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ECOLOGY OF *CULICOIDES* (DIPTERA: CERATOPOGONIDAE) IN SOUTHCENTRAL FLORIDA AND EXPERIMENTAL *CULICOIDES* VECTORS OF THE AVIAN HEMATOZOAN *HAEMOPROTEUS DANILEWSKYI* KRUSE

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ABSTRACT: To determine the vectors of *Haemoproteus danilewskyi* in blue jays (*Cyanocitta cristata*) in southcentral Florida (USA), we conducted a 2 yr study from January 1993 to December 1995 of the presence and seasonal abundance of *Culicoides* spp. Of the 14 species of *Culicoides* captured in Centers for Disease Control light traps, 10 were ornithophilic. Of these, *C. edeni*, *C. knowltoni*, *C. stellifer*, *C. beckae*, and *C. arboricola* were most abundant, representing 46% of the total collection and 99% of the ornithophilic collection. The presence of *C. stellifer* in Bennett trap collections represents a new biting record for this species on passerine birds. We experimentally challenged the most abundant ornithophilic species to determine which were capable of supporting sporogonic development of *H. danilewskyi*. *Culicoides edeni*, *C. knowltoni*, and *C. arboricola* supported sporogonic development of *H. danilewskyi*.

Key words: Blue jay, *Culicoides*, *Cyanocitta cristata*, *Haemoproteus*, vector.

INTRODUCTION

Biting midges (Dipter: Ceratopogonidae) are competent vectors of *Haemoproteus* spp. (Fallis and Wood, 1957; Bennett and Fallis, 1960; Bennett and Combs, 1975) which are common hematozoa in wild birds. Despite a great deal of recent interest in the effect of blood parasites on the evolutionary ecology of birds, little is known of the pathogenicity and ecology of species of *Haemoproteus* because laboratory infections are difficult to establish. Unlike species of *Plasmodium*, infections of *Haemoproteus* cannot be transmitted between birds via inoculation with infected blood because schizogony does not occur in erythrocytes. Therefore, pathogenicity studies of *Haemoproteus* spp. require the determination of arthropod vectors for their use as a source of infective sporozoites.

Haemoproteus danilewskyi is a common blood parasite of blue jays in south Florida (USA; Garvin and Greiner, 2003). In Newfoundland (Canada), Fallis and Bennett (1960, 1961) found that *Culicoides crepuscularis*, *C. stilobezzioides*, and *C. sphagnumensis* were capable of supporting de-

velopment of *H. danilewskyi*. However, of these ceratopogonids, only *C. crepuscularis* is known from previous surveys at our field site in southcentral Florida (W. Wirth pers. comm).

Of the other species captured by Wirth at our field site, five are known to be ornithophilic. *Culicoides arboricola* was reported to feed on chickens (Snow et al., 1957), *C. stellifer* on turkeys (Humphreys and Turner, 1973), *C. crepuscularis* on chickens (Hoffman, 1925), and *C. edeni* on turkeys (Atkinson et al., 1983).

As part of a 3 yr field investigation of the pathology and epizootiology of *H. danilewskyi* in blue jays (*Cyanocitta cristata*) in southcentral Florida, we determined the diversity, relative abundance, seasonal abundance, and vertical distribution of *Culicoides* species as well as the meteorological factors affecting abundance. Using the five most abundant ornithophilic species from these collections, we conducted a laboratory study to determine which were capable of supporting sporogonic development of *H. danilewskyi* in captive blue jays.

METHODS

Study site and vector survey

Field work was conducted at Archbold Biological Station (Archbold; 27°10'N, 81°21'W) at the southern end of the Lake Wales Ridge in Highlands County, southcentral Florida. The 2,024 ha, xeric upland preserve is a relict ancient dune system comprised of a mosaic of seven vegetation types that vary with elevation across a range of 35–65 m (Abrahamson et al., 1984). The climate is subtropical with a rainy season during the hot summer months and dry, mild conditions during the winter months. Average rainfall is 133 cm, with most rain falling between June and September and least during winter and spring months. Temperatures below freezing are recorded several times a year between November and February, but are continuous for only several hours.

Because vector abundance and diversity vary with microhabitat, we established two trap sites 1 km apart to monitor vector populations. We chose sites likely to have similar blue jay abundance (Tarvin and Garvin, 2002), but different elevation and vegetation associations to provide a more complete picture of species diversity and abundance than would be obtained with a single site. Site 1 was located at the Florida Division of Forestry firetower at an altitude of 63 m, the highest point at Archbold. The area was human-modified by mowing with well-drained soil and was surrounded by pine forest. The only known standing water available for *Culicoides* larval development in this area was a 0.5×0.5 m permanent puddle created by a leaking faucet 150 m from the trap site. Tree-holes and the gutters on a small cottage 20 m from the tower also may have served as larval habitat. Slash pines (*Pinus elliotti*) are common and probably provide numerous sites for species such as *C. arboricola* which breed in tree-holes (Blanton and Wirth, 1979), and possibly *C. beckae* and *C. knowltoni*, although larval habitats for these two species are not known. Site 2, elevation 42 m, also was in a human-modified area and next to an old cattle pond with a sandy edge where ground breeding species, such as *C. insignis* and *C. edeni* may deposit eggs (Blanton and Wirth, 1979). The site was mainly unmaintained pasture with numerous large slash pines and patches of oak (*Quercus* spp.) understory. An orange grove that extended several km to the east and south was 150 m from the trap site and scrubby flatwoods was to the north and west.

To determine seasonal abundance of *Culicoides* spp. at Archbold, we made biweekly light trap collections from January through September 1993. Then, to gain a finer resolu-

tion of species abundance, we made weekly collections for the remainder of the study, from October 1994 through December 1995. At each site, we operated five Centers for Disease Control (CDC) incandescent light traps (Model 512, J. W. Hock Co., Gainesville, Florida), each supplemented with approximately 0.5 kg of dry ice as a carbon dioxide source in a 2 l water thermos (Coleman Co., Inc., Wichita, Kansas, USA). To avoid confounding effects of weather, we did not trap on nights that were likely to be windy or rainy. Average monthly abundance of each *Culicoides* spp. was determined by dividing the total number of individuals captured during the month by the number of trap-nights that month.

Because *Culicoides* species vary in the height at which they feed, we used a rope and pulley system to position sets of traps above each other at 2 m increments from 4–12 m high and operated traps from approximately 1 hr before sunset to 1 hr after sunrise. To determine vertical distribution of the most abundant ornithophilic *Culicoides* species, we combined data from both trap sites to give a more general assessment of the overall *Culicoides* population at Archbold. Chi-square contingency analysis was used to compare prevalence of *C. edeni*, *C. arboricola*, and *C. knowltoni* at each trap height and a Kruskal-Wallis One-way ANOVA was used to compare mean abundance of each species at each of the five trap heights.

To identify *Culicoides* that naturally feed on blue jays and in an attempt to infect flies for experimental infection, Bennett traps (Bennett, 1960) were operated in the forest canopy from 1 hr before sunset to 1 hr after sunset at seven sites for 51 nights in 1993 and 1994. As bait, an infected blue jay, sedated with 7.5 mg (ketamine hydrochloride, Fort Dodge Laboratories, Fort Dodge, Iowa) was placed in a 30×15×20 cm cage made of 1.5×1.25 cm hardware cloth that was centered on a 60×60 cm plywood board. With rope and pulley, we hoisted the jay 10–12 m into the forest canopy. After approximately 10 min, the jay was quickly, but carefully, lowered to the ground and covered with a 58×58 cm nylon organdy box. A second jay was then hoisted into the canopy while the first sat undisturbed for 8–10 min to allow *Culicoides* to finish feeding and leave the bird. Flies were aspirated from the organdy box through a sleeve in the top after the cover was in place for 10 min and were placed into fly holding boxes. During some of the trapping sessions, dry ice was hung directly above the cage to provide additional carbon dioxide as an attractant.

The influence of temperature and rainfall on vector abundance was evaluated by comparing

vector activity with weather data collected from two meteorological stations maintained by the scientific staff at Archbold Biological Station. Hourly temperature and windspeed data were recorded at Archbold's Environmental Science Engineering Station at the southern end of the station, and rainfall was monitored daily approximately 100 m east of the station laboratories. To examine the influence of temperature on adult *Culicoides* activity, we compared trapping night temperatures to abundance of *C. arboricola*, *C. edeni*, and *C. knowltoni* for 55 trapping nights from January–June 1994 and January–December 1995. Mean trapping night temperature was calculated for each trapping session by averaging the hourly temperatures during trap operation. The influence of rainfall on *Culicoides* activity was examined by comparing the total rainfall for the 30 days preceding each trap night to the number of *C. arboricola*, *C. edeni*, and *C. knowltoni* captured each trapping night from January 1994–December 1995. We used Spearman's rank correlation coefficient to assess the relationship between mean monthly abundance of *C. edeni*, *C. arboricola*, and *C. knowltoni* and the previous month's rainfall in 1994 and 1995, as well as mean hourly temperature and average windspeed during the trap session.

Experimental infections

To determine which of the five most abundant ornithophilic species of *Culicoides* are capable of sporogonic development of *H. danilewskyi*, individuals of each species were captured in CDC light traps equipped with fine mesh collection bags and supplemented with approximately 0.5 kg of dry ice in a 2 l thermos. Traps were set approximately 1 hr before sunset and collections were made approximately 1 hr after sunrise by aspirating live *Culicoides* from the collection bags into 240 ml cardboard ice cream boxes fixed with nylon mesh lids. Cotton swabs soaked in 5% sucrose solution were placed on the mesh as a source of food and moisture, and replaced daily to prevent molding. A petri dish was placed on each box as a lid to help retain moisture and boxes were stored in the laboratory at 24–28 C.

Individuals of each *Culicoides* species were infected by allowing them to feed on a naturally infected blue jay that was captured and held in captivity. Via thin blood smear, we determined that density of infection in the jay was 30 infected red blood cells (RBCs) per 10,000 RBCs. A representative slide was deposited in the US National Parasite Collection (Beltsville, Maryland, USA; Accession number 091708). We held *Culicoides* in a 10 ml clear plastic vial

and placed the open end of the vial against the breast, from which feathers had been removed to facilitate feeding. Engorged flies were maintained in the laboratory, as described, for 10 days to allow infections to develop to the sporozoite stage (Atkinson et al., 1983). *Culicoides* were chilled and identified to species by wing pattern (Blanton and Wirth, 1979). Species-specific pools were prepared with 1 ml RPMI tissue culture medium in a Tenbrock glass tissue grinder inserted into an ice bath. Each species-specific slurry was recovered with a separate 1 ml tuberculin syringe for inoculation into uninfected 1 yr old blue jays raised in captivity from 10 day old nestlings. Each of the five blue jays was examined daily during the week preceding inoculation to confirm absence of infection. The birds were inoculated in the abdominal cavity with a separate species-specific slurry and maintained in vector proof facilities at Archbold as described by Garvin et al. (2003). Beginning on day 8 post-inoculation we prepared blood smears daily to search for transmission and the timing of onset of detectable parasitemia. Each slide was examined until approximately 100,000 erythrocytes were observed. Jays were declared uninfected if *H. danilewskyi* was not detected by 28 days post-inoculation.

RESULTS

Survey

During 78 nights of light trapping at two sites, we captured 25,101 *Culicoides* representing 14 species (Table 1). *Culicoides insignis*, a mammal feeder, represented over half of the total collection. Of the known ornithophilic species, *C. edeni* was most abundant, followed by *C. knowltoni*, *C. stellifer*, *C. beckae*, *C. arboricola*, *C. crepuscularis*, *C. haematopotus*, and *C. hinmani*. The number of flies captured per trap followed a negative binomial distribution. Of the eight ornithophilic species collected in light traps (Table 1), five species, *C. edeni*, *C. knowltoni*, *C. stellifer*, *C. beckae*, and *C. arboricola* constituted 46% of the total collection and 99.1% of the ornithophilic collection. Three engorged *C. stellifer* were aspirated from a single Bennett trap, representing the only *Culicoides* collected via this method in this study.

The total number of *Culicoides* captured at site 1 was slightly greater than at

TABLE 1. Species of *Culicoides* captured in CDC light traps and their relative numbers and percent at site 1 and site 2. A total of 25,101 individuals were collected during 78 trapping nights from January 1994–December 1995.

Species	Site 1		Site 2	
	Number collected	% total	Number collected	% total
<i>C. insignis</i>	4,271	31.9	9,136	68.1
<i>C. edeni</i>	5,153	84.0	978	16.0
<i>C. knowltoni</i>	1,819	80.9	430	19.0
<i>C. stellifer</i>	915	52.1	842	47.9
<i>C. beckae</i>	848	92.0	74	8.0
<i>C. arboricola</i>	438	89.4	52	10.6
<i>C. crepuscularis</i>	30	53.6	26	46.4
<i>C. haematopotus</i>	35	83.3	7	16.7
<i>C. pusillus</i>	1	5.6	17	94.4
<i>C. baueri</i>	8	66.7	4	33.3
<i>C. hinmani</i>	3	27.3	8	72.7
<i>C. furens</i>	3	100.0	0	0.0
<i>C. nanus</i>	0	0.0	2	100.0
<i>C. bickleyi</i>	0	0.0	1	100.0
Total	13,524		11,577	

site 2 and the relative abundance of each species varied considerably between the sites (Table 1). With the exception of *C. insignis*, the six most common species of *Culicoides* were more abundant at site 1 than at site 2. Over 80% of *C. edeni*, *C. knowltoni*, *C. beckae*, and *C. arboricola*

were captured at site 1. *Culicoides stellifer* also was slightly more abundant at site 1.

Generally, seasonal abundance of *C. edeni*, *C. arboricola*, and *C. knowltoni* was high in spring and summer and low in winter (Fig. 1). Numbers of *C. arboricola* were lowest during the winter months and highest from April through November. The pattern for *C. edeni* was similar to that of *C. arboricola* with the lowest mean monthly abundance in winter. Similarly, abundance of *C. knowltoni* also was lowest during winter months. In 1994 *C. knowltoni* was absent from collections intermittently throughout the year. Unlike *C. arboricola* and *C. edeni*, mean monthly abundance of *C. knowltoni* was significantly lower in 1994 than in 1995, with 186 and 2,063 individuals captured respectively ($t=110.5$, $P=0.024$).

Average hourly temperature during each trap session was positively correlated with abundance of *C. arboricola* ($r_s=0.57$, $P<0.001$), *C. edeni* ($r_s=0.61$, $P<0.001$), and *C. knowltoni* ($r_s=0.40$, $P<0.001$; Fig. 2). During the study period, temperature at Archbold ranged from 8 C in December, 1995 to 32 C in May, 1995. The lowest average weekly temperatures were record-

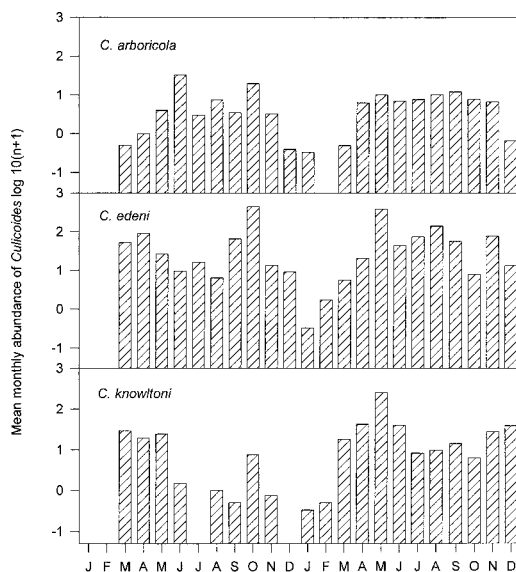


FIGURE 1. Mean monthly number of *C. arboricola*, *C. edeni*, and *C. knowltoni* captured in CDC light traps, all trap sites combined, 1994-95.

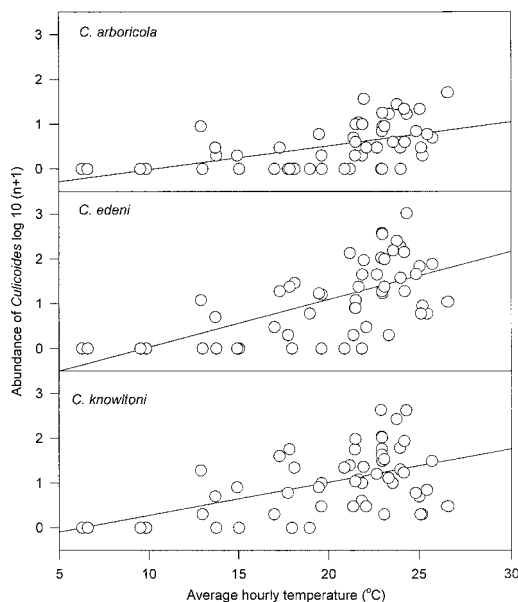


FIGURE 2. Abundance of *C. arboricola*, *C. edeni*, and *C. knowltoni* in relation to average hourly temperature during trap session, 1994–95.

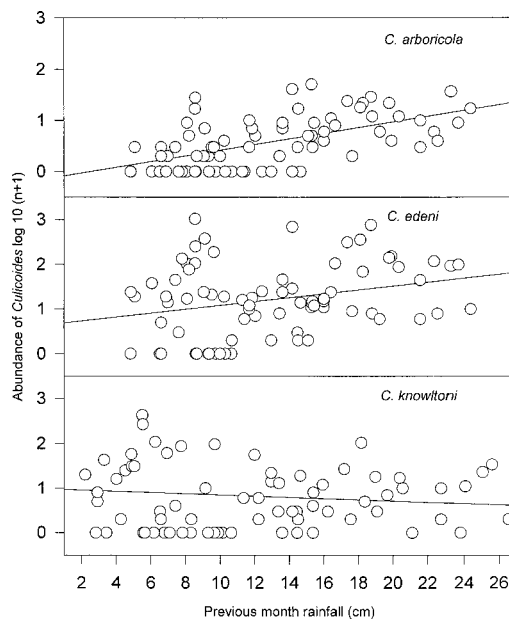


FIGURE 3. Abundance of *C. arboricola*, *C. edeni*, and *C. knowltoni* relative to total rainfall during 1 mo prior to trapping night, 1994–95.

ed in December, January, and February, and the highest average weekly temperatures were recorded in May, June, July, and August.

Average annual rainfall from 1992–95 was 143 cm and peaked during the warmer months in the summer and early fall. The influence of total monthly rainfall on mean vector abundance during the following month varied with species (Fig. 3). Abundance of *C. arboricola* was positively correlated ($r_s=0.58$, $P<0.001$) with previous month's rainfall, however abundance of *C. edeni* and *C. knowltoni* was not ($r_s=0.21$, $P=0.74$, $r_s=-0.13$, $P=0.275$).

Windspeed had a significant negative effect on the abundance of both *C. arboricola* ($r_s=-0.38$, $P=0.006$) and *C. edeni* ($r_s=-0.41$, $P=0.003$), but not on *C. knowltoni* ($r_s=-0.12$, $P=0.402$; Fig. 4).

All three species were most abundant at 12 m above ground and became less abundant with each 2 m reduction in height (Fig. 5). The lowest percent of *C. edeni* (8%) and *C. knowltoni* (3%) was at 4 m, and *C. arboricola* (8%) at 6 m. The influence of height was significant for both *C.*

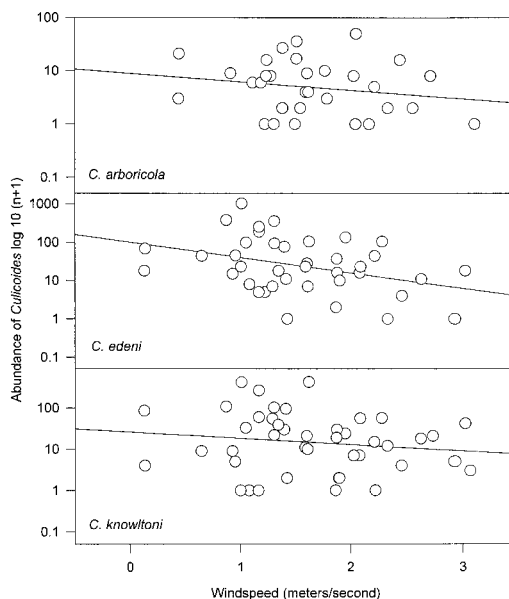


FIGURE 4. Abundance of *C. arboricola*, *C. edeni*, and *C. knowltoni* relative to average hourly wind-speed during trap session, 1994–95.

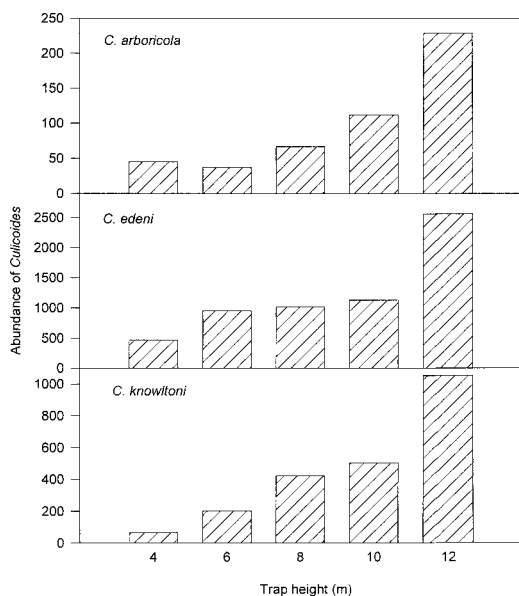


FIGURE 5. Abundance of *C. arboricola*, *C. edeni*, and *C. knowltoni* captured at five trap heights during 78 nights, 1994–95.

arboricola ($F=2.58$, $P=0.037$) and *C. knowltoni* ($F=4.99$, $P=0.001$). Despite a strong trend, we detected no significant effect of height on *C. edeni* abundance ($F=4.99$, $P=0.061$).

Experimental infections

The five most common ornithophilic *Culicoides* species became the focus of experimental transmission studies to identify species capable of supporting development and transmission of *H. danilewskyi*. Blue jays injected with slurries of ground *C. knowltoni*, *C. beckae* and *C. edeni* developed infections of *H. danilewskyi*, although those inoculated with *C. arboricola*

and *C. stellifer* did not develop infections at 28 days post-infection (Table 2).

DISCUSSION

The subtropical climate and heterogeneity of habitat types occurring in central Florida contribute to the high diversity of *Culicoides* species found there. Fourteen species of *Culicoides* reported in this study was low in comparison to more than 50 species known to occur in Florida (Blanton and Wirth, 1979). Overall, the diversity of *Culicoides* species in this study was similar to that found at Archbold by Wirth (pers. comm.), with the addition of three additional species of relatively low abundance: *C. baueri*, *C. hinmani*, and *C. bickleyi*. Because the mosaic of habitat types at Archbold provides a wide variety of oviposition and larval development sites, more detailed sampling is likely to reveal even more species breeding in vegetation and wet areas not sampled in this study.

Culicoides insignis was the most abundant species captured in light trap collections, making up 53.4% of the entire collection. *Culicoides edeni* represented 24.4% of the total collection and was the most abundant ornithophilic species, followed by *C. knowltoni*, *C. stellifer*, *C. beckae*, and *C. arboricola*. These five ornithophilic species represented 46% of the total collection and 99% of the ornithophilic collection. These data support other work in south Florida by Atkinson (1988) who also found that *C. insignis* was the most abundant *Culicoides* species and *C. edeni* was the most abundant ornithophilic spe-

TABLE 2. Results of inoculation of single species of *Culicoides* pools into blue jays. A single pool of each species was inoculated into a single lab reared blue jay.

Species	Number of flies injected	Status of inoculated jay
<i>C. edeni</i>	9	gametocytes observed 14 days post-inoculation
<i>C. knowltoni</i>	19	gametocytes observed 12 days post-inoculation
<i>C. stellifer</i>	18	gametocytes not observed by 28 days post-inoculation
<i>C. arboricola</i>	22	gametocytes observed 12 days post-inoculation
<i>C. beckae</i>	16	gametocytes not observed by 28 days post-inoculation

cies followed by *C. knowltoni*, *C. stellifer*, and *C. arboricola*.

Culicoides stellifer was captured in blue jay-baited Bennett traps, representing the first biting record of this species on a passerine bird. Given the high abundance of *C. edeni*, its absence from Bennett traps is curious. Atkinson et al. (1988) captured both *C. edeni* and *C. arboricola* in Bennett traps baited with turkeys at Fisheating Creek in south Florida. The difference may be because of the relatively greater attraction of *C. edeni* and *C. arboricola* to turkeys over blue jays due to their greater mass and proportionally greater production of carbon dioxide and heat. All of these species do feed on blue jays as indicated in the experimental infection portion of our study.

The seasonal abundance of *C. edeni*, *C. arboricola*, and *C. knowltoni* was generally greatest during the spring and summer and lowest during the winter. These results were similar to the seasonal variation in abundance of these species described by Atkinson et al. (1988). Abundance of both *C. edeni* and *C. knowltoni* was significantly correlated with mean hourly trapping night temperature. Abundance of *C. arboricola* was positively correlated with total rainfall during the month preceding each trapping night, perhaps a result of the accumulation of water in treehole breeding sites following rainy periods. The abundance of *C. edeni* at the well-drained sandhill site might indicate that this species is more of a generalist in its larval habitats than previously thought, possibly depositing eggs in tree holes, other wet depressions in vegetation, or the gutters of a nearby cabin. In contrast, abundance of *C. knowltoni* was not significantly correlated with rainfall. Although the larval habitats for this species are not known, increased rainfall may flood breeding sites and prevent oviposition or development of larvae.

Spatial factors, such as vertical distribution of potential vector species (Tanner and Turner, 1974; Henry and Adkins, 1975) are critical in evaluating their role in

hematozoa transmission. The relative abundance of the three likely vector species, *C. edeni*, *C. arboricola*, and *C. knowltoni* was greatest at 12 m. Therefore, each species forages in close proximity to mean nest height of blue jays at Archbold, which averaged 11 m (Tarvin and Garvin, 2002). Furthermore, although roosting height was not evaluated, on the several occasions when blue jays were observed at dusk, they moved to roost sites in large slash pines, at heights greater than 10 m above the ground. This position in the forest canopy makes it likely that *C. edeni*, *C. arboricola*, and *C. knowltoni* feed regularly on nesting and roosting blue jays. However, roosting height is relevant to vector host interaction only if vector species feed during the crepuscular and nocturnal periods when jays are roosting. Although most *Culicoides* species prefer to feed during crepuscular or nocturnal periods (Kettle, 1965, 1977), peak activity varies among species (Blanton and Wirth, 1979). Atkinson et al. (1988) studied the activity cycles of *C. arboricola*, *C. edeni*, and *C. knowltoni* at Fisheating Creek, 19 km south of Archbold and found that all three species were crepuscular and nocturnal, with most biting activity occurring within the first hour after sunset. This crepuscular and nocturnal activity, combined with vertical stratum preferences of 10–12 m above the ground, places these three species in the forest canopy at a time when they are most likely to encounter roosting blue jays.

Of the five most abundant ornithophilic species of *Culicoides* at Archbold (Table 1), *C. edeni*, *C. knowltoni*, and *C. arboricola* are capable of supporting sporogonic development of *H. danilewskyi* infections, and, therefore, probably serve as the natural vectors in Florida. In addition to these vector species, Bennett and Fallis (1960) found *C. crepuscularis*, *C. stelobezziodes*, and *C. sphagnumensis* also capable of supporting sporogonic development of *H. danilewskyi* in Canada. Both *C. edeni* and *C. arboricola* also were found to support sporogonic development of *H. meleagridis*

(Atkinson et al., 1983). Gametocytes were first observed in the peripheral circulation of jays injected with *C. knowltoni* and *C. arboricola* on day 12 post-infection and in *C. edeni* on day 14 post-infection. This prepatent period is similar to that observed in other studies of *Haemoproteus* species transmitted by *Culicoides* (Fallis and Wood, 1957; Fallis and Bennett, 1960).

The absence of sporogonic development in *C. stellifer* and *C. beckae* may have been due to our methods of preparing or injecting the slurry. Because only one species-specific slurry of *Culicoides* was injected into a single jay, mishandling or damage of sporozoites during slurry preparation may have resulted in false negatives.

Hippoboscids flies also are capable of transmitting species of *Haemoproteus* (Adie, 1925), however, the absence of these flies on hundreds of jays handled during a concurrent study (Garvin and Greiner, 2003) suggests they are either not present, or are at very low abundance at Archbold and, therefore, are not important in the transmission cycle.

Culicoides edeni is likely to be the most important vector of *H. danilewskyi* in blue jays in south Florida. In addition to being physiologically capable of supporting sporogonic development and the most abundant ornithophilic species, its temporal and spatial overlap with nesting and roosting jays indicates that it is probably the most important vector of *H. danilewskyi* in southcentral Florida. Similarly, *Culicoides knowltoni* and *C. arboricola* also are likely to contribute to transmission, but to lesser degrees given their relative abundance. In studies of *H. meleagridis* in wild turkeys in south Florida (Atkinson, 1988) concluded that *C. edeni* was the primary vector involved in transmission, although *C. arboricola* also supported sporogonic development. Other less abundant species of *Culicoides* not included in our experimental infections may also be involved in transmission. These include *C. crepuscularis*,

known to transmit *H. danilewskyi* in Canada and *C. hinmani*, capable of supporting development of *H. meleagridis* (Atkinson et al., 1983) and likely involved in its transmission to wild turkeys in south Florida (Atkinson et al., 1988). Studies of natural transmission of avian haematozoa in other species of birds in south Florida should focus on *C. edeni* as the most likely vector species, but also consider the involvement of the less abundant species.

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