Halobates sericeus*, from southwest to northeast and vice versa, through the continuous motions of the *Kuroshio* and North Equatorial currents [15]. Sea skaters living on these currents could show greater hardiness to temperature fluctuations than those inhabiting the sea surface but with little or no influence from the currents.

This study aims, first, to clarify whether coma caused by low temperatures in these species occurs at a higher temperature than is shown in terrestrial insects and, second, to test the hypothesis that sea skaters inhabiting the sea surface near the *Kuroshio* current show a higher resistance to lower temperatures than those inhabiting places far from the current.

MATERIALS AND METHODS

Samplings

Samplings were performed on June 5 2013, at 24ºN 138ºE during a cruise (cruise number: MR-13-03) and between June 4 to 7 2014, at 25ºN, 160ºE during another cruise (KH-14-02) in the subtropical Pacific Ocean, both using a neuston net (6 m long and with diameter of 1.3 m). One trial consisted of trailing the net three times (for 15 min on each occasion) on the sea surface each night from the starboard side of the R/V MIRAI (8687 tonnes) for the MR-13-03 cruise and the R/V HAKUHOMARU (3991 tonnes) for KH-14-02 cruise. Both research vessels were owned by JAMSTEC (Japan Agency for Marine-earth Science and Technology). Sampling was performed at a ship speed of 2.0 knot, and was repeated twice at each location. The surface area that was swept by the neuston net was evaluated as: [flow-meter value x 1.3 m width of the neuston net].

Treatment of specimens after sampling and before experiments

The sea skaters were trapped in grey plastic bottles fixed at the end of the neuston net and were paralyzed by the physical shock of trailing the net at a speed of 2 knots. The paralyzed sea skaters were transferred to a paper towel to enable them to respire. Some recovered from paralysis within 20 min. When sea skaters were trapped in the jelly of a jellyfish, the jelly was removed by hand very carefully and quickly, to allow recovery from paralysis.

Adults that recovered from paralysis were moved to seawater in an aquarium set up in the laboratory for the cool coma experiments and for measuring the super-cooling point. Several white cube aquaria (30 × 30 × 40 cm) were placed in the laboratory of the ship for rearing adults that recovered from paralysis after capture. Each aquarium contained 10-30 adults of *Halobates sericeus*. Both room temperature and seawater temperature in the aquaria were kept at 28 ± 2 ºC. Prior to the cool coma experiment, the sea skaters were kept in the aquariua for 11 to 12 hours. All the individuals of *Halobates* kept in the aquaria were fed adult flies *Lucillia illustris*. Food was given only for 8 hours, between 1000 and 1800 h, and the seawater in the aquaria was replaced twice, at 0800 and at 1800, to prevent the water from becoming too dirty because of uneaten food. No food was given for at least 12 hours before measuring SCP, because the contents of the alimentary canal could turn into ice-nuclei.

Cool coma experiment

The experimental area was a transparent, round aquarium with seawater maintained at the same temperature (28-29 ºC) as the rearing aquarium. Ten or eleven adult specimens were moved to a low temperature thermostatic water bath (Thomas: T22LA, 55 × 40 × 35 cm). The temperature was decreased stepwise by 1 ºC every 15 min using an automatic cooling system in the water bath until cool coma occurred in all experimental specimens. During this protocol, the temperature was controlled very precisely by an automatic thermostat system in the water bath. The animal is said to be in a cool coma state if one of the following situation occurs: (1) The ventral surface of the animal's body is caught in the water surface film; (2) The animal shows no movement and is seen in an abnormal posture, for example, lying upside down, with one leg sunk in the water, or with the mid-leg stretched backward and attached to the hind leg. The temperature at which COC occurred was recorded as the Cool Coma Temperature (COCT). The gap temperature for cool coma (GTCOC) was calculated as the difference between the baseline temperature (to which specimens had adapted for at least 12 hours) and the COCT.

Determination of super-cooling point (SCP)

Measurement of the super-cooling point (SCP) was performed on the adult specimens of *H. sericeus* during the cruises, immediately after the cool coma experiment. The surface of each adult was dried with filter paper, and thermocouples (which consisted of nickel and bronze) were attached to the ventral surface of the thorax and connected to an automatic temperature digital recorder (Digital Thermometer, Yokogawa Co, LTD, Model 10, Japan). The thermocouple was completely fixed to the ventral surface of abdomen with a tape. The specimen and attached thermocouples were placed in a compressed Styrofoam box (5 × 5 × 3 cm) that was kept inside a larger insulating compressed Styrofoam box, to ensure that the cooling rate was about 1 ºC/min when the apparatus was placed in the freezer (in which the temperature was -35 ºC). The lowest temperature recorded before an exothermic event that occurred due to release of latent heat was regarded as the SCP [16]. All specimens died from freezing by the time the SCP was determined.

RESULTS

Samplings

High population densities of *H. sericeus* of almost 100,000 individuals per km^2 and about 52,000 individuals per km^2 was estimated for the stations at 24ºN, 138ºE and 25ºN, 160ºE, respectively (Mann-Whitney U-test, z = -0.578, p = 0.563) (Table 1). At the locations at 25ºN, 160ºE and 24ºN, 138ºE, a total of 66-148 larvae and 23-54 specimens in each of the 5 larval stages were collected. Eighty four exuviae (wasted skin at molting) were collected at the location at 160ºE and 10 at the location at 138ºE. Therefore, reproductive and growth activities of *H. sericeus* might be high at both locations.

Cool coma experiment and measurement of super cooling point

The adult *H. sericeus* collected at 25ºN, 160ºE exhibited a mean COCT of 19.01 ºC (SD = ±3.39, n = 79), which was significantly higher than that of specimens at 24ºN, 138ºE (17.53 ± 1.87 ºC, n = 49; ANCOVA with sex as a covariate: F = 7.989, df = 1, p = 0.005) (Figure 1). The mean GTCOC shown by the specimens collected at 25ºN, 160ºE was 8.94 ºC (±3.30, n = 79), which was significantly lower than that of specimens at 24ºN, 138ºE (10.88 ºC ± 2.24, n = 49; ANCOVA with sex as a covariate: F = 13.327, df = 1, p < 0.001) (Figure 2). The SCP of the specimens collected at 25ºN, 160ºE was -13.99 ºC (±2.47, n = 78) and tended to be higher than that of specimens collected at

Table 1. The oceanic sea skaters, *Halobates* collected at two sites, 24ºN, 138ºE (Cruise: MR-13-03) and 25ºN, 160ºE (Cruise: KH-14-02) in June, 2013 and 2014, respectively. (N: 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.; L: Larvae; A: Adults, E: No. of exuviae; Date: Sampling date; SS: Area of water surface over which the Neuston net was trailed by the ships, R/V MIRAI and R/V HAKUHOMARU); One unit of sampling was performed for 15 min. Three and 12 units were done for the sites, 24ºN, 138ºE and 25ºN, 160ºE, respectively. A: Number of individuals collected; B: Density of inviduals (number per 1 km^2).

	Cruise No.	Sampling sites	N	L	A	H.m.	H.g.	H.s.	H.spp.	EG	E	WT	AT	Time	Date
A	MR-13-03	24ºN, 138ºE	305	179	126	6	0	299	0	0	10	27.6	27.9	19:04-20:10	5-Jun-13
	KH-14-02	25ºN, 160ºE	847	593	254	0	0	847	0	0	84	26.0-26.8	24.1-26.1	20:30-11:40	4-6 Jun 14
B	**Cruise No.**	**Sampling sites**	**N**	**L**	**A**	**H.m.**	**H.g.**	**H.s.**	**H.spp.**	**EG**	**E**	**WT**	**AT**	**Time**	**Date**
	MR-13-03	24ºN, 138ºE	96468	56657	39811	1899.1	0	94639	0	0	3165.2	27.6	27.9	19:04-20:10	5-Jun-13
	KH-14-02	25ºN, 160ºE	52057	36446	15611	0	0	52057	0	0	5162.6	26.0-26.8	24.1-26.1	20:30-11:40	4-6 Jun 14

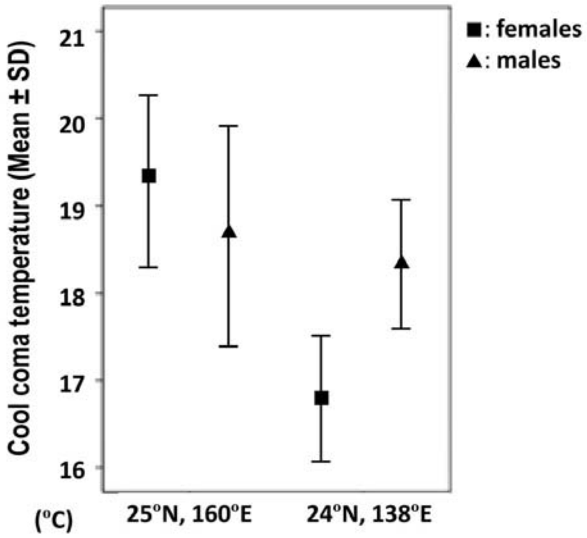

Figure 1. Cool coma temperatures of adult *Halobates sericeus* collected at 24ºN, 138ºE and at 25ºN, 160ºE.

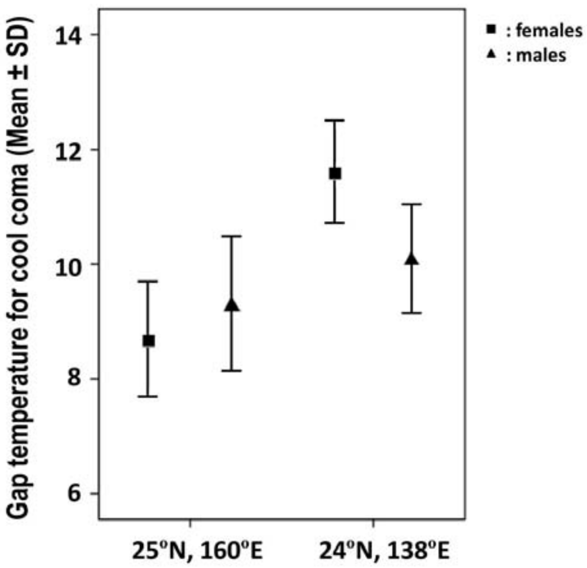

Figure 2. Gap temperature (see text for definition of this) for cool coma of adult *Halobates sericeus* collected at 24ºN, 138ºE and at 25ºN, 160ºE.

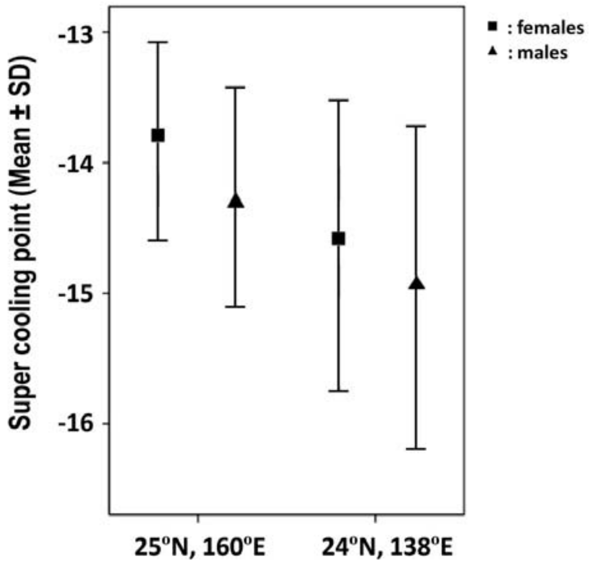

Figure 3. Super-cooling points of adult *Halobates sericeus* collected at 24ºN, 138ºE and at 25ºN, 160ºE.

24ºN, 138ºE (-14.80 ºC ± 2.76, n = 49; ANCOVA with sex as a covariate: F = 2.525, df = 1, p = 0.115) (Figure 3). To investigate the possible relationship between sex and COCT, GTCOC and SCP (Figures 1, 2, 3), ANCOVA with station as a covariate was performed and there were no significant differences between sexes (COCT: df = 1, F = 0.185, p = 0.668; GTCOC: df = 1, F = 0.260, p = 0.611; SCP: df = 1, F = 0.684, p = 0.410).

DISCUSSION

The relationship between cool coma and chill coma temperatures

The remarkable finding in this study that may be new to science is the discovery of a 'cool coma' phenomenon. This occurs around 20 ºC and may be 10 ºC or more higher than the chill coma temperature (defined as the critical temperature for reduced movement or chill stupor) observed in terrestrial insects [1]. This higher critical temperature for stupor or coma may be related to the relatively stable water temperatures that are present in the subtropical Pacific Ocean, which is the natural habitat of *H. sericeus*.

The relationship between the *Kuroshio* current and tolerance to lower temperature in *H. sericeus*

The sampling position at 24ºN, 138ºE is relatively close to the *Kuroshio* current, while the sampling position at 25ºN, 160ºE is far from it (Figure 4). *Halobates sericeus* may be transferred to other locations by the *Kuroshio* current [13, 15]. The relatively high population density and higher

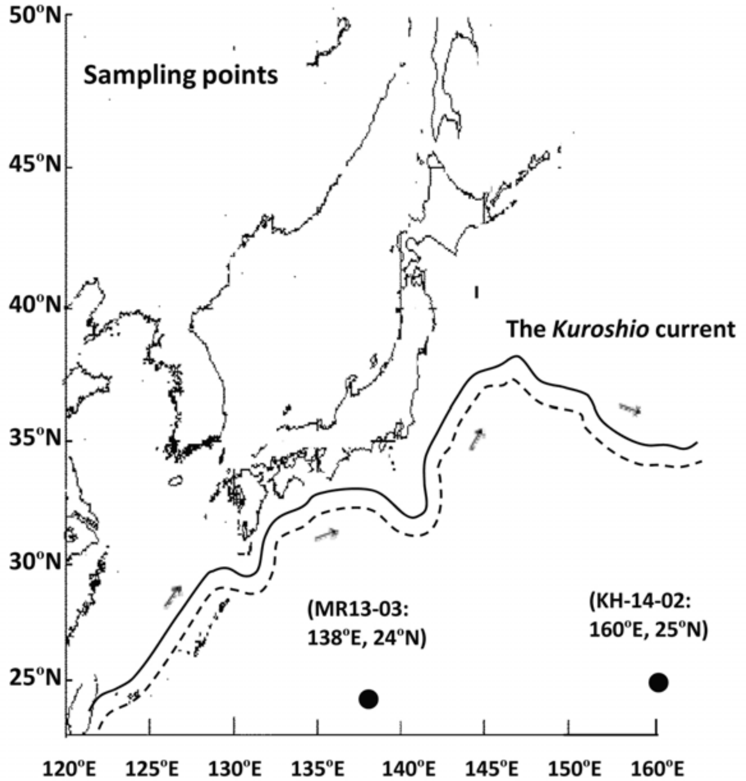

Figure 4. Sampling points (shown by black circles) and the *Kuroshio* current. (This map is an original creation of the corresponding author).

resistance to lower temperature may be related to such transfers by the *Kuroshio* current. In other words, specimens collected at 24ºN, 138ºE may include 'travelers' on the *Kuroshio* current.

CONCLUSION

The hypotheses that the 'cool coma' (COC) occurs at a far higher temperature than chill coma (CC), and that sea skaters collected near the *Kuroshio* current show a higher resistance to lower temperature than those collected far from the current were proven to be true by the results of the experiments. There is a possibility that the population near the *Kuroshio* may be transferred to other locations by this current. If so, this population could experience a temperature difference during the transfer by the *Kuroshio* as well as by the continuous current to the *Kuroshio*, the *North-Equator* currents, and the resistance to temperature change might be developed during the transfer through wide latitude area where temperature changes substantially and seasonably.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest concerning this study.

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