HABITAT ASSOCIATIONS OF MOSQUITO AND COPEPOD SPECIES[1]

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ABSTRACT. Copepods and mosquitoes were collected and identified over a 12-month period from three woodland ponds, discarded tires and a salt marsh. The species distribution of both mosquitoes and copepods varied among habitats and seasonally. *Acanthocyclops vernalis* was the predominant copepod in all of the habitats except the discarded tires, where *Thermocyclops dybowskii* was the predominant species. *Amblyospora* sp.-infected mosquitoes and copepods were found on several occasions in one of the woodland ponds and in the salt marsh. The results indicate that several copepod species have the potential to influence larval mosquito populations either directly as predators or indirectly as intermediate hosts of parasites.

INTRODUCTION

Crustaceans in the subclass Copepoda are almost universally distributed in aquatic habitats, and can be numerous in areas producing mosquito larvae. The nature and extent of the interactions between copepods and mosquitoes are largely unknown. However, copepods serve as intermediate or alternate hosts for organisms parasitic in mosquitoes, such as fungi in the genus *Coelomomyces* (Federici et al. 1985), and Microsporidia in the genus *Amblyospora* (Sweeney et al. 1985, Andreadis 1985). In addition, several copepod species are predatory on first-instar mosquito larvae (Riviere and Thirel 1981, Williamson 1986).

Knowledge of the copepod species that inhabit mosquito habitats is necessary to further investigations of mosquito/copepod interactions. This paper identifies the copepod species coincident with mosquito larvae in several habitats located in southwestern Louisiana. The primary purpose was to identify copepods that, because of their temporal and spatial associations with mosquito larvae, have the most potential as intermediate hosts of *Amblyospora* sp. parasites.

MATERIALS AND METHODS

Copepods and mosquito larvae were collected bimonthly for 12 months beginning in October 1985 in three woodland ponds, and in discarded tires. Bimonthly collections were made for eight months in two salt marsh sites.

Lag Hole woodland pond was an extensive site located in an upland, primarily hardwood forest located near Moss Buff, Louisiana. This site rarely dried out between rainfalls. In addition to mosquitoes and copepods, this pond frequently contained numerous invertebrate (e.g., Odonata nymphs, dytiscid beetle larvae) and vertebrate (e.g., *Gambusia*) predators. The bottom of this pool consisted mainly of dead leaves and pine needles.

Haymark woodland pond was located in a mixed coniferous/live oak forest, adjacent to a salt marsh near Lake Charles, Louisiana. This site consisted of a series of shallow depressions that frequently dried completely between rainfalls. Occasionally, invertebrate predators were found at this site. The bottom of this pool was predominantly dried pine needles and leaf litter.

Breaux Road woodland pond was a small pool formed by tire ruts in the floor of a deciduous forest near Chloe, Louisiana. This site dried out quickly between rainfalls. Predators were never noted at this site. The bottom of the pool was bare soil.

The Lacassine tire site was a pile of discarded tires on the edge of a deciduous forest near Lacassine, Louisiana. The tires consistently contained water throughout the study.

The samples at Haymark salt marsh were taken at two sites; one within 200 m of the marsh edge and the other ca. 2 km into the marsh. The mosquito and copepod species found in the two areas were similar, so the samples were pooled for analysis.

Copepods and mosquito larvae were sampled by dipping large volumes of water from a variety of locations within each site. The water was passed through a series of wire screens. Mosquito larvae were retained by the larger mesh sizes, and the copepods were retained and concentrated by the 125 mesh screen. Live copepods and mosquitoes were placed in separate glass jars and transported to the laboratory.

While still alive, each third- and fourth-instar mosquito larva was examined on a black spot plate for the presence of patent Microsporidia infections. These infections, which are evidence of transovarial transmission of the Microsporidia, are readily seen as large, porcelain-white patches of cells under the cuticle (Hazard 1985).

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Those with patent infections were smeared on a glass slide, fixed for 2 min. in absolute methanol, stained for 10 min. in 10% Giemsa and rinsed with deionized water. The slides of smeared tissue were then observed with a compound microscope to confirm the identity of the Microsporidian species. *Amblyospora* sp. in the mosquito larvae were identified by the characteristic morphology of their vegetative stages and spores (J. Becnel, unpublished data). No attempt was made to screen mosquito larvae for orally acquired Microsporidia infections. Uninfected larvae were preserved in 95% methanol for later identification and counting.

Copepods in each sample were held alive in the laboratory until the mosquito larvae were screened for patent Microsporidian infections. If Microsporidia-infected mosquito larvae were found, 200 copepods from the sample were smeared on glass slides, stained as described above, and observed for the presence and identity of Microsporidia. *Amblyospora* sp. in the copepods were identified by their characteristic spore morphology and the morphology of their vegetative stages (J. Becnel, unpublished data).

Infections in copepods undoubtedly occur at times when infected mosquito larvae are not present. However, screening copepods for infections when transovarially-infected mosquito larvae were present increased the possibility that infective Microsporidia spores were present in the water at that time.

The remaining copepods were placed in 95% methanol and later identified to species using the techniques and keys provided by Pennak (1978). It was not possible to identify the species of copepods smeared for parasite detection. Copepod identification requires extensive dissection, which leaves insufficient material for preparing a tissue smear. As a result, the presence of Microsporidia in smears of copepod tissue indicates only that one or more of the species in the sample were infected.

RESULTS

The seasonal distribution of mosquito and copepod species collected in each site, and the total number of occurrences of each species in the site, are shown in Tables 1–5. The three woodland ponds produced the greatest variety of mosquito and copepod species. The highest number of species was recorded from Haymark woodland pond. Over the course of the season, this site contained 16 mosquito species and 12 copepod species (Table 2).

While the predominant mosquito species varied among the three woodland ponds, *Acanthocyclops vernalis* was the most frequently encountered copepod (Tables 1–3). In the Haymark woodland pond, *Thermocyclops dybowskii* was found as frequently as *Acanthocyclops vernalis* (Table 2). The Calanoid copepod, *Diaptomus moorei,* also was frequently encountered in the Lag Hole woodland pond (Table 1).

In the Lacassine tires, the container-inhabiting mosquitoes *Aedes triseriatus* (Say) and *Orthopodomyia signifera* (Coquillett) were the most frequently identified species. However, several other permanent water mosquitoes were often found. The predominant copepod species in the tires was *T. dybowskii* (Table 4).

The Haymark salt marsh produced eight mosquito species and eight copepod species (Table 5). *Acanthocyclops vernalis* was the most frequently identified copepod species.

Amblyospora sp. parasites were identified in several mosquito species from five samples in the Haymark woodland pond and two samples in the Haymark salt marsh. Infected copepods were found in one sample in the Haymark woodland pond and two samples in the Haymark salt marsh.

In the Haymark woodland pond, the second November sample produced *Amblyospora*-infected *Culex quinquefasciatus* Say (3 larvae infected/14 larvae examined) and *Culiseta inornata* (Williston) (2/160). The first December sample produced infected *Cx. salinarius* Coq. (6/319) and *Cs. inornata* (1/246). In the second December sample, infected *Cx. salinarius* (5/343) were found. Infected *Cx. salinarius* were also found in both January samples (7/160, 4/258). *Amblyospora*-infected copepod smears were found in the first January collection from Haymark woodland pond.

In the first April sample from the Haymark salt marsh, *Amblyospora*-infected *Ae. sollicitans* (Walker) (3/324) and *Cx. salinarius* (1/213) were found. Infected *Cx. salinarius* were also found in the second June sample from the Haymark salt marsh (3/100). *Amblyospora*-infected copepods were present in the first April and second June samples from this site.

The copepod species *A. vernalis* was present in every sample in which *Amblyospora*-infected copepod smears were found. Also, this species was present in 7 of 9 samples when *Amblyospora*-infected mosquito larvae were found. The other copepod species that were present when *Amblyospora* was found in the habitats were: *Cyclops exilis, C. venustoides, Diacyclops bicuspidatus, Macrocyclops albidus, Paracyclops fimbriatus poppei* and *T. dybowskii.* Of these, all but *C. exilis* and *D. bicuspidatus* were present in samples containing infected copepod smears.

DISCUSSION

Mosquito larval habitats support a rather large and diverse community of copepod species. In this study, 16 cyclopoid copepods species,

Table 1. Occurrence of mosquito and copepod species in bimonthly samples taken from Lag Hole woodland pond.

	Sample period																								Total occur-rences*
	Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		
Mosquito species	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
Culex territans					+	+		+	+	+	+	+	+		+	+	+			+	+	+			14
Anopheles crucians					+	+		+	+		+	+	+			+				+	+				10
Culex salinarius					+	+			+		+														4
Culex restuans									+	+	+	+													4
Culiseta melanura									+	+	+	+													4
Anopheles quadrimaculatus												+				+									2
Aedes triseriatus								+																	1
Culex nigripalpus									+																1
Psorophora ferox			+																						1
Psorophora howardii			+																						1
Copepod species																					X				
Acanthocyclops vernalis			+		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+					16
Diaptomus moorei			+		+	+		+	+	+	+	+	+	+	+	+		+	+						14
Microcyclops varicans rubellus								+				+			+	+		+	+	+					8
Thermocyclops dybowskii			+		+	+		+	+	+				+				+	+						8
Harpacticoida sp.					+	+		+	+		+		+												7
Ectocyclops phaleratus			+			+				+		+	+		+										5
Tropocyclops prasinus																			+	+		+			3
Cyclops latipes											+						+								2
Eucyclops agilis																+				+					2
Macrocyclops albidus																				+		+			2
Macrocyclops ater																+		+							2
Cyclops exilis									+																1
Eucyclops speratus																	+								1
Habitat condition**	D	D		N			N							N				N	N				D	D	

* Total occurrences out of 20 samples taken when habitat contained water.
** D denotes habitat dry when sampled, N denotes habitat sampled but no mosquito larvae were collected.
X Copepod samples lost on this date.

Table 2. Occurrence of mosquito and copepod species in bimonthly samples taken from Haymark woodland pond.

	Sample period*																								Total occurrences**
	Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		
Mosquito species	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
Culex restuans			+	+	+	+	+	+	+	+															8
Culex salinarius				+	X	X	X	X	+	+	+														8
Culiseta inornata				X	X	+	+	+	+	+	+														8
Aedes vexans		+		+	+	+			+															+	6
Culex territans				+		+			+		+														4
Aedes fulvus pallens				+		+											+								3
Psorophora howardii		+															+							+	3
Aedes triseriatus								+	+																2
Anopheles crucians						+					+														2
Psorophora ferox		+																						+	2
Aedes atlanticus																								+	1
Aedes sticticus																								+	1
Aedes tormentor		+																							1
Anopheles bradleyi																	+								1
Anopheles quadrimaculatus				+																					1
Culex quinquefasciatus				X																					1
Copepod species																									
Acanthocyclops vernalis		+	+	+	+	+	+	+	+	+	+						+		+						12
Thermocyclops dybowskii					+	+	+			+	+			+		+	+	+	+	+				+	12
Diacyclops bicuspidatus		+		+	+	+																			4
Cyclops venustoides						+	+	+																	3
Harpacticoida sp.					+				+																3
Cyclops exilis								+	+																2
Macrocyclops albidus							+			+															2
Microcyclops varicans rubellus														+				+							2
Ectocyclops phaleratus			+																						1
Eucyclops agilis																			+						1
Halicyclops sp.																				+					1
Paracyclops fimbriatus poppei										+															1
Amblyospora-infected specimens							X																		
Habitat condition***	D											D	D	N	D	N		N	N	N	D	D	D		

* X denotes the presence of *Amblyopspora*-infected specimens.
** Total occurrences out of 17 samples taken when habitat contained water.
*** D denotes habitat dry when sampled. N denotes habitat sampled but no mosquito larvae collected.

Table 3. Occurrence of mosquito and copepod species in bimonthly samples taken from Breaux Road woodland pond.

	Sample period																								Total occurrences*
	Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		
Mosquito species	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
Aedes vexans			+		+	+		+		+		+									+				7
Culex restuans							+	+		+		+													4
Psorophora ferox			+									+					+				+				4
Psorophora howardii															+			+			+				3
Aedes canadensis										+															1
Aedes sollicitans																		+							1
Aedes triseriatus								+																	1
Anopheles crucians										+															1
Culex salinarius												+													1
Psorophora ciliata																		+							1
Psorophora columbiae					+																				1
Copepod species																					X				
Acanthocyclops vernalis		+	+		+	+	+	+	+	+	+	+			+		+	+	+						14
Microcyclops varicans rubellus							+	+	+	+		+			+										6
Cyclops latipes							+				+							+	+						4
Ectocyclops phaleratus			+		+	+																			3
Thermocyclops dybowskii		+															+		+						3
Harpacticoida sp.								+	+																2
Cyclops exilis															+										1
Diaptomus moorei						+																			1
Habitat condition**	D	N		N					N		N		D	D		D			N	D		D	D	D	

* Total occurrences out of 16 samples taken when habitat contained water.
** D denotes habitat dry when sampled, N denotes habitat sampled but no mosquito larvae were collected.
X Copepod samples lost on this date.

Table 4. Occurrence of mosquito and copepod species in bimonthly samples taken from Lacassine tires.

	Sample period																								Total occur-rences*
	Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		
Mosquito species	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
Aedes triseriatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+	22
Orthopodomyia signifera	+	+	+	+		+	+	+	+		+				+	+	+	+	+		+	+	+	+	18
Culex quinquefasciatus	+	+	+	+	+	+						+			+		+	+	+	+	+	+	+	+	16
Culex salinarius		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+								15
Culex restuans		+	+	+			+	+	+	+	+	+	+	+	+										12
Culex territans	+	+					+	+	+		+	+	+			+									8
Toxorhynchites rutilus septentrionalis	+					+									+	+		+				+	+	+	8
Anopheles crucians								+			+	+	+	+											5
Aedes vexans												+					+								2
Copepod species																					X				
Thermocyclops dybowskii	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+		+	+	+	21
Eucyclops agilis			+							+		+			+			+	+	+					7
Eucyclops speratus								+			+		+	+		+	+								6
Cyclops exilis							+	+	+																3
Ectocyclops phaleratus				+	+				+																3
Acanthocyclops vernalis						+	+																		2
Harpacticoida sp.					+																				1

* Total occurrences out of 24 samples taken when habitat contained water.
X Copepod samples lost on this date.

Table 5. Occurrence of mosquito and copepod species in bimonthly samples taken from Haymark salt marsh.

	Sample period*															Total
	Feb	Mar		Apr		May		Jun		Jul		Aug		Sep		occur-
Mosquito species	1	1	2	1	2	1	2	1	2	1	2	1	2	1	2	rences**
Culex salinarius	+	+	+	X	+	+		+	X		+				+	10
Anopheles bradleyi	+	+	+	+		+		+	+						+	8
Aedes sollicitans				X		+	+				+				+	5
Culiseta inornata	+	+	+													3
Psorophora columbiae											+				+	2
Aedes vexans															+	1
Anopheles crucians				+												1
Culex restuans		+														1
Copepod species																
Acanthocyclops vernalis	+	+		+	+	+	+	+	+		+				+	10
Harpacticoida sp.	+	+	+		+	+	+	+	+							8
Cyclops paramensis					+					+	+				+	4
Halicyclops sp.										+	+					2
Microcyclops varicans rubellus	+				+											2
Cyclops venustoides															+	1
Ectocyclops phaleratus						+										1
Paracyclops fimbriatus poppei				+												1
Amblyospora-infected specimens				X					X							
Habitat condition***										N		D	D	D		

* X denotes the presence of *Amblyospora*-infected specimens.
** Total occurrences out of 17 samples taken when habitat contained water.
*** D denotes habitat dry when sampled, N denotes habitat sampled but no mosquito larvae were collected.

representing 11 genera, and one calanoid copepod species were collected from aquatic habitats producing mosquito larvae. The predominant species collected in all of the habitats except the discarded tires was *A. vernalis*. Also, this species was present in at least one of the sampling sites during every month of the year. *Acanthocyclops vernalis* is considered a very common copepod (Pennak 1978). This frequent occurrence in many mosquito habitats over most of the season, along with the observations that it is an intermediate host of *Coelomomyces* (Federici 1980) and of an unidentified *Amblyospora* species (Andreadis 1985), suggest that *A. vernalis* may have a significant effect on mosquito populations. The fact that *A. vernalis* was found in the habitats almost every time that *Amblyospora*-infected mosquito larvae were found, and every time that infected copepod smears were found leads to the speculation that this species may be involved in the life cycle of *Amblyospora* sp. parasites in the field. However, other species may be involved since the methods used did not permit identification of the infected copepods. More extensive sampling procedures and revised copepod identification/parasite screening techniques allowing identification of the smeared copepod species are necessary for confident identification of the copepod species acting as the intermediate host of parasites in the field.

The other frequently occurring copepod species, *T. dybowskii*, has not yet been investigated as an intermediate host of mosquito parasites, but it is a voracious predator of first-instar mosquito larvae, as is the less frequently occurring *M. albidus* (S. G. F. Hare, unpublished data).

Nothing is known of the potential for the other copepod species in the mosquito habitats to influence mosquito populations. Further research should be conducted to determine the extent to which copepods affect mosquito populations both directly through predation, and indirectly as intermediate or alternate hosts of mosquito parasites.

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