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Cross Tolerance between Heat and Cold Stress by Warm Temperate *Aquarius paludum paludum* and Subtropical *Aquarius paludum amamiensis*, Semi-Aquatic Bugs (Gerridae, Heteroptera) 【Research report】

溫帶水黽 *Aquarius paludum paludum* 及亞熱帶水黽 *Aquarius paludum amamiensis* 對於熱壓力及冷壓力的交互耐受性【研究報告】

Tetsuo Harada*, Shin Ikeda, and Takamasa Ishibashi
Tetsuo Harada*, Shin Ikeda, and Takamasa Ishibashi

*通訊作者E-mail: haratets@kochi-u.ac.jp

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Abstract

The effects of geographical location (Kochi-strain [33°N]: *Aquarius paludum paludum*, Naha-strain [26°N]: *A. p. amamiensis*) and/or rearing temperature were examined on the lower and higher lethal temperatures (LLT and HLT) of larvae and also on the super-cooling point (SCP) of the adult water strider. Experiments to determine the lethal temperatures were performed mainly within 24-48 hours after the hatching or molting of the first to 5th (last) instars. The first instars just after hatching were reared at 25°C or 20°C for the Kochi-strain and at 25°C for the Naha-strain. The air temperature inside the incubating chamber was dropped or raised stepwise by 1°C every hour to find the low and high lethal temperatures of the larvae on the water surface, respectively. The higher resistance to both lower and higher temperatures was shown by the first instars of the Naha-strain-25°C group (mean HLT: 45.1°C; LLT: -3.38°C) followed by Kochi-strain-25°C group (HLT: 42.6°C; LLT: -1.40°C). Similar differences in the lower and higher lethal temperatures were also shown by the 3rd instars between the two strains. However, that difference disappeared by the 5th (last) instar stage, probably due to exposure to a constant ambient temperature till the 5th instar stage. On the other hand, in the Kochi-strain the 5th instars grown at 20°C showed both a higher mean HLT (45.34°C) and a lower LLT (-5.30°C) than those grown at 25°C (HLT: 43.71°C, LLT: -3.20°C). The average SCPs in the *A. p. amamiensis* and Hybrid (F15) were significantly lower than those of *A. p. paludum* under long- and short-day photoperiods. Natural selection of heat tolerance in the Naha subtropical strain might also lead to an accompanying higher cold hardiness. The overwintering generation of larvae grow up in relatively low ambient temperature of around 20°C during late October and November in Kochi. This moderate temperature could lead to higher cold hardiness of the last instars just before winter.

摘要

本試驗檢視不同地理品系水黽(高知品系·33oN·*Aquarius paludum paludum*; 那霸品系·26oN·*Aquarius paludum amamiensis*)及飼育溫度對稚蟲對致死低溫(lower lethal temperatures, LLT)與致死高溫(higher lethal temperatures, HLT)及對成蟲過冷卻點(super-cooling point, SCP)的效應。致死溫度試驗乃利用孵化或脫皮後24~48小時內之一至五齡稚蟲進行試驗。高知品系孵化之一齡稚蟲飼養於20°C及25°C之生長箱,那霸品系飼養於25°C之生長箱;生長箱內之氣溫以每小時降低/提高1°C的方式,以檢驗水面幼蟲之致死低溫或致死高溫。結果顯示那霸品系之一齡稚蟲對高低溫具有較高之耐受性(HLT: 45.1°C; LLT: -3.38°C),其次為飼養於25°C之高知品系稚蟲(HLT: 42.6°C; LLT: -1.40°C)。兩品系之三齡稚蟲亦有類似結果。然而這些差異在第五齡稚蟲之前消失,可能由於持續暴露在恆定的周遭環境所致。另一方面,飼養於20°C之高知品系五齡稚蟲較飼養於25°C之稚蟲呈現較高之致死高溫(HLT: 45.34 vs. 43.71°C)及較低的致死低溫(LLT: -5.30 vs. -3.20°C)。在長/短光週期條件下,*Aquarius paludum amamiensis*及其雜交品系(F15)之過冷卻點均低於*Aquarius paludum paludum*。亞熱帶那霸品系水黽對高溫耐受性的天擇可能伴隨產生對低溫的耐受性。在高知地區,從10月下旬至11月,越冬稚蟲在氣溫約20°C下成長,此溫度可能使得最後一齡稚蟲在冬天來齡之前獲得較高之冷耐受性。

Key words: *Aquarius paludum paludum*, *Aquarius paludum amamiensis*, juveniles, higher and lower lethal temperatures, Naha (26°N) and Kochi (33°N)

關鍵詞: *Aquarius paludum amamiensis*、*Aquarius paludum paludum*、幼體、致死高溫及致死低溫、那霸及高知。

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Tetsuo Harada*, Shin Ikeda, and Takamasa Ishibashi

Laboratory of Environmental Physiology, Graduate School of Integrated Studies on Humans and Nature, Kochi 780-8520, Japan

ABSTRACT

The effects of geographical location (Kochi-strain [33°N]: *Aquarius paludum paludum*, Naha-strain [26°N]: *A. p. amamiensis*) and/or rearing temperature were examined on the lower and higher lethal temperatures (LLT and HLT) of larvae and also on the super-cooling point (SCP) of the adult water strider. Experiments to determine the lethal temperatures were performed mainly within 24-48 hours after the hatching or molting of the first to 5th (last) instars. The first instars just after hatching were reared at 25°C or 20°C for the Kochi-strain and at 25°C for the Naha-strain. The air temperature inside the incubating chamber was dropped or raised stepwise by 1°C every hour to find the low and high lethal temperatures of the larvae on the water surface, respectively. The higher resistance to both lower and higher temperatures was shown by the first instars of the Naha-strain-25°C group (mean HLT: 45.1°C; LLT: -3.38°C) followed by Kochi-strain-25°C group (HLT: 42.6°C; LLT: -1.40°C). Similar differences in the lower and higher lethal temperatures were also shown by the 3rd instars between the two strains. However, that difference disappeared by the 5th (last) instar stage, probably due to exposure to a constant ambient temperature till the 5th instar stage. On the other hand, in the Kochi-strain the 5th instars grown at 20°C showed both a higher mean HLT (45.34°C) and a lower LLT (-5.30°C) than those grown at 25°C (HLT: 43.71°C, LLT: -3.20°C). The average SCPs in the *A. p. amamiensis* and Hybrid (F15) were significantly lower than those of *A. p. paludum* under long- and short-day photoperiods. Natural selection of heat tolerance in the Naha subtropical strain might also lead to an accompanying higher cold hardiness. The overwintering generation of larvae grow up in relatively low ambient temperature of around 20°C during late October and November in Kochi. This moderate temperature could lead to higher cold hardiness of the last instars just before winter.

Key words: *Aquarius paludum paludum*, *Aquarius paludum amamiensis*, juveniles, higher and lower lethal temperatures, Naha (26°N) and Kochi (33°N)

*Corresponding email: haratets@kochi-u.ac.jp

Introduction

Many studies have been carried out on the tolerance to severe conditions such as extremely low and extremely high temperatures, desiccation, high osmotic pressure and so on. Cross tolerance in which tolerance to one environmental stressor results in increased tolerance to another is thought to be widespread in insects, particularly with regards to low temperature and drought, longevity and starvation tolerance, and high temperatures and desiccation (Ring and Danks, 1994; Harshman and Hoffman, 2000; Ramløv and Lee, 2000; Wu *et al.*, 2000; Bayley *et al.*, 2001; Vermeuleu and Loeschke, 2007). Because of its ease of rearing and artificial selection, *Drosophila* has been a good model for experiments on cross-tolerance (Harshman and Hoffmann, 2000). Selecting a cold hardy strain can lead to higher tolerance to desiccation (Bubliy and Loeschke, 2005), but the reverse is not true (Ring and Danks, 1994; Sinclair *et al.*, 2007). Such discordance could suggest an independence of the profiles in the mechanism for cold tolerance and desiccation tolerance. Then what about the cross tolerance between cold and heat hardiness? There have been few reports on such cross tolerance (Burton *et al.*, 1988; Ohtsu *et al.*, 1998; Rinehart *et al.*, 2007), although a trade-off relationship has been reported in *Drosophila* (Kellelt *et al.*, 2005; MacMillan *et al.*, 2009) and other ectotherms (Stillman, 2003).

Most studies on cold or heat hardiness of insects living in a temperate zone were carried out on terrestrial species (Loeschke and Sørensen, 2005). There have been few studies done on semi-aquatic insects inhabiting the water surface that defined the critical dimension between air and water body these insects live in. The specific heat value of a body of water is much higher than that of land. Therefore the variation in temperatures is relatively less on a water surface than that on a land

surface. However, all of the species in Gerridae except for the sea skaters (Halobatinae) overwinter as adults on land and thus they have to survive lower temperature. At the same time, even larvae could be forced to live on a land surface like terrestrial insects when the body of water they were located on dries out. In temperate zone, both the larvae and adults of Gerridae need to have a high tolerance to both low and high temperatures. However there has been little information regarding the tolerance to either cold or heat by Gerridae.

Insects can be separated into three categories based on physiological status regarding cold hardiness (Bale, 1993). First, freeze-tolerant species possesses a hemolymphatic circulatory system that provides cellular protection against freezing by making ices “outside” the cells; second, freeze-avoiding species try to avoid the ice formation throughout the whole body for example by expressing Anti-Freezing Proteins (AFPs) which depress the freezing point of the hemolymph in more than 50 insect species (Raymond *et al.*, 1973; Douset *et al.*, 2009); and third, there are the chilling-injury species which have a relatively high lethal temperature of around 0°C because of some kind of critical “injury” in the body and their lethal temperature is far higher than the supercooling points (SCPs: temperature at which body fluid of insects begins to freeze under 0°C, SCPs can be decreased due to the increased concentration of soluble substances) (Harada, 2003).

As an index of cold hardiness, SCPs are being used by many insects (Bale, 1987, 1993; Worland, 2005). The presence of ice-nucleating agents for freeze-tolerance species can often promote higher supercooling points (Milonas and Savopoulou-Soultani, 1999). Super cooling points, however, might be estimated as only the lower limits of supercooling capacity and only a theoretical lower lethal temperature for both “freeze-avoiding” and “chilling-

injury” organisms. Therefore, many insects show considerable non-freezing mortality at temperatures well above the SCPs of a “chilling-injury” species (Carrillo *et al.*, 2005; Liu *et al.*, 2007). Liu *et al.* (2009) showed that SCPs change with the process of winter diapauses, decreasing in Dec-Feb and increasing rapidly in Feb-Apr by making trehalose from glycogen. This relationship supports the possibility of SCPs being an indirect indicator of the cold hardiness of insects.

A semi-cosmopolitan water strider, *Aquarius paludum* (Fabricius) occurs in a wide range of palearctic zones (Andersen, 1990). *Aquarius paludum amamiensis* is a subspecies of *A. paludum* and occurs in Amami-Oshima and the Rhykyu Islands (Andersen, 1990; Harada, 1998). The other subspecies is *A. p. paludum* and can be found throughout the remainder of the distribution including the islands of Japan. The heat tolerance of *A. p. amamiensis* living in subtropical Naha during the summer can be hypothesized to be higher than that of the warm temperate Kochi-strain of *A. p. paludum*. However there have been no studies to test this hypothesis.

The critical lower lethal temperature for the Kochi strain of *A. p. paludum* is around -3°C (Harada, 2003). At -3°C the hours of survival under short-days of 12L-12D was 42 hours on average, whereas it was only 14.5 hours under long-days of 16.5L-7.5D. However, the effects of rearing temperature on cold hardiness remain to be studied in this species. Higher resistance to high temperature was shown in subtropical (Naha and Iriomote) populations and also in the northern cool-temperate population (Sapporo) based on a comparative study on *A. p. paludum* inhabiting 11 latitude points of the islands of Japan (Harada, 2008). This result suggests the possibility not of a trade-off relationship but of a cross between cold and heat hardiness in this species. However, no such study was ever carried out on the larvae of this

species which might have a lower resistance to severe temperatures and desiccation because of their thinner and softer skin cuticle compared to the adults.

The aims of the present study were first to examine whether the cross-tolerance between cold and heat hardiness is shown by the larvae at several levels of growing status in the semi-aquatic bug, *Aquarius paludum*. The second aim was to compare cold and heat tolerance between the two different geographical populations, the warm-temperate Kochi (33.3°N: *A. p. paludum*) and the subtropical Naha (26.0°N: *A. p. amamiensis*). The third aim was to investigate whether the ambient and rearing temperatures affect the development of cold and heat hardiness in the larvae. And finally this study aims to examine the super cooling points, as an index of cold hardiness of three strains of *A. p. paludum* and *A. p. amamiensis* and their hybrid to clarify the heredity of a physiological index related to cold hardiness.

Materials and Methods

Materials for lower and higher lethal temperature experiments

About 40 pairs of adults of *Aquarius paludum paludum* were collected from a water way in Nankoku (33°N), an east-neighbor to Kochi in the middle of September 2005. In mid-October 2004, 26 adult females and 20 adult males of *A. paludum amamiensis* were collected from a pond inside a public park in Naha (26°N). Those specimens were kept at 20°C and under 15.5L-8.5D to promote active reproduction (Harada and Numata, 1993).

The first instars of F1 (Nankoku) and F3 (Naha) were used for the rearing experiments to determine the lower and higher lethal temperatures of the first to fifth instars. The larvae were reared at a density of 25 ± 2 first and second instars, or 15 ± 2 third to fifth instars per plastic transparent container (34 × 23.5 × 4.5 cm).

Six or eight replicates of rearing cases (150 or 200 1st instars in total) were prepared for each experimental group.

Materials for the SCP (Super-cooling point) determination experiment

In 1999, more than 30 pairs of *A. p. amamiensis* and 30 pairs of *A. p. paludum* were collected from a water way in Naha and a water way in Kochi (33°N), respectively and they were kept under 15.5L-8.5D at 20 ± 2°C. The next generation adults then were used to determine SCP.

In July of 1994, more than 30 pairs of adults *A. p. paludum* were collected from a water way in Kochi (*A. p. paludum*) and another 30 pairs of adults were collected from a water way in Naha (*A. p. amamiensis*). Then the 15 pairs of female *A. p. paludum* and male *A. p. amamiensis* and another 15 pairs of female *A. p. amamiensis* and male *A. p. paludum* were reared separately in a transparent plastic container (15 cm in diameter and 5 cm high). The hybridized offspring was kept under 15.5L-8.5D at 20 ± 2°C till the generation F14. The adults of the F15-hybrids were used for the SCP determination experiment.

Rearing conditions prior to examining the lower and higher lethal temperature

The first instars of the next generation were reared at 25°C or 20°C under 15.5L-8.5D, and the first to fifth instars (Kochi-strain-25°C group, Kochi-strain-20°C group) were used for the experiments. The Naha-strain was kept under 15.5L-8.5D at 20°C throughout F1 to F2 generation. The first instars of generation F3 were kept under 15.5L-8.5D at 25°C (Naha-strain-25°C group) until the tolerance experiments were performed.

Experiment to determine the higher and lower lethal temperatures

The larvae of the three experimental groups were transferred from the rearing

incubator to the experimental chamber in which the air temperature could be regulated within a range of -25°C to 60°C. The first to fifth instars were used for lethal experiments 24h-96h after molting to ensure their cuticles had hardened. The larvae were placed in the plastic containers (34 × 23.5 × 4.5 cm) containing fresh water 1-2 cm high at the same density as the rearing regime. They were then exposed to a stepwise temperature increase of 1°C every 1 hour.

The lethal experiments started at 25°C for the Kochi-strain-25°C and the Naha-strain-25°C groups, and at 20°C for the Kochi-strain-20°C group, and were performed for all five instars. At the end of 1 hour at each temperature, all specimens were quickly returned to the laboratory where the air temperature was regulated at 20°C, where they were monitored for 15 minutes to check whether they were still alive. If one or more specimens did not move during the 15 minutes, the current temperature inside the incubating machine was judged to be the lethal temperature for that specimen.

Rearing conditions before determining the SCP

For SCP determination experiment, the first instars of the F1 generation (Naha-strain and Kochi-strain) and the F15 generation (Hybrid-strain) were reared at 20°C under 15.5L-8.5D or 12L-12D until the 40th day after adult emergence when their SCP was determined.

Determination of super cooling points (SCP)

Determining the super cooling points was done for the adults of the three strains (*A. p. paludum*, *A. p. amamiensis*, and the hybrid of the two subspecies) on day 0 or 40 days after adult emergence. The surface of each adult was dried with filter paper. Then the thermocouples consisting of nickel and bronze were attached to the ventral surface of the

Table 1. Distribution of upper and lower lethal temperatures shown by larvae of a water strider, *Aquarius paludum* (Kochi strain) and of a subspecies, *Aquarius paludum amamiensis* (Naha strain) [Median]] and Kruskal-Wallis test (df = 2) on lethal temperatures

	Lower lethal temp			Higher lethal temp		
	Median (n)	χ^2 -cal	p-value	Median (n)	χ^2 -cal	p-value
1 st instar stage						
Kochi-strain-25°C-group	-2 (47)	39.5	<0.001	43 (39)	56.7	<0.001
Naha-strain-25°C-group	-4 (40)			44 (46)		
Kochi-strain-20°C-group	-4 (53)			43 (63)		
2 nd instar stage						
Kochi-strain-25°C-group	-4 (42)	1.5	0.480	47 (20)	17.5	<0.001
Naha-strain-25°C-group	-3 (55)			43 (23)		
Kochi-strain-20°C-group	-3 (21)			43 (44)		
3 rd instar stage						
Kochi-strain-25°C-group	-2 (52)	17.6	<0.001	41 (37)	30.7	<0.001
Naha-strain-25°C-group	-4 (43)			45 (59)		
Kochi-strain-20°C-group	-3 (27)			45 (17)		
4 th instar stage						
Kochi-strain-25°C-group	-2 (51)	21.9	<0.001	42 (57)	15.7	<0.001
Naha-strain-25°C-group	-3 (38)			39 (26)		
Kochi-strain-20°C-group	-5 (45)			43 (22)		
5 th instar stage						
Kochi-strain-25°C-group	-2 (49)	65.5	<0.001	43 (51)	13.0	0.001
Naha-strain-25°C-group	-3 (33)			45 (19)		
Kochi-strain-20°C-group	-6 (54)			45 (35)		
Three-way ANOVA						
		df		F		p
Lower lethal temp						
Ambient temp		1		15.41		<0.001
Age		1		59.99		<0.001
Strain		1		12.98		<0.001
Higher lethal temp						
Ambient temp		1		8.22		0.004
Age		1		17.91		<0.001
Strain		1		35.62		<0.001

thorax and were then connected to automatic temperature recorders (M-unit, Model TCS-52-B/E, M-System Co, LTD, Osaka, Japan). The adult specimen attached to the thermocouples was placed into a compressed-styrofoam box (5 × 5 × 3 cm) which was then set inside another larger insulating compressed-styrofoam box to ensure that the cooling rate was about 1°C/min for recording the SCP. The supercooling point was defined as the

temperature at which body fluid begins to freeze under 0°C (Zhao and Kang, 2000).

Statistical Analysis

Data were analyzed using SPSS, version 11.5 (SPSS Inc., Chicago, IL). The Kruskal-Wallis test was used for the analysis of the lower and higher lethal temperatures among the three experimental conditions (Table 1). The Mann-Whitney *U*-test was used for the

Table 2. Result of Mann-Whitney *U*-test between Kochi-strain-25°C-group (Group A) and one of the other two groups, Naha-strain-25°C-group (Group B) and Kochi-strain-20°C-group (Group C) on upper and lower lethal temperatures shown by larvae of a water strider sub-species, *Aquarius paludum paludum* (Kochi strain) and of another subspecies, *Aquarius paludum amamiensis* (Naha strain)

	Lower lethal temperature		Higher lethal temperature	
	<i>z</i>	<i>p</i> -value	<i>z</i>	<i>p</i> -value
1 st instar stage				
Group A vs Group B	-5.570	<0.001	-8.337	<0.001
Group A vs Group C	-5.292	<0.001	-0.684	0.494
2 nd instar stage				
Group A vs Group B	-1.274	0.203	-3.973	<0.001
Group A vs Group C	-0.553	0.582	-3.202	0.001
3 rd instar stage				
Group A vs Group B	-3.675	<0.001	-5.183	<0.001
Group A vs Group C	-2.807	0.005	-3.530	<0.001
4 th instar stage				
Group A vs Group B	-5.167	<0.001	-1.612	0.107
Group A vs Group C	-2.990	0.003	-3.735	<0.001
5 th instar stage				
Group A vs Group B	-1.129	0.259	-1.916	0.055
Group A vs Group C	-6.861	<0.001	-3.464	0.001

pair-wise analysis of the lower and higher lethal temperatures (Table 2) and also the SCPs between the two ages of 0 and 40 imaginal days and two photoperiods of 15.5L-8.5D and 12L-12D. The Kruskal-Wallis test was used for the analysis of the SCPs among the three strains. Three-way ANOVA was used for the integrated analysis of the independent effect of ambient temperature, age (instars) and strain on the lower and higher lethal temperatures.

Results

Differences between the Kochi and Naha strains

The first instars of the Naha-strain-25°C group were significantly more resistant to both lower and higher temperatures than those of the Kochi-strain-25°C (Table 2) (Fig. 1). Although these differences disappeared with the second instars (Fig. 2), both the higher and lower lethal temperatures of the 3rd

instars in the Naha-strain-25°C group were once again significantly higher and lower, respectively than those in the Kochi-strain-25°C group (Table 2) (Fig. 3). In the fourth instar stage, a significant difference in the lower lethal temperature remained, but the differences in higher lethal temperature disappeared (Table 2) (Fig. 4). In the 5th instar stage, there were no significant differences in either lethal temperature (Table 2) (Fig. 5).

Effect of growing ambient temperature on the development of cross tolerance

The first instars of the Kochi-strain-20°C-group were more resistant than the Kochi-strain-25°C group, but only to the lower temperatures (Table 2) (Fig. 1). There were no significant differences in both the lower and higher lethal temperatures between the Kochi-strain-20°C-group and the Kochi-strain 25°C-group for the second instar stage (Table 2) (Fig. 2). The 3rd to 5th instars of the Kochi-strain-20°C-group again showed

Table 3. Detailed mean and SD in each experimental group due to strain, photoperiod and stage in a semi-aquatic bug, *Aquarius paludum* and following detailed statistical analysis on the effect of each of the three factors on SCPs

	<i>A. p. paludum</i>		<i>A. p. amamiensis</i>		Hybrid	
	0 day	40 days	0 day	40 days	0 day	40 days
Photoperiod						
(a) 16.5L-7.5D	-6.1 ± 1.2 (13)	-7.5 ± 1.4 (12)	-10.4 ± 1.8 (30)	-11.7 ± 5.3 (29)	-9.8 ± 3.4 (25)	-13.4 ± 3.1 (27)
(b) 12L-12D	-7.8 ± 2.6 (15)	-8.3 ± 1.8 (14)	-10.5 ± 2.5 (32)	-13.3 ± 2.4 (30)	-11.6 ± 2.3 (28)	-15.2 ± 3.0 (27)
Exp. Group No.	Group 1 (a, b)	Group 2 (a, b)	Group 3 (a, b)	Group 4 (a, b)	Group 5 (a, b)	Group 6 (a, b)
Kruskal-Wallis test on effect of strain	Group 1 vs 3 vs 5 (a)		Group 1 vs 3 vs 5 (b)		Group 2 vs 4 vs 6 (a)	
χ^2 -value (df)	25.4 (2)		15.8 (2)		24.9 (2)	
<i>p</i> -value	<0.001		<0.001		<0.001	
Mann-Whitney <i>U</i> -test on effect of photoperiod	Group1(a)vs(b)	Group2(a)vs(b)	Group3(a)vs(b)	Group4(a)vs(b)	Group5(a)vs(b)	Group6(a)vs(b)
<i>z</i> -value	-1.98	-1.26	-0.162	-1.32	-2.29	-1.98
<i>p</i> -value	0.047	0.207	0.871	0.187	0.022	0.047

significantly higher and lower lethal temperatures compared to those of the Kochi-strain-25°C-group (Table 2) (Figs 3-5).

Heredity of photoperiodic responses of super cooling points (SCPs)

The Kochi strain of *A. p. paludum* showed a SCP of -7 - -8°C on average for both stages of 0 and 40 days regardless of photoperiod (Table 3) (Fig. 6). The Naha strain of *A. p. amamiensis* and the hybrid between *A. p. paludum* and *A. p. amamiensis* showed significantly lower SCPs of -10 - -14 °C on average compared to the Kochi strain, regardless of stage and photoperiod (Three-Way ANOVA: the effect of strains, df = 1, mean square value = 47188.6, *F*-value = 54.3, *p* < 0.001). A significantly lower SCP was shown under the short-days than under the long-days especially for the hybrid (Three-Way ANOVA: the effect of photoperiod, df = 1, mean square value = 10579.3, *F*-value = 11.5, *p* = 0.001). The 40th day after emergence showed a lower SCP (Three-Way ANOVA: the effect of stage, df = 1, mean square value = 40445.8, *F*-value =

44.1, *p* < 0.001) than that on day 0, regardless of strain and photoperiod.

Discussion

This study seems to show an example of a very rare case of cross-tolerance with regards to “cold and heat stress” (Figs. 1-5) among insects: (1) heat tolerance which may have been selected because of the hot weather in a sub-tropical habitat leads to increased tolerance for the cold stressor; (2) the development of cold tolerance which might have been induced by being reared at the relatively low temperature of 20°C, and resulting in the development of tolerance to the heat stressor (Fig. 5, Table 2). A common physiological mechanism for heat and cold hardiness might be possible in this species.

The Naha-strain was more resistant to both lower and higher temperatures than the Kochi-strain in the relatively early stages of first and third instars. During the 4th and 5th instar stages, however, the Naha-strain was no longer more resistant than the Kochi-strain under the common rearing temperature of

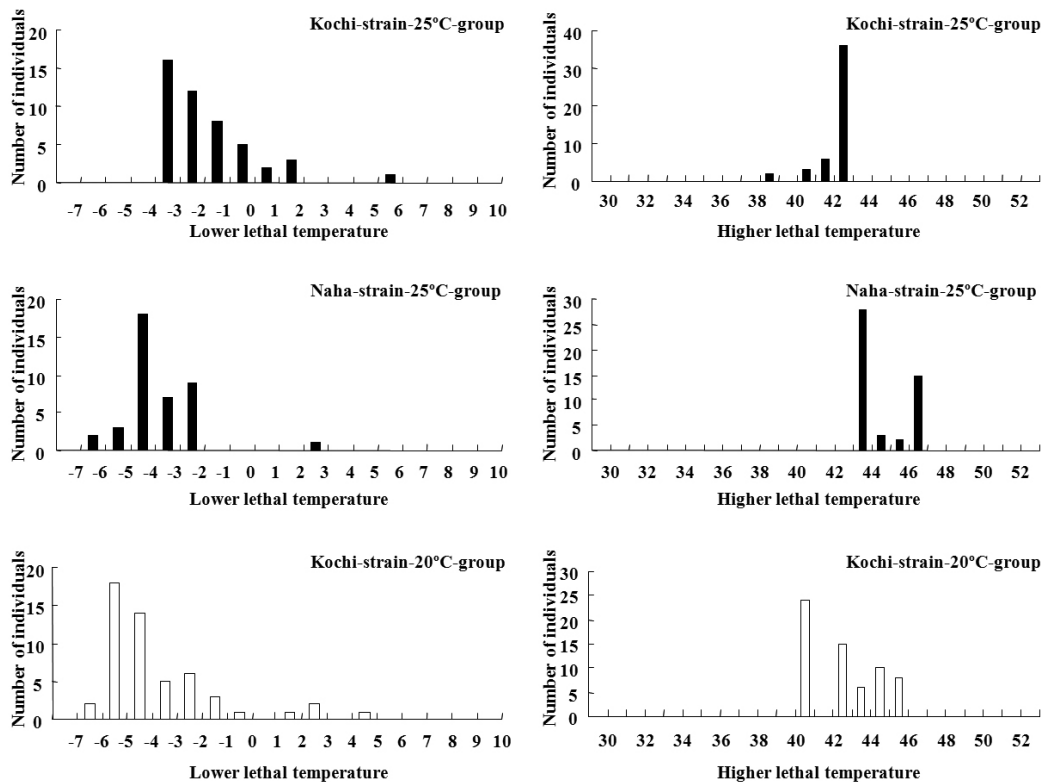


Fig. 1. Distribution of the upper and lower lethal temperatures shown by the first instar larvae of two subspecies of water strider, *Aquarius paludum paludum* (Kochi strain) and *Aquarius paludum amamiensis* (Naha strain).

25°C. Experiencing a constant ambient temperature (25°C) throughout all larval stages might diminish such genetic differences in the tolerance to cold and heat stress.

A relatively low temperature of 20°C seems to promote resistance to both lower and higher temperatures in the larvae of *Aquarius paludum paludum*. Resistance to higher temperatures seems to be selected due to the sub-tropical climate in the case of *A. p. amamiensis*. High resistance to lower temperature might be “cross-selected” accompanied with the natural selection of heat hardiness, due to some physiological linkage in mechanisms regulating cold and heat stress.

When the larvae of *A. paludum paludum* were raised at 20°C, it strengthened their cold and heat

hardiness (Table 2) (Figs. 1-5). Exposure to a low temperature near the lethal value promotes rapid cold hardening (RCH) in many insect species (Meats, 1973; Lee *et al.*, 1987; Coulson and Bale, 1990; Czajka and Lee, 1990; Denlinger and Lee, 1998; Kelty and Lee, 1999). Even the relatively moderate temperature of 20°C might possibly have promoted RCH in the first instars of *A. paludum paludum*.

On the other hand, during the entire larval period, a relatively slow development of cold and heat hardenings might have occurred when the larvae were kept at 20°C which is equivalent to the spring (May) and fall (Oct) seasons in Kochi (33°N) (Kochi Metrological Station Report, 2005). The lower and higher lethal temperatures changed from -3.30°C at the first instar stage to -5.30°C at the last

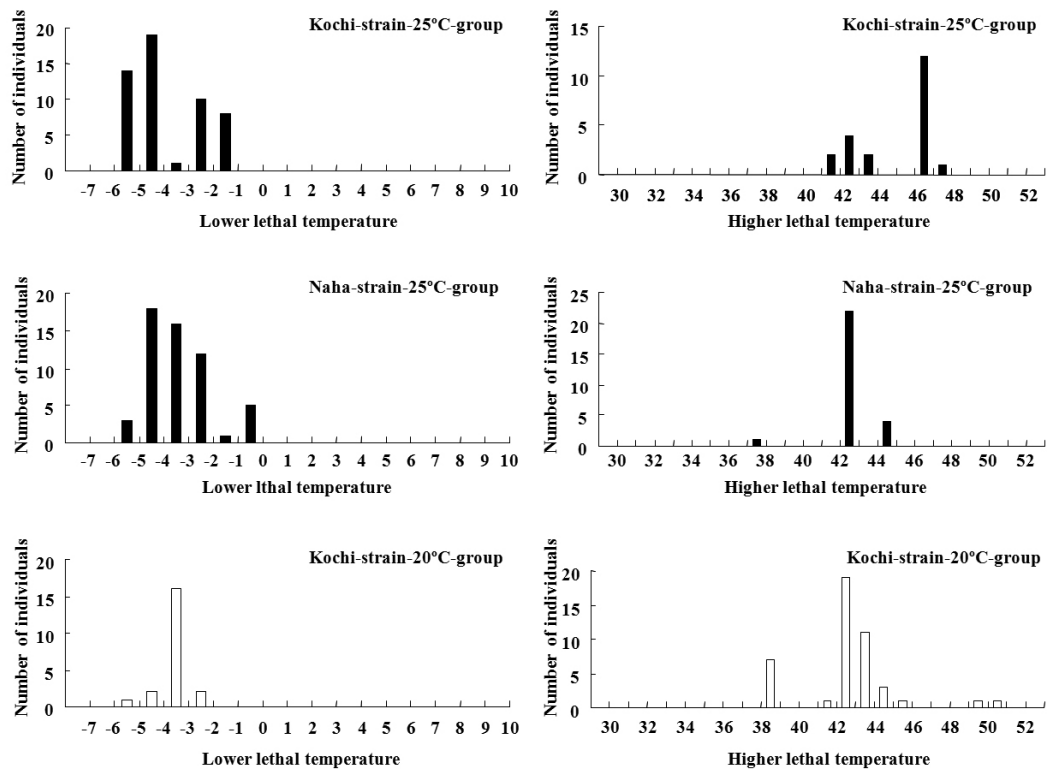


Fig. 2. Distribution of the upper and lower lethal temperatures shown by the second instar larvae of two subspecies of water strider, *Aquarius paludum paludum* (Kochi strain) and *Aquarius paludum amamiensis* (Naha strain).

instar stage, and on average from 43.03°C to 45.34°C, respectively, in *A. p. paludum* (Mann-Whitney *U*-test, higher temp.: $z = -5.043$, $p < 0.001$; lower temp.: $z = -6.234$, $p < 0.001$) (Figs. 1, 5). This cross-tolerance development as a result of the larvae being exposed to moderately low ambient temperatures might be preparative adaptation for the high and low temperatures coming in summer and winter, respectively after adult emergence in Kochi, although such hypothesis remains to be studied in the future.

As a genetic contrast, the first and third instars of the Naha-strain-25°C of *A. paludum amamiensis* were more resistant than those of the Kochi-strain-25°C of *A. paludum paludum* to both lower and higher temperatures. Adults of the Iriomote-strain of *A. paludum amamiensis*

were also more resistant to the low temperatures than the adults of the Kochi-strain of *A. paludum paludum* (Harada, 2003). Subtropical *A. paludum amamiensis* might keep a higher genetic potential of cross tolerance of cold and heat hardness than the warm-temperate *A. paludum paludum*. However, there have been no data presented for cross tolerance in the adult stage of this species, and is the subject to future study.

The super cooling point (SCP) of an invertebrate is the temperature at which spontaneous freezing begins to occur in the hemolymph with a high concentration of solubles (Salt, 1961; Sømme, 1982; Vannier, 1994; Salin *et al.*, 1998). The super cooling point can be a good indicator of cold tolerance, because there is a clear negative correlation between the survival

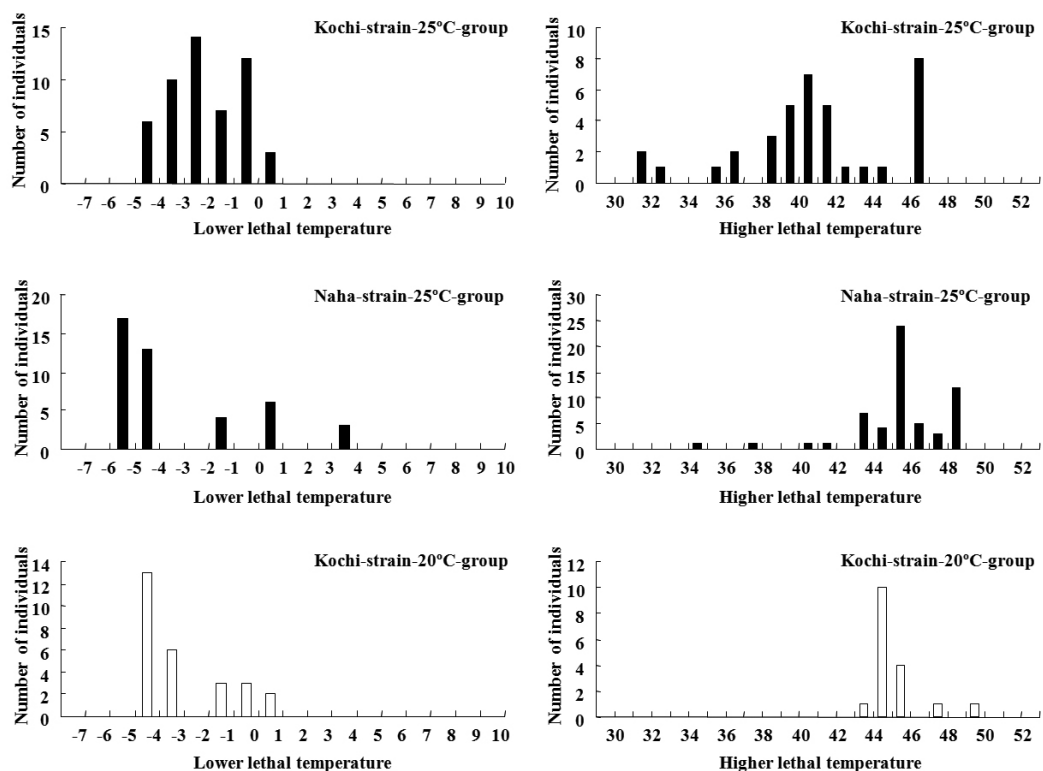


Fig. 3. Distribution of the upper and lower lethal temperatures shown by the third instar larvae of two subspecies of water strider.

rate at a critical low lethal temperature and the SCP for “freeze-avoidance” species such as the lepidopteran species, *Dendrolimus tabulaeformis* (Zeng *et al.*, 2008).

However, in the case of the temperate water strider, *A. paludum paludum* and the subtropical *A. paludum amamiensis*, however, the SCP is far lower than the lethal temperature point (about -1.5 - -5.3°C: Harada, 2003; Table 1). Such species might be identified as “chilling-injury” species.

In the case of sub-tropical *A. paludum amamiensis*, the lower SCP could be regulated by a “dominant” gene (Fig. 6) and might involve a mechanism of cold hardiness promotion. The lower SCPs and the higher tolerances to both high and low temperatures shown by *A. paludum amamiensis* were also due to short-days,

and might be an example of cross tolerance. Such cross tolerance might be selected due to the hot weather in a sub-tropical habitat. The tolerance to high temperature could be accompanied by a high tolerance to the cold stressor, although to date there have been no data on whether there is a positive correlation between the lower lethal temperature and the SCP in *A. paludum amamiensis*.

Lower feeding activity leading to a lower SCP and a slower development are promoted by short-days in the larvae of the clover leaf weevil, *Hypera punctata* which is distributed in the temperate zone (Watanabe, 2000). This means that short-days promote both slower development (in other words, larval diapause) and cold hardiness enhanced. Therefore, photoperiod could possibly regulate the cold hardiness

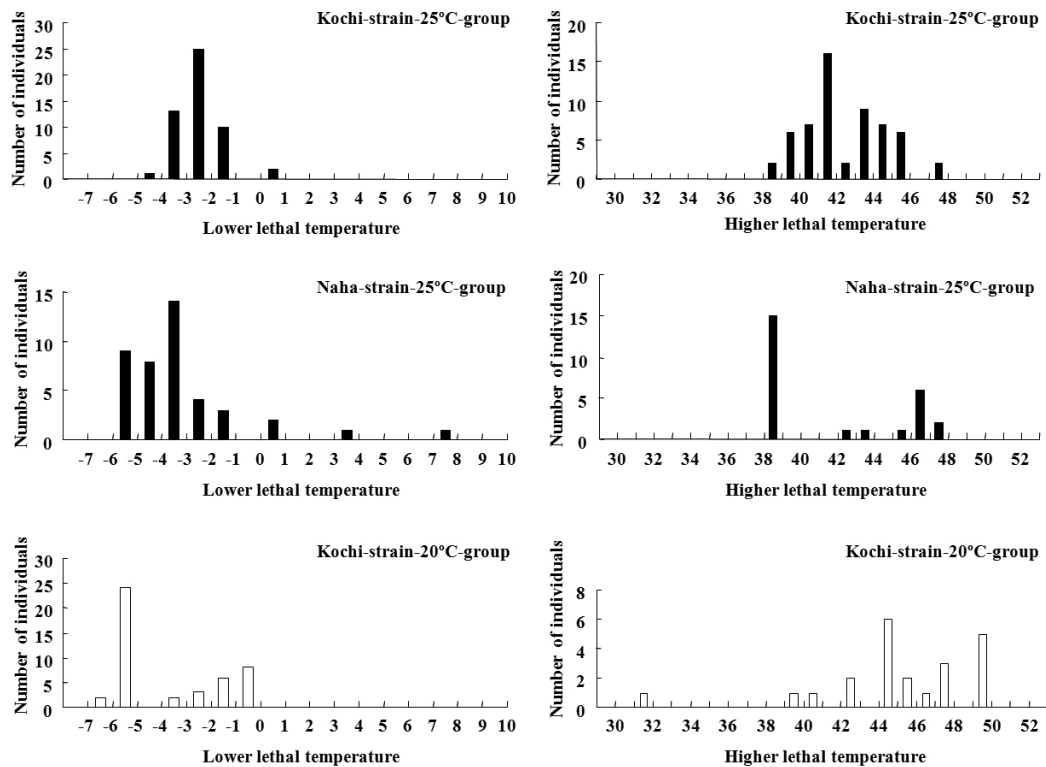


Fig. 4. Distribution of the upper and lower lethal temperatures shown by the fourth instar larvae of two subspecies of water strider.

as a part of the diapause syndrome (a set of characteristics to survive severe environmental conditions: reproductive diapauses, hardiness to low temperature and drought, negative phototaxis, low metabolic rate, high storage of energy and nutritional resources in fat bodies and so on: Hodkova & Hodek, 1997; Harada, 2003) thereby leading to the survival over winter for the temperate *A. paludum paludum* (Harada, 2003) (Fig. 6; Table 3).

The expression of *Hsp* (heat shock protein) 70 which is promoted by the exposure to high-temperature-shock leads to the extreme cold-hardening of *Drosophila melanogaster* (Burton *et al.*, 1988). When the expression of *Hsp* 23 or *Hsp* 70 is suppressed, the extent of cold hardiness becomes extremely low in the flesh fly, *Sarcophaga crassipalpis* (Rinehart

et al., 2007). It can also be hypothesized that in *Aquarius paludum* such cross tolerance between cold and heat hardiness shares a common mechanism via *Hsp* which might be a dominant character. However, to date there is no data on the incorporation of *Hsp* to such cross tolerance in *A. paludum*, and this issue remains to be studied in the future.

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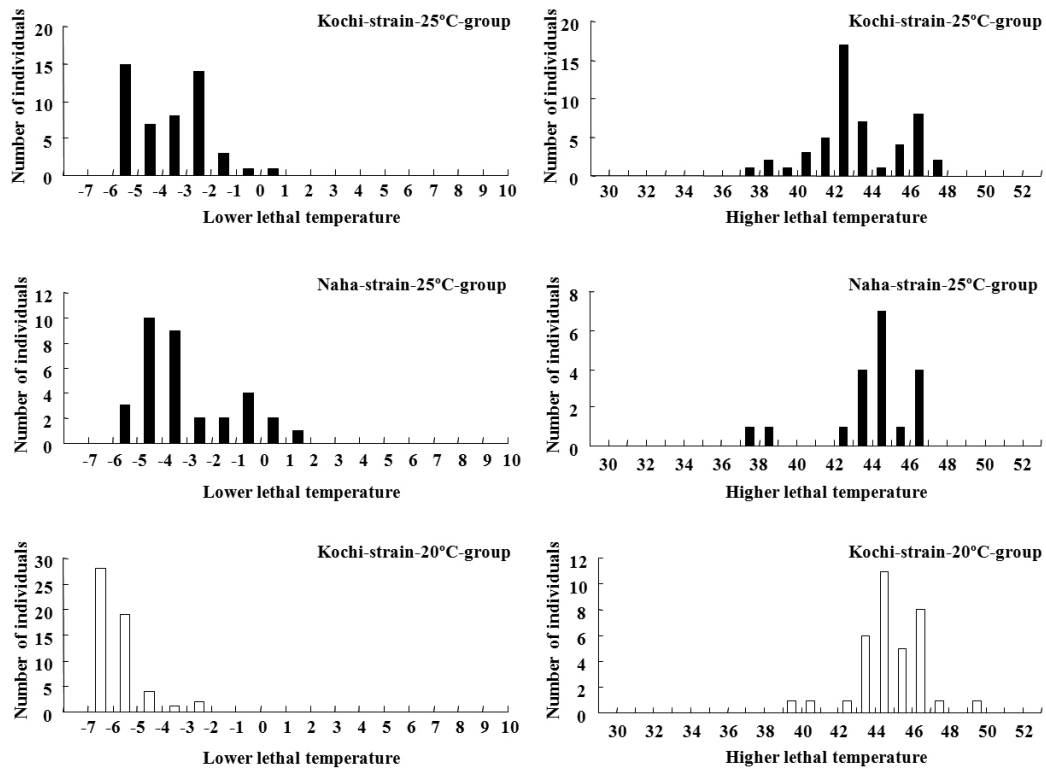


Fig. 5. Distribution of the upper and lower lethal temperatures shown by the fifth instar larvae of two subspecies of water strider.

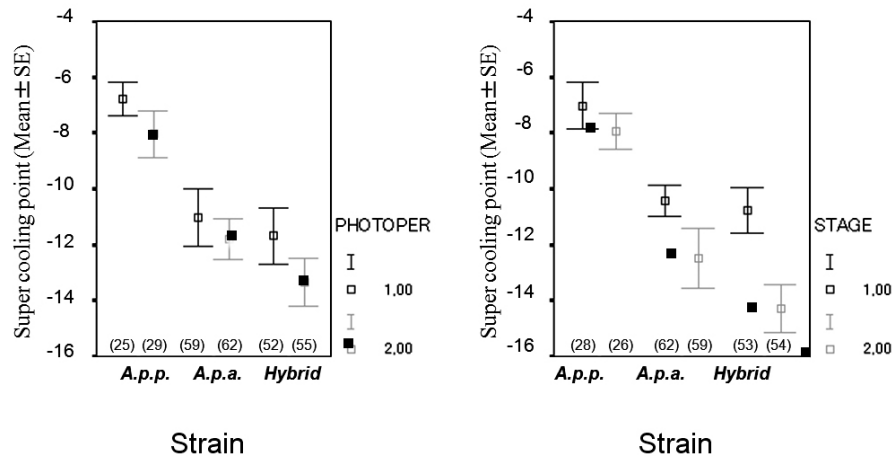


Fig. 6. The effects of the photoperiod (left panel) and age (right panel) on the SCP in three strains of water strider, *Aquarius paludum*. *A. p. p.*: Kochi (33°N) strain of *Aquarius paludum paludum*; *A. p. a.*: Naha (26°N) strain in *Aquarius paludum amamiensis*; Hyb: F15 of hybrid of the Kochi and the Naha strains. "PHOTOPE 1.00" and "PHOTOPE 2.00" mean long-day photoperiod of 16.5h light-7.5h dark and short-day photoperiod of 12h light-12h dark in the larval and adult stages before measuring the SCP, respectively. The numbers in parentheses represent the sample size.

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溫帶水黽 *Aquarius paludum paludum* 及亞熱帶水黽 *Aquarius paludum amamiensis* 對於熱壓力及冷壓力的交互 耐受性

Tetsuo Harada*, Shin Ikeda, and Takamasa Ishibashi

Laboratory of Environmental Physiology, Graduate School of Integrated Studies on Humans and Nature, Kochi 780-8520, Japan

摘 要

本試驗檢視不同地理品系水黽（高知品系，33°N，*Aquarius paludum paludum*；那霸品系，26°N，*Aquarius paludum amamiensis*）及飼育溫度對稚蟲對致死低溫（lower lethal temperatures, LLT）與致死高溫（higher lethal temperatures, HLT），及對成蟲過冷卻點（super-cooling point, SCP）的效應。致死溫度試驗乃利用孵化或脫皮後 24~48 小時內之一至五齡稚蟲進行試驗。高知品系孵化之一齡稚蟲飼養於 20°C 及 25°C 之生長箱，那霸品系飼養於 25°C 之生長箱；生長箱內之氣溫以每小時降低/提高 1°C 的方式，以檢驗水面幼蟲之致死低溫或致死高溫。結果顯示那霸品系之一齡稚蟲對高低溫具有較高之耐受性（HLT: 45.1°C；LLT: -3.38°C），其次為飼養於 25°C 之高知品系稚蟲（HLT: 42.6°C；LLT: -1.40°C）。兩品系之三齡稚蟲亦有類似結果。然而這些差異在第五齡稚蟲之前消失，可能由於持續暴露在恆定的周遭環境所致。另一方面，飼養於 20°C 之高知品系五齡稚蟲較飼養於 25°C 之稚蟲呈現較高之致死高溫（HLT: 45.34 vs. 43.71°C）及較低的致死低溫（LLT: -5.30 vs. -3.20°C）。在長/短光週期條件下，*Aquarius paludum amamiensis* 及其雜交品系（F15）之過冷卻點均低於 *Aquarius paludum paludum*。亞熱帶那霸品系水黽對高溫耐受性的天擇可能伴隨產生對低溫的耐受性。在高知地區，從 10 月下旬至 11 月，越冬稚蟲在氣溫約 20°C 下成長，此溫度可能使得最後一齡稚蟲在冬天來齡之前獲得較高之冷耐受性。

關鍵詞：*Aquarius paludum amamiensis*、*Aquarius paludum paludum*、幼體、致死高溫及致死低溫、那霸及高知。