Infestation intensity and prevalence of an ectosymbiotic midge under variable environmental and host conditions

C.M. Pennuto, C.L. Wooster-Brown, and C.A. Belisle

Abstract: Host–symbiont theory suggests that the abundance of an obligate symbiont will correlate positively with the density and size of its host. We examined these expectations using an obligate chironomid ectosymbiont – host fishfly system in 13 streams of southern Maine over 2 years (1996–1998). In addition, we investigated field conditions and macroinvertebrate-community composition, to search for correlates with symbiont prevalence and mean infestation intensity. Prevalence and infestation intensity were high in both years of the study, but neither infestation intensity nor prevalence was correlated with host population density across sites. Within sites, prevalence increased significantly when host density declined, contrary to our expectation. Symbiont-infestation intensity was not related to host size. No symbiont-population attributes correlated with physicochemical conditions in either sample year but prevalence was positively correlated with infestation intensity. Ectosymbiotic midges exhibited a significant host and attachment-site bias. Collectively, these data suggest that this symbiont–host interaction is obligate and stable, but the dynamics in our streams do not support the general expectation of increasing symbiont-population size with either increasing host-population or body size. Intraspecific interactions between symbionts are thought to have a stronger influence on population dynamics than do host populations or abiotic conditions for this ectosymbiotic midge.

Résumé : La théorie sur les relations hôte–symbionte semble indiquer que l'abondance d'un symbionte obligé est en corrélation positive avec la densité et la taille de ses hôtes. Nous avons examiné ces prévisions dans un système ectosymbionte–hôte, dans 13 ruisseaux du sud du Maine, où le symbionte était un chironomidé et l'hôte, un corydalidé. De plus, nous avons examiné les conditions du milieu et la composition de la communauté de macroinvertébrés afin d'établir des corrélations avec la prévalence des symbiontes et l'intensité moyenne des infestations. La prévalence et l'intensité des infestations ont été élevées les 2 années de l'étude, mais ni l'une ni l'autre de ces deux variables n'était reliée à la densité de la population hôte à aucun des sites. Contrairement à nos prévisions, la prévalence a augmenté significativement aux divers sites lorsque la densité des hôtes a diminué. L'intensité des infestations de symbiontes n'était pas reliée à la taille des hôtes. Aucune des caractéristiques démographiques du symbionte n'était en corrélation positive avec les conditions physicochimiques ni l'une ni l'autre des 2 années d'échantillonnage, mais elles étaient en corrélation positive avec l'intensité des infestations. Les chironomidés ectosymbiontes ont une préférence marquée pour des hôtes particuliers et pour certains points d'attache sur leurs hôtes. Dans leur ensemble, ces données semblent indiquer que l'interaction hôte–symbionte est obligatoire et stable, mais que la dynamique dans nos ruisseaux ne supporte pas la prévision générale d'une augmentation de la taille de la population de symbiontes en fonction de l'augmentation de l'importance de la population hôte ou de la taille du corps des hôtes. Pour ce chironomidé ectosymbionte, les interactions intraspécifiques entre les symbiontes ont probablement une influence plus grande sur la dynamique des populations que les conditions abiotiques ou les populations hôtes.

[Traduit par la Rédaction]

Introduction

Host–symbiont interaction dynamics remain of interest to ecologists, especially in light of global declines in biodiversity (e.g., Wilson 1992; Allan and Flecker 1993). Of the major symbioses (i.e., mutualism, commensalism, and parasitism), commensalisms have received the least attention from ecologists, possibly because of the unidirectional requisite of benefits used in the typological classification scheme defining commensalisms (Thompson 1988; Bronstein 1994*a*). Commensalisms also have the stigma of being evolutionary intermediates between mutualism and parasitism, and thus have

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not commanded great attention (e.g., Whitney 1982; Houck and O'Connor 1991; Houck and Cohen 1995). Several authors, however, suggest that commensal interactions should be viewed more carefully. When examining communities containing >10 interactions, Dodds (1997) suggests that ecologists overemphasize predation and competition when other interactions, like commensalism or amensalism, should be the most common. Thompson (1988) and Bronstein (1994*b*) reveal that species-interaction outcomes can vary from amensalism to mutualism, depending on the ecological conditions present (called "interaction norms" or "context dependency") and Wilson and Knollenberg (1987) suggest that commensalism "might be evolutionarily stable simply because many environments provide no opportunity for commensals to become mutualistic." In addition, studies on trophic dynamics contain many examples of direct and indirect commensalisms (e.g., Dethier and Duggins 1984; Heard 1994, 1995; Paradise and Dunson 1997).

In aquatic habitats, the Chironomidae have evolved symbioses, including parasitism (Weins et al. 1975; Jacobsen 1995; Giberson et al. 1996; Jacobsen 1999) and symphoresy (Steffan 1965, 1967; Gotceitas and Mackay 1980; Svensson 1980; Furnish et al. 1981; Bottorff and Knight 1987; de la Rosa 1992; Pennuto 1997). Symphoresy describes an association where midge larvae live on the body surface of a larger mobile host without apparent benefit or harm to the host but with benefits to the midge (Cranston et al. 1983). Bottorff and Knight (1987) suggest that this relationship can be defined as a commensalism, since hosts are unaffected but the midges receive benefits. Whereas there is ample evidence of symbiotic lifestyles among aquatic-midge species (see reviews by Tokeshi 1993, 1995), few works have examined these interactions in the context of environmental conditions across the landscape, the notable exception being Svensson (1980). In addition, no studies have placed midge symphoreses–commensalisms in the context of general host–symbiont theory.

In southern Maine, the midge *Nanocladius* (*Plecopteracoluthus*) sp. No. 5 (Diptera: Chironomidae) occurs attached to its host, the saw-combed fishfly, *Nigronia serricornis* Say (Megaloptera: Corydalidae), in all seasons (Pennuto 1998). We have never found this midge free-living. This midge exhibits a life cycle very similar to that reported by Bottorff and Knight (1987) for a congeneric species, *Nanocladius* (*Plecopteracoluthus*) *downesi* (Steffan), in a Michigan stream and to that reported by Gotceitas and Mackay (1980) for *Nanocladius* (*Nanocladius*) *rectinervis* (Kieffer) in Ontario (though the *N. rectinervis* of Gotceitas and Mackay may actually be *N.* (*P.*) sp. No. 5 (Epler 2001; R. Jacobsen, personal communication)). It is a common inhabitant of woodland streams of the region when its host is present. It is found attached to hosts in all seasons, constructing a gelatinous tube from which it feeds on particles attached to the surface of the host or its tube. It forms a puparium on the thorax of the host for pupation, and has a peak emergence in May (Pennuto 1997, 1998). Intraspecific competition, possibly for pupation sites, and larval movement behavior have been implicated as important conditions influencing emergence success in this species (Pennuto 2000). Midges removed from their hosts fail to emerge, suggesting an obligate association (C.M. Pennuto, unpublished data).

Several authors have suggested possible mechanisms giving rise to symbiotic lifestyles in the Chironomidae (reviewed by Tokeshi 1993). In general, ectosymbioses by midges could have evolved if fitness was greater for individuals, owing to an association with a host relative to no association. Of the mechanisms proposed as potentially important in the evolution of symbioses in the Chironomidae, substrate instability has often been cited as an environmental condition that could be overcome by a symbiotic association with a larger, presumably stronger and more mobile, host (e.g., Steffan 1967; Gotceitas and Mackay 1980; Bottorff and Knight 1987). We investigated this and several other abiotic conditions as possible correlates to ectosymbiont midge population dynamics (as inferred from infestation intensity and prevalence) across streams and years. Krasnov et al. (2002) suggested that interhost habitat conditions are critical to understanding host–symbiont dynamics for symbionts transmitted horizontally between hosts. In this study, we assessed the physical and chemical conditions in streams to characterize interhost habitat for these midges. We expected to find strong correlates across years for those conditions influencing prevalence and intensity within sites. In addition, whereas we have anecdotal evidence for the obligate nature of this particular symphoretic–commensal association, we investigated host preference and attraction to conspecifics as potential mechanisms leading to host specificity.

Lastly, we assessed the response of midge populations to host-population density, using a survey approach through time. This approach can provide insights into benefits accrued by midges behaving symbiotically. Thus, if the symbiont experiences a fitness increase with increasing density of the host population, the interaction should be favored (e.g., Boucher et al. 1982; Addicott 1984). Measures like symbiont per capita growth rate, body size, or population density are often used as indices of fitness associated with changes in host density. We investigated the relationship between mean ectosymbiont-infestation intensity (number of midges per infected host) and infestation prevalence (percentage of host population harboring ectosymbionts) and host density across streams in southern Maine. We expected an increase in both prevalence and infestation intensity with increasing host-population density, as suggested by Svensson (1980) for midges and as shown for host–ectoparasite symbioses (e.g., Anderson and May 1978; Krasnov et al. 2002).

Materials and methods

Stream sampling

Eight streams were sampled in June 1996 and 12 in June 1998, of which seven were sampled in both years (Table 1). All sites were second- to fourth-order streams flowing through woodland landscapes and were located within a 50-km radius of our campus in southern Maine, U.S.A. (Cumberland County, 43°41′N, 70°29′W). We characterized physicochemical conditions in each stream by measuring conductivity (microsiemen per centimetre; Corning Checkmate 90), total alkalinity (milligram per litre; Hach digital titrator methyl-orange), dissolved oxygen (milligram per litre; YSI Model 55), turbidity (nephelometric turbidity unit (NTU); LaMotte Model 2008), canopy cover (percentage; crown densiometer), maximum width (metres), mean depth (metres), mean velocity (metres per second; Swoffer Model 2100 flow meter), discharge (cubic metres per second), substrate embeddedness

Table 1. Physicochemical characteristics of streams sampled for the presence of midge symbionts (*Nanocladius* (*Plecopteracoluthus*) sp. No. 5) on *Nigronia serricornis* hosts in southern Maine.

	Conductivity (µS/cm)	Total alkalinity (mg/L)	Dissolved oxygen (mg/L)	Turbidity (NTU)	pH	Canopy cover	Max. width (m)	Mean depth (m)	Mean velocity (m/s)	Discharge (m^3/s)	Substrate embeddedness (%)	Temperature (°C)
1996												
Aldens Brook	317	86.5	12.5	1	8.1	5	2.1	5.1	0.11	0.01	2	17.0
Bonney Eagle Brook	75	47.1	8.7	4	7.4	3	7.0	9.9	0.18	0.12	2	20.2
Cooks Brook	86	34.2	10.0	5	7.2	4	8.0	14.5	0.20	0.15	1	20.8
Douglas Brook	96	34.2	9.0	2	7.8	4	6.0	15.7	0.24	0.21	1	15.6
Little River 202	142	45.7	10.0	3	8.4	1	16.2	16.6	0.40	0.39	2	15.4
North Branch	125	43.0	10.0	2	7.7	4	6.2	21.0	0.23	0.29	2	21.0
Pleasant River	138	30.6	9.2	3	7.7	4	12.5	16.5	0.32	0.66	2	18.8
Waterman	111	45.7	9.1	6	8.4	3	9.0	18.3	0.29	0.32	2	16.0
1998												
Aldens Brook	167	39.7	8.5	12	7.7	5	1.8	2.7	0.06	0.01	1	22.5
Black Brook	58	5.8	9.0	1	6.6	5	4.0	12.9	0.34	0.20	2	20.0
Bonney Eagle Brook	57	5.6	8.9	2	6.9	5	7.0	16.2	0.21	0.30	2	22.8
Brown Brook	55	10.1	8.6	1	7.3	5	3.5	22.9	0.48	0.43	2	20.9
Collyer Brook	138	20.2	11.1	4	7.6	2	6.0	39.9	0.31	0.84	2	18.5
Cooks Brook	75	8.2	8.8	7	6.7	5	4.5	16.3	0.36	0.33	2	20.8
Douglas Brook	88	16.9	7.8	3	7.0	4	6.0	20.8	0.06	0.07	2	22.0
Mousam River	34	6.8	9.3	2	6.8	4	5.3	18.5	0.15	0.15	2	18.2
North Branch	83	13.4	10.9	9	7.5	3	5.0	15.6	0.23	0.20	2	16.7
Pendexter Brook	43	11.6	9.3	1	7.7	5	5.2	22.1	0.48	0.69	1	20.6
Pleasant River	75	14.5	9.3	2	7.8	3	19.2	33.8	0.43	3.10	1	20.8
Waterman	89	16.8	8.8	8	6.9	4	6.3	9.8	0.16	0.18	2	20.0

(percentage), and temperature (degree Celsius). All variables except discharge were measured in the field. Discharge was calculated from cross-sectional depth and velocity measurements. Canopy cover was estimated near noon and dissolved oxygen was determined before 10:00.

We collected saw-combed fishfly nymphs, *N. serricornis*, from riffles and snag habitats using kick nets (1-mm mesh). Nymphs were field-sorted from debris and placed individually in sample jars with alcohol (70% EtOH), to facilitate the association of midges with their appropriate host. Availability of potential alternative hosts was estimated from five Surber samples collected in riffle habitats in each stream. Macroinvertebrates were preserved in 70% EtOH and later sorted to genus for estimating the abundance of potential alternative hosts.

Laboratory and statistical analyses

Midge attachment sites and density distribution

Host head capsule width was measured for each nymph using an ocular micrometer on a Wild MZ8 dissecting microscope. The number and location of chironomid ectosymbionts (*N.* (*P.*) sp. No. 5) were recorded. We quantified infestation prevalence (percentage of the host population harboring ≥1 midge(s)), percentage of hosts harboring a single midge, mean infestation intensity (no. of *N.* (*P.*) sp. No. 5 per *N. serricornis* examined), mean abundance (no. of *N.* (*P.*) sp. No. 5 per infected *N. serricornis*), attachment-site distribution, and density distribution on hosts from these data. Ectosymbiont population terminology follows Bush et al. (1997).

We examined the relationship between infestation intensity and prevalence and abiotic conditions using Pearson's product–moment correlations. We also used correlations to examine any relationships between intensity or prevalence and host density (logarithmically transformed). We applied the "sampling model" of Hanski et al. (1993) to sites with >10 hosts, to determine if an observed relationship was a sampling artifact. In this application, hosts equate with "patches" and prevalence equates with "density" as per Hanski et al. (1993). To investigate whether changes in prevalence and infestation intensity correlated with changes in host density, we determined the proportional change in prevalence, infestation intensity, and density using the relationship $(A - B)/A \times 100$, where A and B are the 1996 and 1998 values, respectively. We assessed ectosymbiont attachment site preference at each site within years with a series of χ^2 tests, excluding those sites with <15 midges in a given year. Attachment sites were grouped into four categories: dorsal thorax, dorsal abdomen, ventral thorax, and ventral abdomen. The number of midges observed on each host-body quadrant was compared with the number expected if they were equally distributed over the host. The surface area of a *N. serricornis* abdomen is approximately double the area available on the thorax, thus, expected frequencies on the abdomen were twice those for the thorax.

Between-year differences in attachment-site and density distributions on hosts were investigated using a *G* test. The observed frequency of midge attachment sites was compared between years, and the observed frequency distribution of midges per host (0, 1, 2, 3, or >3 midges) was compared with that expected on the basis of a Poisson distribution prior to comparing years.

Host specificity and attachment trials

Host specificity and attachment behavior were examined in the laboratory using two different methods: single-host trials and paired-host trials. We also assessed host attachment in the field using caged hosts. For laboratory trials, host *N. serricornis* harboring midges were collected from the Little River in April and May, placed in buckets of river water, and returned to the laboratory. These dates preceded midge pupation and coincided with dates previously shown to be active periods for midge movement behavior (Pennuto 2000). Potential alternative hosts were collected at the same site or from Alden's Pond (*Chauloides rastricornis* only), located near the University of Southern Maine campus. In the laboratory, attached midges were removed from their *N. serricornis* hosts and immediately placed in individual beakers with 400 mL of stream water.

For single-host trials, replicate ($n = 11$) beakers were swirled for 24 h on an orbital shaker to simulate current. Each container held a single potential host or no host (control) plus a single midge. Potential hosts included the perlid stonefly, *Acroneuria abnormis* (Newman), the saw-combed fishfly, *N. serricornis*, the fishfly *C. rastricornis* (Rambur), and the alderfly, *Sialis* sp. Beakers held 400 mL of river water and one stone placed in the center for shelter. After 24 h, the fate of the midges was determined. We scored three possible outcomes: eaten, attached, or free. Eaten designated a midge that was missing from the beaker after 24 h. Attached designated a midge that had located an available host and constructed a gelatinous tube to it or, in the case of the midge-only control, to the substrate. Free midges were neither eaten nor attached. All attached and free midges were alive after 24 h.

In paired-host trials, five midges were placed in beakers with two potential hosts, one *N. serricornis* – one *A. abnormis* ($n = 20$) or two *N. serrricornis* ($n = 10$). These trials were similar to single-host trials, except the beakers were not swirled. *Acroneuria abnormis* was used as a potential alternative host in these trials, because it was often the most numerous potential host in all streams sampled in the 1996 and 1998 surveys and it is a reported host for other midge ectosymbionts (e.g., Steffan 1965; Hilsenhoff 1968; Bottorff and Knight 1987). The double-*N. serricornis* trials were used to explore the likelihood that these midges might be attracted to a specific host individual, not just to the species per se. A possible alternative hypothesis to explain an overabundance of midges per individual host, if present, is attraction to conspecifics.

We used a *G* test to examine differences in the observed fate of midges in single-host trials (i.e., Did the frequency of attached, eaten, and free midges differ between treatments?). A χ^2 test was used to determine whether midges preferred one host in the *N. serricornis* – *A. abnormis* paired-host trials and whether midges were attracted to a specific host in the double-*N. serricornis* trials. All analyses were performed using Statistix 95 (Analytical Software, Tallahasee, Fla.), with $\alpha = 0.05$.

For field trials, 25 larval *N. serricornis* were collected on 15 July, placed in buckets of stream water, and brought into

Table 2. Host *N. serricornis* and ectosymbiont (the midge *Nanocladius* (*Plecopteracoluthus*) sp. No. 5) population characteristics in streams sampled over 2 years in southern Maine.

	No. of hosts	No. of midges	Host density (no./m^2)	Prevalence (% *N. serricornis* with ≥1 midge)	Intensity[a] (mean no. of midges per *N. serricornis* examined)	Abundance (mean no. of midges per infected host)	Percentage of hosts with a single midge
1996							
Aldens Brook	25	0	12.9	0	0	0	0
Bonney Eagle Brook	62	42	90.4	53	0.7	1.3	81.8
Cooks Brook	23	17	8.6	65	0.7	1.1	86.7
Douglas Brook	26	50	34.4	88	1.9	2.1	33.3
Little River	5	4	10.8	80	0.8	1.0	100.0
North Branch	10	13	15.1	80	1.3	1.6	50.0
Pleasant River	17	0	7.5	0	0	0	0
Waterman	22	25	2.2	86	1.1	1.3	78.9
Total	187	151					
1998							
Aldens Brook	27	0	10.8	0	0	0	0
Black Brook	24	22	19.4	79	0.9	1.2	66.7
Bonney Eagle Brook	31	40	19.4	81	1.3	1.7	54.2
Brown Brook	24	17	19.4	25	0.7	1.1	93.8
Collyer Brook	5	2	2.2	40	0.4	1.0	100.0
Cooks Brook	18	12	16.1	50	0.7	1.3	77.8
Douglas Brook	32	53	62.4	84	1.7	2.1	32.0
Mousam River	16	13	26.9	63	0.8	1.3	70.0
North Branch	7	14	8.6	86	2.0	2.3	50.0
Pendexter Brook	30	41	32.3	77	1.4	1.8	47.8
Pleasant River	24	0	2.2	0	0	0	0
Waterman	25	55	23.7	84	2.2	2.6	23.5
Total	263	269					

[a] Intensity is inclusive of *N. serricornis* harboring zero midges, whereas abundance is exclusive of *N. serricornis* with zero midges.

the laboratory. Trials were conducted in July, because this is the peak recruitment period for newly colonizing midges. We removed all attached midges and the remains of their gelatinous tubes and placed hosts in plastic cage tubes open at both ends (i.d. = 3 cm, length = 10 cm). Tube ends were sealed with 0.5-mm mesh screen. This mesh size was small enough to prevent host escape but large enough to allow access by midges. The tube cages with potential hosts were returned to the stream within 8 h, wedged under large stones with their open ends parallel to the flow, and left for 10 days to allow colonization.

Results

General field and population observations

The physicochemical conditions of the 13 streams sampled were typical of softwater streams draining forested landscapes in southern Maine (Table 1). All sites had a circumneutral pH, abundant dissolved oxygen, and low buffering capacity. The Little River was the largest stream sampled; with a width of over 16 m, it had the least canopy cover of the sites studied. Stream-discharge values ranged from a minimum of 0.01 m^3/s to a maximum of 0.66 m^3/s in 1996 but varied from 0.01 to 3.10 m^3/s in 1998. No abiotic field parameters correlated with any measure of ectosymbiotic midge populations in 1996 and only one significant correlation was detected in 1998. Infestation intensity was positively correlated with percentage of riffle embeddedness (r = 0.581, P = 0.047). The familywise error rate across 24 correlations was 0.708, suggesting that caution should be used when interpreting this single significant test.

The midge *N.* (*P.*) sp. No. 5 occurred at 11 of 13 sites (~85%). Sites without midges were excluded from analyses of infestation intensity and prevalence, because we were interested in association dynamics following host attachment. Thus, sites without midges were uninformative in post-attachment dynamics. A total of 450 *N. serricornis* hosts were examined harboring 420 ectosymbionts. Host densities varied considerably among streams (CV = 127 and 80% in 1996 and 1998, respectively; Table 2) and between years within sites (CV = 98.7%). Midge prevalence was high across sites and years (grand mean ~70%) but showed no trend in the direction of change within sites across years. Prevalence declined at three sites, whereas it increased at two sites (Table 2). There was no difference in prevalence between years (t = 0.83, P = 0.420, arcsine-transformed). Mean infestation intensity also did not differ between years (t = 0.37, P = 0.717) and varied considerably within sites (CV = 42.9 and 50.3% in 1996 and 1998, respectively). Whereas infestation intensity varied within sites, the mean abundance of midges increased or remained the same at all sites sampled in both years (Table 2). The percentage of hosts harboring a single commensal did not differ between years (t = 0.73, P = 0.476, arcsine-transformed). As with prevalence and infestation intensity, the proportion of hosts harboring a single midge varied greatly between sites in both years (CV = 71.8 and

Fig. 1. Relationship between infestation intensity (no. of midges per *Nigronia serricornis* examined; log(x + 1) transformed) and prevalence (% *N. serricornis* harboring ≥1 midge; logarithmically transformed) for the ectosymbiotic *Nanocladius* (*Plecopteracoluthus*) sp. No. 5 on its host, *N. serricornis*, in streams sampled over 2 years in southern Maine.

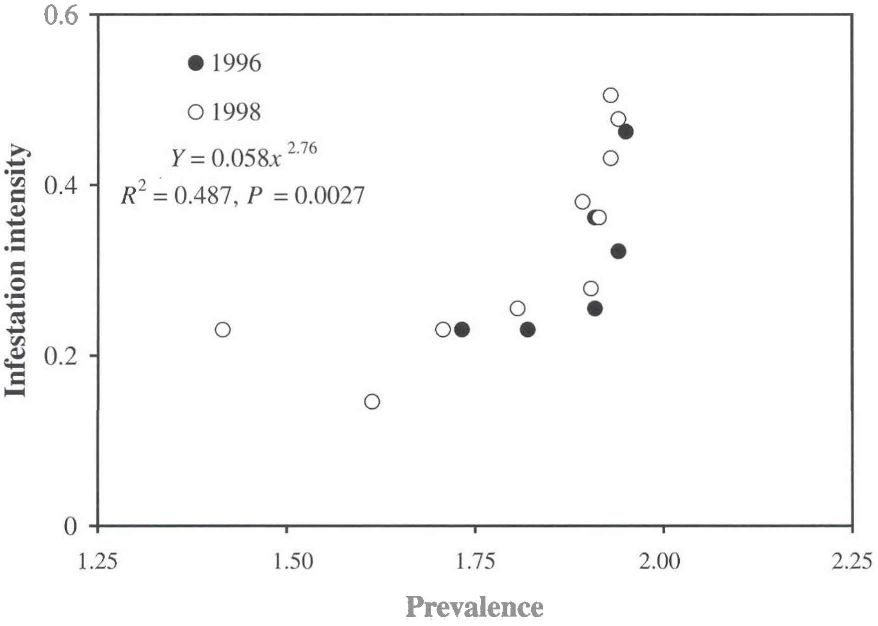
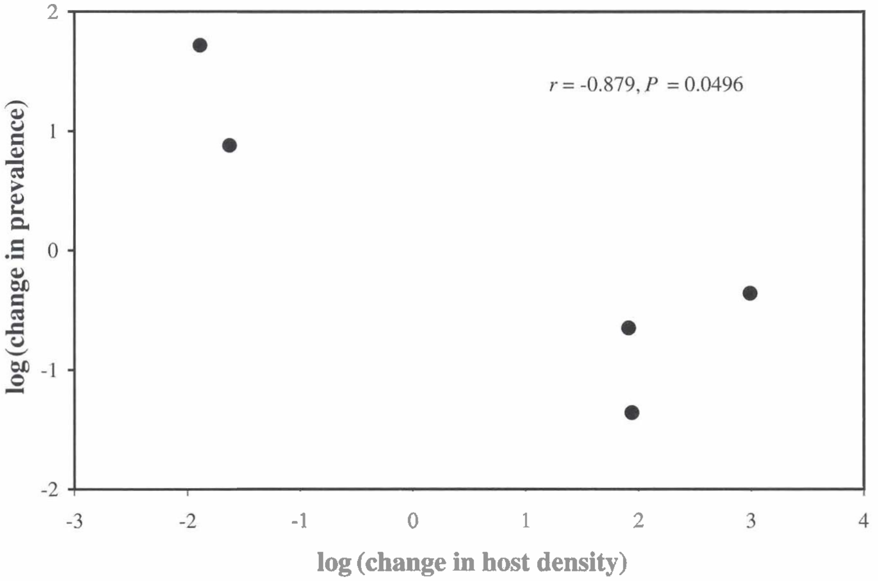


Fig. 2. Relationship between ectosymbiont prevalence and host density at sites sampled in southern Maine in the 2 years of the study.



61.6% in 1996 and 1998, respectively). The proportion of hosts harboring a single midge declined or remained unchanged at all sites sampled in both years (Table 2).

Midge prevalence was significantly correlated with infestation intensity (R^2 = 0.487, n = 16, P = 0.0027; Fig. 1), when examined across all sites in both sample years. The "sampling model" of Hanski et al. (1993) explained almost 95% of the variation in prevalence across sites ($F_{[1,11]}$ = 198.25, R^2 = 0.947, P << 0.001), indicating strong agreement with expectations of a negative binomial distribution. Counter to our expectation, neither infestation intensity nor prevalence was correlated with host density within years. However, there was a significant negative correlation between the change in prevalence and the change in host density (r = –0.879, P = 0.049; Fig. 2).

There was no difference in attachment-site use between years (Fig. 3) but midges exhibited a significant preference in attachment location, selecting the ventral thorax more than expected at ~64% of the sites (9 of 14; Table 3). Host size was significantly larger at sites where midges exhibited an attachment-site preference than at sites where no attachment-site preference was detected (t = 3.70, P = 0.003; mean head capsule width 1.49 vs. 0.92, respectively). Additionally, there was no between-year difference in the density distribution of midges on hosts (Fig. 4), and the distributions within most sites (11 of 14 comparisons) did not differ from a Pois-

Fig. 3. Comparison between years of the attachment site of the ectosymbiotic midge *N.* (*P.*) sp. No. 5 on hosts for the 2 years of the study.

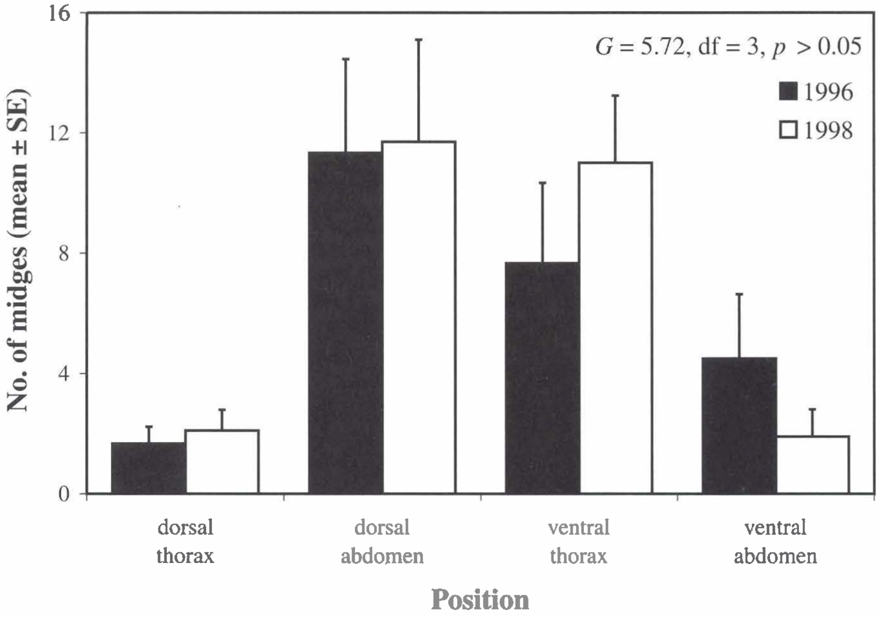


Table 3. Statistical results for ectosymbiotic midge – host dynamics in streams sampled over 2 years in southern Maine.

	Attachment-site preference		Frequency distribution		Host size vs. intensity	
	χ^2	P	χ^2	P	r	P
1996						
Bonney Eagle Brook	18.86	<0.001	4.82	ns	0.44	<0.01
Cooks Brook	7.35	ns	4.98	ns	0.50	<0.05
Douglas Brook	17.38	<0.001	3.63	ns	0.20	ns
North Branch	6.66	ns	0.89	ns	0.50	ns
Waterman	7.43	ns	10.88	<0.025	0.10	ns
1998						
Black Brook	76.80	<0.001	9.83	<0.025	0.14	ns
Bonney Eagle Brook	33.95	<0.001	0.71	ns	–0.12	ns
Brown Brook	45.29	<0.001	8.62	<0.05	0.49	<0.05
Cooks Brook	6.75	ns	1.38	ns	0.26	ns
Douglas Brook	18.88	<0.001	2.44	ns	0.19	ns
Mousam River	10.99	<0.05	1.41	ns	–0.06	ns
North Branch	7.22	ns	1.94	ns	0.29	ns
Pendexter Brook	42.13	<0.001	0.42	ns	–0.09	ns
Waterman	17.94	<0.001	6.49	ns	0.49	<0.05

Note: Site preference data are χ^2 results examining attachment-site preference of symbiotic midges. The test examines whether the number of midges attached to the dorsal thorax, dorsal abdomen, ventral thorax, or ventral abdomen (see text for explanation) differ from expected. In all cases, df = 3, and three sites (Aldens Brook, Little River 202, and Pleasant River in 1996 and Aldens Brook, Collyer Brook, and Pleasant River in 1998) were excluded from analyses, owing to the small number of observed midges. Frequency-distribution data are χ^2 results examining whether the frequency of midges per host differs from a Poisson expectation. Host-size and -intensity data are correlation coefficients between host head capsule width and number of midges per host. The degrees of freedom for correlations are the number of hosts examined minus one from Table 2.

son expectation. Only four sites (~28.5%) showed a significant correlation between host size and the number of midges (Table 3).

Alternative-host occurrence and host-specificity trials

Four potential alternative-host taxa were common in each stream sampled: *Sialis* sp. (Sialidae: Megaloptera), *Boyeria vinosa* Say (Aeshnidae: Odonata), *A. abnormis* Newman (Perlidae: Plecoptera), and *Ephemera* sp. (Ephemeridae: Ephemeroptera). Of the five potential hosts (including *N. serricornis*), none was the most common in all streams and *N. serricornis* was most common in only two (Fig. 5).

Fig. 4. Comparison between years of the frequency of occurrence of different densities of the ectosymbiotic midge *N.* (*P.*) sp. No. 5 on its host, *N. serricornis*, in the 2 years of the study.

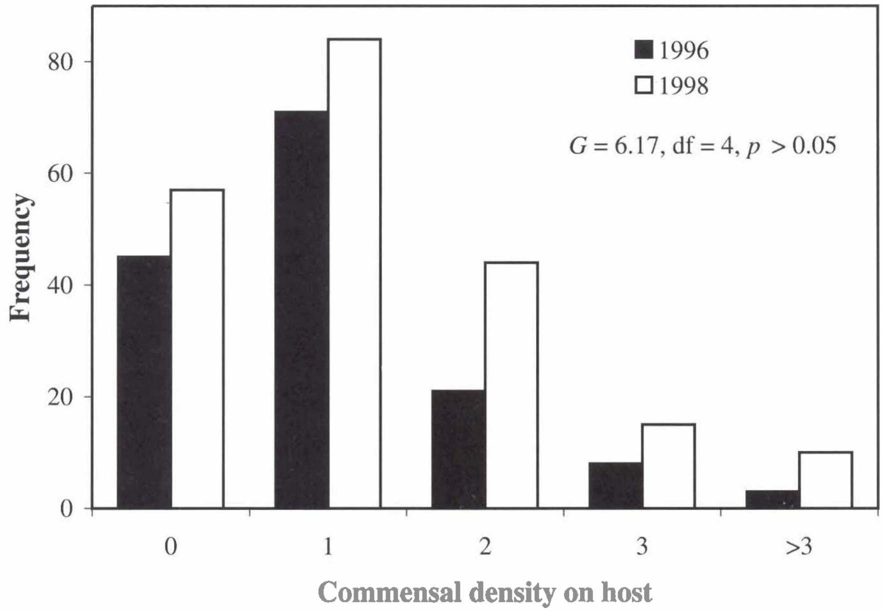
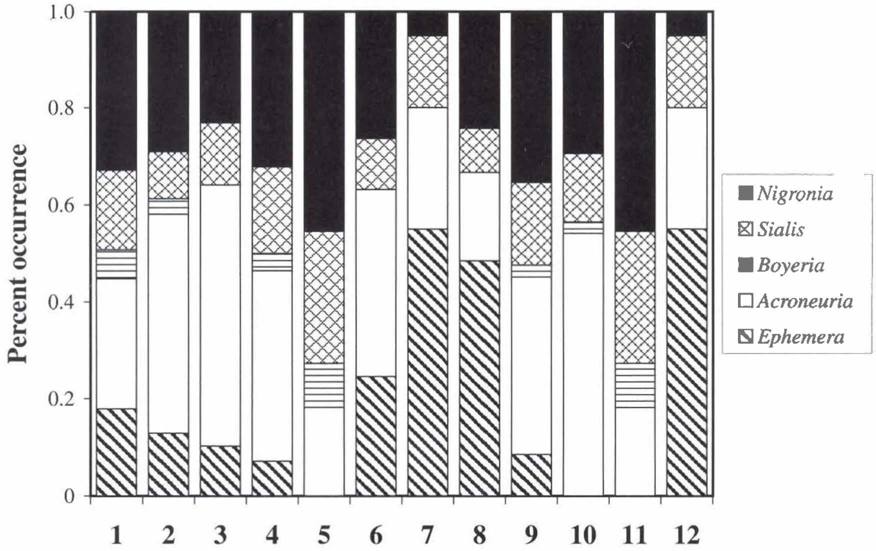


Fig. 5. The proportional abundance of potential alternative hosts (*Sialis* sp., *Boyeria vinosa*, *Acroneuria abnormis*, and *Ephemera* sp.) occurring in riffle habitats with *N. serricornis* hosts. Values are based on number per square metre from five replicate Surber samples in 12 streams with *N.* (*P.*) sp. No. 5 in 1998. The numbers 1 through 12 represent Waterman, Bonney Eagle Brook, Black Brook, Brown Brook, Collyer Brook, Pendexter Brook, Cooks Brook, North Branch, Douglas Brook, Mousam River, Aldens Brook, and Pleasant River, respectively.



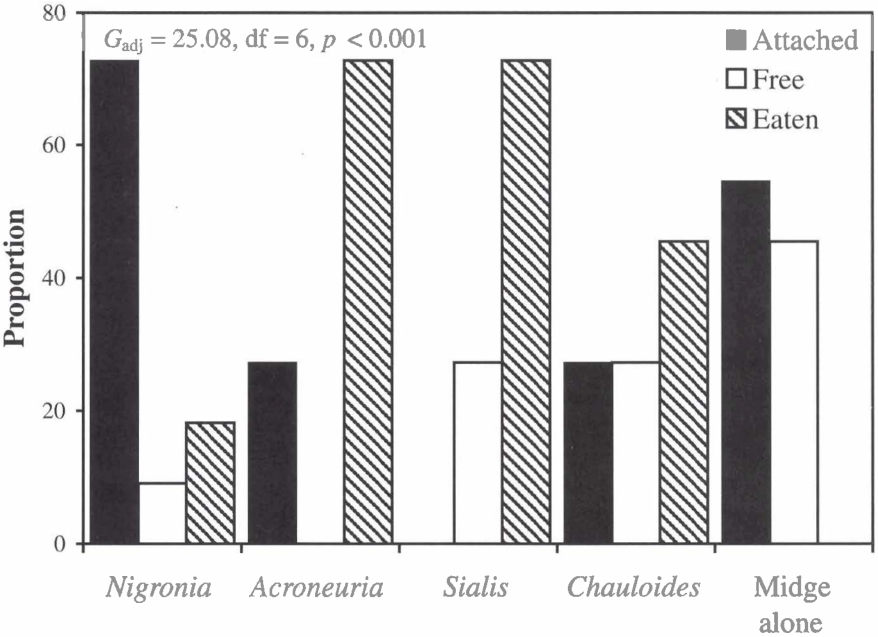
None of the other potential hosts harbored this symbiotic midge, although a single specimen of *N.* (*P.*) *downesi* (Steffan) was observed on a nymphal *A. abnormis*.

There were significant differences in midge fate in the single-host trials (Fig. 6). Most midges attached to *N. serricornis* hosts (~75%), whereas they rarely attached to other potential hosts (all occurrences <30%). "Eaten" was the most common fate of midges in containers with alternative hosts and "free" was the least common fate. In the *N. serricornis* – *A. abnormis* trials, midges attached to *N. serricornis* much more than would be expected by chance (χ^2 = 39.81, df = 19, $P < 0.005$). Midges exhibited no attraction to a specific *N. serricornis* host in the double *N. serricornis* trials (χ^2 = 8.30, df = 9, $P > 0.05$). No midges colonized caged hosts in the 10-day field trials.

Discussion

Ectosymbiotic midges attached to *N. serricornis* hosts were common in the region of southern Maine examined, corroborating an earlier study by Pennuto (1997). In addition, prevalence within sites was generally high (~70% on average) and infestation intensity was higher than reported for other north temperate zone species (e.g., Gotceitas and Mackay 1980;

Fig. 6. Fate of the ectosymbiotic midge *N.* (*P.*) sp. No. 5 in replicate tanks (n = 11) containing a single potential host (*N. serricornis*, *A. abnormis*, *Sialis* sp., *C. rastricornis*) or the midge alone.



Bottorff and Knight 1987). Although several other large potential host taxa were present in the same microhabitat as *N. serricornis* hosts, none harbored *N.* (*P.*) sp. No. 5 in any of the streams examined. Laboratory studies corroborated the field collections; midges attached to *N. serricornis* significantly more than they did to any other offered host. Collectively, these data suggest that the symbiosis between this midge and *N. serricornis* is a stable and potentially host-specific one (although R.E. Jacobsen (personal communication) has observed this species attached to nymphs of the damselfly, *Argia*, in Maryland).

High substrate instability has been proposed as a potentially important condition in the origin of ectosymbiosis within the Chironomidae (e.g., Steffan 1967; Gotceitas and Mackay 1980; Bottorff and Knight 1987). In this study, neither substrate instability nor any other environmental condition evaluated showed a strong correlation with commensal prevalence or intensity across years, counter to our expectation. These results suggests that either (*i*) biotic interactions, compared with abiotic ones, have greater importance in determining symbiont population dynamics; (*ii*) we failed to investigate any important abiotic variables; (*iii*) the conditions that may have been important in the evolution of this midge symbiosis are no longer the selective mechanisms maintaining it, potential ghosts of symbiosis past (sensu Connell 1980); or (*iv*) midge population dynamics may be only weakly controlled by environmental conditions, assuming their hosts occur in the stream. Figuerola (2000) reached a similar conclusion, finding few correlates between prevalence and ecological conditions when investigating feather mites on passerine birds.

Boucher et al. (1982) suggest that insights into symbioses and the strength of biotic interactions can be gleaned by examining changes in the rates of symbiont prevalence and infestation intensity as host density changes. Using this approach, others have shown that symbiont intensity and prevalence often increase with host density (plants, Silander 1978; ectosymbiotic midges, Svensson 1980; internal parasites, Arneberg et al. 1998; ectoparasitic fleas, Krasnov et al. 2002). When we examined the relationship between rates of infestation intensity and prevalence and host density, we did not observe any positive correlations. Possibly we searched for correlations on the wrong time scale in this study. The continuous summer-emergence pattern and year-round occurrence of the midge on its host led us to believe that the population structure in this species was composed of split cohorts, having either a 1- or 2-year life cycle. Thus, our stream surveys were spaced 2 years apart. Maybe midge populations respond to host density more rapidly than we suspected and annual surveys would reveal the expected patterns. Alternatively, perhaps host spatial distribution, not density per se, is an important correlate of midge population dynamics. If hosts aggregate in key microhabitats also occupied by midges, encounter rates with hosts would still be high even if host density was low. Thus, no correlation between host density and either infestation intensity or prevalence would be detected. Further investigations of both host and midge microhabitat use would help address this possibility.

When we limited our observation to only those sites where we documented changes in host density, there was a significant negative correlation with prevalence. This observation was counter to our expectation. One possible explanation for this observation is dependent on midge searching behavior. If midge population size is constant and they actively seek hosts (Svensson 1980), then prevalence should increase as host density decreases, because midges locate all hosts.

We observed a significant relationship between commensal prevalence and infestation intensity on hosts, as has been observed in other host–symbiont systems (Anderson and May 1978; Bush and Holmes 1986; Krasnov et al. 2002). Further, the data are in clear agreement with Hanski et al. (1993) in that a negative binomial distribution best describes this relationship. Habitat patchiness, intraspecific competition, and inequities in host-detection ability among midges could generate this distribution pattern. Alternatively, the positive correlation between prevalence and intensity might arise if these midges, although able to discriminate suitable from nonsuitable hosts (i.e., they selected *N. serricornis* over other potential hosts), attach to hosts in a passive nonsearching manner (Bottorff and Knight 1987). The lack of colonization of caged hosts during peak recruitment supports this contention. If midges do not actively seek hosts but wait for host contact, the proportion of hosts harboring symbionts should increase with midge-population size, because there is an increased likelihood of any midge encountering a host. Thus, a positive correlation between infestation intensity and prevalence would be observed. However, Svensson (1980) suggested that chemosensory cues were likely used for host identification by the midge commensal *Epoicocladius flavens* (Malloch) on burrowing mayflies in Sweden. If chemosensory cues are important and midges actively seek hosts, prevalence should remain constant (because hosts are all located by searching midges), whereas infestation intensity should increase with increasing midge-population size, because more midges are available to colonize hosts. Further work on host identification cues is warranted.

Intraspecific competition has been invoked as a mechanism generating the distribution patterns of ectosymbiotic midges on their hosts (Svensson 1980; Bottorff and Knight 1987; Pennuto 2000). Although only 3 of 14 frequency distributions on hosts indicated competition for attachment sites, all midge populations showed significant attachment-site biases, indicating nonrandom patch (host) use. Once midges attached to a host, they occurred most frequently on the ventral thorax. This pattern has been observed for other symbiotic midges associated with Megaloptera hosts (Furnish et al. 1981; Hayashi 1998). In addition, attachment-site bias has been shown for related midge symbionts on Plecoptera and Ephemeroptera hosts (e.g., Tokeshi 1986; Bottorff and Knight 1987; Jacobsen 1999). Pennuto (2000) suggested that the ventral-thorax location might allow direct access to pupation sites on the thorax, where these larvae construct a puparium prior to emergence. Our observations on host foraging behavior might also explain this attachment-site bias. These fishfly hosts are sit-and-wait predators, often remaining in the same position in a stream for over 24 h. Hayashi and Nakene (1989) reported a similar finding for *Protohermes grandis*, a related corydalid from Japan. In addition, fishfly predatory posture consists of a slightly raised thorax with a downward-jutting head, resulting in the jaws being directed toward the substrate (C.M. Pennuto, personal observation). It is possible that this position "funnels" water under the head and between the legs, bringing potential drift prey into contact with the predator. If true, two beneficial outcomes are apparent for a midge residing on the ventral thorax: constant aeration with incoming currents and access to high quality food resources as a result of spill-over during host feeding (i.e., fishflies are sloppy eaters). Neither of these possibilities has been investigated. Perhaps this symbiosis represents a processing-chain commensalism (Heard 1994), providing benefits to the midge while being neutral for the host.

These ectosymbiotic midges preferred *N. serricornis* when provided with an alternative host, *A. abnormis*, in paired-host trials and they attached to *N. serricornis* more frequently than to any of three potential alternative hosts in single-host trials. In fact, "eaten" was the most common fate of midges in the potential alternative host trials. A small number of midges attached to an alternative host and some constructed tubes on the bottom of the experimental containers, suggesting that these symbionts are not host specific. However, the host-selection trials were very short (only 24 h) and midge survival was not determined. Our laboratory has been unable to rear these midges to adulthood when removed from their host (C.M. Pennuto, unpublished data), indicating some host dependency.

The high prevalence and infestation intensity of ectosymbiotic midges on hosts in both years of the study suggests that this symbiosis is persistent and stable, although neither measure responded to changes in host density as expected. Potentially, the life-history dynamics of this midge reduce its dependency on host-population dynamics, even though its obligate nature indicates some level of dependency. Perhaps extremely high fecundity of adult midges or very high survivorship of early instars ensures contact with hosts, regardless of host density. Alternatively, the 2-year sampling schedule may have missed shorter-scale patterns between the species. Symbiotic associations among aquatic insects remain understudied, but should provide rich information concerning the fitness costs and benefits associated with symbioses. For example, our data on alternative-host choice suggest that there are predation costs associated with host attachment. Comparative studies of free-living, commensal, and parasitic midges in the genus *Nanocladius* would allow assessment of fitness consequences associated with life style.

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