

TOO HOT TO HANDLE? RANGE EXPANSION AND LIFE-HISTORY RESPONSES TO CLIMATE WARMING OF THE SOUTHERN GREEN STINK BUG *NEZARA VIRIDULA* (HETEROPTERA: PENTATOMIDAE)

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ABSTRACT.

We compared the past and the current limits of the distribution range of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae), in central Japan. In the early 1960s, the northern limit of the range was in Wakayama Prefecture and was limited by a +5 °C isothermal line for the mean January temperature. In 2006–2007, a new survey demonstrated that this northern limit had shifted northward by 85 km, at a mean rate of 19.0 km/decade. The shift was likely promoted by milder thermal conditions in winter.

The effect of simulated climate change on *N. viridula* was further studied close to the species' northern range limit. Insects from the same egg masses were reared for 15 months in 10 consecutive series under quasi-natural (i.e. outdoor) conditions and in a transparent incubator, in which climate warming was simulated by adding 2.5 °C to the outdoor temperature. The warming strongly affected all life-history and phenological parameters. In the spring, simulated warming advanced the timing of body colour changes and post-diapause reproduction. In the early summer, it increased egg production and accelerated nymphal development. In the late summer (the hottest season), the effect of the simulated warming was strongly deleterious: nymphs developed slowly, suffered higher mortality and had difficulties during final moulting; the emerged females were smaller, some exhibited abnormal cuticle, produced fewer eggs and had a decreased life span. In the autumn, the warming accelerated nymphal development, resulted in larger female size, affected the timing of the diapause-associated adult body colour change from green to russet and enhanced preparation for overwintering. Larger females had a higher winter survival rate than smaller females. The warming strongly increased survival rate in both size classes and allowed smaller females to reach the same winter survival rate as larger females had under the quasi-natural conditions. The winter survival also differed between the green and dark-coloured females under the quasi-natural, but not under the warming conditions. However, under the warming conditions, green females survived the winter even better than dark-coloured females did under the quasi-natural conditions. Moreover, the warming shortened the life span of females from the summer generations and prolonged it in those from the autumn generation. It is concluded that even a moderate temperature increase (+2.5 °C) in the future is likely to have a complex influence upon insects, strongly affecting many of their life-history and phenological parameters.

INTRODUCTION.

Rapid climate change implies simultaneous and complex changes of many environmental variables – primarily air temperature (IPCC 2007). There is now considerable attention directed to how the global biota are and will be responding to global warming (Bale *et al.* 2002; Deutsch *et al.* 2008; IPCC 2007; Parmesan 2006).

The southern green stink bug, *Nezara viridula*, is apparently responding rapidly to climate warming (Musolin 2007; Musolin *et al.* 2009; Tougou *et al.* 2009; Yukawa *et al.* 2007). The species occurs in an ever-widening range throughout tropical and subtropical regions and Japan is at the northern margin of *N. viridula*'s Asian range. In the north, *N. viridula* is replaced by a congeneric species, the oriental green stink bug, *Nezara antennata* Scott. Both species, and especially *N. viridula*, are major agricultural pests.

In the early 1960s, Kiritani and co-workers conducted a wide-scale field survey and mapped the northern limit of the range of *N. viridula* in central Japan (Fig. 1a; Kiritani *et al.* 1963). They showed that the northern limit of *N. viridula*'s distribution in central Japan occurred in Wakayama Prefecture (latitude approximately 34.1°N). *Nezara antennata* dominated in northern and central parts of the prefecture, whereas coastal and the southern parts of the prefecture were mostly or completely occupied by *N. viridula* (Fig. 1a). In general, *N. viridula* was found to occur sympatrically in warmer parts of the distribution range of *N. antennata*. The area of co-existence of two species was shown to lay on the +5 °C isothermal line for mean temperature of the coldest month (usually January) and, thus, January temperature was proposed as the principal factor determining the northern limit of *N. viridula*'s distribution (Kiritani *et al.* 1963).

In our attempt to understand and predict how insects will respond to further warming, we: (1) compared the past and the current limits of the distribution range of *N. viridula* in central Japan; and (2) studied experimentally the effect of simulated global warming on life-history traits and phenology of *N. viridula* by exposing two groups of insects for a 15 month period to outdoor conditions and conditions simulating a moderate temperature increase (2.5 °C), which is within the likely range of temperature increase (1.1–6.4 °C) predicted for the end of the XXI century (IPCC 2007).

MATERIALS AND METHODS.

Range Change Survey.

To determine the current northern limit of *N. viridula*, an extensive field survey was conducted in six prefectures in central Japan in 2006–2007 (Tougou *et al.* 2009).

Phenology and Life-history Responses to Simulated Climate Change.

Adults of *N. viridula* were collected in Kochi (33.6°N, 133.6°E) and transferred to Kyoto (35.0°N, 135.8°E), where the experiment was conducted. On ten dates in 2006–2007 (i.e. 1 June, 1 July, etc.), egg masses were transferred to plastic containers and placed under two experimental conditions: *quasi-natural* (i.e. *outdoor*) and *simulated warming conditions*. Under the quasi-natural conditions, insects were

reared in plastic containers on metal shelves open from all sides. Under the simulated warming conditions, insects were reared similarly in a specially modified incubator LH-350NSZ (NK Systems, Osaka) placed next to the open shelves. The incubator had transparent glass walls to ensure the insects experienced the same photoperiodic conditions that their outside siblings did. A sensor measured outdoor temperature on the shelves of the quasi-natural conditions and the incubator constantly simulated warming conditions inside by adding 2.5 °C to the outside temperature. In each series and each treatment, all important events such as hatching, moulting, copulation, oviposition, adult colour change, and death were recorded daily. Only data on females are presented. Life-history and phenological parameters were compared between the two conditions and all series to examine whether and how simulated warming affected performance and fitness of *N. viridula* (Musolin *et al.* 2009).

RESULTS.

Range Change Survey.

The survey of 2006–2007 demonstrated that the northern limit of *N. viridula*'s distribution range had shifted northward by 85 km from the early 1960s (Fig. 1).

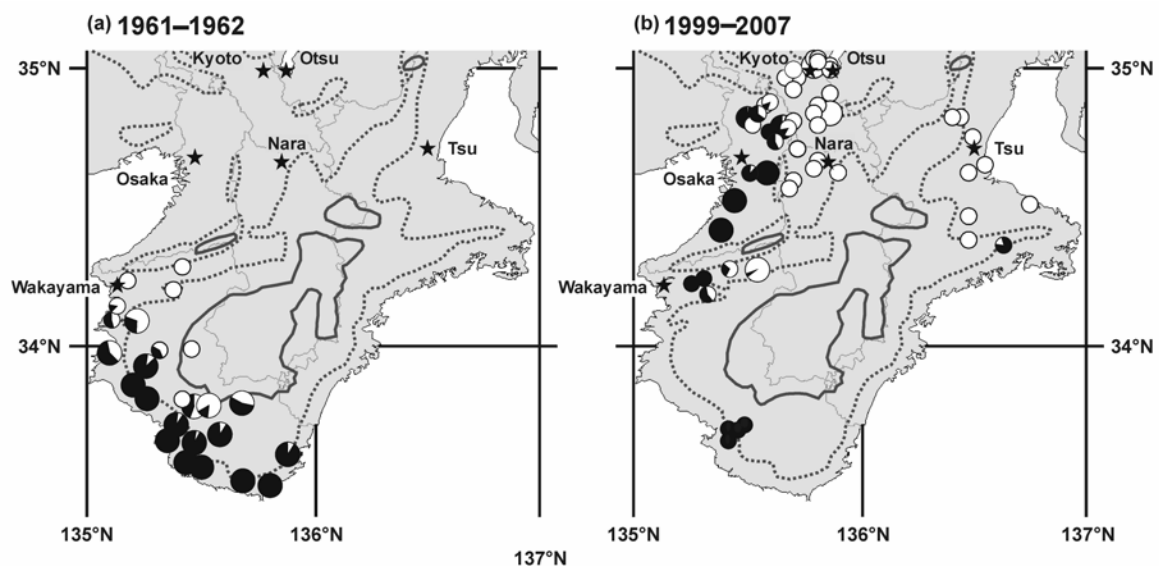


Fig. 1. Distribution and relative abundance of *Nezara* spp. in central Japan: (a) in the early 1960s (Kiritani *et al.* 1963); and (b) in 2007 (Tougou *et al.* 2009). Symbols: black sections, *N. viridula*; white sections, *N. antennata*. Sample size: small circles, 1–50 specimens; large circles, more than 50 specimens. Elevation: dotted line, 500 m above sea level; solid black line, 1,000 m above sea level.

PHENOLOGY AND LIFE-HISTORY RESPONSES TO SIMULATED CLIMATE CHANGE.

Nymphal development. The duration of the nymphal period showed a predictable seasonal trend under the quasi-natural conditions: higher outdoor temperatures in August resulted in a shorter nymphal period in the 1 August series compared to the 1 June and 1 July series and as the season progressed the nymphal period became significantly longer (Fig. 2). The effect of the artificial warming was unexpected. While warming significantly accelerated nymphal development in the 1 June, 1 and 15 September series, it retarded development to varying levels in the 1 July and 1 August series. Thus, while under the quasi-natural conditions the nymphal development tended to be faster in the 1 August series than in the June and July series, under warming conditions it tended to be slower.

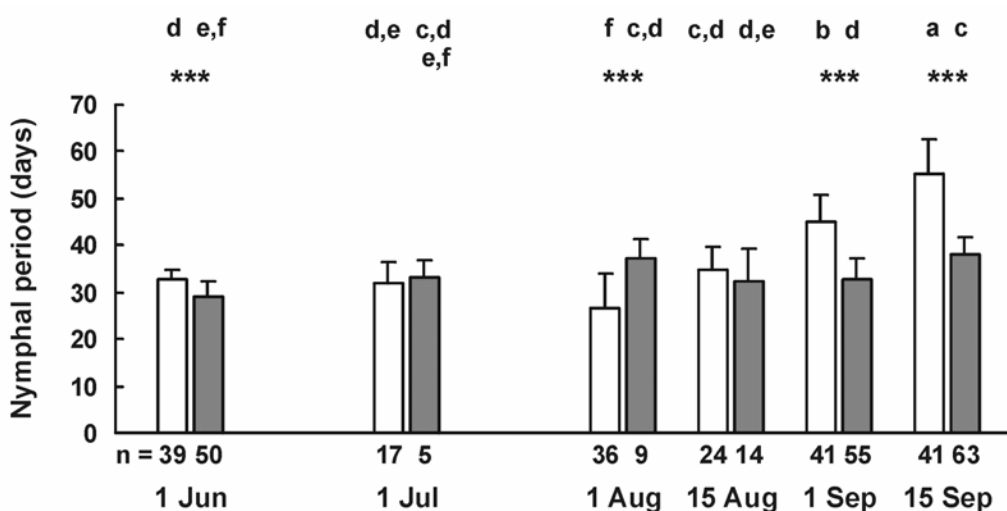


Fig. 2. Mean duration (+SD) of the nymphal period in females of *Nezara viridula*. Bar colour: white, the quasi-natural conditions; grey, the simulated warming conditions. Stars: pair-wise comparison between the two treatments within the same series, *t*-test; ***, $P < 0.001$. Letters above bars: multiple comparison (all series and treatments together), Tukey–Kramer HSD test; means with the same letters are not significantly different ($P > 0.05$) (from Musolin *et al.* 2009).

When the rate of nymphal development was calculated, averaged for the series and plotted against the mean temperature experienced during the nymphal period, most data points fit well to a linear regression line, although three data points corresponding to the warmest season (the 1 July, 1 and 15 August series) were below and far from the line (Fig. 3).

The simulated warming also affected survival of nymphs. In summer it led to increased nymphal mortality, some adults in these series had apparent difficulties while moulting to the adult stage and many of them died during moulting.

Size and physical condition of adults. The body length of females tended to be smaller in the 1 and 15 August series than in most of other series (Fig. 4). The simulated warming strongly enhanced this tendency. When compared pairwise with

the quasi-natural conditions on the same dates, warming significantly reduced the size of adults in August, but increased it in September.

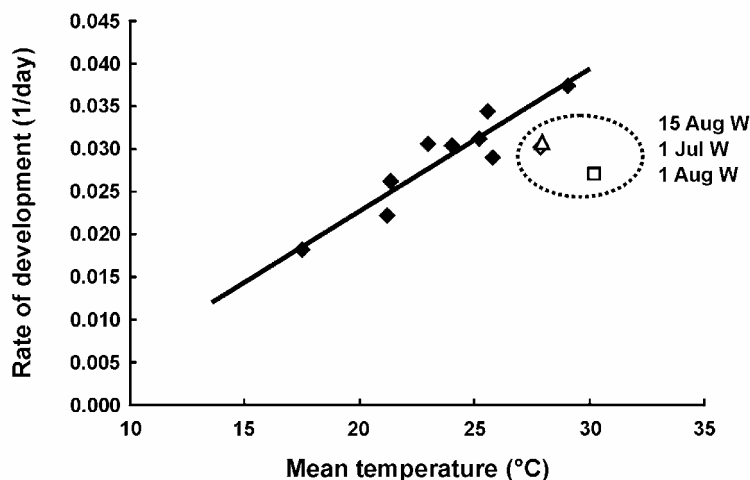


Fig. 3. Effect of temperature on the rates of nymphal development in females of *Nezara viridula*. The linear regression line ($E_{(1,8)} = 47.937$, $P = 0.0002$; ANOVA) is drawn for all series and treatments except three encircled mid-summer series under the simulated warming conditions (from Musolin *et al.* 2009).

In addition to having a smaller size in the warmest season, some adults were apparently in a weaker physical condition under the warming conditions: they had a softer cuticle and a lighter yellowish body colour instead of intensive green.

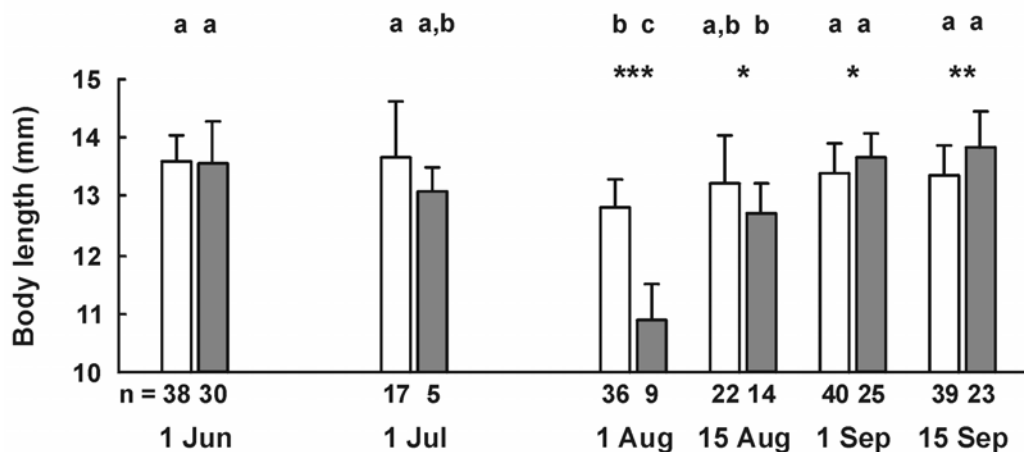


Fig. 4. Mean (+SD) body length of *Nezara viridula* females. Bar colour: white, the quasi-natural conditions; grey, the simulated warming conditions. Stars: pair-wise comparison between the two treatments within the same series, *t*-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Letters above bars: multiple comparison, Tukey–Kramer HSD test; means with the same letters are not significantly different ($P > 0.05$) (from Musolin *et al.* 2009).

Adult size and winter survival. Under both conditions, the series with larger mean body length tended to have a higher winter survival than those with smaller means (Fig. 5a), although, this trend was not statistically significant likely because of the very low survival rate in some series under the quasi-natural conditions. However, when analysed on an individual basis, both the female size and the simulated warming strongly affected the success of overwintering. Larger females had significantly higher winter survival rates under each of the two experimental conditions ($P \leq 0.008$; Fig. 5b). Moreover, females from each size group had higher winter survival rates under the simulated warming conditions than under the quasi-natural conditions ($P \leq 0.06$; Fig. 5b). Finally, the simulated warming allowed the smaller females to reach the same winter survival rate as the larger females had under the quasi-natural conditions.

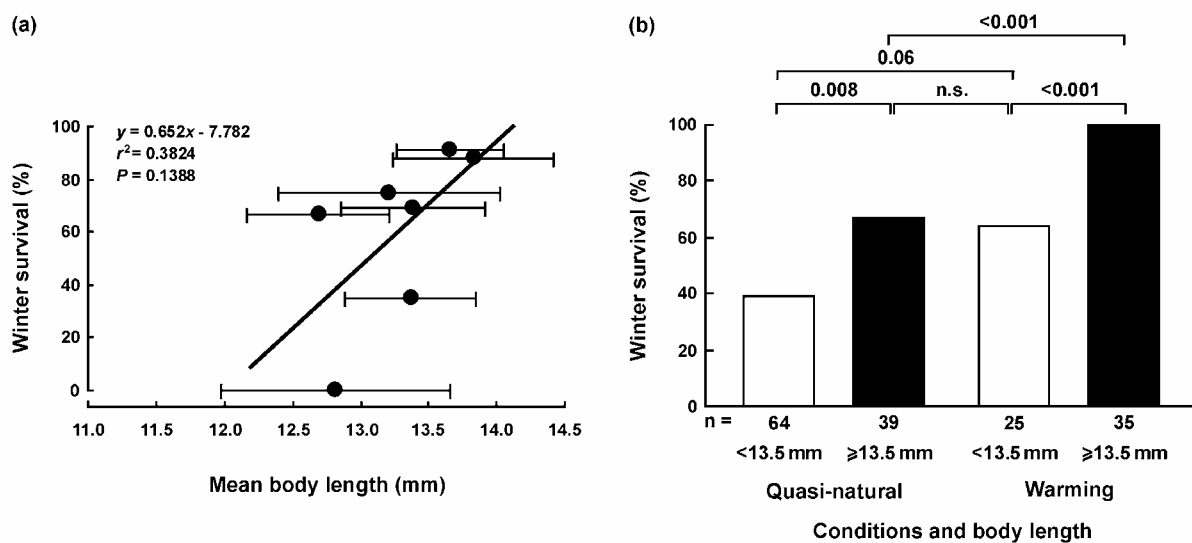


Fig. 5. Effect of body size on winter survival rate of *Nezara viridula* females: (a) The relationship between winter survival and mean (\pm SD) body length. All series and treatments in which females survived until at least 1 December are shown, the linear regression line and statistics after arcsine transformation are shown; (b) Winter survival rate in different body length groups of non-reproductive females under different treatments (all series are combined; P of Fisher exact test after Bonferroni adjustment is shown) (from Musolin *et al.* 2009).

Adult body colour and winter survival. Induction of winter diapause in *N. viridula* is associated with adult body colour change from green to russet. The experiment showed that winter survival of females was strongly correlated with their body colour. When winter survival was analysed separately for each colour grade in the non-reproductive females, it differed significantly between green and russet females, but not between each of those colour grades and the intermediate colour grade (Fig. 6a). The simulated warming strongly influenced the relationship between body colour and winter survival. Under the quasi-natural conditions the dark-coloured females had a significantly higher survival rate than the green females ($P < 0.001$), whereas under the warming conditions the survival rates were higher in both colour grade groups

and did not differ significantly (Fig. 6b). The comparison of winter survival within each colour grade but between the conditions showed that the green females had a significantly higher survival rate under the simulated warming conditions ($P = 0.01$). Survival of the dark-coloured females was also higher under the warming conditions, although insignificantly (Fig. 6b).

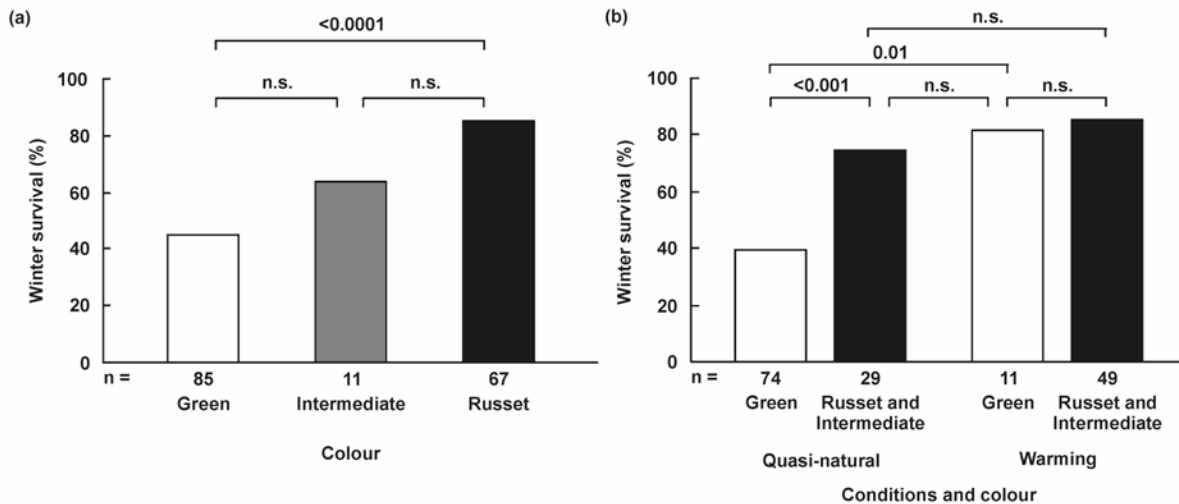


Fig. 6. Effect of colouration on winter survival of *Nezara viridula* females. Winter survival in: (a) different colour groups of non-reproductive females (all series and treatments combined; P of χ^2 test is shown); and (b) different colour groups of non-reproductive females and under different treatments (all series are combined; P of Fisher exact test is shown) (from Musolin *et al.* 2009).

Timing of reproduction. Before overwintering, females tended to start both copulation and oviposition earlier under the simulated warming than under quasi-natural conditions, although the difference was not significant likely due to the small number of reproducing females under the warming conditions. In the hottest mid-summer season, the start of reproduction tended to be delayed. After overwintering, the trend to advance reproduction was more pronounced: in all the series, copulation and oviposition started earlier under the simulated warming conditions than under quasi-natural conditions and in most cases the difference was statistically significant.

Fecundity. In the early summer, the simulated warming significantly increased egg production, whereas later in the season fecundity tended to be lower under the simulated warming than under the quasi-natural conditions, although the difference was not significant. After overwintering, no common patterns were detected and only in the 15 September series did the warming significantly increase post-diapause egg production.

Longevity. The simulated warming significantly reduced longevity of females in the summer series ($P < 0.001$). In the autumn series, most females survived until the next spring and early summer and the simulated warming increased their longevity likely by optimizing thermal overwintering conditions.

DISCUSSION.

Range Change.

The survey demonstrated that the northern limit of *N. viridula*'s distribution had shifted northward by 85 km from the early 1960s to 2006–2007, at a mean rate of 19.0 km/decade (Fig. 1). Analysis of climatic data shows that the mean January–February temperature in the region was 1.03–1.91 °C higher in 1998–2007 than in 1960–1969. The number of cold days (with the mean temperature below +5 °C) also significantly decreased, while the annual lowest temperature significantly increased. *Nezara viridula* was found mostly close to those locations where: (1) the mean January temperature exceeded +5 °C, (2) the mean number of cold days did not exceed 26 in January–February, and (3) where the mean annual lowest temperature did not drop below –3.0 °C. The general linear model shows that the mean January temperature and number of cold days are the most important factors controlling the northern limit of distribution of *N. viridula*. Altogether, the climatic data suggest that over the last 45 years environmental conditions have become more favourable for overwintering of *N. viridula* at many locations in central Japan. This has likely promoted the northward spread of the species, representing a direct response to climate warming. A sympatrically distributed congeneric *N. antennata* seems to respond to the warming by a retreat from the ocean coast towards cooler elevated habitats, which might be a complex response to elevated temperature and interspecific mating with *N. viridula* (Musolin *et al.* 2009).

Phenology and Life-history Responses to Simulated Climate Change.

As in many species (Parmesan 2006), temperature elevation is expected to affect numerous phenological events in *N. viridula*. For example, in this experiment, the simulated warming advanced the start of the spring colour change and reproduction. A further temperature increase is likely to stimulate an earlier start of reproduction in *N. viridula* and perhaps other insects, provided that they can find enough food.

The simulated warming strongly affected the timing of nymphal development. The nymphal growth was significantly accelerated by the warming in the early summer and autumn series, but retarded in the late summer series (Fig. 2). The late summer is the warmest period in central Japan: in August 2006, daily maximum temperature reached +40.0 °C under our experimental quasi-natural conditions and, thus, +42.0 °C under the simulated warming conditions. The relationship between the rate of nymphal development and temperature (Fig. 3) further shows that the simulated warming made the mid-to-late summer conditions unfavourable for nymphal development. As a result, nymphs developed slower, suffered higher mortality, and had difficulties during the final moulting.

The simulated warming also affected many life-history traits. In general, under the quasi-natural conditions, females of *N. viridula* had a smaller body size in the 1 August series than earlier in summer or later in autumn (Fig. 4), a seasonal tendency that has been reported in some other insect species. The simulated

warming strongly enhanced this tendency: females that emerged in the 1 and 15 August series were not only the smallest in the entire experiment, but on each of these dates females were significantly smaller under the simulated warming conditions than under the quasi-natural conditions. These findings show that the response to further warming will not be the same through out the year and will differ by season. Difference in size, in turn, is likely to affect other life-history traits such as survival or reproductive performance.

Winter is a critical season for insects in the temperate zone. Under both experimental conditions, larger females had significantly higher winter survival rates than smaller females (Fig. 5). More importantly, an additional 2.5 °C of the simulated warming during the winter strongly increased the likelihood of winter survival in both size classes and allowed smaller females to reach the same winter survival rate as larger females had under the quasi-natural conditions. Thus, if warming continues in the future, not only larger but also smaller females of *N. viridula* will survive winters more successfully and this will likely provide a basis for faster population growth.

The simulated warming strongly affected the functional relationship between the colour change in autumn and overwintering success. Under the warming conditions green females survived the winter even better than dark-coloured females did under the quasi-natural conditions (Fig. 6). This finding indirectly implies that the further climate warming will increase chances of successful winter survival even in those individuals that failed to change body colour and, thus, properly prepare for diapause before the winter.

Whereas it is believed that many species in the temperate zone will benefit from the temperature rise in one or another way (e.g., Deutsch *et al.* 2008), the present experiment shows that the situation is more complicated. The strong retardation of nymphal development, smaller size, softer cuticle and lighter yellowish body colour of *N. viridula* adults as well as their dramatically reduced life span in the 1 August series suggest that the elevated temperature experienced by nymphs and young adults exceeded their thermal optima. The mechanism underlying the abnormality of the cuticle colour and structure is so far unknown, although it might be related not only to thermal stress experienced by the bugs but also to the malfunction of their gut symbiotic bacterial fauna caused by the daily high temperature extremes (T. Fukatsu, pers. comm.). The design and the results of our experiment suggest that the warming-mediated suppression of performance might be a direct effect of temperature rather than an indirect one (via altered precipitation or condition of host plants). On the other hand, in contrast to plants, active stages of insects can possibly minimize the deleterious effect of elevated temperature, for example, by active selection of microhabitats. This might be especially useful in coping with daily temperature extremes. Some insect species will probably be able to mitigate the negative hot season effects of warming by evolving a summer diapause.

The data accumulated so far suggest that the effect of the rapid climate change is likely to be complex and differ among species and regions. The current study demonstrates that, even within the same species or population, responses will be different for different life-history traits and seasons. Thus, for instance, warming might negatively affect nymphal development during the hot season, stimulate development in autumn and/or strongly enhance survival of adults in winter. All this together, in a

complex way, will affect a species population dynamics, voltinism, relationships with other members of the community and likely pest status.

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REFERENCES.

- Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J., Good, J.E.G., Harrington, R., Hartley, S., Jones, T.H., Lindroth, R.L., Press, M.C., Symrnioudis, I., Watt, A.D., and Whittaker, J.B. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology* 8, 1-16.
- Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., and Martin, P.R. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America* 105, 6668-6672.
- IPCC (Intergovernmental Panel on Climate Change). 2007. Climate Change 2007: The Physical Science Basis. Summary for Policymakers. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland, 18 pp. <http://www.ipcc.ch/index.htm> (last accessed Sept. 25 2008)
- Kiritani, K., Hokyo, N., and Yukawa, J. 1963. Co-existence of the two related stink bugs *Nezara viridula* and *N. antennata* under natural conditions. *Researches on Population Ecology* 5, 11-22.
- Musolin, D.L. 2007. Insects in a warmer world: ecological, physiological and life-history responses of true bugs (Heteroptera) to climate change. *Global Change Biology* 13, 1565-1585.
- Musolin, D.L., Tougou, D., and Fujisaki, K. 2009. Too hot to handle? Phenological and life-history responses of the southern green stink bug *Nezara viridula* (Heteroptera: Pentatomidae) to the simulated rapid climate change. Submitted to *Global Change Biology*.
- Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* 37, 637-669.
- Tougou, D., Musolin, D.L., and Fujisaki, K. 2009. Some like it hot! Rapid climate change promotes changes in distribution ranges of *Nezara viridula* and *Nezara antennata* in Japan. Submitted to *Entomologia Experimentalis et Applicata*.
- Yukawa, J., Kiritani, K., Gyoutoku, N., Uechi, N., Yamaguchi, D., and Kamitani, S. 2007. Distribution range shift of two allied species, *Nezara viridula* and *N. antennata* (Hemiptera: Pentatomidae), in Japan, possibly due to global warming. *Applied Entomology and Zoology* 42, 205-215.