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Effect of temperature on woodwasp (*Sirex noctilio* F.) development and parasitism by the entomopathogenic nematode, *Deladenus siricidicola*

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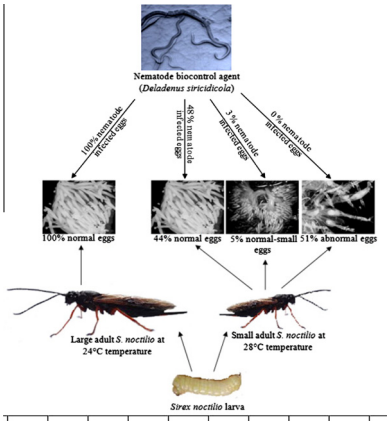
HIGHLIGHTS

- Elevated temperature reduces the developmental period and size of *S. noctilio*.
- Elevated temperature disrupts egg development and maturation of *S. noctilio*.
- Elevated temperature lowers biocontrol agent infection rate of eggs in *S. noctilio*.

GRAPHICAL ABSTRACT



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ABSTRACT

The woodwasp, *Sirex noctilio*, is a significant global pest of exotic pine plantations in the Southern Hemisphere and now threatens native pine forests in North America. Management in Australia relies on biocontrol using the nematode, *Deladenus* (= *Beddingia*) *siricidicola* (Bedding), which infects and sterilises females who then further disperse the nematode. This pest is spreading into warmer regions in Australia and South America and coupled with the threat of global climate change, there is uncertainty as to how increasing temperatures will affect the biocontrol program. *S. noctilio* within nematode-inoculated wood were reared at four temperatures (24, 25.3, 26.6 and 28 °C) to investigate the effects of elevated temperatures on wasp development (emergence time, sex ratio and size), development of eggs (number, size, and maturation) and infection by the nematode. At 24 °C, which reflects current field temperature, *S. noctilio* were bigger in size and all the eggs were normal and all were infected with nematodes. Modest rises in temperature reflecting climate change scenarios resulted in smaller sized *S. noctilio*, disrupted egg development and maturation, and lowered the nematode sterilisation rate in females. Reduced *S. noctilio*

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female body size and egg infection will likely compromise biocontrol by *D. siricidicola* in its current distribution, but disrupted egg development may act directly on the pest, limiting dispersal of *S. noctilio* into subtropical pine plantations and adaptation to climate change.

1. Introduction

The woodwasp, *Sirex noctilio* Fabricius (Hymenoptera: Siricidae), is native to Eurasia and Northern Africa and has invaded several regions of the world such as Australasia (Madden, 1988), South America (Iede et al., 1998), South Africa (Tribe, 1995) and North America (Hoebeke et al., 2005; de Groot et al., 2006). *S. noctilio* primarily attacks stressed trees but can also attack healthy and vigorous trees if the pest population density is high. It has become one of the most economically significant pests of softwood forestry in the Southern Hemisphere (Hurley et al., 2007; Madden, 1988). *S. noctilio* kills trees when it deposits a phytotoxic mucus and a symbiotic fungus, *Amylostereum areolatum* (Chaillet) Boiden (Russulales: Amylostereaceae) during oviposition.

Many biocontrol agents have been used against *S. noctilio* (Taylor, 1976). The most widely used agent is a parasitic nematode, *Deladenus siricidicola* (Nematoda: Sphaerulariidae) (Bedding, 1984), which effectively controls *S. noctilio* when used inundatively (Slippers et al., 2012b). This agent is used in every Southern Hemisphere continent in which *S. noctilio* is a known pest: Australasia, South America and southern Africa. During the flight season, *S. noctilio* adults disperse and females lay eggs in suitable trees (Neumann and Minko, 1981; Ryan and Hurley, 2012). The biocontrol program begins when plots of 10–12 trees are selected where the *S. noctilio* population is likely to be high and treated with a weak herbicide to make them attractive to ovipositing *S. noctilio* (Carnegie and Bashford, 2012; Neumann et al., 1987). Nematodes are then inoculated into these trap trees (Bedding and Iede, 2005; Slippers et al., 2012a), where they infect *S. noctilio* larvae (Bedding, 1967). During pupation, mature female *D. siricidicola* release several thousand juveniles into the haemocoel of the host pupa. These enter eggs of *S. noctilio* before chorions harden, ultimately resulting in adult female *S. noctilio* subsequently distributing non-viable, nematode-filled eggs once they emerge (Bedding, 1972; Bedding and Iede, 2005). The penetration of eggs by the nematodes depends upon host reproductive development and may be regulated by hormonal changes or changes in haemolymph biochemistry taking place within its host body (Bedding, 1972; Lawrence, 1986; Riddiford, 1975). The biocontrol program depends on a high rate (>90%) of nematode infection of eggs (Bedding and Akhurst, 1974). Larger female oviposits more and survive longer thus favouring wide dispersal of the agent in the local pest population (Bedding, 2009; Bruzzone et al., 2009).

In Australia, *S. noctilio* is spreading north into warmer climates (Carnegie and Bashford, 2012) and is predicted to be able to establish in these warmer temperatures (Carnegie et al., 2006). Coupled with expected increases in temperature attributed to climate change, there is uncertainty how increasing temperatures will affect *S. noctilio* biocontrol. An increase in global average surface temperature of 0.74 °C (1906–2005) (Solomon et al., 2007) has already caused shifts in the structure and distribution of ecological communities at different levels (Parmesan and Yohe, 2003; Walther et al., 2002). Temperature is the primary driver of insect developmental rates, digestion, diapause and voltinism and also has a strong influence on larval and adult behaviour, including flight and fecundity (e.g. Heinrich, 1993; Hou and Lee, 1984; Speight et al., 2008). In insects, metabolic rate generally doubles with a 10 °C increase in temperature (Clarke and Fraser, 2004; Gillooly et al., 2001) and this may reduce developmental time and may result in higher population growth rates if voltinism, survivorship or fecundity are increased (e.g. Bale et al., 2002; Caldeira et al., 2002; Gan, 2004; Wermelinger and Seifert, 1999). Epidemics of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins in British Columbia (Raffa et al., 2008) and the pine processionary moth, *Thaumetopoea pityocampa* Den & Schiff, in Europe (Battisti et al., 2005, 2006) are recent examples. More specifically, Villacide and Corley (2012) have shown that the spread of *S. noctilio* in Argentina is related to higher mean temperature and increased frequency of drought periods. Recently, Lantschner et al. (2014) showed the rate of spread of *S. noctilio* increased with increasing mean annual temperature and isothermality.

Temperature affects insect growth and development directly via insect physiology and indirectly by effects on plant food quality, including increased tree stress (Lexer et al., 2002) and subsequent susceptibility to attack (Mattson and Haack, 1987; Rouault et al., 2006). Temperature also affects insect size, leading to smaller insects at higher temperatures (Atkinson, 1994). Temperature may also affect insect reproductive development, for example, yellow dung flies, *Scathophaga stercoraria* L. laid smaller eggs when reared at higher temperatures (Blanckenhorn, 2000). In another study on rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, elevated temperatures delayed reproductive development (Shi et al., 2007).

The life cycle of *S. noctilio* is temperature dependent (Madden, 1981). Temperature also affects the developmental rates of *S. noctilio*. In warmer climates, *S. noctilio* completes its life cycle in as little as three months where it is observed to exhibit up to two generations per year (Neumann et al., 1987). In colder climates, the life cycle takes several years. At temperatures below 6.8 °C, egg hatching is delayed and larval development is prolonged for up to three years (Corley and Villacide, 2012; Madden, 1981). The temperature range for complete development of *S. noctilio* is 12.5–33.5 °C, with a 60% mortality rate at 33.5 °C (Madden, 1981). Accelerated development of *S. noctilio* at higher temperatures may also affect its reproductive development by accelerating egg chorion hardening or by more general changes to egg development and maturation. This may lead to asynchrony between the timing of the release of biocontrol nematodes and the availability of eggs at a suitable stage for infection.

The aim of this study was to determine if elevated temperature affected nematode parasitism of *S. noctilio* eggs, as well as examining the direct effect of temperature on *S. noctilio* adult emergence, body size and egg production. To achieve this aim, sections of trunk ('billets') from trap trees infested with *S. noctilio* were incubated at 24 °C, reflecting typical maximum field temperature, and at three higher temperatures representing warmer climates and possible climate change conditions. Emergence time, sex ratio, size, nematode parasitism and egg development and maturation of adult *S. noctilio* were determined. The effect of temperature on egg size and number of eggs produced by each adult female was also investigated.

2. Materials and methods

2.1. Study site and trap tree plot selection

A total of 15 trap tree plots (= 150 trees) were established between December 2011 and January 2012 in six commercial

P. radiata plantations (Canobolas State Forest, 33.39°S 149.02°E; Gurnang State Forest, 33.96°S 149.85°E; Jenolan State Forest, 33.75°S 150.04°E; Pennsylvania State Forest, 33.81°S 149.20°E; Sunny Corner State Forest, 33.30°S 149.87°E; Vittoria State Forest, 33.40°S 149.32°E) in the Central Tablelands region of New South Wales, Australia. Mean maximum temperature for the localities was 24 °C (Bureau of Meteorology, Australia). Trees were treated with herbicide in a manner to mimic standard forestry practice for the establishment of trap tree plots (see Carnegie and Bashford, 2012). Briefly, an axe cut was made at the base of each tree approximately 100 cm from the ground and 5 ml of DiCamba (3,6-dichloro-2-methoxybenzoic acid), a broad-spectrum systemic herbicide injected into the wound. The herbicide caused the trees to die slowly, making them attractive oviposition sites for gravid *S. noctilio*. In May–June 2012 trees were cut and felled and nematodes (Kamona strain supplied by Ecogrow Environment Pty Ltd.) were then inoculated into these trap trees according to standard procedures (see Bedding and Iede, 2005). Briefly, holes (10 mm deep) were made every 30 cm with one row along the entire length of the tree trunk using hammer punch. Nematodes suspended in 1% polyacrylamide gel were inoculated into these holes (approximately 2000 nematodes/hole). Inoculated trees were then left in the forests until early summer.

2.2. Collection of billets

S. noctilio infested billets (50–60 cm long) were collected from trap tree plots. Criteria for selecting trees included presence of resin beads on the bark of the trees (typical external symptoms of *S. noctilio* oviposition) and observation of *S. noctilio* larvae in a sub-sample of the billets from each tree after being split with an axe. Selected trees were then cut using a chainsaw and a total of 336 billets were collected from 84 trap trees. This occurred by early September 2012 when *S. noctilio* larvae were at an early stage of development, and approximately 4 months after trap trees were inoculated with nematodes. Billets were transferred to the laboratory and the cut ends of each were sealed with paraffin wax to prevent moisture loss and then stored at 4–7 °C and 90 ± 5% relative humidity to slow the growth rate of insect larvae until used in temporally replicated experiments.

2.3. Billet allocation and temperature selection

Four constant temperatures (24, 25.3, 26.6 and 28 °C) were used. The lowest temperature was selected to reflect the mean maximum field temperature whilst higher temperatures reflected possible global warming change and to indicate how temperature will affect the capacity for *S. noctilio* to spread into warmer climates in Australia. Humidity in all incubators was 75 ± 5% (mean ± SE) with 12L:12D lighting. Twenty-eight billets were placed in each incubator on two shelves in an upright position. Individual billets were covered with a wire mesh sleeve to confine wasps on emergence. The billets were examined every day for *S. noctilio* adults until no emergence had occurred for three weeks. Each run was completed within 13 weeks. In all temporal runs same aged billets were used.

2.4. Emergence and measurement of S. noctilio

Emerging adult *S. noctilio* from each incubator-temperature were collected individually in labelled insect collecting tubes and the date of emergence and sex recorded. Prothorax width of *S. noctilio* was measured with a digital Vernier Calliper (Kincrome, Scoresby, Australia).

2.5. S. noctilio parasitism

Insects were placed in a freezer for approximately five minutes to immobilize them and then dissected with micro scissors and tweezers under stereoscopic microscopy (10×). Magnification was then increased to 20× to examine for the presence or absence of nematodes within the haemocoel, testis, ovaries and eggs and location of nematodes in the ovaries of females (within or outside of eggs). Males were considered parasitised if the nematodes were present in the haemocoel and testis. For females nematode parasitism was categorised as: (i) fully sterilised (nematodes present in haemocoel, ovaries and in all eggs), (ii) partially sterilised (nematodes present in haemocoel, ovaries and only in half (53.3198% ± 7.4483) of the eggs), (iii) unsterilised (nematodes absent from all eggs but present in haemocoel and ovaries). Male and female *S. noctilio* with no nematode penetration/infection were considered unparasitised.

Eggs found within the ovaries of female *S. noctilio* were of different age and morphology, so eggs were categorised as: (i) normal (fully developed mature eggs with a clearly visible chorion), (ii) normal-small (developed mature eggs with visible chorion but smaller in size (approximately 1000 × 250 μm) and (iii) abnormal (poorly developed eggs with no visible chorion) (see Fig. 4). Abnormal eggs were variable and irregular in shape and size (e.g. bulb-like structures) and not separated within the ovarioles to qualify as a separate egg. Eggs were counted at 40× magnification. For abnormal eggs, the whitish bulb-like structure (see Fig. 4d and e) within a single ovariole was considered to be one egg.

Length and maximum width of 10 randomly selected eggs from each female were measured at 40× magnification. All eggs from parasitised *S. noctilio* were dissected at 60× magnification under transmitted light to check for the presence of nematodes.

2.6. Experimental design and statistical analysis

The experiment used a randomised block design in which the four temperatures were assigned randomly to four incubators in each of three temporal runs. Multiple billets ($n = 28$) from trees at different localities were randomly assigned to each incubator though each tree was represented by a billet within each incubator for a given run. Trees and localities were regarded as random effects. Analyses indicated that 45.8% of the variance in days to emergence was attributable to the temporal runs. One way ANOVA was used to test for an effect of temperature on *S. noctilio* days to emergence, sex ratio, prothorax width, and nematode parasitism of male and female *S. noctilio*, egg production in parasitised and unparasitised *S. noctilio*, differences in egg development, nematode infection and size of eggs in different categories. Effect of nematode parasitism on egg development and maturation in response to temperature was tested using two way ANOVA for parasitised and unparasitised *S. noctilio*. Bonferroni was used to separate the temperature means.

3. Results

3.1. Emergence and measurement of S. noctilio

There was a significant effect of temperature on the emergence timing of *S. noctilio* ($F_{3,6} = 12.03$, $p = 0.006$) (Fig. 1). There was a significant difference in the days to emergence of males and females ($F_{1,8} = 5.62$, $p = 0.045$). At all temperatures males (56.96 ± 0.94, mean ± SE) began to emerge earlier than females (60.01 ± 0.97, mean ± SE) (Fig. 1). There was no significant interaction between temperature and sex on the days to emergence of *S. noctilio* ($F_{3,8} = 1.39$; $p = 0.315$).

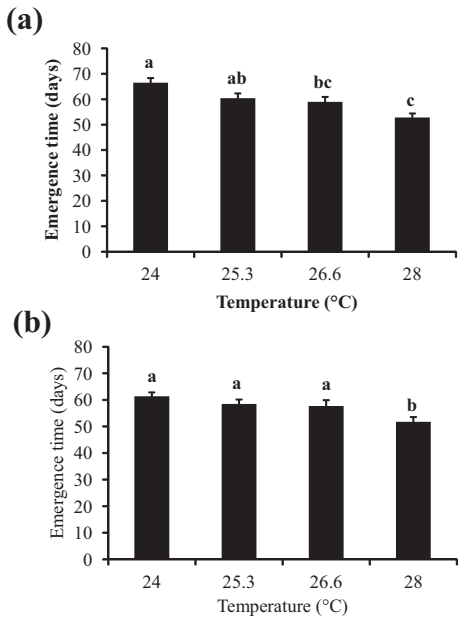

Fig. 1. Effect of temperature on adult *S. noctilio* emergence. (a) Female; (b) male.

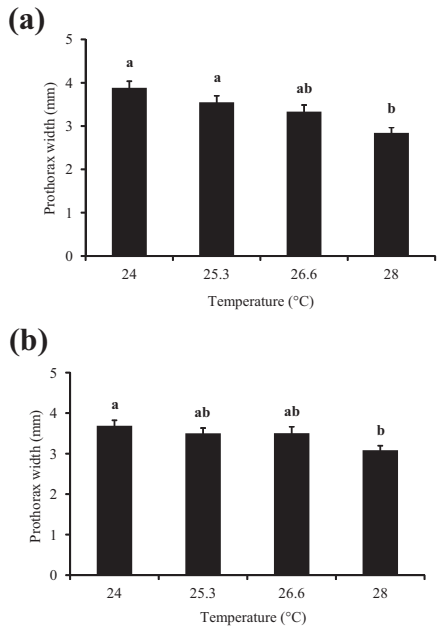

Fig. 2. Effect of temperature during larval development and metamorphosis on adult *S. noctilio* prothorax width (mean ± SE): (a) female; (b) male.

The male to female ratio was above 1 for all temperatures, 1.07 ± 0.11 (mean ± SE) at 24 °C; 1.45 ± 0.49 at 25.3 °C; 1.26 ± 0.16 at 26.6 °C and 1.14 ± 0.03 at 28 °C but there was no significant ($F_{3,6}$ = 1.22; p = 0.382) effect of temperature on the sex ratio.

Prothorax width of female *S. noctilio* from 24 and 25.3 °C was significantly greater than from 28 °C ($F_{3,165}$ = 10.34; p < 0.0001) (Fig. 2a). Prothorax width of male *S. noctilio* from 24 °C was significantly larger than those from 28 °C ($F_{1,105}$ = 5.00; p = 0.027) (Fig. 2b).

3.2. S. noctilio parasitism

There was no significant effect of temperature on the percent parasitism of female ($F_{3,6}$ = 0.98; p = 0.463) relative to male ($F_{3,6}$ = 0.85; p = 0.506) *S. noctilio*. However, there was a significant effect of temperature on the incidence of nematodes within the three categories of parasitised female *S. noctilio* ($F_{3,6}$ = 57.90; p < 0.0001). The percentage of fully sterilised female *S. noctilio* was significantly ($F_{3,6}$ = 10.38; p = 0.009) lower at 28 °C than at lower temperatures (Fig. 3a). Percentage of partially sterilised female *S. noctilio* increased significantly ($F_{3,6}$ = 7.02; p = 0.013) with an increase in temperature (Fig. 3b). Similarly, the percentage of unsterilised female *S. noctilio* was significantly ($F_{3,6}$ = 9.09; p = 0.012) higher at 28 °C than at lower temperatures (Fig. 3c). No partially or unsterilised *S. noctilio* emerged from 24 °C (Fig. 3b and c).

All female *S. noctilio* from 24 °C had ovaries with normal eggs (Fig. 4a; Table 1), whereas females from 25.3, 26.6 and 28 °C had ovaries with normal, normal-small and abnormal eggs (Fig. 4c–e; Table 1). Some (n = 4) females from 28 °C had ovaries with no clearly separated ovarioles, and eggs were absent (e.g. Fig. 4f).

The number of eggs in parasitised (n = 134) and unparasitised (n = 35) *S. noctilio* decreased significantly ($F_{3,130}$ = 18.05; p < 0.0001 and $F_{3,31}$ = 5.88; p = 0.003, respectively) with increase in temperature (Fig. 5). At 24 °C and 28 °C eggs in parasitised *S. noctilio* were significantly ($F_{1,41}$ = 6.45; p = 0.015 and $F_{1,135}$ = 4.10; p = 0.048, respectively) less than in unparasitised *S. noctilio* (Fig. 5, Table 1). However, the numbers of eggs did not differ at intermediate temperatures 25.3 and 26.6 °C (Fig. 5).

Temperature also affected development of eggs in both parasitised and unparasitised *S. noctilio* but the effect of elevated temperatures was more noticeable in parasitised *S. noctilio* ($F_{3,165}$ = 23.82; p < 0.0001) (Table 1). The number of normal eggs in unparasitised *S. noctilio* was significantly higher than parasitised *S. noctilio* at 24 °C ($F_{1,40}$ = 6.447; p = 0.015) and 28 °C ($F_{1,51}$ = 9.382; p = 0.003) (Table 1). Number of normal eggs did not differ at intermediate temperatures. The number of normal-small eggs did not differ

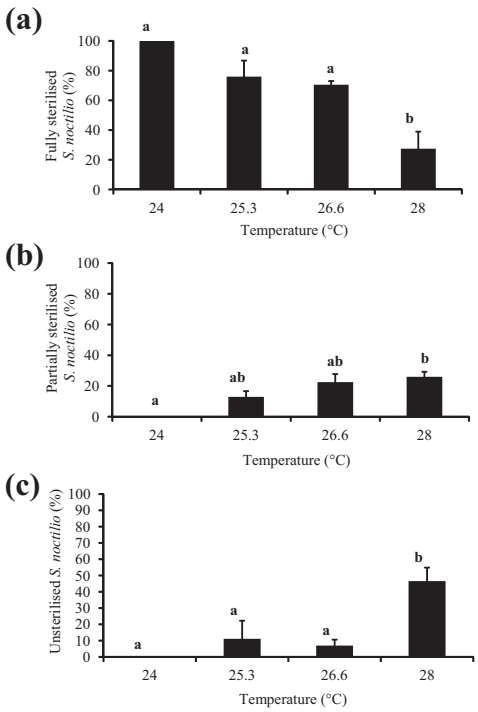

Fig. 3. Effect of temperature during larval development and metamorphosis on parasitism (mean ± SE) of *S. noctilio*: (a) fully sterilised – nematodes within haemocoel, ovaries and in all eggs; (b) partially sterilised – nematodes within haemocoel, ovaries and in some eggs; (c) Unsterilised – nematodes within haemocoel and ovaries, but absent from eggs.

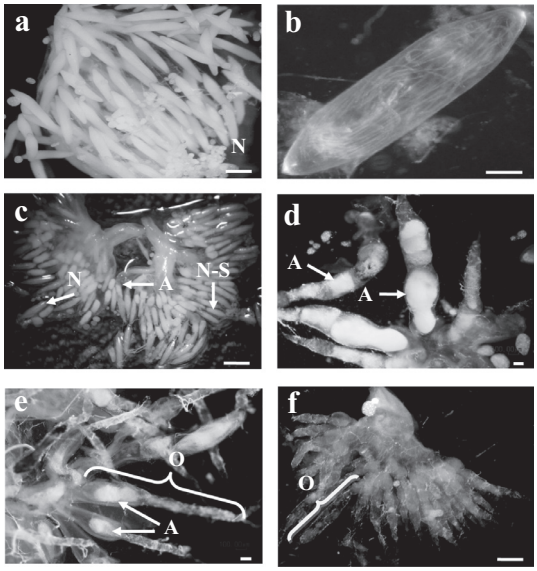

Fig. 4. Eggs of *S. noctilio*; (a) normal eggs; (b) parasitised egg; (c) ovaries with combination of normal (N), normal-small (N-S) and abnormal (A) eggs; (d and e) ovarioles (O) that contain abnormal eggs and no mature eggs can be seen; (f) ovaries with no eggs within the ovarioles (Scale bar = 100 μm).

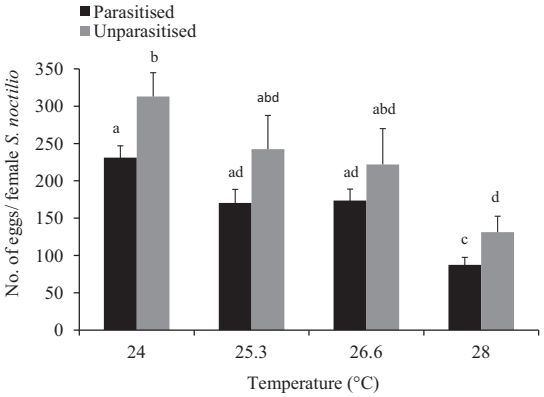

Fig. 5. Effect of temperature on total number of eggs per parasitised and unparasitised (mean ± SE) *S. noctilio*.

significantly ($F_{1,7}$ = 0.86; p = 0.538) between parasitised and unparasitised *S. noctilio* (Table 1). However, the number of abnormal eggs in parasitised *S. noctilio* was significantly higher than unparasitised *S. noctilio* at three elevated temperatures ($F_{1,7}$ = 6.66; p < 0.0001) (Table 1).

Egg development within unparasitised *S. noctilio* differed significantly differed at all the temperatures ($F_{15,140}$ = 26.3311; $p \leqslant 0.0001$) (Table 1). The number of normal eggs at 24 °C did not differ from intermediate temperatures but significantly (p < 0.0001) higher than 28 °C. The number of normal-small and abnormal eggs did not at any temperature (Table 1). There was no difference recorded in the total number of egg at 24, 25.3 and 26.6 °C, whereas significant (p < 0.0001) difference was noticed at 28 °C (Table 1).

Egg development within parasitised *S. noctilio* significantly differed at all the temperatures ($F_{15,538}$ = 53.919; p = < 0.0001) (Table 1). The number of normal eggs at 24 °C was significantly (p < 0.0001) higher than other three elevated temperatures. There was no difference in the number of normal-small and abnormal eggs at three elevated temperatures (Table 1). Total number of egg at 24 °C was significantly (p < 0.0001) higher than 28 °C (Table 1).

Temperature influenced the ability of the nematodes to infect eggs in parasitised *S. noctilio*. Total percentage of nematode infection of eggs decreased significantly with increase in temperature ($F_{3,130}$ = 27.46; p < 0.0001) (Table 1). There was a significant difference in the nematode infection of normal eggs in parasitised *S. noctilio* at different temperatures ($F_{3,130}$ = 12.53; p < 0.0001) (Table 1). At 24 °C all the normal eggs were 100% infected by the nematodes but this percentage decreased to 48.88% at 28 °C. There was also a significant ($F_{3,130}$ = 4.12; p = 0.008) difference in the nematode infection of normal-small eggs with increase in temperature (Table 1). The highest number of normal-small eggs infected by the nematodes was at 26.6 °C, and the lowest at 28 °C. None of the abnormal eggs were penetrated or infected by the nematodes (Table 1).

The size of eggs in the normal, normal-small and abnormal categories differed significantly in length ($F_{2,347}$ = 658.83, p < 0.0001) and width ($F_{2,347}$ = 13.09, p < 0.0001) (Fig. 6a and b). Abnormal eggs were significantly shorter (p < 0.0001) and wider (p < 0.0001) than normal and normal-small eggs (Fig. 6a and b).

Table 1
Effect of temperature on the development of eggs in parasitised and unparasitised *S. noctilio* and incidence of the nematode in host eggs for parasitised *S. noctilio*.

Temperature (°C)	Eggs category	Eggs per female (unparasitised)	Eggs per female (parasitised)	Eggs infected by the nematode in parasitised *S. noctilio* (%)
24	Normal	313.00 (31.77) a∗	231.16 (15.60) a	100
	Normal-small	0.00 b	0.00 f	–
	Abnormal	0.00 b	0.00 f	–
	Total eggs	313.00 (31.77) a∗	231.16 (15.60) a	100
25.3	Normal	239.33 (44.44) ad	148.63 (19.14) c	82.25 (6.70)
	Normal-small	3.17 (3.17) be	17.53 (10.82) b	8.46 (4.77)
	Abnormal	0.00 b∗	4.18 (2.91) b	0.00
	Total eggs	242.50 (45.14) ac	170.34 (18.16) c	85.22 (6.18)
26.6	Normal	208.33 (46.12) ag	144.83 (17.74) c	84.73 (6.29)
	Normal-small	12.50 (11.53) bh	13.93 (8.56) b	18.84 (6.25)
	Abnormal	1.17 (1.17) b∗	14.90 (6.81) b	0.00
	Total eggs	222.00 (47.86) af	173.67 (15.23) ac	84.02 (5.02)
28	Normal	105.33 (24.91) bfg∗	38.27 (9.40) be	48.88 (7.77)
	Normal-small	11.25 (6.72) b	4.32 (2.72) b	3.36 (2.47)
	Abnormal	14.67 (6.32) b∗	44.85 (8.78) bd	0.00
	Total eggs	131.25 (21.29) cdefgh∗	87. 44 (9.93) de	35.44 (7.00)

Note: Values are given as mean with SE in parentheses.
Bonferroni is applied separately for each column (unparasitised and parasitised female *S. noctilio*).

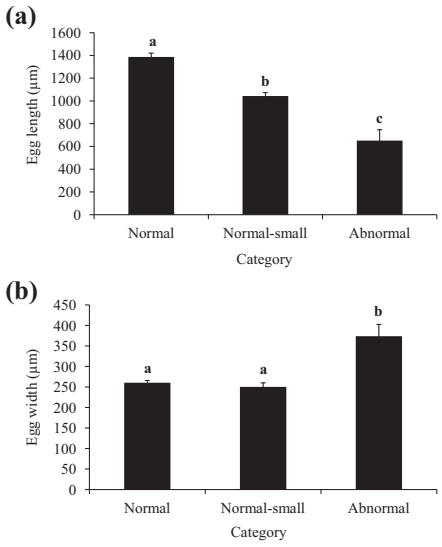

Fig. 6. Size of different categories of egg (mean ± SE) of *S. noctilio* (see text for detail): (a) length; (b) width.

4. Discussion

This study is the first to demonstrate the negative effects of higher temperature on the development of *S. noctilio*, development and maturation of eggs and nematode parasitism and infection. Temperature influenced timing of emergence of *S. noctilio*, development, maturation and nematode infection of eggs. Nematode infection of normal and normal-small eggs decreased with increase in temperature. Egg development and maturation in parasitised *S. noctilio* was negatively influenced by temperature increase.

We found that *S. noctilio* emerged earlier when reared at temperatures higher than 24 °C. The early emergence rate and decrease in developmental time could be due to increase in insect metabolic rate (e.g. Bale et al., 2002; Gillooly et al., 2001), possibly reflecting faster cell division (Van der Have and de Jong, 1996). Male *S. noctilio* emerged prior to females within each temperature treatment in our study, reflecting male protandry (Rawlings, 1948; Morgan and Stewart, 1966). More males emerged than females at all temperature treatments but there was no effect of temperature on the sex ratio of *S. noctilio*. The sex ratio was close to one which is consistent with the findings of Carnegie et al. (2005) in NSW, Australia and Eskiviski et al., 2004 (1.4:1–2:1) in Argentina but different from other studies (where *S. noctilio* is also invasive) reporting a male biased sex ratio such as 2:1 in Victoria, Australia (Collett and Elms, 2009), 20:1 in New Zealand (Zondag and Nuttall, 1977), 1.5:1–32:1 in Brazil (Iede et al., 1998) and 12:1 (Hurley et al., 2008) from South Africa. Sex ratio in *S. noctilio* depends upon haplodiploid females that produce female off-spring only if mated (Neumann et al., 1987). A ratio closer to one is preferable for the spread of the nematodes because nematodes are only spread by females (Collett and Elms, 2009).

Results show that elevated temperature negatively affected size (prothorax width) of *S. noctilio*. This effect, known as the "temperature-size rule" is common in ectotherms, where insects' size reduces at higher temperatures and increases at lower temperatures (Atkinson, 1994; Kingsolver and Huey, 2008). At higher temperatures rate of metabolism in insects increases (Von Bertalanffy, 1960) which affects somatic growth (Perrin, 1995) leading to smaller size. Rapid developmental rate, small body size and weight are often also related to lower fecundity (Kant et al., 2012). In *S. noctilio*, adults do not feed and depend on the nutrients acquired during the immature stages of their development to sustain reproductive activities. Larger females are preferable for the success of the biocontrol program (Collett and Elms, 2009) as they make longer flights, can survive longer, probably visit more trees, and are more fecund (e.g. Bedding and Iede, 2005; Bruzzone et al., 2009), which is important for optimum distribution of nematodes by parasitised *S. noctilio* (Bedding, 2009). A decrease in *S. noctilio* size also influenced egg development at higher temperatures which may directly, negatively, influence biocontrol success. High temperatures are not conducive to the population expansion of *S. noctilio*.

Temperature did not affect levels of *S. noctilio* parasitism (= nematode within *S. noctilio*) and this may be because nematodes had already penetrated larvae before sections of trees were exposed to different temperatures. However, increased temperature directly affected the ability of nematodes to fully sterilise the host (= nematodes in eggs). At 24 °C all of the eggs within parasitised *S. noctilio* were infected by nematodes but the percentage decreased as the temperature increased. Higher temperatures resulted in more partially sterilised and unsterilised *S. noctilio* compared to lower temperatures. More partially and unsterilised *S. noctilio* were present at 28 °C than at lower temperatures. This could be due to the disrupted reproductive development of *S. noctilio* at elevated temperatures. Findings show that increased temperature affects egg production, development and maturation of eggs in *S. noctilio*. Egg numbers decreased as the temperature increased from 24 to 28 °C. Furthermore, egg development and maturation in *S. noctilio* was also affected. At 24 °C all the eggs within *S. noctilio* ovaries were normal, whereas the number of normal eggs gradually decreased with increase in temperature and at the highest temperature of 28 °C fewer normal eggs and more abnormal eggs were found. At intermediate temperatures more normal-small eggs were found in ovaries of *S. noctilio*. Abnormal eggs may be already sterile and/or unviable and may not be laid in trees during oviposition.

Insect size and fecundity at maturity are positively correlated and this could be one of the factors that impacted egg production and development in *S. noctilio* at higher temperatures. Reduction in numbers of normal eggs could be due to the small size of adults that emerged from higher temperatures because the temperature size rule may also apply to eggs, such that females maintained at lower temperatures produce larger egg sizes, and that larger adult size is correlated with large egg size (Honek, 1993). Elevated rearing temperature increases development and growth rates, thus shortening development time (thus time to adult or reproduction). At elevated temperatures metabolic rates increase which may cause depletion of resources affecting insect size and reproductive output (Steigenga and Fischer, 2007), as well as potentially decrease the rate of egg development (e.g. Isenhour and Yeargan, 1981).

The biocontrol nematode, *D. siricidicola*, did not penetrate or infect abnormal eggs and was present only within eggs that were normal or normal-small. This could affect the success of biocontrol program by limiting the availability of nematode infected eggs for inoculation by *S. noctilio*. At 24 °C, nematodes had infected all the normal eggs, but with an increase in temperature nematode infection of normal eggs also decreased. Furthermore, the incidence of nematode presence within normal-small eggs declined with increased temperature. The capacity of the nematodes to infect eggs depends upon host reproductive development. Juvenile nematodes migrate towards host ovaries and enter eggs just before the egg chorion hardens. This response of nematodes may be mediated by hormonal changes taking place within the host body (Bedding, 1972; Lawrence, 1986; Riddiford, 1975). A recent study by Kroll et al. (2013) showed that nematodes failed to enter eggs of *S. noctilio* with smaller pronatum width and with lower egg numbers which indicates that nematode activity is dependent on its

host development. Elevated temperatures might have disrupted the synchrony between the timing of the release of biocontrol nematodes and the availability of eggs at a suitable stage for infection either by affecting the biology of *S. noctilio*, nematodes or both. The life cycle of *D. siricidicola* is also temperature dependent; the eggs of the free-living stage (found in wood) cannot survive at 27 °C, while 30 °C was found to be lethal for all nematode life stages including those found inside host insects (Akhurst, 1975). A direct effect of temperature on the reproductive development of *S. noctilio* and the response of the nematodes has yet to be reported and the mechanisms involved require further study.

Reproductive development in unparasitised *S. noctilio* was also affected by temperature. Egg number and development also decreased with increase in temperature in unparasitised *S. noctilio*. However, the effect of temperature on egg number and development was more severe in parasitised *S. noctilio* than unparasitised *S. noctilio*. There were more eggs in unparasitised *S. noctilio* than parasitised *S. noctilio* at all temperatures. The numbers of normal eggs were lower in parasitised *S. noctilio* than unparasitised at all temperatures. Abnormal eggs were higher in parasitised *S. noctilio* than unparasitised at elevated temperatures. This could be due to the presence of nematodes within the insect body where they not only infect host reproductive organs but also consume fat reserves that influence reproductive development by suppressing ovarial development and also influence size, total flight distance and velocity (Bedding and Iede, 2005; Villacide and Corley, 2008; Corley and Villacide, 2012). Therefore, the precise mechanism underlying the temperature dependent changes in *S. noctilio* reproductive development and nematode parasitism remain to be identified.

5. Conclusion

Our work is the first to show the effect of temperature on *S. noctilio* emergence, egg number, development and maturation and its effect on nematode parasitism. The development of *S. noctilio* is temperature dependent and elevated temperatures (particularly 28 °C) negatively affected egg number and development, influencing fitness and population dynamics of *S. noctilio*. Furthermore, elevated temperature affected the ability of the biocontrol nematode, *D. siricidicola*, to fully infect *S. noctilio* eggs, thus fewer nematode-infected eggs are produced for inoculation into new trees by dispersing females. Coupled with the negative effect on size of adult *S. noctilio,* this could have the important consequence of leading to a more general diminution in the spatial and temporal distribution of nematode inoculum. This finding, though based on laboratory rather than field studies, gives important information on the effect of temperature on *S. noctilio* parasitism by *D. siricidicola* and signals a potentially adverse effect on biocontrol by this globally important nematode agent. Since an estimated increase in temperature of +4 °C is predicted in Australia over the next century (Christoff, 2013), these effects could be realised. Irrespective of any effect of climate change, the present results indicate that if *S. noctilio* is able to adapt to and establish in warmer regions, new strains or even new species of biocontrol agents will need to be deployed.

Notwithstanding the implications of these results for biocontrol, the observation of direct, negative effects of elevated temperature on *S. noctilio* demand further investigation. If the observed negative effects on size and, more especially, egg development are evident in future field studies there are important implications for the pest. First, its geographical range could shift towards the poles and lower elevation gradients in response to warming temperatures. Second, recent reports of range expansion into warmer areas and risk to sub-tropical forestry species may be curtailed by climate change. Related to these two major conclusions, it is important to conduct work to gain a better understanding of the mechanisms responsible for these temperature-dependent changes, i.e., whether these changes are due to change in the biochemical composition of the haemocoel of *S. noctilio* or the synthesis of proteins linked to reproduction. It is also important to know whether female *S. noctilio* with ovaries carrying normal-small and abnormal eggs can oviposit these eggs into trees or whether elevated temperature results in aborted oviposition. It is also of interest to know whether nematodes which are located outside the eggs are deposited into trees during oviposition as occurs with other species of siricid wasps and *Deladenus* nematodes.

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