Investigation of Relationships Between Temperature and Developmental Rates of Tick *Ixodes scapularis* (Acari: Ixodidae) in the Laboratory and Field

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ABSTRACT Relationships between temperature and preoviposition, preeclosion, and premolt developmental periods for the tick *Ixodes scapularis* Say were investigated by holding field-collected ticks in the laboratory at temperatures of 0 to 32°C at constant daylength. The duration of these developmental periods decreased significantly with increasing temperature. Host of origin, prior storage at 4°C, and season of collection of the ticks were also significantly associated with variations in the duration of the preoviposition period. For each developmental stage, the effect of temperature on development rate was best described as a power relationship. Laboratory-derived relationships were used to predict dates for molting, oviposition, and eclosion of engorged larvae and nymphs, engorged adult females and egg masses, respectively, placed in the field during 1989–1992. Predicted dates for oviposition by adult females, eclosion of eggs, and molting of engorged larvae were within 2 wk of the observed dates, and field-observed seasonal activity of questing larvae and nymphs also was predicted well by laboratory data. Molting of engorged nymphs and seasonal activity of questing adult ticks were, however, poorly predicted. Our findings suggest that duration of development in the field, of larvae from engorged adult females, and of nymphs from engorged larvae, may be explained largely by temperature effects alone, whereas emergence of adult *I. scapularis* from engorged nymphs may depend on temperature-independent diapause phenomena. The significance of these findings for understanding current and future distributions of *I. scapularis*, and of the pathogens it transmits, is discussed.

THE IXODID TICK *Ixodes scapularis* Say is the vector of a number of tick-borne zoonoses, including Lyme borreliosis, human granulocytic ehrlichiosis, and human babesiosis (Thompson et al. 2001). Our power to predict the geographic and temporal occurrence of these pathogens, and the potential for changes in their distribution with projected climate changes, depends in part on our understanding of the phenology of the tick. The timing of the seasonal activity of different tick instars can be crucial to the existence of endemic cycles of zoonotic tick-borne pathogens. For example, coincident seasonal activity of uninfected larval and infected nymphal *Ixodes ricinus* Linné ticks favors the occurrence of endemic cycles of tick-borne encephalitis virus, because infections are very short-lived in rodent reservoir hosts (Randolph et al. 1999). In contrast, it is thought that the occurrence of endemic cycles of *Borrelia burgdorferi* s.s. (the agent of Lyme borreliosis), which persistently infects rodent reservoir hosts, may be favored where infective *I. scapularis* nymphs are active earlier in the year than are uninfected larvae (Yuval and Spielman 1990).

Interstadial development rates of ixodid ticks are determined in part by temperature, with rates generally increasing, usually nonlinearly, with increasing temperature (Branagan 1973, Chilton and Bull 1994, Peavey and Lane 1996, Randolph 1997, Randolph et al. 2002). However, simple predictions of the seasonality of ticks from relationships between developmental rates and temperature are often confounded by diapause phenomena, i.e., delayed development (morphogenetic diapause) or delayed host-seeking activity (behavioral diapause). Cues for the onset and termination of diapause may be complex and include changes in daylength and exposure to different maxima and minima of temperature (Madder et al. 1999, Belozerov et al. 2002, Randolph et al. 2002). It is important to elucidate the extent to which diapause

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phenomena, rather than simpler relationships between tick development and temperature, control the phenology of an ixodid tick species. The relative importance of these factors may determine the potential for the tick to extend its geographic range in response to climate change, and the potential consequences of climate change in regions where the tick already exists.

It is thought that the development of engorged nymphal *I. scapularis* to adult ticks is in part controlled by diapause induced by effects of daylength on either host seeking or engorged nymphs (Belozerov and Naumov 2002). The direct effects of temperature on developmental rates of *I. scapularis*, however, have not been fully investigated. In this study, we investigated in the laboratory the effects of temperature on developmental rates of each stage of *I. scapularis* (preoviposition period of engorged adult females, preeclosion period of egg masses, and development of engorged larvae and nymphs to the next instar) under conditions of constant daylength (12-h light:dark). We then compared the power of these laboratory findings to predict the patterns of tick development observed at a field site in Ontario, Canada, at the northern edge of the geographic range of *I. scapularis.*

Materials and Methods

Incubation of Ticks in the Laboratory. Adult *I. scapularis* females were field collected from an established population at Long Point, Ontario, from autumn 1989 through autumn 1992. The ticks engorged on raccoons, *Procyon lotor*, and dogs as described previously (Lindsay et al. 1998). Engorged adult females were temporarily stored in 18.5-ml polystyrene vials for 8–28 d (mean 10.5 d) at 4°C and ≥95% humidity before being transferred to experimental conditions.

The effect of temperature on deposition of eggs by adult *I. scapularis* females was evaluated by allocating ticks to individual containers placed in environmental chambers operated within ± 1°C of the following temperatures: −10°C (n = 12), 0°C (n = 9), 4°C (n = 13), 8°C (n = 14), 10°C (n = 4), 12°C (n = 28), 16°C (n = 14), 20°C (n = 112), 24°C (n = 28), 28°C (n = 16), and 32°C (n = 16). A greater number of ticks was incubated at 20°C to expedite production of larvae for other experiments. Humidity was maintained at ≥95% and diel periods were maintained at a photoperiod of 12:12 (L:D) h. All adult females remained at the initial temperature of exposure until death occurred. Tick survival was assessed daily, except for adult females held at −10°C, which were observed at 1- or 2-h intervals for up to 12 h. Adult females were considered dead if movement (of legs or internal organs) was not observed after ticks were moved to room temperature (20°C) and exposed to 10 short breaths from the observer. Adult females also were observed daily for the presence of eggs. The interval from introduction of adult females to a given temperature to when the first eggs were observed was considered the preoviposition period (POP). The date when the first eggs were observed therefore marked the beginning of the oviposition period.

Eggs produced by each adult female were gently removed each week until the ticks died. The egg masses produced each week from each female were placed in a new, labeled vial, and then randomly allocated to chambers operated at 8, 10, 12, 16, 20, 24, 28, and 32°C. All chambers were at ≥95% humidity with diel periods of 12:12 (L:D) h. Egg masses were observed daily for evidence of hatching. Observations continued until all eggs within each egg mass seemed to have hatched. Any egg masses that failed to produce larvae were discarded 2 mo after eggs held under comparable conditions had emerged. The preeclosion period (PEP) was defined as the interval from when eggs were first deposited to when larvae were first observed.

Unfed *I. scapularis* larvae were hatched in the laboratory from eggs deposited by adult females that engorged on dogs during November 1991 and April 1992. These larvae were allowed to engorge on laboratory-reared white-footed mice and hamsters. Nymphs were obtained from fed larvae that had been held at 24°C until the molt; unfed nymphs were then fed on laboratory-reared mice or hamsters. Engorged larvae and nymphs were transferred to environment chambers maintained at 8, 12, 16, 20, 24, 28, 30, or 32°C, all at ≥95% RH with diel periods of 12:12 (L:D) h. Fed larvae and nymphs were observed daily for ecdysis; the interval from when ticks were placed at a given temperature until molting was observed was considered the premolt period.

Statistical Analysis. Four outcome variables were investigated: the length in days of the preoviposition period for engorged adult females, the preeclosion period for egg masses, and the premolt periods for engorged larvae and nymphs. Temperature was the explanatory variable in all cases. Multivariable models that included tick collection period (spring or autumn for each collection year), host species, and duration of tick storage at 4°C before the start of experiments as additional explanatory variables, were used when preoviposition and preeclosion periods were the outcomes. Regression models were fitted in STATA version 6.0 for Windows (STATA Corporation, College Station, TX), and temperature and outcome were compared directly and after logarithmic and natural logarithmic transformations to investigate potential power and exponential relationships. The relationships that give the best fit were considered as those that had the highest R^2 values and did not violate regression model assumptions (StataCorp 1999). To predict tick molting dates in the field (see below), we used the equations from "best fit" relationships between duration of development and temperature. The estimates for these equations were based on regression models in which temperature only was the explanatory variable, because ticks placed in the field were obtained from the same range of hosts during the same seasons and subject to the same variations in storage and handling as ticks investigated in the laboratory. Temperature also was investigated as a fac-

torized variable for each outcome in multivariable regression models that also included the nontemperature variables when POP and PEP were the outcomes. In these models, forward and backward substitution and elimination of dummy variables for each incubation temperature, yielded an F-test statistic for the significance of differences in developmental rates at different temperatures. The level of statistical significance was $P < 0.05$.

Comparison between Developmental Rates in the Laboratory and Field. Lindsay et al. (1998) performed detailed studies of *I. scapularis* POP, PEP, and developmental rates for engorged larvae and nymphs, at field sites at Long Point, Ontario (42° 36′ N; 80° 5′ W). The ticks used in that field study and the laboratory studies described herein were collected from the same sources, at the same time, and for the most part, from the same hosts. Engorged larvae and nymphs placed in the field had fed on the same laboratory animals as those described for larvae and nymphs in the current study. This provided an opportunity to compare the simple relationships between temperature and rates of development observed in the laboratory, with rates observed in the field. The ticks placed in the field would have been exposed to varying temperatures and stimuli such as varying daylength that may have additional effects on development via diapause phenomena (Belozerov and Naumov 2002).

The inverse of the developmental rate, from the temperature–development relationships obtained in the laboratory studies described above, gives the daily fraction of development possible if the tick is held at a particular temperature, assuming temperature is the only determinant of developmental rates. Weekly mean ground-level temperatures to which the ticks placed in the field by Lindsay et al. (1998) were exposed were obtained only during the years 1991 and 1992. Each day of each week of these years was assigned the mean temperature recorded for that week. For periods earlier and later than 1991–1992, we used, as an approximation, the mean monthly normal temperatures recorded for 1961–1990 at the Port Dover meteorological station (42° 47′ N; 80° 13′ W), the station nearest to the Long Point study site for which such data are available from Environment Canada (http://www.msc.ec.gc.ca/climate/climate_normals_1990/index_e.cfm). For these periods, each day of each month of the year was assigned the mean normal temperature for that month. Using these temperatures we calculated the fraction of tick development (for each stage: POP, PEP, engorged larva to nymph, and engorged nymph to adult) that would occur on each day of the year. For each day that ticks were placed in the field by Lindsay et al. (1998), we were then able to calculate a predicted date for completion of each developmental stage. This was achieved by adding the fraction of development predicted to take place on the release date to the fraction occurring in each subsequent day, until the sum equaled unity. Lindsay et al. (1998) examined ticks in the field at ≈2-wk intervals and for our comparisons between predicted and observed dates for completion of development, each "predicted date" we quote in the Results is the first possible field observation date after the actual date predicted for development.

Lindsay et al. (1998) used their field data on tick development, and those of Yuval and Spielman (1990), to explain how the tick phenology may have given rise to observed seasonal tick activity in the same field site (Lindsay et al. 1998, 1999a,b). During 1991 and 1992, questing tick and temperature data were collected simultaneously from the same field site at Long Point, Ontario (Lindsay et al. 1999a). Therefore, using the same method as described above, we also were able to obtain predictions for the emergence of cohorts of larvae, nymphs, and adults (knowing the seasonal activity periods for the previous tick stage) by using contemporaneously collected temperature data. These were then compared with the field observations. Tick activity periods detected by dragging for questing ticks were largely corroborated by counts of ticks on hosts (Lindsay et al. 1999a).

Throughout all comparisons (field development and questing activity), we used field data from the maple forest habitat at Long Point, the habitat in which tick survival was greatest (Lindsay et al. 1998).

Results

Analysis of Laboratory Data. All engorged adult female ticks held at −10°C died within 4 h. None of the adult females held at 0°C laid eggs but they survived a mean 133 (±51.9) days. Overall, 69% of adult female ticks held at temperatures >0°C survived to lay eggs. The POP declined from a mean 230 d at 4°C to a mean 12 d at 32°C (Table 1). The eggs produced by adult females maintained at 32°C were misshapen and none produced larvae. This observation suggested that maintenance at 32°C induced pathological effects in the ticks, and the data were not included in statistical analyses. Variations in POP with temperature were significant in all models ($P < 0.001$ in all). When temperature was treated as a continuous variable, a power relationship (i.e., $\log_{10}$ transformation of both temperature and POP) proved the best fit ($R^2 = 0.68$) compared with exponential ($R^2 = 0.61$) and linear ($R^2 = 0.51$) relationships, and the latter significantly violated model assumptions (Cook–Weisberg $X^2 = 166$, $P < 0.001$). In multivariable models $\log_{10}$ POP varied significantly with $\log_{10}$ temperature (coefficient = −1.772, SE = 0.078, $P < 0.001$), the POPs of adult females fed on raccoons were significantly shorter than the POPs of adult females that fed on dogs (coefficient = −0.128, SE = 0.024, $P < 0.001$), the POPs of adult females collected in autumn were significantly shorter than those of adults collected in spring (coefficient = −12.85, SE = 1.85, $P < 0.001$), and storage was associated with significantly longer POPs (coefficient = 0.016, SE = 0.003, $P < 0.001$). Examination of residuals indicated that POP increased linearly with duration of storage over the range of storage periods to which ticks were exposed in this study. When season was accounted for, year of col-

Table 1. Survival of engorged adult female *I. scapularis* ticks and egg masses, and the durations of the preoviposition and preeclosion periods (POP and PEP) at different temperatures

Incubation temp. (°C)	No. females surviving to oviposition (%)	POP (mean ± SE)	No. egg masses producing larvae (%)	PEP (mean ± SE)
−10	0/12 (0)	—	—	—
0	0/9 (0)	—	—	—
4	3/13 (23)	230.00 (8.00)a	0/3 (0)	—
8	12/14 (86)	86.36 (3.59)b	0/12 (0)	—
10	4/4 (100)	44.75 (5.06)c	0/4 (0)	—
12	24/28 (83)	34.40 (2.18)d	20/24 (83)	122.03 (1.60)a
16	13/14 (96)	19.75 (1.87)e	9/13 (69)	58.13 (2.44)b
20	73/112 (65)	21.82 (1.18)f	58/73 (79)	49.07 (3.29)c
24	17/28 (60)	17.76 (3.19)f	17/21 (81)	24.60 (1.06)d
28	12/16 (75)	12.75 (1.46)f	2/12 (17)	18.50 (3.50)d
32	4/4 (100)	12.00 (2.40)	0/4 (0)	—

Values in the same column that are followed by the same letter were not significantly different from one another ($P < 0.05$).

lection was not significant ($F = 1.86$, $P = 0.17$). In a multivariable model where temperature was factorized (and season of collection, storage and tick host were accounted for), the POPs at each temperature were significantly different from one another ($P < 0.001$), except for POPs at 20, 24, and 28°C, which were not significantly different from one another ($F = 1.46$, 0.05, and 1.42; $P = 0.23$, 0.82, and 0.23 respectively; Table 1). In the bivariate relationship as used in comparisons with field observations, POP (in days) = $1{,}300 \times \text{Temp}^{-1.427}$ (SEs 1.26 for intercept and 0.083 for coefficient, $P < 0.001$; Fig. 1a).

The duration of oviposition varied with incubation temperature, from 4 to 5 wk at 20°C or more, to ≥7 wk at temperatures <20°C. Egg masses produced by ticks held at 4 and 8°C were small, suggesting that oviposition was incomplete. None of the egg masses maintained at 8 or 10°C produced larvae after 329 d of observation, nor did any of the egg masses maintained at 32°C. However, eggs deposited at 10°C did eventually produce larvae after having been transferred to a temperature of 24°C. The proportion of surviving egg masses declined with increasing temperature, but death of egg masses was associated with infection by an unidentified fungus. After the first larvae were observed, emergence generally proceeded rapidly and was completed in 2–3 wk at all temperatures. The PEP declined from a mean of 122 d at 12°C to a mean of 18.5 d at 28°C (Table 1). Variations in PEP with temperature were significant in all models ($P < 0.001$ in all). When temperature was treated as a continuous variable rather than as a factor, a power relationship (i.e., $\log_{10}$ transformation of both temperature and PEP) proved the best fit ($R^2 = 0.91$) compared with exponential ($R^2 = 0.82$) and linear ($R^2 = 0.83$) relationships, and the latter significantly violated model assumptions (Cook–Weisberg $X^2 = 8.2$, $P < 0.01$). In a multivariable model $\log_{10}$ PEP varied significantly with $\log_{10}$ temperature (coefficient = −2.42, SE = 0.09 $P < 0.001$), PEPs of egg masses laid by adult females collected in autumn were significantly shorter than those of adults collected in spring (coefficient = −12.88, SE = 2.06, $P < 0.001$), but there were no significant differences in PEP associated with the host species on which the adult females fed, nor with storage times of adults preincubation ($F = 1.67$ and 0.24 and $P = 0.20$ and 0.63, respectively). In a multivariable model in which temperature was factorized (and in which collection season was accounted for), the PEPs at each temperature were significantly different from one another ($P < 0.001$), except for PEPs at 24 and 28°C, which were not significantly different from one another ($F = 0.44$ and $P = 0.51$ for both; Table 1). In the bivariate relationship as used in comparisons with field observations, PEP (in days) = $34{,}234.4 \times \text{Temp}^{-2.271}$ (SEs 1.30 for intercept and 0.090 for coefficient, $P < 0.001$; Fig. 1b).

The molting success of engorged larvae and nymphs varied with temperature, none of the larvae or nymphs maintained at 8 or 32°C molted, and none of the nymphs maintained at 12°C molted by the last observation day (day 200 for nymphs) from the start of incubations. Molting success was greatest among larvae held at 28°C and nymphs held at 24°C (Table 2). The premolt period decreased with increasing temperature, although the premolting periods of both larvae and nymphs held at 30°C were longer that premolting periods of these ticks when maintained at 28°C (Table 2). This suggested that maintenance of the ticks at 30°C had pathological effects on the ticks, and these data were not included in statistical analyses of relationships between temperature and premolt period.

In all models, the premolt periods for larvae and nymphs varied significantly with incubation temperature ($P < 0.001$ in all). In the models where temperatures were factorized, premolt periods were significantly different at each incubation temperature ($P < 0.001$ for all), except for the premolt periods of larvae held at 24 and 28°C, which were not significantly different ($F = 0.47$ and $P = 0.49$ for both). When temperature was treated as a continuous variable rather than as a factor, power relationships (i.e., log transformation of both temperature and premolt periods) proved the best fit ($R^2 = 0.88$ and 0.83 for larvae and nymphs, respectively) compared with exponential ($R^2 = 0.83$ and 0.80) and linear ($R^2 = 0.76$ and 0.78) relationships. The latter relationships significantly vi-

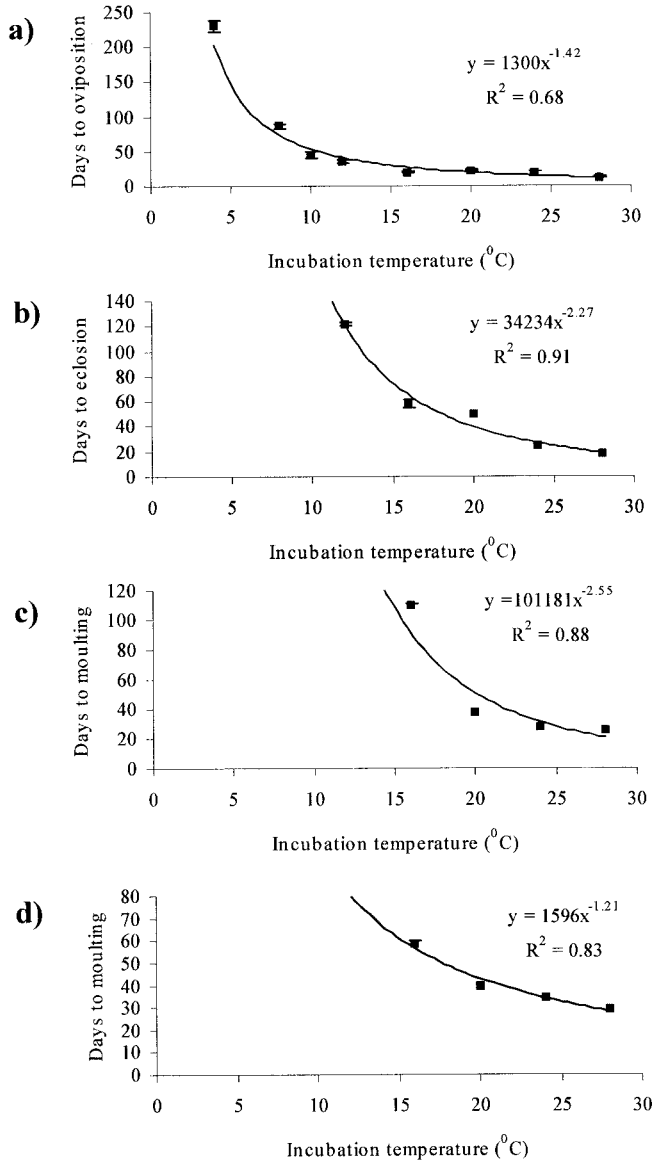

Fig. 1. Duration of development for *I. scapularis* ticks held at different temperatures in the laboratory. (a) Preoviposition period of engorged adult females. (b) Preeclosion period for egg masses. (c) Premolt period of engorged larvae. (d) Premolt period of engorged nymphs. The fitted curves, equations for the relationship between temperature and development (in days), and R^2 values are shown. Values are shown ± SE.

olated model assumptions (Cook–Weisberg $X^2 = 16.6$ and 4.0, $P < 0.001$ and = 0.04, respectively). The bivariate relationship as used in comparisons with field observations were, premolt period (in days) = $101{,}181 \times \text{Temp}^{-2.547}$ for larvae (SEs 1.38 for intercept and 0.104 for coefficient, $P < 0.001$; Fig. 1c), and premolt period = $1{,}596 \times \text{Temp}^{-1.208}$ for nymphs (SEs 12.44 for intercept and 0.080 for coefficient, $P < 0.001$; Fig. 1d).

Predicted versus Observed Data for Tick Development in Field Experiments. Due to the nature of the relationships between temperature and development observed in the laboratory, and because ticks under snow are not subject to air temperatures (Lindgren and Gustafson 2001), the proportion of development that would occur on days when the air temperature was 0°C or less was set at zero. In nearly all cases, predicted dates for oviposition by adult female ticks, and eclosion of their egg masses, came within 2 wk of the dates observed in the field (Table 3). The exception was the preoviposition period for adult females placed in the field on 15 November 1990, which were first produced eggs on 11 May 1991, whereas the predicted date was 6 June 1991.

Table 2. Survival of engorged larval and nymphal *I. scapularis* ticks and the durations of their premolt periods at different temperatures

Incubation temp. (°C)	No. larvae that molted (%)	Premolt period for larvae (± SE)	No. nymphs that molted (%)	Premolt period for nymphs (± SE)
8	0/30 (0)	—	0/30 (0)	—
12	8/30 (27)	198.9 (5.8)a	0/30 (0)	—
16	8/30 (27)	109.6 (8.1)b	4/30 (16)	59 (0.6)a
20	12/30 (40)	38.1 (2.2)c	5/30 (17)	39.8 (0.8)b
24	31/45 (69)	27.5 (0.6)d	32/45 (71)	34.4 (0.5)c
28	23/30 (77)	25.7 (1.1)d	17/30 (57)	29.0 (1.0)d
30	14/30 (46)	27.9 (1.6)	4/30 (13)	76.0 (1.4)
32	0/30 (0)	—	0/30 (0)	—

Values in the same column that are followed by the same letter were not significantly different from one another ($P < 0.05$).

Engorged larvae placed in the field between 22 April and 3 July 1992 all molted between 31 July and 22 September of the same year. The predicted dates for the start of molting of these ticks were either within the range of dates observed or, at worst, 2 wk early (Table 4; Fig. 2a). All of the engorged larvae placed in the field from 12 August to 9 December 1992 delayed molting until the following year, and nymphs were first observed between 18 June and 19 August 1993. The predicted dates for molting of these ticks were all within the observed period except for engorged larvae placed in the field on 12 August 1992, when the predicted date for molting was 1 mo earlier than that observed (Table 4; Fig. 2a). Some of the engorged larvae placed in the field on 15 July and 28 1992 molted in the same year, and the predicted dates fell within the observed molting periods. However, some of these ticks did not molt until the following year, and (for ticks placed in the field on 28 July) such delayed molting was anticipated when predictions were made using the lowest standard error for the relationship between temperature and development. No engorged larvae were predicted to molt between 19 November 1992 and 1 April 1993.

All engorged nymphs placed in the field between 22 April 1992 and 4 June 1992 molted between 14 August and 20 October of the same year. Predicted dates for molting of these ticks were at best 2 wk early but at worst 2 mo early (Table 5; Fig. 2b). All of the engorged nymphs placed in the field between 12 August and 9 December 1992 molted between 7 July and 19 August of the following year. Predicted dates for molting of these ticks were between 2 wk and 3 mo earlier than those observed, whereas ticks placed in the field on 12 August 1992 were all predicted to molt by 5 November of the same year at the latest. Some of the engorged nymphs placed in the field from 17 June to 28 July 1992 delayed molt until summer 1993, when the predicted molting dates all fell within 1992.

Comparison of Observed and Predicted Seasonal Abundance of Questing Ticks. In the studies of Lindsay et al. (1999a,b), larvae were active at the field site from April to October with two peaks: one from mid-May to early July and the other from mid-July to September. The later peak was thought to comprise newly hatched ticks, whereas the earlier peak was thought to comprise overwintered larvae that had failed to find a host the previous year. Nymphs were active from April to late September with peak activity in June and July. It was thought that most nymphs were overwintered, either as engorged larvae or as molted nymphs that had failed to find a host the previous year. Some nymphs active later in the year

Table 3. Comparison of observed and predicted preoviposition and preeclosion periods for engorged adult females and egg masses, respectively, placed in the field at Long Point, Ontario

Date ticks were placed in the field	Date development was first observed in the field	Predicted date for development (SE)
	Preoviposition period	
2 Nov. 1989	8 May 1990	23 May 1990 (23 May–10 June 1990)*
15 Nov. 1990	11 May 1991	6 June 1991 (22 May–6 June 1991)*
18 April 1991	22 May 1991	6 June 1991 (22 May–6 June 1991)
15 Nov. 1991	8 May 1992	21 May 1992 (21 May–5 June 1992)
18 Nov. 1992	22 May 1993	5 June 1993 (20 May–4 June 1993)*
	Preeclosion period	
8 May 1990	19 July 1990	6 July 1990 (6 July–19 July 1990)*
11 May 1991	16 July 1991	4 July 1991 (4 July–16 July 1991)
22 May 1991	16 July 1991	16 July 1991 (4 July–16 July 1991)
8 May 1992	31 July 1992	17 July 1992 (17 July–31 July 1992)
22 May 1993	24 July 1993	9 July 1993 (7 July–24 July 1993)*

SE describes the range of predicted dates possible using the lowest and highest values of the standard errors of the relationships between temperature and development observed in the laboratory.

* indicates that predicted dates for completion of development were obtained using temperature data from Port Dover meteorological station.

Table 4. Comparison of observed and predicted dates for molting of engorged larvae placed in the field at Long Point, Ontario

Date ticks were placed in the field	First and last dates development was observed in the field	Predicted date for development (SE)
22 April 1992	31 July 1992 to 28 Aug. 1992	31 July 1992 (17 July–14 Aug. 1992)
6 May 1992	14 Aug. 1992 to 11 Sept. 1992	31 July 1992 (17 July–14 Aug. 1992)
19 May 1992	14 Aug. 1992 to 22 Sept. 1992	31 July 1992 (17 July–28 Aug. 1992)
4 June 1992	14 Aug. 1992 to 11 Sept. 1992	14 Aug. 1992 (31 July–11 Sept. 1992)
17 June 1992	14 Aug. 1992 to 22 Sept. 1992	28 Aug. 1992 (14 Aug.–11 Sept. 1992)
3 July 1992	28 Aug. 1992–22 Sept. 1992	11 Sept. 1992 (14 Aug.–22 Sept. 1992)
15 July 1992†	11 Sept. 1992 to 20 Oct. 1992 & 18 June 1993 to 19 Aug. 1993	11 Sept. 1992 (28 Aug.–10 Oct. 1992)
28 July 1992†	11 Sept. 1992 to 22 Sept. 1992 & 18 June 1993 to 19 Aug. 1993	10 Oct. 1992 (11 Sept.–19 Nov. 1992 & 6 May–20 May 1993*)
12 Aug. 1992	18 June 1993 to 19 Aug. 1993	20 May 1993 (10 Oct.–19 Nov. 1992 & 1 April–18 June 1993)*
26 Aug. 1992	18 June 1993 to 19 Aug. 1993	18 June 1993 (4 June–7 July 1993)*
9 Sept. 1992	18 June 1993 to 19 Aug. 1993	7 July 1993 (18 June–24 July 1993)*
21 Sept. 1992	18 June 1993 to 19 Aug. 1993	7 July 1993 (7 July–24 July 1993)*
7 Oct. 1992	18 June 1993 to 19 Aug. 1993	24 July 1993 (7 July–19 Aug. 1993)*
20 Oct. 1992	18 June 1993 to 19 Aug. 1993	24 July 1993 (7 July–19 Aug. 1993)*
4 Nov. 1992	18 June 1993 to 19 Aug. 1993	24 July 1993 (7 July–19 Aug. 1993)*
17 Nov. 1992	18 June 1993 to 19 Aug. 1993	24 July 1993 (7 July–19 Aug. 1993)*
9 Dec. 1992	18 June 1993 to 19 Aug. 1993	24 July 1993 (7 July–19 Aug. 1993)*

SE describes the range of predicted dates possible using the lowest and highest values of the standard errors of the relationships between temperature and development observed in the laboratory.

* indicates that predicted molting dates were obtained using temperature data from Port Dover meteorological station.

† indicates that some of the engorged larvae placed in the field on the specified date did not molt until the following year.

were thought to have been newly molted from larvae that fed in the same year. Adults were active in two peaks: newly molted adults being active from late September to early December, with those that failed to find a host at this time being active from March through June of the following year.

The 1991 cohort of adult ticks was active from 10 October 1991 to 7 July 1992. Predictions of POP, PEP, with oviposition periods of 1 mo and 2 wk for autumn- and spring-feeding adults, respectively, resulted in a predicted period for larval hatching of 6 July to 7 August 1992 for this cohort of adults. The observed period of questing activity for this cohort of larvae was 31 July to 25 September 1992 (Fig. 3a).

Larvae feeding from 2 June to 18 August 1991 were predicted to produce nymphs between 18 July and 17 November of the same year, i.e., for the most part the nymphs arising from these larvae could have joined the tail end of questing nymph activity observed in late summer and autumn of the same year. Spring feeding larvae in 1992 (from 2 June to 27 July) also were predicted to molt into nymphs in the same year during the period 8 August to 23 September, corresponding with a small second peak observed in

Table 5. Comparison of observed and predicted molting dates for engorged nymphs placed in the field at Long Point, Ontario

Date ticks were placed in the field	First and last dates development was observed in the field	Predicted date for development (SE)
22 April 1992	14 Aug. 1992 to 11 Sept. 1992	03 July 1992 (19 June–17 July 1992)
06 May 1992	11 Sept. 1992 to 09 Oct. 1992	03 July 1992 (03 July–17 July 1992)
19 May 1992	28 Aug. 1992 to 22 Sept. 1992	17 July 1992 (03 July–31 July 1992)
04 June 1992	14 Aug. 1992 to 20 Oct. 1992	31 July 1992 (17 July–31 July 1992)
17 June 1992†	28 Aug. 1992 to 20 Oct. 1992	31 July 1992 (31 July–14 Aug. 1992)
03 July 1992†	28 Aug. 1992 to 22 Sept. 1992	14 Aug. 1992 (14 Aug.–28 Aug. 1992)
15 July 1992†	11 Sept. 1992 to 20 Oct. 1992	28 Aug. 1992 (28 Aug.–11 Sept. 1992)
28 July 1992†	21 Sept. 1992 to 09 Oct. 1992	11 Sept. 1992 (11 Sept.–10 Oct. 1992)
12 Aug. 1992	07 July 1993 to 19 Aug. 1993	10 Oct. 1992 (22 Sept.–05 Nov. 1992)
26 Aug. 1992	07 July 1993 to 19 Aug. 1993	05 Nov. 1992 (20 Oct.–9 Dec. 1992 and 03 April–23 April 1993*)
09 Sept. 1992	07 July 1993 to 19 Aug. 1993	06 May 1993 (23 April–20 May 1993)*
21 Sept. 1992	07 July 1993 to 19 Aug. 1993	04 June 1993 (06 May–04 June 1993)*
07 Oct. 1992	07 July 1993 to 19 Aug. 1993	04 June 1993 (04 June–18 June 1993)*
20 Oct. 1992	07 July 1993 to 19 Aug. 1993	18 June 1993 (04 June–07 July 1993)*
04 Nov. 1992	07 July 1993 to 19 Aug. 1993	18 June 1993 (18 June–07 July 1993)*
17 Nov. 1992	07 July 1993 to 19 Aug. 1993	18 June 1993 (18 June–07 July 1993)*
09 Dec. 1992	07 July 1993 to 19 Aug. 1993	18 June 1993 (18 June–07 July 1993)*

SE describes the range of predicted dates possible using the lowest and highest values of the standard errors of the relationships between temperature and development observed in the laboratory.

* indicates that predicted molting dates were obtained using temperature data from Port Dover meteorological station.

† indicates that some of the engorged nymphs placed in the field on the specified date did not molt until the following year.

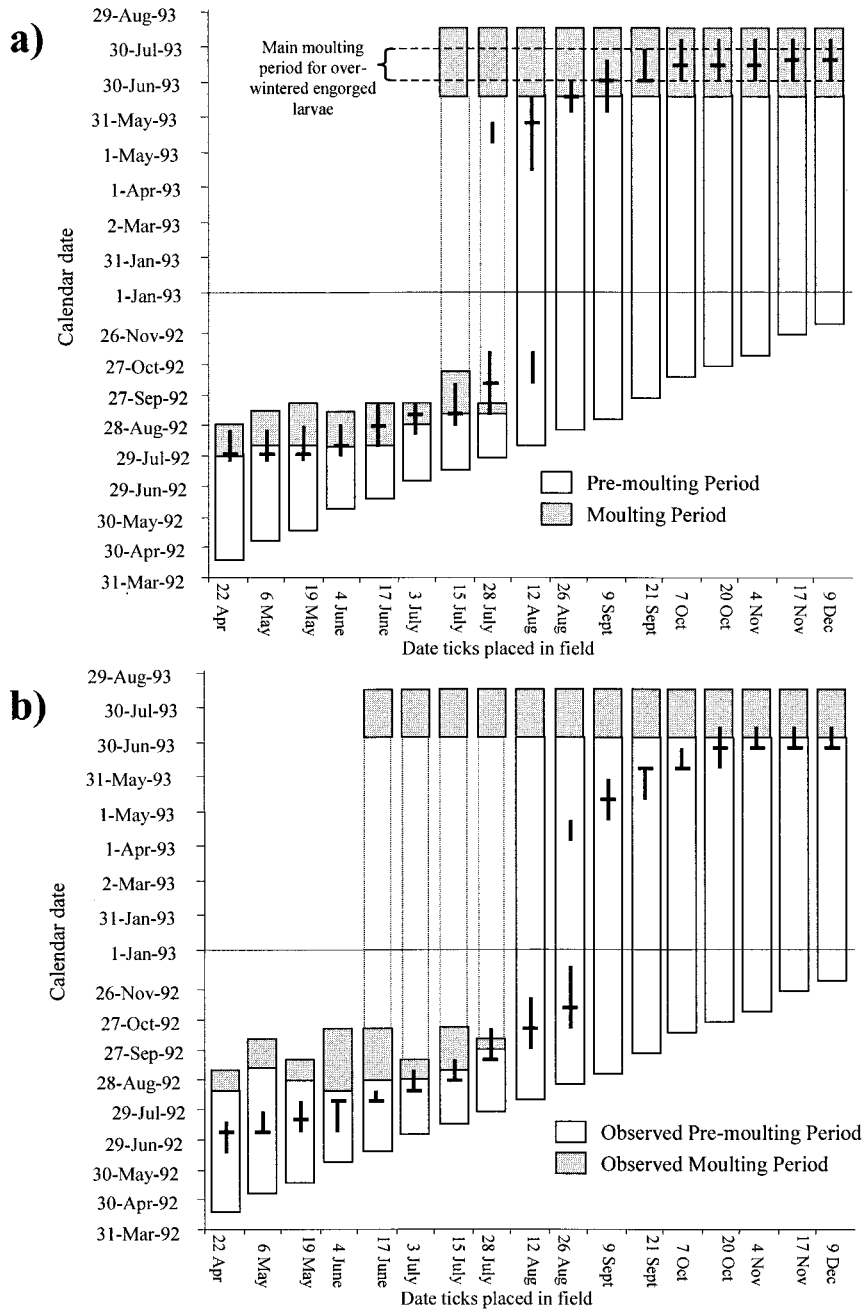

Fig. 2. Comparisons between observed and predicted molting dates of engorged *I. scapularis* larvae and nymphs. The observed premolt periods of engorged larvae (a) and nymphs (b), placed in a field site at Long Point, Ontario, during 1992 (Lindsay et al. 1998), are indicated by unfilled boxes. Their observed molting periods are indicated by stippled boxes for each date on which ticks were placed in the field. Bold horizontal lines within these boxes indicate predicted molting dates using laboratory data. Bold vertical bars within the boxes indicate the range of predicted dates possible using the lowest and highest values of the standard errors of the relationships between temperature and development observed in the laboratory. Predicted molting periods in 1992 were obtained using temperature data from the field site. Predicted molting periods in 1993 were obtained using temperature data from Port Dover meteorological station.

nymphal activity from 24 August to 22 September 1992 (Fig. 3b). Larvae feeding from 19 August to 25 September 1991 were predicted to molt into nymphs between 20 April and 17 May 1992, i.e., at the start of the observed activity period of questing nymphs in 1992 (Fig. 3b).

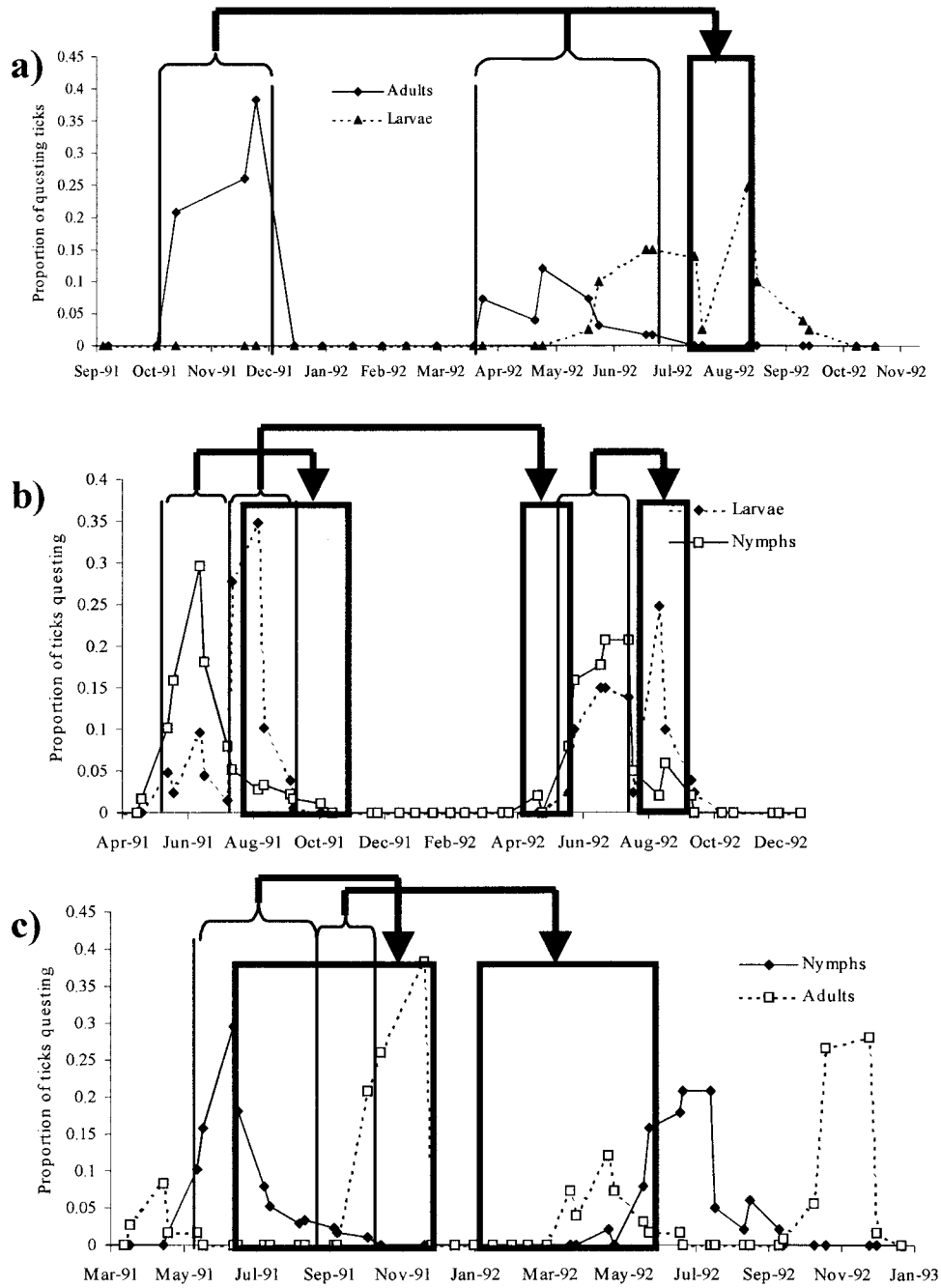

Fig. 3. Comparison of observed and predicted seasonal activity of questing *I. scapularis* ticks for 1991 and 1992 at Long Point, Ontario. (a) Seasonal activity periods for adults and larvae. (b) Seasonal activity of larvae and nymphs. (c) Nymphal and adult. The graphs indicate the number of ticks collected on each sampling occasion, by drag sampling, expressed as a proportion of the total number of that instar collected in the same year. Boxes with bold surround indicate periods of molting (of engorged larvae or nymphs) or hatching (of larvae) predicted for ticks of the previous instar that fed at times of the year delineated by the brackets to which the shaded boxes are linked.

Nymphs that fed from the start of the observed 1991 activity period on 9 May up to those feeding on 7 September 1991 were all predicted to molt into adults in the same year (between 27 June and 14 December 1991). Nymphs that fed after 7 September 1991 were predicted to molt into adults the following year between 11 January and 2 June 1992 (Fig. 3c). In each case, the predicted periods for emer-

gence of adults were considerably different from the periods when questing adults were active on the field site.

Discussion

For each stage of tick development (POP, PEP, and development of engorged immature ticks into the next instar), a power relationship gave the best fit for the decline in development duration with increasing temperature. Such relationships may, however, be approximations of a more complex combination of different relationships (linear or exponential) at different temperature ranges (López-Urrutia 2003). In some cases, duration of development did not differ significantly at different temperatures at the higher end of the range studied. The mean duration of development did decline, however, with increasing temperature across the range studied, suggesting that lack of statistical significance may have been due to sample sizes too low to detect small differences in the duration of development.

The duration of POPs were significantly shorter for ticks collected from raccoons compared with ticks collected from dogs, possibly due to the readiness with which raccoons acquire resistance to *I. scapularis* ticks (Craig et al. 1996). Acquired host resistance to ticks may result in reduced size of developing ticks (Trager 1939). In the laboratory, the developmental times of *I. scapularis* followed the general relationship between body mass and developmental rates (Gillooly et al. 2002) and increased with increasing size of the tick stage (from fertilized ovum to engorged nymph). Why there should be differences between activity seasons in the duration of both POP and PEP is not clear but could relate to seasonal variations in tick size (Ogden et al. 2002, Randolph et al. 2002). The duration of the POP at temperatures >4°C increased linearly with the duration of preincubation storage at 4°C. This suggests a carryover effect of low temperature that retards increases in developmental rates when the ticks' environment warms. The effects of storage, host species and season of collection were, however, small compared with the effects of temperature.

Observed and predicted dates for the emergence of eggs, larvae, and nymphs from engorged ticks placed in the field were the same or differed by no >2 wk, except in one case. Due to the sensitivity of detection of oviposition, hatching and molting used in the field, differences between predicted and observed dates were maximally 14 d for these developmental stages. Delayed development of eggs and larvae from adults, and nymphs from engorged larvae placed in the field in late summer and autumn, until spring or summer of the following year, was predicted by the temperature-development relationships alone. The errors around our estimates for the temperature-development relationship predicted to some extent the field observation that at certain times of the year, a proportion of ticks may develop directly, whereas the rest delay development until the following year. Small errors in our predictions could have been due to differences in recorded temperatures and those to which ticks placed in the field were actually exposed, or to small errors in the temperature–development relationship obtained in the laboratory. Nevertheless, the temperature–development relationships derived from the laboratory data seemed to explain well the duration of development of larvae from engorged adult females and of nymphs from engorged larvae.

Temperature data collected contemporaneously with observations of tick development in the field provided better predictions than did the approximated temperatures from Port Dover meteorological station. The approximated temperature data often did, however, predict development with reasonable accuracy, which underlines the robustness of the power relationship, between temperature and developmental times, to short-term fluctuations in temperature. At higher temperatures (>20°C), large increases in temperature would produce small changes in developmental times. At lower temperatures (<5°C), small reductions in temperature would produce large changes in the developmental times, but such large changes become biologically unimportant when the developmental times more than span the likely duration of periods in each year when temperatures remain <5°C. Variation in temperatures within relatively narrow ranges over which temperature–development relationships change from approximately exponential to approximately linear (5–10°C for POP and 10–20°C for PEP and molting of engorged larvae) would produce larger and biologically meaningful variations in developmental rates. Variations between geographic locations in the duration of the warmer spring and autumn temperatures compared with the duration of cold winter temperature may therefore have a greater impact on seasonality of immature *I. scapularis* than variations in temperature maxima and minima. The degree of latency in the response of *I. scapularis* developmental rates to changes in temperature observed in the current study also would tend to reduce potential effects of diurnal or even daily fluctuations in temperature. Complex accounting for such temperature fluctuations, as used in some models of ixodid tick development (Gardiner and Gettinby 1981), may not be appropriate for *I. scapularis.*

Predictions for the emergence of larvae and nymphs at Long Point, Ontario, obtained from field observations of the seasonal activity of adults and larvae, also fitted well with observations in the field. The predicted period for emergence of larvae (in summer) and nymphs (in spring) had particularly tight patterns. Larvae were predicted to molt into nymphs in April and May 1991 slightly in advance of the observed period of questing, but temperatures in April and early May of that year (being <15°C) are likely to have delayed host seeking activity of nymphs (Vail and Smith 2002) until the end of May when temperatures increased. A postmolting or hatching delay in questing larvae of up to a month has been observed in *I. scapularis* in the field (Daniels et al. 1996).

Although the development of *I. scapularis* larvae from engorged adult females, and nymphs from en-

gorged larvae, (at Long Point) were largely explained by temperature–development relationships alone, predictions for the emergence of adult ticks from engorged nymphs were much less precise. Predictions for molting of nymphs were always considerably in advance of molting observed in the field and seasonal adult tick activity was poorly predicted from the field-observed seasonal occurrence of questing nymphs. To some extent, the detection of ticks in the field may have been affected by responses of questing adults to temperature (Duffy and Campbell 1994, Vail and Smith 2002), but our findings may suggest that diapause driven by daylength as described by Belozerov and Naumov (2002) is an important determinant of rates at which nymphal *I. scapularis* develop into adults.

The data in the current study suggest that, as temperatures fall, the duration of tick development (when unaffected by diapause) becomes asymptotic with the y-axis (Fig. 1), so predicting precise threshold temperatures at which development ceases is difficult. Nevertheless, molting dates for *I. scapularis* ticks have frequently been predicted with some success by using threshold temperatures (the x-axis intercept of the linearized relationship between temperature and the inverse of developmental duration) and cumulative degree-days (Lindsay et al. 1996, Mount et al. 1997). Such methods have been used in tick population models to generate age-specific life tables (Mount et al. 1997). Our findings, however, do, suggest that seasonal dynamics of *I. scapularis* populations could be modeled by a dynamic simulation model approach (Randolph and Rogers 1997).

Our findings may have implications for understanding the distribution of *I. scapularis*, and the pathogens this tick may transmit, now and in the future in the face of predicted climatic changes. Incubation temperatures >30°C had a consistently detrimental effect on developing ticks, a factor that may constrain the southern range of the tick. Elsewhere, if the seasonal activity periods for larval and nymphal ticks vary with temperature alone, then predicted climate changes may impact on endemic cycles of *I. scapularis*-transmitted pathogens, most of which are maintained in transmission cycles involving small mammal or avian hosts, infective nymphs, and uninfected larvae (Thompson et al. 2001). There is some evidence for variation in immature tick seasonality in the United States that may be consistent with temperature effects on developmental rates. Recorded peaks of seasonal activity of larval *I. scapularis* occur in August and September in the northern United States (in Illinois, Siegel et al. 1991; in Massachusetts, Wilson and Spielman 1985, Lyon et al. 1996; in New Jersey, Schulze et al. 1985; in New York, Daniels et al. 1996, Ostfeld et al. 1996), but from May to July in more southern states (in Georgia, Oliver et al. 1993, Lavender and Oliver 1996; in Maryland, Hofmeister et al. 1999; in Missouri, Kollars et al. 1999).

Any variations in seasonality of nymphs and larvae may be constrained by effects of daylength on diapause and development of nymphs to adults, and the response of questing ticks to temperature (Vail and Smith 2002). Belozerov and Naumov (2002) quote evidence for daylength-sensitive diapause in engorged larval *I. scapularis* not seen in our study. Susceptibility to diapause may vary with location of different populations of the same tick species (Madder et al. 1999), however, and our studies on *I. scapularis* development in the field were at the northern edge of the range of this tick, where long developmental times may swamp developmental diapause of engorged larvae influenced by daylength. Further studies are, therefore, required to investigate the full potential for generalization of our findings to *I. scapularis* throughout its range.

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