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Field ecology of Hunterellus hookeri (Hymenoptera: Encyrtidae), and population dynamics of its host, Ixodes scapularis (Acari: Ixodidae) in southeastern Massachusetts.

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SUZANNE M. LYON

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DEDICATION

To Ken, who has given me so many wonderful reasons to be finished with this work.

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CHAPTER 1

INTRODUCTION

Lyme disease was first recognized in Lyme, Connecticut in 1975 (Steere et al. 1977). Six years later, it was discovered that sera from Lyme disease patients reacted significantly to a spirocheate cultured from the midgut diverticula of the tick *Ixodes dammini* Spielman, Clifford, Piesman and Corwin, collected in New York (Burgdorfer et al. 1982). This spirochaete, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner, is now known to be the etiologic agent of Lyme disease (Johnson et al. 1984). Recently it has been established that *I. dammini* is synonymous with *Ixodes scapularis* Say (Oliver et al. 1993), and that this tick serves as the vector of Lyme disease in the eastern and mid-western United States.

From 1982 through 1992, 47,708 cases of Lyme Disease were reported to the Centers for Disease Control (Centers for Disease Control 1991, 1992 & 1993). With 8,884 Lyme disease cases reported to the C.D.C. in 1991, and 7,863 cases reported in 1992, this disease continues to be a serious public health problem. Incidence of Lyme disease has been highest in the eastern U.S., where 89% of cases of the disease were reported in 1992 (C.D.C. 1993).

Ixodes scapularis is a three host tick with a life cycle that is a minimum of two years in duration. Eggs are laid in the spring, but larvae do not eclose until summer. Larvae obtain blood meals and either overwinter as fed larvae or molt and overwinter as unfed nymphs. Nymphs obtain their blood meal in late spring or summer of the following year and then molt to adults, which feed either in the fall or the following spring (Yuval & Spielman 1990).

White-tailed deer, *Odocoileus virginianus* (Zimmerman) serve as the principal host for adult *I. scapularis* (Piesman et al. 1979, Anderson & Magnarelli 1980, Main et al. 1981). Other important hosts for adult *I. scapularis* are medium-sized mammals such as Virginia opposum, *Didelphis virginiana* (Kerr) and raccoon, *Procyon lotor* (L.) (Fish & Dowler 1989). In numerous studies in the northeast, the white-footed mouse, *Peromyscus leucopus* Rafinesque, has been shown to be the most important mammalian host for *I. scapularis* larvae and nymphs, in part due to the relative abundance of *P. leucopus* (Anderson & Magnarelli 1980, Carey et al. 1980, Piesman & Spielman 1979). Immature *I. scapularis* also feed on a broad range of other hosts (Anderson & Magnarelli 1980, Carey et al. 1980, Main et al. 1982), including *O. virgianus* (Piesman & Spielman 1979, Telford et al. 1988). In addition to serving as an important

tick host, *P. leucopus* is the main reservoir of *B. burgdorferi* in nature (Levine et al. 1985).

Borrelia burgdorferi is transmitted during blood-feeding by *I. scapularis* nymphs and adults via saliva (Riberio et al. 1987). As transovarial transmission of *B. burgdorferi* has been detected in less than 1% of the *I. scapularis* larvae examined (Piesman et al. 1986, Magnarelli et al. 1987) larvae do not pose a significant risk of transmitting Lyme disease. Since *I. scapularis* nymphs and adults vector Lyme disease, there has been much interest in reducing their numbers. Tick control methods include applying acaricides, altering tick habitat, managing tick hosts, and biological control. While each of these control methods has some utility in certain situations, no one method is applicable in all settings. In some circumstances, acaricides can be costly to apply, and non-target effects, and the potential for development of resistance must be considered. In extensive, wooded areas, a self-perpetuating biological control agent would be an environmentally benign and possibly more economically feasible way to reduce human exposure to *I. scapularis*.

There have been no reports of significant invertebrate predators or pathogens of *I. scapularis*. The only known parasitoid of *I. scapularis*, the encyrtid wasp *Hunterellus hookeri* Howard, was first noted parasitizing nymphs of *Rhipicephalus sanguineus*

(Latreille) in Texas (Howard 1908). Another tick parasitoid, *Ixodiphagus caucurtei*, was described in 1912 by DuBuysson from specimens found in *Ixodes ricinus* (L.) nymphs near Paris, France (DuBuysson 1912). In 1934, it was determined that *H. hookeri* and *I. caucurtei* are synonymous (Gahan 1934).

Hunterellus hookeri is now considered to have a cosmopolitan but discontinuous distribution. Collections have been made in the following geographic areas: North America (Howard 1908, Bishopp 1934, Mather et al. 1987, Hu et al. 1993), Central and South America (Wood 1911, da Costa Lima 1915), Africa (Philip 1931, Cooley 1934), Europe (Du Buysson 1912, Klyushkina 1958), India (Timberlake 1922, Shastri 1984) and Asia (Hassan 1976, Cheong et al. 1978).

Species of ticks that have been found to be parasitized by *H. hookeri* in nature include: *R. sanguineus* (Howard 1908, da Costa Lima 1915, Philip 1931, Bishopp 1934, Cheong et al. 1978), *I. scapularis* (Larrousse et al. 1928, Hu et al. 1993), *I. ricinus* (DuBuysson 1912), *Ixodes crenulatus* Koch (Klyushkina 1956), *Hyalomma aegyptium* L. (Cooley 1934), *Hyalomma anatolicum* Koch (Shastri 1984), *Haemaphysalis concinna* Koch (Brumpt 1913), and *Dermacentor variabilis* Say (Larrousse et al. 1928, Bishopp 1934).

The majority of the information that exists on the field ecology of *H. hookeri* involves *D. variabilis* or

Dermacentor andersoni Stiles as hosts. In order to gauge the utility of *H. hookeri* as a biological control agent for *I. scapularis*, I investigated a number of aspects of the field ecology of this parasitoid, relative to that of *I. scapularis*. Factors that were evaluated include: seasonal phenology and host preferences of *H. hookeri*, and parasitism rates of *I. scapularis* nymphs at a site where *H. hookeri* is established.

In a biological control program, it is essential to make accurate estimates of the population density of the targeted species before and after the release of a beneficial agent. With this need in mind, I devised a new technique for estimating absolute numbers of immature *I. scapularis* obtaining blood meals on *P. leucopus* per tick generation. This technique was compared with drag sampling for ticks at three sites in southeastern Massachusetts.

CHAPTER 2

FIELD ECOLOGY OF *HUNTERELLUS HOOKERI* (HYMENOPTERA: ENCYRTIDAE) AND EVALUATION OF ITS IMPACT ON *IXODES SCAPULARIS* (ACARI: IXODIDAE) IN SOUTHEASTERN MASSACHUSETTS

Ixodes scapularis Say is the vector of Lyme disease in the eastern United States, where 89% of the cases of this disease reported to the Centers for Disease Control occurred in 1992 (Centers for Disease Control 1993). Efforts to control ticks have included habitat alteration and use of acaricides. While acaricides continue to be employed, current awareness of problems such as effects on non-target organisms, and the development of multiple- and cross-resistance by arthropods to pesticides adds importance to the investigation of alternative control measures. No significant pathogens or predators of *I. scapularis* have been reported. *Hunterellus hookeri* Howard (Hymenoptera: Encyrtidae) is one of seven known species of parasitoids of ticks (Geevarghese 1977, Bowman et al. 1986). A French strain of *H. hookeri* was released on the Elizabeth Islands in southeastern Massachusetts in 1926 in an attempt to reduce numbers of *Dermacentor variabilis* (Say) (Larrouse et al. 1928). The parasitoid became established and has persisted at the release site (Cobb 1942, Mather et al. 1987).

An attempt to introduce *H. hookeri* to Montana as a biological control agent of *Dermacentor andersoni* Stiles

began in 1927. That program was not successful, possibly due to climatic incompatibility (Cooley & Kohls 1934, Cole 1965). In 1929, the French strain of *H. hookeri* was released on Martha's Vineyard in late summer (Hertig & Smiley 1937) but did not result in establishment. It is possible that these releases were made too late in the season. Also, follow-up work was not done in the years immediately following the releases (Hertig & Smiley 1937). Subsequently, additional releases of *H. hookeri* were made on Martha's Vineyard in 1937-39, using a strain from Texas. A laboratory experiment run concurrently indicated that the Texan strain of *H. hookeri* showed poor survival under maritime New England temperature regimes (Smith & Cole 1943).

Mather et al. (1987) found that the parasitism rate of host-seeking *I. scapularis* nymphs on the Elizabeth Islands decreased from May (37%) to August (13%). Hu et al. (1993) observed the same trend on Prudence Island in Rhode Island. They suggested that either parasitized nymphs begin to quest earlier than unparasitized ones, or that parasitized nymphs leave the pool of questing nymphs sooner than unparasitized ones because they find hosts, or die, more quickly than unparasitized nymphs (Hu et al. 1993). Graf (1979), working with an undescribed species of *Hunterellus* in Ivory Coast, reported enhanced

mortality of unfed *Amblyomma nuttalli* Dönitz nymphs that had been exposed to wasps, as compared with unexposed nymphs.

Cooley (1930) found that when larvae and nymphs of *Dermacentor andersoni* Stiles feeding on rabbits were exposed to *H. hookeri* simultaneously, 59.4% of larvae and 80.9% of the nymphs became parasitized. It was not known if *H. hookeri* would exhibit a similar preference for nymphal *I. scapularis*.

Cooley (1928) reported that if a larval tick is parasitized, wasp eggs do not embryonate until the nymphal blood meal begins. At 22°C, adult wasp emergence occurred an average of an 45.5 days after *D. andersoni* nymphs detached from hosts (Cooley 1928). However, when fed nymphs were maintained at 14°C, parasitoid development required nearly one year (Cooley & Kohls 1934). Parker & Butler (1929) found that in Montana several parasitized *D. andersoni* nymphs, which detached from hosts in late September and were held outdoors, yielded wasps late the following May. In southeastern Massachusetts, if some *H. hookeri* overwinter in nymphs that obtain blood meals late in the season, and emerge as adult wasps in May or June, they would constitute a second generation of the parasitoid. Such a second generation would be difficult to detect in field samples of unfed nymphs.

Adults of *H. hookeri* are short-lived, but females are able to oviposit within minutes of emergence (Cooley 1927). In a biological control program, in order for the full reproductive potential of this parasitoid to be realized, releases would need to be targeted such that the wasps made rapid contact with ticks feeding on hosts. Immature deer ticks feed on a broad array of mammals. In many locations, the white-footed mouse, *Peromyscus leucopus* (Rafinesque) (Piesman & Spielman 1979, Carey et al. 1980, Main et al. 1982) and white-tailed deer, *Odocoileus virginianus* (Zimmerman) (Piesman & Spielman 1979, Main et al. 1981) are the principal hosts for *I. scapularis* larvae and nymphs. In Rhode Island, Hu (1990) found that the rate of parasitism of host-seeking *I. scapularis* nymphs was significantly higher than the parasitism rate of fed nymphs from wild rodents. He found no parasitism in *I. scapularis* larvae fed on wild rodents.

The first objective of this study was to clarify the impact of *H. hookeri* on nymphal *I. scapularis*. This was achieved by reexamining the seasonal trend in parasitism of *I. scapularis* nymphs on the Elizabeth Islands, exploring the possibility of enhanced mortality of parasitized nymphs, and evaluating the potential for this parasitoid to be bivoltine in southeastern Massachusetts. The second objective was to investigate the host

preferences of *H. hookeri*, to gain information on how releases of this parasitoid might be optimized temporally and spatially.

Materials & Methods

Study Site. Work was conducted in a forested area on Nonamesset Island, the northernmost of the Elizabeth Islands, near Cape Cod in southeastern Massachusetts, USA. Vegetation at the site consisted of beech (*Fagus grandifolia* Ehrh.) and oak (*Quercus* spp.) overstory with little or no woody or herbaceous understory vegetation due to grazing by a locally dense population of white-tailed deer.

Seasonal Trend in Parasitism Rate of Questing *I. scapularis* Nymphs on Nonamesset. In 1990-1992, unfed nymphs were collected by dragging on Nonamesset. Approximately weekly collections were made in 1990 between 4 June - 28 July, in 1991 between 30 May - 28 August and in 1992, from 11 May - 27 August and again on 17 September. A corduroy cloth (1 m x 1 m) was used as a drag to sample a minimum distance of 240 m per week in 1990 and 500 m per week in 1991 and 1992. Each week's samples encompassed all types of habitat at the site. Ticks were collected 1-3 days per week, for a total of 2-6 hours per week. Random sub-samples of nymphs collected each week were identified and dissected in 75% ethanol

under a stereo microscope at 50x magnification to detect parasitoid eggs. A slit was cut along the posterior margin of the nymph, and the dorsum was removed. Ticks were dissected within two weeks after collection. Until dissection, ticks were held at 21° C, 93% RH and a photoperiod of 14:10 (L:D).

Mortality Rate of Stored, Unfed *I. scapularis* Nymphs. Cohorts of *I. scapularis* nymphs collected by dragging on Nonamesset on 17 June and 15 July 1992 were held at 21°C and 93% RH and a photoperiod of 14:10 (L:D) until dissected. Sub-samples of each cohort were dissected serially, as described above. Portions of the set of nymphs collected on 17 June were dissected on 20 June, 7 September and 12 October. Similarly, portions of the set of nymphs collected on 15 July were dissected on 26 July, 22 August and 12 October. A z test for proportional data (Daniel 1987) was performed to determine if the proportion of nymphs containing wasp eggs differed significantly between collection dates .

Voltinism of *H. hookeri* in Southeastern Massachusetts. The threshold temperature for *H. hookeri* development was determined by analyzing developmental rates for this parasitoid as reported by Cooley (1928). On average, it took 86.5 days from parasitized nymph detachment from hosts until adult wasp eclosion at 17°C, 45.5 days at 22°C, and 38.5 days at 27°C (Cooley 1928). A linear model was fitted to these data. A

hygrothermograph was used to record temperatures at ground level in the forest on Nonamesset from 1 May - 31 October 1991. Average daily temperatures were calculated from the daily minimum and maximum temperature readings. The day-degree model constructed from the literature was used to predict the emergence dates of adult wasps at the field site. Seasonal phenology of wasp emergence was then compared to temporal patterns of larval and nymphal blood feeding on hosts, to see what host resources would be available to wasps on various dates.

Tick Life Stage Preferences of *H. hookeri*. Pairs of laboratory mice were anesthetized with a 10:1 mixture of acepromazine maleate (10 mg/ml) and ketamine HCl (100 mg/ml), 0.07 cc per mouse, IP. After injection, each mouse was infested with 50 unfed *I. scapularis* larvae and 15 nymphs. Each mouse was placed inside a hardware cloth cage (10 x 5 x 5 cm), and pairs of caged mice were placed in a muslin bag (24 x 21 cm). The bag was sealed with masking tape, leaving a temporary opening through which 12 female and 2 male *H. hookeri* were introduced with an aspirator, and the opening was sealed.

Tests were started in the early afternoons of days in September and October on which wasps emerged from laboratory-fed nymphs (collected from the field). After eight hours, mice were removed from bags and placed in individual 12 x 7 x 7 cm hardware cloth cages over water pans. For the next six days, detached ticks were removed

from water pans daily. Fed ticks were dissected within one week to minimize the effect of post-detachment mortality. A z test for proportional data (Daniel 1987) was performed, to determine if the proportion of nymphs containing wasp eggs differed significantly from the proportion of larvae containing wasp eggs.

***H. hookeri* Parasitism of Immature *I. scapularis* Feeding on Mice.** On 7 and 27 August and 18 September 1992, *P. leucopus* were trapped on Nonamesset using Sherman live traps (H.B. Sherman, Inc. Tallahassee, FL) baited with Pacer® sweet feed for horses (Blue Seal Co. Richford, VT). Mice were held over water pans in individual hardware cloth cages (17 x 13 x 11 cm) in a predator-proof field enclosure for approximately 24 h. Fed larvae and nymphs were collected from water pans and were held at 21°C, 14:10 (L:D) until they could be identified and dissected. Dissections were performed as described above.

***H. hookeri* Parasitism of Immature *I. scapularis* Feeding on Mice in a Laboratory Arena.** A 90 x 40 x 60 cm plexiglass arena was maintained under a light regime of 14:10 (L:D). The arena was connected by PVC tubing to a dark 15 x 15 x 12 cm plywood and plexiglass nesting box situated below. On days when wasps emerged from laboratory-fed, parasitized *I. scapularis* nymphs, a single *P. leucopus* was anesthetized as described above. The mouse was then infested with either 125 *I. scapularis*

larvae, or with 30 nymphs, and placed in the nesting box. The entrance to the box was blocked with 1/4 inch mesh hardware cloth to prevent the mouse from ascending. Tests began in the late afternoon, when 12 newly emerged wasps (9 females and 3 males) were aspirated either into the nesting box or into the upper arena. After 48 hours, the mouse was transferred into a small cage over a water pan to collect fed ticks as they detached. Engorged ticks were dissected, as described above, to determine the rate of parasitism. Exposure of larvae to wasps released in the nesting box was replicated twice, exposure of larvae to wasps released in the plexiglass arena, and exposure of nymphs were each replicated four times. During periods when experiments were not being conducted, a single *P. leucopus* was maintained in the arena, ensuring that both chambers and the tube connecting them contained fresh mouse scent at the onset of each experimental run.

***H. hookeri* Parasitism of Fed, Detached *I. scapularis* Larvae.** Twenty *I. scapularis* larvae that had recently fed on laboratory mice were placed in a petri dish (100 x 15 mm) with a small piece of filter paper moistened with distilled water. The dish was sealed with parafilm, and ventilation holes were made in the parafilm with an insect pin. Six newly emerged wasps (4 female and 2 male) were aspirated into the dish via a hole in the lid which was then sealed with parafilm. Tests were set-up

in the afternoon, and dishes were held at 21°C, under a light regime of 14:10 (L:D) for 24 hours. Four groups of fed larvae were exposed in this manner. Larvae were then dissected as described above. Fed larvae that were not exposed to wasps were dissected as controls.

Results

Seasonal Trend in the Parasitism Rate of Questing *I. scapularis* Nymphs on Nonamesset. Sample sizes of nymphs collected each week by dragging varied due to weather conditions and the seasonal phenology of ticks. Numbers of nymphs dissected ranged from 14 (13 August 1991) to 84 (30 June 1992). The average sample was 54 ±3 nymphs. The parasitism rate of *I. scapularis* nymphs collected by dragging ranged from 6% (7 August 1991) to 36% (2 July 1991) (Fig. 2.1). Peak levels of parasitism were noted on 26 June 1990 (27%), 2 July 1991 (36%) and 11 May 1992 (32.7%). In general, the parasitism rate declined gradually throughout the summer.

Mortality Rate of Stored, Unfed *I. scapularis* Nymphs. The parasitism rate of the cohort of nymphs collected on 17 June 1992 decreased from 30.8% (n= 65) on 20 June to 11.1% (n= 63) on 7 September, and 9.1% (n= 22) on 12 October. The parasitism rate of the cohort of nymphs collected on 15 July 1992 decreased from 20.9% (n= 67) on 26 July to 10.3% (n= 68) on 22 August and 3.2%

(n=62) on 12 October (Fig. 2.2). There was a significant decrease in the proportion of surviving nymphs that contained wasp eggs between the first and second dissection dates for both groups ($P < .05$), but not between the second and third dissections.

Voltinism of *H. hookeri* in Southeastern Massachusetts. Analysis of the rates of development of *H. hookeri* at 17, 22 and 27°C suggests that the threshold temperature for development for this parasitoid is 8.2°C. It was found that 703 degree days are required from detachment of a fed, parasitized tick from its host to adult wasp emergence. Analysis of daily temperature data recorded on Nonamesset indicated that the earliest possible wasp emergence in 1991 occurred in late July. Nymphs that became parasitized in late July in 1991 would yield adult wasps in mid-September.

Tick Life Stage Preferences of *H. hookeri*. Detached ticks from fourteen mice infested with both larvae and nymphs were collected. Some ticks were lost due to mouse grooming and post-detachment mortality. Of the larvae and nymphs originally placed on mice, 76% of nymphs and 43% of larvae were recovered and dissected. The parasitism rates were 55% for nymphs, and 30% for larvae The z test for proportional data showed this difference to be significant at $P < 0.05$.

Parasitism Rates of Fed Ticks Collected from *P. leucopus*. A total of 321 *I. scapularis* larvae and 18 *D. variabilis* larvae were collected from trapped *P. leucopus* held over water pans. Only four (1.2%) *I. scapularis* larvae and one (5.6%) *D. variabilis* larva contained wasp eggs. Additionally, 19 *I. scapularis* nymphs and 10 *D. variabilis* nymphs were collected from water pans and dissected. Two of the *I. scapularis* nymphs (10.5%) and three of the *D. variabilis* nymphs (30%) contained developing wasps. Parasitized nymphs were collected on 7 August and 18 September (*I. scapularis*) and 27 August (*D. variabilis*).

Parasitism Rate of Immature *I. scapularis* Feeding on *P. leucopus* in a Laboratory Arena. Of 250 larvae exposed to wasps that were aspirated directly into the nesting box, 72 were recovered after engorgement, and 6% of them contained wasp eggs. Five hundred other larvae were exposed to wasps that were released into the Plexiglas arena. Of those larvae, 1% of the 211 that were recovered contained wasp eggs. One hundred and twenty nymphs were exposed to wasps that were released into the Plexiglas arena. Only one of the 75 nymphs (1.3%) that were recovered contained wasp eggs.

***H. hookeri* Parasitism of Fed, Detached *I. scapularis* Larvae.** All exposed larvae were recovered, and 7/80 (9%) contained wasp eggs. None of the control larvae were found to contain eggs.

Fig. 2.1. Parasitism rates of *I. scapularis* nymphs collected by dragging on Nonamesset in 1990-1992.

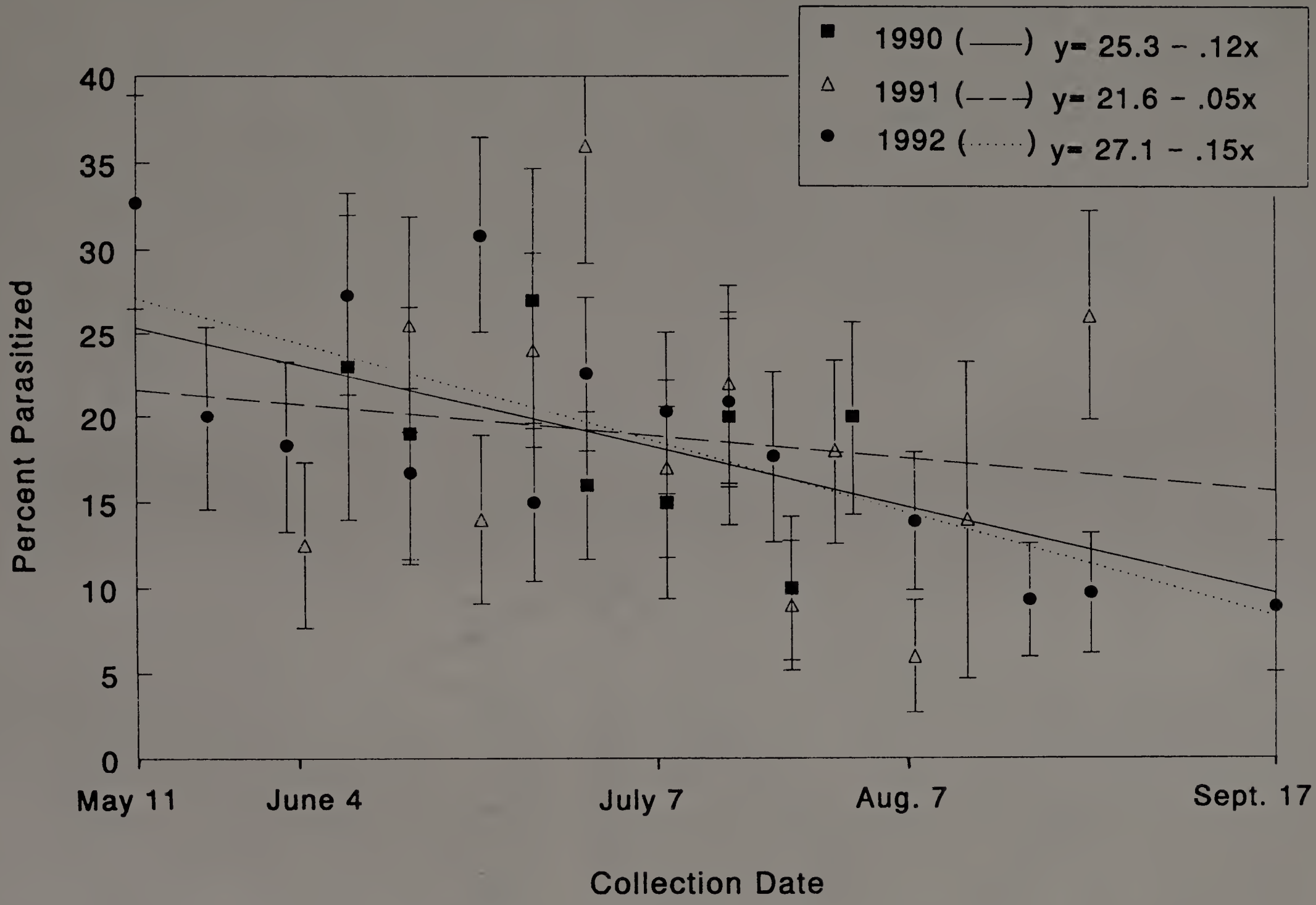
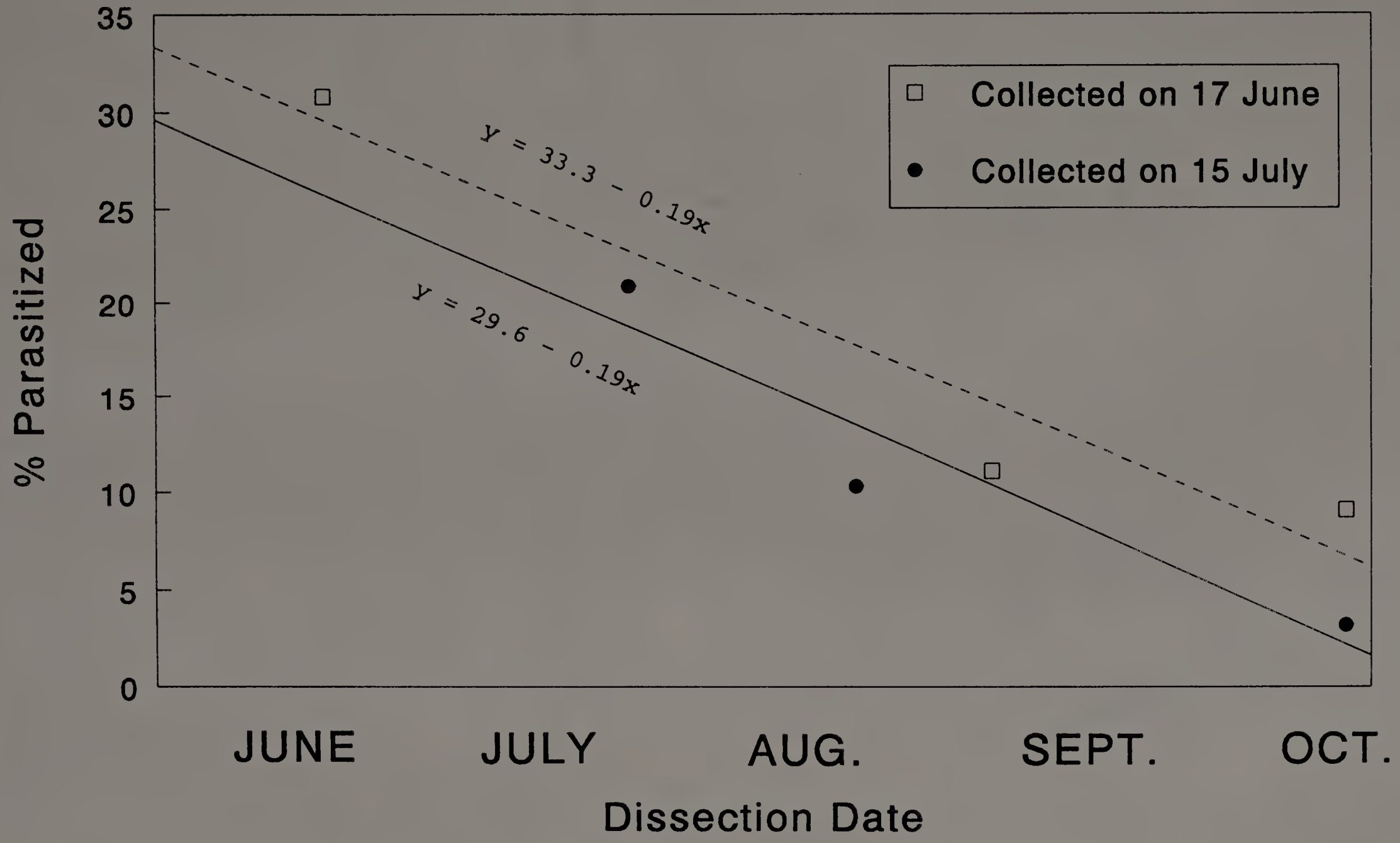

1990 (——) y= 25.3 - .12x
1991 (– – –) y= 21.6 - .05x
1992 (·····) y= 27.1 - .15x
Percent Parasitized
40
35
30
25
20
15
10
5
0
May 11
June 4
July 7
Aug. 7
Sept. 17
Collection Date

Fig. 2.2. Parasitism rates of two cohorts of *I. scapularis* nymphs collected by dragging on Nonamesset in 1992, and dissected serially.


35
30
25
20
15
10
5
0
% Parasitized
Collected on 17 June
Collected on 15 July
y = 33.3 - 0.19x
y = 29.6 - 0.19x
JUNE
JULY
AUG.
SEPT.
OCT.
Dissection Date

Discussion

Seasonal Rates of Parasitism. As the season progressed, in each year of this study, there was a downward trend in the observed parasitism rate of questing *I. scapularis* nymphs. The similarity in slopes of the regression lines run for parasitism levels for immediately dissected and for stored nymphs (Figs. 2.1 & 2.2) suggests that the decline in the parasitism rate of questing nymphs in the field is at least in substantial part due to enhanced mortality of parasitized nymphs.

Wasp Generations per Year. Should a second generation of *H. hookeri* occur, it would be difficult to quantify. There are three ways *H. hookeri* could be bivoltine: (1) Parasitized nymphs that succeeded in obtaining their blood meals early might produce wasps by mid-July. If these wasps parasitized late-feeding nymphs, they would yield a second generation in mid-September. These wasps could then oviposit into either blood feeding or already fed larvae. Although blood-feeding larvae would be relatively scarce, fed larvae should be abundant. It is not known if fed larvae become parasitized by *H. hookeri* in nature. (2) Later-feeding, parasitized nymphs would yield wasps in August. If some of these parasitized very late feeding nymphs, parasitoids might overwinter in the fed nymphs yielding wasps in the spring, when the next generation of nymphs

are questing abundantly. (3) Larvae that feed early (in June) could be parasitized either during blood feeding or afterwards. These larvae could molt to nymphs and take their nymphal blood meal late in the summer, and possibly yield wasps the following spring. Each of these scenarios requires that *I. scapularis* nymphs are obtaining blood meals after mid-July. I did find parasitized nymphs on mice in late summer on Nonamesset. However, on Nonamesset by mid-July of 1991, all but 15% of the *I. scapularis* nymphs that ultimately obtained blood meals from mice that year had already done so (unpublished data, see Chapter 3). This percentage would be the upper limit of ticks capable of producing second generation wasps. Such a cohort would be too small to greatly alter the overall impact of *H. hookeri* on *I. scapularis* populations.

In evaluating the impact of a parasitoid, if samples can be taken at a time when a host generation has experienced all the parasitism that is going to occur, and parasitoids have not begun to emerge, a single peak sample can accurately express the amount of mortality caused by the parasitoid (Van Driesche 1983). It appears that such a well-timed sample can be taken by collecting and dissecting questing *I. scapularis* nymphs as early in the season as ticks can be collected.

Host Preferences. Although a small number (1.2%) of fed *I. scapularis* larvae collected from mice on Nonamesset did contain wasp eggs, dissections of larvae that detached from wild mice, together with laboratory arena observations, suggest that the majority of parasitism of *I. scapularis* larvae that occurs on Nonamesset must not take place in association with *P. leucopus*. In fact, mouse populations on Nonamesset were extremely low during the years when these collections were made (approximately 3 per hectare).

In the laboratory, *H. hookeri* preferred to parasitize *I. scapularis* nymphs over larvae when both were feeding on the same host. In southeastern Massachusetts, the majority of *I. scapularis* nymphs obtain their blood meal in May and June (Piesman & Spielman 1979). In Connecticut, blood feeding by *D. variabilis* larvae peaks in May, while blood feeding by *D. variabilis* nymphs peaks in June and July (Anderson & Magnarelli 1980). It is possible that the greater availability of *D. variabilis* nymphs at the time of the season when a preference for nymphs has been observed indicates that *H. hookeri* is better adapted to parasitize *D. variabilis* than *I. scapularis*. For this reason, *H. hookeri* may have less impact on populations of *I. scapularis* in areas where *D. variabilis*, or other tick species with late-feeding nymphs, are abundant. In the

future it may be useful to sample ticks on medium and large mammals at wasp-endemic sites, to explore the roles of ticks other than *I. scapularis,* feeding on hosts other than *P. leucopus,* in supporting populations of *H. hookeri.*

Alternatively, parasitism may occur during periods when ticks are not attached to hosts. Cooley (1928) reported that *H. hookeri* would parasitize larvae that had fed on *D. andersoni* after they detached from hosts. Cooley and Kohls (1934) reported that *D. andersoni* nymphs also could become parasitized prior to obtaining blood meals. Although in the present study 9% parasitism of fed *I. scapularis* larvae was achieved in petri dishes, it would be useful to test the ability of *H. hookeri* to locate and oviposit into fed larvae in a more natural setting.

H. hookeri is an important mortality factor for *I. scapularis* on Nonamesset, where it is responsible for preventing 20-30% of nymphs from entering the pool of questing adult ticks. The impact of this parasitoid is best estimated by early sampling. Where mammalian hosts of *I. scapularis* are abundant, *H. hookeri* may be employed in combination with other control techniques in order to achieve adequate tick suppression. In addition, it is possible that higher levels of parasitism may be attained by making augmentive releases of *H. hookeri* during periods when questing tick nymphs are abundant.

CHAPTER 3

THE DROP-OFF METHOD: A NEW TECHNIQUE FOR COLLECTING LIFE TABLE DATA FOR TICKS

Ixodes scapularis Say is the principal vector of Lyme disease in North America. With 7,863 cases reported to the Centers for Disease Control in 1992 (Centers for Disease Control 1993), Lyme disease continues to be the most frequently reported arthropod-borne disease in the U.S. Since 1982, when it was discovered that *I. scapularis* vectors *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner, the etiological agent of Lyme Disease (Johnson et al. 1984), numerous studies have been conducted in order to determine the seasonality of transmission of this disease, and to elucidate the host relationships of the vector.

Most past studies have involved the collection of ticks that are either questing for a blood meal or are attached to a host, since quiescent ticks between blood meals are difficult to sample. *I. scapularis* nymphs and adults transmit the Lyme disease spirochete while taking blood meals. Seasonal trends of numbers of nymphs and adults questing for blood meals provide information about risks of disease transmission at particular times and localities (Carroll et al. 1992, Stafford & Magnarelli 1993, Kramer & Beesley 1993). Relative density data,

such as numbers of questing ticks collected by dragging, (Milne 1943) can also assist in evaluation of control measures used against ticks (Schulze et al. 1987, Daniels et al. 1991).

Three sampling methods have been employed in these types of studies (Gray 1985): (1) Numbers of ticks questing per m^2 can be determined by dragging or flagging along a transect line, in random fashion, or for fixed time periods (Macleod 1932, Milne 1943, Falco & Fish 1988, Ginsberg & Ewing 1989, Carroll & Schmidtmann 1992). (2) Numbers of questing ticks responding to carbon dioxide per unit of time can be assessed through the use of traps baited with dry ice which attracts ticks over short distances (Wilson et al. 1972, Anderson & Magnarelli 1980, Falco & Fish 1989). (3) Numbers of ticks blood feeding per host can be assessed by live-trapping and anesthetizing or killing vertebrate hosts and removing ticks from them (Main et al. 1982, Wilson & Spielman 1985, Schulze et al. 1986, Falco & Fish 1988). Collection methods that are employed less frequently include caging live-trapped hosts over water pans and collecting ticks that detach after feeding (Levine et al. 1985) and walking random transects in an area and collecting ticks which attach to the collector's clothing (Schulze et al. 1986, Ginsberg & Ewing 1989).

The majority of field research on *I. scapularis* has consisted of measurements of relative densities of ticks

collected in differing habitats, or in areas where different control practices have been applied. Alternatively, studies may be aimed at determining the factors that shape long-term tick population dynamics, using a life table perspective to evaluate the importance of specific mortality factors (Bellows et al. 1992). To construct stage-specific life tables, total numbers of individuals per generation entering each life stage must be determined. If all members of a generation enter a life stage in synchrony and if they can be counted before mortality factors begin to act upon them, a single, well-timed sample can measure total recruitment to the stage. In populations which gain and lose members simultaneously, total recruitment to a life stage cannot be estimated from a single well-timed sample or from the types of periodic sampling used to determine the seasonality of a population without further analyses. (See Bellows et al. 1992 for a summary of the stage-frequency analysis literature). In such situations, recruitment analysis can be used for life table construction. This entails making an unbroken series of estimates of the numbers of individuals entering a life stage, in specific, sequential periods (Van Driesche et al. 1991).

This paper presents the results of a study in which a new sampling method for *I. scapularis* was used to measure the total numbers of ticks per hectare that

entered the larval and nymphal stages, and which were associated with the important mammalian host of immature *I. scapularis*, the white-footed mouse, *Peromyscus leucopus* (Rafinesque). Our technique, termed "the drop-off method", consisted of measuring the number of engorged ticks dropping per day from trapped mice, for the entire active season of the ticks. Coupled with mark-recapture estimates of mice density per hectare, these data allow total numbers per hectare of ticks in each life stage which successfully feed on mice to be calculated. For comparative purposes, dragging samples of nymphs also were taken.

Materials and Methods

Study Sites. Work was conducted at two field sites on Martha's Vineyard Island (Menemsha Hills Reservation, owned by the Trustees of Reservations, and a Martha's Vineyard Land Bank site "Five Corners" on Chappaquiddick), and at one site on Nonamesset Island, the northernmost of the Elizabeth Islands. Both islands are located off the southwest end of Cape Cod, Massachusetts, USA. Vegetation at the Martha's Vineyard sites consisted of oak (*Quercus* spp.) overstory, with huckleberry (*Gaylussacia* sp.), high bush and low bush blueberry (*Vaccinium* spp.), bayberry (*Myrica pensylvanica* Loisel.) and greenbrier (*Smilax* sp.) shrub

understory. Understory vegetation was more dense at Chappaquiddick than at Menemsha. Vegetation at the Nonamesset site consisted of beech (*Fagus grandifolia* Ehrh.) overstory with little or no woody or herbaceous understory vegetation due to grazing by a locally dense population of white-tailed deer [*Odocoileus virginianus* (Zimmerman)].

Dragging for Nymphal Ticks. Drag sampling was conducted using standard 1 m x 1 m, double-sided, white corduroy drag cloths with a pocket for a wooden support in the hem of the leading edge and a metal chain sewn into the trailing edge for weight to keep the cloth close to the ground. Twine attached to the wooden ends was used to pull the drag cloth behind the collector, trailing over leaf litter and low vegetation. The paced dragging method was used, with tick nymphs being collected from both sides of the drag cloth with forceps at 20 meter intervals. A distance of 800 m or more was sampled at each site on each sampling occasion in 1991. In 1990, at Nonamesset the minimum sampling length was 240 m. In 1991, the Martha's Vineyard sites were drag sampled on one day per week. In some weeks on Nonamesset drag sampling was performed on more than one day. In those cases, data were pooled for each week. Ticks collected by dragging were placed in moistened plaster-lined vials for later identification. The relative humidity in the containers holding the vials was

maintained at an average of 93% with a saturated solution of potassium monophosphate (Winston & Bates 1960).

Larvae on drags were not collected because larvae are known to have a highly contagious distribution and to disperse poorly (Daniels & Fish 1990). In Connecticut it has been observed that after larval dispersal, the majority of larvae were recovered within 40 cm of their egg mass (Stafford 1992). Consequently, drag collections of larvae are highly variable, and are not satisfactory as a means of estimating larval density.

Data from dragging were converted to per hectare values by taking into account the distance covered and an estimate of the collection efficiency of the technique. Sampling efficiency of the method has been estimated at 10% for *I. scapularis* nymphs (D. Fish, NY Medical College, personal communication). Standard errors were also converted to per hectare values.

The Drop-off Method. The drop-off method has two components. The first is the enumeration of the average number of immature ticks completing blood meals on mice per day at a particular site in a given week. The second is the estimation of the number of mice per hectare at the site.

For the first component, *P. leucopus* were trapped weekly (Martha's Vineyard sites) or bi-weekly (Nonamesset) using Sherman live traps (H.B. Sherman Traps, Inc., Tallahassee, FL) baited with Pacer® sweet

feed for horses (Blue Seal Co., Richford, Vermont). Three 300 meter trap lines were established at each research site. Since an estimate of the upper size of the home range of *P. leucopus* is 1585 square meters (Fitch 1958), trap lines were spaced 125 m or more apart, in order to minimize the chance of trapping the same mouse in two consecutive weeks. This precaution was taken to insure that tick loads were not underestimated due to changes in mouse behavior caused by recent trapping. At the Martha's Vineyard sites, 30 traps were set on one 100 meter segment of each of the three trap lines each week, for a total of 90 traps set per week. Segments were chosen randomly with removal, such that a segment was never trapped in two consecutive weeks.

At each site, all trapping was done on the same night each week, with only one trap night per week. On Nonamesset, because the *P. leucopus* population was less dense than that at the Martha's Vineyard sites, it was necessary to set traps along all 300 meters of each trap line on each trapping occasion. For this reason, trapping was only conducted on Nonamesset every second week. It has been shown that tick loads on white-footed mice are increased by ear tagging (Ostfeld et al. 1993). Accordingly, mice trapped for drop-off work were not ear tagged, in order to ensure typical tick loads.

Trapped mice were placed individually in hardware cloth cages (17 x 13 x 11 cm) over water pans in

predator-proof field enclosures. Enclosures were 2.5m x 1.3m x 2m with a roof for rain protection, and chicken wire sides to maintain temperature near that of the outside air temperature. Twenty-four hours after mice were placed over water pans, they were released at the locations where they were trapped, and tick larvae and nymphs that had detached were collected from the water pans. Nymphs collected in 1990 and larvae and nymphs collected in 1991 were preserved in 75% ethanol for later identification. Published keys were used to identify ticks to species (Clifford et al. 1961, Sonenshine 1979). Random subsamples of at least 50% of the larvae collected per trap date in 1991 were identified to species and the percentage of *I. scapularis* in each sample was noted and used to calculate the numbers of *I. scapularis* engorging per day per mouse in each trap week. Because larvae collected in 1990 were not identified to species, the 1991 seasonal average for the same site was used instead.

To estimate the number of mice per hectare, mark-recapture trapping was performed at Chappaquiddick and Menemsha in July and August in 1990, and in May, July and August in 1991. At Nonamesset, mark-recapture work was done in July in 1990 and in May and August in 1991. Sixty-four Sherman live traps baited with Pacer® sweet feed for horses were set on an 8x8 grid. At the Martha's Vineyard sites, traps were spaced 10 meters apart. On

Nonamesset, preliminary work on a grid with 10 meter spacing indicated that it was necessary to set traps at 30 meter intervals in order to trap enough mice for data analysis via Program CAPTURE (White et al. 1982). Traps were pre-baited for one night, followed by four consecutive nights of trapping. Each day, newly trapped mice were ear tagged with sequentially numbered tags (National Band & Tag Co., Newport, KY) and released where trapped. Mark-recapture data were analyzed using Program CAPTURE, which assesses the fit of the data to assumptions made by competing mark-recapture models (White et al. 1982) and chooses the model whose assumptions are met best.

Integration of Mark-recapture and Drop-off Data.

For Menemsha and Chappaquiddick, the entire period during which drop-off sampling occurred was divided into equal portions, two in 1990 and three in 1991, and the mark-recapture estimate of *P. leucopus* population density taken in each period was used as the estimated mouse density per hectare for that period. Average numbers of larvae and nymphs completing blood meals on mice per week (calculated as seven times the observed daily rate from the sample day in the week), were multiplied by the corresponding estimate of the number of mice per hectare at the site for that portion of the season. Standard errors of the product of these two parameters (ticks detaching per mouse per week and mice per hectare) were

calculated. Since trapping for drop-off work was done every second week at Nonamesset, the averages of numbers of ticks detaching per mouse in the bounding weeks were used as estimates for the intervening weeks. The same extrapolation technique was used for one week in May and September at Menemsha in 1991 and for two weeks in September at Chappaquiddick in 1991 when mice were not trapped due to logistical constraints. The resulting weekly totals of immature ticks per hectare completing blood meals on mice were summed for seasonal totals. For comparison, subtotals of ticks feeding per mouse and per hectare in corresponding portions of the trapping season (5 June - 30 August) in 1990 and 1991 were also calculated.

Results

Nymphal Ticks Collected by Dragging. In 1990 at Nonamesset, drag sampling began on 24 June and the highest density of questing nymphs was seen on 17 July (Fig. 3.1). At the time of peak questing, there were an estimated 26,630 questing nymphs per hectare. At Menemsha and Chappaquiddick, drag samples were not taken in 1990.

At Nonamesset in 1991, the average number of questing nymphs was highest in the first sample, taken in the last week in May (Fig. 3.1), when there were an

estimated 29,400 questing nymphs per hectare. In 1991, at both Menemsha and Chappaquiddick the numbers of questing nymphs were more variable, with highest numbers observed at both sites in the first week of June and again in late June (Chappaquiddick), or late July (Menemsha) (Fig. 3.1). At both sites the later peak was slightly higher than the earlier one. At the later peak, at Menemsha there were an estimated 3,111 questing nymphs per hectare. At the corresponding peak at Chappaquiddick there were an estimated 305 questing nymphs per hectare (Fig. 3.1).

Estimates of Numbers of Ticks Engorging Per Mouse Per Day. Average numbers of mice trapped per site per occasion for tick drop-off work ranged from 36 ± 2.6 at Menemsha in 1990 to 10 ± 1.4 at Nonamesset in 1991 (Table 3.1). In 1990, numbers of nymphs successfully engorging per mouse per day were greatest for both research sites between 14-28 June, with peak values of 0.8 ± 0.3 (Chappaquiddick), and 0.8 ± 0.2 (Menemsha) (Figs. 3.2 & 3.3). Numbers of larvae engorging on mice peaked on 16 August in 1990, with 8.4 ± 1.4 (Chappaquiddick) and 15.6 ± 2.2 (Menemsha) (Figs. 3.2 & 3.3).

In 1991, numbers of nymphs successfully engorging per mouse per day were greatest for the three research sites between 4-13 June, with peak values of 6.0 ± 2.0 (Nonamesset), 1.1 ± 0.3 (Chappaquiddick) and 7.7 ± 1.4

(Menemsha) (Figs. 3.2-3.4). Numbers of larvae engorging on mice were greatest between 5-27 August in 1991, with peak values of 24.3 ± 7.6 (Nonamesset), 6.3 ± 1.3 (Chappaquiddick) and 22.0 ± 3.2 (Menemsha) (Figs. 3.2-3.4). In 1991, at Chappaquiddick and Menemsha an early peak of larvae engorging per mouse, per day was observed on 5 June, with 4.7 ± 1.2 at Chappaquiddick and 10.2 ± 1.7 at Menemsha (Figs. 3.2 & 3.3). At Menemsha in July of 1991 there was a small increase in the number of nymphs detaching per mouse (Fig. 3.3), but generally, nymphal blood feeding occurred at a nearly constant, low level throughout July and August at all sites.

Estimates of Seasonal Totals of Ticks Feeding Per Mouse. At Menemsha, nearly twice as many larvae completed blood meals per mouse between 5 June and 30 August in 1991 as did so in the same period in 1990. For nymphs at Menemsha, the corresponding increase was six-fold (Table 3.1). At Chappaquiddick, total larvae and nymphs completing blood meals per mouse for the same period in 1990 and 1991 were similar (Table 3.1). In 1990, nearly twice as many larvae detached per mouse at Menemsha as at Chappaquiddick between 5 June and 30 August. In the same period in 1991, four and four and one-half times as many larvae detached per mouse at Menemsha and Nonamesset as did at Chappaquiddick (Table 3.1). Numbers of nymphs completing blood meals per mouse at Menemsha and Chappaquiddick in 1990 were equivalent.

Between 5 June and 30 August in 1991, four and one-half times as many nymphs completed blood meals on mice at Nonamesset as at Chappaquiddick, and the corresponding difference between Menemsha and Chappaquiddick was six-fold (Table 3.1).

Mark-Recapture Estimates of *P. leucopus* Density Per Hectare. Program capture could not be used to estimate *P. leucopus* populations at Nonamesset in July 1990 or May 1991 because too few mice were trapped. Estimates of numbers of *P. leucopus* per hectare ranged from 3.3 ± 0.8 at Nonamesset in August, 1991 to 162.7 ± 40.3 at Menemsha in July, 1990 (Table 3.2). There was little within-year fluctuation in the estimates for Menemsha and Chappaquiddick, but year-to-year changes were large for each site. At Chappaquiddick the *P. leucopus* population estimate increased by 90% from August 1990 to August 1991. The corresponding decrease observed at Menemsha was 56%.

Absolute Numbers of Ticks Feeding on *P. leucopus* Per Hectare. The highest numbers of larvae and nymphs feeding on mice per hectare between June 5 and August 30 were seen at Menemsha in both 1990 and 1991 (Table 3.3). The lowest numbers of larvae and nymphs feeding on mice per hectare were seen at Chappaquiddick in 1990 and Nonamesset in 1991 (Table 3.3).

Table 3.1 Numbers of *I. scapularis* larvae and nymphs completing blood meals on *P. leucopus* per trapping season per mouse at two sites in 1990 and three sites in 1991.

Site	1990 Chappaquiddick	Menemsha	1991 Nonamesset	Chappaquiddick	Menemsha
Trapping Period	June 5- Aug. 23	June 5- Aug. 23	May 1- Sept. 24	May 22- Sept. 17	May 4- Sept. 17
Seasonal Total Mice Trapped	215	428	97	439	313
# of Trapping Occasions	12	12	10	15	17
Avg. # Mice Trapped/Occasion	18 ±1.7	36 ±2.6	10 ±1.4	29 ±1.8	18 ± 1.4
Seasonal Total[a] Larvae/Mouse	230.9 ±20.6	411.4 ±26.3	1512.7 ±72.8	288.0 ±20.3	1128.6 ±65.1
Total Larvae/Mouse[b] June 5 - Aug. 30	285	439	1021	262	881
Seasonal Total[a] Nymphs/Mouse	34.5 ±4.9	31.8 ±2.8	249.9 ±16.1	43.0 ±4.2	321.0 ±21.6
Total Nymphs/Mouse[b] June 5 - Aug. 30	34.5	32	134	38	192

[a] Seasonal total is daily averages multiplied by 7, and summed across all weeks in the trapping season. Extrapolated values were calculated for weeks in which trapping was not done.

[b] Totals for this common period include extrapolated values for the last week in August in 1990.

Table 3.2. Estimates of *P. leucopus* population densities per hectare, based on five day mark-recapture trapping at three sites in MA in 1990-1991.

Site	Week of Estimate	Model[a]	Estimate of Mice/Hectare ($\bar{x}$, SE)
Chappaquiddick	16 July 1990	M(o)	32.9 ±11.3
	20 Aug. 1990	M(b)	30.2 ±1.0
	20 May 1991	M(o)	38.0 ±7.3
	1 July 1991	M(o)	55.4 ±14.7
	26 Aug. 1991	M(o)	57.6 ±15.3
Menemsha	2 July 1990	M(h)	162.7 ±40.3
	13 Aug. 1990	M(h)	122.6 ±34.0
	5 May 1991	M(h)	17.7 ±6.6
	1 July 1991	M(h)	21.0 ±5.9
	26 Aug. 1991	M(h)	42.7 ±18.0
Nonamesset	12 Aug. 1991	M(o)	3.3 ±0.8

[a] The assumptions on which these models are based are as follows: M(o) there is a constant probability of capture for all individuals on all trapping occasions. M(b) behavioral responses to capture cause probabilities of capture to vary. M(h) individual animals have heterogeneous probabilities of capture due to factors such as age or sex.

Table 3.3. Estimates of total numbers of *I. scapularis* larvae and nymphs completing blood meals on *P. leucopus* per hectare for the entire trapping period, and for the period from June 5 - August 30 at three sites in MA in 1990-1991.

Site	Year	Total Larvae	Total Nymphs
Chappaquiddick	1990		
	June 5 - August 23	7,069 ±662	1,113 ±209
	June 5 - August 30	8,506	1,113
Chappaquiddick	1991		
	May 23 - Sept. 17	14,677 ±1659	1,969 ±253
	June 5 - August 30	12,211	1,435
Menemsha	1990		
	June 5 - August 23	52,301 ±7077	4,862 ±651
	June 5 - August 30	55,682	4,914
Menemsha	1991		
	May 8 - Sept. 16	35,454 ±5433	6,564 ±770
	June 5 - August 30	28,452	4,174
Nonamesset	1991		
	April 30 - Sept. 22	4,992 ±328[a]	825 ±68[a]
	June 5 - August 30	3,368	441

[a] The standard errors for Nonamesset only reflect half of the weekly estimates, because data for every second week were extrapolated.

Table 3.4. Per hectare estimates of seasonal totals of *I. scapularis* nymphs questing or completing blood meals on *P. leucopus* at three sites in 1991.

	Nonamesset	Menemsha	Chappaquiddick
Questing (Estimated by dragging)	29,400 ±4,600	3,111 ±800	305 ±100
Completing meals on mice (Estimated by drop-off)	825 ±68	6,564 ±770	1,969 ±253

Fig. 3.1. Numbers of questing I. scapularis nymphs per hectare, based on drag sampling at three sites in southeastern Massachusetts.

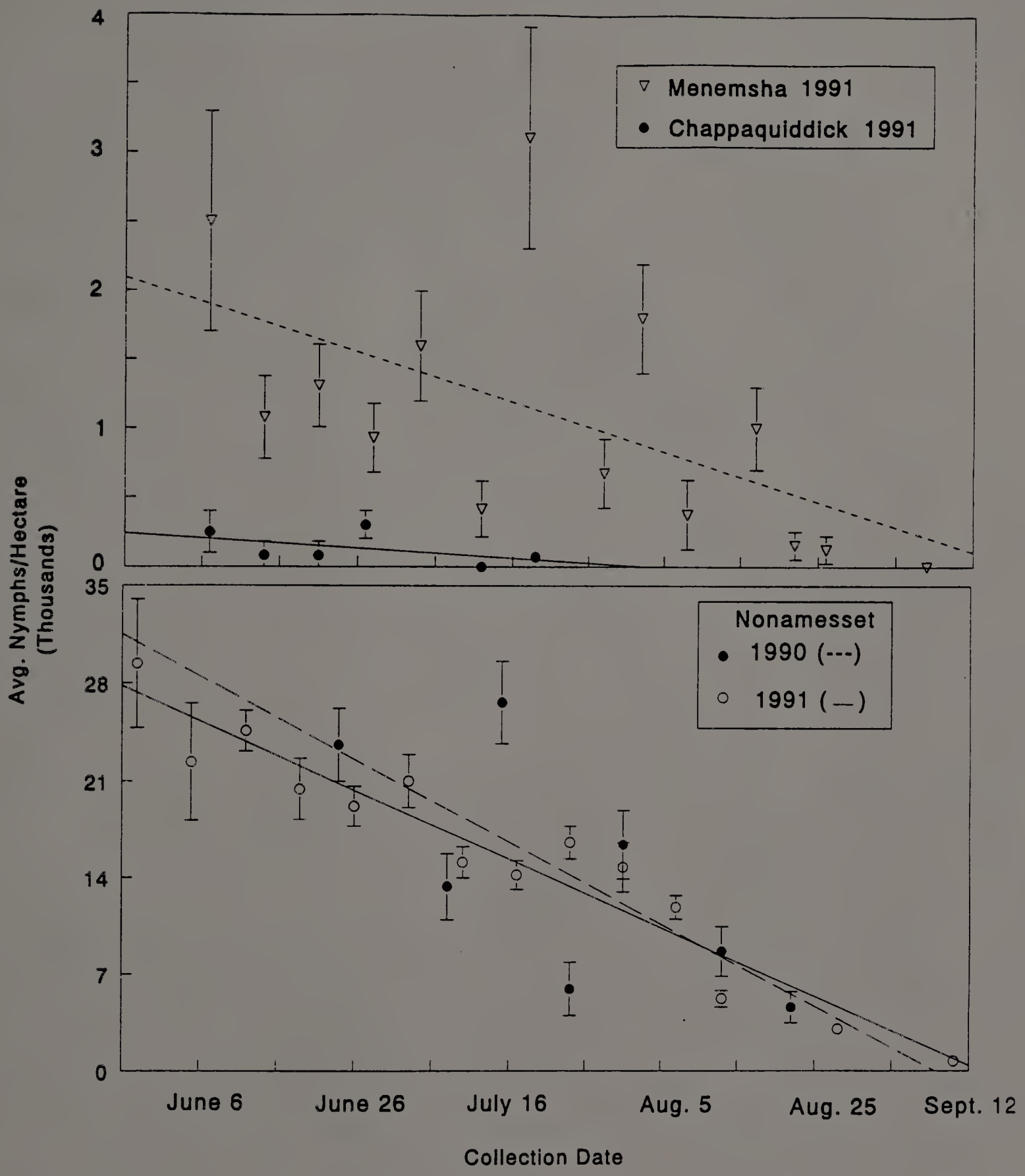

Menemsha 1991
Chappaquiddick 1991
Nonamesset
1990 (---)
1991 (—)
Avg. Nymphs/Hectare (Thousands)
4
3
2
1
0
35
28
21
14
7
0
June 6
June 26
July 16
Aug. 5
Aug. 25
Sept. 12
Collection Date

Fig. 3.2. Average numbers of *I. scapularis* larvae and nymphs completing blood meals on mice per 24 h at Chappaquiddick in 1990 & 1991.

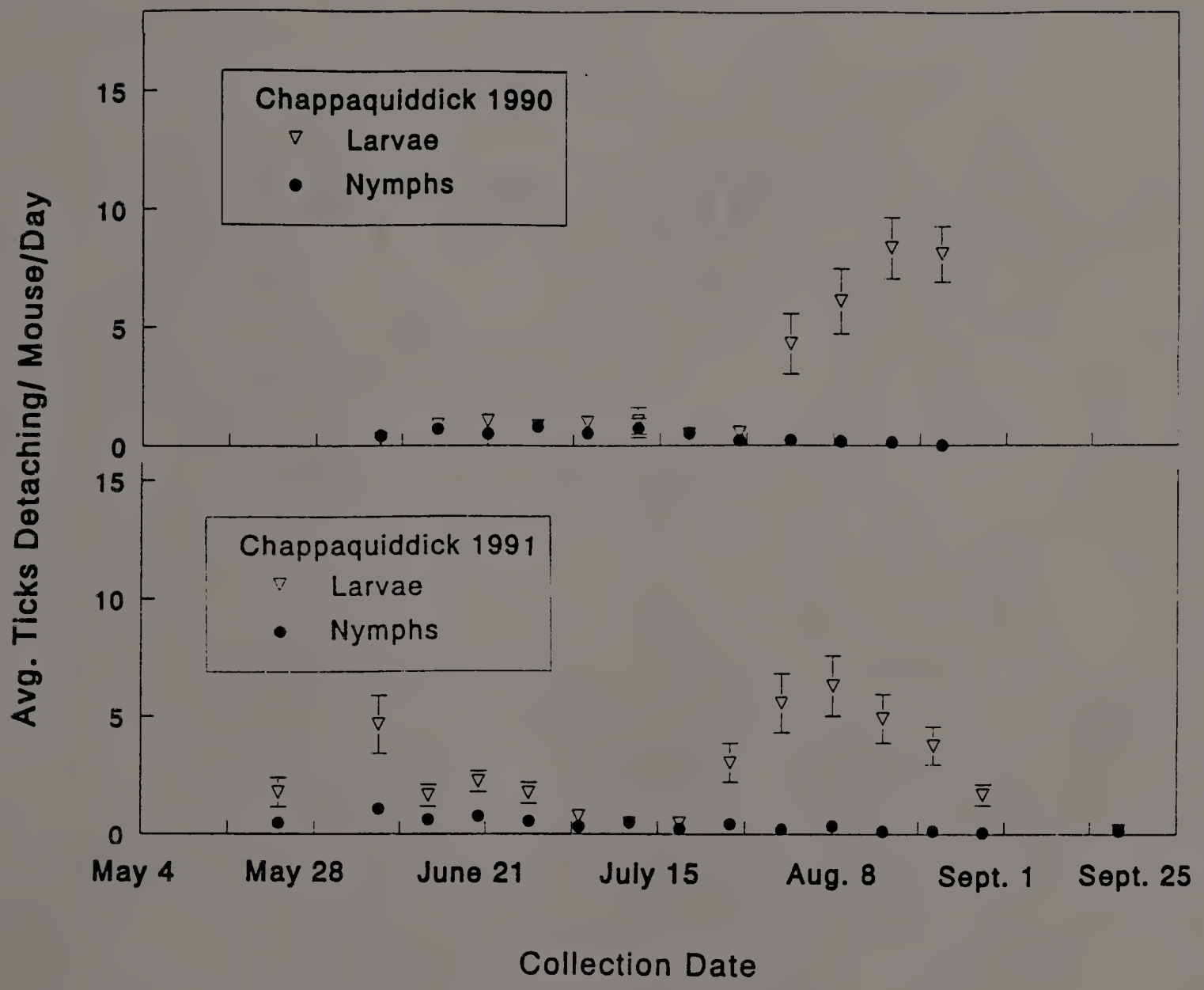

Chappaquiddick 1990
Larvae
Nymphs
Chappaquiddick 1991
Larvae
Nymphs
Avg. Ticks Detaching/ Mouse/Day
0
5
10
15
May 4
May 28
June 21
July 15
Aug. 8
Sept. 1
Sept. 25
Collection Date

Fig. 3.3. Average numbers of *I. scapularis* larvae and nymphs completing blood meals on mice per 24 h at Menemsha in 1990 & 1991.

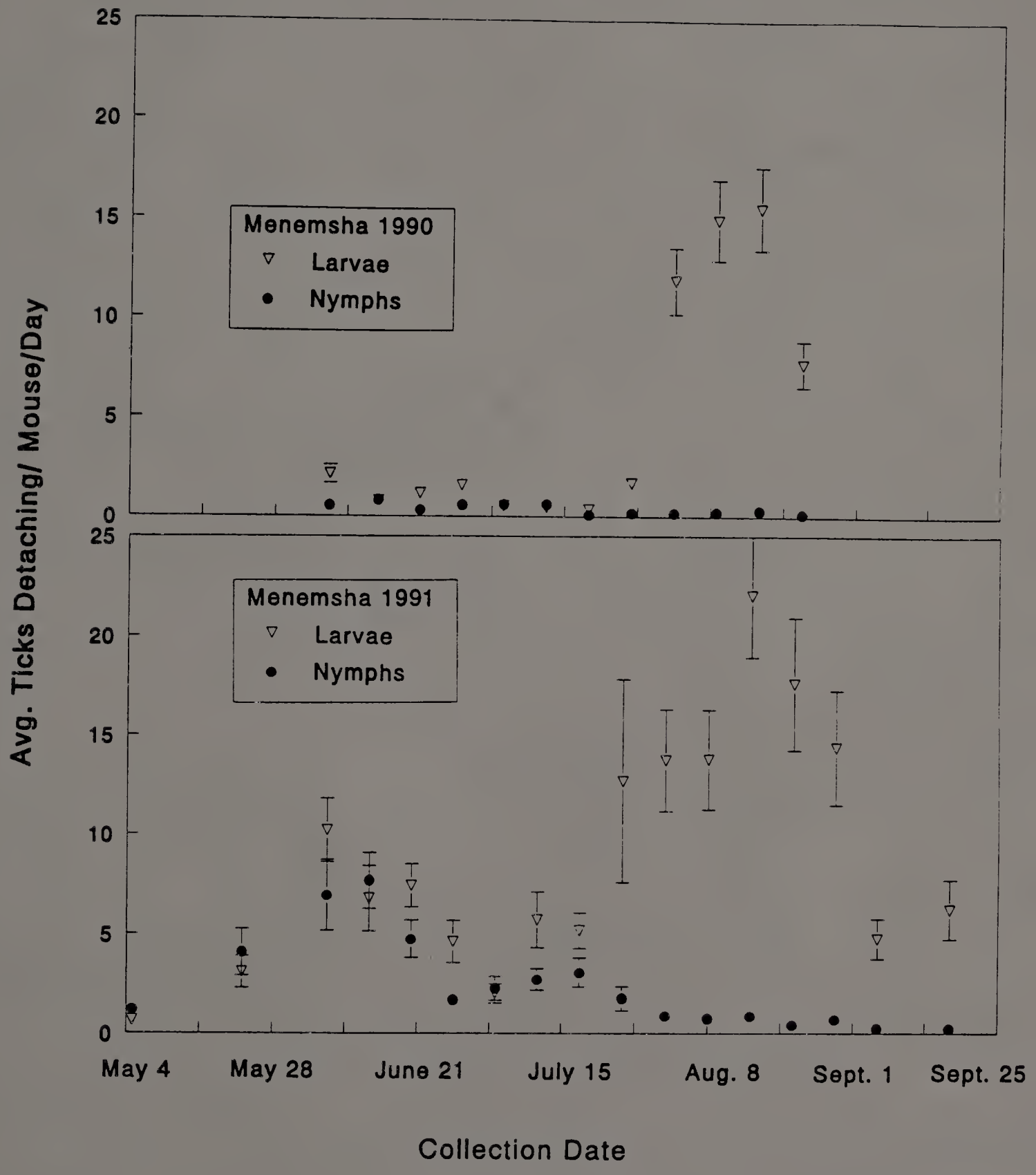
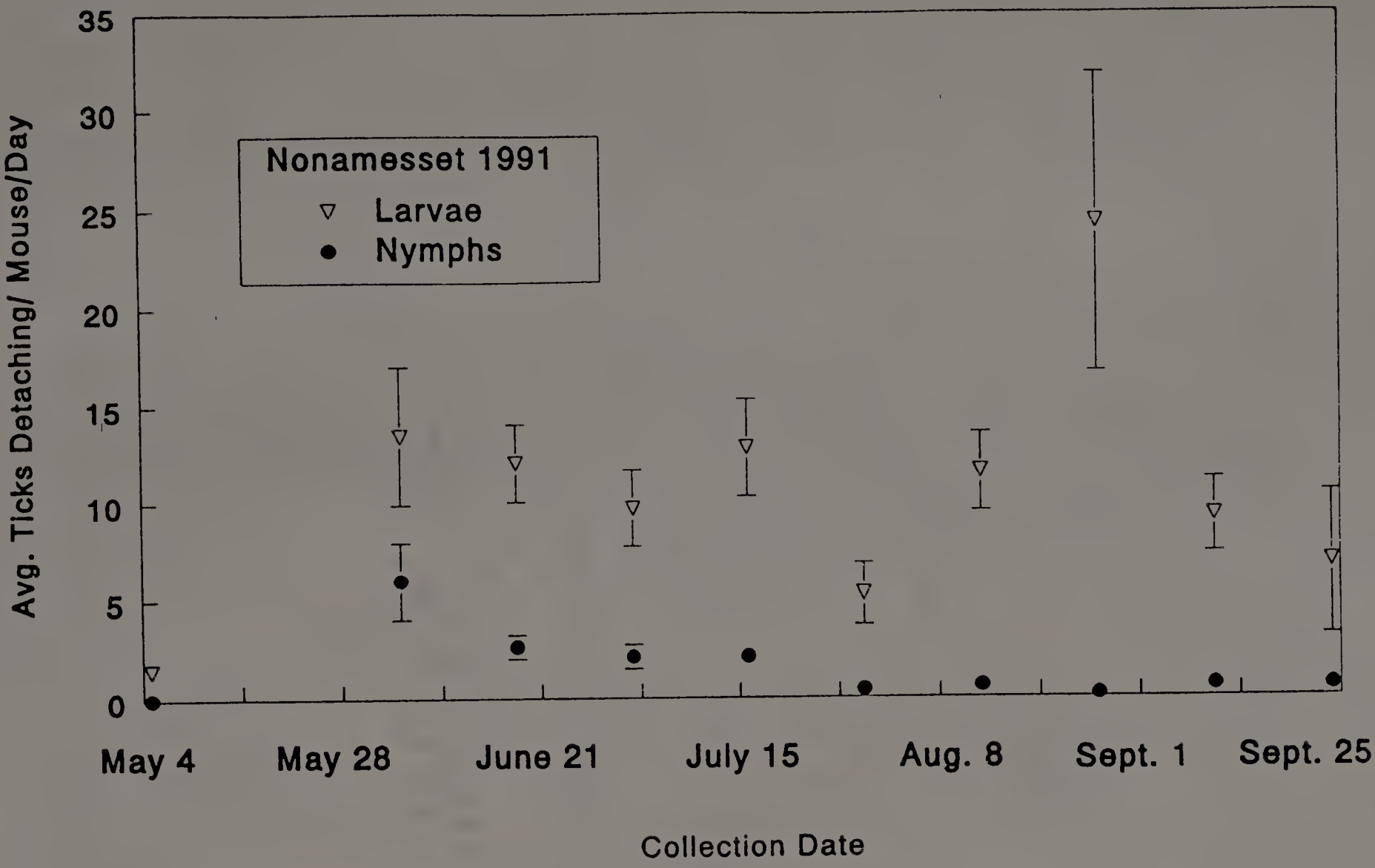

Menemsha 1990
Larvae
Nymphs
Menemsha 1991
Larvae
Nymphs
Avg. Ticks Detaching/ Mouse/Day
25
20
15
10
5
0
May 4
May 28
June 21
July 15
Aug. 8
Sept. 1
Sept. 25
Collection Date

Fig. 3.4. Average numbers of *I. scapularis* larvae and nymphs completing blood meals on mice per 24 h at Nonamesset in 1991.


Nonamesset 1991
Larvae
Nymphs
Avg. Ticks Detaching/ Mouse/Day
35
30
25
20
15
10
5
0
May 4
May 28
June 21
July 15
Aug. 8
Sept. 1
Sept. 25
Collection Date

Discussion

Evaluation of Sampling Methods. Within-site differences between seasonal totals of *I. scapularis* nymphs completing blood meals on *P. leucopus* determined by the drop-off method, and totals of questing nymphs collected by dragging at the time of peak questing are large (Table 3.4). At Nonamesset, where the density of the *P. leucopus* population was very low in 1991, only 3% (825/29,400) of the estimated pool of questing nymphs completed blood meals on mice. However, at Menemsha, the estimate for nymphs completing blood meals on mice is more than twice the seasonal estimate of nymphs questing at that site. The corresponding difference for Chappaquiddick is greater than six-fold. At Menemsha, in the first three weeks of June in 1991, approximately 2,700 fed *I. scapularis* nymphs detached from mice per hectare. This amount nearly equals the entire per hectare drag estimate for questing nymphs that year at Menemsha, yet drag samples taken in July suggest that more than 2,000 questing nymphs per hectare remained. Drop-off data for Chappaquiddick in 1991 further imply that drag sampling yielded artificially low estimates of numbers of questing nymphs at sites with relatively dense understory vegetation. There, approximately 608 nymphs per hectare obtained blood meals on mice in the first

three weeks of June. This number is two times the seasonal estimate of total questing nymphs.

Numbers of nymphs collected by dragging varied by several orders of magnitude between sites (Table 3.4). These large differences between sites in total questing nymphs per hectare were not reflected in per mouse per hectare drop-off rates. In fact, numbers of both larvae and nymphs detaching per mouse at Menemsha and Nonamesset in 1991 were nearly equivalent (Table 3.1). An explanation for these differences may be found by considering the effect of vegetation structure in the efficiency of dragging for collecting ticks.

Milne (1943) found that tick dragging efficiency was influenced by the degree of uniformity of the vegetation surface at a site. He cautioned that numbers of ticks collected by dragging could only be compared between sites with similar uniformity of vegetation surface. Carroll & Schmidtmann (1992) compared dragging with sweeping for ticks in three types of habitat: open oak-beech woodland, spruce-hardwood forest with many fallen trees, and mixed-hardwood forest with blueberry understory. In the latter two habitats, where the drag cloth was only intermittently in contact with the leaf litter, the tick sweep collected significantly more nymphs than did the drag cloth. The habitat at the Nonamesset site was similar to the oak-beech forest with open understory described by Carroll & Schmidtmann

(1992). Menemsha had a combination of areas of relatively open understory and areas of dense huckleberry. Chappaquiddick had thicker understory vegetation than Menemsha.

The correction factor employed to estimate total numbers of nymphs actually questing per hectare is based on observations made in a forest with a relatively open understory (D. Fish, personal communication). For that reason, use of this correction factor is likely to cause underestimation of *I. scapularis* abundance at sites such as Chappaquiddick and Menemsha. Determining drag efficiency at each research site and creating site-specific correction factors would allow more accurate site-to-site comparisons via drag sampling. Alternatively, sweeping for ticks, rather than dragging, may be advisable when attempting to compare questing tick populations at sites with heterogeneous vegetation. However, even for sweeping, vegetation structure may be an important determinant of efficiency.

Since, by a large margin, *P. leucopus* was the most frequently trapped small mammal at all 3 study sites (in 1990, percentages of trapped small mammals that were *P. leucopus* were 96% at Menemsha, 88% at Chappaquiddick and 77% at Chappaquiddick), this study focused on ticks completing blood meals on *P. leucopus*. In coastal Massachusetts, Levine et al. (1985) found that *P. leucopus* was the predominant host (among small mammals)

for *I. scapularis* larvae and nymphs. In south-central Connecticut Carey et al. (1980) also found that *P. leucopus* was the most abundant host of *I. scapularis,* but they reported comparable numbers of *I. scapularis* larvae and nymphs per host on *P. leucopus*, Eastern chipmunk (*Tamias striatus* L.), Eastern gray squirrel (*Sciurus carolinensis* Gmelin) and raccoon (*Procyon lotor* L.). At another location in south-central Connecticut, Main et al. (1982) found that *P. leucopus* and Eastern gray squirrels were the principal hosts for *I. scapularis* larvae, while both prevalence and incidence of nymphs were higher on Eastern gray squirrels, Eastern chipmunks and raccoons than on *P. leucopus*. In view of the broad host range of *I. scapularis*, when using the drop-off method for this tick, more accurate tick population estimates could be obtained by sampling from both small and medium-sized mammals.

Phenology of Questing by Immature *I. scapularis*. Yuval & Spielman (1990) observed that autumn-fed *I. scapularis* adults laid eggs in June whereas spring-fed adults did so in July. However, all eggs appeared to hatch synchronously in August. They also found that unfed larvae were able to overwinter, but survived for less than 12 months. Stafford (1992) reported that larvae resulting from fall-fed ticks began to disperse in mid-July in Connecticut. While Wilson & Spielman (1985) found a unimodal seasonal distribution of *I. scapularis*

larvae and nymphs in Massachusetts, in Connecticut, Main et al. (1982) observed bimodal frequencies of abundance of *I. scapularis* larvae and nymphs on mice. In the latter study, the peak average number of larvae per mouse in June was greater than half the peak number observed in August. The nymphal peak Main et al. (1982) observed in early September was less than half the size of the June peak. Schulze et al. (1986) reported a bimodal pattern of *I. scapularis* larvae feeding on small and medium-sized mammals in New Jersey. The average number of larvae per host in May was approximately one-fifth the number observed in August and September. In Illinois, minor peaks of questing larvae (Siegel et al. 1991) and blood-feeding larvae (Kitron et al. 1991) in late June, were followed by major peaks in mid-August.

In the present study, a bimodal distribution of numbers of larvae completing blood meals on mice was detected at Menemsha and Chappaquiddick in 1991. At Chappaquiddick, the June peak was greater than half the size of the August one (Fig. 3.2). At Menemsha, the early peak was nearly half the size of the late one.

Yuval & Spielman (1990) found that larvae that fed in May or June and were confined in containers in the field in southeastern Massachusetts all had molted to nymphs two months after the larval blood meal. In southeastern Massachusetts, if early-feeding larvae molt promptly and quest for hosts as nymphs there would be an

infusion of new individuals into the questing nymph population in late July and early August. In the present study, a second peak in nymphal blood feeding of comparable magnitude to the one that Main et al. (1982) reported was not observed. However, there was a small possible second peak of nymphs feeding on mice at Menemsha in July in 1991 (Fig. 3.3). In addition, there was an increase in the number of questing nymphs collected by dragging in early July of 1990 at Nonamesset, and in mid-July of 1991 at Menemsha (Fig. 3.1). These observations are consistent with a bimodal pattern of larval questing.

Overall, the drop-off method was used to estimate absolute numbers of immature *I. scapularis* per generation completing blood meals on mice. This method overcomes a shortcoming associated with drag sampling, since it can be used for making site-to-site comparisons. It should be possible to apply the drop-off method in settings where *P. leucopus* is not a principal host, as long as sufficiently large numbers of hosts are sampled on a weekly basis.

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