

Too hot to handle? Phenological and life-history responses to simulated climate change of the southern green stink bug *Nezara viridula* (Heteroptera: Pentatomidae)

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Abstract

The effect of simulated climate change on *Nezara viridula* was studied close to the species' northern range limit in Japan. 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, the simulated warming advanced the timing of postdiapause body colour changes and 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 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. The warming also 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.

Keywords: diapause, direct and indirect responses to climate warming, environmental change, Hemiptera (Heteroptera), invasive species, overwintering, pest insects, range expansion, seasonal adaptations

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Introduction

Global climate warming is commonly accepted as a reality by both scientists and the general public, and the attention is mostly focused on the responses of biota

to the recent and predicted temperature increase and associated environmental changes (Walther *et al.*, 2002; Helmuth *et al.*, 2005; Parmesan, 2006; Deutsch *et al.*, 2008). It is clear that the responses differ among different taxa in nature (e.g., changes in distribution, phenology, abundance, population structure and dynamics), magnitude (from organisms to local and global communities), and strength (from undetectable or no responses to dramatic) (Bale *et al.*, 2002; Parmesan, 2007). Despite trends that seem to be general and common (e.g., spring

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advances in phenology, extension of growing seasons in the temperate zone, and range shifts), all responses are species- (or even population-) specific.

Whereas the data accumulated on range changes and phenology of certain taxa have provided some basis for preliminary generalizations (e.g., Walther *et al.*, 2001; Reemer *et al.*, 2003; Hickling *et al.*, 2006; Parmesan, 2007; Lawler *et al.*, 2009), very little is known about the underlying mechanisms and eco-physiological responses to climate warming (Bradshaw & Holzapfel, 2008; Deutsch *et al.*, 2008; Helmuth, 2009). For instance, do particular species expand ranges northward because it is becoming warm enough for their survival towards more northern latitudes? Or, are temperatures within the species' range simply becoming intolerably hot in the summer or insufficiently cold in the winter? Is it critical that overwintering survival rates have changed or is it the timing of events that matters most? And, perhaps, most importantly: How will the species respond to the further – probably even more accelerated – climate warming?

There is a growing demand for accurate forecasts of biotic responses to climate change and concern about the effects on biological diversity as well as the future of humankind's well-being (Botkin *et al.*, 2007; IPCC, 2007; Lawler *et al.*, 2009). The insufficiency of data suitable for generalizations and predictions of further responses might partly be explained by difficulties in obtaining such data and a lack of accepted experimental protocols (Helmuth, 2009). To put it simply, should we heat *a bug* or *a whole ecosystem*, how do we measure responses and, just as importantly, how do we interpret the data?

Climate change implies simultaneous and complex changes of many environmental variables – primarily air temperature (IPCC, 2007). In an attempt to understand and predict how insects will respond to further warming, we exposed 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 predicted for the end of the XXI century (1.1–6.4 °C; IPCC, 2007).

As a model we used the southern green stink bug *Nezara viridula* (Heteroptera: Pentatomidae), a species that has been shown to respond to climate warming as evidenced by the northward expansion of its range (Musolin & Numata, 2003a, b, 2004; Shardlow & Taylor, 2004; Kiritani, 2007; Musolin, 2007; Yukawa *et al.*, 2007; Tougou *et al.*, 2009). This major agricultural pest occurs in an ever-widening range throughout the tropics and subtropics; Japan is at the northern margin of *N. viridula*'s range in Asia (Panizzi *et al.*, 2000; Tougou *et al.*, 2009).

It has been suggested that the northern limit of the *N. viridula*'s distribution in central Japan is limited by the +5 °C isothermal line for mean temperature of the coldest month and that the winter mortality is strongly

influenced by temperature (Kiritani, 1971, 2007; Musolin, 2007; Tougou *et al.*, 2009). This observation suggests that further global warming might influence the overwintering success, performance and/or fitness of the species. The objective of the study was to determine in a simulated climate warming experiment which life-history traits and how are likely to be affected if there is a further temperature increase.

Materials and methods

Field collection and stock culture

Adults of *N. viridula* were collected in June–August 2006 and May 2007 in Kochi (33.6°N, 133.6°E) and transferred to Kyoto (35.0°N, 135.8°E), where the experiment was conducted. The stock culture was kept under long-day conditions (LD 16:8 h) at +25 ± 1 °C and supplied with dry soybean *Glycine max* seeds, shelled raw peanuts *Arachis hypogaea* and water containing 0.05% sodium L-ascorbate and 0.025% L-cystein (Musolin & Numata, 2003a).

Experimental design and setup

On eight dates in 2006 (Fig. 1), egg masses from the stock culture were transferred to smaller transparent plastic containers (diameter 90 mm, depth 45 mm; lid openings covered with mesh for aeration) and placed under two experimental conditions. To increase genetic uniformity of the material between and its heterogeneity within the conditions, 5–16 egg masses (200–550 eggs in total) were used for each series. Each egg mass was divided into two roughly equal parts to be allocated into both conditions.

The two experimental conditions were identified as *quasi-natural* (i.e. *outdoor*) and *simulated warming conditions*. Under the quasi-natural conditions, egg masses and subsequently nymphs and adults were reared in transparent plastic containers (as described above) on metal shelves open from all sides (Fig. 2a). Under the simulated warming conditions, insects were reared similarly in a specially modified incubator LH-350NSZ (NK Systems, Osaka, Japan; height 1875 mm, width 840 mm; Fig. 2b) placed next to the open shelves. Both the incubator and the open shelves were sheltered from rain, direct sunlight, and artificial illumination. The incubator had transparent walls on three sides to ensure the insects experienced the same day-length conditions that their outside siblings did. A sensor measured outdoor temperature on the shelves of the quasi-natural conditions every 5 s, then a computer calculated an average temperature for the past 2 min, and based on these data, the incubator constantly

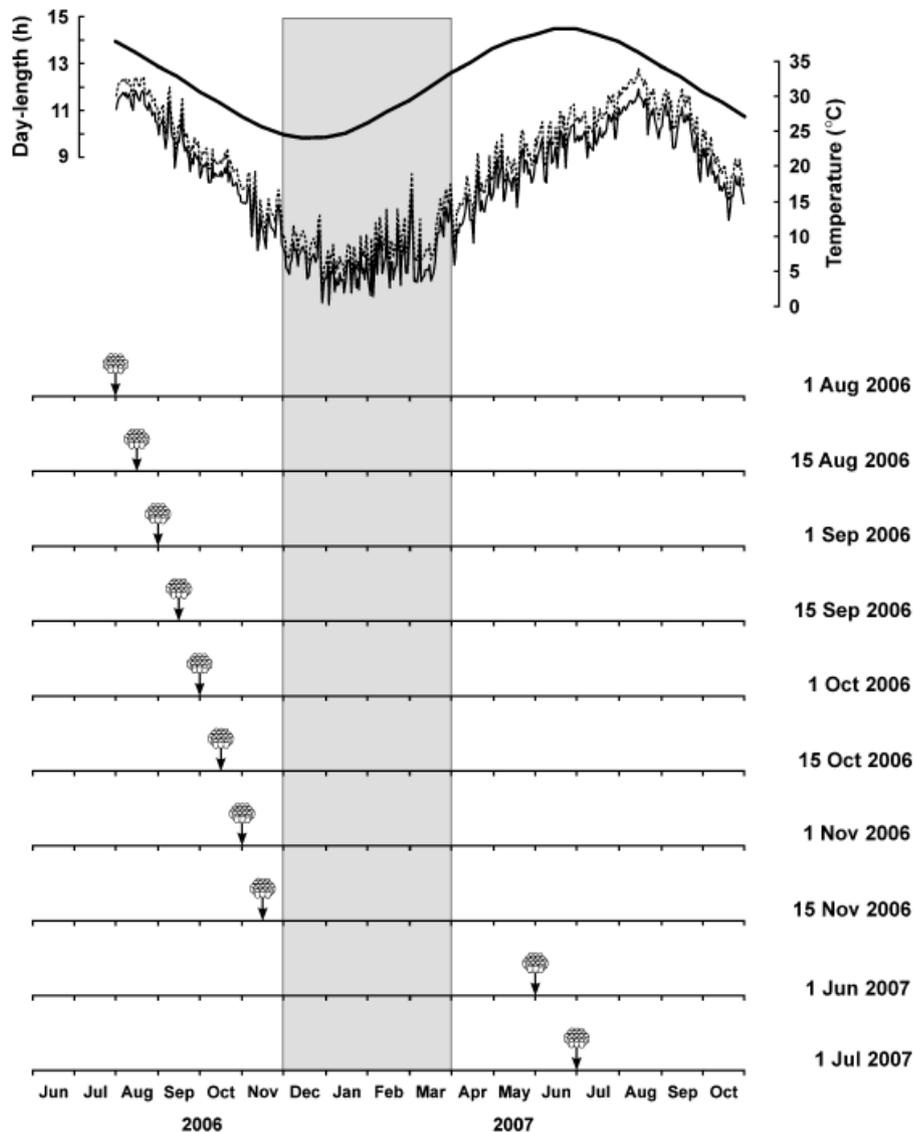


Fig. 1 The outline of the experiment. The egg mass symbol indicates the dates when egg masses of *Nezara viridula* were placed under the two experimental conditions. Environmental conditions (at the top of the figure): thick line, natural day-length; solid line, temperature outdoors; broken line, temperature in the simulated warming incubator. Shaded area, an overwintering period.

simulated warming conditions inside by adding 2 °C to the outside temperature.

Additionally temperature and humidity were recorded using a Thermo Recorder RTR-53 logger (Ondori Jr; T&D Co., Nagano, Japan). The records show that the actual temperature in the incubator was slightly higher than our planned artificial warming (+2.53 °C on average for 15 months). These logger records were used for data analysis. The relative humidity in the incubator was not more than 10% lower than the outside humidity.

Two additional series were set the next summer (Fig. 1). These series are analysed together with those of 2006 because the weather conditions were similar in both years. The 1 June, 1 July, and 1 August series

represent summer nondiapause (i.e. directly breeding) generations, whereas the three subsequent autumn series (15 August, 1 and 15 September) represent an autumn diapause generation with reproduction occurring only after overwintering (see Fig. 6).

In each series and each treatment, hatching of nymphs and then emergence of adults was checked daily. Nymphs and later adults were provided with the same diet as in the culture. Female–male pairs were transferred to new containers and reared under the same conditions as before. If too many adults emerged, only the earlier 25–40 pairs were kept, although all available data were used for nymphal development analysis. The start of copulation, dates of oviposition, changes of body colour,

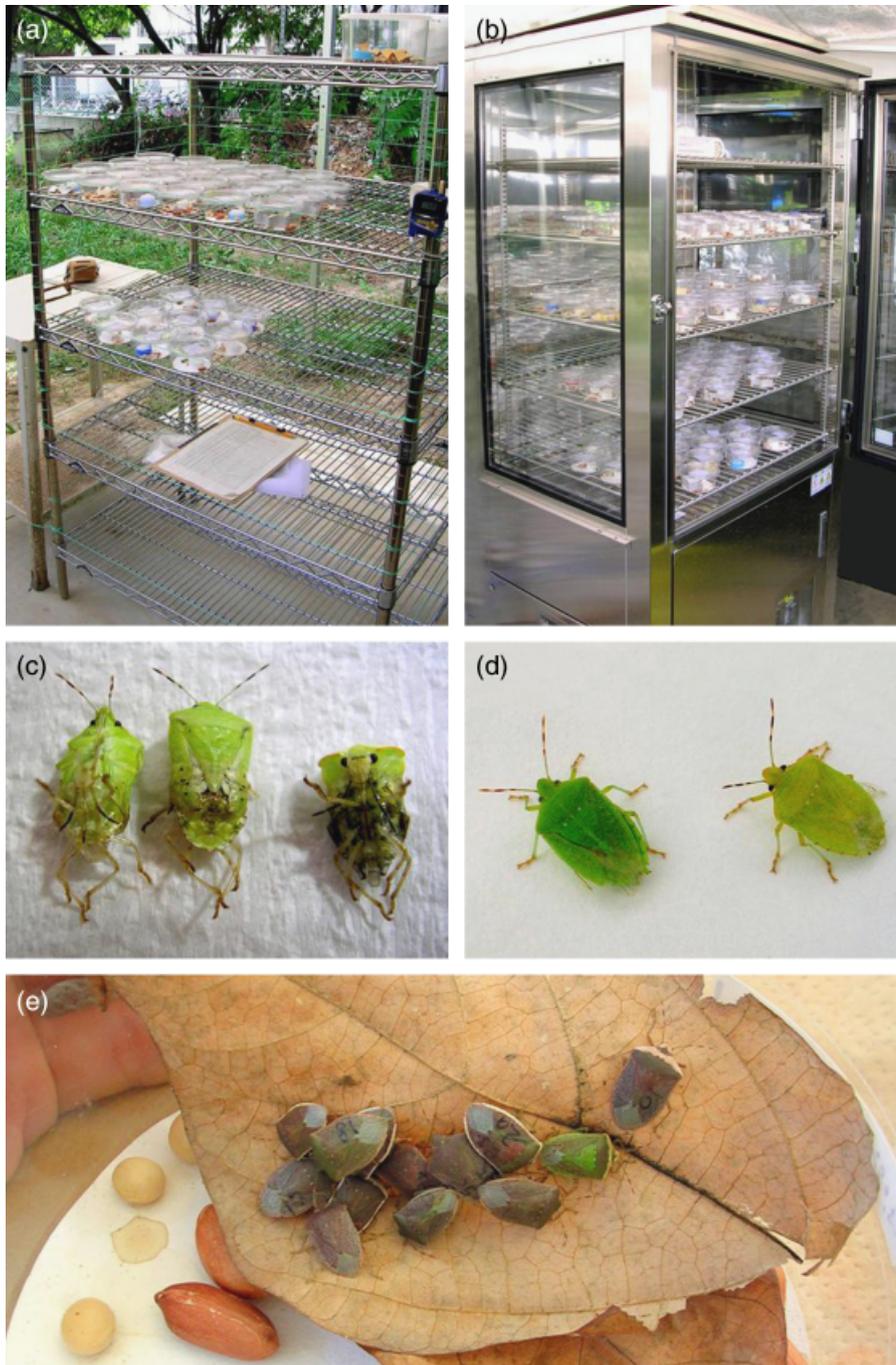


Fig. 2 The experimental setup: open metal shelves of the quasi-natural conditions (a) and an incubator with transparent walls of the simulated warming conditions (b). The 1 August series: difficulties during the moulting of *Nezara viridula* to the adult stage under the simulated warming conditions (c), adults from the quasi-natural conditions (d, left) and the simulated warming conditions (d, right). The 1 September series: numbered dark-coloured adults in diapause at the beginning of overwintering in a group container (e).

and mortality were recorded every other day. All eggs were counted. Because colouration of adults is correlated with their diapause status, which, in turn, is critical for overwintering, the following grades of females' body colour were used: *green*, *intermediate* (approximately

50% of the body surface is light russet), and *russet* (Musolin & Numata, 2003a, b, 2004).

On 10 December 2006, in each series, all russet females were numbered using permanent ink, transferred together with males into larger transparent plastic

containers (diameter 150 mm, depth 90 mm) at a density of 8–15 pairs per container, provided food and water. Two–three large leaves were added as shelter for overwintering (Fig. 2e). When mating was observed in the spring 2007, each mating pair was transferred into a smaller container. Couples with females that were green and intermediately coloured on 10 December 2006 were reared in smaller containers throughout their lives.

Data analysis

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. For brevity we report data on females only. We used *t*-test and Tukey–Kramer HSD test to compare different quantitative traits (between the two treatments and for multiple comparisons between all series, respectively) and χ^2 test and Fisher's exact test (in some cases after Bonferroni's adjustment) to compare proportions (JMP 5.1.2 statistical package). Particular tests and *P* levels are specified in each figure legend or in the text.

Results

Nymphal development

Nymphs hatched and many of them successfully completed development in the June–September series, although the low temperature even under the simulated warming conditions did not allow the nymphs to reach adulthood in the October–November series. These late-season series were excluded from further analysis.

In the early-season series the duration of the nymphal period showed a predictable seasonal trend under the quasi-natural conditions: higher outdoor temperatures in August resulted in a significantly shorter nymphal period in the 1 August series compared with the 1 June and 1 July series and as the season progressed the nymphal period became significantly longer (Fig. 3; results for males were similar, but not shown for brevity). The effect of the artificial warming was unexpected. It significantly accelerated nymphal development in the 1 June, 1 and 15 September series, but significantly retarded development in the 1 August series (Fig. 3). As a result, whereas the nymphal development was significantly faster in the 1 August series than in the June and July series under the quasi-natural conditions, it was slower under the simulated warming conditions.

The rate of nymphal development was calculated for each nymph that reached adulthood as a reciprocal of its nymphal period, averaged for the series and plotted against the mean temperature experienced during the

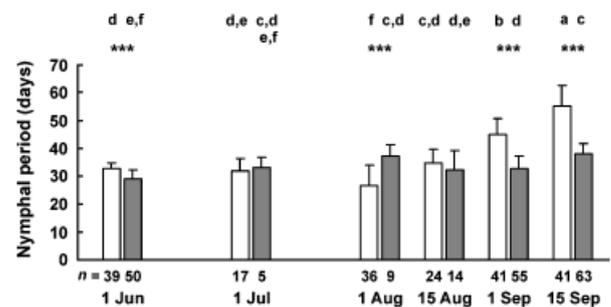


Fig. 3 Mean duration (\pm SD) of the nymphal period of *Nezara viridula* females. Bar colour: white, the quasi-natural conditions; grey, the simulated warming conditions. Stars: pair-wise comparison between the two treatments within the same series (i.e. on the same date), *t*-test; ****P* < 0.001. Letters above mean bars: multiple comparison (all series and treatments together), Tukey–Kramer HSD test; means with the same letters are not significantly different (*P* > 0.05). Sample size *n* is shown below the axis.

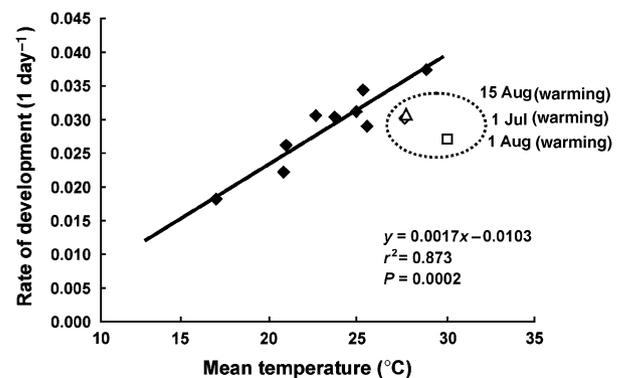


Fig. 4 Effect of temperature on the rates of nymphal development of *Nezara viridula* females. The mean rate of nymphal development is plotted against the mean temperature during the nymphal period in each series and treatment. The linear regression line and statistics are calculated for all series and treatments except three encircled mid-summer series under the simulated warming conditions. Sample size: see in Fig. 3.

nymphal period. Most data points fit well to a linear regression line ($F_{1,8} = 47.937$, $P = 0.0002$; ANOVA), although, three data points corresponding to the warmest season (the 1 July, 1 and 15 August series) were below and far from the lines (Fig. 4; results for males are not shown for brevity).

The simulated warming also affected survival of nymphs. Whereas the number of hatched nymphs was equal under both conditions, the number of emerged adults was more than three times lower under the simulated warming than quasi-natural conditions in the 1 July and 1 August series, strongly suggesting that warming in summer led to increased nymphal mortality (see the number of nymphs that attained the adult

stage in Fig. 3). Under the simulated warming conditions, some adults in these series had apparent difficulties while moulting to the adult stage and many of them died during moulting (Fig. 2c). In contrast, warming in the September series decreased both nymphal mortality and incidence of difficulties during the ecdysis.

Size and physical condition of adults

The body length of females was significantly smaller in the 1 August series than in other series (Fig. 5). The simulated warming strongly enhanced this tendency. When compared pair-wise with the quasi-natural conditions on the same dates, warming significantly reduced the body length of females in August, but significantly increased it in September. At the same time, the dependence of the mean body length on mean duration of the nymphal period was weak and not significant ($F_{1,11} = 0.01$, $P = 0.922$, $r^2 = 0.001$; regression analysis). Results for pronotum width were similar (not shown for brevity).

In addition to having a smaller size in the warmest season, some adults were apparently in a weaker physical shape under the warming conditions: they had a softer cuticle and a lighter yellowish body colour instead of intensive green (Fig. 2d; not to be confused with genetic colour morphs).

Seasonal development and adult body colour change before overwintering

All females that emerged in the summer series were reproductive (Fig. 6a). Under the quasi-natural conditions, 71.4–100% of them copulated and oviposited, although reproduction was suppressed under the warming conditions. Females in all the summer series

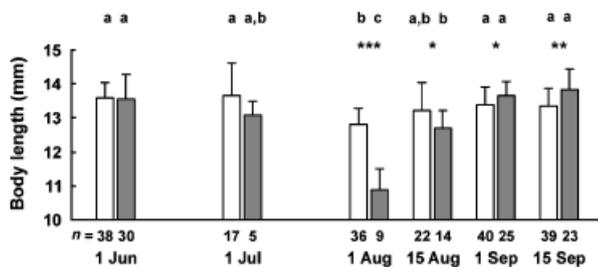


Fig. 5 Mean (+SD) body length (from the tip of the head to the end of the abdomen) of *Nezara viridula* females. Bar colour: white, the quasi-natural conditions; grey, the simulated warming conditions. Stars: pair-wise comparison between the two treatments within the same series (i.e. on the same date), *t*-test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Letters above mean bars: multiple comparison (all series and treatments together), Tukey–Kramer HSD test; means with the same letters are not significantly different ($P > 0.05$). Sample size n is shown below the axis.

and under both treatments did not change body colour and all died before the next summer.

In the autumn series (Fig. 6b), only 6.1% of females started reproduction before the winter, whereas almost all females in different series reproduced only after overwintering. Only 2.9% of females in the 15 August and 1 September series reproduced both before and after overwintering.

In the 15 August series under both conditions, 68.8–72.7% of females went through the diapause-associated body colour change from green to intermediate and russet by November–December and remained dark-coloured until the next spring (Fig. 6b). In the next series (1 September), the colour change pattern differed between the two conditions: 44.1% of females remained green under the quasi-natural conditions, whereas 91.3% of females successfully changed body colour and overwintered being dark-coloured under the warming conditions. The difference was even more pronounced in the last series: none of the females managed to change their body colour under the quasi-natural conditions, whereas 76% of females became dark-coloured by the late November under the warming conditions (Fig. 6b).

Adult body colour and winter survival

The winter survival of adults was strongly correlated with their body colour. When the proportion of females that survived winter in each series and under each condition was plotted against the proportion of dark-coloured individuals, a significant positive relationship was found: a higher proportion of dark-coloured individuals resulted in their higher rate of winter survival ($P = 0.006$; $r^2 = 0.811$; regression analysis; Fig. 7a). Females of the later series (15 September) remained green through the winter and the following spring and suffered the highest winter mortality (62.0%; Fig. 6b). When winter survival was analysed separately for each colour grade in the nonreproductive females, it differed significantly between green and russet females ($P < 0.0001$; χ^2 test; Fig. 7b).

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 ($P = 0.644$; Fisher's exact test; Fig. 7c). 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 warming conditions ($P = 0.01$). Survival of the dark-coloured females was also

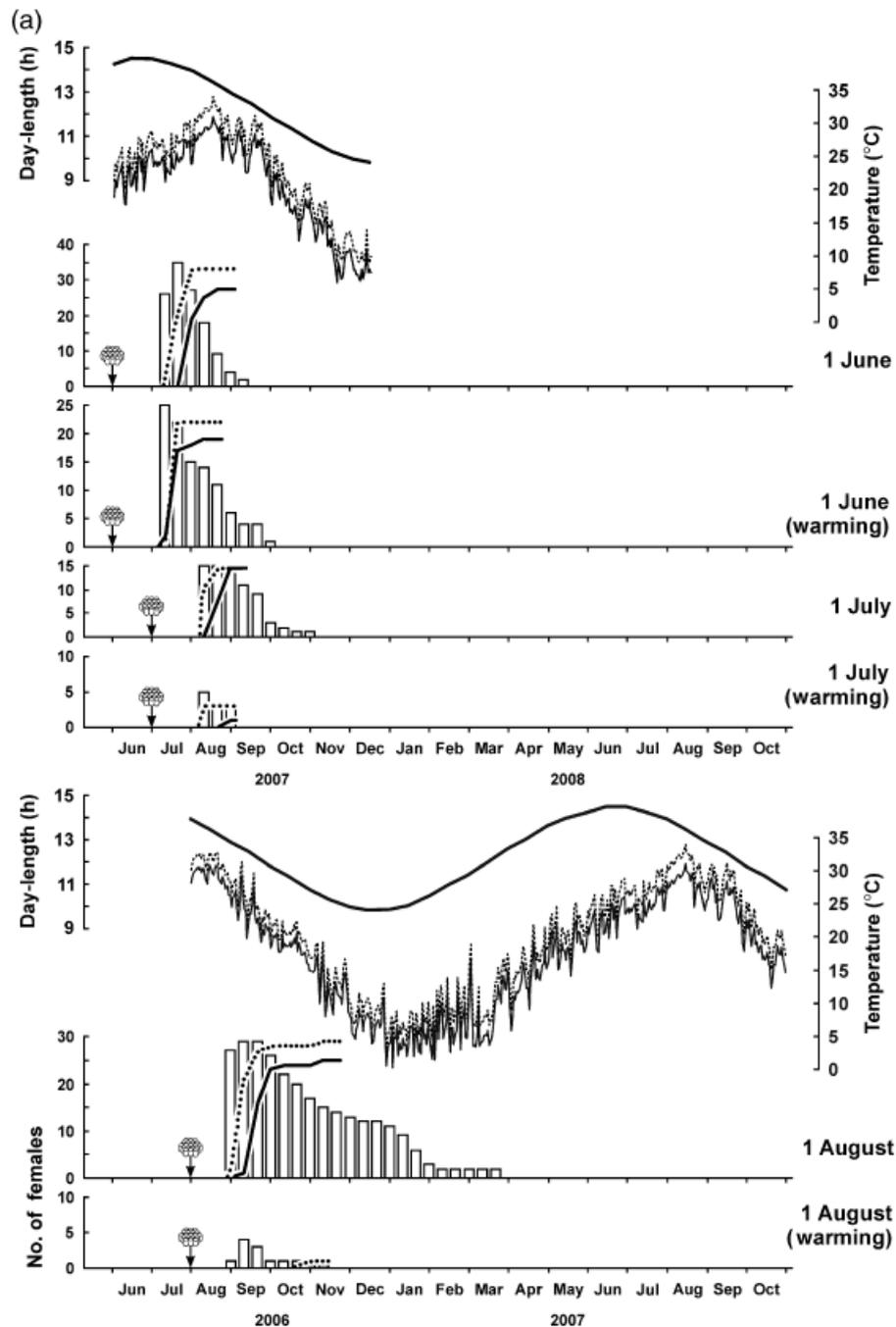


Fig. 6 Seasonal development of the summer (a) and autumn (b) series of *Nezara viridula* females under the quasi-natural and the simulated warming conditions (only the series in which insects reached the adult stage are shown). The egg mass symbol indicates the dates when the series started. Histograms denote the adult female emergence, survival, and relative abundance of colour grades: *green* (white bars), *intermediate* (grey bars), and *russet* (black bars). The dotted line and solid line overlaid on the histograms denote cumulative copulation and cumulative oviposition, respectively. Environmental conditions (at the top of the figure): thick line, natural day-length; solid line, temperature outdoors; broken line, temperature in the simulated warming incubator. See text for details.

higher under the warming conditions, although insignificantly ($P = 0.362$; Fisher's exact test; Fig. 7c).

Finally, the colour of females at the beginning of the winter was strongly linked to the ability of females to

survive until reproduction in the next season. Thus, among all the females that survived until 1 April, those females that had been dark-coloured on 1 December had significantly higher likelihood to reproduce during

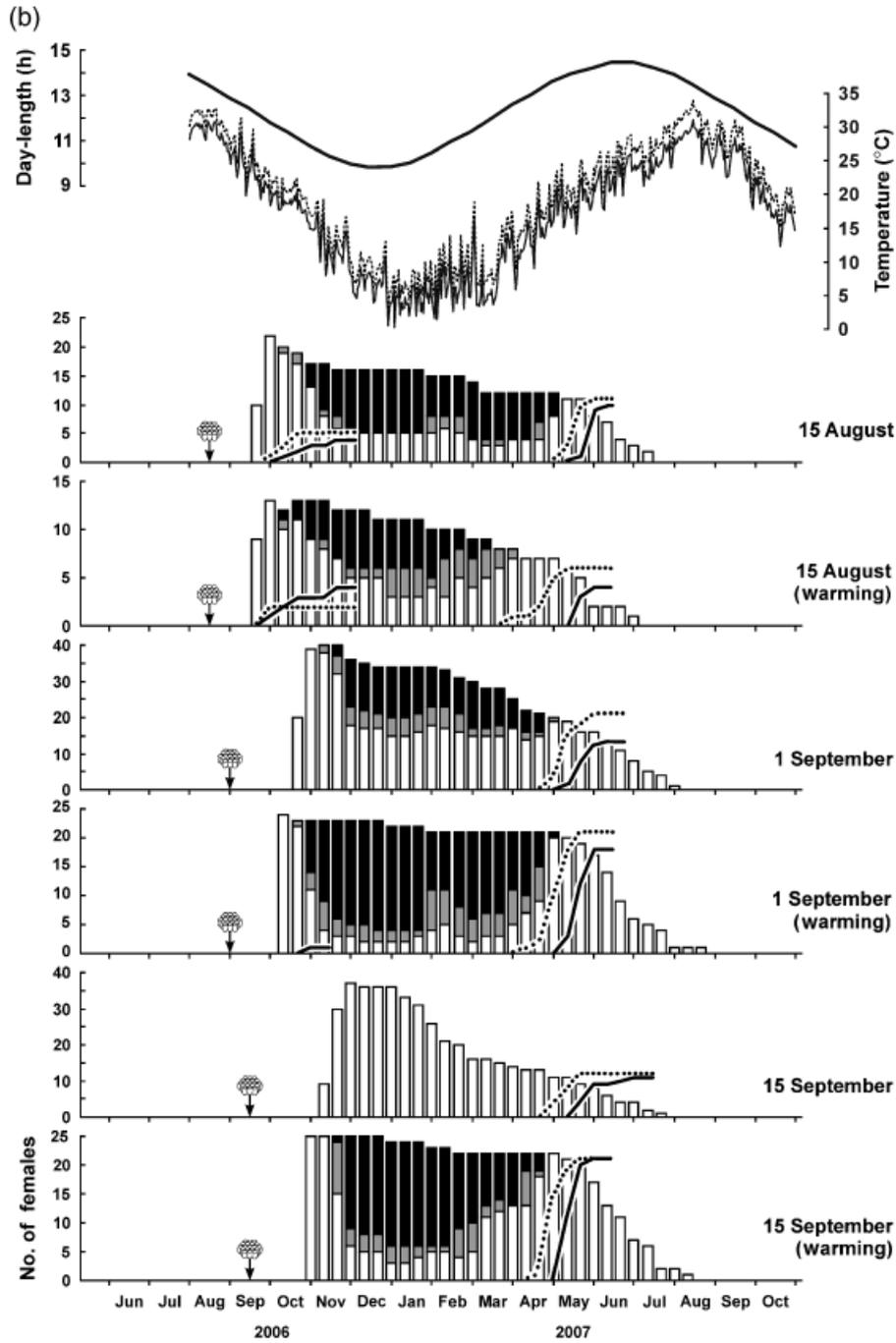


Fig. 6 Continued

the next season than those females that had been green on 1 December ($P = 0.008$; Fisher's exact test; not shown for brevity).

Adult size and winter survival

Both the female size and the simulated warming strongly affected the success of overwintering. For that

analysis, each female was classified as larger or smaller relative to the grand total mean size (13.5 mm for body length) and winter survival was then calculated separately for each size group and condition. Larger females had significantly higher winter survival rates under each of the two experimental conditions ($P \leq 0.008$; Fisher's exact test; Fig. 8). Moreover, females from each size group had significantly higher winter

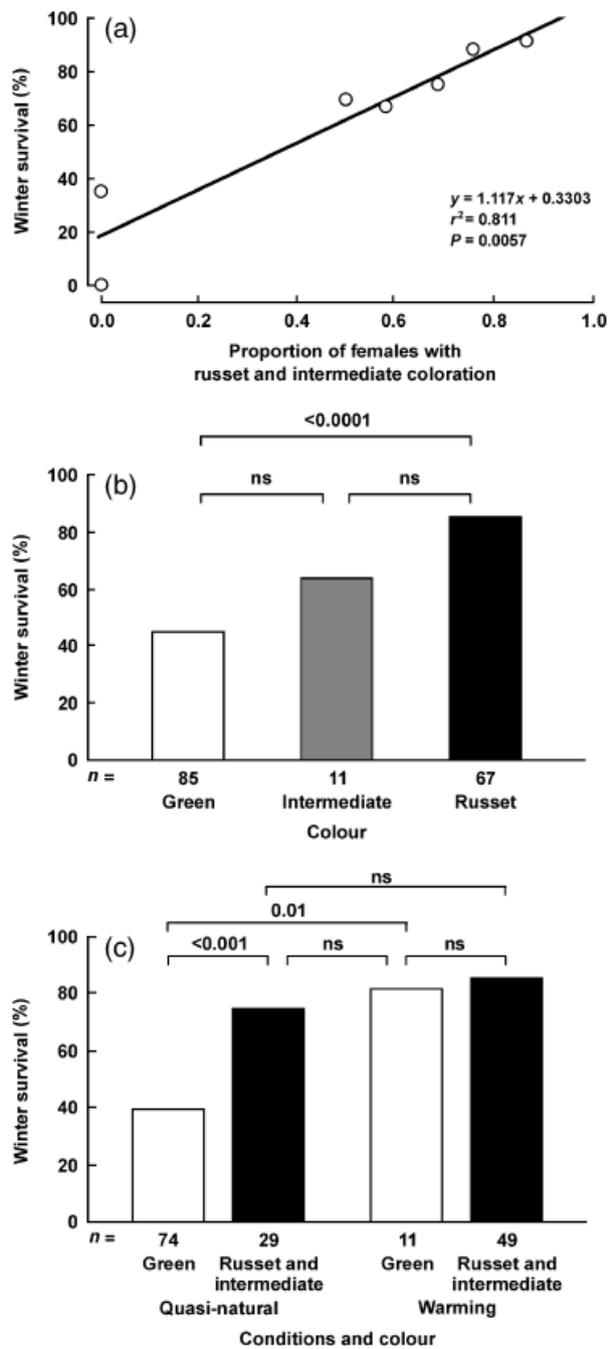


Fig. 7 Effect of colouration on winter survival (from 1 December to 1 April) of *Nezara viridula* females. The relationship between winter survival and proportion of together *russet* and *intermediately* coloured females in the total number of nonreproductive females in each series and treatment on 1 December (all series and treatments in which females survived the winter are included; the linear regression line and statistics after arcsine transformation are shown) (a). Winter survival in different colour groups of nonreproductive females (all series and treatments combined; P of χ^2 test is shown) (b). Winter survival in different colour groups of nonreproductive females and under different treatments (all series are combined; P of Fisher exact test is shown) (c). Sample size n is shown below the axes (in b and c).

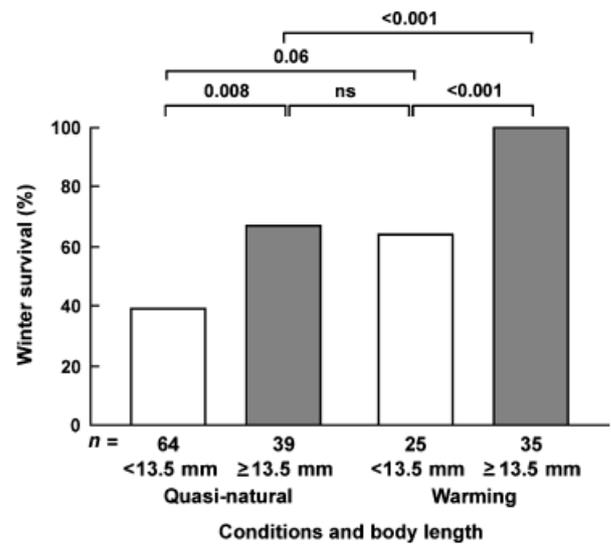


Fig. 8 Winter survival (from 1 December to 1 April) in different body length groups of nonreproductive *Nezara viridula* females under different treatments (all series are combined; P of Fisher exact test after Bonferroni adjustment is shown). Sample size n is shown below the axis.

survival under the simulated warming conditions than under the quasi-natural conditions ($P \leq 0.06$; Fisher's exact test; Fig. 8). 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. 8; the mean difference of temperature was $+2.6^\circ\text{C}$ for December–March). The data on pronotum width showed similar trends (not presented for brevity).

Timing of the spring colour change

In spring, the dark-coloured females of the 15 August series became green significantly earlier under the warming conditions than under the quasi-natural conditions (comparison of the Julian dates: $P = 0.047$; t -test; Fig. 6b). In the next series (1 September), however, the dates of the spring colour change did not differ ($P = 0.222$; t -test).

Timing of reproduction

The effect of warming on the start of copulation and oviposition was more pronounced after overwintering than before it. In the spring, in all the series, copulation and oviposition started earlier under the simulated warming conditions than under the quasi-natural conditions and in four of six cases the difference (2.0–7.9 days) was significant (Figs 6 and 9a, b).

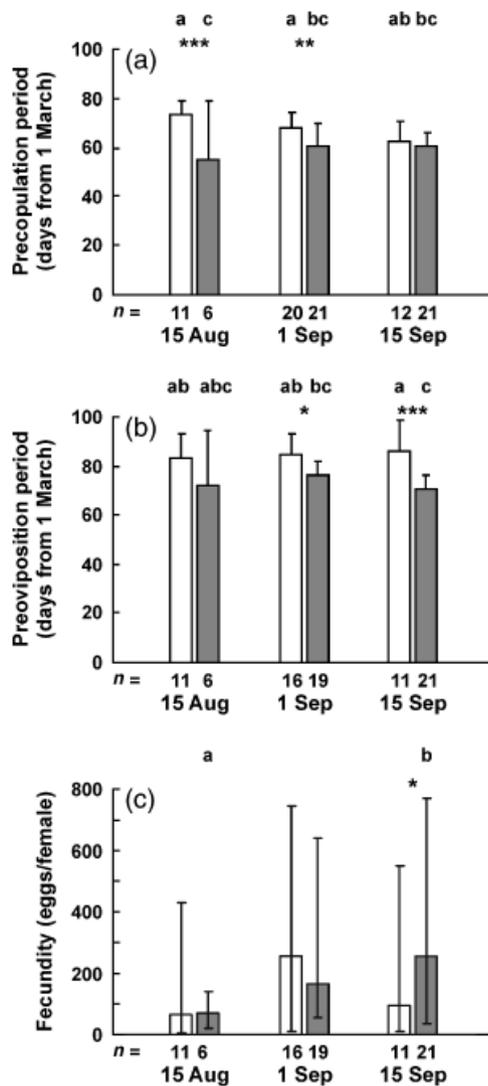


Fig. 9 Mean (+SD) precopulation (a) and preoviposition (b) periods and median (with range) fecundity (c) after the winter (1 December to 1 April) in *Nezara viridula* females. Bar colour: white, the quasi-natural conditions; grey, the simulated warming conditions. Only ovipositing females are analysed (in b and c). Stars: pair-wise comparison between the two treatments within the same series (i.e. on the same date), *t*-test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Letters above mean bars: multiple comparison for each parameter (all series and both treatments together), Tukey–Kramer HSD test; means (a and b) and medians (c) with the same letters are not significantly different ($P > 0.05$). Sample size n is shown below the axes.

Fecundity

Fecundity measured in the reproductive females as the total number of produced eggs showed a complicated pattern. The simulated warming significantly increased egg production in the directly breeding females in the early summer (the 1 June series) and in the females

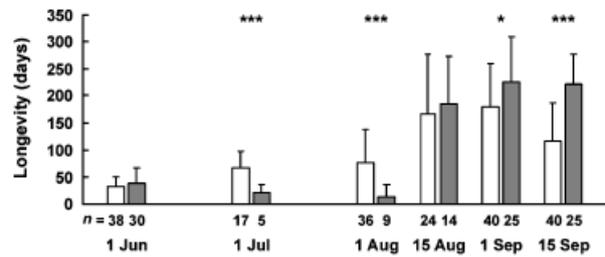


Fig. 10 Mean (+SD) longevity of *Nezara viridula* females. Bar colour: white, the quasi-natural conditions; grey, the simulated warming conditions. Stars: pair-wise comparison between the two treatments within the same series (i.e. on the same date), *t*-test; * $P < 0.05$; *** $P < 0.001$. Sample size n is shown below the axis.

reproducing after overwintering (the 15 September series; Fig. 9c).

Longevity

No females from the summer series survived until the end of the next spring (Fig. 6a) and the simulated warming significantly reduced the mean longevity of females in the 1 July and 1 August series ($P < 0.001$; *t*-test; Fig. 10). In the autumn series, most females survived until the next spring and summer and the simulated warming increased their longevity (in two series significantly: $P \leq 0.034$; *t*-test; Fig. 10) likely by optimizing thermal overwintering conditions.

Discussion

The current climate change is affecting numerous species, although response to the change is far from simple in its nature and very difficult to study. Different techniques and approaches developed so far allow detecting different particular responses and in some cases understanding their physiological or genetic basis (Helmuth *et al.*, 2005; Parmesan, 2007), although none of them have managed to show the whole integral response of a species, population or even an organism to climate change. Experiments simultaneously analysing multiple ecological and physiological features and life-history traits are very rare but would be especially useful for a better understanding of the current responses and forecast what should be expected in the future (Crozier, 2004; Helmuth, 2009). *N. viridula* represents an excellent experimental model for such an approach. The species has steadily expanded its wide range from the tropics and subtropics towards the temperate zones in both hemispheres and climate change has been shown to play a key role in this process (Musolin & Numata, 2003b; Musolin, 2007; Ohno &

Table 1 Summary of potential effects of temperature increase on different phenological and life-history parameters of *Nezara viridula* in different seasons

Phenological and life-history parameters	Seasons					References
	Spring	Early summer	Late summer	Autumn	Winter	
Postdiapause resumption of activity and start of reproduction	+ *					Figs 6 and 9
Nymphal development	+	+	–	+		Figs 3 and 4
Physical conditions of nymphs and adults	+ / 0	+ / 0	–	+		Fig. 2
Size of emerging adults	0	0	–	+		Fig. 5
Reproductive output	+	+ / 0	– / 0	+		Text
Diapause-associated adult body colour change	+ / 0			+	+	Fig. 6
Longevity of adults	+	– / 0	–	+	+	Fig. 10
Survival of adults	+ / 0	– / 0	–	+	+	Figs 6–8

Effects of temperature increase: +, beneficial; –, detrimental; 0, neutral.

*Depending on availability of food.

Nakamura, 2007; Yukawa *et al.*, 2007; Tougou *et al.*, 2009).

In the present study, insects experienced the same environmental conditions (food, day-length, rearing density, humidity, etc.), with temperature being the only factor, which constantly differed between the two treatments. The 15-month-long experiment apparently had a number of limitations, the main of which were: (1) only one true replicate, (2) exclusion of natural enemies, competitors and host plant from the experimental system, and (3) a fixed temperature increase throughout the whole duration of the experiment, whereas it has been predicted that the temperature differences will vary month to month and the warming will be more pronounced in winter than summer in the northern hemisphere (Kato, 1996; Houghton *et al.*, 2001). Such monthly variance can to some extent dampen or amplify the observed effects in different seasons. The findings, nevertheless, strongly suggest that if there is a further increase in air temperature many phenological events and life-history traits of *N. viridula* and likely of other species might be affected during all seasons, although the effects may be beneficial or detrimental to particular species depending on the time of year (Table 1; summarized below). At the same time, it should be kept in mind that the responses and their combinations are often species-specific and findings from one species can be generalized only with a great caution.

Phenology

Temperature elevation is expected to affect numerous phenological events in many species (Bale *et al.*, 2002; Parmesan & Yohe, 2003; Root *et al.*, 2003), although this response has been seldom demonstrated experimentally (Helmuth *et al.*, 2005; Parmesan, 2007). In this

study, the simulated warming advanced the resumption of the postdiapause development (as indicated by the spring body colour change) and the start of the spring reproduction of *N. viridula* (Figs 6b and 9a, b). These results strongly suggest that a further temperature increase will stimulate an earlier start of activity and reproduction in this as well many other insect species, provided that they can find enough food in spring.

The simulated warming strongly affected the nymphal development. Its growth was significantly accelerated by the warming in the early summer and autumn, but retarded in the late summer (Fig. 3). The late summer is the warmest period in the region: the mean August temperature in Kyoto in 1997–2007 was +28.3 °C (Japan Meteorological Agency, 2008). In August 2006, daily maximum temperature reached +40.0 °C under our quasi-natural conditions and, thus, +42.5 °C under the simulated warming conditions (Fig. 6a). The relationship between the rate of nymphal development and temperature (Fig. 4) further shows that the simulated warming made the mid-to-late summer conditions unfavourable for nymphal development of *N. viridula*. As a result, nymphs developed slower, suffered higher mortality, and had difficulties during the final moulting (Fig. 2c). Significance of this response will be discussed below.

In autumn, when temperature was progressively decreasing and, thus, naturally slowing down the nymphal development, the simulated warming significantly (by 12–18 days) shortened the nymphal period and advanced emergence of adults (Figs 3 and 6b). The proper timing of both reaching the overwintering stage and diapause induction is vitally important for winter survival of insects (Danks, 1987; Musolin & Numata, 2003b; Bradshaw & Holzapfel, 2008). The timing of adult emergence is also crucial for the diapause-associated adult colour change: almost no females that emerged as

adults after 1 November could successfully change colour from green to russet as most of females that had emerged earlier did. The colour change, in turn, is very important for overwintering in this species: the dark-coloured adults have significantly higher likelihood to survive the winter (Figs 7a and b). The complex relationship between diapause, winter colouration, and winter survival existing in many species will remain valid even if the climate becomes warmer in the temperate zone, although the species will have to adjust their physiological traits such as timing of dormancy-associated changes or ranges of tolerance (Bradshaw & Holzapfel, 2008).

Adult body size

In general, under the quasi-natural conditions, females of *N. viridula* were smaller in late summer than in early summer or later in autumn (Fig. 5), a seasonal trend typical of some insect species (see Danks, 1987, pp. 214–216). The simulated warming strongly enhanced this tendency: females that emerged in the two August series were not only the smallest in the entire experiment, but on each of these dates they were significantly smaller under the simulated warming conditions than under the quasi-natural conditions (Fig. 5). A few studies have found evidence of a decline in body size over historical time and considered it a response to climate change (e.g., Yom-Tov *et al.*, 2006; Teplitsky *et al.*, 2008 and references therein). Our findings experimentally show that this can happen quickly and might be caused even by a moderate increase of the ambient temperature. The results also show that this type of the response will not be the same throughout the year and will differ by season. In general, differences in size of animals and plants, in turn, are likely to affect many of their life-history traits, e.g., survival or reproductive performance and, thus, be of considerable ecological importance.

Winter survival

Winter is a critical season for insects in the temperate zone (Danks, 1987). For some of the species that currently expand their ranges, winter warming is considered a prerequisite for such an expansion (Crozier, 2004; Tougou *et al.*, 2009). Under both our experimental conditions, larger females of *N. viridula* had significantly higher winter survival rates than smaller females (Fig. 8). More importantly, an additional 2.6 °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 (Fig. 8). 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. These results are likely to reflect a general trend and under further warming conditions higher winter survival should be expected in many animal species.

The simulated warming also strongly affected an ecologically important functional relationship between the colour change and overwintering success in *N. viridula*. The winter survival differed significantly between the green and dark-coloured females under the quasi-natural conditions, but not under the warming conditions (Fig. 7c). Moreover, under the warming conditions green females survived the winter even better than dark-coloured females did under the quasi-natural conditions (Fig. 7c). These findings suggest that the further climate warming will increase likelihood of winter survival even in those individuals that failed to properly prepare for diapause before the winter. Similarly to the case of body size discussed above, this trend is not likely to be limited by only one species: under warmer conditions even less physiologically prepared individuals of many species will increase likelihood of winter survival.

Altogether our data suggest that moderate warming can profoundly affect and likely enhance winter survival of insects and other organisms, at least at the northern edges of their ranges where overwintering thermal conditions are most likely to be critical (Crozier, 2004).

Reproductive performance and longevity

The simulated warming significantly enhanced the egg production in the early summer by both the overwintered (Fig. 9c) and directly breeding females. Furthermore, under the warming conditions, females lived and reproduced longer (Fig. 10) likely because of more favourable overwintering conditions. These findings experimentally support the earlier speculation that further warming might promote early-season reproduction of insects and raise the pest status of some of these insects (Kiritani, 2007).

Voltinism

It has been suggested that the higher temperatures due to climate change should allow faster development of insects and, in some cases, together with the prolonged growing season this might lead to production of additional generation(s) within a year (e.g., Yamamura & Kiritani, 1998; Bale *et al.*, 2002; Kiritani, 2007). The application of Yamamura & Kiritani's formula (1998) using thermal constants from different studies (Kariya, 1961; Nonaka & Nagai, 1978; Musolin & Numata,

2003a) suggests that a temperature increase of 2.0–2.5 °C might result in production of a full additional annual generation of *N. viridula* in central Japan (0.97–1.34 generations for different data sets). The situation, however, is more complicated. The present experiment revealed that warming might induce not only acceleration of development in spring and autumn, but also suppression of development in mid-summer (Figs 3 and 4; Table 1). If four series of the present experiment (1 June, 1 July, 1 August, and 1 September) are considered as an approximate representation of four annual generations of *N. viridula* in the region (cf. Kiritani, 1971), then the total accumulated acceleration due to the simulated warming would amount to approximately 31 days (i.e. advanced start of oviposition after overwintering, shorter nymphal periods in the 1 June and 1 September series and shorter preoviposition period in the 1 June series). The total time, in which development would be delayed, however, might be about 24 days (i.e. prolonged nymphal period in the 1 July and 1 August series and prolonged preoviposition period in the 1 July series). Thus, the data suggest that even though the warming is expected to expand the activity season, production of a full additional generation is unlikely because of the pronounced suppression of development during the hot mid-summer season. The present results, however, were obtained under conditions when insects experienced almost equal heating during the whole year, whereas the currently observed regional trends (Kato, 1996) and most of the global climatic models (Houghton *et al.*, 2001) indicate that the warming will be more pronounced in winter than summer. Thus, the observed effects might be amplified in winter (mostly beneficial for *N. viridula*) and dampen in summer (mostly detrimental). Prediction of changes in species voltinism and performance are extremely difficult and unreliable without profound knowledge of the species' ecophysiology and adaptability.

Deleterious effect of climate warming

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 and weaker physical condition of *N. viridula* adults as well as their dramatically reduced life span in the early August series (Figs 2–5 and 10, Table 1) suggest that the elevated temperature experienced by nymphs and young adults exceeded their thermal optima and potential of heat shock tolerance is limited. One would not expect the warm-adapted species or populations distributed close to the species'

northern limits to be under the threat of temperature increase. Our results show that in fact this might be the case. In more general terms, the findings emphasize that the response to climate change might be unexpected and complex in the regions with hot summers. The underlying mechanism of the recorded deleterious effect is so far unknown, although it might be related not only to acute or chronic thermal stress experienced by the individuals but also to the malfunction of their gut symbiotic bacterial fauna caused by the daily high temperature extremes (T. Fukatsu, pers. comm.).

The suppression of performance reported here is not the first evidence to illustrate the deleterious effect of warming. For instance, a dramatic decline in abundance of cereal aphids in Britain is predicted by the end of this century, chiefly due to the change in temperature and rainfall (Newman, 2005). It has also been reported that increased temperature results in physiological stress and reduced reproductive success of a montane leaf beetle (Dahlhoff *et al.*, 2008), decreased rice yields (Peng *et al.*, 2004), decelerating growth in temperate zone (Jump *et al.*, 2006) and tropical (Feeley *et al.*, 2007) forest trees. Further climate warming is expected to have a strong influence on species' fitness, which will differ depending on latitude (Deutsch *et al.*, 2008). The design and results of the present experiment suggest that the warming-mediated suppression of performance might be a direct effect of temperature rather than indirect one (via altered precipitation or condition of host plants, as suggested in some other studies, see, e.g., Newman, 2005; Feeley *et al.*, 2007). 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.

Conclusion

The data accumulated so far suggest that effect of climate change is likely to be complex and differ among species and regions (Walther *et al.*, 2002; Reemer *et al.*, 2003; Hickling *et al.*, 2006; Deutsch *et al.*, 2008). The current study demonstrates that even within the same species or population responses will be different for different life-history traits and seasons (Table 1). Thus, for instance, warming might negatively affect nymphal development during the hot season, accelerate development in autumn and/or strongly enhance survival of adults in winter. All this together, in a complex way, will affect species' population dynamics, relationship with other members of the community and likely pest status.

While interpreting the results, it is important to acknowledge that herbivores will not experience the climate change on their own, temperature is not the only environmental factor that will change, and the change will not be equal during a year. Host plants, competitors, predators, parasites and symbionts will respond to all seasonally variable environmental changes individually as well as affect each other making the precise overall prediction extremely difficult if possible at all. These complex relationships and responses must be taken into consideration in further studies.

It is known that species can potentially respond to climate change through phenotypic flexibility or rapid evolutionary (genetic) responses to strong selection (Bale *et al.*, 2002). As noted by Thomas *et al.* (2001), 'improving environmental conditions at existing margins ... are likely to initiate range expansions purely on the basis of ecological, physiological and population-dynamic processes – requiring no evolutionary change' (p. 579). This is likely what has been observed in the case of *N. viridula*: the improved overwintering conditions in central Japan have stimulated northward range expansion of the species (Musolin, 2007; Yukawa *et al.*, 2007; Tougou *et al.*, 2009). The recent analysis, however, showed that all known responses to climate warming involve genetic changes related to seasonality and none of them involves an increase in thermal optimum or in heat tolerance (Bradshaw & Holzapfel, 2008). Careful monitoring of performance of *N. viridula* and other model species in the recently colonized areas as well as detailed examination of the plant–insect complex's response will be essential for understanding of adaptation mechanisms to climate change.

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References

- Bale JS, Masters GJ, Hodkinson ID *et al.* (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, **8**, 1–16.
- Botkin DB, Saxe H, Araújo MB *et al.* (2007) Forecasting the effects of global warming on biodiversity. *BioScience*, **57**, 227–236.
- Bradshaw WE, Holzapfel CM (2008) Genetic response to rapid climate change: it's seasonal timing that matters. *Molecular Ecology*, **17**, 157–166.
- Crozier L (2004) Warmer winters drive butterfly range expansion by increasing survivorship. *Ecology*, **85**, 231–241.
- Dahlhoff EP, Fearnley SL, Bruce DA *et al.* (2008) Effects of temperature on physiology and reproductive success of a montane leaf beetle: implications for persistence of native populations enduring climate change. *Physiological and Biochemical Zoology*, **81**, 718–732.
- Danks HV (1987) *Insect Dormancy: An Ecological Perspective (Monograph Ser No. 1)*. Biological Survey of Canada, Ottawa.
- Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (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.
- Feeley KJ, Wright SJ, Nur Supardi MN, Kassim AR, Davies SJ (2007) Decelerating growth in tropical forest trees. *Ecology Letters*, **10**, 461–469.
- Helmuth B (2009) From cells to coastlines: how can we use physiology to forecast the impacts of climate change? *Journal of Experimental Biology*, **212**, 753–760.
- Helmuth B, Kingsolver JG, Carrington E (2005) Biophysics, physiological ecology, and climate change: does mechanism matter? *Annual Review of Physiology*, **67**, 177–201.
- Hickling R, Roy DB, Hill JK, Fox R, Thomas CD (2006) The distributions of a wide range of taxonomic groups are expanding polewards. *Global Change Biology*, **12**, 450–455.
- Houghton JT, Ding Y, Griggs DJ *et al.*, eds (2001) *Climate Change 2001: The Scientific Basis*. Cambridge University Press, Cambridge.
- 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. Available at <http://www.ipcc.ch/index.htm> (accessed 19 March 2009).
- Japan Meteorological Agency (2008) Available at <http://www.jma.go.jp/jma/indexe.html> (accessed 19 March 2009).
- Jump AS, Hunt JM, Peñuelas J (2006) Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*. *Global Change Biology*, **12**, 2163–2174.
- Kariya H (1961) Effect of temperature on the development and the mortality of the southern green stink bug, *Nezara viridula* and the oriental green stink bug, *N. antennata*. *Japanese Journal of Applied Entomology and Zoology*, **5**, 191–196.
- Kato H (1996) Statistical method for separating urban effect trends from observed temperature data and its application to Japanese temperature records. *Journal of the Meteorological Society of Japan*, **74**, 639–653.
- Kiritani K (1971) Distribution and abundance of the southern green stink bug, *Nezara viridula*. In: *Proceedings of the Symposium on Rice Insects*, pp. 235–248. Tropical Agricultural Research Center, Tokyo.
- Kiritani K (2007) The impact of global warming and land-use change on the pest status of rice and fruit bugs (Heteroptera) in Japan. *Global Change Biology*, **13**, 1586–1595.

- Lawler JJ, Shafer SL, White D, Kareiva P, Maurer EP, Blaustein AR, Bartlein PJ (2009) Projected climate-induced faunal change in the Western Hemisphere. *Ecology*, **90**, 588–597.
- Musolin DL (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 DL, Numata H (2003a) Photoperiodic and temperature control of diapause induction and colour change in the southern green stink bug *Nezara viridula*. *Physiological Entomology*, **28**, 65–74.
- Musolin DL, Numata H (2003b) Timing of diapause induction and its life-history consequences in *Nezara viridula*: is it costly to expand the distribution range? *Ecological Entomology*, **28**, 694–703.
- Musolin DL, Numata H (2004) Late-season induction of diapause in *Nezara viridula* and its effects on adult coloration and post-diapause reproductive performance. *Entomologia Experimentalis et Applicata*, **111**, 1–6.
- Newman JA (2005) Climate change and the fate of cereal aphids in Southern Britain. *Global Change Biology*, **11**, 940–944.
- Nonaka K, Nagai K (1978) Studies on the biology of pentatomids and their control. 6. Developmental rate of the southern green stink bug. *Proceedings of the Association of Plant Protection of Kyushu*, **24**, 80–81 (in Japanese).
- Ohno H, Nakamura K (2007) Distribution of *Nezara viridula* and *N. antennata* in Okayama Prefecture and Shikoku Island, Japan. *Naturalistae*, **11**, 1–8 (in Japanese with English abstract).
- Panizzi AR, McPherson JE, James DG, Javahery M, McPherson RM (2000) Stink bugs (Pentatomidae). In: *Heteroptera of Economic Importance* (eds Schaefer CW, Panizzi AR), pp. 421–474. CRC Press, Boca Raton.
- Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637–669.
- Parmesan C (2007) Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, **13**, 1860–1872.
- Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37–42.
- Peng S, Huang J, Sheehy JE *et al.* (2004) Rice yields decline with higher night temperature from global warming. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 9971–9975.
- Reemer M, van Helsdingen PJ, Kleukers RMJC, eds (2003) *Changes in Ranges: Invertebrates on the Move*. European Invertebrate Survey, Leiden, *Proceedings of the 13th International Colloquium of the European Invertebrate Survey*, Leiden, 2–5 September 2001, 137 pp.
- Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA (2003) Fingerprints of global warming on wild animals and plants. *Nature*, **421**, 57–60.
- Shardlow MEA, Taylor R (2004) Is the Southern Green Shield Bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) another species colonising Britain due to climate change? *The British Journal of Entomology and Natural History*, **17**, 143–146.
- Teplitsky C, Mills JA, Alho JS, Yarrall JW, Merilä J (2008) Bergmann's rule and climate change revisited: disentangling environmental and genetic responses in a wild bird population. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 13492–13496.
- Thomas CD, Bodsworth EJ, Wilson RJ, Simmons AD, Davies ZG, Musche M, Conrard L (2001) Ecological and evolutionary processes at expanding range margins. *Nature*, **411**, 577–581.
- Tougou D, Musolin DL, Fujisaki K (2009) Some like it hot! Rapid climate change promotes shifts in distribution ranges of *Nezara viridula* and *N. antennata* in Japan. *Entomologia Experimentalis et Applicata*, **30**, 249–258.
- Walther G-R, Burga CA, Edwards PJ, eds (2001) *"Fingerprints" of Climate Change: Adapted Behaviour and Shifting Species Ranges*. Kluwer Academic/Plenum Publishers, New York.
- Walther G-R, Post E, Convey P *et al.* (2002) Ecological responses to recent climate change. *Nature*, **416**, 389–395.
- Yamamura K, Kiritani K (1998) A simple method to estimate the potential increase in the number of generations under global warming in temperate zone. *Applied Entomology and Zoology*, **33**, 289–298.
- Yom-Tov Y, Yom-Tov S, Wright J, Thorne CJR, du Feu R (2006) Recent changes in body weight and wing length among some British passerine birds. *Oikos*, **112**, 91–101.
- Yukawa J, Kiritani K, Gyoutoku N, Uechi N, Yamaguchi D, 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.